Eco-epidemiología, evolución y filogenética de la tribu Rhodniini: Vectores de la enfermedad de Chagas

Diana Carolina Hernández Castro

Documento de tesis presentado como requisito para optar al título de Doctora en Ciencias Biomédicas y Biológicas

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TABLA DE CONTENIDO

1. LISTA DE PUBLICACIONES

Todos los artículos se encuentran anexos a este documento, la información suplementaria/o tablas serán anexadas en archivos comprimidos siguiendo el número de artículos que se mencionan a continuación:

- **Artículo 1: Hernández C,** Salazar C, Brochero H, Teherán A, Buitrago LS, Vera M, Soto H, Florez-Rivadeneira Z, Ardila S, Parra-Henao G, Ramírez JD. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. Parasit Vectors. 2016 Dec 1;9(1):620.
- **Artículo 2:** Velásquez-Ortiz N, **Hernández C**, Herrera G, Cruz-Saavedra L, Higuera A, Arias- Giraldo LM, et al,.*Trypanosoma cruzi* infection, discrete typing units and feeding sources among *Psammolestes arthuri* (Reduviidae: Triatominae) collected in eastern Colombia. Parasit Vectors. 2019 Apr 8;12(1):157. doi: 10.1186/s13071-019-3422-y
- **Artículo 3:** Arias-Giraldo LM, Muñoz M, **Hernández C**, et al. Identification of blood-feeding sources in *Panstrongylus, Psammolestes, Rhodnius* and *Triatoma* using amplicon- based next-generation sequencing. *Parasit Vectors*. 2020;13(1):434. Published 2020 Aug 31. doi:10.1186/s13071-020-04310-z
- **Artículo 4:** Rincón-Galvis HJ, Urbano P, **Hernández C**, Ramírez JD. Temporal Variation of the Presence of *Rhodnius prolixus* (Hemiptera: Reduviidae) Into Rural Dwellings in the Department of Casanare, Eastern Colombia. J Med Entomol. 2019 Sep 27. pii: tjz162. doi: 10.1093/jme/tjz162.
- **Artículo 5:** Velásquez-Ortiz, N., Herrera, G., **Hernández C**, Muñoz M, Ramírez JD. Discrete typing units of *Trypanosoma cruzi*: Geographical and biological distribution in the Americas. *Sci Data* 9, 360 (2022). https://doi.org/10.1038/s41597-022-01452-w
- **Artículo 6:** Nascimento JD, da Rosa JA, Salgado-Roa FC, **Hernández C**, Pardo-Diaz C, Alevi KCC, et al,. Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species? PLoS One. 2019 Feb 7;14(2):e0211285. doi: 10.1371/journal.pone.0211285. eCollection 2019.
- **Artículo 7: Hernández, C.,** Alvarado, M., Salgado-Roa F.C., Ballesteros N, Rueda -M N., *et al.* Phylogenetic relationships and evolutionary patterns of the genus *Psammolestes* Bergroth, 1911 (Hemiptera: Reduviidae: Triatominae). *BMC Ecol Evo* 22, 30 (2022). https://doi.org/10.1186/s12862-022-01987-x
- **Artículo 8: Hernández C,** da Rosa JA, Vallejo GA, Guhl F, Ramírez JD. Taxonomy, Evolution, and Biogeography of the Rhodniini Tribe (Hemiptera: Reduviidae). Diversity 2020, 12(3), 97; https://doi.org/10.3390/d12030097
- **Artículo 9: Carolina Hernández**, Mateo Alvarado, Fabian C. Salgado-Roa, Joao Aristeu da Rosa, Carolina Pardo, Nathalia Ballesteros, Mateo Alvarado, Jader

Oliveira, Clever Galvao, Simone Freitas, Jose Calzada, Juliana Damieli Nascimento, Lineth Garcia, Mario Grijalva, Anita Villacis, Hernan Carrasco, Maikell Segovia,Cesar Gomez Hernandez, Plutarco Urbano, Omar Cantillo, Marina Muñoz, Felipe Guhl, Gustavo Vallejo, Julio cesar Carranza, Luz Stella Buitrago, Marina Stella Gonzalez, Kaio Cesar Chaboli Alevi, Andres Cuervo, Claudia Sandoval, Camilo Salazar, Juan David Ramírez. Enfoque multilocus en la resolución de conflictos filogenéticos y evolutivos de la tribu Rhodniini (por someter).

- Link Verificación:

https://scholar.google.com/citations?hl=es&user=gXh8vqEAAAAJ&view_op=list_works&sortby=pubdate

2. LISTA DE ANEXOS

2.1. Anexos de artículos

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2.2. Anexos de productos de la tesis

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Anexos_Reconocimientos

Anexos_Presentación en eventos

Anexo_Pasantía

3. LISTA DE FIGURAS Y TABLAS

3.1. LISTA DE FIGURAS

3.2. LISTA DE TABLAS

4. LISTA DE ABREVIATURAS

RAPD: Random Amplified Polimorphic DNA (Fragmentos polimórficos amplificados al azar)

ADN: Ácido desoxirribonucleico

NJ: (Por sus siglas en inglés Neighbor-Joining)

MP: Máxima parsimonia

MV: Máxima verosimilitud

IB: Inferencia Bayesiana.

DTU: Discrete Typing Units (Unidades de tipificación discretas).

18S: *18S rRNA*

28S: 28S rRNA

Cyt b: Citocromo b

12S: 12S rRNA

COI: Citocromo oxidasa I

COII: Citoctomo oxidasa II

rDNA: ADN ribosomal

5. RESUMEN

La subfamilia Triatominae comprende 158 especies existentes y tres fósiles, esta subfamilia presenta importancia en salud pública, debido a que algunas especies son capaces de transmitir el parasito protozoario *Trypanosoma cruzi*, responsable de la enfermedad de Chagas, la cual es de gran impacto en países de Latinoamérica. Existen diferentes mecanismos de transmisión de la enfermedad, entre ellos se encuentran: la transmisión vectorial, transfusional, congénita, mediante trasplante de órganos, accidental en el laboratorio y la transmisión oral. Sin embargo, el principal mecanismo de transmisión es el vectorial, que se produce por el contacto de humanos con heces de triatominos infectados.

El control vectorial requiere de varios aspectos clave para mitigar la transmisión de *T. cruzi*, entre estos la adecuada identificación de especies de insectos vectores, estudiar la dinámica de su distribución actual, delimitar claramente estás y aportar información como su adaptación a las diferentes variantes del parasito y hospederos, su historia evolutiva, sus patrones de diversificación, diferencias genéticas dentro y entre especies, así como la determinación del flujo genético y su posible aporte en la especiación y adaptación de estas a diferentes condiciones ambientales.

Dentro de los principales vectores incriminados en enfermedad de Chagas se encuentran especies de la tribu Rhodniini, por ende, las medidas y estrategias de control actuales en su mayoría están dirigidas a varias especies de este grupo, a pesar de ello su panorama evolutivo y taxonómico no es claro, y por ende tampoco su impacto en su rol como vectores. Por lo anterior, esta tesis se encamina a describir características epidemiológicas (tasas de infección, preferencias alimenticias y DTUs: Unidades de tipificación discretas) de algunas especies de la tribu Rhodniini, determinar patrones filogenéticos y evolutivos en estas especies en una amplia distribución geográfica de Latinoamérica (Colombia, Brasil, Argentina, Venezuela, Panamá y Ecuador) mediante el uso y diseño de diferentes de herramientas moleculares. Nuestros resultados reconfirman el rol de las especies consideradas domiciliadas dentro de la tribu en las que se han detectado y muestran que existe el riesgo de que otras especies consideradas vectores secundarios o no vectores del parásito puedan involucrarse en escenarios de transmisión al huésped. Sumado esto a la complejidad taxonómica de la tribu pone en riesgo el éxito de los programas de control

vectorial dirigidos a las especies generadoras de riesgo, por esto con nuestro estudio proporcionamos evidencia filogenética de la clasificación de la tribu en dos géneros, el estatus de algunas especies del grupo *prolixus* y las relaciones interespecíficas del grupo *pallescens*, aspectos que en conjunto son de gran importancia en el diseño de estrategias de control vectorial dirigido a dichas especies.

6. MARCO TEÓRICO

1. Enfermedad de Chagas

La enfermedad de Chagas es causada por el parasito hemoflagelado *Trypanosoma cruzi*, afecta de 6 a 8 millones de personas en el mundo y es endémica en 21 países donde produce más de 10.000 muertes al año [1,2]. Existen diferentes mecanismos de transmisión de la enfermedad, entre ellos se encuentran: la transmisión vectorial, transfusional, congénita, mediante trasplante de órganos, accidental en el laboratorio y la transmisión oral [3]. Sin embargo, el principal mecanismo de transmisión es el vectorial que se produce por el contacto de humanos con heces de insectos infectados, de la subfamilia Triatominae (Hemiptera: Reduviidae) y aproximadamente 70 millones de personas están en riesgo de adquirir la infección de *T. cruzi* por transmisión vectorial [1].

2. Subfamilia Triatominae

La subfamilia Triatominae (Hemiptera: Reduviidae), se compone de 158 especies (155 especies existentes y tres fósiles) dentro de 18 géneros y 5 tribus [4–7]. Los triatominos pertenecen a la familia Reduviidae, conformada por insectos predadores, fitófagos y hematófagos, estos últimos se agrupan en la subfamilia Triatominae, siendo el hábito hematofágico su principal característica, ya que incluye los insectos de este grupo en el ciclo de vida del parásito *T. cruzi* [8].

La subfamilia Triatominae, se ha clasificado en 5 tribus, basándose en sus características ecológicas, biológicas y morfológicas [9–12]. Las tribus Alberproseniini y Cavernicolini, que están compuestas por un género y dos especies, la tribu Boldoderini con 4 géneros y 14 especies. Estas tres tribus han sido poco estudiadas desde el punto de vista taxonómico y filogenético, debido a que las especies que las integran no se consideran importantes como vectores de la enfermedad de Chagas [7,10,13,14]. Sin embargo, cabe resaltar que todas las especies que integran la subfamilia se consideran potencialmente vectores del parásito *T. cruzi* [10,15]. Las dos tribus restantes, son la tribu Triatomini con 7 géneros y 116 especies en las que se encuentran los tres fósiles reportados a la fecha (*Triatoma dominicana*,

Panstrongylus hispanolae y *Paleotriatoma metaxytaxa*) y la tribu Rhodniini compuesta por 2 géneros y 24 especies. Estas dos tribus son las más diversas en términos de especies, las más relevantes epidemiológicamente y por ende las más estudiadas [15–17]. A pesar de la gran relevancia epidemiológica de los insectos de la subfamilia Triatominae, su estatus taxonómico y el de las tribus que la integran es aún controversial [10,11,18–21].

3. Tribu Rhodniiini

La tribu Rhodniini está compuesta por dos géneros, el género *Rhodnius* que consta de 21 especies y el género *Psammolestes* con 3 especies (Tabla 1). Inicialmente, se propuso que las tres especies que pertenecen al género *Psammolestes* debían ser agrupadas en una tribu que fue llamada *Psammolestini,* esto dado las marcadas diferencias morfológicas con el género *Rhodniu*s [22]. Posteriormente los dos géneros fueron agrupados en la tribu Rhodniini, principalmente con base en la presencia de tuberosidades posteriores a los ojos y teniendo en cuenta que en su mayoría son especies principalmente arborícolas con excepción de algunas especies de *Rhodnius* [9,10,23].

La tribu Rhodniini ha sido una de las más estudiadas debido a su importancia epidemiológica. La especie *R. prolixus* es uno de los principales vectores de la enfermedad de Chagas, debido a su amplia distribución geográfica que abarca desde Centroamérica y se extiende por los países andinos del norte, su capacidad de domiciliación, su alta frecuencia de dispersión y buena capacidad vectorial. Se han encontrado tres especies domiciliadas dentro de la tribu Rhodniini: *R. ecuadoriensis* en la zona norte de Perú y Ecuador, *R. stali* en Bolivia y *R. pallescens* en Panamá. Adicionalmente, *R. prolixus* presenta una tasa de infección por *T. cruzi* entre el 12.0% - 82.0% [16,24–27], *R. ecuadoriensis* entre el 10.0% – 42.0.% [28–30], *R. pallescens* entre 42.0% -87.4% [25,31–35] y *R. stali* del 7.7% [36]. Adicional a las especies domiciliadas, las especies *R. robustus, R. neglectus, R. neivai, R.nasutus, R.brethesi, R. pictipes* y *R. colombiensis* del género Rhodnius y *P. arthuri* del género *Psammolestes* se han descrito infectadas con *T. cruzi* y sus diferentes variantes genéticas [37,38].

El género *Rhodnius* de la tribu ha sido clasificado en 3 grupos: *pictipes*, *pallescens* y *prolixus*, con base en su distribución geográfica, biogeografía y morfología. Inicialmente estos grupos fueron llamados linajes, sin embargo, dado que se ha cuestionado su origen monofilético actualmente se usa el termino grupo [10,11,19,20].

Tabla 1. Géneros y especies de la tribu Rhodniini

Género	Grupo	Especie
Rhodnius	***pictipes***	***R. amazonicus, R. brethesi, R. paraensis, R. pictipes, R. stali, R. zeledoni, R. micki*** [39]
	pallescens	***R. colombiensis, R. ecuadoriensis, R. pallescens***
	prolixus	***R. barretti, R. dalessandroi, R. domesticus, R. milesi, R. marabaensis, R. montenegrensis, R. nasutus, R. neglectus, R. neivai, R. prolixus, R. robustus , R. taquarussuensis****
Psammolestes		***P. arthuri, P. coreodes, P. tertius***

*** Sinonimizada como variante fenotípica de *R. neglectus*** [40]

3.1 Distribución geográfica

La tribu Rhodniini presenta una amplia distribución geográfica la cual va desde centro América hasta el cono sur **(Figura 1)**. La distribución de la riqueza de especies de la tribu es unimodal, con un número mayor de especies hacia el hemisferio norte en latitudes bajas y algunas especies en el hemisferio sur, alcanzando el nivel de latitud 30° sur [4,5].

Figura 1. Distribución geográfica de la Tribu Rhodniini. Mapa realizado con la base de datos reportada por Cecarelli y colaboradores. Los puntos color naranja corresponden a los ejemplares de la tribu Rhodniini reportados a la fecha [5].

El género *Psammolestes*, presenta una distribución geográfica muy interesante, esto dado que cada una de las tres especies de esté genero tienen una distribución particular (**Figura 2**). Se encuentra principalmente en nidos de aves (Furnariidae y Psittacidae), aunque se ha capturado también en cuevas y palmeras [9,41,42]. La especie *P. arthuri* se encuentra distribuida en Colombia y Venezuela. En Colombia, se ha descrito en los departamentos de la Orinoquia (Casanare, Meta y Arauca) y en Venezuela se ha descrito en 15 estados diferentes principalmente en la región de los llanos[4,5,23].

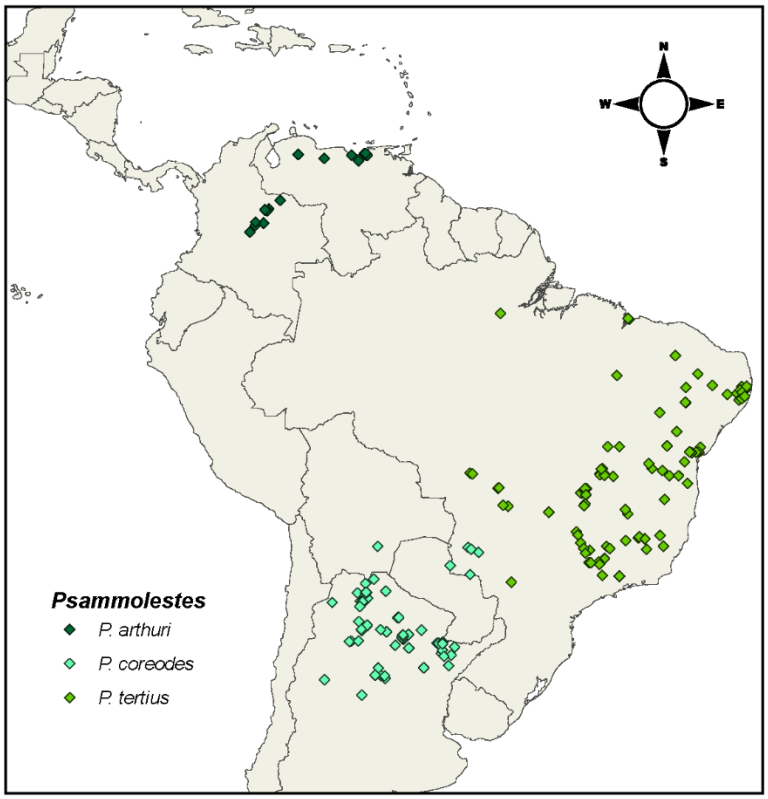

Figura 2. Distribución geográfica de especies del género *Psammolestes*. Mapa realizado con la base de datos reportada por Cecarelli y colaboradores[5].

Para el género *Rhodnius* la distribución geográfica es mayor, abarcando parte de Centroamérica y gran parte de América del sur. La distribución geográfica es una de las características que ha permitido la clasificación de los grupos previamente mencionados, así las especies del grupo *pallescens* se encuentran distribuidas al oeste de la cordillera de los Andes (Figura 3A), mientras que las especies de los grupos *prolixus* y *pictipes* se encuentran distribuidas al este de los Andes (Figuras 3B y 3C) [10].

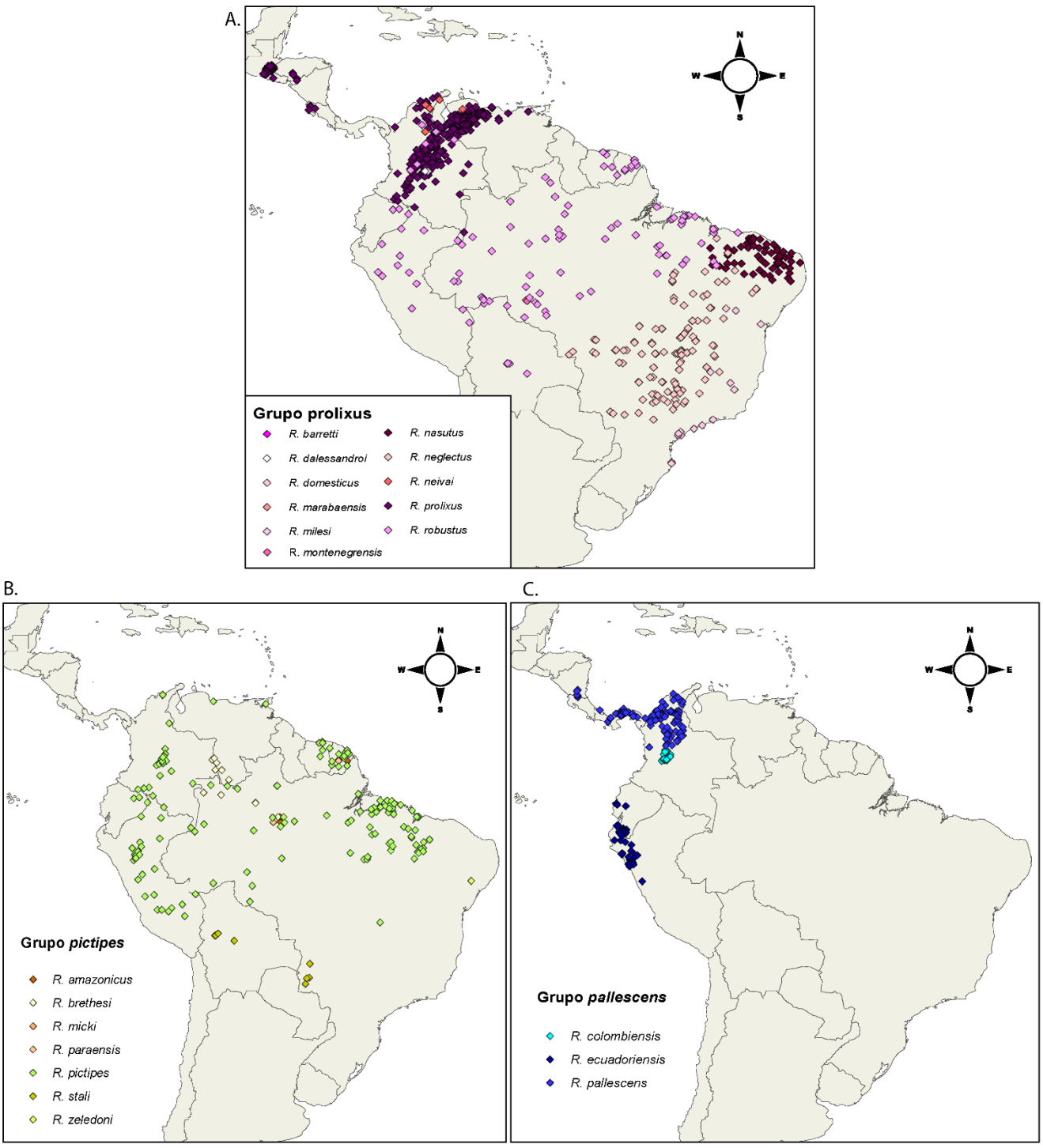

Figura 3. Distribución de especies del género *Rhodnius*. A. Distribución geográfica de especies del grupo *prolixus* **B.** Distribución geográfica de especies del grupo *pictipes* **C.** Distribución geográfica de especies del grupo *pallescens.* Mapas realizados con la base de datos reportada por Cecarelli y colaboradores [5].

3.2 **Estudios taxonómicos y filogenéticos de la tribu Rhodniini**

La taxonomía y sistemática de la tribu Rhodniini ha sido muy compleja, los esfuerzos por clasificar las especies de esta tribu se remontan a mediados del siglo XVIII, en los que se inició la descripción de las especies *R. prolixus, R. pictipes* y *R. nasutus*. La especie más estudiada es *R. prolixus* por ser un importante vector de la enfermedad de Chagas y dado que su ciclo de vida es relativamente corto en comparación con los demás triatominos ha sido utilizada como modelo biológico para estudios de fisiología y biología de los Triatominae [16,20]

3.2.1. Morfología de la tribu Rhodniini

Los estudios dirigidos a la clasificación taxonómica de la tribu Rhodniini, inicialmente se basaron en el estudio de las similitudes y diferencias morfológicas entre especies e incluyeron también aspectos biogeográficos. Sin embargo, una de las principales limitaciones especialmente de la tribu Rhodniini es la escasa variabilidad morfológica entre especies [9,11]. Los caracteres que han sido utilizados clásicamente para la clasificación de la tribu Rhodniini son a nivel general, el tamaño, el color o patrones de coloración en algunas partes del insecto y el aspecto de la cutícula. Adicionalmente, se utilizan diversos caracteres a nivel de la cabeza, tórax, abdomen y las piernas [11,12,20]. Por otro lado, se han propuesto nuevos caracteres para su uso en la identificación de triatominos, que se han utilizado en la tribu Rhodniini, tales como la espermateca, morfometría geométrica de las alas, morfología de los segmentos abdominales IX y X, coloración de las glándulas salivales y morfología de la genitalia; algunos de los cuales han proporcionado diferenciación a nivel de la tribu (presencia de nitroforinas que dan coloración roja a las glándulas salivales de la tribu Rhodniini y la morfometría de las alas) y a nivel intra-especifico del género *Rhodnius* (Forma de la genitalia femenina) [11,43–46].

La inclusión de los géneros *Rhodnius* y *Psammolestes* dentro de la tribu Rhodniini obedece al comportamiento principalmente arborícola y la presencia de tuberosidades post-oculares,

estas últimas exclusivas en los dos géneros [9,19,47,48]. Entre los dos géneros se observan diferencias en la morfología de la cabeza y la forma de los fémures de las piernas, sin embargo, los caracteres observados no fueron suficientes para reconstruir con precisión un cladograma e investigar la relación entre los géneros (Figura 4) [10,11].

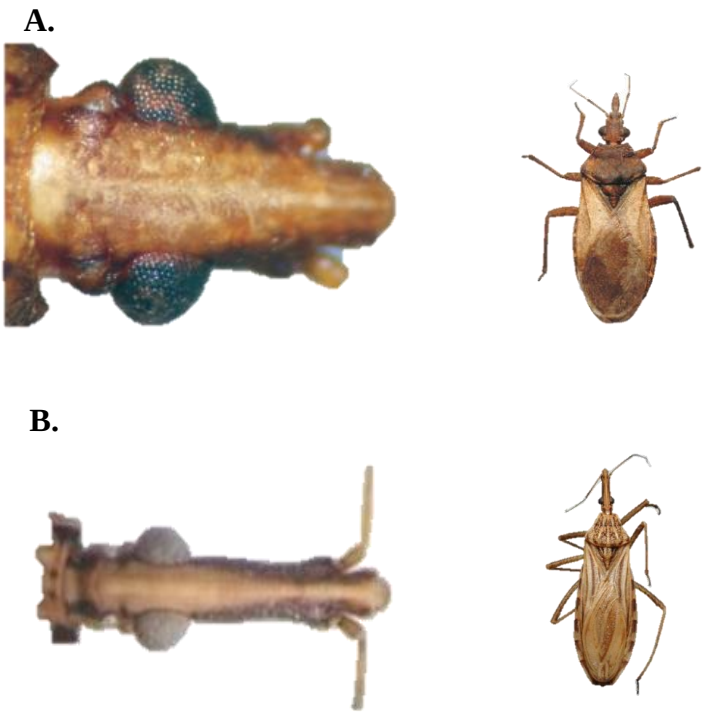

Figura 4. Morfología de la cabeza y cuerpo completo de los géneros de la tribu Rhodniini. A. Morfología del género *Psammolestes* (*P. arthuri*) **B.** Morfología del género *Rhodnius* (*R. pallescens*).

En la tribu *Rhodniini*, adicional a los estudios morfológicos, se han aplicado estudios citogenéticos y moleculares, proporcionando diferentes niveles de resolución en el estudio de las relaciones interespecíficas de la tribu. Sin embargo, cabe resaltar que muchos de estos estudios se han enfocado solo en la diferenciación o análisis de algunas especies y son pocos los que tienen como objetivo aclarar las relaciones de todos los integrantes de la tribu [20,49].

3.2.2. Estudios citogenéticos en la tribu Rhodniini

En cuanto a los estudios citogenéticos aplicados en la tribu Rhodniini, al igual que en toda la subfamilia Triatominae, se han podido observar aspectos diferenciales: *i.* los cromosomas son holocéntricos (No poseen constricción primaria) *ii.* El cinetocoro es difuso a lo largo de los brazos cromosómicos *iii.* La segregación de los cromosomas durante la mitosis ocurre mediante movimiento "holocinetico" (las cromatidas se dirigen a los polos en forma paralela al plano ecuatorial) *iv.* Durante la meiosis la asociación de microtúbulos se halla restringida a los extremos cromosómicos, estos son opuestos entre primera y segunda división meiótica *v.* Los cromosomas sexuales segregan ecuacionalmente durante la primera división meiótica y reduccionalmente durante la segunda *vi*. Los cromosomas tienen un tamaño pequeño [50–55].

En la tribu Rhodniini se ha observado que el número de cromosomas es homogéneo en todas las especies analizadas hasta el momento (17 especies: 14 especies de *Rhodnius* y 3 especies de *Psammolestes*), en las que se encuentran representados los dos géneros y los tres grupos del género *Rhodnius*, así el genoma diploide de la tribu está conformado por 20 autosomas y los cromosomas sexuales XY, que en primera instancia diferencian esta tribu de la tribu Triatomini en la que se observan variaciones en cuanto al número de autosomas y cromosomas sexuales, presente en sus especies y complejos [51,54–56]
Adicionalmente, todas las especies de la tribu Rhodniini analizadas hasta el momento evidencian bandeo de heterocromatina C en el cromosoma Y [55].

También se ha observado persistencia nucleolar durante la meiosis, la cual consiste en la presencia de nucléolos o corpúsculos nucleolares durante la metafase meiótica en 15 miembros de la tribu (*P. tertius*, *R. brethesi*, *R. colombiensis*, *R. domesticus*, *R. ecuadoriensis*, *R. milesi*, *R. montenegrensis*, *R. nasutus*, *R. neglectus*, *R. neivai*, *R. pallescens*, *R. pictipes*, *R. prolixus*, *R. robustus*, *R. stali*) [57,58]. En cuanto al tamaño del genoma, se ha documentado la medición mediante citometría de flujo en cuatro especies de la tribu, *R. ecuadoriensis*, *R. colombiensis*, *R. pallescens* y *R. prolixus* que mostraron un tamaño de 0.72, 0.58, 0.73 y 0.75 picogramos de contenido haploide de ADN respectivamente, las cuales son menores en comparación con todos los demás triatominos [50,51,55,59].

Los estudios a nivel de género han permitido evidenciar en *Psammolestes* que en las tres especies se presentan heterocromatina y repeticiones de AT en el cromosoma Y, el cromocentro formado por los cromosomas sexuales y ausencia de heterocromatina autosómica [47,51,55,56,60]. Los estudios del género *Rhodnius* han permitido evidenciar la presencia de heterocromatina autosómica en *R. pallescens, R.colombiensis, R. domesticus, R. nasutus, R. taquarussuensis* (*R. neglectus*) y la ausencia de está en las especies *R. brethesi, R. ecuadoriensis, R. pictipes, R. neglectus, R. prolixus, R. robustus, R. montenegrensis*. En las especies que se encuentra la heterocromatina C normalmente se observan bandas muy diminutas [51,56,61,62]. Sin embargo, a pesar de la homogeneidad en los caracteres citogenéticos mencionados en la tribu, se han encontrado diferencias a nivel intra e interespecíficas de la tribu en la ubicación cromosómica de los genes ribosomales. Se han observado dos patrones de ubicación, uno en los dos cromosomas sexuales X e Y (*P. tertius, R. domesticus, R. neglectus, R. neivai, R. milesi, R. pictipes, R. pallescens y R. stali*) y otro solamente en el cromosoma X (*R. nasutus, R. prolixus, R. robustus, R. colombiensis* y *R. ecuadoriensis*), en *R. ecuadoriensis* se observaron los dos patrones, uno en poblaciones de Perú y otro en poblaciones de Ecuador. Lo anterior dejo en evidencia que la detección de genes ribosómicos mediante técnicas citogenéticas puede ser un marcador útil para detectar la divergencia reciente de especies o poblaciones y permitió formular dos hipótesis con respecto a la evolución y presencia de los patrones encontrados que pueden deberse a: *i*. Un mecanismo de perdida de los loci en el cromosoma Y, siendo el patrón XY el más ancestral *ii*. La transferencia parcial de genes desde el cromosoma X al Y, mediante mecanismos de transposición o recombinación ectópica entre cromosomas sexuales [61,63].

Se ha realizado el análisis de heterocromatina C a nivel intraespecífico en diferentes ejemplares de *R. pallescens* obtenidos varias localizaciones geográficas en Colombia y Panamá, observándose variabilidad intraespecífica de la especie con la presencia de dos citotipos con diferencias en el tamaño, número y distribución de heterocromatina C durante la mitosis y meiosis, cuyas frecuencias fueron diferentes en relación a las características ecológicas y geográficas de los sitios de recolección. Adicionalmente, los citotipos

concuerdan con diferencias morfológicas en cuanto al tamaño, morfometría de las alas y caracteres de la cabeza [59,64]

Finalmente, también se han utilizado caracteres citogenéticos (cantidad y tamaño de cromosomas, heterocromatina autosómica y en cromosomas sexuales, ubicación de genes ribosomales) para evaluar la variabilidad intraespecífica de diferentes poblaciones de las especies *R. prolixus* y *R. neglectus*, sin embargo, no se ha encontrado variación de estos con respecto a las características ecológicas y geográficas de las poblaciones analizadas [65](106).

3.2.3. Análisis moleculares en especies de la Tribu Rhodniini

Diferentes análisis moleculares han sido implementados, para estudiar las relaciones filogenéticas y diversidad intraespecífica de especies la tribu Rhodniini. Estos estudios han utilizado isoenzimas, RAPD, microsatélites, secuenciación de marcadores de ADN nucleares y mitocondriales y secuenciación de genomas [11,20]. Sin embargo, en cuanto a las relaciones filogenéticas de la tribu, se han detectado diferentes aspectos que han sido controversiales en los estudios publicados: *i.* Se ha observado parafilia de especies del grupo *prolixus* del género *Rhodnius* con respecto a *Psammolestes ii.* La agrupación de linajes dentro del género *Rhodnius* ha sido incongruente y a su vez la ubicación de algunas especies dentro de los grupos *iii.* La identidad de algunas especies es cuestionable debido a la presencia de incongruencias en algunas filogenias. Estos aspectos controversiales, se deben a que en muchos estudios no se han utilizado todas las especies de la tribu, la mayoría de los estudios son intraespecíficos, el número de marcadores en los estudios es escaso y que la mayoría de ellos se limitan a describir linajes, pero no delimitar correctamente todas las especies y a entender los procesos evolutivos que llevaron a su formación [10,11,19,20].

Tabla 2. Características de estudios filogenéticos que han evaluado la relación entre grupos del género *Rhodnius*

Agrupación	Marcador Nuclear	Marcador Mitocondrial	Taxones	Método Reconstrucción	Referencia
pictipes + pallescens		16S	4	MP	Stohard et al.,1998
		16S, Cyt b	8	NJ	Lyman et al.,1999
		16S, Cyt b	15		Schofield y Dujardin, 1999
	28S	16S, Cyt b	13	MP, NJ	Monteiro et al.,2000
		Cyt b	18	NJ	Monteiro et al.,2018
	28S	Cyt b	9	NJ	Marquez et al.,2011
		Cyt b	12	MP	Maia da Silva et al., 2007
		Cyt b	5	MV, IB	Da Rosa et al.,2012
	28S, Wg	16S	13	MV, NJ	Da Paula et al.,2021
	51 marcadores	Genoma mitocondrial	36	MV, IB	Filee et al.,2022
pictipes + prolixus		16S, 12S	14	MP, NJ	Hypsa et al.,2002
		16S	14	MP, MV	De Paula et al., 2007
		16S	14	MP, MV	De Paula et al.,2005
	18S, 28S	16S, Cyt b, COI, COII	10	MV, IB	Justi et al.,2014
	18S, 28S, Wg	16S	11	MV, IB	Justi et al.,2016
	28S, Wg	16S	13	IB	Da Paula et al.,2021
		Cytb	ND	MV	Filee et al.,2022
pallescens + prolixus	Elementos ultraconservados y ribosomales (Illumina)		12	MV	Kieran et al.,2021

*En este estudio se consolidaron varios marcadores: RAPD, Isoenzimas, morfometría y secuenciación de marcadores. **NJ:** (Por sus siglas en inglés Neighbor-Joining), **MP:** Máxima parsimonia, **MV**: Máxima verosimilitud, **IB**: Inferencia Bayesiana.

Debido a que los datos morfométricos, citogenéticos y marcadores moleculares han generado resultados contradictorios en varios aspectos sistemáticos y filogenéticos de la tribu Rhodniini. Existen varias hipótesis contradictorias acerca del origen y diversificación de los géneros, grupos y especies de esta tribu [19,20,66].

Los estudios más recientes han utilizado concatenados de genes y secuenciación de genomas en un amplio número de especies de la tribu Rhodniini con el fin de solucionar las incongruencias presentadas. El análisis mediante secuenciación de Ilumina de secuencias ultraconservadas y rDNA en ejemplares de entre 8 y 16 especies, evidenció nuevamente la parafilia del grupo *prolixus* y *Psammolestes.* Además, se propone una nueva agrupación

entre grupos conformada por las especies de los grupos *prolixus* y *pallescens* como clados hermanos [21]. Por otro lado, el concatenado tres marcadores (dos nucleares y uno mitocondrial) en ejemplares entre 2 y 13 especies de la tribu, recupero el género *Psammolestes* como un clado monofilético dentro del grupo *prolixus* del género *Rhodnius* en algunas reconstrucciones [66].

Finalmente, el estudio más reciente realiza secuenciación de genomas de 17 especies de 36 ejemplares (incluyendo como especie: *R. taquarussuensis*), particularmente en este estudio se detectan incongruencias entre los tipos de marcadores utilizados (mitocondriales, nucleares y ribosomales), específicamente observaron incongruencias entre las agrupaciones de los grupos del género *Rhodnius* y la parafilia del del grupo *prolixus* del género *Rhodnius* con *Psammolestes tertius* que fue la única especie del género incluida en el estudio. En cuanto a las especies del género *Rhodnius*, en esté estudio se propone que exista un nuevo complejo de especies conformado por *R. marabaensis*, *R. montenegrensis*, *R. robustus* y *R. prolixus.* Además, se cuestiona el estatus de la especie *R. milesi* y se propone que *P. tertius* se incluya en el género *Rhodnius* [49]

Persisten incongruencias entre las relaciones taxonómicas recuperadas mediante taxonomía clásica y la taxonomía molecular, siendo las más relevantes: (*i*) la parafilia del género *Rhodnius* con respecto a *Psammolestes*, (*ii*) diferencias en las agrupaciones de especies del género *Rhodnius* y (*iii*) estatus taxonómico de algunas especies del grupo *prolixus* en el género *Rhodnius* [20,21,49]. Por lo anterior, las hipótesis acerca del origen, evolución y dispersión de las especies de la tribu Rhodniini también han sido contradictorias, es por eso por lo que se requieren estudios en los que se mejoré el muestreo y tipo de datos para dar solución a las incongruencias y con ello formular hipótesis más cercanas a la realidad taxonómica, evolutiva y biogeográfica de la tribu Rhodniini y de esta manera contribuir al control vectorial de la enfermedad de Chagas [7,67].

7. OBJETIVOS

7.1. General

Describir la eco-epidemiologia, estructura filogenética y evolutiva de especies pertenecientes a la tribu Rhodniini usando herramientas moleculares.

7.2. Específicos

7.2.1. Describir la eco-epidemiología molecular asociada a la transmisión vectorial de las especies de la tribu Rhodniini en Colombia.

7.2.2. Diseñar y evaluar marcadores moleculares nucleares a partir del genoma de *R. prolixus* para estudios evolutivos en todas las especies de la tribu Rhodniini.

7.2.3. Realizar la reconstrucción filogenética molecular de los taxones (géneros y especies) y resolución de conflictos de la tribu Rhodniini mediante un enfoque multilocus.

8. INTRODUCCIÓN A LOS CAPÍTULOS

La enfermedad de Chagas pertenece al grupo de enfermedades tropicales desatendidas, es decir, enfermedades que afectan principalmente a las poblaciones más vulnerables y con el menor acceso a los servicios de salud. Esta enfermedad causa la pérdida de aproximadamente 2 millones de años de vida ajustados por discapacidad [68]. La infección con *T. cruzi*, ocurre principalmente por el mecanismo de transmisión vectorial que consiste en el contacto del humano con heces de triatominos infectados [3].

Dentro de las estrategias de control de la enfermedad de Chagas, el control vectorial es una de las más importantes en todos los países de Latinoamérica. Con el fin de fortalecer los programas de control vectorial se hace necesario realizar la correcta identificación de especies de insectos vectores, estudiar la dinámica de su distribución actual, delimitar claramente estás especies y aportar información como su adaptación a las diferentes variantes del parasito y hospederos, su historia evolutiva, sus patrones de diversificación, diferencias genéticas dentro y entre especies, así como la determinación del flujo genético y su posible aporte en la especiación y adaptación de estas [7,13,17,19] . Así son los insectos justamente una de las fuentes de información más completa, ya que mediante técnicas moleculares, se pueden realizar análisis filogenéticos y evolutivos de los vectores, detección/genotipificación de *T. cruzi* y fuentes alimenticias en estos y por lo tanto identificar focos de infección, factores de riesgo, y distribución de mamíferos en los ciclos de transmisión del parásito [15,16,69,70].

Dentro de los principales vectores incriminados en enfermedad de Chagas se encuentran especies de la tribu Rhodniini, por ende, las medidas y estrategias de control están dirigidas a varias especies de este grupo [15,17,71]. Uno de los pasos primordiales en los programas de control es la correcta identificación de las especies de vectores y el entendimiento de su estructura filogenética y biogeográfica. Sin embargo, para el caso de la tribu Rhodniini es aún confusa dicha estructura debido a incongruencias filogenéticas y taxonómicas que persisten a pesar de la existencia en la literatura de varios estudios con la finalidad de resolverlas.

Por lo anterior, los capítulos de esta tesis se encaminan a describir características eco-epidemiológicas (tasas de infección, preferencias alimenticias y DTUs: Unidades de tipificación discretas) de algunas especies de la tribu, determinar patrones filogenéticos de las especies de la tribu Rhodniini, recolectados en una amplia y representativa distribución geográfica (Colombia, Brasil, Argentina, Venezuela, Panamá y Ecuador). En conjunto, todo lo anterior proporcionará información que podrá ser utilizada en programas de control vectorial de la enfermedad de Chagas dirigidos a especies de la tribu Rhodniini [20].

CAPITULO 1. Características eco-epidemiológicas de especies de la tribu Rhodniini en Colombia

Este capítulo consiste en el cumplimiento del objetivo 1, por lo que en primera instancia se realizaron varios estudios en Colombia en los que se recolectaron diferentes triatominos en zonas endémicas para la enfermedad de Chagas, se caracterizaron aspectos eco-epidemiológicos como su distribución geográfica, infección por *T. cruzi* y con sus diferentes variantes genéticas; y preferencias alimenticias, con el fin de conocer estos aspectos en especies la tribu Rhodniini a nivel local. Para ello, se realizó una exploración inicial recolectando triatominos en diferentes zonas endémicas de Colombia, en los que se detectaron tres especies representativas de cada uno de los grupos del género *Rhodnius* (*R. pictipes*, *R. prolixus* y *R. pallescens*) y la especie *P. arthuri* del género *Psammolestes*, infectadas con *Trypanosoma cruzi*, distribuidas en diferentes ciclos epidemiológicos de transmisión y se analizaron las preferencias alimenticias simultáneamente para evaluar su rol en la transmisión del parásito en el marco de la enfermedad de Chagas en Colombia (Resultados artículo 1). Los resultados obtenidos dejaron en evidencia que efectivamente las especies encontradas de la Tribu Rhodniini, juegan un papel importante en la transmisión del parásito en el país (Resultados Artículos 1 y 2). Así, se encontró infección en las especies *R. pictipes, R. pallescens* y *P. arthuri* con *T. cruzi* (en la mayoría de los casos con la DTU TcI), y fuentes alimenticias principalmente asociadas con ciclos epidemiológicos silvestres y con sangre humana, dejando en evidencia que, si bien no son

vectores primarios, pueden generar intrusiones en ciclos domésticos y por ende pueden ser responsables de transmisión del parasito al humano (Resultados Artículos 1, 2 y 3).

Adicionalmente, nuestros resultados confirman el rol de la especie *R. prolixus* en Colombia como vector primario de *T. cruzi*, ya que se encontraron altas tasas de infección en ejemplares recolectados en diferentes zonas del país, elevada variabilidad de fuentes alimenticias especialmente asociadas con ciclos domésticos de transmisión, alta frecuencia de infección con sangre humana e infección con el parásito de forma simultánea, corroborando la importancia de esta especie como vector (Resultados Artículos 1, 3 y 4). Por ende, se analizó el comportamiento de la especie *R. prolixus* en ambientes domésticos, caracterizándose su presencia en las viviendas en una de las zonas endémicas más afectadas del país (departamento de Casanare), encontrándose variación temporal durante el año asociada con la variación en los periodos de lluvia (Resultados artículo 4).

A la par se realizó una revisión en la literatura en la que se colecto información acerca de todos los registros bibliográficos del parásito *T. cruzi* y sus variantes genéticas, en la que se evidencio infección en varias especies de la tribu Rhodniini con distribución geográfica en diez países de centro y sur América, encontrándose 1260 registros de infección con las diferentes variantes genéticas del parásito tanto en ciclos selváticos como silvestres. Todo lo anterior deja en relevancia la importancia que presenta la tribu Rhodninii en el marco de la transmisión de *T. cruzi* tanto en Colombia como en América Latina (Resultados artículo 5).

A continuación, las publicaciones que muestran los resultados relacionados en el capítulo 1.

- **Artículo 1: Hernández C**, Salazar C, Brochero H, Teherán A, Buitrago LS, Vera M, Soto H, Florez-Rivadeneira Z, Ardila S, Parra-Henao G, Ramírez JD. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. Parasit Vectors. 2016 Dec 1;9(1):620.
- **Artículo 2:** Velásquez-Ortiz N, **Hernández C**, Herrera G, Cruz-Saavedra L, Higuera A, Arias- Giraldo LM, et al,.*Trypanosoma cruzi* infection, discrete

typing units and feeding sources among Psammolestes arthuri (Reduviidae: Triatominae) collected in eastern Colombia. Parasit Vectors. 2019 Apr 8;12(1):157. doi: 10.1186/s13071-019-3422-y

- **Artículo 3:** Arias-Giraldo LM, Muñoz M, **Hernández C**, et al. Identification of blood-feeding sources in *Panstrongylus, Psammolestes, Rhodnius and Triatoma* using amplicon- based next-generation sequencing. *Parasit Vectors*. 2020;13(1):434. Published 2020 Aug 31. doi:10.1186/s13071-020-04310-z
- **Artículo 4:** Rincón-Galvis HJ, Urbano P, **Hernández C**, Ramírez JD. Temporal Variation of the Presence of *Rhodnius prolixus* (Hemiptera: Reduviidae) Into Rural Dwellings in the Department of Casanare, Eastern Colombia. J Med Entomol. 2019 Sep 27. pii: tjz162. doi: 10.1093/jme/tjz162.
- **Articulo 5:** Velásquez-Ortiz, N., Herrera, G., **Hernández C**, Muñoz M, Ramírez JD. Discrete typing units of *Trypanosoma cruzi*: Geographical and biological distribution in the Americas. *Sci Data* 9, 360 (2022). https://doi.org/10.1038/s41597-022-01452-w

Capítulo 2. Diseño de marcadores para análisis de la variabilidad genética de especie de la tribu Rhodniini

En vista de la importancia de las especies de la tribu Rhodniini en la transmisión de *T. cruzi* en centro y Sur América; y de algunas de sus especies como vectores de la enfermedad de Chagas y sumado a las falencias que existen en cuanto a la taxonomía de la tribu y que son de crucial importancia resolver para el fortalecimiento de estrategias de control vectorial de la enfermedad, para cumplir con los objetivos 2 y 3 se realizó el diseño de 40 marcadores moleculares que corresponden a genes de copia única a partir del genoma de *R. prolixus*. Posteriormente, se realizó la amplificación y secuenciación de dichos marcadores en ADNs obtenidos a partir de 5 ejemplares de *R. prolixus,* con el fin de verificar su adecuada amplificación. Se seleccionó un conjunto de 8 marcadores moleculares nucleares que fueron utilizados junto con los marcadores mitocondriales *Citocromo b* y ND4 (subunidad de NADH deshidrogenasa 4).

En este capítulo con el fin de cumplir el objetivo número dos, se describen dos estudios en los que se llevó a cabo el uso de los marcadores seleccionados con el fin de evaluar su utilidad para resolver conflictos taxonómicos y realizar estudios evolutivos en los dos géneros de la tribu Rhodniini.

En el género *Rhodnius* fueron utilizados 4 marcadores de los 8 diseñados y dos reportados previamente en la literatura (ND4 y *Citocromo b*) con el fin de verificar el estatus taxonómico de una especie descrita recientemente en el grupo *prolixus,* denominada *R. taquarussuensis* [45], está ultima presenta alta similitud morfológica y co-localización geográfica con la especie *R. neglectus*, por tanto, su descripción como especie se consideró un conflicto taxonómico que requería verificación. En consecuencia, en este estudio se analizaron cruces inter e intraespecíficos y marcadores moleculares para verificar el estatus taxonómico de *R. taquarussuensis.* Los resultados obtenidos en este estudio evidenciaron que *R. taquarussuensis* no corresponde a una nueva especie, bajo el concepto de definición de especie biológica ya que al realizar los cruces interespecíficos con *R. neglectus* se observa viabilidad de los huevos, sumado a esto los análisis filogenéticos de diversidad

genética y delimitación de especies, permitieron observar que *R. taquarussuensis* corresponde a una variante fenotípica de *R. neglectus* y no a una nueva especie dentro del género *Rhodnius*, por esta razón *R. taquarussuensis* ha sido sinonimizada dentro de *R. neglectus* [11] (Resultados artículo 6).

Adicionalmente, los cuatro marcadores restantes y dos marcadores reportados previamente en la literatura (28S y *Citocromo b*) fueron analizados como herramienta para dilucidar las relaciones filogenéticas y patrones evolutivos de las tres especies del género *Psammolestes*, esto dado a que se ha cuestionado el estatus monofilético del género ya que se ha descrito ampliamente la parafilia de especies del género *Rhodnius* y del grupo *prolixus* con respecto a *Psammolestes.* Sin embargo, los estudios a la fecha solo incluyen una o dos especies del género en las reconstrucciones filogenéticas y análisis evolutivos (. Los resultados obtenidos en este capítulo permitieron recuperar las especies del género *Psammolestes* en un clado monofilético con respecto a *R. prolixus, P. coreodes* y *P. tertius* en un clado monofilético hermano de *P. arthuri.* El análisis de delimitación de especies y diferentes análisis de variabilidad genética permitieron recuperar los tres linajes como especies diferentes en concordancia con las tres morfoespecies. Adicionalmente, se realizó un modelo demográfico que evidenció un escenario de divergencia sin flujo genético en el Mioceno tardío, impulsado por el aislamiento geográfico y que sugiere que los haplotipos mixtos pueden ser el resultado de la variación ancestral compartida desde la divergencia de las especies subtropicales-templadas (*P. coreodes/P. tertius*). Así, la cuenca amazónica es una barrera climática que promueve la especiación alopátrica después de la dispersión a largo plazo (Resultados artículo 7).

Finalmente, mediante el desarrollo de este capítulo se aportan conocimientos sobre los patrones de especiación y dispersión de algunas especies de la tribu Rhodniini que serán de gran utilidad para fortalecer los programas de vigilancia vectorial de la enfermedad de Chagas. Por otro lado, podemos evidenciar la utilidad de los marcadores moleculares diseñados utilizando enfoques multilocus para evaluar la relaciones filogenéticas y patrones evolutivos de la tribu Rhodniini en el siguiente capitulo.

A continuación, las publicaciones que muestran los resultados relacionados en el capítulo:

- Nascimento JD, da Rosa JA, Salgado-Roa FC, **Hernández C**, Pardo-Diaz C, et al. (2019) Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species?. PLOS ONE 14(2): e0211285. https://doi.org/10.1371/journal.pone.0211285
- **Hernández, C.,** Alvarado, M., Salgado-Roa, F.C. *et al.* Phylogenetic relationships and evolutionary patterns of the genus *Psammolestes* Bergroth, 1911 (Hemiptera: Reduviidae: Triatominae). *BMC Ecol Evo* **22**, 30 (2022). https://doi.org/10.1186/s12862-022-01987-x

Capítulo 3. Utilidad de enfoque multilocus en la resolución de conflictos filogenéticos y evolutivos de la tribu Rhodniini

En general la subfamilia Triatominae presenta varios desafíos a nivel taxonómico y su panorama evolutivo aún no es claro, lo que dificulta el entendimiento de su comportamiento vectorial y con ello el diseño de estrategias de control la enfermedad de Chagas, teniendo en cuenta que el principal mecanismo de transmisión es el vectorial. La tribu Rhodniini está compuesta por dos géneros, el género *Rhodnius* que consta de 21 especies y el género *Psammolestes* con 3 especies. Inicialmente, se propuso que las tres especies que pertenecen al género *Psammolestes* debían ser agrupadas en una tribu que fue llamada *Psammolestini,* esto dado las marcadas diferencias morfológicas con el género *Rhodniu*s (Figura 4). Posteriormente los dos géneros fueron agrupados en la tribu Rhodniini, principalmente con base en la presencia de tuberosidades posteriores a los ojos y teniendo en cuenta que en su mayoría son especies principalmente arborícolas con excepción de algunas especies de *Rhodnius.* La tribu Rhodniini ha sido una de las más estudiadas debido a su importancia epidemiológica y amplia distribución geográfica. Adicionalmente, el género *Rhodnius* de la tribu ha sido clasificado en 3 grupos: *pictipes*, *pallescens* y *prolixus*, con base en su distribución geográfica, biogeografía y morfología. Inicialmente estos grupos fueron llamados linajes, sin embargo, dado que se ha cuestionado su origen monofilético actualmente se usa el termino grupo. Alrededor de estás clasificaciones dentro de la tribu se han presentado diferentes conflictos, siendo entre ellos tres los de mayor importancia: la parafilia de los géneros, las diferentes agrupaciones de los linajes/grupos del género *Rhodnius* y el estatus taxonómico de algunas especies, principalmente las que integran el grupo *prolixus.*

En este capítulo con el fin de cumplir el objetivo tres, se presentan dos artículos, uno de revisión en el que se describen aspectos generales de la taxonomía de la subfamilia Triatominae y se hace énfasis en la tribu Rhodniini. Se realiza una descripción de los aspectos que influyen en la taxonomía y evolución de la tribu Rhodniini, desde su distribución geográfica, características morfológicas y todos los estudios citogenéticos,

moleculares y omicos cuyo objetivo han sido el estudio de las relaciones taxonómicas de las especies que integran la tribu Rhodniini (Artículo 8).

Al conocer la complejidad taxonómica de la tribu Rhodniini y los aspectos relacionados con esta se plantea un enfoque multilocus en él que se realizó énfasis en disminuir los sesgos que pueden generar incongruencias taxonómicas: muestreo insuficiente y no representativo de la distribución de las especies, tipo y cantidad de marcadores, cantidad de datos, tipos de análisis multilocus y de reconstrucción filogenética en diferentes sets de datos. Por lo tanto, se utilizó un muestreo representativo de los géneros, grupos y especies de la tribu Rhodniini con el fin de evitar el sesgo que puede generarse por muestreo y falta representatividad de la distribución geográfica de las especies por lo que se utilizó un set de 500 muestras obtenidas en diferentes países de América latina.

Se realizó la aplicación de un enfoque multilocus que integra marcadores nucleares (diseñados en el capítulo 2), ribosomal (28S) y mitocondrial (Cytb) reportados previamente en la literatura, esto dado que las incongruencias ocurren en diferentes niveles taxonómicos de la tribu y los estudios previos no eran comparables entre si porque los diferentes marcadores eran usados en sets de muestras y especies diferentes. Los tipos de resolución molecular de los marcadores incluidos permitieron abordar los distintos niveles taxonómicos que presentan problemas en la tribu y comprender los mecanismos y/o razones que producen las incongruencias descritas previamente. Adicionalmente, dentro del enfoque multilocus se realizó concatenación y análisis de coalescencia para comprender las incongruencias entre árbol de genes y árbol de especies, así como el análisis por cada marcador de manera independiente.

Sumado a lo anterior, se utilizaron diferentes tipos de reconstrucción (MV e IB) usando diferentes algoritmos y de esta manera determinar las reconstrucciones mejor soportadas y mediante un análisis de credibilidad de topología se logró seleccionar la reconstrucción filogenética que mejor representa las relaciones taxonómicas de las especies de la tribu y mediante la cual se proporciona solución a los problemas taxonómicos descritos previamene. Sumado a esto se realizaron análisis de estructura genética y estimación de

tiempos de divergencia que permitieron comprender los posibles mecanismos asociados con las incongruencias filogenéticas de la tribu (Resultados Articulo 9). Finalmente, mediante el desarrollo de este capítulo se lograron resolver y comprender las diferentes incongruencias y problemas taxonómicos de la tribu Rhodniini.

A continuación, las publicaciones que muestran los resultados relacionados en el capítulo:

- **Artículo 8: Hernández C,** da Rosa JA, Vallejo GA, Guhl F, Ramírez JD. Taxonomy, Evolution, and Biogeography of the Rhodniini Tribe (Hemiptera: Reduviidae). Diversity 2020, 12(3), 97; https://doi.org/10.3390/d12030097
- **Artículo 9: Carolina Hernández**, Mateo Alvarado, Fabian C. Salgado-Roa, Nathalia Ballesteros, Carolina Pardo, Joao Aristeu da Rosa, Jader Oliveira, Clever Galvao, Simone Freitas, Jose Calzada, Juliana Damieli Nascimento, Lineth Garcia, Mario Grijalva, Anita Villacis, Hernan Carrasco, Maikell Segovia,Cesar Gomez Hernandez, Plutarco Urbano, Omar Cantillo, Marina Muñoz, Felipe Guhl, Gustavo Vallejo, Julio cesar Carranza, Luz Stella Buitrago, Marina Stella Gonzalez, Kaio Cesar Chaboli Alevi, Andres Cuervo, Claudia Sandoval, Camilo Salazar, Juan David Ramírez. Enfoque multilocus en la resolución de conflictos filogenéticos y evolutivos de la tribu Rhodniini (por someter).

CAPITULO 1. Características eco-epidemiológicas de especies de la tribu Rhodniini en Colombia

Hernández et al. Parasites & Vectors (2016) 9:620
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Parasites & Vectors

RESEARCH Open Access

Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units

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Abstract

Background: *Trypanosoma cruzi* is the causative agent of Chagas disease. Due to its genetic diversity has been classified into six Discrete Typing Units (DTUs) in association with transmission cycles. In Colombia, natural *T. cruzi* infection has been detected in 15 triatomine species. There is scarce information regarding the infection rates, DTUs and feeding preferences of secondary vectors. Therefore, the aim of this study was to determine *T. cruzi* infection rates, parasite DTU, ecotopes, insect stages, geographical location and bug feeding preferences across six different triatomine species.

Methods: A total of 245 insects were collected in seven departments of Colombia. We conducted molecular detection and genotyping of *T. cruzi* with subsequent identification of food sources. The frequency of infection, DTUs, TcI genotypes and feeding sources were plotted across the six species studied. A logistic regression model risk was estimated with insects positive for *T. cruzi* according to demographic and eco-epidemiological characteristics.

Results: We collected 85 specimens of *Panstrongylus geniculatus*, 77 *Rhodnius prolixus*, 37 *R. pallescens*, 34 *Triatoma maculata*, 8 *R. pictipes* and 4 *T. dimidiata*. The overall *T. cruzi* infection rate was 61.2% and presented statistical associations with the departments Meta (OR: 2.65; 95% CI: 1.69–4.17) and Guajira (OR: 2.13; 95% CI: 1.16–3.94); peridomestic ecotope (OR: 2.52: 95% CI: 1.62–3.93); the vector species *P. geniculatus* (OR: 2.40; 95% CI: 1.51–3.82) and *T. maculata* (OR: 2.09; 95% CI: 1.02–4.29); females (OR: 2.05; 95% CI: 1.39–3.04) and feeding on opossum (OR: 3. 15; 95% CI: 1.85–11.69) and human blood (OR: 1.55; 95% CI: 1.07–2.24). Regarding the DTUs, we observed TcI (67. 3%), TcII (6.7%), TcIII (8.7%), TcIV (4.0%) and TcV (6.0%). Across the samples typed as TcI, we detected TcIDom (19%) and sylvatic TcI (75%). The frequencies of feeding sources were 59.4% (human blood); 11.2% (hen); 9.6% (bat); 5.6% (opossum); 5.1% (mouse); 4.1% (dog); 3.0% (rodent); 1.0% (armadillo); and 1.0% (cow).

Conclusions: New scenarios of *T. cruzi* transmission caused by secondary and sylvatic vectors are considered. The findings of sylvatic DTUs from bugs collected in domestic and peridomestic ecotopes confirms the emerging transmission scenarios in Colombia.

Keywords: Chagas disease, Secondary vectors, *Trypanosoma cruzi*, DTUs, Feeding sources, Colombia

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Background

Chagas disease caused by the protozoan parasite *Trypanosoma cruzi*, affects about six million people in Latin America. The main transmission mechanism is by insect vectors (stercoralian route). The insects responsible for vector transmission belong to the subfamily Triatominae (Hemiptera: Reduviidae), composed by approximately 140 species of 5 tribes [1]. The natural habitats of triatomines include palm trees, tree holes, cracks in rocks, small caves and other animal shelters [2]. The main vectors of *T. cruzi* in the Southern Cone countries are *Triatoma infestans*, *Triatoma brasiliensis* and *Panstrogylus megistus*; *Rhodnius prolixus* and *Triatoma dimidiata* in the Andean region and parts of Central America, and *Triatoma dimidiata* and *T. barberi* in Mexico [3]. A total of 26 species have been reported in Colombia; of these 15 have been shown to be naturally infected with *T. cruzi* [4]. Thus, *R. prolixus* and *T. dimidiata* are considered primary vectors, whereas *P. geniculatus*, *T. maculata*, *R. pictipes* and *R. pallescens* are considered secondary vectors, among others that have been found naturally infected with *T. cruzi*. In Colombia, there are approximately 436,000 people infected with *T. cruzi*, with an annual incidence of 5,250 vector-borne cases per population [5]. In addition, some studies revealed that species at greatest risk of transmission are *R. prolixus*, *T. dimidiata*, *T. maculata* and *T. venosa* [4]. However, vector control programs have focused on domiciled species as *R. prolixus* and *T. dimidiata*.

Trypanosoma cruzi transmission mostly occurs in three epidemiological cycles: sylvatic (enzootic), domestic and peridomestic, where the parasite circulates among triatomines, mammal reservoirs and human hosts. Around 180 sylvatic and synanthropic species of mammal have been described to date, which act as reservoirs of *T. cruzi* inhabiting places near human settlements [6]. *Trypanosoma cruzi* exhibits remarkable genetic diversity and has been classified by international consensus in six Discrete Typing Units (DTUs) (TcI-TcVI), plus a new genotype associated to anthropogenic bats (TcBat) [7]. The DTUs present associations with transmission cycles, geography, vector species and clinical manifestation to some extent [7–12]. The DTU with the broadest geographical distribution is TcI and due to its high genetic diversity has been divided into two genotypes, associated to domestic and sylvatic foci (TcIDom and Sylvatic TcI, respectively) [13–16].

Current molecular tools have improved the detection and genotyping of the parasite in the vectors as well as the identification of the vectors' feeding sources involved in the transmission of the parasite. However, in Colombia there is scarce information about the relationship between different vector species, *T. cruzi* infection and eco-epidemiological aspects of the transmission cycles. Control programs in this country have focused on domiciled vectors while epidemiological and scientific research has been restricted to the Caribbean region. Therefore, the study of vectors in other areas of the country with a holistic perspective that considers *T. cruzi* infection rates, genotyping and feeding sources will help to identify the hosts and understand the dynamics of parasite transmission. This information will be useful to generate vector control strategies and accurate surveillance of Chagas disease. Thus, the objective of this study was to apply this holistic perspective to six triatomine species collected in different transmission cycles from seven departments in Colombia.

Methods

Study area and collection of triatomines

A total of 245 specimens corresponding to six species (85 *P. geniculatus*, 77 *R. prolixus*, 37 *R. pallescens*, 34 *T. maculata*, 8 *R. pictipes* and 4 *T. dimidiata*) were collected in the departments Guajira, Antioquia, Cesar, Norte de Santander, Meta, Casanare and Huila (Fig. 1; Additional file 1: Table S1). Insect capture in the sylvatic cycle was performed using two techniques: manual search and modified Noireau baited chicken traps in palms and distant to housing areas. Additionally, insects inside houses and in the peridomestic ecotope were collected. All insects were stored in separate jars, including a description of the site of collection and georeferenced using GPS. The insects were identified using taxonomic keys and stored in 100% ethanol until processing [17]. All of the specimens except *T. dimidiata* were collected from domestic, peridomestic and sylvatic cycles of transmission (Additional file 1: Table S1; Fig. 2).

Molecular detection of *T. cruzi* and genotyping

DNA extraction of the complete body of each insect was conducted using the ZR Tissue & Insect miniprep DNA Zymo ™ kit (Zymo Research, Irvine, USA), then endpoint qPCR was performed for detecting the satellite DNA of *T. cruzi* using primers cruzi1 (5′-AST CGG CTG ATC GTT TTC-3′), cruzi2 (5′-AAT TCC TCC AAG CAG CGG ATA-3′) and cruzi3 probe (FAM-CAC ACA CTG GAC ACC AA-NFQ-MGB) using the conditions previously reported [18]. In the interpretation, since no *T. cruzi* DNA quantitation was performed, it is interpreted as positive DNA amplification in Cts < 38 and negative amplification as absence. 12S subunit ribosomal gene of triatomines was used as internal amplification control under the conditions and primers previously described [19]. Subsequently, the insects with positive results by qPCR were submitted to kinetoplast DNA amplification using primers 121 (5′-AAA TAA TGT ACG GGK GAG ATG CAT GA-3′) and 122 (5′-GGT TCG ATT GGG GTT GGT GTA ATA TA-3′) to discriminate *T. cruzi* and *T. rangeli* infections as previously reported [20]. DNA from strains MHOM/CO/01/DA and RHO/CO/82/Durán were used as positive controls of *T. cruzi* and

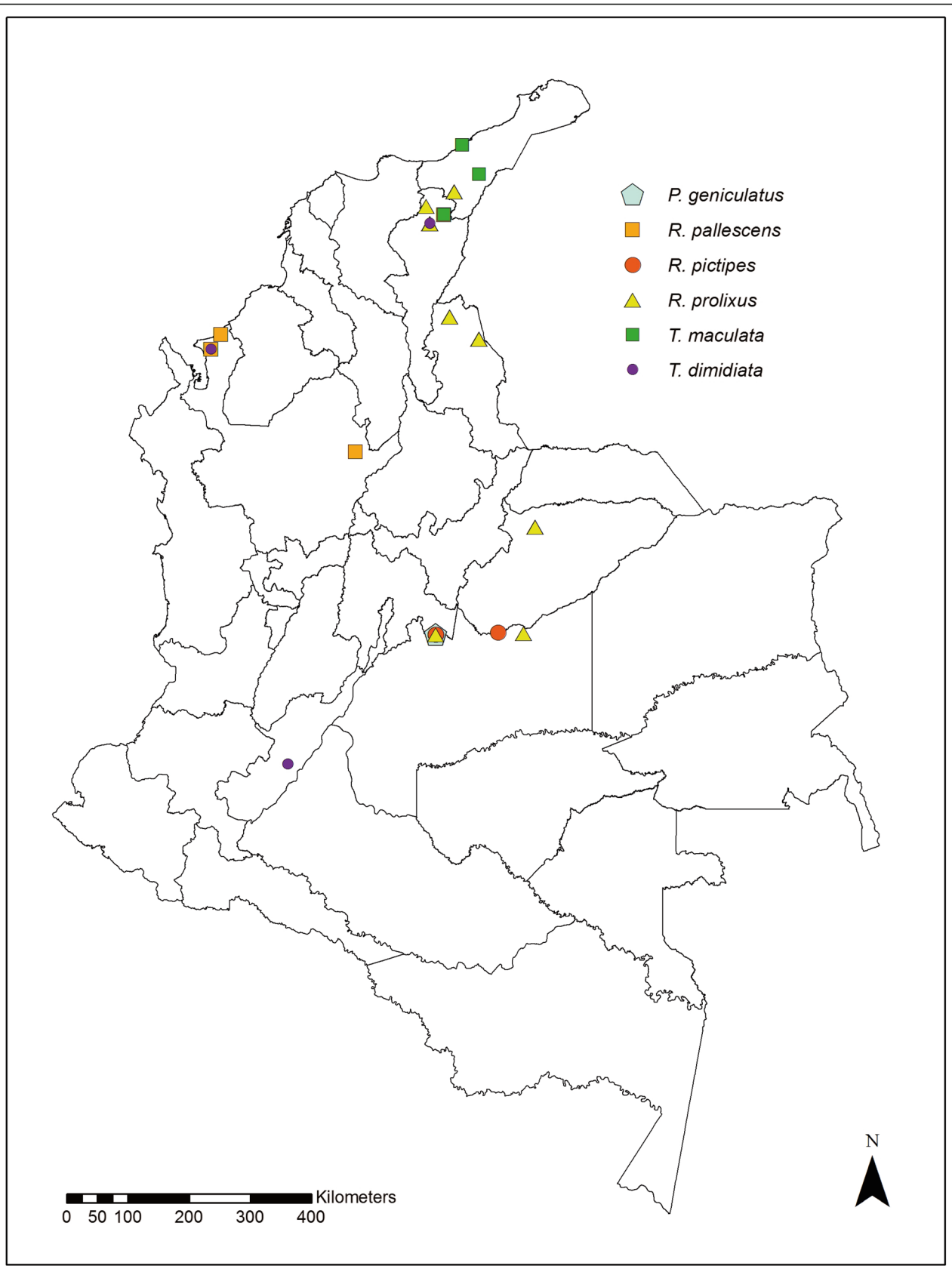

Fig. 1 Geographical distribution of 245 triatomines collected across Colombia and included in this study

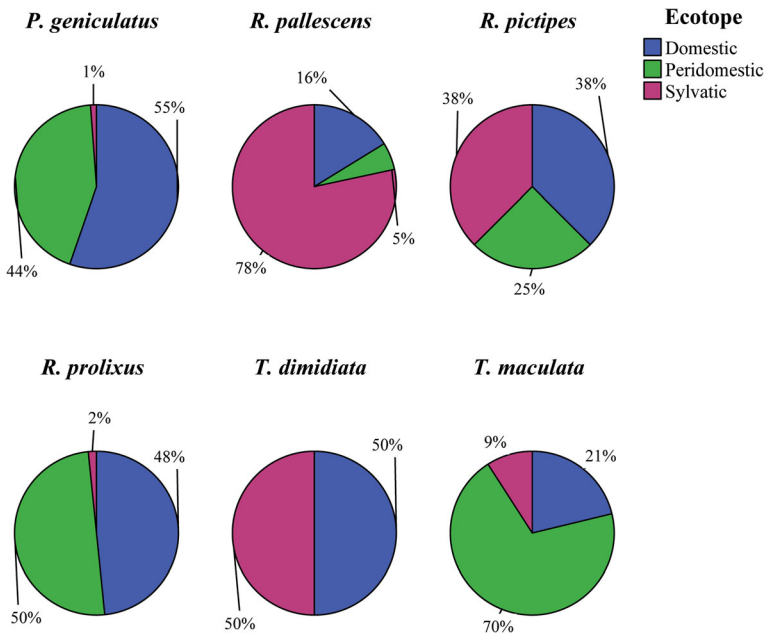

Fig. 2 Frequency of ecotopes for the species studied. Samples were collected in sylvatic, peridomestic and domestic ecotopes

T. rangeli, respectively. The identification of DTUs was accomplished by conventional PCR using the SL-IR, 18 s, 24 s and A10 targets as previously described [11, 21, 22]. We employed reference strains from each DTU as follows: TcIDom (DA), TcISylvatic (GC), TcII (Y), TcIII (CM17), TcIV (YLY), TcV (Tulahuen) and TcVI (CLBrener).

Molecular characterization of blood sources

All the 245 specimens were submitted to identification of feeding preferences by PCR-HRM (Polymerase chain reaction-High resolution melting) as previously reported [23]. Also, the feeding preferences were corroborated by direct sequencing of *cytb* using the primers CytbFw (5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′) and CytbRv (5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′). The resulting sequences were edited in MEGA 6.0 [24] and submitted to BLASTn similarity search. This methodology allows the detection of 14 host species involved in the epidemiological cycles of Chagas disease as previously reported [23].

Statistical analyses

We calculated the frequency of *T. cruzi* infection, DTUs, TcI genotypes and feeding preferences across species and ecotopes (transmission cycles). To establish the association between the variables, Chi-square test was implemented with Monte Carlo adjustment with 10,000 simulations and G-test including pairwise comparison. G-test was not applied in cases where the contingency table had zeros (Additional file 2: Table S2). Additionally, using an unconditional logistic regression, without including the intercept, the risk for infection with *T. cruzi* was estimated (OR, 95% IC) according to the demographic and ecoepidemiological characteristics using EpiInfo V.3.5.4 software and R package version 3.3.1.1. Statistical significance was established with a P-value < 0.05.

Results

Trypanosoma cruzi detection in six species of triatomines

The information regarding the frequency of infection with *T. cruzi* by geographical location, ecotope and insect stage is shown in Table 1. The overall *T. cruzi* infection rate was 61.2% ($n = 150$). The species with the highest percentage of infection with *T. cruzi* was *P. geniculatus*, followed by *R. prolixus* and *T. maculata* (Fig. 3a). In addition, one *R. prolixus* was positive for *T. rangeli*.

The frequency of *T. cruzi* infection within each species was as follows: *T. maculata*: 67.6% (23/34); *P. geniculatus*: 70.6% (60/85); *R. pallescens*: 45.9% (17/37); *R. prolixus*: 55.8% (43/77); *R. pictipes*: 87.5% (7/8); and *T. dimidiata*: 25.0% (1/4) as shown in Fig. 3b. Association between species and the infection with *T. cruzi* was found ($\chi^2 = 13.35$, $df = 5$, $P = 0.0171$; G-test, $G = 13.33$, $P = 0.0175$), therefore statistical analysis was performed within each species using logistic regression (Table 2) and by Chi-square and G-test analysis (Additional file 2: Table S2; Additional file 3: Table S3). The species that showed association with the infection by *T. cruzi* were *P. geniculatus* and *T. maculata*. *Panstrongylus geniculatus*

Table 1 Frequency of infection with *T. cruzi* by geographical location, ecotope and insect stage

Department	*n*	% of infection with *T. cruzi*	95% CI
Meta	69	46.0	38.0–53.9
Guajira	32	21.3	14.7–27.8
Cesar	24	16.0	9.5–21.1
Antioquía	12	8.0	3.6–12.3
Norte de Santander	9	6.0	2.2–9.8
Casanare	3	2.0	0.4–5.7
Huila	1	0.7	0.02–3.6
Ecotope			
Peridomestic	68	45.3	39.7–56.1
Domestic	52	34.7	28.7–44.5
Sylvatic	22	14.7	9.5–21.4
Stage			
Female	76	50.7	44.3–60.5
Male	43	28.7	22.2–37.1
Nymph	26	17.3	11.7–24.2

Abbreviation: n, number of positive samples

showed association with *T. cruzi* infection by the three statistical analyses. G-test revealed significant pairwise differences specifically with *R. pallescens* and *R. prolixus*. *Trypanosoma maculata* showed association with positive rate by Chi-square and logistic regression. Additionally, *R. pictipes* showed association only by Chi-square analysis. However, this is because the sample size is very small, which is reflected in the wide confidence interval in the logistic regression that invalidates the significant OR (Table 2; Additional file 2: Table S2; Additional file 3: Table S3). Furthermore, the association between species and ecotopes was statistically significant (χ^2 = 142.82, df = 8, $P < 0.0001$; G-test, G = 123.17, $P < 0.0001$). By analyzing G-pairwise comparison test, significant differences were observed among all species except for *R. prolixus* compared to *P. geniculatus* and *R. pictipes* compared to *R. pallescens* (Additional file 4: Table S4).

Regarding the infection with *T. cruzi* and the variables listed in Table 1, statistically significant association was evident with geographical location (χ^2 = 16., df = 6, P = 0.0066; G-test, G = 16.93, P = 0.0095). As for the geographical location, two departments (Guajira and Meta) exhibited a positive association with *T. cruzi* (Table 2). In both cases, these departments showed differences with departments Antioquia and Cesar; additionally, there were differences between Meta and Norte de Santander (Additional file 5: Table S5). No association with infection with *T. cruzi* was found regarding ecotope (χ^2 = 5.75, df = 2, P = 0.0542; G-test, G = 5.83, P = 0.0539) and life stage (χ^2 = 2.051, df = 2, P = 0.3586; G-test, G = 2.050, P = 0.3864). However, logistic regression detected association between the peridomestic ecotope, female stage and *T. cruzi* infection (Table 2).

Feeding preferences across the six species of triatomines

A total of 9 feeding sources were detected in 197 insects corresponding to six species. The frequencies were as follows: human blood: 59.4% (117); hen: 11.2% (22); bat: 9.6% (19); opossum: 5.6% (11); mouse: 5.1% (10); dog: 4.1% (8); rodent: 3.0% (6); armadillo: 1.0% (2); and cow: 1.0% (2). Association of the feeding sources and *T. cruzi* infection was observed specifically with human blood and opossum (χ^2 = 12.917, df = 8, P = 0.1002) (Table 2). Additionally, we evaluated the feeding preferences across 122 insects that were *T. cruzi*-positive and found human blood in 58.2% (71), hen in 7.4% (9), bat in 9.8% (12), opossum in 7.4% (9), mouse in 4.9% (6), dog in 6.6% (8), rodent in 3.3% (4), armadillo in 0.8% (1) and cow in 1.6% (2). Regarding the feeding preferences by species, statistically significant association was observed (χ^2 = 99.56, df = 40, P = 0.0015). The insect vectors with the greatest variety of feeding sources were *P. geniculatus*, *R. prolixus* and *R. pallescens* (Fig. 4a). When feeding preferences were evaluated by ecotope, statistically significant association was found (χ^2 = 25.74, df = 16, P = 0.0468) and the most common sources were human, hen, bat and opossum (Fig. 4b). Finally, the species positive for *T. cruzi* and blood-feeding on humans are shown in Table 3.

Trypanosoma cruzi DTUs and TcI genotypes

DTUs and TcI genotypes characterization was performed on 149 samples that were positive for *T. cruzi*. The frequencies were analyzed according to species and ecotope (Fig. 5a). We found cases of single and mixed infections observing TcI in 67.8% (101), TcII in 6.7% (10), TcIII in 8.7% (13), TcIV in 4.0% (6), TcV in 6.0% (9) and mixed infections in 6.7% (10/149). No association was found between DTUs and species (χ^2 = 22.29, df = 20, P = 0.3419), feeding sources (FD: 32, χ^2 = 34.08, df = 32, P = 0.6476) and/or ecotopes (χ^2 = 13.88, df = 8, P = 0.1733).

Regarding the TcI genotypes, we detected TcIDom in 19.0% (19/100), sylvatic TcI in 75.0% (75/100) and TcIDom/TcIsylvatic in 6% (6/100) of the mixed infections corresponded to TcI sylvatic + TcII, TcIDom + TcII, TcIsylvatic + TcIII, TcIsylvatic + TcIII + TcIV, TcIsylvatic + TcIV and TcIsylvatic + TcV. We determined the infecting DTU and TcI genotypes discriminated by the species and ecotopes (Fig. 5b). Also, association between the TcI genotypes and the feeding sources (χ^2 = 94.21, df = 16, P = 0.0137), ecotopes (χ^2 = 17.32, df = 4, P = 0.0013) and species (χ^2 = 29.46, df = 10, P = 0.0049).

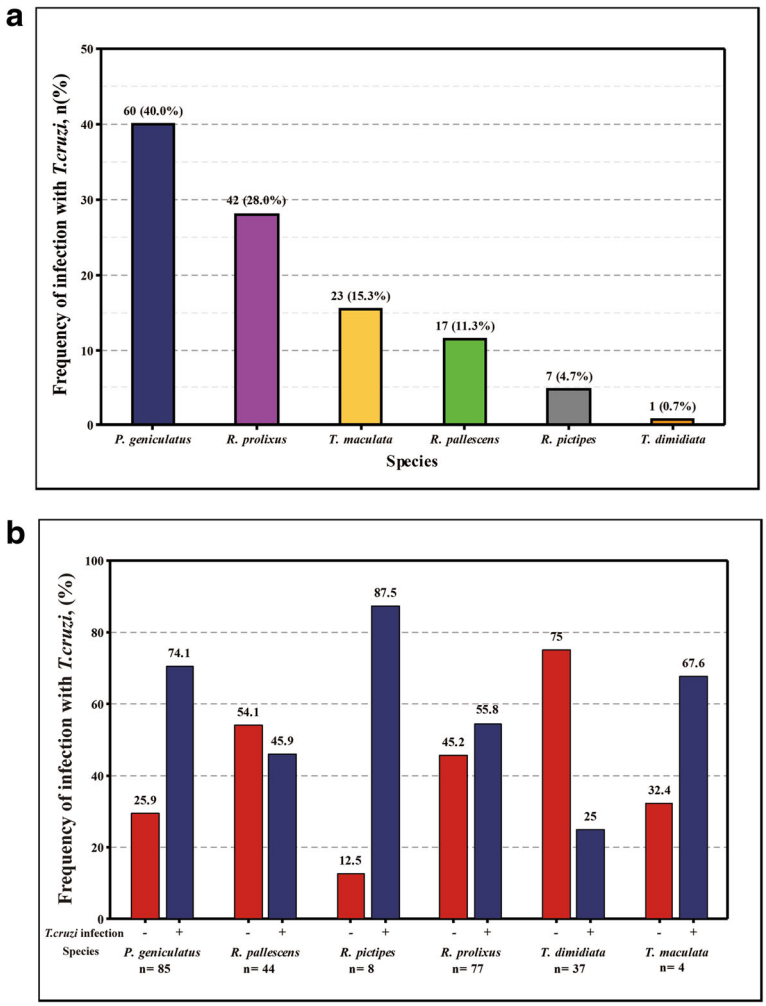

Fig. 3 Frequency of infection with *T. cruzi* in the triatomines collected. **a** Frequency of infection with *T. cruzi* in all samples. **b** Frequency of insects negative (–) and positive (+) for *T. cruzi* in all species collected

Discussion

Triatomine species collected in this study with the exception of *R. prolixus* and *T. dimidiata* are considered sylvatic and secondary vectors. Nevertheless, they were mainly collected in domestic ecotopes (Fig. 2), and there was an association between the ecotopes and species; and the main dietary source was human blood mainly in the domestic ecotope. Feeding sources with sylvatic reservoirs blood was the lowest in our dataset. These findings reflect the intrusion of vectors from sylvatic habitats to domestic habitats and their adaptation to the available feeding sources.

The high percentage of infection with *T. cruzi* and its relationship to the specific tested variables (peridomestic ecotope, feeding with human and opossum blood), together with the occurrence of sylvatic DTUs (sylvatic TcI and TcIII); *P. geniculatus* and *T. maculata* with high percentages of infection and feeding with human blood. They all suggest the existence of possible new transmission scenarios caused by intrusion of secondary vectors (mainly *P. geniculatus* and *T. maculata* in Meta and Guajira departments, respectively). The association of TcI genotypes with ecotopes, feeding sources and species is relevant given that the higher frequencies corresponded to sylvatic TcI, domestic ecotopes, feeding sources of humans, domestic and sylvatic animals. Therefore, since the statistically significant associations are the evidence of parasite population's movement from sylvatic to "domestic" populations with sylvatic strains is confirmed. This is of paramount relevance due to the impact of sylvatic *T. cruzi* in the acute phase and outbreaks of oral transmission. Additionally, statistically significant association between food sources (human and opossum) and *T. cruzi* infection reaffirms the potential of *D. marsupialis* as an important reservoir of the parasite [25, 26].

Table 2 Variables associated with infection with *T. cruzi* across the insect vectors studied

Characteristic		Infection with *T. cruzi*	
	Odds Ratio	95% CI	*P*-value
Species			
P. geniculatus	**2.40**	**1.51–3.82**	**0.0001**
T. maculata	**2.09**	**1.02–4.29**	**0.044**
R. pictipes	7.0	0.86–56–8	0.068
R. prolixus	1.20	0.76–1.88	0.420
R. pallescens	0.85	0.44–1.62	0.622
T. dimidiata	0.33	0.03–3.20	0.341
Department			
Casanare	1.50	0.25–8.98	0.657
Cesar	1.00	0.56–1.78	1.000
Guajira	**2.13**	**1.16–3.94**	**0.016**
Huila	1.00	0.06–15.99	1.000
Meta	**2.65**	**1.69–4.17**	**0.0001**
Norte de Santander	0.82	0.34–1.97	0.655
Feeding source			
Armadillo	1.00	0.06–15.99	1.000
Canine[a]	1473120.79	7.00–>1.0e^{12}	0.957
Opossum	**3.15**	**1.85–11.69**	**0.087**
Hen	0.60	6.26–1.40	0.235
Human	**1.55**	**1.07–2.24**	**0.022**
Bat	1.47	0.60–3.60	0.400
Mouse	1.50	5.42–5.32	0.530
Rodent	2.00	0.37–10.92	0.424
Ecotopes			
Sylvatic	1.38	0.72–2.62	0.332
Domestic	1.24	0.82–1.86	0.303
Peridomestic	**2.52**	**5.61–3.93**	**0.0001**
Stage			
Female	**2.05**	**1.39–3.04**	**0.0001**
Male	1.39	0.87–2.20	0.165
Nymph	1.18	0.67–2.09	0.564

[a]The result is due to the low sample size
Significant values are indicated in bold

Our results are in accordance with other studies conducted in the Caribbean region of Colombia, which showed that secondary vectors play an important role in the different epidemiological transmission cycles of *T. cruzi*. These studies have shown that the frequency of patients with positive serology in the presence of sylvatic vectors is similar to the frequency in the presence of domestic vectors [4, 27]. Furthermore, our findings reinforce the role of *R. prolixus* as a domestic vector in Colombia given that among the triatomine species collected in this study, *R. prolixus* had the highest frequency of feeding with human blood and at the same time showed high rate of *T. cruzi* infection (33.8%). The frequency of *R. prolixus* specimens collected in domestic habitats was 98.4% (Fig. 3a). The feeding sources of *R. prolixus* were mainly humans and domestic animals (Fig. 4a) and the DTUs detected were primarily associated with domestic cycles (TcI and TcII), and mostly infected with TcIDom (Fig. 5). These findings must be a support for the vector control programs in the country. Mainly, because most of the efforts have been focused on domestic vectors such as *R. prolixus* and *T. dimidiata* and our findings evidenced the potential risk of *T. cruzi* transmission by secondary vectors. Therefore, monitoring and control strategies specifically designed for sylvatic vectors are required in Colombia [28].

Surprisingly, we detected TcV in specimens of *P. geniculatus* and *R. pictipes* (sylvatic vectors). This DTU has been reported in domestic cycles from southern Latin-American countries [7, 29]. However, insects infected with TcV have been reported in domestic habitats in Colombia including some reports of human infections [10, 30]. TcV associated with sylvatic cycles has been reported with a frequency of 3.5% [29]. Consistent with our findings of TcV, a recent study [31] reported the presence of TcV in Colombian isolates obtained from *P. geniculatus*, *R. prolixus*, *T. venosa* and armadillos, using high-resolution markers: MLST, MLMT and ten mitochondrial markers. Messenger et al. [31] have also shown that the Colombian TcV isolates are due to migration processes from Southern Cone countries and not to local hybridization processes.

Triatoma maculata has a wide geographical distribution in Colombia. We observed a high invasion of domestic ecotopes by this species (Fig. 2), consistent with other studies in Colombia, Brazil and Venezuela where this species even presents morphological and genetic changes across individuals collected in domestic ecotopes [26, 27, 32–34]. *Triatoma maculata* has not been included in the vector control programs because its diet is mainly composed of bird blood [32, 35–37] and some studies have reported low frequency of infection in Brazil and Venezuela [36, 38]. By contrast, herein the frequency of *T. cruzi* infection was 67.6% and the percentage of feeding with human blood was 75.0% with the presence of TcIDom in some specimens collected in peridomiciliary habitats. Recent studies in Colombia and Venezuela have revealed infection frequencies between 38.0 and 75.0% and the presence of "TcIb" genotype that is associated with the peridomestic cycle [26, 27, 33, 34, 39, 40]. Regarding the DTUs herein detected, most of the specimens were infected with TcI and TcIII (sylvatic DTUs) and domestic specimens harbored TcIII suggesting how *T. maculata* can connect domestic and sylvatic transmision cycles (Fig. 4). Our results and

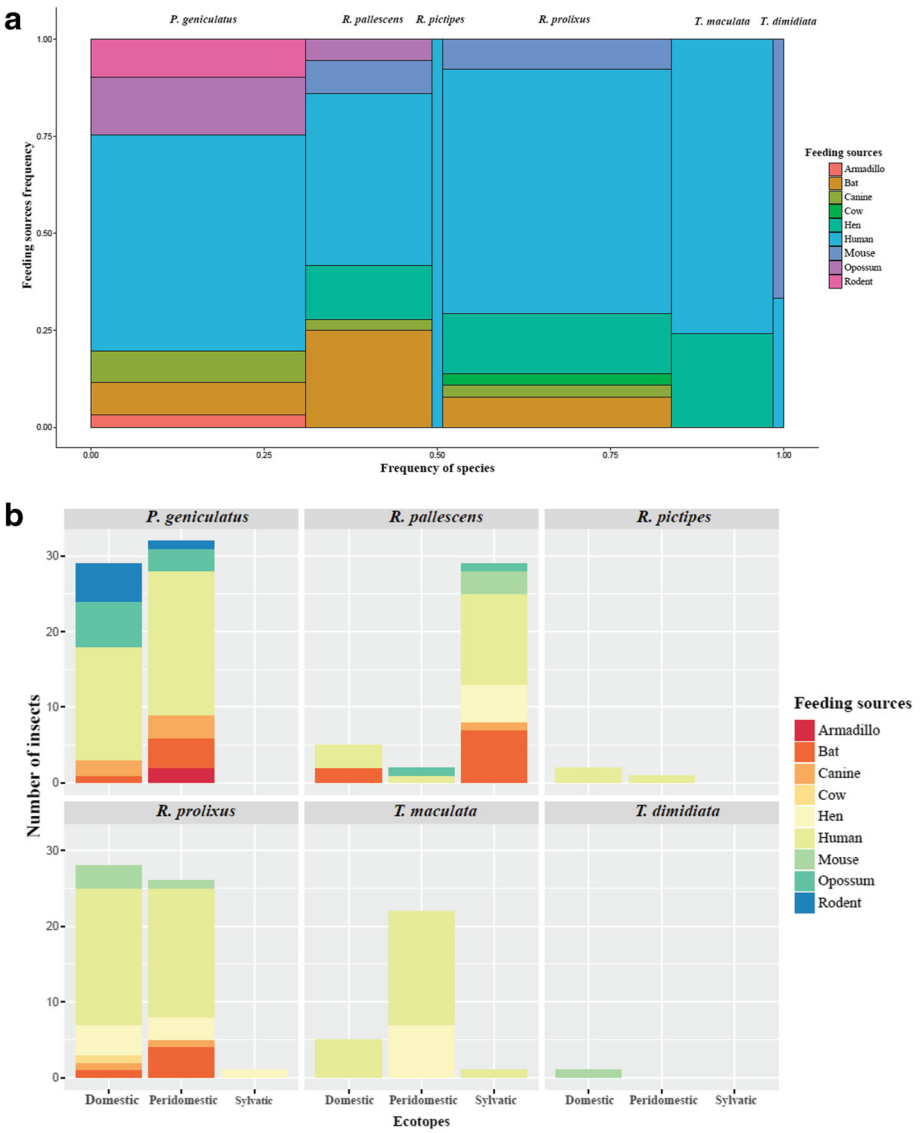

Fig. 4 Feeding sources in triatomines collected. **a** Feeding preferences across the six species of vector collected. **b** Feeding sources in species of triatomines collected by ecotope

previous reports highlight the relevance of *T. maculata* as a potential vector in Colombia suggesting the need to prioritize this species in vector control programs, and additionally, to be cautious about the potential risk of domiciliation that this species may have.

Panstrongylus geniculatus is the most widely distributed in Latin America species of the genus *Panstrongylus* [41]. In Colombia this species has the widest geographical distribution and is recorded in 25 departments including the Department of Meta [4]. In this study, most of the specimens were collected from the domestic ecotope (Fig. 2; Additional file 3: Table S3). *Panstrongylus geniculatus* is considered a sylvatic vector that inhabits in burrows primarily associated with armadillos, opossums, rodents and bats [41]. Accordingly, there are several reports in Latin America and Colombia demonstrating the intrusion of adult specimens of *P. geniculatus* in domestic habitats [42–45] and also findings of different nymphal stages in human dwellings mainly in Brazil, Venezuela and Colombia (Amalfi, Antioquia) [46–50]. Colonization in domestic habitats may be due to changes generated by housing construction and alteration of the ecosystems in the municipalities analyzed and/or by the attraction generated by the artificial light [51].

Our results showed *P. geniculatus* ranking first in *T. cruzi* infection (Fig. 3a; Table 2; Additional file 3: Table S3), similar to previous reports in Brazil, Venezuela

Table 3 Frequency of infection with *T. cruzi* and human blood-feeding within each species

Species	*n*	*N*	Frequency of infection with *T. cruzi* (%)	Frequency of infection with *T. cruzi* and human blood-feeding (%)
P. geniculatus	60	85	70.59	27.06
T. maculata	23	34	67.65	47.06
R. pallescens	17	37	45.95	16.22
R. prolixus	43	77	55.84	32.47

Abbreviations: *n* Number of infected with *T. cruzi*, *N* Total number

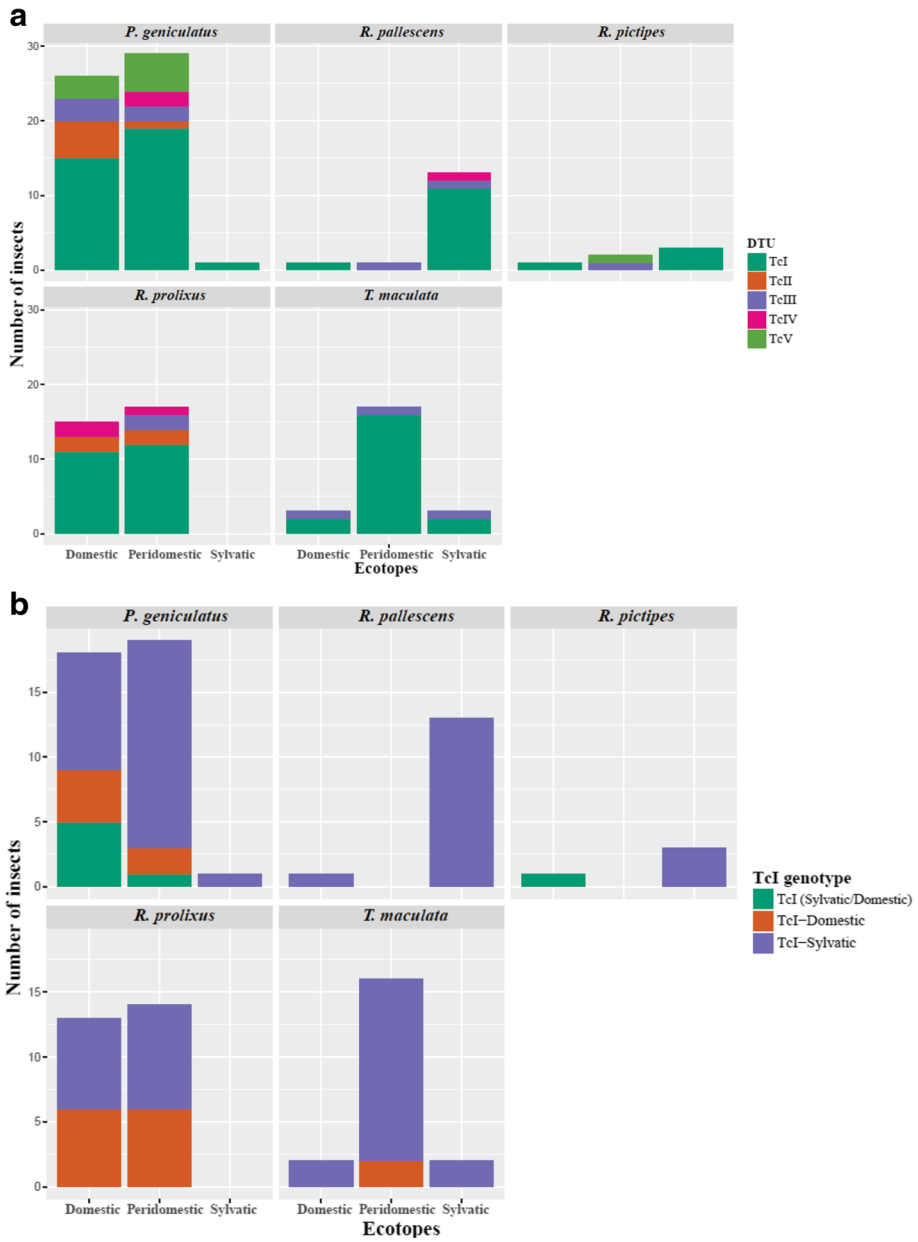

Fig. 5 Distribution of *T. cruzi* (DTUs) and TcI genotypes according to species and ecotope. **a** Distribution of DTUs (TcI-TcVI) in the six species collected and ecotopes. **b** Distribution of TcI genotypes (TcIDom, TcI sylvatic and TcI sylvatic/TcIDom across species and ecotopes

and the Colombian Caribbean region [40, 46, 48, 49]. We detected five DTUs in this species (TcI-TcV) that is in accordance with previous reports in Brazil, Venezuela and the Colombian Orinoco, and showing the interesting permissivity of this species facilitating the transmission of a wide variety of DTUs [10, 46, 48, 51]. In contrast with other reports, we report a wide range of feeding sources with mammals of different epidemiological cycles and human blood. These results demonstrate the strong adaptive ability to different food sources and possibly explaining the high rate of infection and variety of DTUs. Human and canine blood constitute an important food source for *P. geniculatus* and similar to that observed in Venezuela, where also ten outbreaks of *T. cruzi* oral transmission involving this species were reported [48, 49, 52]. A small quantity of *P. geniculatus* fed on sylvatic sources (opossums, rodents, bats and armadillos) explaining the detection of sylvatic DTUs (TcI sylvatic, TcIII and TcIV) across our dataset [2, 41, 53] (Fig. 5a). All these findings could be a signal of an intrusive process from sylvatic ecotopes to domestic ones facilitated by adaptation, occurring in parallel in Colombia, Venezuela and Brazil [42–45], even accompanied by morphological changes in insects [54, 55]. This pattern of ecotope intrusion explains the incrimination of *P. geniculatus* in oral outbreaks in Colombia [28, 56]; it is mandatory that surveillance strategies are deployed not only to avoid incrimination but also a possible domiciliation process.

Another sylvatic species is *R. pallescens*, given that its habitats are tree palms of *Attalea butyracea* and the invasion in domestic ecotopes is seen through intrusion [2, 57–60]. Herein, insects were found in the three ecotopes and most specimens (both nymphs and adults) were collected in sylvatic habitats mainly in wine (*A. butyracea*) and oil (*Elaeis guineensis*) palm trees, while 21.6% (adults) were collected in domestic habitats. This is consistent with previous studies in Colombia [27, 57, 58, 60, 61] and Panama [40, 58, 62–65], where the percentage of *T. cruzi* infection is similar to our results. Moreover, it was observed that the main feeding source was human blood across the three ecotopes. This might be due to agriculture at the collection sites, where palms are nearby to homes and this can facilitate contact between the vectors and human hosts. We report that *R. pallescens* also fed on different mammals such as *D. marsupialis* in the peridomicile habitat and bats, mice and dogs in the sylvatic ecotope, showing an interplay between peridomestic and sylvatic cycles [59, 62–64, 66, 67]. Finally, in the domestic ecotope the sylvatic TcI was only detected, showing that in fact, the specimens of *R. pallescens* might correspond to intrusions in homes from the sylvatic habitat. However, in contrast Cantillo et al. [58], detected "TcIb" in specimens of *R. pallescens* collected in domestic habitats, confirming that in Colombia there is a risk of *R. pallescens* intrusion supported by the high presence blood-feeding on humans and *T. cruzi* infection.

Conclusions

To the best of our knowledge, we conducted the first robust study sampling secondary vectors of *T. cruzi* in Colombia from different locations of the country. We used a broad variety of techniques to detect *T. cruzi* infection, DTUs, TcI genotypes and feeding sources that allowed us to understand the transmission dynamics in secondary vectors such as *P. geniculatus*, *T. maculata* and *R. pallescens*. Our findings reinforce the epidemiological relevance of these species and highlight the need to include them in the vector control programmes as well as in the entomological surveillance systems. Most of the secondary insects captured harbored sylvatic DTUs and fed on human blood highlighting the importance of these species. Our results demonstrate the need of the government to invest on the control of them in the light of their effort to interrupt *T. cruzi* transmission in Colombia.

Additional files

Additional file 1: Table S1: Geographical coordinates and ecotopes in vectors collected. (DOC 376 kb)

Additional file 2: Table S2: *T. cruzi* infection rates in the triatomine species studied. (DOCX 16 kb)

Additional file 3: Table S3: Pairwise G-test (*T. cruzi* infection rates by species). (DOCX 13 kb)

Additional file 4: Table S4: Pairwise G-test (Species *vs* Ecotopes). (DOCX 13 kb)

Additional file 5: Table S5: Pairwise G-test (*T. cruzi* infection rates *vs* Geographical location). (DOCX 14 kb)

Abbreviations

DTU: Discrete typing unit; OR: Odds ratio; PCR-HRM: Polymerase chain reaction-high resolution melting; qPCR: Quantitative polymerase chain reaction

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Availability of data and material

The datasets supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions
JDR and CH wrote the manuscript. JDR, CH, AT and CS analyzed the data. CH performed molecular techniques. HB, LSB, HS, ZFR, SR and GPH collected and identified insects. HB and MV contributed reagents/materials/analysis tools. All authors read and approved the final version of the manuscript.

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The authors declare that they have no competing interests.

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References

1. Bargues MD, Schofield CJ, Dujardin JP. Classification and phylogeny of the Triatominae. First Edition. American Trypanosomiasis. Elsevier Inc.; 2010. p. 117–47. Available from: http://dx.doi.org/10.1016/B978-0-12-384876-5.00006-X.
2. Waleckx E, Gourbière S, Dumonteil E. Intrusive *versus* domiciliated triatomines and the challenge of adapting vector control practices against Chagas disease. Mem Inst Oswaldo Cruz. 2015;110(3):324–38.
3. Rassi A, Rassi A, Marcondes De Rezende J. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am. 2012;26(2):275–91.
4. Guhl F, Aguilera G, Pinto N, Vergara D. Actualización de la distribución geográfica y ecoepidemiología de la fauna de triatominos (Reduviidae: Triatominae) en Colombia. Biomedica. 2007;27:143–62.
5. WHO. Weekly epidemiological record - Chagas diseases in Latin America: an epidemiological update based on 2010 estimates. 2015. p. 5–13.
6. Herrera L. Una revisión sobre reservorios de *Trypanosoma* (*Schizotrypanum*) *cruzi* (Chagas, 1909), agente etiológico de la Enfermedad de Chagas. Bol Malariol y Salud Ambient. 2010;50(1):3–15.
7. Zingales B, Miles M, Campbell D, Tibayrenc M, Macedo AM, Teixeira MMG, et al. The revised *Trypanosoma cruzi* subspecific nomenclature: Rationale, epidemiological relevance and research applications. Infect Genet Evol. 2012;12(2):240–53.
8. Guhl F, Ramírez JD. *Trypanosoma cruzi* I diversity: Towards the need of genetic subdivision? Acta Trop. 2011;119(1):1–4.
9. Burgos JM, Begher SB, Freitas JM, Bisio M, Duffy T, Altcheh J, et al. Molecular diagnosis and typing of *Trypanosoma cruzi* populations and lineages in cerebral chagas disease in a patient with AIDS. Am J Trop Med Hyg. 2005;73(6):1016–8.
10. Guhl F, Ramírez JD. Retrospective molecular integrated epidemiology of Chagas disease in Colombia. Infect Genet Evol. 2013;20:148–54.
11. Ramírez JD, Guhl F, Rendón LM, Rosas F, Marin-Neto JA, Morillo C. Chagas cardiomyopathy manifestations and *Trypanosoma cruzi* genotypes circulating in chronic Chagasic patients. PLoS Negl Trop Dis. 2010;4(11), e899.
12. Messenger LA, Miles MA, Bern C. Between a bug and a hard place: *Trypanosoma cruzi* genetic diversity and the clinical outcomes of Chagas disease. Expert Rev Anti Infect Ther. 2015;13(8):995–1029.
13. Zumaya-Estrada F, Messenger L, Lopez-Ordonez T, Lewis MD, Flores-Lopez C, Martínez-Ibarra AJ, et al. North American import? Charting the origins of an enigmatic *Trypanosoma cruzi* domestic genotype. Parasit Vectors. 2012;5(1):226.
14. Llewellyn MS, Miles MA, Carrasco HJ, Lewis MD, Yeo M, Vargas J, et al. Genome-scale multilocus microsatellite typing of *Trypanosoma cruzi* Discrete Typing Unit I reveals phylogeographic structure and specific genotypes linked to human infection. PLoS Pathog. 2009;5(5), e1000410.
15. Ramírez JD, Guhl F, Messenger L, Lewis MD, Montilla M, Cucunuba Z, et al. Contemporary cryptic sexuality in *Trypanosoma cruzi*. Mol Ecol. 2012;21(17):4216–26.
16. Ramírez JD, Tapia-calle G, Guhl F. Genetic structure of *Trypanosoma cruzi* in Colombia revealed by a High-throughput Nuclear Multilocus Sequence Typing (nMLST) approach. BMC Genet. 2013;14:96.
17. Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. Bull Am Mus Nat Hist. 1979;163:123–520.
18. Duffy T, Cura CI, Ramirez JC, Abate T, Cayo NM, Parrado R, et al. Analytical performance of a Multiplex Real-Time PCR Assay using TaqMan Probes for quantification of *Trypanosoma cruzi* satellite DNA in blood samples. PLoS Negl Trop Dis. 2013;7(1), e2000.
19. Uehara LA, Moreira OC, Oliveira AC, Azambuja P, Lima APCA, Britto C, et al. Cruzipain promotes *Trypanosoma cruzi* adhesion to *Rhodnius prolixus* midgut. PLoS Negl Trop Dis. 2012;6:12.
20. Ramírez JD, Guhl F, Umezawa ES, Morillo C, Rosas F, Marin-Neto J, et al. Evaluation of adult chronic Chagas' heart disease diagnosis by molecular and serological methods. J Clin Microbiol. 2009;47(12):3945–51.
21. Burgos JM, Diez M, Vigliano C, Bisio M, Risso M, Duffy T, et al. Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. Clin Infect Dis. 2010;51(5):485–95.
22. Villa LM, Guhl F, Zabala D, Ramírez JD, Urrea DA, Hernández DC, et al. The identification of two *Trypanosoma cruzi* I genotypes from domestic and sylvatic transmission cycles in Colombia based on a single polymerase chain reaction amplification of the spliced-leader intergenic region. Mem Inst Oswaldo Cruz. 2013;108(7):932–5.
23. Peña VH, Fernández GJ, Gómez-Palacio AM, Mejía-Jaramillo AM, Cantillo O, Triana-Chávez O. High-resolution melting (HRM) of the cytochrome B gene: A powerful approach to identify blood-meal sources in Chagas disease vectors. PLoS Negl Trop Dis. 2012;6:2.
24. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30(12):2725–9.
25. Deane MP, Lenzi HL, Jansen A. *Trypanosoma cruzi*: vertebrate and invertebrate cycles in the same mammal host, the opossum *Didelphis marsupialis*. Mem Inst Oswaldo Cruz. 1984;79:513–5.
26. Cantillo-Barraza O, Garcés E, Gómez-Palacio A, Cortés LA, Pereira A, Marcet PL, et al. Eco-epidemiological study of an endemic Chagas disease region in northern Colombia reveals the importance of *Triatoma maculata* (Hemiptera: Reduviidae), dogs and *Didelphis marsupialis* in *Trypanosoma cruzi* maintenance. Parasit Vectors. 2015;8(1):482.
27. Cantillo-Barraza O, Chaverra D, Marcet P, Arboleda-Sánchez S, Triana-Chávez O. *Trypanosoma cruzi* transmission in a Colombian Caribbean region suggests that secondary vectors play an important epidemiological role. Parasit Vectors. 2014;7(1):381.
28. Ramírez JD, Montilla M, Cucunubá ZM, Floréz AC, Zambrano P, Guhl F. Molecular epidemiology of human oral Chagas disease outbreaks in Colombia. PLoS Negl Trop Dis. 2013;7(2):1–7.
29. Brenière SF, Waleckx E, Barnabé C, Tibayrenc M, Kjellberg F, Ayala F, et al. Over six thousand *Trypanosoma cruzi* strains classified into Discrete Typing Units (DTUs): Attempt at an inventory. PLoS Negl Trop Dis. 2016;10(8), e0004792.
30. Hernández C, Cucunubá Z, Flórez C, Olivera M, Valencia C, Zambrano P, et al. Molecular diagnosis of Chagas disease in Colombia: Parasitic loads and Discrete Typing Units in patients from acute and chronic phases. PLoS Negl Trop Dis. 2016;10(9), e0004997.
31. Messenger LA, Ramirez JD, Llewellyn MS, Guhl FMM. Importation of hybrid human-associated *Trypanosoma cruzi* strains of southern South American origin, Colombia. Emerg Infect Dis. 2016;22:8.

32. Luitgards-Moura JF, Vargas AB, Almeida CE, Magno-Esperança G, Agapito-Souza R, Folly-Ramos E, et al. A *Triatoma maculata* (Hemiptera, Reduviidae, Triatominae) population from Roraima, Amazon Region, Brazil, has some bionomic characteristics of a potential Chagas disease vector. Rev Inst Med Trop Sao Paulo. 2005;47(3):131–7.
33. García-Alzate R, Lozano-Arias D, Reyes-Lugo RM, Morocoima A, Herrera L, Mendoza-León A. *Triatoma maculata*, the vector of *Trypanosoma cruzi*, in Venezuela. Phenotypic and genotypic variability as potential indicator of vector displacement into the domestic habitat. Front public Heal. 2014;2:170.
34. Rojas ME, Várquez P, Villarreal MF, Velandia C, Vergara L, Morán-Borges YH, et al. An entomological and seroepidemiological study of Chagas' disease in an area in central-western Venezuela infested with *Triatoma maculata* (Erichson 1848). Cad saude publica/Minist da Saude, Fund Oswaldo Cruz, Esc Nac Saude Publica. 2008;24(10):2323–33.
35. Espinola H, Rodríguez Md BRT. Informaciones sobre la biología y el ciclo de vida de *Triatoma maculata* (Erichson, 1848) (Hemiptera, Reduviidae, Triatominae), en condiciones de laboratorio. Bol Dir Malariol San Amb. 1981;21:141–2.
36. Tonn R, Otero E, Mora H, Carcavallo R. Aspectos biológicos,ecológicos y distribución geográfica de *Triatoma maculata* (Erichson, 1848), (Hemiptera, Reduviidae), en Venezuela. Bol Dir Malariol San Amb. 1978;18:16–24.
37. Feliciangeli M, Rabinovich J. Vital statistics of triatominae (Hemiptera: Reduviidae) under laboratory conditions. J Med Entomol. 1985;22:43–8.
38. Luitgards-Moura JF, Borges-Pereira J, Costa J, Zauza PL, Rosa-Freitas MG. On the possibility of autochthonous chagas disease in Roraima, Amazon Region, Brazil, 2000–2001. Rev Inst Med Trop Sao Paulo. 2005;47(1):45–54.
39. Gómez-Melendro EN, Hernández C, González-Uribe C, Brochero H. First record of *Triatoma maculata* (Erichson, 1848) (Hemiptera: Reduviidae: Triatomini) in the Municipality of Riohacha. La Guajira Colombia Front Public Heal. 2014;2(219):1–9.
40. Escalante ME, Gomez D, Silvera LA, Sánchez G, Venegas J. Detection of high percentage of *Trypanosoma cruzi* infection, the etiologic agent of Chagas disease, in wild populations of Colombian Caribbean triatomines. Acta Parasitol. 2015;60(2):315–21.
41. Patterson JS, Barbosa SE, Feliciangeli MD. On the genus *Panstrongylus* Berg 1879: Evolution, ecology and epidemiological significance. Acta Trop. 2009; 110(2–3):187–99.
42. Angulo VM, Esteban L, Luna KP. *Attalea butyracea* próximas a las viviendas como posible fuente de infestación domiciliaria por *Rhodnius prolixus* (Hemiptera: Reduviidae) en los Llanos Orientales de Colombia. Biomedica. 2012;32(2):277–85.
43. Serrano O, Mendoza F, Suárez B, Soto A. Seroepidemiología de la enfermedad de Chagas en dos localidades del municipio Costa de Oro, Estado de Aragua, Venezuela. Biomédica Rev Del Inst Nac Salud. 2008; 28(1):108–15.
44. Maeda FY, Cortez C, Alves RM, Yoshida N. Mammalian cell invasion by closely related *Trypanosoma* species *T. dionisii* and *T. cruzi*. Acta Trop. 2012; 121(2):141–7.
45. Reyes-lugo M. Panstrongylus geniculatus Latreille 1811 (Hemiptera : Reduviidae : Triatominae), vector de la enfermedad de Chagas en el ambiente domiciliario del centro-norte de Venezuela. Medicina (B Aires). 2009;20(3):180–205.
46. Valente VC, Valente SA, Noireau F, Carrasco HJ, Miles MA. Chagas disease in the Amazon Basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with domestic pigs. J Med Entomol. 1998;35(2):99–103.
47. Feliciangeli MD, Carrasco H, Patterson JS, Suarez B, Martínez C, Medina M. Mixed domestic infestation by *Rhodnius prolixus* Stål, 1859 and *Panstrongylus geniculatus* (Latreille, 1811), vector incrimination, and seroprevalence for *Trypanosoma cruzi* among inhabitants in El Guamito, Lara State, Venezuela. Am J Trop Med Hyg. 2004;71(4):501–5.
48. Carrasco HJ, Torrellas A, García C, Segovia M, Feliciangeli MD. Risk of *Trypanosoma cruzi* I (Kinetoplastida: Trypanosomatidae) transmission by *Panstrongylus geniculatus* (Hemiptera: Reduviidae) in Caracas (Metropolitan District) and neighboring States, Venezuela. Int J Parasitol. 2005;35(13):1379–84.
49. Carrasco HJ, Segovia M, Londoño JC, Ortegoza J, Rodríguez M, Martínez CE. *Panstrongylus geniculatus* and four other species of triatomine bug involved in the *Trypanosoma cruzi* enzootic cycle: high risk factors for Chagas' disease transmission in the Metropolitan District of Caracas, Venezuela. Parasit Vectors. 2014;7(1):1003–28.
50. Maestre-Serrano R, Eyes-Escalante M. Actualización de la presencia y distribución de triatominos en el departamento del Atlántico-Colombia: 2003–2010. Bol Malariol y Salud Ambient. 2012;52(1):125–8.
51. Jácome-Pinilla D, Hincapie-Peñaloza E, Ortiz MI, Ramírez JD, Guhl F, Molina J. Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit Vectors. 2015;8(1):600.
52. De Noya BA, Díaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Muíoz-Calderón A, et al. Update on oral chagas disease outbreaks in Venezuela: Epidemiological, clinical and diagnostic approaches. Mem Inst Oswaldo Cruz. 2015;110(3):377–86.
53. Molinari J, Aldana E, Nassar JM. *Panstrongylus geniculatus* (Heteroptera: Reduviidae: Triatominae): Natural infection with *Trypanosoma cruzi* under cavernicolous conditions in Paraguaná Peninsula, Venezuela. J Cave Karst Stud. 2007;69(2):285–7.
54. Aldana E, Heredia-Coronado E, Avendaño-Rangel F, Lizano E, Concepción JL, Bonfante-Cabarcas R, et al. Morphometric analysis of *Panstrongylus geniculatus* (Heteroptera: Reduviidae) from Caracas City, Venezuela. Biomedica. 2011;31:108–17.
55. Mejía-Jaramillo AM, Fernández GJ, Montilla M, Nicholls RS, Triana-Chávez O. Sensibilidad al benzonidazol de cepas de *Trypanosoma cruzi* sugiere la circulación de cepas naturalmente resistentes en Colombia. Biomédica, Rev del Inst Nac Salud. 2012;32(2):196–205.
56. Soto H, Tibaduiza T, Montilla M, Triana-Chávez O, Suárez DC, Torres Torres M, et al. Investigación de vectores y reservorios en brote de Chagas agudo por posible transmisión oral en Aguachica, Cesar, Colombia. Cad Saúde Pública. 2014;30(4):746–56.
57. Romaña CA, Pizarro JC, Rodas E, Guilbert E. Palm trees as ecological indicators of risk areas for Chagas disease. Trans R Soc Trop Med Hyg. 1999; 93(Jan 2016):594–5.
58. Cantillo-Barraza O, Gómez-Palacio A, Salazar D, Mejía-Jaramillo AM, Calle J, Triana O. Distribution and ecoepidemiology of the triatomine fauna (Hemiptera: Reduviidae) in Margarita Island, Bolívar, Colombia. Biomedica. 2010;30(3):382–9.
59. Gottdenker NL, Chaves LF, Calzada JE, Saldaña A, Carroll CR. Host life history strategy, species diversity, and habitat influence *Trypanosoma cruzi* vector infection in changing landscapes. PLoS Negl Trop Dis. 2012;6(11):5–7.
60. Jaramillo N, Schofield CJ, Gorla D, Caro-Riaño H, Moreno J, Mejia E, et al. The role of *Rhodnius pallescens* as a vector of Chagas disease in Colombia and Panama. Res Rev Parasitol. 2000;60(3–4):75–82.
61. Parra-henao G, Angulo V, Jaramillo N, Restrepo M. Triatominos (Hemiptera: Reduviidae) de la Sierra Nevada de Santa Marta, Colombia. Aspectos epidemiológicos, entomológicos y de distribución. Rev CES Med. 2009;23(1):17–26.
62. Pipkin AC. Domiciliary reduviid bugs and the epidemiology of Chagas disease in Panama (Hemiptera: Reduviidae: Triatominae). J Med Entomol. 1968;5(1):107–24.
63. Pineda V, Montalvo E, Alvarez D, Santamaría AM, Calzada JE, Saldaña A. Feeding sources and trypanosome infection index of *Rhodnius pallescens* in a Chagas disease endemic area of Amador County, Panama. Rev Inst Med Trop Sao Paulo. 2008;50(2):113–6.
64. Calzada JE, Pineda V, Montalvo E, Alvarez D, Santamaría AM, Samudio F, et al. Human trypanosome infection and the presence of intradomicile *Rhodnius pallescens* in the western border of the Panama Canal, Panama. Am J Trop Med Hyg. 2006;74(5):762–5.
65. Hurtado LA, Calzada JE, Pineda V, González K, Santamaría AM, Cáceres L, et al. Conocimientos y factores de riesgo relacionados con la enfermedad de Chagas en dos comunidades panameñas donde *Rhodnius pallescens* es el vector principal. Biomedica. 2014;34:260–70.
66. De Vasquez AM, Samudio FE, Saldaña A, Paz HM, Calzada JE. Eco-epidemiological aspects of *Trypanosoma cruzi*, *Trypanosoma rangeli* and their vector (*Rhodnius pallescens*) in Panama. Rev Inst Med Trop Sao Paulo. 2004;46(4):217–22.
67. Zeledón R, Marín F, Calvo N, Lugo E, Valle S. Distribution and ecological aspects of *Rhodnius pallescens* in Costa Rica and Nicaragua and their epidemiological implications. Mem Inst Oswaldo Cruz. 2006;101(1):75–9.

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Parasites & Vectors

RESEARCH Open Access

Trypanosoma cruzi infection, discrete typing units and feeding sources among *Psammolestes arthuri* (Reduviidae: Triatominae) collected in eastern Colombia

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Abstract

Background: Chagas disease (CD) is caused by the protozoan parasite *Trypanosoma cruzi*, and is transmitted by hematophagous insects of the family Reduviidae. *Psammolestes arthuri* is a sylvatic triatomine distributed in Colombia and Venezuela which feeds on birds and there are a few studies that have reported *Ps. arthuri* naturally infected with *T. cruzi*. In Colombia, *Ps. arthuri* has been found in dwellings, making it important to evaluate its possible role in the *T. cruzi* transmission cycle. We aimed to evaluate the presence of *T. cruzi* and feeding sources of *Ps. arthuri* to elucidate new possible scenarios of *T. cruzi* transmission in the country.

Methods: A total of 60 *Ps. arthuri* were collected in Arauca and Casanare, Colombia. We detected and genotyped *T. cruzi* and identified feeding sources. The frequency of the presence of *T. cruzi* was obtained and compared with different eco-epidemiological variables. Multiple correspondence analysis was conducted to explore associations between eco-epidemiological variables and the presence of *T. cruzi*; with these results, a logistic regression was used to determine statistical associations.

Results: The infection rate of *T. cruzi* was 70.7% and was mostly associated with insect stage, sex, bird nest and feeding source. Regarding discrete typing units (DTUs), TcI was found in 54.7% samples, of which 21.7% (5/23) were TcI_{Dom}, 52.1% (12/23) had mixed infection (TcI_{Dom}-TcI_{Sylv}), and single infection with TcI_{Sylv} was not detected. Mixed infections (TcI/TcII-TcVI) were found in 9.52% (4/42) of the samples; of these, 14.2% (6/42) were TcII-TcVI. A total of 15 feeding sources were identified and the most frequent were: *Cranioleuca baroni* (35.85%), *Homo sapiens* (26.42%), *Thraupis episcopus* (11.32%) and *Serinus albogularis* (3.77%).

Conclusions: Although *Ps. arthuri* is mainly ornithophilic, this species may be feeding on other animals that can be infected with *T. cruzi*, possibly playing a role maintaining the zoonotic cycle of the parasite. Further studies with molecular techniques and wider sampling are needed to improve information regarding infection rates, ecotopes and habits with the aim of evaluating whether *Ps. arthuri* could be a potential *T. cruzi* vector.

Keywords: Chagas disease, *Trypanosoma cruzi*, *Psammolestes arthuri*, Colombia

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Background

Trypanosoma cruzi is a flagellated protozoan that causes Chagas disease (CD), and is mainly transmitted by insects of the family Reduviidae (Order: Hemiptera) through their feces [1]. CD affects about 6–7 million people around the world and is especially important in Latin America, where it is considered a public health problem [2]. Based on 2010 estimates, in Colombia there are 437,960 infected people, with an estimated 5274 new cases annually due to vectorial transmission [3]. CD has been reported in different geographical regions of the country, with the departments of Casanare, Arauca, Santander, Boyacá, Norte de Santander and Cundinamarca being endemic for the disease [4]. Due to the high genetic diversity of *T. cruzi*, the parasite has been subdivided into discrete typing units (DTUs): TcI to TcVI [5, 6] and a genotype associated to bats (TcBat) [7–9]. Each DTU presents different characteristics based on its geographical distribution, clinical manifestations of the disease and epidemiological associations. TcI is the most widely distributed DTU in the Americas, and because of its genetic diversity, it has been divided into domestic and sylvatic genotypes (TcI_{Dom} and TcI_{Syl}) [10].

Triatomines of the family Reduviidae are widely distributed in the Americas, mainly in the Neotropical region at different altitudes, they present a high adaptation to different feeding sources, especially sylvatic and domestic mammals [11, 12]. There are about 180 species of synantropic, sylvatic and domestic animals that have been reported as potential *T. cruzi* reservoirs, mainly rodents and marsupials (Didelphimorphia) [13], rodents, bats, dogs, birds (hens), cows, armadillos and other mammals (anteaters and humans) [13–16]. In Colombia there are 26 registered triatomine species of which 15 have been reported as naturally infected with *T. cruzi* [17, 18]. *Rhodnius prolixus* and *Triatoma dimidiata* are the main species that transmit *T. cruzi* and are considered primary vectors of the parasite; therefore, surveillance programs focus mainly on these two species [15, 18]. Recently, some species, such as *Panstrongylus geniculatus*, *R. pallescens* and *Eratyrus mucronatus*, have begun to be considered important in the transmission of this parasite because of their domiciliation process due to deforestation and urbanization [18, 19] that disturbs triatomine ecology [11, 15]. Furthermore, defaunation caused by ecological disturbances, such as anthropogenic deforestation and land-use conversion, directly impacts biotic interactions [20]. In this case, fauna migration and extinction can indirectly affect triatomine populations due to their hematophagic habits, forcing them to find other ecosystemic units and feeding sources.

There are some triatomine species considered as secondary vectors for the transmission of *T. cruzi* to humans, which include *Psammolestes arthuri*, *Panstrongylus lignarius*, *Cavernicola pilosa*, *Belminus rugulosus*, *Rhodnius colombiensis*, *Triatoma dispar* and *Microtriatoma trinidadensis*. *Psammolestes arthuri* is a sylvatic triatomine distributed in Colombia and Venezuela which feeds mainly on birds, and is frequently found in nests [21]. There are a few studies that report *Ps. arthuri* naturally infected with *T. cruzi* [22–24]. One study conducted in Venezuela in 2014 by Cruz-Guzmán et al. [25] reported *Ps. arthuri* naturally infected with *T. cruzi* and coexisting in the same geographical space with *R. prolixus* and *T. maculata*, but it is still not considered important in the transmission of *T. cruzi* for humans [26]. Despite of this, due to recent evidence in Venezuela, the possibility that *Ps. arthuri* is feeding on infected mammals thus contributing to the maintenance of the sylvatic cycle of *T. cruzi* [25, 27] in Colombia cannot be dismissed. In Colombia, *R. prolixus*, *E. mucronatus* and *Ps. arthuri* have been reported inside houses, associated with the nearby presence of *Attalea butyracea* [28, 29]. This fact is relevant because *Ps. arthuri* could be participating in the domestic cycle of *T. cruzi*. Then, considering the ability of triatomines to be adaptive depending on the feeding sources available, it is important to include sylvatic triatomines in eco-epidemiology studies of CD [30] because they can represent a connection between domestic and sylvatic transmission cycles of the parasite [15].

Therefore, in the light of the current absence of studies investigating the plausible presence of *T. cruzi* in sylvatic triatomines such as *Ps. arthuri*, we evaluated the presence of *T. cruzi*, the DTUs and feeding sources of *Ps. arthuri* collected in the departments of Arauca and Casanare, Colombia (eastern departments of the country), with the aim to elucidate new possible scenarios of *T. cruzi* transmission in the country.

Methods

Sampling, DNA extraction and detection of *T. cruzi*

A total of 60 *Ps. arthuri* were collected in the departments of Arauca (2 specimens) and Casanare (58 specimens) (Fig. 1a). In Casanare, specimens were collected in the municipalities of Mani (Mararabe: 4°49′59.88″N, 72°20′60″W), Monterrey (Marenao: 4°53′4.0992″N, 72°45′19.08″W), Paz de Ariporo (Caño chiquito: 5°45′0″N, 71°28′59.988″W), Pore (El verde: 5°40′34.1976″N, 72°4′31.0368″W) and Tamara (La picacha: 5°50′40.632″N, 72°9′49.176″W). In Arauca, the specimens were collected in Arauquita (Arauca: 6°58′5.8512″N, 71°11′47.6124″W). Casanare specimens were collected in trees near human houses (Fig. 1b) and captured in *Cacicus cela* (yellow-rumped cacique) and *Phacellodomus rufifrons* (rufous-fronted thornbird) nests using manual capturing (Fig. 1c). Furthermore, Arauca

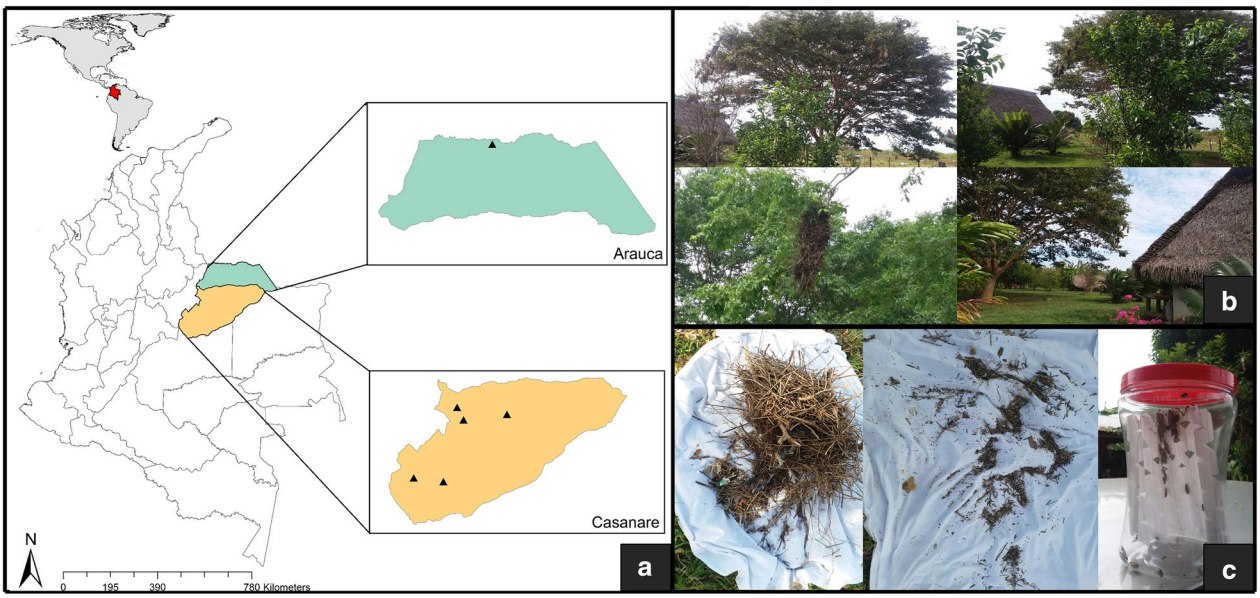

Fig. 1 **a** Geographical distribution of 60 *Ps. arthuri* collected in the departments of Arauca and Casanare, Colombia. **b** Collection sites in Casanare. **c** Capturing methods in the field

insects were collected in the domestic ecotope (inside the houses). All specimens were stored and conserved in ethanol until processing. DNA extraction of the insects' guts was conducted using a Qiagen Dneasy Blood & Tissue kit (Qiagen, Berlin, Germany). Detection of *T. cruzi* was conducted by end-point qPCR using TaqMan Fast Advanced Master Mix 2× (Roche Diagnostics GmbH, Mannheim, Germany), water and the primers cruzi1 (10 µM) (5′-AST CGG CTG ATC GTT TTC-3′), cruzi2 (10 µM) (5′-AAT TCC TCC AAG CAG CGG ATA-3′) and a cruzi3 probe (5 µM) (FAM-CAC ACA CTG GAC ACC AA-NFQ-MGB) to detect the satellite tandem repeat DNA of the parasite (166 bp) following the conditions previously reported [31]. A Ct value < 38 was considered as positive amplification [15]. For insects with a positive qPCR result, a conventional PCR for kinetoplast DNA amplification was conducted using Buffer Taq 10×, $MgCl_2$ 50 mM, dNTPs 25 mM, Taq Platinum 5 U/µl, water and the primers 121 (50 pmol/µl) (5′-AAA TAA TGT ACG GGK GAG ATG CAT GA-3′) and 122 (50 pmol/µl) (5′-GGT TCG ATT GGG GTT GGT GTA ATA TA-3′) to discriminate between *T. cruzi* (330 bp) and *T. rangeli* (400–450 bp) as reported elsewhere [32].

Genotyping of *T. cruzi*

Parasite genotyping was accomplished by the amplification of the spliced leader intergenic region of miniexon gene (SL-IR), dividing DTUs in two groups: TcI (350 bp) and TcII-TcVI (300 bp). The reaction mix consisted of Go Taq Green Master Mix 2×, water and primers TCC (10 nM) (5′-CCC CCC TCC CAG GCC ACA CTG-3′), TC1 (10 nM) (5′-GTG TCC GCC ACC TCC TTC GGG CC-3′) and TC2 (10 nM) (5′-CCT GCA GGC ACA CGT GTG TGT G-3′) [33]. Then, insects with mixed infection (TcI/TcII-TcVI) and TcII-TcVI group results were submitted to Sanger sequencing using the TCC primer as reported elsewhere [34]. PCR products were cleaned using ExoSAP-IT® Express PCR Product Cleanup 75001/75002 (Affymetrix, USB, California, USA) and then submitted to Sanger sequencing. Sequences were aligned in MEGA X software [35] and submitted to BLASTn for similarity search.

Feeding source characterization

A 215 bp fragment of the *12S* gene fragment was amplified using Go Taq Green Master Mix, water and primers L1085 (10 nM) (5′-CCC AAA CTG GGA TTA GAT ACC C-3′) and H1259 (10 nM) (5′-GTT TGC TGA AGA TGG CGG TA-3′) as reported by Dumonteil et al. [35]. PCR products were cleaned using ExoSAP-IT® Express PCR Product Cleanup 75001/75002 (Affimetrix) and then submitted to Sanger sequencing. Resulting sequences were edited in MEGA X software [36] and submitted to BLASTn for similarity search.

Statistical analysis

We determined the frequency of *T. cruzi* infection, as well as DTUs and feeding sources, regarding variables such as ecotope (sylvatic or domestic), insect stage (adult or nymph), sex (male or female) and bird nests

(*P. rufifrons* or *C. cela*) to find any plausible associations among them. We carried out a multiple correspondence analysis (MCA) using 3 dimensions to explore the proximity of the variables with the presence of *T. cruzi*. The MCA was made with 51 samples, since 9 samples were excluded due to their lack of information for the evaluated variables. Cronbach's alpha coefficient and inercy were calculated to establish dimension consistency in the model and to determine the proportion of total variability contributed by each variable in the matrix, respectively.

A binary logistic regression, without including the intercept, was made to determine the statistical associations between *T. cruzi* infection and the explicative variables: locality, ecotope, insect stage, sex, bird nest and feeding source (x_1, x_2, x_3....), previously identified in the MCA. Logistic regression was selected between five multivariate models that adjusted to type of data (Additional file 1: Figure S1). Statistical analyses were performed in Statistical Package for the Social Sciences (SPSS) v.25 and a P-value < 0.05 was considered statistically significant.

Results

Frequency of *T. cruzi* infection and DTUs

Of the 60 *Ps. arthuri* collected, we found that 70.7% (42/60) were positive by end-point qPCR for *T. cruzi*. Of these, TcI was found in 54.7% of the samples (23/42), of which 21.7% (5/23) were TcI_{Dom}, 52.1% (12/23) had mixed infection (TcI_{Dom}-TcI_{Sylv}) and 26% (6/23) were not able to be typed. Mixed infection (TcI/TcII-TcVI) was found in 9.52% (4/42) of the samples, 14.2% (6/42) were TcII-TcVI and 21.4% (9/42) samples were not able to be typed. Table 1 shows the eco-epidemiological information evaluated by ecotope, insect stage, sex and bird nests. As shown in Table 1, 97.6% of the *Ps. arthuri* were positive for *T. cruzi* belonging to sylvatic ecotope, whereas just one domestic insect was positive for *T. cruzi* presence. No nymphs were infected with *T. cruzi* and 70% of the insects were found in *Phacellodomus rufifrons* nests. No insects were found infected with *T. rangeli.*

Table 1 Frequency of infection with *T. cruzi* among eco-epidemiological variables

Eco-epidemiological variable	*T. cruzi*		
	Positive (%)	Negative (%)	All (95% CI)
Ecotope			
Sylvatic	41 (97.6)	17 (94.4)	58 (89.7–99.3)
Domestic	1 (2.4)	1 (5.6)	2 (0.7–10.3)
Insect stages			
Adults	42 (100)	15 (83.3)	57 (87.3–98.6)
Nymphs	0 (0)	3 (16.7)	3 (1.4–12.7)
Sex			
Male	22 (52.4)	7 (46.7)	29 (38.1–63.5)
Female	20 (47.6)	8 (53.3)	28 (16.0–38.1)
Bird nest			
Phacellodomus rufifrons	29 (70.7)	14 (82.4)	43 (61.9–84.0)
Cacicus cela	12 (29.3)	3 (17.6)	15 (16.0–38.1)

Feeding sources of *Ps. arthuri*

A total of 15 feeding sources of *Ps. arthuri* were found (Fig. 2). The frequencies were: *Cranioleuca baroni* (Baron's spinetail) (35.85%, $n = 19$), *Homo sapiens* (human) (28.30%, $n = 15$), *Thraupis episcopus* (blue-gray tanager) (11.32%, $n = 6$), *Serinus albogularis* (white-throated canary) (3.77%, $n = 2$) and 11 other species corresponding to 1.89% [*Arremon aurantiirostris* (orange-billed sparrow), *Chrysolophus amherstiae* (Lady Amherst pheasant), *Geospiza magnirostris* (large ground-finch), *Icterus mesomelas* (yellow-tailed oriole), *Melospiza melodia* (song sparrow), *Phasianus colchicus alaschanicus* (common pheasant), *Pipilo maculatus* (spotted towhee), *Pan paniscus* (bonobo), *Pyrrhula pyrrhula* (common bullfinch), *Serinus canaria* (wild canary) and *Varanus flavescens* (yellow monitor)].

Additionally, we found that the majority of insects feeding on different bird species were *T. cruzi* positive, especially those who fed on *C. baroni*, and mostly presented TcI DTU. Additionally, we found that insects that were *T. cruzi* positive fed on humans presented TcI DTU (6/14) and just one insect had TcII. Mixed infection was found when *Ps. arthuri* fed on 2 species: *C. baroni* (2) and *S. albogularis* (1). Two species, *Arremon aurantiirostris* and *Phasianus colchicus alaschanicus*, presented only TcII. Some feeding sources were *T. cruzi* positive but no information about DTUs was available (Fig. 3).

Proxy associations between *T. cruzi* infection and eco-epidemiological variables

Two subgroups of variables were identified through the MCA. The first was associated with the variables ecotope and locality, and the second was associated with the variables *T. cruzi* infection, insect stage, sex, bird nest and feeding source. Additionally, in dimension 2 we identified a geometric relation between the variable ecotope and *T. cruzi* presence (Table 2). Once MCA values were obtained, a proximity bidimensional plot (bi-plot) was made to elucidate relations between variables by representing the geometric distribution in three dimensions of evaluated variables (Fig. 4). It can be observed that, generally, *T. cruzi* presence was mostly associated with insect stage, sex, bird nest and feeding source. Logistic regression results showed that Pore municipality, adult insect stage, male *Ps. arthuri*, *C. cela* bird nest and *C. baroni* feeding preference were related the most with *T. cruzi*

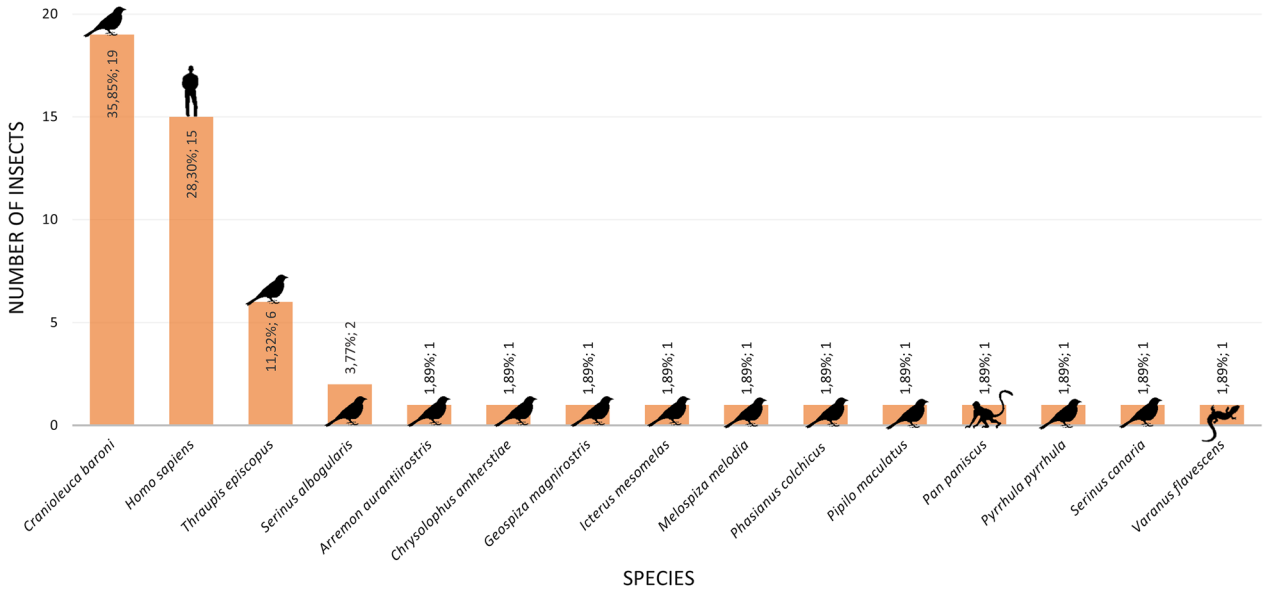

Fig. 2 Frequency of the 15 feeding sources found in the evaluated *Ps. arthuri*

presence (Table 3). This analysis is consistent with results obtained in the MCA (Fig. 4).

Discussion

In Colombia 15 of the 26 registered triatomines have been reported as naturally infected with *T. cruzi* and some are considered secondary vectors for *T. cruzi* transmission [18]. This last group includes *Ps. arthuri*, a triatomine with sylvatic habits [21]. *Psammolestes arthuri* has been reported in three departments of Colombia, Meta, Arauca and Casanare, with no available data about *T. cruzi* presence [18]. Its presence has been associated with *Phacellodomus rufifrons* nests [37]. Furthermore, there are some studies that report triatomine species with sylvatic habits in a domiciliation process due to deforestation and ecosystemic fragmentation. Therefore, these triatomines have begun to be considered an important factor in parasite transmission [18, 19], especially in Arauca and Casanare, where it has been found that sylvatic triatomine species such as *Ps. arthuri*, *P. geniculatus*, *T. maculata* and *T. venosa*, are moving to human houses [15, 28].

In this study, the percentage of *Ps. arthuri* infected with *T. cruzi* was high (70%), which might suppose a possibility that *Ps. arthuri* is changing its ornithophilic behavior to feed on other blood sources, including human blood (Fig. 2). It is important to highlight that niche changing or adaptation to other niches starts with a behavioral change [41], which is relevant in the context of new transmission scenarios of *T. cruzi*. By feeding solely on birds, triatomines cannot get infected with the parasite, because birds are naturally resistant to *T. cruzi* infection due to their innate immune mechanism against the parasite that quickly eliminates infective forms from their system [42]. Schaub [43] showed that chicken blood had a lytic effect for trypanosomes after an incubation period of 60 minutes, but transmission could still occur because of lysis-resistant epimastigotes in the triatomine gut. Otherwise, it is known that specific feeding preferences of triatomines can influence the transmission dynamics of *T. cruzi* [44]. A study showed that mice infected with *T. cruzi* isolates previously exposed to bird blood presented a high survival rate. This suggests that ornithophilic behavior of triatomines could modulate the parasite and bird blood does not prevent parasite development and transmission [45].

On the other hand, direct transmission of *T. cruzi* between insects can occur through entomophagy behavior such as cannibalism, coprophagy and cleptohaematophagy. Cannibalism in triatomines is rare and poorly demonstrated but coprophagic behavior is associated with the acquisition of microbe symbionts and is a possibility of infection that cannot be excluded [43]. Coprophagy has been described for many insects, such as Isoptera (termites), Hemiptera (true bugs) and Blattaria (cockroaches) as a predominant route of beneficial bacteria transmission [46], and the possibility of *T. cruzi* transmission should also be considered. Otherwise, some triatomine species are able to feed on the hemolymph of other insects if starving [47]. Sandoval et al. [48] showed that *Belminus herreri* in early stages was unable to feed on vertebrate hosts but successfully fed on replete *R.*

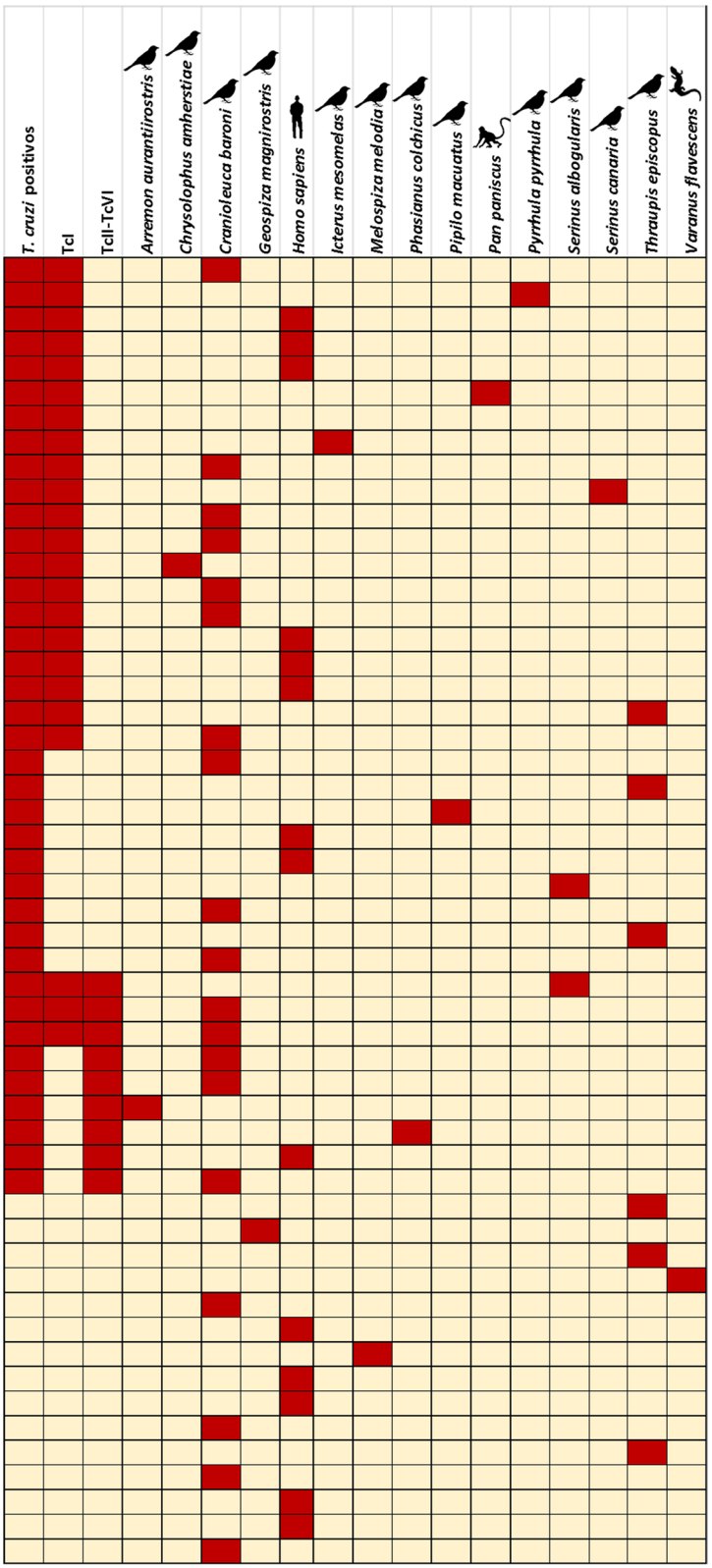

Fig. 3 *T. cruzi* presence, DTUs and feeding sources of 53/60 *Ps. arthuri* collected

Table 2 MCA values for each variable in 3 dimensions (Dim) as previously described

Variable	Dim 1	Dim 2	Dim 3
Locality (municipality)	1.349[a]	0.804	0.661
Ecotope	1.311[a]	0.041[c]	0.009
Insect stage	0.008[b]	0.116	0.613
Sex	0.190[b]	0.162	0.048
Bird nest	0.033[b]	0.590	0.050
Feeding sources	0.049[b]	0.524	0.371
T. cruzi	0.020[b]	0.074[c]	0.452

[a] First subgroup of variables

[b] Second subgroup of variables

[c] Relation between ecotope and *T. cruzi* presence

Note: α-Cronbach values: dim 1, 0.772; dim 2, 0.662; dim 3, 0.637. Inercy values: dim1, 0.423; dim 2, 0.330; dim 3, 0.315

prolixus, showing a cleptohaematophagy behavior. This behavior has also been reported with *R. prolixus* nymphs [49]. Therefore, this can be considered as another possible way by which *Ps. arthuri* could become infected with *T. cruzi*, taking into account that this triatomine has been found in sympatry with *R. prolixus* [25, 28], but further studies are required to prove this hypothesis.

Most of the *T. cruzi*-positive insects (70.7%, 29/43) were found in *P. rufifrons* nests (Table 2). The same as reported by Cruz-Guzmán et al. [25]. However, in Table 3, logistic regression indicates that the *C. cela* bird nest is statistically more significant with respect to the presence of *T. cruzi* (OR 4.00; 95% CI: 1.12–14.17) but this could be explained by the sample size in these nests. Additionally, other variables such as adult insect stage (OR 2.8; 95% CI: 1.55–5.04), male sex (OR 3.14; 95% CI: 1.34–7.35) and feeding on *C. baroni* (OR 3.75; 95% CI: 1.24–11.29) appear to be associated with the presence of *T. cruzi* in *Ps. arthuri*. Adult life stage association with *T. cruzi* presence in *Ps. arthuri* could be explained if it is considered as the mobile stage, in which triatomines can move around to take different meals. Regarding the *C. baroni* relation with *T. cruzi* presence, we consider it plausible that insects feeding on this bird species are more likely to feed on other animals that might be infected because these birds leave their nests (migrate), forcing triatomines to move and find other feeding sources [25].

In a study made in Venezuela in 2014, Cruz-Guzmán et al. [25] found a few *Ps. arthuri* naturally infected with *T. cruzi*, despite the fact that these triatomines fed on birds. This feeding preference is not a coincidence, because *Psammolestes* genus is closely related with *Rhodnius* (Rhodniini Tribe) [38], and many species of this genus feed on birds, due to their associations with palm trees in which there are bird nests [39]. Something similar occurs with *T. maculata*, a triatomine with wide distribution in Colombia, whose diet also consist mainly of bird blood. Because of that, this triatomine is excluded from vector control programs [40]. However, in 2016 Hernández et al. [15] reported *T. maculata* feeding on humans with a frequency of 75% and also infected with *T. cruzi* with a frequency of 67%. They also found TcI and TcII in some specimens, suggesting a connection between parasites' domestic and sylvatic transmission cycles by this triatomine. Finally, they highlighted *T. maculata* as a potential vector for CD and underlined the importance of prioritizing secondary vectors in vector surveillance due to their capacity of domiciliation. These findings may suggest a future behavior that *Ps. arthuri* could develop, but further studies about its potential as a *T. cruzi* vector are needed. The MCA results highlight that *T. cruzi* presence appears to be highly associated with Pore municipality (Fig. 4). This is due to the sample size, because this was the only municipality in which all of the specimens, except one, were positive for *T. cruzi* presence. However, the obtained frequency for *T. cruzi* presence in Pore, Casanare, could be explained by taking into account other triatomines present in this area such as *R. prolixus*, a triatomine species with a wide distribution in Colombia and the one that presents the highest rate of infection with *T. cruzi* [18].

Triatomines are known as nest-dwelling insects, and their usual hosts are tree-dwelling animals such as birds, reptiles, marsupials and burrow vertebrates (e.g. bats, rodents and armadillos) [50]. Additionally, their alimentary habits may be influenced by the density and availability of new feeding sources [30] which is relevant in the context of human urbanization. Here, we highlight that 28.3% of *Ps. arthuri* that were positive for *T. cruzi* had fed on humans (Fig. 2), being the first study in Colombia and, to our knowledge, in the region to report it. This is a fact to underline, because there is an option where, besides the fact that *Ps. arthuri* is reaching dwellings, human activities near houses mean that people are accessible to these triatomines. Further studies are required to evaluate the possibility of *Ps. arthuri* effectively transmitting *T. cruzi* to humans. In this case, these findings become relevant for CD transmission; particularly in an endemic region for CD such as Casanare, which shows the highest incidence for this pathology in the country and where most of the specimens were collected.

The wide variety of feeding sources of *Ps. arthuri* found in this study could be explained because other animals often use *P. rufifrons* nests [51], which may explain the non-bird feeding sources found in some of the insects evaluated, such as *Varanus flavescens* and *Pan paniscus* (Fig. 2). Additionally, *Ps. arthuri* has been found in other bird nests and under the bark of dead trees [52],

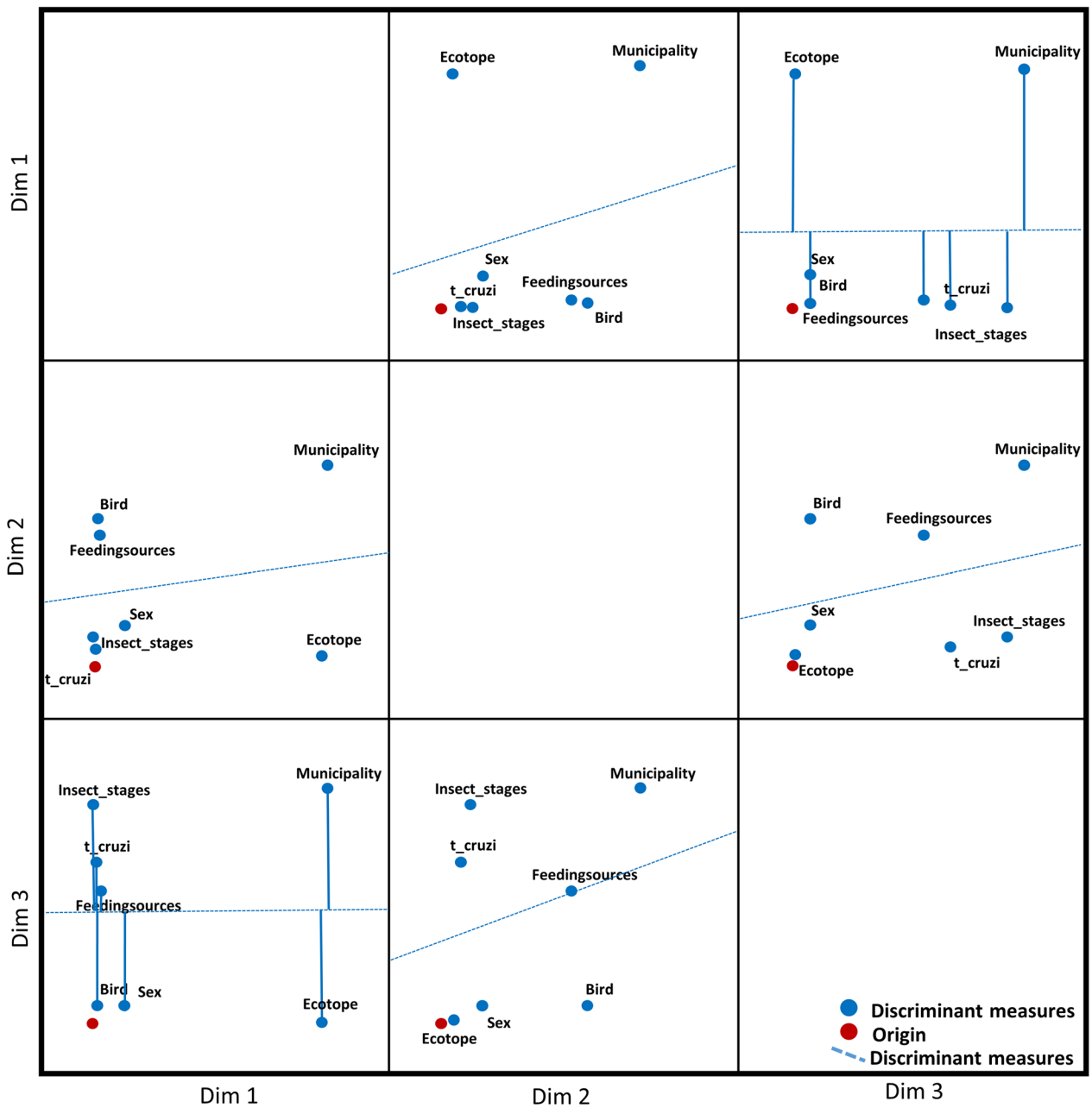

Fig. 4 Proximity bi-plot between *T. cruzi* and the eco-epidemiological variables evaluated

suggesting they are moving through different niches looking for blood meals. The description of feeding patterns of sylvatic triatomines is relevant for a good understanding of *T. cruzi* transmission and its circulation among different hosts. Furthermore, the presence of the parasite in a sylvatic vector considered as secondary for humans is highly relevant in the context of CD vector control programs, because in a hypothetic scenery where *R. prolixus* is eliminated, other triatomines with similar feeding behaviors could take their niche and then possibly transmit *T. cruzi* [30]. *In vivo* studies of *Ps. arthuri* are required to evaluate the progression and development of *T. cruzi* life-cycle. Additionally, it is important to evaluate defecation patterns, insect densities, parasite-triatomine interactions, triatomine microbiota, immune response and ecology, because these are main factors to determine if a triatomine could be a potential vector for *T. cruzi* [44, 53].

Table 3 Significative variables obtained with logistic regression

Variable	Characteristic	β-coefficient	*P*-value	OR (Exp[B])	95% CI
Municipality	Pore, Casanare	2.398	0.022	11.0	1.42–85.2
Ecotope	Sylvatic	0.880	0.002	2.41	1.37–4.24
Insect stage*	Adult	1.030	0.001	2.80	1.55–5.04
Sex*	Male	1.145	0.008	3.14	1.34–7.35
Sex	Female	0.916	0.028	2.50	1.10–5.67
Bird nest*	*C. cela*	1.386	0.032	4.00	1.12–14.17
Bird nest	*P. rufifrons*	0.728	0.025	2.07	1.09–3.92
Feeding source*	*C. baroni*	1.322	0.019	3.75	1.24–11.29

Note: Response variable was presence (1) or ausence (2) of *T. cruzi*. Explicative variables are the ones shown in the table. A stratified simulation (1000 samples) was made for the response variable (*T. cruzi* presence) in each executed model. Wald estimator was used to determine the OR 95% confidence intervals

*Most statistically significant variables

It is important to highlight the presence of the TcI_{Dom} genotype in 40.4% of the *Ps. arthuri* which were positive for *T. cruzi*, knowing that 97.6% of insects were collected in a sylvatic ecotope, indicating the intrusion of domestic DTUs in the sylvatic cycle of the parasite. In contrast, Cruz-Guzmán et al. [25] reported the presence of TcIII in an adult specimen of *Ps. arthuri*. This DTU belongs to the sylvatic cycle of the parasite and has been found in armadillos and didelphimorphos [54], animals that are frequently found near dwellings. Another study of the secondary vectors of CD, reported *T. maculata* and *P. geniculatus* infected with TcI/TcIII and TcI-TcV, respectively, concluding that this triatomine represents an important connection between sylvatic and domestic transmission cycles, facilitating the circulation of many DTUs [15]. In this study we did not discriminate DTUs from the TcII-TcVI group. Further studies are required to determine truly circulating genotypes. Futhermore, TcI is associated with arboricoral mammals such as *Didelphis marsupialis* and others like *Rattus rattus* and *Canis lupus familiaris* [5, 15, 16], while TcIII and TcIV are related to armadillos [55]. *Psammolestes arthuri* may be feeding on these reservoirs associated with the domestic cycle, explaining the presence of TcI and TcII-TcVI DTUs. Furthermore, the presence of domestic DTUs may be explained considering that collection sites are located near human settlements (Fig. 1b) and that *Ps. arthuri* could be moving through domestic and sylvatic transmission cycles, as reported in vectors and synanthropic reservoirs for the parasite [10]. These triatomines could be circulating between sylvatic and domestic ecotope not only because of the need to find new feeding sources, but through active dispersal, because they might be attracted to the artificial light of human dwellings [56]. This phenomenon has important epidemiological significance, because if they are truly attracted to this light, the probability of triatomines attracted to a dwelling increases [47]. Jácome-Pinilla et al. [57] studied the associated risks among dispersive nocturnal flights of sylvatic triatomines because of artificial lights in northeastern Colombia. They reported *Ps. arthuri*, *T. maculata*, *P. geniculatus* and *R. prolixus* being attracted to light traps, highlighting a potential risk of active dispersion of sylvatic triatomines and their implications in the introduction of sylvatic DTUs into the domestic transmission cycle. Moreover, a study in Brazil [58] about the attraction of Chagas disease vectors to artificial light found that almost all of the known vectors of CD in the zone were attracted by artificial light sources, and they propose this as a possible route by which triatomines can reach dwellings and become involved in *T. cruzi* transmission.

Conclusions

We present herein the first *Ps. arthuri* study in Colombia regarding *T. cruzi* infection and its feeding preferences. Our findings indicate that *Ps. arthuri* is feeding on other potential reservoirs for *T. cruzi* aside from birds and could possibly be maintaining the zoonotic cycle of the parasite. Furthermore, the finding that these triatomines are feeding on humans may be highly relevant for the epidemiology and control of CD, but further studies are needed to evaluate *Ps. arthuri* as a potential vector for *T. cruzi*. These should consider factors such as the biology of the parasite inside the triatomine, changes that the parasite can trigger in the host and feeding and defecation behavior, because these are important factors to better understand *T. cruzi* transmission. Moreover, studies using other molecular techniques, such as deep sequencing, are required to improve feeding source detection and *T. cruzi* genotyping. Additionally, a wider sampling is needed to determine true associations of *Ps. arthuri* and the presence of *T. cruzi*. Finally, we encourage the scientific community to keep including other triatomine species into CD eco-epidemiological studies. This might help to develop a better understanding of transmission dynamics of the parasite. Also, we encourage the government to pay special attention to *Ps. arthuri* in eastern Colombia, considering the ability of triatomines to adapt to new environments and the findings of the present study.

Additional file

Additional file 1: Figure S1. Model selection using SPSS software modeler.

Abbreviations
CD: Chagas disease; DTU: discrete typing unit; PCR: polymerase chain reaction; qPCR: real-time PCR; SL-IR: spliced leader-intergenic region; MCA: multiple correspondence analysis; OR: odds ratio.

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Availability of data and materials
The data supporting the conclusions of this article are included within the article and its additional file.

Authors' contributions
NVO wrote the manuscript. JDR, GH, CH and NVO analyzed the data. AT carried out statistical tests. GH, LMAG, AH, LCS and NVO carried out experimental procedures. AC and PU collected insects in Arauca and Casanare, respectively. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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References
1. Rassi A Jr, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am. 2012;26:275–91.
2. WHO. Chagas disease (American trypanosomiasis). 2018. https://www.who.int/en/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis). Accessed 20 Nov 2018.
3. WHO. Weekly epidemiological record Relevé épidémiologique hebdomadaire. 2015. http://www.who.int/wer/2015/wer9006.pdf?ua=1. Accessed 20 Nov 2018.
4. OPS. Guía de Atención Clínica de la enfermedad de Chagas. Ministerio de la Protección Social, Republica de Colombia, Organización Panamericana de la Salud; 2010. Accessed 20 Nov 2018.
5. Guhl F, Ramírez JD. *Trypanosoma cruzi* I diversity: towards the need of genetic subdivision? Acta Trop. 2011;119:1–4.
6. Zingales B, Miles MA, Campbell DA, Tibayrenc M, Macedo AM, Teixeira M, et al. The revised *Trypanosoma cruzi* subespecific nomenclature: rationale, epidemiological relevance and research applications. Infect Genet Evol. 2012;12:240–53.
7. Marcili A, Lima L, Cavazzana M, Junqueira AC, Veludo HH, Da Silva M, et al. A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using *SSU* rDNA, cytochrome b and Histone H2B genes and genotyping based on ITS1 rDNA. Parasitology. 2009;136:641–55.
8. Pinto CM, Kalko EKV, Cottontail I, Wellinghausen N, Cottontail VM. TcBat a bat - exclusive lineage of *Trypanosoma cruzi* in the Panama Canal Zone, with comments on its classification and the use of the *18S* rRNA gene for lineage identification. Infect Genet Evol. 2012;12:1328–32.
9. Ramírez JD, Hernández C, Montilla M, Zambrano P, Flórez AC, Parra E, Cucunubá ZM. First report of human *Trypanosoma cruzi* infection attributed to TcBat genotype. Zoonoses Public Health. 2014;61:477–9.
10. Ramírez JD, Hernández C. *Trypanosoma cruzi* I: towards the need of genetic subdivision? Part II. Act Trop. 2018;184:53–8.
11. Parra-Henao G, Suárez-Escudero LC, González-Caro S. Potential distribution of Chagas disease vectors (Hemiptera: Reduviidae, Triatominae) in Colombia, based on ecological niche modeling. J Trop Med. 2016;2016:1439090.
12. Gordon ERL, Georgieva AY, Weirauch C. Sylvatic host associations of Triatominae and implications for Chagas disease reservoirs: a review and new host records based on archival specimens. PeerJ. 2017;5:e3826.
13. Herrera L. Una revisión sobre reservorios de *Trypanosoma* (*Schizotrypanum*) *cruzi* (Chagas, 1909), agente etiológico de la enfermedad de Chagas. Bol Malariol y Salud Ambient. 2010;50:3–15.
14. Rendón LM, Guhl F, Cordovez JM, Erazo D. New scenarios of *Trypanosoma cruzi* transmission in the Orinoco region of Colombia. Mem Inst Oswaldo Cruz. 2015;110:283–8.
15. Hernández C, Salazar C, Brochero H, Teherán A, Buitrago LS, Vera M, et al. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. Parasit Vectors. 2016;9:620.
16. León CM, Hernández C, Montilla M, Ramírez JD. Retrospective distribution of *Trypanosoma cruzi* I genotypes in Colombia. Mem Inst Oswaldo Cruz. 2015;110:387–93.
17. Cantillo-Barraza O, Gómez-Palacio A, Salazar D, Mejía-Jaramillo AM, Calle J, Triana O. Distribución geográfica y ecoepidemiología de la fauna de triatominos (Reduviidae: Triatominae) en la Isla Margarita del departamento de Bolívar, Colombia. Biomédica. 2010;30:382–9.
18. Guhl F, Aguilera G, Pinto N, Vergara D. Actualización de la distribución geográfica y ecoepidemiológica de la fauna de triatominos (Reduviidae: Triatominae) en Colombia. Biomédica. 2007;27:143–62.
19. Wolff M, Castillo D. Evidencias de domesticación y aspectos biológicos de *Panstrongylus geniculatus* (Latreille, 1811) (Hemiptera: Reduviidae). Acta Ent Chilena. 2000;24:77–83.
20. Ramírez-Mejía D, Mendoza E. El papel funcional de la interacción planta-mamífero en el mantenimiento de la diversidad tropical. Biológicas. 2010;12:8–13.
21. Abad Franch F, Monteiro F, Jaramillo N, Gurgel-Gonçalves R, Braga Stehling Dias F, Diotaiuti L. Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). Act Trop. 2009;110:159–77.
22. Pifano C. Anotaciones acerca de *Psammolestes arthuri* Pinto, 1926 (Hemiptera, Heteroptera: Triatominae), reduvideo hematófago encontrado en nidos de cucarachero de monte (probablemente Dendrocolaptidae) en un sector del valle del Yaracuy. Su importancia como posible vector en la naturaleza del *Schizotripanum cruzi* Chagas, 1909. Gac Méd Caracas. 1938;45:241–5.
23. Pifano F. Nota sobre la infestación experimental y en la naturaleza del *Psammolestes arthuri* (Pinto, 1926) por el *Schizotrypanum cruzi* Chagas, 1909. 440 *Psammolestes arthuri* naturalmente infectado con *Trypanosoma cruzi*. Gac Med Caracas. 1909;1939(47):433–5.
24. Sherlock I, Carcavallo R, Galíndez I. List of natural and experimental flagellate infections in several triatominae species. In: Carcavallo R, Galíndez I, Jurberg J, Lent H, editors. Atlas of Chagas disease vectors in the Americas, vol. II. Rio Janeiro: Editora Fiocruz; 1997. p. 289–97.
25. Cruz-Guzmán PJ, Morocoima A, Chique JD, Ramonis-Quintero J, Toquero-Uzacátegui M, Carrasco HJ. *Psammolestes arthuri* naturally infected with *Trypanosoma cruzi* found in sympatry with *Rhodnius prolixus* and *Triatoma*

maculata on bird nests in Anzoátegui, Venezuela. Saber Univ Oriente. 2014;26:428–40.
26. Molina J, Gualdrón L, Brochero H, Olano V, Barrios D, Guhl F. Distribución actual e importancia epidemiológica de las especies de triatominos (Reduviidae: Triatominae) en Colombia. Biomédica. 2000;20:344–60.
27. Kierszembaum F, Ivanyi J, Budzco D. Mechanisms of natural resistance to trypanosomal infection. Role of complement in avian resistance to *T cruzi* infection. Immunology. 1976;30:1–6.
28. Angulo VM, Esteban L, Luna KP. *Attalea butyracea* próximas a las viviendas como posible fuente de infestación domiciliaria por *Rhodnius prolixus* (Hemiptera: Reduviidae) en los Llanos Orientales de Colombia. Biomédica. 2012;32:277–85.
29. Cazorla-Perfetti D. *Psammolestes arthuri* naturally infected with *Trypanosoma cruzi* found in sympatry with *Rhodnius prolixus* and *Triatoma maculata* on bird nests in Anzoátegui, Venezuela. Saber Univ Oriente Venezuela. 2015;27:324–7.
30. Rabinovich L, Kitron U, Obed Y, Yoshioka M, Gottdenker N, Chaves L. Ecological patterns of blood-feeding by kissing-bugs (Hemiptera: Reduviidae: Triatominae). Mem Inst Oswaldo Cruz. 2011;106:479–94.
31. Duffy T, Cura CI, Ramírez JC, Abate T, Cayo NM, Parrado R, et al. Analytical performance of a multiplex real-time PCR assay using TaqMan probes for quantification of *Trypanosoma cruzi* satellite DNA in blood samples. PLoS Negl Trop Dis. 2013;7:e2000.
32. Ramírez JD, Guhl F, Umezawa ES, Morillo C, Rosas F, Marin-Neto J. Evaluation of adult chronic Chagas' heart disease diagnosis by molecular and serological methods. J Clin Microbiol. 2009;47:3945–51.
33. Souto RP, Fernandes O, Macedo AM, Campbell D, Zingales B. DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. Mol Biochem Parasitol. 1996;83:141–52.
34. Herrera C, Bargues MD, Fajardo A, Montilla M, Triana O, Vallejo GA, Guhl F. Identifying four *Trypanosoma cruzi* I isolate haplotypes from different geographic regions of Colombia. Infect Genet Evol. 2007;7:535–9.
35. Dumonteil E, Ramírez-Sierra MJ, Pérez-Carillo S, Teh-Poot C, Herrera C, Gourbiére S, Waleckx E. Detailed ecological associations of triatomines revealed by metabarcoding and next-generation sequencing: implications for triatomine behavior and *Trypanosoma cruzi* transmission cycles. Sci Rep. 2018;8:4140.
36. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9.
37. Barreto M, Barreto P, D'Alessandro A. *Psammolestes arthuri* (Hemiptera: Reduviidae) and its parasite *Telenomus capito* (Hymenoptera: Scelionidae) in Colombia. J Med Entomol. 1984;21:703–5.
38. Schofield Christopher J. Biosystematics and evolution of the Triatominae. Cad Saúde Públ. 2000;16:S89–92.
39. Gaunt M, Miles MA. The ecotopes and evolution of triatomine bugs (Triatominae) and their associated trypanosomes. Mem Inst Oswaldo Cruz. 2000;95:557–65.
40. Luitgards-Moura JF, Vargas AB, Almeida CE, Magno-Esperança G, Agapito-Souza R, Folly-Ramos E, et al. A *Triatoma maculata* (Hemiptera: Reduviidae, Triatominae) population from Roraima, Amazon Region, Brazil, has some bionomic characteristics of a potential Chagas disease vector. Rev Inst Med Trop Sao Paulo. 2005;47:131–7.
41. Mayr E. Animal species and evolution. Harvard: Harvard University Press; 1963.
42. Kierszembaum F, Gottlieb CA, Budzko DB. Antibody-independent, natural resistance of birds to *Trypanosoma cruzi* infection. J Parasitol. 1981;67:565–660.
43. Schaub GA. Direct transmission of "*Trypanosoma cruzi*" between vectors of Chagas' disease. Acta Trop. 1988;45:11–9.
44. De Fuentes-Vicente J, Gutiérrez-Cabrera A, Flores-Villegas A, Lowenberg C, Benelli G, Salazar-Schettino P, Córdoba-Aguilar A. What makes an effective Chagas disease vector? Factors underlying *Trypanosoma cruzi*-triatomine interactions. Acta Trop. 2018;183:23–31.
45. Calderon-Arguedas O, et al. Variaciones biológicas de *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatiade) asociadas con la ingestión de diferentes tipos de sangre por el vector *Triatoma dimidiata* (Hemiptera: Reduviidae). Parasitol Latinoam. 2003;58:3–10.
46. Salem H, Florez L, Gerardo N, Kaltenpoth M. An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. Proc R Soc B. 2015;282:20142957.
47. Lazzari CR, Pereira MH, Lorenzo MG. Behavioural biology of Chagas disease vectors. Mem Inst Oswaldo Cruz. 2013;108:34–47.
48. Sandoval CM, Joya MI, Gutierez R, Angulo VM. Cleptohaematophagy of the triatomine bug *Belminus herreri*. Med Vet Entomol. 2000;14:100–1.
49. Piñero DF, Carcavallo RU, Fernandez E. Canibalismo y transmisión directa de *Trypanosoma cruzi* entre ninfas de *Rhodnius prolixus*. Chagas. 1988;5:18–22.
50. Otálora-Luna F, Pérez-Sánchez AJ, Sandoval C, Aldana E. Evolution of hematophagous habit in Triatominae (Heteroptera: Reduviidae). Rev Chil Hist Nat. 2015;88:4.
51. Skutch AF. A study of the rufous-fronted thornbird and associated birds, Part II: birds which breed in thornbirds' nests. Wilson Bull. 1969;81:123–39.
52. Lent H, Wygodzinsky P. Revision of the triatomine (Hemiptera: Reduviidae) and their significance as vectors of Chagas' disease. Bull Am Mus Nat Hist. 1979;163:123–220.
53. Zeledón R, Alvarado R, Jirón LF. Observations on the feeding and defecation patterns of three triatomine species (Hemiptera: Reduviidae). Acta Trop. 1977;34:65–77.
54. Morocoima A, Carrasco HJ, Boadas J, Chique JD, Herrera L, Urdaneta-Morales S. *Trypanosoma cruzi* III from armadillos (*Dasypus novemcinctus novemcinctus*) from northeastern Venezuela and its biological behavior in murine model. Risk of emergency of Chagas' disease. Exp Parasitol. 2012;132:341–7.
55. Yeo M, Acosta N, Llewellyn M, Sánchez H, Adamson S, Miles GA, et al. Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. Int J Parasitol. 2005;35:225–33.
56. Vazquez-Prokopec GM, Ceballos LA, Kitron U, Gurtler RE. Active dispersal of natural populations of *Triatoma infestans* (Hemiptera: Reduviidae) in rural northwestern Argentina. J Med Entomol. 2004;41:614–21.
57. Jácome-Pinilla D, Hincapie-Peñaloza E, Ortiz MI, Ramírez JD, Guhl F, Molina J. Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit Vectors. 2015;8:600.
58. Castro, Marcelo CM, Barrett, Toby V, Santos, Walter S, Abad-Franch F, Rafael JA. Attraction of Chagas disease vectors (Triatominae) to artificial light sources in the canopy of primary Amazon rainforest. Mem Inst Oswaldo Cruz. 2010;105:1061–4.

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Parasites & Vectors

RESEARCH Open Access

Identification of blood-feeding sources in *Panstrongylus*, *Psammolestes*, *Rhodnius* and *Triatoma* using amplicon-based next-generation sequencing

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Abstract

Background: Triatomines are hematophagous insects that play an important role as vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease. These insects have adapted to multiple blood-feeding sources that can affect relevant aspects of their life-cycle and interactions, thereby influencing parasitic transmission dynamics. We conducted a characterization of the feeding sources of individuals from the primary circulating triatomine genera in Colombia using amplicon-based next-generation sequencing (NGS).

Methods: We used 42 triatomines collected in different departments of Colombia. DNA was extracted from the gut. The presence of *T. cruzi* was identified using real-time PCR, and discrete typing units (DTUs) were determined by conventional PCR. For blood-feeding source identification, PCR products of the vertebrate *12S* rRNA gene were obtained and sequenced by next-generation sequencing (NGS). Blood-meal sources were inferred using blastn against a curated reference dataset containing the *12S* rRNA sequences belonging to vertebrates with a distribution in South America that represent a potential feeding source for triatomine bugs. Mean and median comparison tests were performed to evaluate differences in triatomine blood-feeding sources, infection state, and geographical regions. Lastly, the inverse Simpson's diversity index was calculated.

Results: The overall frequency of *T. cruzi* infection was 83.3%. TcI was found as the most predominant DTU (65.7%). A total of 67 feeding sources were detected from the analyses of approximately 7 million reads. The predominant feeding source found was *Homo sapiens* (76.8%), followed by birds (10.5%), artiodactyls (4.4%), and non-human primates (3.9%). There were differences among numerous feeding sources of triatomines of different species. The diversity of feeding sources also differed depending on the presence of *T. cruzi*.

Conclusions: To the best of our knowledge, this is the first study to employ amplicon-based NGS of the *12S* rRNA gene to depict blood-feeding sources of multiple triatomine species collected in different regions of Colombia. Our findings report a striking read diversity that has not been reported previously. This is a powerful approach to unravel transmission dynamics at microgeographical levels.

Keywords: Chagas disease, *Trypanosoma cruzi*, Triatominae, Feeding sources, NGS, Colombia

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Background

Triatomines (Hemiptera: Reduviidae) are hematophagous insects that play an important role as vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease [1], which is a neglected tropical disease (NTD). Over 8 million people are considered infected with *T. cruzi*, and more than 200,000 new cases are identified each year [2, 3]. The parasite *T. cruzi* boasts tremendous genetic diversity and has been divided into six discrete typing units (DTUs) from TcI to TcVI [4], which are associated with various clinical manifestations, geographical distribution, and ecotopes [5]. This difference in ecotopes results in the ability of invading both "domestic" and "sylvatic" environments, which is facilitated by its vectors, that have adapted to multiple blood-feeding sources [6], including various vertebrates, such as rodents, humans, non-human primates, bats, marsupials, dogs, armadillos, porcupines, cows, goats and birds. This has been reported over the years *via* scientific studies using Sanger sequencing techniques [7–10].

Feeding habits can affect relevant aspects of insect life-cycles and interactions. For example, cellulase activity within digestion is affected by the feeding habits of termites [11]; in addition, infection with *Mycobacterium ulcerans* is conditioned by the feeding behavior of water bugs due to a possible symbiotic relationship between the host and insect [12]. Moreover, the bacterial and fungal communities in the gut of certain insects are defined by their feeding habits [9, 13], which can affect the effectivity of insects as vectors, as has been shown for *Anopheles* in previous studies [14–20]. Due to the aforementioned effects, these variables have an impact on transmission dynamics [21, 22], which makes knowledge of feeding habits important for the development of effective prevention and control strategies for tropical diseases.

In Colombia, 15 triatomine species are found to be naturally infected with *T. cruzi*, with *Rhodnius prolixus*, *Triatoma dimidiata* and *Panstrongylus geniculatus* being the most relevant [23]. The infection rate within these triatomines can surpass 40% [8, 24–27], making it higher than that within other triatomines [28, 29], and explains the relevance of these particular triatomine species, along with their capability of colonizing human dwellings [23]. The feeding preferences of these insects include humans; arboreal mammals, such as New World monkeys, sloths, opossums, and coatis; domestic mammals, such as cats, dogs, cows and rodents, as well as other animals such as reptiles and bats [7–10, 30].

Latter studies have explored the feeding habits and interactions of triatomines, such as the work by Dumonteil et al. [9], in which triatomines were observed simultaneously feeding on different vertebrates. These authors also constructed a possible transmission network for the parasite, involving the 14 vertebrate hosts elucidated in this study [9]. Recently, Erazo et al. [27] identified 18 vertebrate species as a feeding source for *R. prolixus*. According to this study, the infection rate varied among triatomines feeding upon different vertebrates in a way suggesting that diet specialization plays a pivotal role in defining the transmission dynamics of Chagas disease. Although the described range of triatomine feeding sources is wide [7, 8, 10, 22, 31] and is known to affect crucial aspects of their life-cycles and interactions, this aspect has not been vastly evaluated in order to completely understand the dynamics involved. Furthermore, only a few studies have been conducted using next-generation sequencing (NGS), particularly amplicon-based sequencing [9, 32, 33], despite its capacity to reveal multiple host species simultaneously and characterize many more samples than traditional techniques [32].

Depicting the complexity of feeding preferences among triatomine bugs is of pivotal importance for building efficient control strategies for these vectors, given that these preferences can define the behavior and explain the presence of the insects under certain conditions (i.e. modify parasite transmission routes). Ultimately, all this information could be important for completely understanding the Chagas disease transmission and potentially improving the current measures established against it. For this, it is also necessary to assess the *T. cruzi* presence in the vector, alongside the characteristics of the parasite, such as its genetic diversity. Therefore, we herein conducted a robust characterization of feeding sources using amplicon-based NGS from available individuals of the primary triatomine genera found in Colombia (*Panstrongylus*, *Rhodnius* and *Triatoma*) and included *Psammolestes* due to its recent evidence of *T. cruzi* infection. This study was also complemented with detecting *T. cruzi* infection and assessing the genetic diversity of *T. cruzi*.

Methods

Insect sampling, dissection and DNA extraction

Forty-two triatomines (see Additional file 1: Table S1) collected between 2012 and 2018 in different districts of Colombia (Arauca, Bolívar, Boyacá, Casanare, La Guajira, Magdalena, Meta and Santander) were used in this study (Fig. 1). These specimens were collected in the framework of previous studies using different entomological surveillance techniques for each ecotope (i.e. domestic, peridomestic and sylvatic) as described elsewhere [34]. In total, the triatomines used consisted of 6 *P. arthuri*, 15 *R. prolixus*, 7 *R. pallescens*, 8 *P. geniculatus*, 3 *T. maculata* and 3 *T. venosa*. Manipulation of triatomine individuals was carried out, taking into account the field permit from Autoridad Nacional de Licencias Ambientales (ANLA) 63257-2014 provided by Universidad del Rosario. The

collection of all triatomines was conducted on public land. Insects were stored in Eppendorf tubes with 100% ethanol and, upon arrival at the laboratory, were frozen at −20 °C until dissection. The abdominal region was excised and washed 3 times with ultra-pure water in preparation for posterior use. DNA from the gut was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), and DNA concentrations were determined using a NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Detection and genotyping of *T. cruzi*

The presence of *T. cruzi* parasites within triatomines was detected using real-time PCR with the primers Cruzi1 (5′-AST CGG CTG ATC GTT TTC GA-3′) and Cruzi2 (5′-AAT TCC TCC AAG CAG CGG ATA-3′), as well as probe Cruzi3 (5′-CAC ACA CTG GAC ACC AA-3′), as described elsewhere [8, 35]. Samples were considered positive when the amplification exceeded the threshold of fluorescence of 0.01. For insects yielding positive results by initial qPCR, it was necessary to discriminate if detection was due to the presence of *T. cruzi* or *Trypanosoma rangeli*, another trypanosome species circulating in the Neotropics transmitted by triatomine bugs, which do not have a pathogenic effect over its mammalian hosts [36], and therefore, a kinetoplast fragment DNA amplification was performed using primers 121 (5′-AAA TAA TGT ACG GGK GAG ATG CAT GA-3′) and 122 (5′-GGT TCG ATT GGG GTT GGT GTA ATA TA-3′) as described elsewhere [37]. For insects identified as being positive for *T. cruzi*, TcI and non-TcI DTUs were discriminated, *via* the usage of part (PCR directed to the SL-IR region only) of the algorithm implemented by Hernández et al. [8]. Finally, for the TcI-positive samples, we discriminated between TcIDom and TcISylv, also adopting part of the algorithm used by Hernández et al. [8].

Feeding sources

PCR products of the vertebrate *12S* rRNA gene were obtained through amplification of a 215-bp fragment using primers L1085 (5′-CCC AAA CTG GGA TTA GAT ACC C-3′) and H1259 (5′-GTT TGC TGA AGA TGG CGG TA-3′) [9]. These fragments were pooled for sequencing after independent library construction, and 2 × 300 paired-end sequencing was performed on an Illumina HiSeq flow cell (Illumina, San Diego, USA). Amplicon sequencing was conducted by Novogene (Beijing, China).

The *12S* rRNA sequences produced by the Illumina HiSeq went through a quality control (QC) step applied with aim of reducing the technical bias (PCR or sequencing related) and to assure that the diversity detected truthfully reflected the biological scenario. During this QC step, sequences with incongruences in the barcode or without the correct primer sequence as well as short (< 200 bp in length) and low quality (with a minimal average quality score of 25) reads were discarded. The less frequent sequences were not removed in order to capture possible rare food sources [33]. The quality filtering was made using QIIME software [38]. The high-quality sequences were used to describe the feeding source preferences of the triatomines. Blood meals were inferred using BLASTn against a curated dataset (see 'Reference dataset construction' below), considering a minimum of 95% identity and an e-value of 10 as a match. The 5 best vertebrate matches for each read were narrowed down to select the best result per read selecting the highest identity percentage and the lowest e-value. Matches with different vertebrate species with the same similarity were detected with a reduced number of reads (0.87% on average); these data were excluded for subsequent analyses given their ambiguity.

The number of reads corresponding to each vertebrate in the reference dataset was recorded and used as a proxy of its abundance within the triatomine diet as reported elsewhere [9]. Additionally, we used the online tool CIRCOS (http://circos.ca/) to graphically represent the relative abundance and distribution of feeding sources for all triatomines per species [39].

Reference dataset construction

To build this database, we considered all the sequences contained in the NCBI Nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore/). For this, we conducted an advanced search where "*12S* rRNA" was targeted as the gene name and "vertebrates" was targeted as the organism. The geographical distribution of each non-human species was checked online to select the vertebrates present in Latin America (Mexico, Central America and South America) taking into account the dispersion capacity of numerous vertebrates and the consequent potential of their presence in the regions where we collected the insects. The only vertebrates excluded from this should be the ones with biological restrictions that limit their distribution (e.g. endemic species in regions different from Latin America). Therefore, for the geographical authentication we accessed the following major public repositories: https://www.fishbase.in for fish; https://bioweb.bio/faunaweb/mammaliaweb/ for mammals; http://reptile-database.reptarium.cz for reptiles; http://amphibiaweb.org/ for amphibians; https://www.hbw.com/ for birds; and https://www.iucnredlist.org/ and https://www.naturalista.mx/ for any of the above (in case the information was not available in the previous databases); where available maps and literature information about the distribution of each vertebrate were used. The

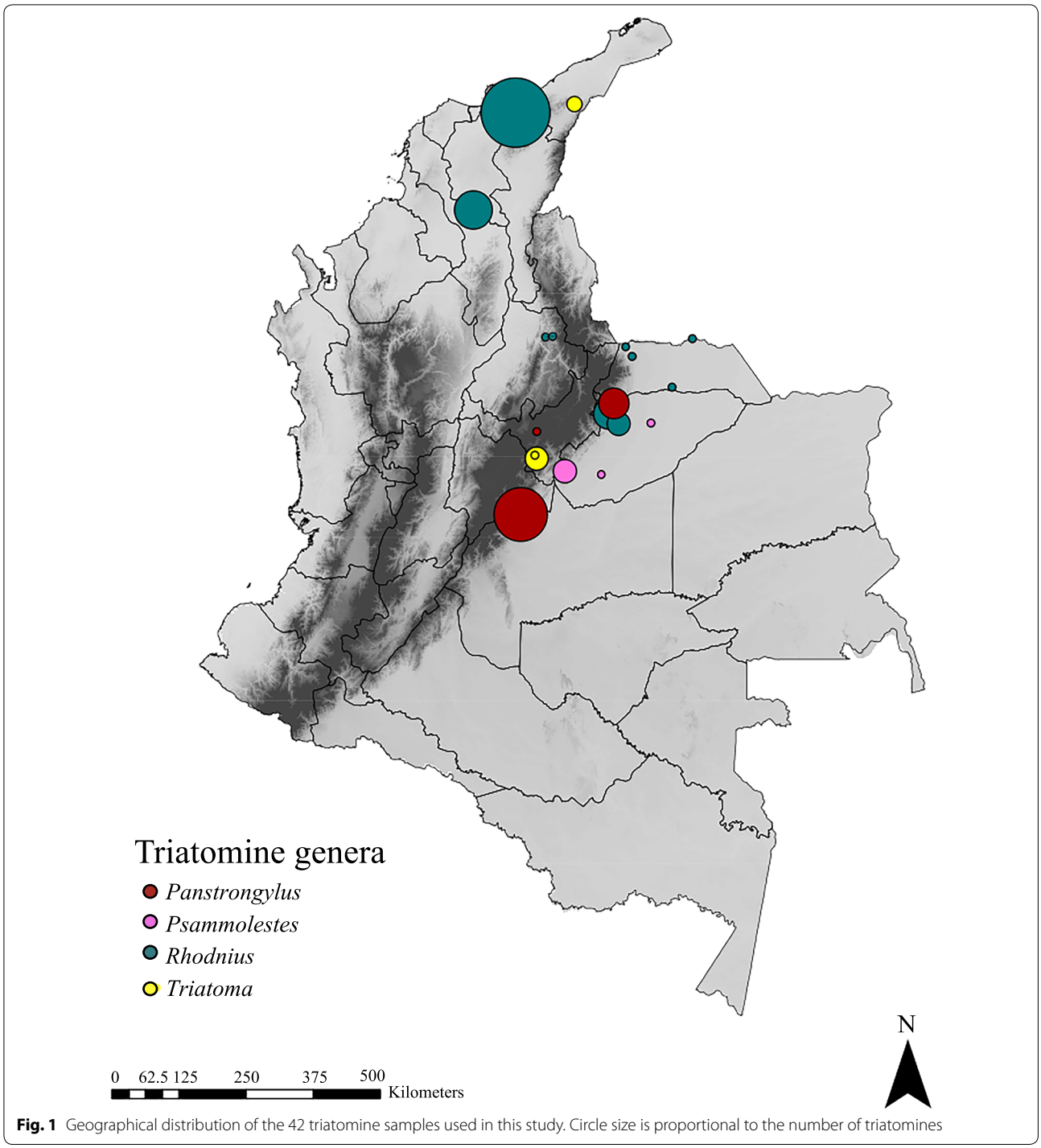

Fig. 1 Geographical distribution of the 42 triatomine samples used in this study. Circle size is proportional to the number of triatomines

geographical distribution of each non-human species did not have to be limited to Latin America, introduced species were also considered, and the *Homo sapiens* sequence with GenBank accession number X62996.1 was included without taking the geographical factor into consideration. The length and pertinence of all the sequences used in this reference file were double-checked applying the search criteria by different members of the team, and with an additional step where BLAST was executed against the reference dataset using all the *H. sapiens* sequences available in the NCBI, to verify the absence of wrongly assigned sequences. Throughout the whole

process, 3851 sequences were initially evaluated and a total of 397 definitive sequences comprised our reference FASTA file (Additional file 2: Alignment S1).

Statistical analyses

Median and mean comparison tests, according to the normality of the data, were implemented to evaluate differences within blood-feeding sources among triatomines in terms of (i) the reads of each vertebrate category within individual triatomine species, (ii) the alpha diversity index calculated for each triatomine species, and (iii) the *T. cruzi* infection state. These analyses were conducted using the R software version 3.6.1, fixing a 0.05 significance level for all hypothesis tests. The normality of data was verified implementing a Shapiro-Wilk normality test. When normality was met and the comparison evaluated was of multiple grouping (e.g. triatomine species), mean values were compared using ANOVA, and when normality was not met, median values were compared implementing the Kruskal-Wallis chi-square test. When normality was met and the comparison evaluated was between 2 groups (i.e. *T. cruzi* infection state), mean values were compared using the Welch two sample t-test, and for the opposite cases, the Wilcoxon test was used to compare median values. When necessary, individual t-tests were performed to further explore the statistical differences detected. All this was performed using R Commander (Rcmdr) [40]. Alpha diversity was estimated with the inverse Simpson diversity index, which was calculated for each triatomine species using the same software.

Results

Detection and genotyping of *T. cruzi*

The overall *T. cruzi* infection frequency was 83.3% ($n=35$). The frequency of *T. cruzi* infection within *P. geniculatus* and *R. prolixus* was 87.5% (7/8) and 73.3% (11/15), respectively. In the case of *Ps. arthuri*, *R. pallescens*, *T. maculata* and *T. venosa*, the frequency of infection was 100%. No insects were positive for *T. rangeli.*

TcI and TcII–TcVI were detected with TcI being the predominant DTU (80%, $n=28/35$), taking into account that mixed cases are also considered as positive for TcI (Additional file 1: Table S1). Among the positive samples for this DTU, we found 7 *P. geniculatus* (25%), 4 *Ps. arthuri* (14.3%), 6 *R. pallescens* (21.4%), 7 *R. prolixus* (25%), 1 *T. maculata* (3.6%) and 3 *T. venosa* (10.7%). The only case of TcII–TcVI found alone (i.e. not within a mixed infection case) (2.9%, $n=1/35$) belonged to a single *T. maculata*. One additional category was established for those individuals in which the DTU could not be determined as there was either inadequate DNA available for PCR or, despite the result being *T. cruzi*-positive, the parasitic load appeared too low to be identified. In this case, we found 6 ND (not detected) cases (17.1%, $n=6/35$) which corresponded to 1 *R. pallescens*, 4 *R. prolixus* and 1 *T. maculata*. Regarding the previously mentioned mixed infection (TcI + TcII–TcVI), we identified 5 cases (14.3%, $n=5/35$) corresponding to 3 *P. geniculatus*, 1 *Ps. arthuri* and 1 *R. prolixus*.

Within the cases detected as positive for TcI, TcIDom and TcISylv were also detected, with 9 (32.1%, $n=9/28$) found to be TcIDom, of which 3 corresponded to *P. geniculatus*, 1 to *Ps. arthuri*, 3 to *R. pallescens*, 1 to *R. prolixus* and 1 to *T. venosa* (Additional file 1: Table S1). In addition, 5 cases were detected as positive for TcISylv (17.9%, $n=5/28$), where 3 belonged to *R. prolixus* samples and 2 belonged to *P. geniculatus*. We also detected 5 (17.9%, $n=5/28$) mixed infection cases in which TcIDom and TcISylv are found together within a single sample; these consisted of 1 *P. geniculatus*, 3 *Ps. arthuri* and 1 *R. prolixus*. There were 9 ND cases (32.1%, $n=9/28$) corresponding to 1 *P. geniculatus*, 3 *R. pallescens*, 2 *R. prolixus*, 1 *T. maculata* and 2 *T. venosa*.

Feeding sources identification

A total of 67 feeding sources were detected within the 42 collected insects as a result of analyses of approximately 7 million total reads. The predominant feeding source was found to be *H. sapiens* (76.8%), followed by birds (10.5%), artiodactyls (4.4%), and non-human primates (3.9%) (Additional file 3: Figure S1). The totality of detected vertebrate species is presented in Additional file 4: Figure S2. These species were arbitrarily grouped to facilitate their graphic display (shown in Additional file 5: Table S2). This grouping aimed to maintain a maximum of 15 categories, therefore, species were grouped by family, order, or broad range (i.e. bats and birds) and the category "Other mammals" contained vertebrates that did not share one of the previous taxonomic categories with any other species.

We found that, despite all the collected triatomine species fed on almost every group of vertebrate detected, they did it in apparent different proportions (Figs. 2, 3). While the reads belonging to *H. sapiens* seem to be equally present in every triatomine species, this is the only case where it is as clear. For instance, and without considering *H. sapiens*, we observed that *Ps. arthuri* seems to feed mostly on birds, while *T. maculata* has the highest proportion of reads belonging to bats. Also, *T. venosa* was the species for which the highest number of reads corresponding to rodents was found, and more than 50% of reads corresponding to anteaters (Vermilingua) were found in *R. prolixus*. It is also worth

noting than more than 80% of reads corresponding to non-human primates were found in *R. pallescens* and *R. prolixus* together. Lastly, despite the low number of reads, the totality of amphibian reads was found in *R. pallescens* bugs (Fig. 2). These preferences were graphically represented by the CIRCOS plot containing read frequencies of the vertebrate hosts detected within each triatomine genus (Fig. 3), where *H. sapiens* was not plotted given its predominance and homogeneous distribution among triatomine species. The CIRCOS plot showed that more than 50% of the reads identified as sequences belonging to artiodactyls showed an association with *R. prolixus*, and all reads corresponding to amphibians showed an association with *R. pallescens* (Fig. 3).

In terms of vertebrate species, different proportions were found in each triatomine species, and only some of these were detected in all triatomine species. A more detailed summary of the vertebrate host species and their relative abundances within triatomines is shown in Additional file 4: Figure S2, where we found that *Eudromia elegans* and *Numida meleagris* were observed only in *Ps. arthuri*; *Didelphis albiventris*, *Philander opossum* and *Telmatobius* sp. only in *R. pallescens*; and *Chironectes minimus*, *Coccyzus americanus*, *Sciurus flammifer* and *Tremarctos ornatus* only in *R. prolixus* bugs.

As to whether there were differences among triatomines of the same species in varying geographical locations, for most of the cases, we observed that the read proportions found for each group of vertebrate was variable. Most of the reads corresponding to non-human primates found in *R. pallescens* belonged to individuals sampled in Bolívar; nearly all reads for bats found in *T. maculata* belonged to insects sampled in Casanare; and nearly all reads for Equidae found in *P. geniculatus* belonged to insects sampled in Boyacá, among other cases (Fig. 4a). Also, given that there was one department in which all triatomine genera were sampled, we compared these data and observed that each triatomine genus exhibits different non-human preferences despite the shared location (Fig. 4b), with *Psammolestes* showing a preference for birds, *Triatoma* for bats, *Rhodnius* for artiodactyls and non-human primates, and *Panstrongylus* showing a preference for canids and rodents.

Additionally, since some samples were positive for TcI-Dom and TcISylv, we evaluated if the presence of this DTU could correspond to vertebrate habitat (e.g. domestic or sylvatic). For the latter, we divided the detected vertebrates in domestic and sylvatic ones (this is shown in Additional file 5: Table S2). We observed that the TcI-Dom findings did not imply that vertebrates from which

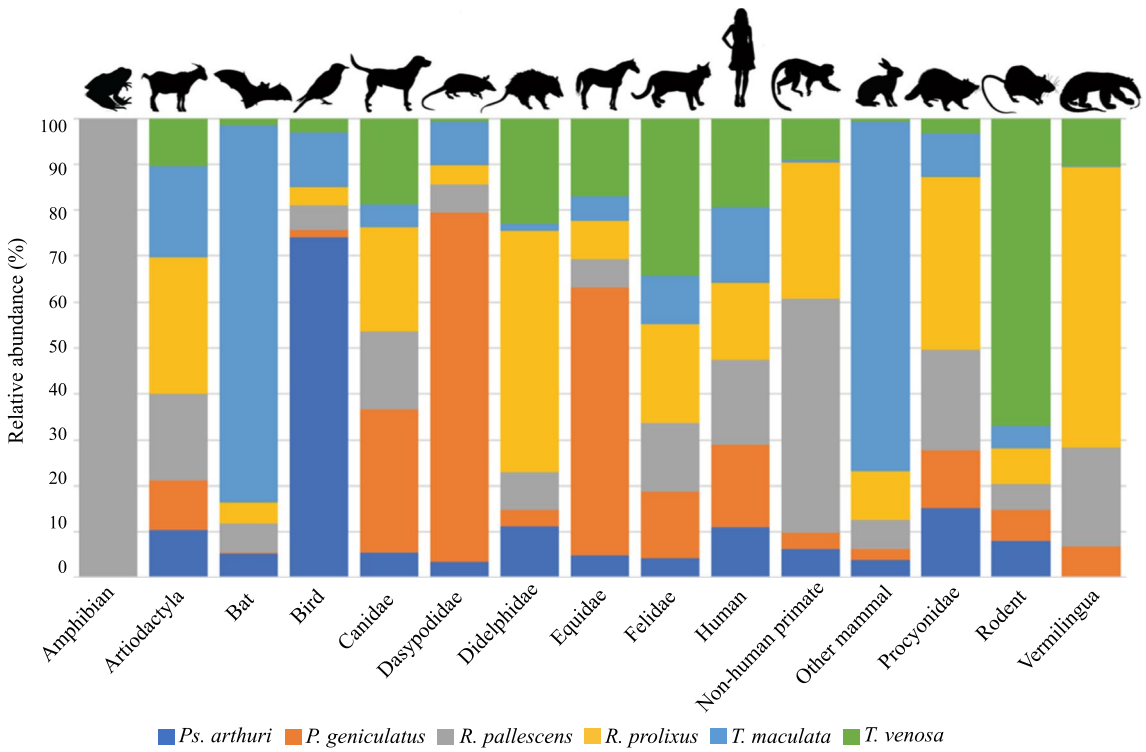

Fig. 2 Number of reads found for each vertebrate group detected. Bar colors represent the triatomine species in which these reads were found. The total number of reads was 7,178,645

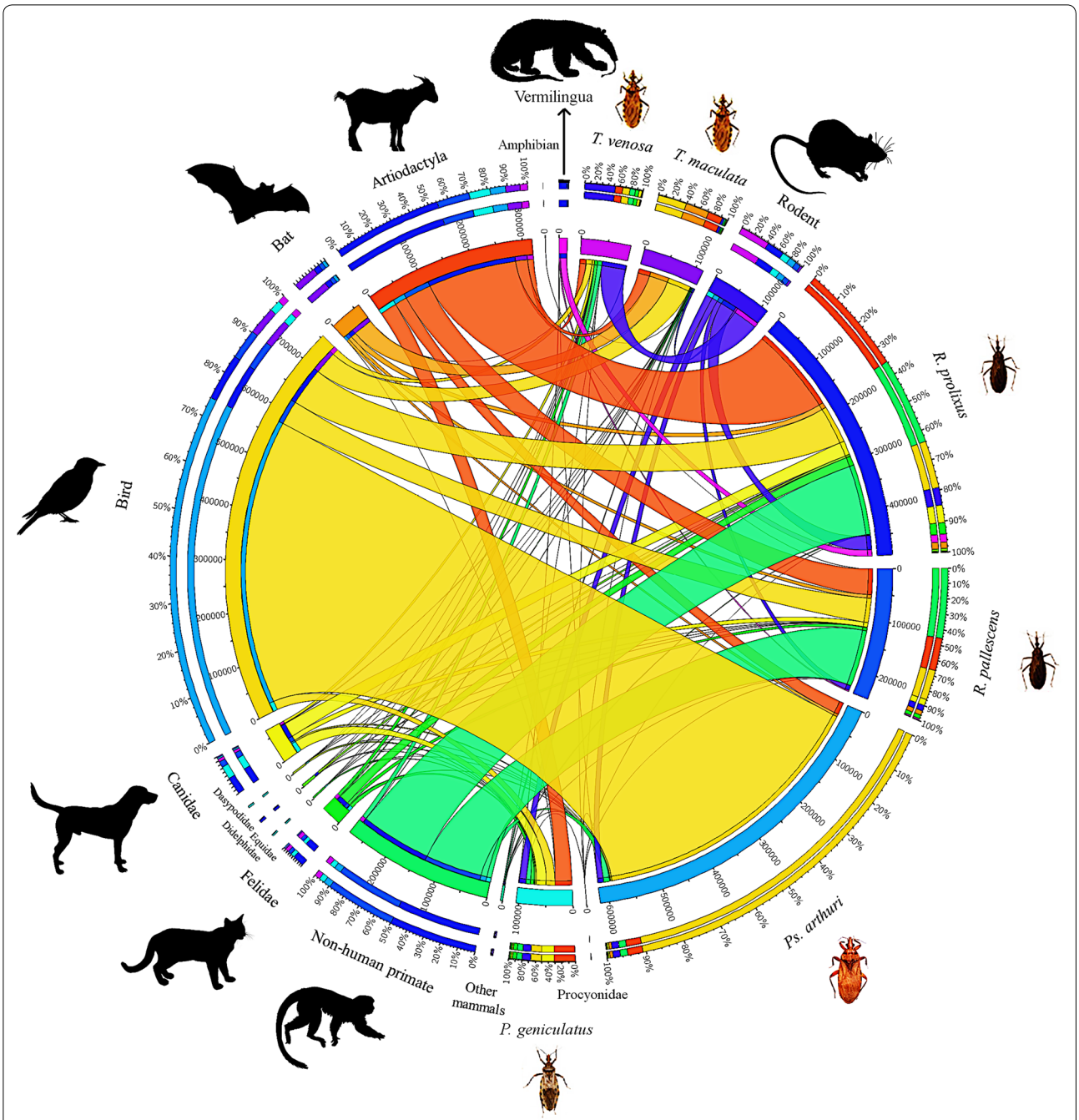

Fig. 3 Circular web made with CIRCOS online tool representing the relative abundance of the non-human feeding sources detected in each of the evaluated triatomine species. Vertebrate feeding sources are conveniently shown in the arbitrary grouping established for this study. Since humans are not shown, the total number of reads here was 1,662,701

the triatomines fed were domestic, given that for every triatomine detected with TcI, the vertebrates composing their diet were both domestic and sylvatic (Additional file 6: Figure S3).

Statistical analysis

Kruskal-Wallis tests revealed statistically different median values among triatomine species. According to these tests, significantly different medians were found among triatomine species for reads belonging to the Felidae ($\chi^2 = 11.959$, $df = 5$, $P = 0.035$), Didelphidae

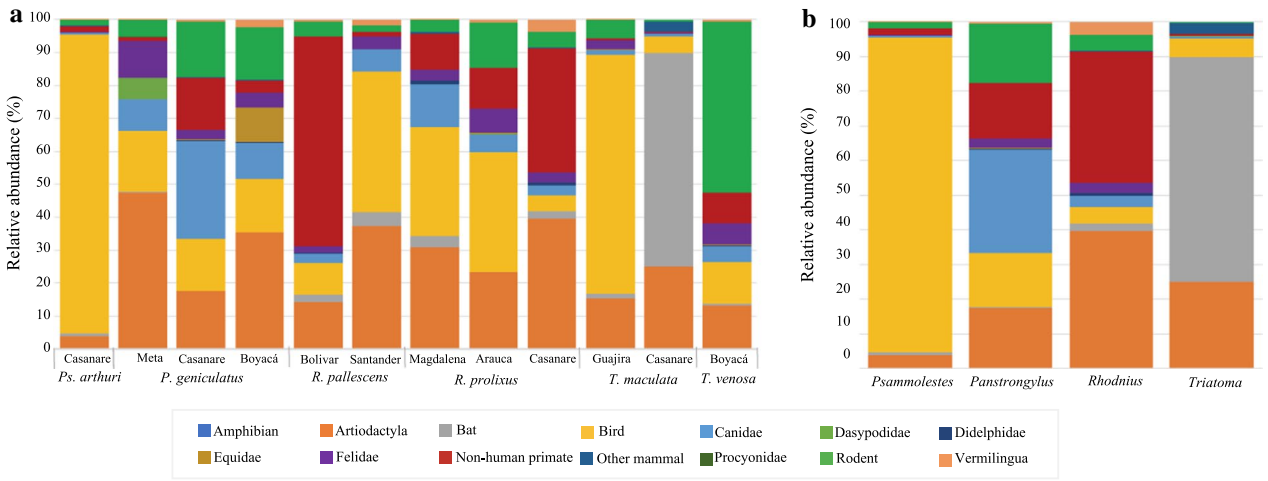

Fig. 4 Vertebrate feeding sources depending on geographical location. **a** Relative abundance of feeding sources for each triatomine species and all the regions in which the insects were collected. **b** Relative abundance of feeding sources for each triatomine genus in the department of Casanare. Since the genera *Rhodnius* and *Triatoma* each consisted of two species, we clarify that *R. prolixus* and *T. maculata* are the representatives of their corresponding genus in this case. For both graphs, vertebrate feeding sources are conveniently shown in the arbitrary grouping established for this study

($\chi^2 = 14.558$, $df = 5$, $P = 0.012$), Dasypodidae ($\chi^2 = 12.778$, $df = 5$, $P = 0.026$), birds ($\chi^2 = 12.568$, $df = 5$, $P = 0.028$) and bats ($\chi^2 = 17.277$, $df = 5$, $P = 0.004$), which can also be observed graphically (Figs. 2, 3). Exploring the values for these particular vertebrates by performing individual t-tests, we found that *Ps. arthuri* median value for bird reads is significantly different from each of the other triatomine bugs, *T. maculata* median value for the Dasypodidae reads is significantly different from each of the other insects, and that both of these triatomine species have significantly different median values for the Felidae regarding the rest of the triatomines. Also, *P. geniculatus* and *R. prolixus* have significantly different median values for Felidae, and the same happens for *T. venosa* and *P. geniculatus* with bat reads.

For the alpha diversity analysis, we calculated the inverse Simpson diversity index for vertebrate species hosts delineated by triatomine species. We found that this index exhibited the highest (1.77) and lowest (1.19) median values for *T. maculata* and *P. geniculatus*, respectively (Fig. 5a), whereas the median value obtained for *Ps. arthuri* was 1.38, 1.45 for *R. pallescens*, 1.54 for *R. prolixus*, and 1.31 for *T. venosa*. Nonetheless, no statistically significant differences were detected when triatomine species were compared with Kruskal-Wallis tests. The overall diversity index of vertebrate species as feeding sources for triatomines was 1.45, which can be considered as being a typical value obtained for this test. We observed a statistical significance in the difference between the diversity index of *T. cruzi*-positive and *T.*

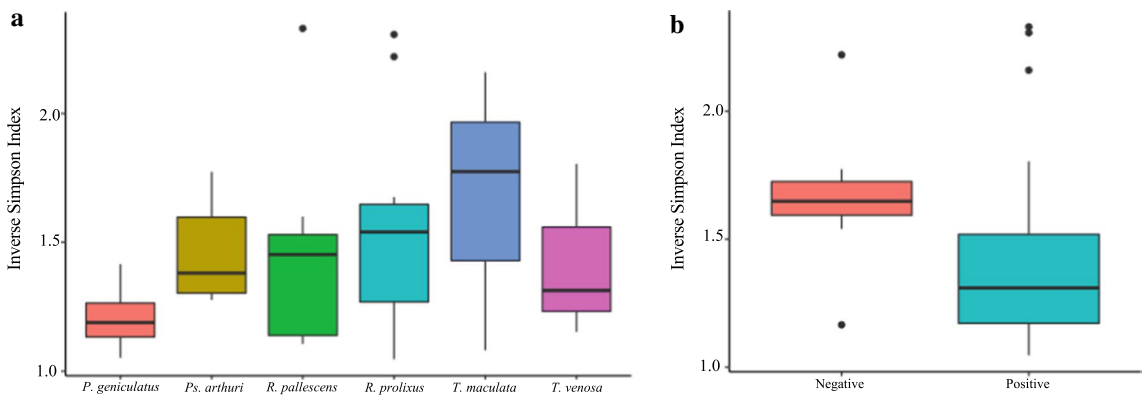

Fig. 5 Boxplots displaying inverse Simpson index results for each triatomine genus evaluated (**a**), and for *T. cruzi*-positive and negative samples (**b**)

cruzi-negative samples ($W = 188$, $P = 0.02588$), where the diversity index was higher for *T. cruzi* negative samples (Fig. 5b).

Discussion

An understanding of the feeding source patterns for the vectors of human-borne diseases is pivotal for elucidating the relationship of these vectors with their hosts and with the parasites they transmit. To the best of our knowledge, this is the first study to use NGS technologies for several *T. cruzi* vector species to describe their feeding sources in Colombia. Our results suggest that NGS technologies can be used to identify a vast diversity of feeding sources of triatomines. Thus, these technologies could play a crucial role in understanding the ecology of Chagas disease, especially the parasite transmission dynamics, as it has been suggested previously [41].

Herein, we identified 67 animal species as constituents of triatomine feeding habits (Additional file 3: Figure S1, Additional file 4: Figure S2), a higher value than those previously reported [9, 18, 22]. We also found multiple vertebrate feeding sources per triatomine (Figs. 2, 3), which agrees with previous reports assuring that the feeding source of individual insects was not restricted to a single vertebrate host, like recently shown by Dumonteil et al. [9]. Our study identified a greater number of feeding sources than previously reported, possibly because more numerous species were analyzed and larger sample size was used (despite our sample size was relatively low, it was higher than previous reports). We also highlight that the number of reads obtained per sample was around 170,000, which surpasses that reported in Mexico and Colombia [9, 27]. This suggests that read depth should be considered when identifying blood sources across triatomines in order to fully unravel its usefulness within epidemiological studies.

The predominant feeding source detected, found in all triatomines, was *H. sapiens* (Fig. 2), and around 80% of the samples evaluated were infected with *T. cruzi*, which is a similar result to that reported in previous studies on *Triatoma* and *Rhodnius* [42–45]. Human blood was the main feeding source of *R. prolixus* and *P. geniculatus* (Additional file 3: Figure S1), as has been previously reported for these and other triatomine species [8, 9, 31]. Moreover, reads belonging to *H. sapiens* were abundant regardless of the geographical location or the triatomine species, possibly due to the already reported preference of triatomines for the blood of *H. sapiens* [46]. This suggests a highly dynamic transmission in the areas where these insects were collected, which emphasizes the need to intensify prevention and control measures in said areas. A possible explanation for such high abundance of *H. sapiens* reads among the triatomine feeding sources includes the presence of human settlements, even within sylvatic ecotopes throughout the country. Severe deforestation has been reported, and even oral *T. cruzi* transmission outbreaks have occurred in the sampled areas due to human invasion into sylvatic environments [47]. Humans may, therefore, be a common component within the diet of triatomines, even more so than other vertebrates. In our findings, all the triatomine species were found to harbor a remarkable number of reads belonging to *H. sapiens* in their guts (Additional file 3: Figure S1). The previous findings again highlight the importance of these vector species in maintaining the human role in the life-cycle of *T. cruzi* and its importance in public health. Moreover, secondary vectors, such as *T. maculata* and *T. venosa*, should not be discarded, as we also detected reads associated with *H. sapiens* in their guts. In the Andean region, *R. prolixus* has been the focus of vector control programmes, but we herein additionally highlight the need to include other species, particularly *P. geniculatus* and also due to its importance in oral transmission outbreaks in Venezuela and Colombia [48, 49].

Although this is a descriptive study, it is interesting to observe the overall patterns in terms of feeding source diversity (Figs. 2, 3). In most individuals collected in domestic environments, we also found sylvatic-like feeding sources, including non-human primates, artiodactyls, didelphids and chiropterans (Fig. 2). These findings suggest a high dispersal ratio of the studied species, which challenges the current vector control programmes in Colombia. This agrees with previous findings in our country and other countries [8, 9, 50, 51] but contradicts other reports in El Salvador where domestic *T. dimidiata* fed mainly on *H. sapiens*, *C. lupus familiaris* and domestic birds [43].

In the present study, birds were the second most frequent feeding source (Additional file 3: Figure S1), with 10.5% of the total reads detected. This is an interesting finding that shows how challenging it can be to attempt to control the transmission of *T. cruzi*, given that hosts with such a remarkable capacity for dispersion are involved. It is also noteworthy of mention that many of the triatomine species used in this study were once considered to have feeding habits restricted to birds, such as *Ps. arthuri* [52] and *T. maculata* [46]. Since a broader feeding behavior has been observed in the last years [8, 53] as well as in this study, it is possible that the feeding behavior of these triatomine species has suffered changes throughout time (which could have been facilitated by the domiciliation processes they seem to endure [53–55]) or that the detection methods for feeding sources have improved during the last years, allowing researchers to discover an increasingly amount of vertebrates that compose the diet of these insects. Whichever the correct

assumption, it is important that this feeding source and its impact on the transmission of the parasite are further studied in the future.

Another interesting observation was finding non-human primates among the most frequent feeding sources (Fig. 3, Additional file 3: Figure S1), implying an interesting scenario in terms of Chagas disease control in the studied regions. It is important to mention that non-human primate genera have been found infected with all DTUs except for TcV and TcBat in various countries within the Americas, thereby incriminating them in the parasite transmission cycle [56–58]. In our results, *R. pallescens*, *P. geniculatus* and *R. prolixus* contained the highest number of reads associated with blood belonging to non-human primates, and this is particularly important due to the ecological landscape of oil plantations in the east of the country (i.e. *Attalea butyracea* and *Elaeis guineensis*). These oil-producing plant forests are highly infested with *R. pallescens*, *P. geniculatus*, and *R. prolixus* and are also frequented by non-human primates. Several studies highlight that these forests containing plants from which oils are derived represent a risk for *T. cruzi* transmission and our findings reinforce this hypothesis [27, 59, 60].

Some trends were identifiable in triatomine species with respect to feeding sources (Fig. 4), supported by the statistically significant difference found in this study. Here, we want to highlight that our aim was to describe the feeding sources and not finding associations. Furthermore, we identified *T. maculata* as the triatomine species that fed mostly on bats. This is an important finding given the importance of the Chiroptera in the cycle of transmission of *T. cruzi* and their usual presence within human dwellings, which can ultimately lead to transmission to humans. Besides, since many bat species have omnivorous feeding habits and can feed on small mammals and triatomines [61, 62] the probability of infection with *T. cruzi* could be slightly higher for these animals and this could translate in more *T. cruzi* transmissions that reach humans.

When evaluated by the Wilcoxon test, only *H. sapiens* showed a statistically significant difference between *T. cruzi*-positive and negative samples ($W=64$, $P=0.04873$). This could suggest that the presence of *T. cruzi* in a triatomine can modify the feeding behavior of the bug, but we consider this finding is not enough to reach a conclusion; we therefore encourage further studies focusing in this aspect of the feeding dynamic of triatomines. Despite this, and the absence of significant differences in feeding behavior between these two groups in the rest of vertebrate groups, evaluating differences in feeding sources between *T. cruzi*-positive and *T. cruzi*-negative samples was considered to be worth displaying in this study, as it may prove to be important for our understanding of the eco-epidemiology of Chagas disease since it offers insight into the role of vertebrates within the transmission cycle for both domestic and sylvatic ecotopes, and for this we encourage the development of future studies that further explore this variable. We also found a statistically significant difference between the inverse Simpson diversity index of *T. cruzi*-positive and *T. cruzi*-negative samples (Fig. 5b). The aforementioned highlights the need to detect the presence of *T. cruzi* in feeding-source studies, due to the possibility of identifying vertebrates that could be considered as more relevant in terms of the transmission dynamics of the parasite. Given that the triatomine species did not seem to have an effect in this differentiation, potential behavioral changes in the insect caused by the presence of *T. cruzi* might explain this difference.

Some limitations existed in our study, including a relatively small number of samples, number of regions of the provenance of triatomines, and the fact that the majority of the tested samples were positive for *T. cruzi*, which precluded the possibility of establishing a pattern of *T. cruzi* infections associated with the triatomine diet. It is also worth noting that not all the specimens had the same weight in the overall diet, given that the resulting number of reads detected was different for all of them and the percentages shown throughout this manuscript were calculated in terms of relative abundance (taking the number of reads detected per vertebrate for a sample and dividing them by the total amount of reads of the sample). Additionally, there was no control samples or abundance threshold to evaluate the level of possible cross-contamination and secure that the read diversity detected was indeed an accurate depiction of reality, therefore the blood-source diversity could have been artificially increased and future studies with controls and adequate abundance thresholds are needed. Likewise, a relationship exists between time elapsed and the number of reads capable of being detected, in which the more recently the triatomine has fed, the higher the number of reads that could be obtained. Therefore, considering the transversal nature of this study, not detecting a certain feeding source is not conclusive evidence for a lack of this feeding source within the triatomine diet, but could be a consequence of the amount of time passed since the collection of the insect. Nonetheless, it is important to take into consideration that on certain occasions, it can be problematic to extract intestinal contents from insects, especially when they have been starved for long, which could have been the case in our study, given that we had no information concerning the dietary status of the collected samples [5, 45]. Additionally, given the differences between erythrocyte structure, some feeding sources

tend to persist longer in the insect gut, which could alter detected read proportions [63]. We suggest that future studies attempt to overcome these limitations in order to improve the quality of the provided information.

When analyzing the feeding sources at a microgeographical scale, several interesting patterns were depicted in our study (Fig. 4). For example, in the case of *R. pallescens*, insects were collected in Mompox, Bolívar, within the sylvatic ecotope and at two different localities from Santander (i.e. inside houses). The ecological landscape of Bolívar seems complex as individual reads from both domestic and sylvatic vertebrates were detected; however, individuals in Santander, despite presenting the same vertebrate groups, revealed a higher number of reads belonging to domestic vertebrates. The landscape of Bolívar is full of forests, and the draining Magdalena River, as well as the Atlantic Ocean, allows an enzootic cycle as compared with the more urban transmission cycle present in Santander [50]. In recent years, *R. pallescens* has gained importance as vector due to its intrusion into human dwellings and high rates of *T. cruzi* infection [8]. Our findings also suggest the high capacity of this species to adapt and switch from sylvatic to domestic blood preferences. This characteristic is particularly important for defining a good vector within medical entomology [64] and could also explain the lack of association of TcIDom with domestic vertebrates, and TcISylv with sylvatic ones (Additional file 6: Figure S3).

In the case of *R. prolixus* and *P. geniculatus*, human blood was the main feeding source (Additional file 3: Figure S1), as has been previously reported [9, 22, 31]. The ecology of these species is complex as they have been found in armadillo nests as well as within human dwellings [48, 65]. Our findings reinforce the epidemiological importance of *P. geniculatus* in the transmission dynamics of Chagas disease within Colombia. In fact, several oral transmission outbreaks have been linked to this species in Colombia, Venezuela and Brazil [8, 26, 48, 49]. The finding that *P. geniculatus* utilizes several different feeding sources interposes a challenge for Chagas disease vector control in the light of the extreme adaptation this insect may exhibit; in addition, previous reports suggest its conspicuous capability of transmitting TcI, TcII, TcIII and TcIV DTUs [8].

As stated, our methodological approach allowed us to elucidate up to 67 different feeding sources, which, to our knowledge, is the highest reported level of diversity. We calculated the inverse Simpson index per species in terms of reads and observed interesting and particular patterns (Fig. 5a). Despite *T. maculata* showing the highest values for the diversity index, the other triatomine species had similar values, which consequently suggests great adaptation of all triatomine species to different blood sources. Therefore, the triatomine species evaluated here could be considered as insects that maintain the epizootic and enzootic cycles of *T. cruzi*, as previously reported for some of them [27, 66]. These patterns reiterate the great usefulness of identifying blood sources in vectors of infectious diseases caused by parasites, such as *T. cruzi*, in order to understand the ecological behavior and varying adaptation mechanisms. This is one of the greatest advantages of amplicon-based NGS, in which the description of the global diversity of feeding sources within one individual can be elucidated.

In this study, it became apparent that some triatomine interactions have changed over time, and this is important because alterations in these interactions can affect disease transmission. For instance, *T. maculata* was once considered to be one of the triatomine species that fed solely on birds [46], and even though reads belonging to birds were found, this feeding source was not the most abundant one for the triatomine species. More importantly, we found that the diet of this triatomine species also contained domestic vertebrates, suggesting that *T. maculata* could be involved in a domiciliation process, something that had not been previously considered. Of note, *R. pallescens* has been widely associated with palm trees [67]; however, given the presence of *H. sapiens* reads in this bug, it seems likely that this species is capable of intruding within human dwellings and therefore possesses greater mobility than previously thought. Additionally, *Ps. arthuri* were found to feed on humans and were positive for *T. cruzi*, which has also been reported by other authors recently [68] and highlights the urgency of evaluating the vectorial capacity of this triatomine species, which was not considered to be a vector in the past. Interestingly, we found Artiodactyla and Perissodactyla (Equidae) reads in this species, which was unexpected. One possible explanation might be that *Ps. arthuri* might visit different vertebrate settlements or roam in the surroundings occasionally feeding on other vertebrates, which may explain the non-bird feeding sources found in some of these bugs. Also, this NGS approach is much more sensitive than Sanger sequencing, which was used in a previous study from our group [68]. Nevertheless, a larger sample size is needed to fully understand the transmission dynamics in *Ps. arthuri.*

Conclusions

To our knowledge, this is the first study to employ amplicon-based NGS of the *12S* rRNA region to depict blood-feeding sources of various triatomine species collected in different regions of Colombia. Our findings report a striking diversity of blood-feeding sources that had never been previously reported. Despite being a mainly descriptive study, we highlight the generalist

behavior of the insects evaluated, and the differences existing among the diets of triatomine species. Consequently, and considering that our methodology does not impose a threat to the studied vertebrates, we propose the performance of similar studies to examine new regions considered as non-endemic for Chagas disease and to profoundly investigate triatomine interactions with possible hosts with the intention of improving control strategies for this disease in Colombia.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-020-04310-z.

Additional file 1: Table S1. Complete dataset of the 42 triatomines used in this study. This dataset contains ID code, triatomine species, qPCR result, DTU information obtained through conventional PCR, geographical information (region and town), and correlating information for the insect (ecotope and sex).

Additional file 2: Alignment S1. FASTA file with the 543 vertebrate species used as reference in this study.

Additional file 3: Figure S1. Relative abundance of the 15 vertebrate arbitrary groups within each collected triatomine species.

Additional file 4: Figure S2. Relative abundance of reads corresponding to each triatomine species, regarding their division among the 15 arbitrary vertebrate groups.

Additional file 5: Table S2. Arbitrary grouping of the vertebrate species. The 67 vertebrate species detected were grouped in 15 different categories, which are used consistently through this study. This file also displays which categories were considered as domestic or sylvatic when this division was considered necessary for the analysis: an asterisk (*) indicates the groups with domestic species, while a plus (+) indicates the groups with sylvatic species. If the vertebrate group was considered to contain both domestic and sylvatic species, the asterisk was placed next to the species considered as domestic, understanding from this that the unmarked species are considered sylvatic.

Additional file 6: Figure S3. Number of reads found for each type of TcI DTU (Dom, Sylv and Dom-Sylv). Bar colors represent the habitat of the detected vertebrate (i.e. domestic or sylvatic).

Abbreviations

NTD: neglected tropical disease; DTU: discrete typing unit; NGS: next-generation sequencing; PCR: polymerase chain reaction; qPCR: quantitative (real-time) polymerase chain reaction; rRNA: ribosomal ribonucleic acid; QC: quality control; QIIME: quantitative insights into microbial ecology; NCBI: National Center for Biotechnology Information; nd: not detected.

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Authors' contributions

LAG and JDR wrote the manuscript. JDR, GH, CH, MM and LAG analyzed the data. MM and GH carried out statistical tests. GH, LAG, OC, PU, AC, NVO and JDR carried out experimental procedures. All authors read and approved the final manuscript.

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Availability of data and materials

The data supporting the conclusions of this article are included within the article and its additional files. The dataset generated during the present study was deposited at DDBJ/ENA/GenBank under the study accession number: PRJEB38830.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Rassi AJ, Rassi A, Marin-Neto JA. Chagas disease. Lancet. 2010;375:1388–402.
2. Molyneux DH, Savioli L, Engels D. Neglected tropical diseases: progress towards addressing the chronic pandemic. Lancet. 2017;389:312–25.
3. Anis R, Anis R, Rassi SG. Predictors of mortality in chronic Chagas disease. Circulation. 2007;115:1101–8.
4. Zingales B, Andrade SG, Briones MRS, Campbell DA, Chiari E, Fernandes O, et al. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz. 2009;104:1051–4.
5. Ramirez JD, Hernandez C. Trypanosoma cruzi I: towards the need of genetic subdivision? Part II. Acta Trop. 2018;184:53–8.
6. Parra-Henao G, Suarez-Escudero LC, Gonzalez-Caro S. Potential distribution of Chagas disease vectors (Hemiptera, Reduviidae, Triatominae) in Colombia, based on ecological niche modeling. J Trop Med. 2016;2016:1439090.
7. Georgieva AY, Gordon ERL, Weirauch C. Sylvatic host associations of Triatominae and implications for Chagas disease reservoirs: a review and new host records based on archival specimens. PeerJ. 2017;5:e3826.
8. Hernandez C, Salazar C, Brochero H, Teheran A, Buitrago LS, Vera M, et al. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. Parasit Vectors. 2016;9:620.
9. Dumonteil E, Ramirez-Sierra M-J, Perez-Carrillo S, Teh-Poot C, Herrera C, Gourbiere S, et al. Detailed ecological associations of triatomines revealed by metabarcoding and next-generation sequencing: implications for triatomine behavior and *Trypanosoma cruzi* transmission cycles. Sci Rep. 2018;8:4140.
10. Valença-Barbosa C, Fernandes FA, Santos HLC, Sarquis O, Harry M, Almeida CE, et al. Molecular identification of food sources in triatomines in the Brazilian Northeast: roles of goats and rodents in Chagas disease epidemiology. Am J Trop Med Hyg. 2015;93:994–7.
11. Li ZQ, Liu BR, Zeng WH, Xiao WL, Li QJ, Zhong JH. Character of cellulase activity in the guts of flagellate-free termites with different feeding habits. J Insect Sci. 2013;13:37.
12. Meyin A, Ebong S, Garcia-Pena GE, Pluot-Sigwalt D, Marsollier L, Le Gall P, Eyangoh S, et al. Ecology and feeding habits drive infection of water bugs with *Mycobacterium ulcerans*. Ecohealth. 2017;14:329–41.
13. Kudo R, Masuya H, Endoh R, Kikuchi T, Ikeda H. Gut bacterial and fungal communities in ground-dwelling beetles are associated with host food habit and habitat. ISME J. 2019;13:676–85.

14. Ekoko WE, Awono-Ambene P, Bigoga J, Mandeng S, Piameu M, Nvondo N, et al. Patterns of anopheline feeding/resting behaviour and *Plasmodium* infections in North Cameroon, 2011–2014: implications for malaria control. Parasit Vectors. 2019;12:297.
15. Machani MG, Ochomo E, Sang D, Bonizzoni M, Zhou G, Githeko AK, et al. Influence of blood meal and age of mosquitoes on susceptibility to pyrethroids in *Anopheles gambiae* from western Kenya. Malar J. 2019;18:112.
16. Lyimo IN, Haydon DT, Russell TL, Mbina KF, Daraja AA, Mbehela EM, et al. The impact of host species and vector control measures on the fitness of African malaria vectors. Proc Biol Sci. 2013;280:20122823.
17. Day JF. Host-seeking strategies of mosquito disease vectors. J Am Mosq Control Assoc. 2005;21(Suppl. 4):17–22.
18. Killeen GF, McKenzie FE, Foy BD, Bogh C, Beier JC. The availability of potential hosts as a determinant of feeding behaviours and malaria transmission by African mosquito populations. Trans R Soc Trop Med Hyg. 2001;95:469–76.
19. Asale A, Duchateau L, Devleesschauwer B, Huisman G, Yewhalaw D. Zooprophylaxis as a control strategy for malaria caused by the vector *Anopheles arabiensis* (Diptera: Culicidae): a systematic review. Infect Dis Poverty. 2017;6:160.
20. St Laurent B, Burton TA, Zubaidah S, Miller HC, Asih PB, Baharuddin A, et al. Host attraction and biting behaviour of *Anopheles* mosquitoes in South Halmahera, Indonesia. Malar J. 2017;16:310.
21. Becker DJ, Streicker DG, Altizer S. Using host species traits to understand the consequences of resource provisioning for host-parasite interactions. J Anim Ecol. 2018;87:511–25.
22. Gottdenker NL, Chaves LF, Calzada JE, Saldaña A, Carroll CR. Host life history strategy, species diversity, and habitat influence *Trypanosoma cruzi* vector infection in changing landscapes. PLoS Negl Trop Dis. 2012;6:e1884.
23. Guhl F, Aguilera G, Pinto N, Vergara D. Updated geographical distribution and ecoepidemiology of the triatomine fauna (Reduviidae: Triatominae) in Colombia. Biomedica. 2007;27(Suppl. 1):143–62.
24. Wong YY, Sornosa Macias KJ, Guale Martinez D, Solorzano LF, Ramirez-Sierra MJ, Herrera C, et al. Molecular epidemiology of *Trypanosoma cruzi* and *Triatoma dimidiata* in costal Ecuador. Infect Genet Evol. 2016;41:207–12.
25. Zuriaga MA, Blandon-Naranjo M, Valerio-Campos I, Salas R, Zeledon R, Bargues MD. Molecular characterization of *Trypanosoma cruzi* and infection rate of the vector *Triatoma dimidiata* in Costa Rica. Parasitol Res. 2012;111:1615–20.
26. Carrasco HJ, Torrellas A, Garcia C, Segovia M, Feliciangeli MD. Risk of *Trypanosoma cruzi* I (Kinetoplastida: Trypanosomatidae) transmission by *Panstrongylus geniculatus* (Hemiptera: Reduviidae) in Caracas (Metropolitan District) and neighboring States, Venezuela. Int J Parasitol. 2005;35:1379–84.
27. Erazo D, Gottdenker NL, González C, Guhl F, Cuellar M, Kieran TJ, et al. Generalist host species drive *Trypanosoma cruzi* vector infection in oil palm plantations in the Orinoco region, Colombia. Parasit Vectors. 2019;12:274.
28. Ramirez-Sierra MJ, Dumonteil E. Infection rate by *Trypanosoma cruzi* and biased vertebrate host selection in the *Triatoma dimidiata* (Hemiptera: Reduviidae) species complex. J Med Entomol. 2016;53:20–5.
29. Dumonteil E, Gourbiere S. Predicting *Triatoma dimidiata* abundance and infection rate: a risk map for natural transmission of chagas disease in the Yucatan Peninsula of Mexico. Am J Trop Med Hyg. 2004;70:514–9.
30. Galvao C, Justi SA. An overview on the ecology of Triatominae (Hemiptera:Reduviidae). Acta Trop. 2015;151:116–25.
31. Peña-Garcia VH, Gomez-Palacio AM, Triana-Chavez O, Mejia-Jaramillo AM. Eco-epidemiology of Chagas disease in an endemic area of Colombia: risk factor estimation, *Trypanosoma cruzi* characterization and identification of blood-meal sources in bugs. Am J Trop Med Hyg. 2014;91:1116–24.
32. Montoya-Porras LM, Omar TC, Alzate JF, Moreno-Herrera CX, Cadavid-Restrepo GE. *16S* rRNA gene amplicon sequencing reveals dominance of Actinobacteria in *Rhodnius pallescens* compared to *Triatoma maculata* midgut microbiota in natural populations of vector insects from Colombia. Acta Trop. 2018;178:327–32.
33. Pompanon F, Deagle BE, Symondson WOC, Brown DS, Jarman SN, Taberlet P. Who is eating what: diet assessment using next generation sequencing. Mol Ecol. 2012;21:1931–50.
34. Noireau F, Abad-Franch F, Valente SAS, Dias-Lima A, Lopes CM, Cunha V, et al. Trapping Triatominae in silvatic habitats. Mem Inst Oswald Cruz. 2002;97:61–3.
35. Duffy T, Cura CI, Ramirez JC, Abate T, Cayo NM, Parrado R, et al. Analytical performance of a multiplex real-time PCR assay using TaqMan probes for quantification of *Trypanosoma cruzi* satellite DNA in blood samples. PLoS Negl Trop Dis. 2013;7:e2000.
36. Espinosa-Alvarez O, Ortiz PA, Lima L, Costa-Martins AG, Serrano MG, Herder S, et al. *Trypanosoma rangeli* is phylogenetically closer to Old World trypanosomes than to *Trypanosoma cruzi*. Int J Parasitol. 2018;48:569–84.
37. Ramirez JD, Guhl F, Umezawa ES, Morillo CA, Rosas F, Marin-Neto JA, et al. Evaluation of adult chronic Chagas' heart disease diagnosis by molecular and serological methods. J Clin Microbiol. 2009;47:3945–51.
38. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335–6.
39. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19:1639–45.
40. Fox J, The R. Commander: a basic-statistics graphical user interface to R. J Stat Softw. 2005;14:142607.
41. Hernández-Andrade A, Moo-Millan J, Cigarroa-Toledo N, Ramos-Ligonio A, Herrera C, Bucheton B, et al. Metabarcoding: a powerful yet still underestimated approach for the comprehensive study of vector-borne pathogen transmission cycles and their dynamics. London: IntechOpen; 2019.
42. Lucero DE, Ribera W, Pizarro JC, Plaza C, Gordon LW, Peña R Jr, et al. Sources of blood meals of sylvatic *Triatoma guasayana* near Zurima, Bolivia, assayed with qPCR and *12S* cloning. PLoS Negl Trop Dis. 2014;8:e3365.
43. Orantes LC, Monroy C, Dorn PL, Stevens L, Rizzo DM, Morrissey L, et al. Uncovering vector, parasite, blood meal and microbiome patterns from mixed-DNA specimens of the Chagas disease vector *Triatoma dimidiata*. PLoS Negl Trop Dis. 2018;12:e0006730.
44. Escandón-Vargas K, Muñoz-Zuluaga CA, Salazar L. Blood-feeding of Rhodnius prolixus. Biomédica. 2017;37:299–302.
45. Peña VH, Fernandez GJ, Gomez-Palacio AM, Mejia-Jaramillo AM, Cantillo O, Triana-Chavez O. High-resolution melting (HRM) of the cytochrome *b* gene: a powerful approach to identify blood-meal sources in Chagas disease vectors. PLoS Negl Trop Dis. 2012;6:e1530.
46. Luitgards-Moura JF, Vargas AB, Almeida CE, Magno-Esperanca G, Agapito-Souza R, Folly-Ramos E, et al. A *Triatoma maculata* (Hemiptera, Reduviidae, Triatominae) population from Roraima, Amazon region, Brazil, has some bionomic characteristics of a potential Chagas disease vector. Rev Inst Med Trop Sao Paulo. 2005;47:131–7.
47. Hernandez C, Vera MJ, Cucunuba Z, Florez C, Cantillo O, Buitrago LS, et al. High-resolution molecular typing of *Trypanosoma cruzi* in 2 large outbreaks of acute Chagas disease in Colombia. J Infect Dis. 2016;214:1252–5.
48. Carrasco HJ, Segovia M, Londono JC, Ortegoza J, Rodriguez M, Martinez CE. *Panstrongylus geniculatus* and four other species of triatomine bug involved in the *Trypanosoma cruzi* enzootic cycle: high risk factors for Chagas' disease transmission in the Metropolitan District of Caracas, Venezuela. Parasit Vectors. 2014;7:602.
49. de Noya BA, Díaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Muñoz-Calderón A, et al. Update on oral Chagas disease outbreaks in Venezuela: epidemiological, clinical and diagnostic approaches. Mem Inst Oswaldo Cruz. 2015;110:377–86.
50. Cantillo-Barraza O, Garces E, Gomez-Palacio A, Cortes LA, Pereira A, Marcet PL, et al. Eco-epidemiological study of an endemic Chagas disease region in northern Colombia reveals the importance of *Triatoma maculata* (Hemiptera: Reduviidae), dogs and *Didelphis marsupialis* in *Trypanosoma cruzi* maintenance. Parasit Vectors. 2015;8:482.
51. Moo-Millan JI, Arnal A, Pérez-Carrillo S, Hernandez-Andrade A, Ramírez-Sierra MJ, Rosado-Vallado M, et al. Disentangling *Trypanosoma cruzi* transmission cycle dynamics through the identification of blood meal sources of natural populations of *Triatoma dimidiata* in Yucatán. Mexico. Parasit Vectors. 2019;12:572.
52. Abad-Franch F, Monteiro FA, Jaramillo ON, Gurgel-Goncalves R, Dias FBS, Diotaiuti L. Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). Acta Trop. 2009;110:159–77.
53. Reyes-Lugo M, Reyes-Contreras M, Salvi I, Gelves W, Avilan A, Llavaneras D, et al. The association of *Triatoma maculata* (Ericsson 1848) with the gecko

Thecadactylus rapicauda (Houttuyn 1782) (Reptilia: Squamata: Gekkonidae): a strategy of domiciliation of the Chagas disease peridomestic vector in Venezuela? Asian Pac J Trop Biomed. 2011;1:279–84.
54. Klotz SA, Shirazi FM, Boesen K, Beatty NL, Dorn PL, Smith S, et al. Kissing bug (*Triatoma* spp.) intrusion into homes: troublesome bites and domiciliation. Environ Health Insights. 2016;10:45–9.
55. Ricardo-Silva A, Goncalves TCM, Luitgards-Moura JF, Lopes CM, da Silva SP, Bastos AQ, et al. *Triatoma maculata* colonises urban domicilies in Boa Vista, Roraima, Brazil. Mem Inst Oswaldo Cruz. 2016;111:703–6.
56. Vitelli-Avelar DM, Sathler-Avelar R, Mattoso-Barbosa AM, Gouin N, Perdigao-de-Oliveira M, Valerio-Dos-Reis L, et al. Cynomolgus macaques naturally infected with *Trypanosoma cruzi*-I exhibit an overall mixed pro-inflammatory/modulated cytokine signature characteristic of human Chagas disease. PLoS Negl Trop Dis. 2017;11:e0005233.
57. Hodo CL, Wilkerson GK, Birkner EC, Gray SB, Hamer SA. *Trypanosoma cruzi* transmission among captive nonhuman primates, wildlife, and vectors. Ecohealth. 2018;15:426–36.
58. Guhl F, Ramirez JD. *Trypanosoma cruzi* I diversity: towards the need of genetic subdivision? Acta Trop. 2011;119:1–4.
59. Jácome-Pinilla D, Hincapie-Peñaloza E, Ortiz MI, Ramírez JD, Guhl F, Molina J. Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit Vectors. 2015;8:600.
60. Urbano P, Poveda C, Molina J. Effect of the physiognomy of *Attalea butyracea* (Arecoideae) on population density and age distribution of *Rhodnius prolixus* (Triatominae). Parasit Vectors. 2015;8:199.
61. Thomas ME, Rasweiler Iv JJ, D'Alessandro A. Experimental transmission of the parasitic flagellates *Trypanosoma cruzi* and *Trypanosoma rangeli* between triatomine bugs or mice and captive neotropical bats. Mem Inst Oswaldo Cruz. 2007;102:559–65.
62. Wray AK, Olival KJ, Moran D, Lopez MR, Alvarez D, Navarrete-Macias I, et al. Viral diversity, prey preference, and *Bartonella* prevalence in *Desmodus rotundus* in Guatemala. Ecohealth. 2016;13:761–74.
63. Kato H, Jochim RC, Gomez EA, Tsunekawa S, Valenzuela JG, Hashiguchi Y. Salivary gland transcripts of the kissing bug, *Panstrongylus chinai*, a vector of Chagas disease. Acta Trop. 2017;174:122–9.
64. de Fuentes-Vicente JA, Gutiérrez-Cabrera AE, Flores-Villegas AL, Lowenberger C, Benelli G, Salazar-Schettino PM, et al. What makes an effective Chagas disease vector? Factors underlying *Trypanosoma cruzi*-triatomine interactions. Acta Trop. 2018;183:23–31.
65. Thankaswamy-Kosalai S, Sen P, Nookaew I. Evaluation and assessment of read-mapping by multiple next-generation sequencing aligners based on genome-wide characteristics. Genomics. 2017;109:186–91.
66. Teixeira AR, Monteiro PS, Rebelo JM, Argañaraz ER, Vieira D, Lauria-Pires L, et al. Emerging Chagas disease: trophic network and cycle of transmission of *Trypanosoma cruzi* from palm trees in the Amazon. Emerg Infect Dis. 2001;7:100–12.
67. Cantillo-Barraza O, Gómez-Palacio A, Salazar D, Mejía-Jaramillo AM, Calle J, Triana O. Distribución geográfica y ecoepidemiología de la fauna de triatominos (Reduviidae: Triatominae) en la Isla Margarita del departamento de Bolívar, Colombia. Biomédica. 2010;30:382–9.
68. Velasquez-Ortiz N, Hernandez C, Herrera G, Cruz-Saavedra L, Higuera A, Arias-Giraldo LM, et al. *Trypanosoma cruzi* infection, discrete typing units and feeding sources among *Psammolestes arthuri* (Reduviidae: Triatominae) collected in eastern Colombia. Parasit Vectors. 2019;12:157.

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Research

Sampling, Distribution, Dispersal

Temporal Variation of the Presence of *Rhodnius prolixus* (Hemiptera: Reduviidae) Into Rural Dwellings in the Department of Casanare, Eastern Colombia

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Abstract

Rhodnius prolixus (Stål, 1859) is the major vector of *Trypanosoma cruzi* in Colombia and Venezuela. The species is strongly associated with high-altitude ecotopes, such as sylvatic palms (*Attalea butyracea*), where spatially and temporally stable infestations are established. We investigated temporal variation in regards to the presence of *R. prolixus* in rural dwellings in the department of Casanare (eastern Colombia) over a period of 12 mo. Thirty houses were sampled from January to December 2017 by installing Maria sensors, collecting triatomines through community entomological surveillance, and conducting a monthly search in each house. The collection of specimens from the houses varied significantly by month with the higher number of collections occurring in the low-rainfall season and the lower number of collections occurring in the months of increased precipitation. The proportions of males, females, and nymphs also varied significantly throughout the time period: nymphs (fifth instar only) were reported only during May, July, and September and significantly greater numbers of females than males were reported in the inspected dwellings in all months. Density, crowding, and colonization indices varied according to the season. A bloodmeal analysis revealed 17 different hosts. A total of 42 randomly selected *R. prolixus* specimens were subjected to molecular analyses for detection of *T. cruzi* DNA with 22 found positive (infection prevalence of 52%). In conclusion, we observed a high presence of *R. prolixus* (infected with *T. cruzi*) in dwellings close to native palm plantations. These findings indicate a high risk of vector transmission of *T. cruzi* for people in the study areas and challenges for the current vector control schemes in the region.

Key words: Colombia, temporal variation, *Rhodnius prolixus*, density, dispersion

American trypanosomiasis, Chagas disease, is a parasitic infection caused by the protozoan *Trypanosoma cruzi* (Cortés and Suárez 2005, Montilla et al. 2011, Llano et al. 2014). This parasite is transmitted by insects of the subfamily Triatominae, when mucous membranes or wounded skin comes into contact with the parasite-infected feces of the bug (Guhl et al. 2007, Becerril et al. 2010). In Colombia, the department of Casanare (eastern Colombia) has the second highest reported prevalence of *T. cruzi* infection in humans (10%), after Arauca (21.1%) (Cortés and Suárez 2005, Guhl et al. 2007, Parra-Henao et al. 2009). Casanare is recognized as a focus of vector and oral transmission of Chagas disease as well (Angulo et al. 2012, Zuleta et al. 2017, Hernández et al. 2016).

This disease is endemic on the American continent (Dias et al. 2002), where millions of people are affected. In Colombia, it has been estimated that 5% of the population are infected and 23% live in areas with high risk of transmission (Hoyos et al. 2007, Llano et al. 2014). Vector transmission can occur through the domestic, peri-domestic, and sylvatic cycles (Montilla et al. 2011, Angulo et al. 2012, Guhl and Ramírez 2013). In a process of domicilation that is defined as sylvatic triatomines entering dwellings and then propagating as evidenced by the presence of at least three developmental stages (Guhl et al. 2007, Esteban et al. 2017) sylvatic triatomines can enter dwellings where both shelter and blood of humans and/or domestic animals can be obtained. This capacity of triatomines

to disperse from sylvatic ecotopes to human habitations represents a critical factor in the population dynamics of triatomines and the epidemiology of Chagas disease as previously reported in Brazil and Argentina (Zeledon and Rabinovich 1981, Pizarro and Romaña 1998, Canale et al. 1999, Dias-Lima and Sherlock 2000).

The distribution of the 149 known species of triatomines varies according to their ecological requirements (Justi and Galvão 2017). In Colombia, 27 species have been recorded, of which 17 have been found infected with *T. cruzi* (Parra-Henao et al. 2015, Ayala et al. 2019, Velásquez-Ortiz et al. 2019). In the eastern plains of Colombia (extending into Venezuela), *Triatoma dimidiata* (Latreille, 1811), *Triatoma. maculata* (Erichson, 1848) and *Rhodnius prolixus* (Stål, 1859) have been recorded as the main vectors of *T. cruzi*. Of these, *R. prolixus* is the most important in Colombia and Venezuela because of occurrence in sylvatic, domestic, and peri-domestic ecotopes (Dias et al. 2002, Angulo and Esteban 2011, Angulo et al. 2012, Urbano et al. 2015, Esteban et al. 2017, Urbano et al. 2018).

Forests of *Attalea butyracea* (wine palm) are considered the main ecological entities for sustaining and establishing colonies of triatomines, particularly *R. prolixus* in the eastern plains of Colombia (Teixeira et al. 2001, Abad-Franch et al. 2005, Angulo et al. 2012, Urbano et al. 2018). Rueda et al. (2014), observed higher densities of triatomines in palms located near houses, banana plantations, and fruit trees, than in palms located in secondary forests, possibly because the former provide conditions (food resource in the blood of humans and domestic animals) that facilitate domiciliation of the vector (Guhl et al. 2007, Angulo et al. 2013, Urbano et al. 2015, Urbano et al. 2018). Consequently, humans play an important role in altering the cycle of transmission of the trypanosome parasite and the vector, increasing the probability of transmission of the parasite (Guhl et al., 2007). It is important to clarify the capacity of this vector to adapt to different ecotopes and to intrude into homes under different environmental conditions. Precipitation is one of the most relevant factors involved in the presence of triatomines in human dwellings; a higher population density was found in wild populations of *R. prolixus* (Urbano et al. 2018), *Rhodnius neglectus* (Lent, 1954), and *Rhodnius robustus* (Larouse, 1927) during periods of low rainfall (Gurgel-Gonçalves et al. 2004, Longa and Scorza 2005).

Such information is required to define rational surveillance interventions and to implement control programs in areas where sylvatic populations of triatomines are potentially involved in the transmission of Chagas disease (Abad-Franch et al. 2005). Most rural dwellings in the department of Casanare are immersed in landscape matrices whose main structural component consists of sylvatic palms. This area provides biological, ecological, and environmental conditions, which favor the eco-epidemiological cycles of the parasite. The aim of this study was to determine the temporal variation of *R. prolixus* in rural dwellings of the Casanare department over a 12-mo period. We also identified the *T. cruzi* infection and the blood feeding sources of a random subsample of collected *R. prolixus*.

Materials and Methods

Study Area

The study was carried out in the villages of Agualinda in the municipality of Pore (5.66278°N, –71.9908°W) and Sabanetas in the municipality of Paz de Ariporo (5.872663°N, –71.84714°W) of the department of Casanare (Fig. 1). The area comprises forest matrices composed of grazing and silvo-pastoral areas (trees, forage, and the grazing of domesticated animals), in addition to natural and introduced pastures currently used for extensive cattle ranching. Plantain, cassava, corn, and cacao crops are the most important economic activities in these municipalities (Bejarano 2012). These areas have an average annual temperature of 28 ± 8°C and average annual rainfall of 2,000–2,700 mm. According to the climatic classification of Köppen (Köppen 1918), a tropical climate is represented with a marked unimodal seasonality having a period of high rainfall that extends from May to November with average monthly precipitation of 329 mm, and a period of low rainfall during the remaining 5 mo with average monthly precipitation of 89 mm, and an altitude range of 280 ± 10 m above sea level. The dwellings selected for monitoring were typically located in grazing areas surrounded by small cultivated areas and scattered palms near riverine forests, where the main structural component was sylvatic palms. A high index of infestation by *R. prolixus* (3.3%) and cases of infection in humans (7.2%) had previously been reported for the area (Guhl et al. 2007, Zuleta et al. 2017).

Sampling

Sampling and monitoring were carried out on a total of 30 houses, 12 selected from the village of Sabanetas (Paz de Ariporo) and 18 from the village of Agualinda (Pore). Maria sensors were installed in these houses, according to the methodology proposed by Wisnivesky-Colli et al. (1992) and triatomines were collected through community entomological surveillance by families trained in recognition of, searching for, and collection of the triatomine insects. The 'María Sensors' (in-house made) are cardboard boxes with absorbent paper folded inside that, fixed to the walls of the house, can passively detect the presence of triatomines. Direct collection of the insects from the Maria Sensors occurred when periodic monitoring of the boxes was carried out, or by evidence of the triatomines such as feces, eggs, or exuvia. Visits were made monthly during 2017, when the numbers of triatomines recorded by the installed sensors was checked and specimens captured by the families were collected. In addition, an active-man-hour search was carried out inside each house, with a sampling effort of 16 h per month, and triatomines that were attracted by light during 2 d per month from 1,800 to 2,200 were collected (Jácome-Pinilla et al. 2015). Triatomines were placed in labeled plastic containers, with filter paper folded inside, covered with a tulle cloth and fastened with rubber band, allowing movement and absorbing excess moisture, for later identification and processing in the laboratory. Specimens were identified using the keys of Lent and Wygodzinsky (1979). In addition, a subsample (42 randomly selected individuals) was selected to carry out molecular analysis for identification of the infective agent; these specimens were placed in absolute ethanol and labeled with the household and the month sampled.

Statistical Analysis

Shapiro–Wilk tests were performed to analyze data normality assumptions and Levene tests to determine the homoscedasticity of the variables. Subsequently, Kruskal–Wallis nonparametric tests were applied to determine significant differences in the population densities of *R. prolixus* among sampling months, and among nymphs, females, and males. Trends in the density of individuals captured per month were analyzed using a multiple Dunn test ($P > 0.05$). The relationships between the density of *R. prolixus* and the average monthly precipitation and temperature in the inspected dwellings were examined using the Spearman correlation coefficient ($P < 0.05$). Density (number of triatomines collected/number of houses examined), crowding (number of triatomines collected/number of positive houses), infestation (number of positive houses/number of houses

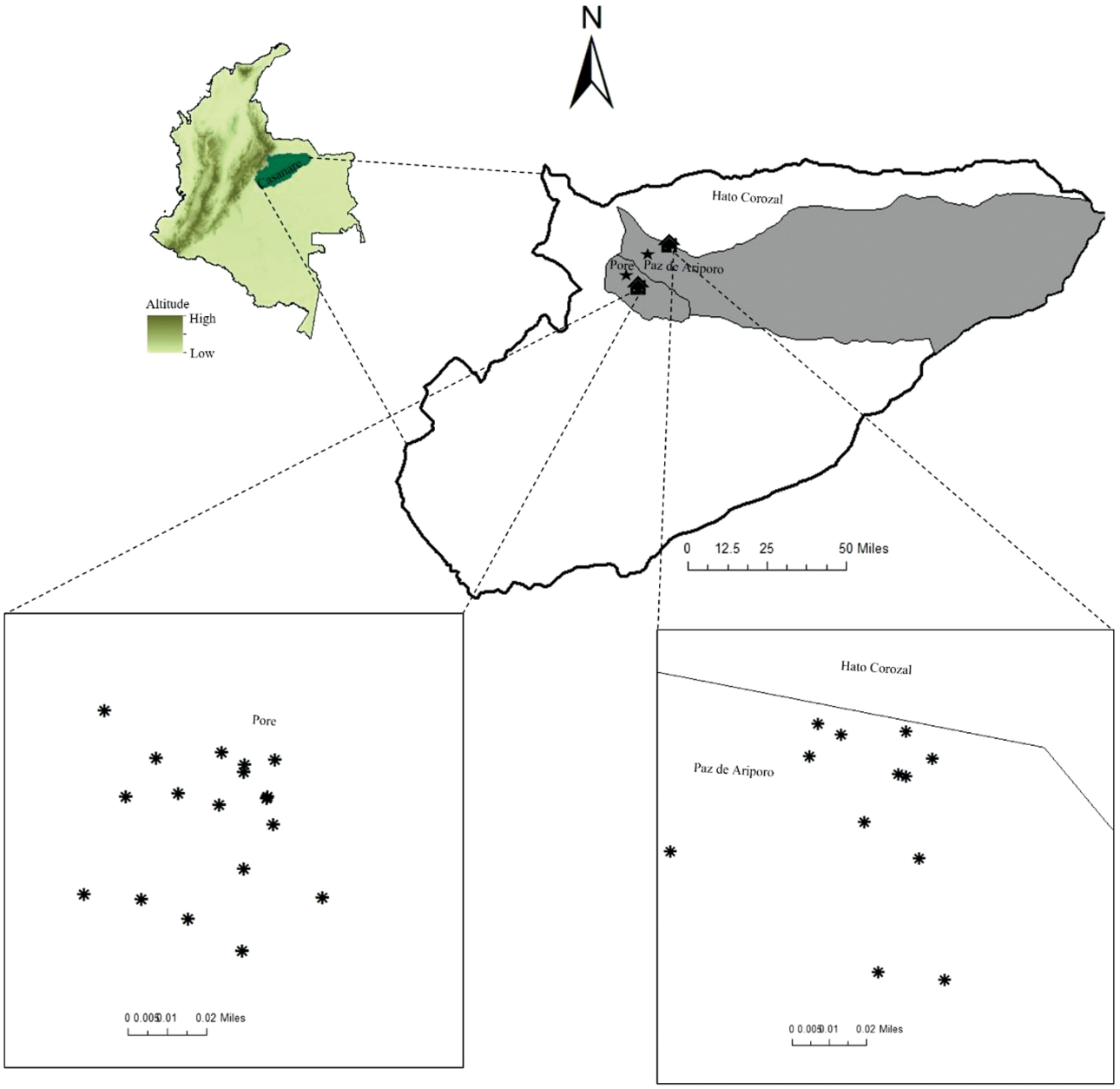

Fig. 1. Geographic location of the villages of Agualinda and Sabanetas in the municipalities of Pore and Paz de Ariporo, respectively (Casanare). Symbols in the form of houses represent the locations of the villages and stars indicate the urban centers of the two municipalities. Asterisks represent houses that were monitored in each municipality.

examined × 100), and colonization (number of houses with nymphs/number of positive houses × 100) were quantified using the methodological parameters proposed by Suarez-Davalos et al. (2010). Statistical analyses were performed using RStudio software and the graphics were made with the Origin 5.0 program.

Detection of *T. cruzi* DNA, Parasite Genotyping, and Determination of Feeding Sources

A total of 42 randomly selected *R. prolixus* were subjected to molecular analyses for detection of *T. cruzi* DNA, parasite genotyping, and identification of feeding sources. All specimens were stored and conserved in ethanol until processing. DNA extraction of the insects' guts was conducted using a Qiagen Dneasy Blood & Tissue kit (Qiagen, Berlin, Germany). Detection of *T. cruzi* was conducted by end-point quantitative polymerase chain reaction (qPCR) using TaqMan Fast Advanced Master Mix 2× (Roche Diagnostics GmbH, Mannheim, Germany), water and the primers cruzi1 (10 µM) (5′-AST CGG CTG ATC GTT TTC-3′), cruzi2 (10 µM) (5′-AAT TCC TCC AAG CAG CGG ATA-3′), and a cruzi3 probe (5 µM) (FAM-CAC ACA CTG GAC ACC AA-NFQ-MGB) to detect the satellite tandem repeat DNA of the parasite (166 bp), following the procedure previously reported (Hernández et al. 2016). A Ct value <38 was considered as positive amplification. For insects with a positive qPCR result, a conventional PCR for kinetoplast DNA amplification was conducted using Buffer Taq 10×, $MgCl_2$ 50 mM, dNTPs 25 mM, Taq Platinum 5 U/µl, water and the primers 121 (50 pmol/µl) (5′-AAA TAA TGT ACG GGK GAG ATG CAT GA-3′) and 122 (50

pmol/µl) (5′-GGT TCG ATT GGG GTT GGT GTA ATA TA-3′) to discriminate between *T. cruzi* (330 bp) and *T. rangeli* (400–450 bp), as reported elsewhere (Ramírez et al. 2009). Parasite genotyping was accomplished by amplification of the spliced leader intergenic region of the miniexon gene (SL-IR), dividing discrete typing units into two groups: TcI (350 bp) and TcII–TcVI (300 bp) as reported elsewhere (Ramírez et al. 2010).

To determine the blood feeding sources, a 215-bp fragment of the 12S gene fragment was amplified using Go Taq Green Master Mix, water and primers L1085 (10 nM) (5′-CCC AAA CTG GGA TTA GAT ACC C-3′) and H1259 (10 nM) (5′-GTT TGC TGA AGA TGG CGG TA-3′), as reported by Dumonteil et al. (2018). PCR products were cleaned using ExoSAP-IT Express PCR Product Cleanup 75001/75002 (Affymetrix, Thermo Fisher Scientific Inc., Waltham, MA) and then submitted to Sanger sequencing. Resulting sequences were edited in MEGA X software and submitted to BLASTn for similarity search.

Results

A total of 2,240 specimens of *R. prolixus* were collected with density variation recorded according to the month sampled. Despite the use of Maria sensors to ensure the maximum collection of insects, these sensors did not collect or detect any specimens. Triatomines were detected only in each of the inspected homes by communitarian surveillance and active-man-hour search. The months, organized from lowest to highest with respect to the numbers of individuals collected, were: 125 in July, 130 in September, 132 in August, 136 in June, 141 in October, 149 in November, 185 in May, 205 in March, 210 in April, 223 in December, 289 in January, and 316 in February.

Presence

The population density of captured *R. prolixus* varied significantly among months (Kruskal–Wallis, $\chi^2 = 51.12$, $P < 0.05$). The highest densities of presence of triatomines into homes were observed in February, January, December, April, and March, and the lowest densities were observed in May, November, October, June, August, September, and July (Dunn test, $P < 0.05$). The density of individuals captured per dwelling was significantly higher in the period of low rainfall than during high rainfall (Dunn test, $P < 0.05$). However, the period of transition from low to high rainfall (April and May) also recorded high densities of individuals in the dwellings (Fig. 2). In the case of temperature, this variable was homogenous across the year with an average temperature of 26.4°C. Therefore, we were not able to find an association between insect's density and temperature.

Statistically significant differences were found among the monthly samples in the densities of males, females, and nymphs (fifth instar) of *R. prolixus* (Kruskal–Wallis, $\chi^2 = 9{,}573.12$, $P < 0.05$). The proportion of females recorded in the inspected houses was higher than that of males in all months. The highest presence densities of females relative to males were recorded in February, January, December, April, and March. Nymphs were reported only in the months of May, July, and September (Fig. 3).

Entomological Indexes

The months of low rainfall had higher density and crowding indexes. The colonization index estimates the number of micro-habitats in which immature stages of development are found with respect to the number of habitats in which an individual is collected. There was evidence of possible colonization only in the months of May, July, and September (Table 1). In contrast, the index of infestation was relatively high throughout the year; although the number of individuals arriving per dwelling fluctuated according to the season, there was continuous presence of individuals throughout the year.

Presence and Precipitation

As the average monthly precipitation levels increased, the number of individuals arriving at the dwellings decreased (Spearman coefficient = –0.6643) indicating greater presence into dwellings during the months of low rainfall ($P = 0.0021$) (Fig. 4). During the 7 mo from May to November with the greatest precipitation, the detection of triatomines was low as evidenced by only 998 specimens (44.5% of the total for the year) collected, while the 5 mo of lower rainfall from December to April recorded 1,243 insects (55.4%). In the case of temperature, detections did not vary significantly.

Detection of *T. cruzi* Infection, Parasite Genotyping, and Determination of Blood Sources

We analyzed 20 *R. prolixus* individuals from Paz de Ariporo. Eight (40%) were found infected with *T. cruzi* and subsequently four were

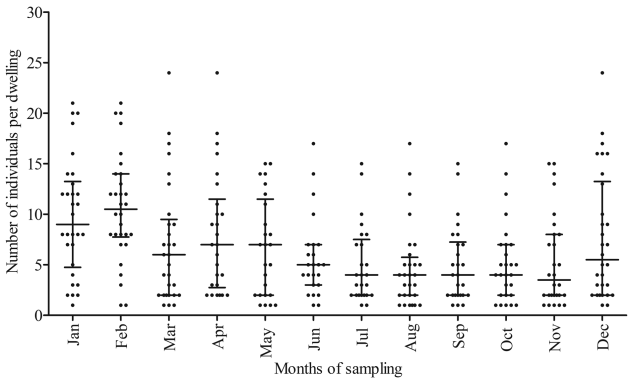

Fig. 2. Numbers of *Rhodnius prolixus* specimens (adults and nymphs) collected per dwelling during the sampling months in the villages of Agualinda (Pore) and Sabaneta (Paz de Ariporo) in the department of Casanare. Error bars are shown during each month of sampling.

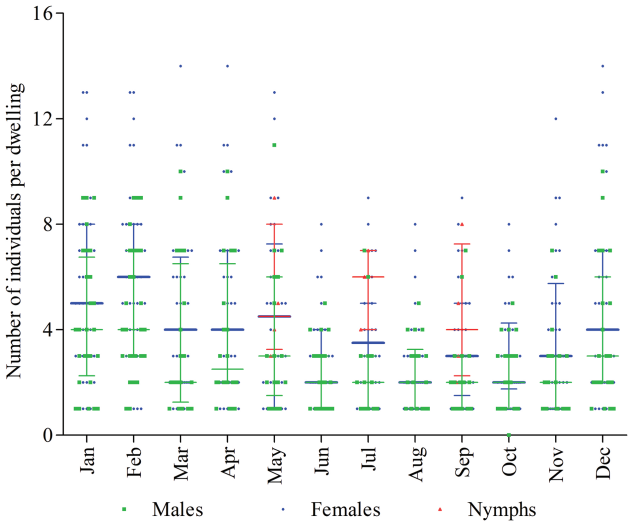

Fig. 3. Numbers of *Rhodnius prolixus* males (Green), females (Blue), and nymphs (red) collected per dwellings of the villages of Agualinda (Pore) and Sabaneta (Paz de Ariporo) in the department of Casanare. Error bars are shown during each month of sampling.

Table 1. Monthly changes of density, crowding indexes, infestation, and colonization indexes of *Rhodnius prolixus* in dwellings in the department of Casanare, during the year 2017

Index	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Density	9.6	10.6	6.8	7.0	6.2	4.5	4.2	4.4	4.3	4.7	5.0	7.4
Crowding	10.3	10.9	7.6	8.1	7.4	5.7	5.0	4.7	5.0	5.2	5.3	8.0
Infestation (%)	93.3	96.7	96.7	86.7	83.3	80.0	83.3	93.3	86.7	90.0	93.3	93.3
Colonization (%)	0.0	0.0	0.0	0.0	16.7	0.0	10.7	0.0	15.4	0.0	0.0	0.0

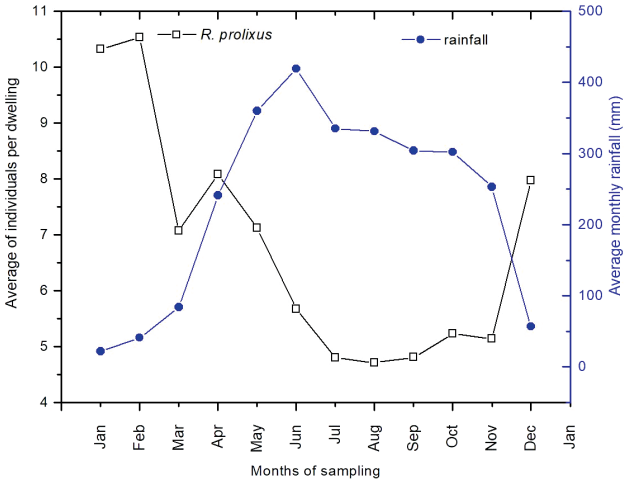

Fig. 4. Changes in the density of *R. prolixus* (adults and nymphs) in rural dwellings in the municipalities of Pore and Paz de Ariporo Casanare, and in the average monthly rainfall reported at the Paz de Ariporo meteorological station (36011501 of 2017).

typed as TcI, and two with mixed infections (TcI/TcII–TcVI). At Pore, we analyzed 22 *R. prolixus* individuals of which 14 (64%) were found infected with *T. cruzi*, of which six were typed as TcI and four as mixed infections (TcI/TcII–TcVI). In terms of blood sources, we identified the following BLAST hits: *Coendou melanurus* (porcupine) (seven hits), *Homo sapiens* (Human) (eight hits), *Alouatta caraya* (Howler monkey) (seven hits), *Ovis candensis* (Sheep) (one hit), *Equus caballus* (Horse) (seven hits), *Sus scrofa* (Pig) (seven hits), *Oryctolagus cuniculus* (Rabbit) (two hits), *Felis catus* (Cat) (five hits), *Rattus norvegicus* (Rat) (one hit), *Monodelphis domestica* (Opposum) (four hits), *Canis lupus familiaris* (Dog) (seven hits), *Didelphis marsupialis* (Opposum) (seven hits), *Mus musculus* (Mouse) (three hits), *Ochotona koslowi* (Kozlov's Pika) (one hit), *Myrmecophaga tridactyla* (Giant anteater) (six hits), *Xenothrix mcgregori* (The Jamaican monkey) (four hits), and *Talpa occidentalis* (Iberian Mole) (two hits). No individuals were positive for *T. rangeli*.

Discussion

Human habitations provide suitable environments for some triatomines to easily establish infestations (Gurgel-Gonçalves et al. 2004). Once triatomines establish in dwellings, or in adjacent locations, they are able to feed on animals and/or people, increasing the transmission risk of *T. cruzi*, which in turn would be reflected in the incidence of Chagas disease and the maintenance of triatomines in the home and peri-domiciliary areas (Rueda et al. 2014, Hernández et al. 2016). This could explain the high indexes of infestation and crowding found in this investigation (Table 1). However, the colonization index found was very low and in only a small proportion of dwellings, which suggests that the species was not establishing populations within the dwellings. Rather, there were continuous events of presence throughout the year (Fig. 2), with an index of home infestation higher than previously recorded for *R. prolixus* in other areas of the department (Angulo et al. 2012, Zuleta et al. 2017), and also higher than reported for other species: *Rhodnius ecuadoriensis* (Lent & León, 1958) in Ecuador (47.4%) (Grijalva et al. 2017) and *Triatoma infestans* (Klug, 1834) in Argentina (2.9–14.4%) (Espinoza et al. 2017, Cavallo et al. 2018). This is consistent with the high prevalences of *T. cruzi* infection (40% in Paz de Ariporo and 64% in Pore), which were higher than those reported for intradomiciliary triatomines in Brazil (1.8–24.7%) (Ferro e Silva et al. 2018), Venezuela (0.06%) (Carrasco et al. 2014), and the Colombian Orinoco (15.78%) (Angulo et al. 2012).

The understanding regarding the feeding sources and *T. cruzi* infection in triatomines found in human dwellings has relevance in terms of transmission dynamics and vector control programs. For example, in Mexico, the feeding sources confirmed a significant dispersal of *T. dimidiata* between habitats (domestic and sylvatic) (Torres-Montero 2012). In addition, this has been reported in *T. brasiliensis* from Brazil where the authors could hypothesize with the mammal feeding source information previous concerns about the potential of several animals to link the sylvatic and domiciliary *T. cruzi* cycles (Almeida et al. 2016). Recent work in the Colombian Orinoco region concluded that *A. butyracea* palms found in altered areas provide a similar quality habitat for *R. prolixus* populations in terms of bloodmeal availability. Both habitats showed similarities in vector infection prevalence and potential host species, representing a single *T. cruzi* transmission scenario at the introduced oil palm plantation and native *Attalea* palm interface (Erazo et al. 2019). These studies augment our findings of 17 feeding sources and high *T. cruzi* infection indicating high risk for Chagas disease transmission in the study area.

Although a high frequency of presence in houses was observed throughout the year (12-mo period of sampling) (Fig. 2), there was no evidence of colonization, because the presence of immature stages was sporadic (Fig. 3, Table 1). This could perhaps be explained by the first to fourth instars not having the capacity to disperse the distance from the palms to the houses. Nevertheless, a study conducted in Venezuela showed the presence of first to fourth instar infesting houses but in the absence of palm surroundings (Feliciangeli et al. 2004). Future studies should consider the sampling of the palms and houses to unveil the true entomological indexes. In addition, this highlights one limitation of our study as *R. prolixus* individuals were removed from the environment each month, reducing the effective sample size of the population. In certain houses, the monthly surveys may have reduced the presence of *R. prolixus* to near zero. Thus the next month survey would have been new individuals that moved in since the previous survey, representing a sample bias. As we are aware of this possible limitation, the collection of new individuals each month supports our hypothesis of strong dispersion of *R. prolixus* into the houses, which poses a challenge for the vector control programs in the region.

In the case of the adults collected, Ayala et al. (2019) recorded the highest number of triatomine presences into houses in the months of April and May, and lower densities in the months of higher rainfall. These observations are consistent with ours, in which we recorded the largest numbers of *R. prolixus* individuals reaching homes in the months of lower average rainfall (Fig. 4), and higher frequency of arrival at homes in the months of April and May (Fig. 2). This reinforces the importance of precipitation in terms of triatomine invasion and entomological indexes. In our case, temperature was not an important factor as this was constant during the year. In the present study, an inverse relationship was noted between the number of individuals per dwelling and the average monthly rainfall (Fig. 4), corroborating earlier studies (Pizarro and Romaña 1998, Longa and Scorza 2005, Vásquez et al. 2013, Esteban et al. 2017, Ayala et al. 2019). These changes represent a response of the species to environmental changes, habitat disturbances, and inter- and intraspecific competition. However, triatomines mitigate exposure of their populations to these adverse conditions by developing adaptive physiological and ethological responses to seasons, which may also be reflected in variations in the incidence of developmental stages (Schowalter 2006). The difference noted in the proportions of nymphs and adults collected in the dwellings could be determined, in the first instance, by forced dispersion from wild and peri-domiciliary ecotopes and, secondly, by physiological adaptations of Triatominae, consisting of variations of nymphal periods, molting and hatching rates of eggs, longevity of adults, and the numbers of instars and periods of starvation (Zeledon and Rabinovich 1981, Arévalo et al. 2007). In addition, *R. prolixus* tends to migrate from its original ecotope in search of food or shelter in some seasons (Noireau and Dujardin 2001), and the immature stages have smaller dispersion distances than adults (Zeledon and Rabinovich 1981). Likewise, other authors have suggested that the density of triatomines captured in the months of low rainfall is significantly higher than during the high rainfall season (Noireau and Dujardin 2001, Hernández et al. 2010, Vásquez et al. 2013, Reyes et al. 2017), possibly because the dispersion of triatomines is related to their nutritional status during the dry season. When their wild food sources are reduced, adult triatomines may migrate toward artificial ecotopes in search of alternatives sources of blood. This is consistent with our observations of an inverse relationship between the density of triatomines that reach dwellings and the average monthly precipitation in the study area, giving rise to a higher density of triatomines in dwellings in the dry season (Fig. 4). Similarly, higher population densities were found in wild populations of *R. prolixus* (Urbano et al. 2018), *R. neglectus*, and *R. robustus* during periods of low rainfall (Gurgel-Gonçalves et al. 2004, Longa and Scorza 2005).

Rhodnius prolixus is a species with a wide climatic and altitudinal range. It is frequently found in houses in localities where the opportunity to colonize domestic structures is present (Noireau et al. 1994, Esteban et al. 2017). However, according to Esteban et al. (2017), adaptation of this vector to ecotopes such as palms means that the presence of immature stages in dwellings could result from displacement from nearby wild habitats rather than a colonization event. The presence of the species in homes has been associated with decrease in vegetation coverage, the distance to the forest, and lights from houses at night (Angulo et al. 2012, Erazo and Cordovez 2016). This could explain the low colonization frequency, which was observed in only three months (Table 1). These results differed from those found for *R. ecuadoriensis* and *Panstrongylus rufotuberculatus* (Latreille, 1811), which exhibited rates of intradomiciliary colonization up to 77% (Grijalva et al. 2017). Interestingly, the average densities of males, females, and nymphs within dwellings showed the opposite trend to those reported by Esteban et al. (2017) for the department of Santander (eastern Colombia), where a greater proportion of males were reported to reach homes. A significant difference between collections of females and males (Fig. 3) was detected, suggesting the composition of populations that enter dwellings is variable due to previous reports that demonstrate that the male/female ratio in sylvatic populations is similar (Grijalva et al. 2012, Urbano et al. 2015).

The high dispersal capacity of *R. prolixus* is linked to a high adaptability to invade and colonize different micro-habitats in particular areas (Heger et al. 2006, Feliciangeli et al. 2007). This was evidenced in the present investigation by the observation that 100% of the homes inspected contained triatomines in at least 1 mo, and that the index of infestation throughout the hydrological period was relatively constant (Table 1). Conversely, since 100% of the houses contained triatomines, the presence of the triatomines may also have been the result of propagation within the houses. *Rhodnius prolixus* was recorded in houses of the municipalities of both Pore and Paz de Ariporo, suggesting that the wide geographical distribution of this species in Casanare results from its successful colonization of many species of palm and types of housing. Dispersion of *R. prolixus* may occur toward anthropogenic environments where some vertebrate refuges are present or relict palm species occur (Urbano et al. 2015); later they behave as intruders in homes in search of food. These factors have been evaluated for several species of triatomines in other countries (Fitzpatrick et al. 2008, Grijalva et al. 2014, Ferro e Silva et al. 2018). An understanding of the mammals that inhabit the palm forests is important to unveil the blood sources for the triatomines. However, we do not have information about the diversity of animals in these palms and therefore cannot formulate a hypothesis in this regard. The only available information was the high diversity of blood sources demonstrating that despite having food available, *R. prolixus* is attracted to human dwellings, possibly due to light attraction (Jácome-Pinilla et al. 2015).

Finally, control strategies for vector transmission of Chagas disease in the department of Casanare have been directed towards housing interventions with insecticides (Palomino et al. 2007, Silva et al. 2007, Rendón et al. 2015, Zuleta et al. 2017). However, our data show that the months in which the probability of dispersion and presence of insects into houses is greatest corresponds to the low rainfall period. It is important to keep this in mind in the implementation of control strategies and epidemiological surveillance in the department.

Conclusion

The high values of the indices and trends in the presence frequencies of triatomines in rural dwellings in the department of Casanare indicate a high risk of *T. cruzi* vectorial transmission in the study areas. However, the low colonization indexes recorded for *R. prolixus* indicate that this species is unlikely to exhibit domiciliation. This does not rule out this process in the future, given that the largest proportion of individuals entering the houses in an intrusive or sporadic manner were females.

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Competing Interests

The authors assert no conflict of interest. All authors have read and approved the manuscript and its analyses.

References Cited

Abad-Franch, F., F. S. Palomeque, H. M. Aguilar, and M. A. Miles. 2005. Field ecology of sylvatic *Rhodnius* populations (Heteroptera, Triatominae): risk factors for palm tree infestation in western Ecuador. Trop. Med. Int. Health. 10: 1258–1266.

Almeida, C. E., L. Faucher, M. Lavina, J. Costa, and M. Harry. 2016. Molecular individual-based approach on *Triatoma brasiliensis*: inferences on Triatomine Foci, *Trypanosoma cruzi* natural infection prevalence, parasite diversity and feeding sources. Plos Negl. Trop. Dis. 10: e0004447.

Angulo, V. M., and L. Esteban. 2011. Nueva trampa para la captura de triatominos en hábitats silvestres y peridomésticos. Biomedica. 31: 264–268.

Angulo, V. M., L. Esteban, and K. Luna. 2012. Attalea butyracea próximas a las viviendas como posible fuente de infestación domiciliaria por *Rhodnius prolixus* (Hemiptera: Reduviidae) en los Llanos Orientales de Colombia. Biomedica. 32: 277–285.

Angulo, V. M., L. Esteban, P. Urbano, E. Hincapié, and L. Nuñez. 2013. Comparación de métodos para la captura de triatominos (Hemiptera: Reduviidae) en palmas *Attalea butyracea* en los Llanos Orientales de Colombia. Biomedica. 33: 653–659.

Arévalo, A., J. C. Carranza, F. Guhl, J. A. Clavijo, and G. A. Vallejo. 2007. Comparación del ciclo de vida de *Rhodnius colombiensis* Moreno, Jurberg & Galvão (1999) y *Rhodnius prolixus* Stal (1872) (Hemiptera, Reduviidae, Triatominae) en condiciones de laboratorio. Biomédica. Supl. 27: 119–129.

Ayala, C. J., C. M. Hernández, M. Eyes, L. R. Romero, R. A. Álvarez, and P. Blanco. 2019. Detección de infección natural por *Trypanosoma cruzi* (Trypanosomatidae) en triatominos del municipio de Colosó, Colombia. Acta biol. Colomb. 24: 180–184.

Becerril, M. A., V. A. Perez, J. Garcia, and J. Palafox. 2010. Riesgo de transmisión de *Trypanosoma cruzi* en el Municipio de Metztitlán, Estado de Hidalgo, México, Mediante la Caracterización de Unidades Domiciliares y sus Índices Entomológicos. Neotropical Entomology. 39: 810–817.

Bejarano, G. E. 2012. Plan de desarrollo Municipal: "ciudad de orden y progreso". Alcaldía municipal-Paz de Ariporo. (https://vdocuments.pub/informe-gestion-2012-2015-paz-de-ariporo-ciudad-de-orden-y-progreso.html).

Canale, B., J. Jurberg, R. Carcavallo, C. Galvao, G. I. Galindez, M. Segura, S. Rocha, and A. Martinez. 1999. Bionomics of some species, pp. 839–890. *In* R. U. Carcavallo, I. G. Giron, J. Jurberg, H. Lent. Atlas of Chagas disease vectors in the Americas. Editorial Fiocruz, Rio de Janeiro, Brasil.

Carrasco, H. J., M. Segovia, J. C. Londoño, J. Ortegoza, M. Rodríguez, and C. E. Martínez. 2014. *Panstrongylus geniculatus* and four other species of triatomine bug involved in the *Trypanosoma cruzi* enzootic cycle: high risk factors for Chagas' disease transmission in the Metropolitan District of Caracas, Venezuela. Parasit. Vectors. 7: 602.

Cavallo, M. J., I. Amelotti, L. Abrahan, G. Cueto, and D. E. Gorla. 2018. Rural houses infestation by *Triatoma infestans* in northwestern Argentina: vector control in a high spatial heterogeneous infestation area. Plos One. 13: e0201391.

Cortés, L. A., and H. A. Suárez. 2005. Triatominos (Reduviidae: Triatominae) en un foco de enfermedad de Chagas en Talaigua Nuevo (Bolívar, Colombia). Biomédica. 25: 568–574.

Dias, J. C., A. C. Silveira, and C. J. Schofield. 2002. The impact of Chagas disease control in Latin America: a review. Mem. Inst. Oswaldo Cruz. 97: 603–612.

Dias-Lima, A. G., and I. A. Sherlock. 2000. Sylvatic vectors invading houses and the risk of emergence of cases of Chagas disease in Salvador, State of Bahia, Northeast Brazil. Mem. Inst. Oswaldo Cruz. 95: 611–613.

Erazo, D., and J. Cordovez. 2016. The role of light in Chagas disease infection risk in Colombia. Parasit. Vectors. 9: 9.

Erazo, D., N. L. Gottdenker, C. González, F. Guhl, M. Cuellar, T. J. Kieran, T. C. Glenn, J. D. Umaña, and J. Cordovez. 2019. Generalist host species drive *Trypanosoma cruzi* vector infection in oil palm plantations in the Orinoco region, Colombia. Parasit. Vectors. 12: 274.

Espinoza, J. E., A. Nogales-Rodriguez, M. Rojas-Cortez, L. Gonçalves-Diotaiuti, and D. E. Gorla. 2017. Spatial and temporal distribution of house infestation by *Triatoma infestans* in the Toro Toro municipality, Potosi, Bolivia. Parasit. Vectors 10: 58–64.

Esteban, L., J. M. Montes, and V. M. Angulo. 2017. Diversity of Triatominae (Hemiptera: Reduviidae) in Santander, Colombia: epidemiological implications. Biomedica. 37: 42–52.

Feliciangeli, M. D., H. Carrasco, J. S. Patterson, B. Suarez, C. Martínez, and M. Medina. 2004. Mixed domestic infestation by *Rhodnius prolixus* Stal, 1859 and *Panstrongylus geniculatus* Latreille, 1811, vector incrimination, and seroprevalence for *Trypanosoma cruzi* among inhabitants in El Guamito, Lara State, Venezuela. Am. J. Trop. Med. Hyg. 71: 501–505.

Feliciangeli, M. D., M. Sanchez-Martin, R. Marrero, C. Davies, and J. P. Dujardin. 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). Acta Trop. 101: 169–177.

Ferro e Silva, A. M., T. Sobral-Souza, M. H. Vancine, R. L. Muylaert, A. P. De Abreu, S. M. Pelloso, M. D. De Barros-Carvalho, L. De Andrade, M. C. Ribeiro, and M. J. Ornelas-Toledo. 2018. Spatial prediction of risk areas for vector transmission of *Trypanosoma cruzi* in the State of Paraná, southern Brazil. PLoS Negl Trop Dis 12: e0006907.

Fitzpatrick, S., M. D. Feliciangeli, M. J. Sanchez-Martin, F. A. Monteiro, and M. A. Miles. 2008. Molecular genetics reveal that silvatic *Rhodnius prolixus* do colonise rural houses. Plos Negl. Trop. Dis. 2: e210.

Grijalva, M. J., V. Suarez-Davalos, A. G. Villacis, S. Ocaña-Mayorga, and O. Dangles. 2012. Ecological factors related to the widespread distribution of sylvatic *Rhodnius ecuadoriensis* populations in southern Ecuador. Parasit. Vectors. 5: 17.

Grijalva, M. J., D. Terán, and O. Dangles. 2014. Dynamics of sylvatic Chagas disease vectors in coastal Ecuador is driven by changes in land cover. Plos Negl. Trop. Dis. 8: e2960.

Grijalva, M. J., A. G. Villacís, A. L. Moncayo, S. Ocaña-Mayorga, C. A. Yumiseva, and E. G. Baus. 2017. Distribution of triatomine species in domestic and peridomestic environments in central coastal Ecuador. Plos Negl. Trop. Dis. 11: e0005970.

Guhl, F., G. Aguilera, N. Pinto, and D. Vergara. 2007. Updated geographical distribution and ecoepidemiology of the triatomine fauna (Reduviidae: Triatominae) in Colombia. Biomedica. 27 (Suppl 1): 143–162.

Guhl, F., and J. D. Ramírez. 2013. Retrospective molecular integrated epidemiology of Chagas disease in Colombia. Infect. Genet. Evol. 20: 148–154.

Gurgel-Gonçalves, R., M. A. Duarte, E. D. Ramalho, A. R. T. Palma, C. A. Romaña, and C. A. Cuba-Cuba. 2004. [Spatial distribution of Triatominae populations (Hemiptera: Reduviidae) in *Mauritia flexuosa* palm trees in Federal District of Brazil]. Rev. Soc. Bras. Med. Trop. 37: 241–247.

Heger, T., P. Guerin, and W. Eugster. 2006. Microclimatic factors influencing refugium suitability for *Rhodnius prolixus*. Physiol. Entomol. 31: 248–256.

Hernández, J. L., E. A. Rebollar-Téllez, F. Infante, A. Morón, and A. Castillo. 2010. Indicators of infestation, colonization and infection of *Triatoma dimidiata* (Latreille) (Hemiptera: Reduviidae) in Campeche, México. Neotrop. Entomol. 39: 1024–1031.

Hernández, C., C. Salazar, H. Brochero, A. Teherán, L. S. Buitrago, M. Vera, H. Soto, Z. Florez-Rivadeneira, S. Ardila, G. Parra-Henao, et al. 2016. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. Parasit. Vectors. 9: 620.

Hoyos, R., L. Pacheco, L. A. Agudelo, G. Zafra, P. Blanco, and O. Triana. 2007. Seroprevalence of Chagas disease and associated risk factors in a population of Morroa, Sucre. Biomedica. 27 (Suppl 1): 130–136.

Jácome-Pinilla, D., E. Hincapie-Peñaloza, M. Ortiz, J. D. Ramírez, F. Guhl, and J. Molina. 2015. Risks associated with dispersive nocturnal flights

of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit. Vectors. 8: 2–11.

Justi, S. A., and C. Galvão. 2017. The evolutionary origin of diversity in Chagas disease vectors. Trends Parasitol. 33: 42–52.

Köppen, W. 1918. Klassification der Klimate nach Temperatur, Niederschlag and Jahreslauf". Petermanns Geographische Mitteilungen. 64: 193–203.

Lent, H., and P. Wygodzinsky. 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. Bull. Am. Mus. Nat. Hist. 163: 123–520.

Llano, M., P. Pavía, A. C. Flórez, A. Cuéllar, J. M. González, and C. Puerta. 2014. Preliminary evaluation of the commercial kit Chagas (*Trypanosoma cruzi*) IgG-ELISA ® in Colombian individuals. Biomedica. 34: 228–236.

Longa, A., and J. Scorza. 2005. *Acrocomia aculeata* (Palmae), hábitat silvestre de *Rhodnius robustus* en el Estado Trujillo, Venezuela. Parasitología Latinoamericana. 60: 17–24.

Montilla, M., H. Soto, E. Parra, M. Torres, P. Carrillo, L. Lugo, J. Colorado, and M. T. Arias. 2011. Infestation by triatomine bugs in indigenous communities of Valledupar, Colombia. Rev. Saude Publica. 45: 773–780.

Noireau, F., and J. P. Dujardin. 2001. Flight and nutritional status of sylvatic *Triatoma sordida* and *Triatoma guasayana*. Mem. Inst. Oswaldo Cruz. 96: 385–389.

Noireau, F., M. F. Bosseno, F. Vargas, and S. F. Breniere. 1994. Apparent trend to domesticity observed in *Panstrongyus rufotuberculatus* Champion, 1899 (Hemiptera: Reduviidae) in Bolivia. Res Rev Parasitol. 54: 263–264.

Palomino, M., W. León, P. Valencia, F. Cárdenas, and J. Ancca. 2007. Evaluación de campo del efecto residual de la deltametrina sobre la mortalidad y knockdown en triatoma infestans, según tipo de supeficie en Arequipa, Perú. Rev Peru Med Exp Salud Publica. 24: 136–43.

Parra-Henao, G., V. Angulo, N. Jaramillo, and M. Restrepo. 2009. Triatominae (Hemiptera: Reduviidae) in the Sierra Nevada of Santa Marta, Colombia: epidemiological, entomological and distributional aspects. CES Med. 23: 17–26.

Parra-Henao, G. J., M. Flórez, and V. M. Angulo. 2015. Vigilancia de triatominae (Hemiptera: Reduviidae) en Colombia. 1ª edición, pp. 1–127. Editorial Sic Ltda., Bogotá, D.C.

Pizarro, J. C., and C. Romaña. 1998. Variación estacional de una población silvestre de *Rhodnius pallescens* Barber 1932 (Heteroptera: Triatominae) en la costa Caribe colombiana. Boletin del Instituto Francés de Estudios Andinos. 27: 309–325.

Ramírez, J. D., F. Guhl, E. S. Umezawa, C. A. Morillo, F. Rosas, J. A. Marin-Neto, and S. Restrepo. 2009. Evaluation of adult chronic Chagas' heart disease diagnosis by molecular and serological methods. J. Clin. Microbiol. 47: 3945–3951.

Ramírez, J. D., F. Guhl, L. M. Rendón, F. Rosas, J. A. Marin-Neto, and C. A. Morillo. 2010. Chagas cardiomyopathy manifestations and *Trypanosoma cruzi* genotypes circulating in chronic Chagasic patients. Plos Negl. Trop. Dis. 4: e899.

Rendón, L. M., F. Guhl, J. M. Cordovez, and D. Erazo. 2015. New scenarios of *Trypanosoma cruzi* transmission in the Orinoco region of Colombia. Mem. Inst. Oswaldo Cruz. 110: 283–288.

Reyes, M., Á. Torres, L. Esteban, M. Flórez, and V. M. Angulo. 2017. Risk of transmission of Chagas disease by intrusion of triatomines and wild mammals in Bucaramanga, Santander, Colombia. Biomedica. 37: 68–78.

Rueda, K., J. E. Trujillo, J. C. Carranza, and G. A. Vallejo. 2014. Transmisión oral de *Trypanosoma cruzi*: una nueva situación epidemiológica de la enfermedad de Chagas en Colombia y otros países suramericanos. Biomédica. 34: 631–641.

Schowalter, T. D. 2006. Insect ecology. An ecosystem approach, 2nd ed. 153–176. Academic Press, London, UK.

Silva, N., M. Pachón, B. Ortegón, C. Wilches, H. Tarquino, F. Guhl, N. Pinto, J. Naranjo, L. Castro, and C. Pérez. 2007. Programa de control de la enfermedad de Chagas en viviendas rurales de los municipios de Hato Corozal, Nunchía, Paz de Ariporo y Támara (Casanare). Biomédica. 27: 127. 23.

Suarez-Davalos, V., O. Dangles, A. G. Villacis, and M. J. Grijalva. 2010. Microdistribution of sylvatic triatomine populations in central-coastal Ecuador. J. Med. Entomol. 47: 80–88.

Teixeira, A. R., P. S. Monteiro, J. M. Rebelo, E. R. Argañaraz, D. Vieira, L. Lauria-Pires, R. Nascimento, C. A. Vexenat, A. R. Silva, S. K. Ault, et al. 2001. Emerging Chagas disease: trophic network and cycle of transmission of *Trypanosoma cruzi* from palm trees in the Amazon. Emerg. Infect. Dis. 7: 100–112.

Torres-Montero, J., López-Monteon, A., Dumonteil, E., Ramos-Ligonio, A. 2012. House infestation dynamics and feeding sources of *Triatoma dimidiata* in central Veracruz Mexico. Am. J. Trop. Med. Hyg. 86: 677–682

Urbano, P., C. Poveda, and J. Molina. 2015. Effect of the physiognomy of *Attalea butyracea* (Arecoideae) on population density and age distribution of *Rhodnius prolixus* (Triatominae). Parasit. Vectors 8: 199.

Urbano, P., E. Hincapié, V. M. Angulo, L. Esteban, and A. Núñez-Avellaneda. 2018. Variación poblacional de *Rhodnius prolixus* (Reduviidae: Triatominae) en *Attalea butyracea* (Arecaceae) en la Orinoquia colombiana. Revista Colombiana de Entomología. 44: 211–216.

Vásquez, C., S. Robledo, J. Calle, and O. Triana. 2013. Identificación de nuevos escenarios epidemiológicos para la enfermedad de Chagas en la región momposina, norte de Colombia. Biomédica. 33: 526–537.

Velásques-Ortiz, N., L. C. Hernández, G. Herrera, L. Cruz-Saavedra, A. Higuera, L. Arias-Giraldo, P. Urbano, A. Cuervo, A. Teherán, and J. D. Ramírez. 2019. *Trypanosoma cruzi* infection, discrete typing units and feeding sources among *Psammolestes arthuri* (Reduviidae: Triatominae) collected in eastern Colombia. Parasit. Vectors 12: 1–157.

Wisnivesky-Colli, C., N. J. Schweigmann, A. Alberti, S. M. Pietrokovsky, O. Conti, S. Montoya, A. Riarte, and C. Rivas. 1992. Sylvatic American trypanosomiasis in Argentina. *Trypanosoma cruzi* infection in mammals from the Chaco forest in Santiago del Estero. Transacciones de la real sociedad de Medicina Tropical e Higiene. 86: 38–41.

Zeledon, R., and J. E. Rabinovich. 1981. Chagas disease: and ecological appraisal with special emphasis on its insect vectors. Annu. Rev. Entomol. 26, 101–133.

Zuleta, L. P., Á. J. Lopez, F. Torrez, and O. Castañeda. 2017. Posible transmisión oral de la enfermedad de Chagas en trabajadores del sector de los hidrocarburos en Casanare, Colombia, 2014. Biomédica. 37: 218–232.

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Discrete typing units of *Trypanosoma cruzi*: Geographical and biological distribution in the Americas

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Chagas disease caused by *Trypanosoma cruzi* is a public health issue in Latin America. This highly diverse parasite is divided into at least seven discrete typing units (DTUs) TcI-TcVI and Tcbat. Some DTUs have been associated with geographical distribution in epidemiological scenarios and clinical manifestations, but these aspects remain poorly understood. Many studies have focused on studying the parasite and its vectors/hosts, using a wide variety of genetic markers and methods. Here, we performed a systematic review of the literature for the last 20 years to present an update of DTUs distribution in the Americas, collecting ecoepidemiological information. We found that the DTUs are widespread across the continent and that there is a whole gamma of genetic markers used for the identification and genotyping of the parasite. The data obtained in this descriptor could improve the molecular epidemiology studies of Chagas disease in endemic regions.

Background & Summary

Chagas disease (CD) is a neglected tropical disease considered a public health concern in Latin America[1]. World Health Organization reports that between 15 and 17 million people get infected, and around 50.000 die out of 100 million people at risk of infection[1–3]. CD is caused by the protozoan parasite *Trypanosoma cruzi*, which is transmitted by kissing bugs, members of the subfamily Triatominae, through their faeces, where the infective forms of the parasite are present[4]. *T. cruzi* is divided into at least seven discrete typing units (DTUs) from TcI to TcVI and Tcbat[5,6]. TcI presents an extensive genetic diversity and is divided according to the transmission cycle in domestic (TcI_{Dom}) and sylvatic (TcI_{Sylv}) genotypes[7]. The DTUs are commonly associated with epidemiological and ecological scenarios, but no actual associations have been found. Also, some DTUs are related to oral outbreaks in Brazil, Colombia, Venezuela, Bolivia, and French Guiana (TcI, TcV, TcIII, TcIV)[8]. This transmission type makes CD one of the most important foodborne diseases, but the genotypes, epidemiology, and clinical traits remain poorly understood because each geographical zone presents its epidemiological characteristics[8].

Through the years, many genetic markers and methods have been used to identify, and genotype *T. cruzi* in the lack of a consensus regarding the two aspects previously mentioned, considering that a single genetic marker is not enough to solve the issues of the parasites classification[6]. Even with all the new technologies recently developed, some researchers still choose old-established but more widely used techniques for their investigations, such as band size PCR or RFLP[9–11]. Moreover, considering the vast diversity of the parasite's DTUs and hosts[6,12–15], one could imagine the amount of different genetic markers used through time for identification: Spliced-leader intergenic region (SL-IR), microsatellites, kinetoplast DNA (kDNA), heat shock proteins (HSP), 18 S ribosomal RNA subunit (18 S rRNA), cytochrome c oxidase subunit 2 (COII), cytochrome b (Cytb), Glycosylphosphatidylinositol (GPI) and 24Sα rDNA/rDNA subunits (24Sα), to name a few[16–27]. This is a problem for the nomenclature used to classify the DTUs, especially for TcI genotypes, leading to a discussion due to biases of some markers that can be more accurate than others for *T. cruzi* classification[7]. Nevertheless,

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this debate continues after 12 years that this nomenclature was established. Even with the plethora of studies unveiling the genomic architecture and plasticity of *T. cruzi*[26].

Some studies describe the geographical distribution of *T. cruzi*'s DTUs to identify epidemiological associations among the genotypes[28,29]. Others focus on the parasite dynamics by performing phylogeographic studies to understand their evolution and the risk of infection to humans[30], but the last update of DTUs distribution and epidemiology at a continental level was published in 2016 by Izeta-Alberdi and colleagues[30]. Since then, many researchers have studied the parasite and its vectors and hosts using new methodologies. Hence, here we present an update of DTU's distribution in the Americas, its ecoepidemiological information such as the transmission cycle, hosts, vectors, and the methods and genetic markers used for their identification and genotyping. To accomplish this, we made a systematic review of the literature available on those above using the PubMed database, hoping this can provide insights that lead to the standardization for DTU's identification to improve future research regarding molecular epidemiology of CD. We published a similar review in 2020, where a database and an interactive map were built and used as a reference for the surveillance of *Leishmania* in the Americas[31]. Therefore, we encourage the scientific community to keep studying the molecular epidemiology of *T. cruzi* for accurate management and surveillance of CD in endemic regions.

Methods

Systematic review. For the construction of this metadata, two researchers independently selected the articles following the same instructions as described in the **Information about the databases used as sources** section below; then, a third investigator made another revision to avoid any discrepancy between the results, followed by a three-step debugging process. We extracted the following information from each article: Original code, Sample type, DTU, TcI DTU/genotype, Coordinates (sexagesimal degrees system), Latitude and longitude (decimal degrees system), Country, Continental division, Upper-division (state/province/department/region), Belong to Amazon basin (yes/no), Lower division (department/municipality/community), Local division (municipality/community/village), Date of isolation, Year of isolation/detection, Species of the host, Common name, Source sample, Order of the host, Tribe (only Triatominae), Genus of the host, Cycle (transmission cycle), Genetic marker (for genotyping), Method of identification (of the parasite) and Genes examined. The articles with no complete/clear information regarding sample collection, hosts/vector species, and methods were excluded from the database. Some coordinates were obtained manually using the web page https://www.gps-coordinates.net if the article specified the place-name where the samples were collected. The coordinate system used was WGS84. For the DTUs distribution, we used the software QGIS 3.16 Hannover (https://www.qgis.org/es/site/) to create and edit the maps, and we used the figures from the software R version 3.6.3 with the library "ggplot2".

Inclusion and exclusion criteria. Herein, we considered those articles with clinical (Identification method, sample type, and species identified) and complete geographical information. Three languages were considered (Spanish, English, and Portuguese). Information was searched for in the abstract and full article. We excluded articles without the full (.pdf) version or with incomplete information, such as coordinates, source of the sample, vector/hosts from where the parasite was recovered, or reported techniques that did not fulfil the correct identification of the parasite.

Information about the databases used as sources. For the database construction, we did a PubMed Advanced Search and employed an algorithm using the words "DTU" and "Trypanosoma cruzi" with the Boolean "AND". The search was done without establishing a time frame. We downloaded the result file and performed a manual depuration to discard articles unrelated to our interests (*i.e.*, pharmacological studies, including another trypanosomatids such as *Leishmania spp*, studies related to another hemipteran species). After reading and refining the articles implementing the previously mentioned criteria, we constructed a database by country to debug. Then, those articles were collected in a metadata database. Furthermore, three more independent debugging processes were carried out to check if the articles comply with the required parameters. Finally, a standardization process of the database fields was performed to verify that their content was all in the same format.

Database fields information. *Original code.* Refers to the code of the samples assigned by the authors of each article.

Sample type. This refers to the type of sample from where the parasite was isolated. We considered the following categories: a) Blood, b) Complete Insect, c) Faeces, d) Food, e) Gut, f) Heart, g) Rectal Ampoule, h) Serum, i) Strain, j) Tissues and k) Xenodiagnoses.

DTU. This refers to *Trypanosoma cruzi*'s DTU per sample. The categories used were: a) TcI, b) TcII, c) TcIII, d) TcIV, e) TcV, f) TcVI, g) Tcbat, h) TcII or TcV, i)TcII or TcVI, j) TcII to TcVI, k) TcIII or TcIV, l) TcIII to TcVI, m) TcIV or TcVI, n) TcIV to TcVI, o) Unknown).

TcI Genotype. Refers to TcI genotyping. They were categorized as follows: a) Sylv (sylvatic), b) Dom (domestic), c) TcIDom/TcISylv and d) Unknown.

Source sample. Refers to the organism from where the sample was isolated. We considered the following categories: a) Food, b) Humans, c) Reservoir (non-human animals), and d) Vector.

Sample origin	Number of studies
Argentina	31
Belize	1
Bolivia	42
Brazil	81
Chile	31
Colombia	52
Costa Rica	3
Ecuador	11
El Salvador	4
French Guiana	5
Guatemala	6
Honduras	5
Mexico	21
Nicaragua	1
Panama	6
Paraguay	16
Peru	14
Surinam	1
USA	20
Uruguay	2
Venezuela	19
Spain*	1
	373

Table 1. Summary of the number of studies per sample origin. *Samples from patients born in Spain, children of Bolivian immigrants.

Species. Regarding the species of the host, we divided the database into a) species of the host (complete scientific name), b) common name, c) order of the host, d) tribe (only for Triatominae), e) genus of the host (only Genus) and f) cycle (refers to the transmission cycle of the host: Domestic/Sylvatic/Peridomestic/NA (No data)).

Genetic marker. Refers to the nature of the marker: Nuclear, Mitochondrial, Antigen or NA (no data).

Method of identification. For optimization, we categorized the tests/methods/techniques as follows: a) Blotting, b) Electrophoretic, c) PCR-based, d) Real-time PCR, e) Sequencing and f) Serologic. Each category includes subcategories described in Table 2.

Genes examined. Refers to the genes used in each study for the parasite identification and genotyping (Supplementary Figure 2).

Geographical location. We have nine categories in the database: a) Coordinates (in the sexagesimal degree system of coordinates), b) Latitude, c) Longitude, d) Country (where the samples were collected), e) Continental division (South or North America), f) Upper-division (state/province/department/region), g) Belong to the Amazon basin (if the division is in the Amazon basin), h) Lower division (department/province/municipality/community) and i) Local division (municipality/community/village).

Dates. Refers to a) Date isolation (Date of the sample collection) and b) Year isolation/detection (Year in which the parasite was detected).

Data Records

The metadata files are available as a tab file on Universidad del Rosario repository[32].

We found a total of 373 articles (data published between 1980 and 2020) from 21 countries in the Americas and two samples from Spain that register the identification of *T. cruzi* DTUs in different hosts and/or vectors (Table 1). Of these, 63.5% of the studies contained Brazil, Colombia, Bolivia, Chile, and Argentina (Table 1). We found a wide distribution of DTUs registered in the continent (Fig. 1a). We also made a distribution map for each DTU where it can be observed that all DTUs are present broadly, especially in South America (Fig. 1). In some studies, DTUs could not be differentiated. Therefore, we opted to put them in a separate category (Fig. 1c,d,e,f,g). Also, it can be noticed that mixed infections between TcI_{Dom} and TcI_{Sylv} were only reported in some countries in the north of South America (Fig. 1b, red points). Moreover, we made an additional map for those categories that comprise a range of DTUs and those that cannot be determined in the studies (Supplementary Figure 4). Finally, in Supplementary Figure 3, there is a distribution map for Tcbat, registered predominantly in Colombia and Brazil (In light of lack of consensus for defining it as a new DTU).

Category	Method	Number of samples
Blotting	Western Blot	137
	Southern Blot	226
	TOTAL	363
Electrophoretic	PCR-RFLP	1658
	MLEE	1564
	Size polymorphism of cruzipain CHEF	1
	TOTAL	3223
PCR-based	LSSP-PCR	160
	PCR band size	6181
	RAPD	1395
	Multilocus conventional PCR	154
	Nested PCR	113
	Multiplex PCR	58
	Heminested PCR	76
	PCR-DNA hybridization	1423
	RT-PCR	5
	TOTAL	9565
Real-time PCR	qPCR	375
	Multiplex real-time PCR	79
	Duplex TaqMan qPCR	22
	TOTAL	476
Sequencing	FFLB	47
	MLMT	922
	MLST	657
	Sequencing	1480
	TOTAL	3106
Serologic	ELISA	187
	IFAT	29
	Chagas Sero K-SeT RDT + TSSApep-II/V/VI	65
	TOTAL	281

Table 2. Summary of the different methods used for the identification and genotyping of *T. cruzi*.

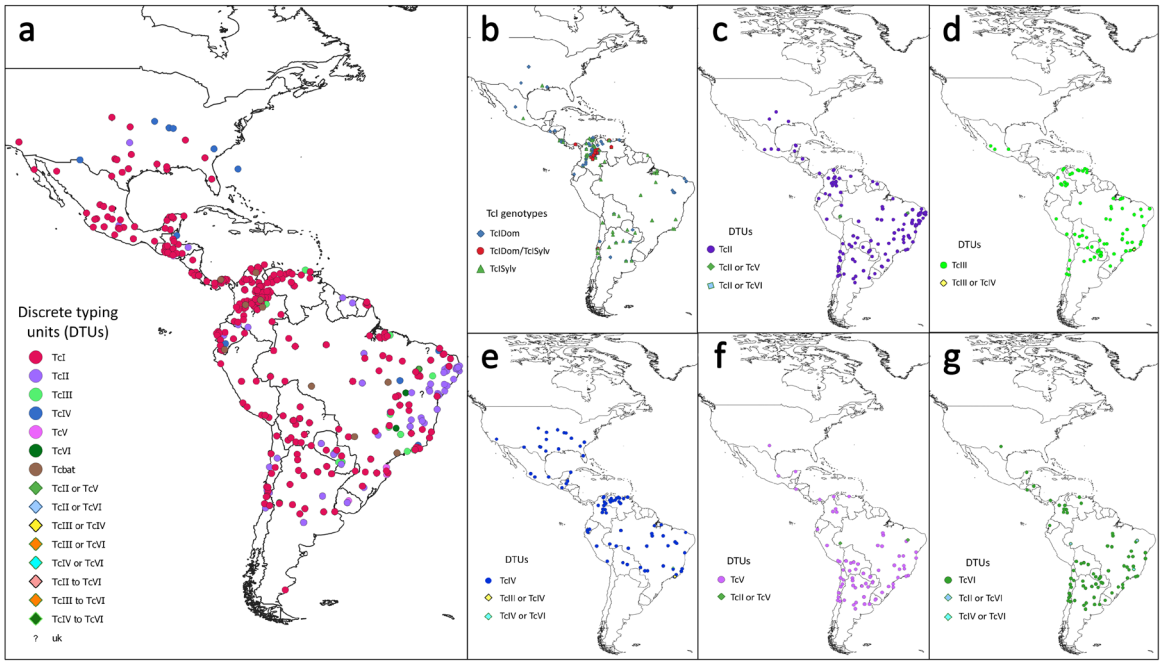

Fig. 1 Distribution of *T. cruzi* DTUs in the Americas. (**a**) Consensus map comprising all 15 categories (shown in the legend), (**b**) TcI and its genotypes distribution, (**c**) TcII, (**d**) TcIII, (**e**) TcIV, (**f**) TcV and (**g**) TcVI.

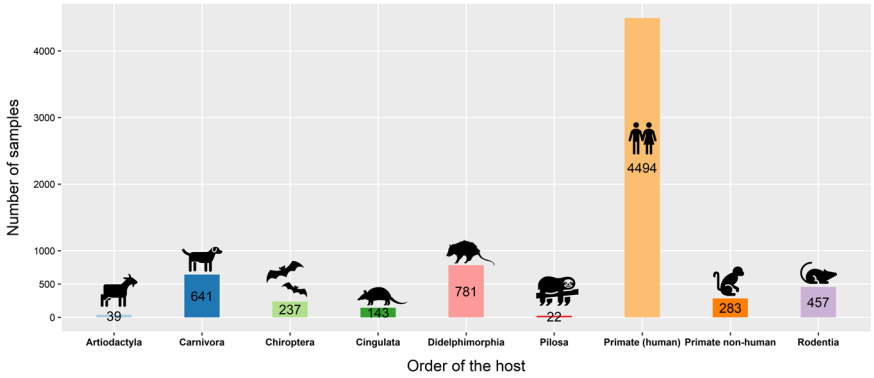

Fig. 2 Number of samples obtained from a wide range of hosts (by Order).

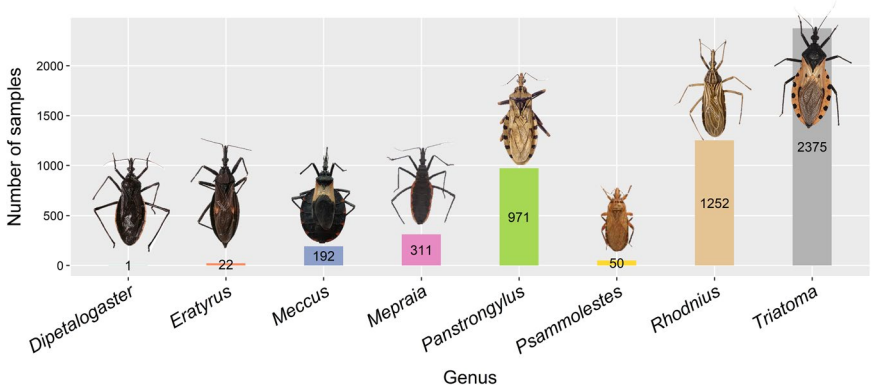

Fig. 3 Frequency of samples obtained from different genera of kissing bugs (Hemiptera: Triatominae).

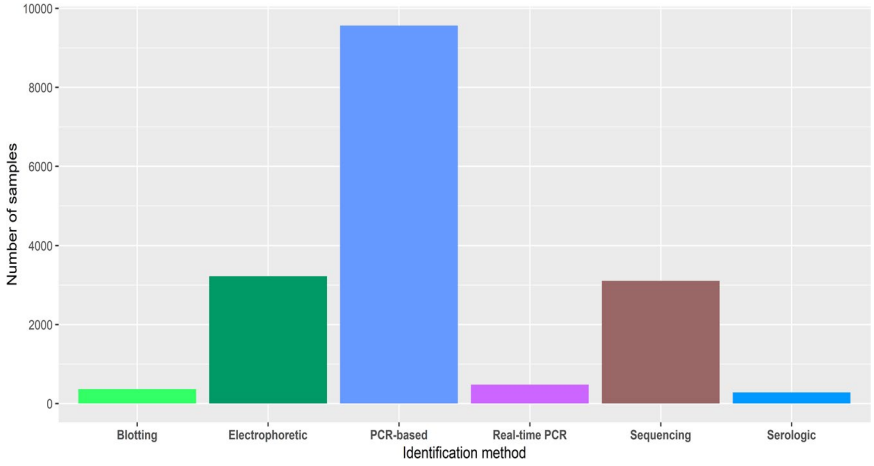

Fig. 4 Methods used for the identification and genotyping of *T. cruzi*.

Most of the samples were obtained from Primates (humans), followed by Didelphimorphia, Carnivora, and Rodentia (Fig. 2). Surprisingly, we found two studies where *T. cruzi* was found in food, Açai palm (Arecales) and sugarcane (Poales) (Supplementary Table 1). Moreover, the most common vectors belong to the Genus *Triatoma*, followed by *Rhodnius* and *Panstrongylus* (Fig. 3). Supplementary Figure 1 shows the transmission cycle of the vectors.

Regarding the methods used for the identification and genotyping of the parasite, we found PCR-based methods as the most widely used, followed by electrophoretic methodologies and sequencing (Fig. 4, Table 2). Furthermore, we counted and manually chose the most common gene algorithms or gene sets used for the identification and genotyping of *Trypanosoma cruzi* (Table 3). Supplementary Figure 2 shows a barplot containing all the different genetic markers used for the above mentioned purpose. In addition, we made a figure that relates

Most common gene algorithms	Number of studies
Gene loci/microsatellites/mitochondrial loci/primers/probes	58
24Sα + COII/SL-IR/cytb/GPI/18 S rRNA/A10/HSP60/microsatellites	82
SL-IR	31
SL-IR + GPI/cytb/COII/ND1/18 S/24Sα/kDNA/1f8	27
18 S + SL-IR/COII/A10/cytb/gGAPDH/	46
kDNA maxi/minicircle	32
kDNA maxi/minicircle + SL-IR/cytb/COII/GPI/1f8	8
Total	284

Table 3. Most common gene sets or algorithms used for the identification and genotyping of *T. cruzi*.

the most common genes with the Method of identification/genotyping, where it can be noted that PCR-based methods are the most widely used for most of the genes (Supplementary Figure 5).

Technical Validation

Once we obtained the final version of the database, we made the debugging process to assure the correct selection of the data included and their reliability. The first debug was to verify the presence of the parasite (*Trypanosoma cruzi*) in the title or summary of each article. Then, it was made a second debug of the articles but this time considering all the fields information previously described in the methods to define the inclusion or exclusion of the article. Finally, a third debug where a review of the geographical coordinates in detail was conducted. This process allowed us to find any typographical or coordinates errors.

Besides, we decided to treat each sample individually to analyse the DTUs distribution because there were too many categories for this field (DTU). This means that for samples with more than two DTUs (expressed in the database field as *p.e.* TcI/TcII-TcVI), we had to duplicate that sample row or a TcI/TcII/TcV in a single sample, we had to triplicate the row, and so on. To clarify the nomenclature used in the original database, the forward-slash (/) means "and," and the hyphen (-) means a range (we change it for the expression "to" for the maps). Also, in some studies, the authors report uncertainty between two DTUs (reported as TcIII "or" TcIV). Therefore, we went from 45 DTU categories to 15 (including the unknown) and then put the modified database in the software QGIS to elaborate the map. This new database was used only for this step while keeping the original database for the figures and tables. Finally, due to the high volume of data retrieved, we should divide the original database into four individual archives: hosts, vectors, genes, and methods, each one filtered by the database fields required for the respective analysis. All the plots were created using the packages ggplot2 v3.3.5, circlize v0.4.14 and Biocircos v0.3.4 in RStudio.

Usage Notes

Due to the high volume of data, we grouped some fields into more general categories. Also, because some geographical coordinates were assigned by searching the place's name, their precise coordinates may vary.

As explained before, because, in the database, we put many variables in one cell for some fields, we should divide them into individual archives to analyze and make each figure. For the genes examined, used the function filter in Excel to count each gene by selecting the boxes that contained them and put the information in a table along with the marker type (nuclear, mitochondrial, or antigen). The exact process was made for the methods, but in this case, we additionally grouped each Method in a general category to optimize the graphic representation of the results. To make the gene algorithms table, we first looked for the most common genes that we defined as the "principal" ones. Then, we wrote down those other genes that are generally used together with the principal one in the studies and using the filter function, and we checked the boxes containing the principal and complementary genes. Finally, we count the corresponding number of articles (Reference field in the database).

Such as our previous data descriptor of *Leishmania* in the Americas[32], we now provide an updated *Trypanosoma cruzi* database with ecoepidemiological information to provide a new powerful tool to improve molecular epidemiology research and surveillance in this case for Chagas disease. Contrary to our *Leishmania* data descriptor, here we did not consider a time period for data collection, and also included new categories for hosts like the common name, order and the source (vector, reservoir or human).

We hope this database will be helpful in future research in the field, focusing on achieving a consensus in which are the most reliable genetic markers and methods to identify/genotype *T. cruzi* and keep on trying to understand the transmission dynamics of the parasite.

Code availability

We did not use any custom code to process the data described in the manuscript.

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References

1. Word Health Organization [WHO]. Chagas disease (American trypanosomiasis). Prevention of chagas disease. Recovered from https://www.who.int/chagas/disease/prevention/en/ (2016).
2. Ministerio de Salud [MinSalud]. Enfermedad de Chagas. Memorias Ministerio de Salud y Protección Social Federación Médica Colombiana (2013).

3. Ministerio de Salud [MinSalud]. Guía-protocolo para la vigilancia en salud pública de chagas. Instituto Nacional de Salud. República de Colombia (2013).
4. Rassi Jr., A., Rassi, A., Marcondes de Rezende, J. American trypanosomiasis (chagas disease). *Infect. Dis. Clin. N. Am*. https://doi.org/10.1016/j.idc.2012.03002 (2012).
5. Zingales, B. *et al*. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. *Mem. Inst. Oswaldo Cruz* **104**, 1051–1054 (2009).
6. Zingales, B. *et al*. The revised *Trypanosoma cruzi* subspecific nomenclature: Rationale, epidemiological relevance and research applications. *Infect. Genet. Evol.* **12**, 240–253 (2012).
7. Ramírez, J. D. & Hernández, C. *Trypanosoma cruzi* I: towards the need of genetic subdivision? Part II. *Acta Trop.* **184**, 53–58, https://doi.org/10.1016/j.actatropica.2017.05.005 (2013).
8. Velásquez-Ortiz, N. & Ramírez, J. D. Understanding the oral transmission of *Trypanosoma cruzi* as a veterinary and medical foodborne zoonosis. *Research in Veterinary Science* **132**, 448–461, https://doi.org/10.1016/j.rvsc.2020.07.024 (2020).
9. Souto, R. P. & Zingales, B. Sensitive detection and strain classification of *Trypanosoma cruzi* by amplification of a ribosomal RNA sequence. *Mol. Biochem. Parasitol.* **62**, 45–52 (1993).
10. Souto, R. P., Fernandes, O., Macedo, A. M., Campbell, D. A. & Zingales, B. DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* **83**, 141–152 (1996).
11. Rozas, N. *et al*. Evolutionary history of *Trypanosoma cruzi* according to antigen genes. *Parasitology* **135**, 1157–1164 (2008).
12. Herrera, L. Una revisión sobre reservorios de *Trypanosoma (Schizotrypanum) cruzi* (Chagas, 1909), agente etiológico de la enfermedad de chagas. *Bol. Malariol. Salud Ambient* **50**, 3–15 (2010).
13. Rendón, L. M., Guhl, F., Cordovez, J. M. & Erazo, D. New scenarios of *Trypanosoma cruzi* transmission in the Orinoco region of Colombia. *Mem. Inst. Oswaldo Cruz* **110**, 283–288 (2015).
14. León, C. M., Hernández, C., Montilla, M. & Ramírez, J. D. Retrospective distribution of *Trypanosoma cruzi* I genotypes in Colombia. *Mem. Inst. Oswaldo Cruz* **110**, 387–393 (2015).
15. Hernández, C. *et al*. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. *Parasit. Vectors* **9**, 620 (2016).
16. Brenière, S. F. *et al*. Genetic characterization of *Trypanosoma cruzi* DTUs in wild *Triatoma infestans* from Bolivia: predominance of TcI. *PLoS Negl Trop Dis.* **6**(5), e1650 (2012).
17. Guhl, F., Auderheide, A. & Ramírez, J. D. From ancient to contemporary molecular eco-epidemiology of chagas disease in the Americas. *Int J Parasitol.* **44**(9), 605–12 (2014).
18. Pérez, E. *et al*. Predominance of hybrid discrete typing units of *Trypanosoma cruzi* in domestic *Triatoma infestans* from the Bolivian Gran Chaco region. *Infect Genet Evol.* **13**, 116–23 (2013).
19. Mejía-Jaramillo, A. M. *et al*. Geographical clustering of *Trypanosoma cruzi* I groups from Colombia revealed by low-stringency single specific primer-PCR of the intergenic regions of spliced-leader genes. *Parasitol Res.* **104**(2), 399–410, https://doi.org/10.1007/s00436-008-1212-0 (2009).
20. Monteiro, W. M. *et al*. *Trypanosoma cruzi* TcIII/Z3 genotype as agent of an outbreak of chagas disease in the brazilian western Amazonia. *Trop Med Int Health. Sep* **15**(9), 1049–51, https://doi.org/10.1111/j.1365-3156.2010.02577.x (2010).
21. Ramírez, J. D., Duque, M. C. & Guhl, F. Phylogenetic reconstruction based on Cytochrome b (cytb) gene sequences reveals distinct genotypes within Colombian *Trypanosoma cruzi* I populations. *Acta Trop.* **119**(1), 61–5 (2011).
22. Rocha, F. L. *et al*. *Trypanosoma cruzi* infection in neotropical wild carnivores (Mammalia: Carnivora): At the top of the *T. cruzi* transmission chain. *PLoS One* **8**(7), e67463 (2013).
23. Santana, R. A. *et al*. *Trypanosoma cruzi* strain TcI is associated with chronic Chagas disease in the Brazilian Amazon. *Parasit Vectors.* **7**, 267 (2014).
24. Ribeiro, G. *et al*. Wide distribution of *Trypanosoma cruzi*-infected triatomines in the State of Bahia, Brazil. *Parasites Vectors* **12**, 604, https://doi.org/10.1186/s13071-019-3849-1 (2019).
25. Velásquez-Ortiz, N. *et al*. *Trypanosoma cruzi* infection, discrete typing units and feeding sources among *Psammolestes arthuri* (Reduviidae: Triatominae) collected in eastern Colombia. *Parasites Vectors* **12**, 157, https://doi.org/10.1186/s13071-019-3422-y (2019).
26. Dumonteil, E. *et al*. Detailed ecological associations of triatomines revealed by metabarcoding and next-generation sequencing: implications for triatomine behaviour and *Trypanosoma cruzi* transmission cycles. *Sci Rep* **8**, 4140 (2018).
27. Hodo, C. L. *et al*. *Trypanosoma cruzi* transmission Among captive non-human primates, wildlife, and vectors. *EcoHealth* **15**(2), 426–436, https://doi.org/10.1007/s10393-018-1318-5 (2018).
28. Bizai, M. *et al*. Geographic distribution of *Trypanosoma cruzi* genotypes detected in chronic infected people from Argentina. Association with climatic variables and clinical manifestations of chagas disease. *Inf Gen and Evol*, 104128. https://doi.org/10.1016/j.meegid.2019.104128 (2019).
29. Carrasco, H. J. *et al*. Geographical Distribution of *Trypanosoma cruzi* genotypes in Venezuela. *PLoS Negl Trop Dis* **6**(6), e1707, https://doi.org/10.1371/journal.pntd.0001707 (2012).
30. Izeta-Alberdi, A. *et al*. Geographical, landscape and host associations of *Trypanosoma cruzi* DTUs and lineages. *Parasites Vectors* **9**, 631, https://doi.org/10.1186/s13071-016-1918-2 (2016).
31. Herrera, G. *et al*. An interactive database of *Leishmania* species distribution in the Americas. *Sci Data* **7**, 110, https://doi.org/10.1038/s41597-020-0451-5 (2020).
32. González, R. & David, J. DTU_DB. *Universidad del Rosario* https://doi.org/10.34848/FK2/6QGLCT (2022).

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Author contributions

N.V.O., C.H. and M.M. performed the systematic review and filled the database. N.V.O. and J.D.R. wrote the manuscript. G.H. was the third reviewer and made the distribution map. J.D.R. and C.H. designed the study.

Competing interests

The authors declare no competing interests.

Additional information

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Capítulo 2. Diseño de marcadores para análisis de la variabilidad genética de especie de la tribu Rhodniini

RESEARCH ARTICLE

Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species?

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Abstract

The use of subtle features as species diagnostic traits in taxa with high morphological similarity sometimes fails in discriminating intraspecific variation from interspecific differences, leading to an incorrect species delimitation. A clear assessment of species boundaries is particularly relevant in disease vector organisms in order to understand epidemiological and evolutionary processes that affect transmission capacity. Here, we assess the validity of the recently described *Rhodnius taquarussuensis* (da Rosa et al., 2017) using interspecific crosses and molecular markers. We did not detect differences in hatching rates in interspecific crosses between *R. taquarussuensis* and *R. neglectus* (Lent, 1954). Furthermore, genetic divergence and species delimitation analyses show that *R. taquarussuensis* is not an independent lineage in the *R. prolixus* group. These results suggest that *R. taquarussuensis* is a phenotypic form of *R. neglectus* instead of a distinct species. We would like to stress that different sources of evidence are needed to correctly delimit species. We consider this is an important step in understanding vectorial Chagas disease spread and transmission.

Introduction

The study of the speciation process requires a complete understanding of the phenotypic variation present across the range of the study taxa. This is particularly challenging in organisms where morphological differences are subtle or not obvious, and where other aspects of their biology such as reproduction, ecology, phenology and life traits are also unknown. An increasing number of studies have documented "cryptic" speciation throughout the tree of life (i.e. taxa that cannot readily be distinguished morphologically, yet evidence indicates they are on different evolutionary trajectories). However, such descriptions have been done in absence of a clear definition of what a cryptic species is, and often using alpha taxonomy as the sole approach for detecting and classifying new species [1–4]. This can lead to false species diagnosis when unreliable traits (those lacking discontinuous, nonoverlapping patterns of variation) are used [5], which is particularly important when delimiting vector species with medical relevance, as this directly impacts the control of the diseases transmitted by them.

The subfamily Triatominae has 18 genera, with *Panstrongylus* (Berg, 1879), *Rhodnius* (Stål, 1859) and *Triatoma* (Laporte, 1832) being the most epidemiologically important genera, since they are the main species responsible for the transmission of *Trypanosoma cruzi* (Chagas, 1909), the etiologic agent of Chagas disease [6, 7]. The identification of these three genera is straightforward and is based on the insertion of the antennae on the head, which is macroscopically perceptible: in *Panstrongylus* the antennae are inserted near the eyes, in *Rhodnius* these appendages are on the anterior portion of the head, and in *Triatoma* they are located on the middle portion of the head [8, 9]. Nonetheless, the most recent Triatominae phylogeny showed that the only monophyletic genus is *Rhodnius* [9–11]. Also, species delimitation within these genera remains problematic [12]. In particular, species of *Rhodnius* show low morphological variation and their complex identification relies on few morphological traits and/or mtDNA divergence [11, 13–16]. For example, it is difficult to differentiate between *R. neglectus* and *R. prolixus* (Stål, 1859) [17], *R. robustus* (Larrousse, 1827) and *R. montenegrensis* (da Rosa et al., 2012) [18], *R. amazonicus* (Almeida, Santos and Sposina, 1973) and *R. pictipes* (Stål, 1872) [19], *R. pictipes* and *R. stali* (Lent, Jurberg and Galvão) [20], among many other examples.

Moreover, the classic division of *Rhodnius* presents additional challenges. The genus is divided into three groups: *prolixus*, *pictipes* and *pallescens*. The first two are found east of the Andes (*cis*-Andean), while the third is distributed west of the Andes (*trans*-Andean) [21–23]. The phylogenetic relationships among these groups are still under debate, especially the position of the *pictipes* group that was initially considered closer to the *pallescens* group, but recent evidence found it as sister to the *prolixus* group [23–26].

Because *Rhodnius* has an intrinsic relation with the propagation of *T. cruzi* and *T. rangeli* (Tejera, 1920), resolving its phylogenetic relationships and accurately differentiating its species is a first step to determine the epidemiological threat associated to each species, as well as to understand their ecology and population dynamics [8, 23, 27].

Recently, a new species of the genus *Rhodnius*, *R. taquarussuensis*, was described based on phenotypic and cytogenetic traits [22]. This is the only species of the *prolixus* group that has dispersed heterochromatin throughout the nucleus and autosomes, and it is morphologically similar to *R. neglectus* [22, 28]. However, the specific status of *R. taquarussuensis* requires a more rigorous confirmation that implements both genetic data and tests of reproductive isolation. Here, we used six molecular markers and performed crosses between *R. taquarussuensis* and *R. neglectus* in order to address whether the former is a valid species.

Methods

Sampling and DNA extraction

Individuals of *R. taquarussuensis* were collected in Taquarussu, Mato Grosso do Sul, Brazil (-22.48 Lat, -53.35 Long; Table 1) and those of *R. neglectus* were collected in Formoso, Goiás, Brazil (-13.65 Lat, -48.88 Long; Table 1) and maintained in the Triatominae insectary of the School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil. *Rhodnius prolixus* were collected in Arauca (7.08 Lat, -70.75 Long), Fortul (6.78 Lat, -71.76 Long), Puerto Rondón (6.28 Lat, -71.10 Long) and Saravena (6.95 Lat, -71.87 Long) in Colombia (Table 1). UNIVERSIDAD DEL ROSARIO provided the field permit from ANLA (Autoridad Nacional de Licensias ambientales) 63257–2014. DNA was extracted from the head, legs and intestine using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's protocol. The DNA concentration was determined using a NanoDrop 1000 Spectrophotometer V3.7 (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at −20˚C.

Loci amplification and sequencing

We amplified and sequenced two mitochondrial gene fragments, Cytochrome b (CYTB) and Mitochondrially Encoded NADH Dehydrogenase 4 (ND4) using the conditions reported elsewhere [29]. We also designed primers to develop new coding nuclear markers in *Rhodnius*. In order to do this, we used the *R. prolixus* genome available in VectorBase (https://www.vectorbase.org/organisms/rhodnius-prolixus) and, from the GFF file, we selected four large exon markers (≥700 bp) using a custom script. We then used BLASTn to compare these exons

Table 1. Genes, primer information and accession numbers.

Symbol	Gene name	*Rn*	*Rp*	*Rt*	Primers (5'-3')	Tm (˚C)	Fragment size (pb)	Accession numbers
CYTB	Cytochrome b**	6	5	8	R: GCW CCA ATT CAR GTT ART AA F: GGA CGW GGW ATT TAT TAT GGA TC	50	659	MH704746—MH704764
ND4	NADH dehydrogenase 4**	5	5	15	F: TAA TTC GTT GTC ATG GTA ATG F: TCA ACA TGA GCC CTT GGA AG	53	560	MH704765—MH704779
PCB	Putative chitin binding peritrophin-a domain protein	8	5	5	R: CAC TAC GGG TCG TGA AGG TT F: ACA TCC TTG GCC ACA AGA AC	55	757	MH704780—MH704797
TOPO	DNA topoisomerase	5	6	5	F: CAA CAC TTG TAA CCC GAG CA F: ATC ATT GGC CGC ATC TTT AG	56	604	MH704798—MH704813
URO	Uroporphyrinogen decarboxylase	11	6	6	R: TTA AGG GCA GCA AGA GGA GA F: AAC ACA TTT CCT GGC CAA AG	54	563	MH704814—MH704828
ZNFP	Toll-like-2. Transmembrane receptor with TIR domain binding	5	5	5	F: TCC TTG CGG TAA TGA TGT GA F: CTC GAA TGG TGT ACG TGG TG	54	588	MH704829—MH704852

Gene IDs correspond to those in the *Rhodnius* genome GFF file annotation.

**Published before. *Rn*: *R. neglectus; Rp*: *R. prolixus; Rt*: *R. taquarussuensis*

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to the *R. prolixus* transcriptome and thus confirm they were single copy markers. Then, we verified the identity of the selected exons in Uniprot with the ID codes registered in the genome. Finally, we designed primers for these loci using Primer 3 [30]. The resulting nuclear markers are Putative chitin binding peritrophin-a (PCB), DNA topoisomerase (TOPO), Uroporphyrinogen decarboxylase (URO) and Toll-Like-2. Transmembrane receptor with TIR domain binding (ZNFP) (Table 1 and Table 2).

PCR reactions had a final volume of 25 μl, consisting of 12.5 μl of GoTaq Green Master Mix (Promega, Madison, WI, USA), 1.25 μL (10 μM) of each primer and, 5.0 μl of DNA (20 ng) and 5μL of H_2O. Amplification was conducted in a Thermal Cycler 4000 (Bio-Rad La-boratories, Inc., Hercules, CA, USA). The following PCR cycling conditions were used: 94˚C for 5 min; 40 cycles of 94˚C for 1 min, 50–56˚C for 1 min (Table 1), and a final extension at 72˚C for 10 min. PCR success was verified by electrophoresis on agarose gel stained with Fast SYBR Green (Applied Biosystems, Foster City, CA, USA) and a molecular weight marker (Promega) adding 2μl of each PCR product. The samples were purified using the PCR kit ExoSAP-IT Product Cleanup (Affymetrix, Santa Clara, CA, USA) and sequenced at Macrogen Inc. (Seoul, Korea).

Sequence analyses

Gene sequences were read, edited and aligned with CLC Main Workbench (Qiagen). For nuclear loci, haplotype inference for heterozygous calls was conducted using the PHASE algorithm implemented in DnaSP v5 [31], accepting haplotypes with a confidence higher than 90% after running 5,000 interactions per simulation. Then, we created alignments for each locus using MUSCLE [32] with the default parameters. These alignments were visualized and corrected by hand in MEGA X [33]. Finally, we translated the sequences to proteins in order to verify for stop codons using MESQUITE 3.04 [34].

Molecular phylogenetics and species delimitation

In order to assess the position of *R. taquarussuensis* within the group *prolixus*, we downloaded from the Genbank all CYTB sequences available for this group and one from *Triatoma infestans* (outgroup; S1 Table) using the following Entrez line: "esearch -db nucleotide -query "<organism> CYTB" | efetch -format fasta" [35]. We combined these data with our sequences and estimated a phylogenetic tree for the group *prolixus* using a Maximum likelihood (ML) optimization in IQ-TREE [36]. The substitution model for CYTB was established in the same software, selecting the model with the lowest BIC score. Node support was calculated with 1,000 ultrafast bootstrap replicates.

Table 2. Nuclear markers (single copy exons) designed in this study.

Gen	Annotation in the *R. prolixus* genome							Region amplified		
	Gene ID		Scaffold	Strand	Start	End	Size (bp)	Location	Start	End
ZNFP	RPRC009262-RA	Tl-like-2: Toll-like-2. Transmembrane receptor with TIR domain binding	KQ034161	+	481476	486977	5501	Exon 1	481599	482146
URO	RPRC013534-RA	UROD: Uroporphyrinogen decarboxylase	KQ034105	-	970351	971418	1067	Exon 1	970699	971261
TOPO	RPRC012703-RA	DNA topoisomerase	KQ034259	+	391034	406927	15893	Exon 3	404730	405333
PCB	RPRC001863-RA	Putative chitin binding peritrophin-a	KQ034056	+	8334541	8342490	7949	Exon 3	8335296	8336052

Gene IDs correspond to those in the *Rhodnius* genome GFF file annotation.

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We also explored the phylogenetic relationships between *R. prolixus*, *R. neglectus* and *R. taquarussuensis*, concatenating all loci (nuclear and mitochondrial; 3731 bp long alignment) in Mesquite 3.04 [34] and estimating a ML phylogenetic tree with in IQ-TREE [36]. We allowed each locus to have its own substitution model, and node support was accessed as above. We also conducted a Bayesian analysis independently for each locus using BEAST 2.5, implementing linked and unlinked tree models [37]. We inferred the nucleotide substitution model, range of the rate of heterogeneity, and proportion of invariant positions during the MCMC analysis with the bModelTest package [38], with transition-transversion split option and empirical frequencies. We ran 10'000,000 generations sampling every 1,000 generations and used TRACER [39] to confirm the coverage of the chain (i.e. effective sample size >200). TreeAnnotator [37] was used to construct a consensus tree per locus and the initial 10% trees were discarded as burn-in. We superimposed and plotted consensus gene trees constructing a Multiphylo object with the densiTree function in R [40].

As the resulting ML and Bayesian topologies were identical, we used the ML tree as input for a species delimitation analysis intended to determine the species boundaries between *R. taquarussuensis*, *R. neglectus* and *R. prolixus*. This analysis was carried out under a phylogenetic species concept using the Bayesian and ML version of PTP with 500,000 MCMC generations, thinning = 100 and burn-in = 0.1 [41]. PTP implements a non-ultrametric phylogeny to model speciation rate as the number of substitutions reflected as branch lengths, assuming that the number of substitutions between species are significantly higher than the number of substitutions within species.

Genetic differentiation analysis and haplotype networks

We calculated segregating sites (SS), nucleotide diversity (π), haplotype diversity (Hd), number of synonymous and non-synonymous substitutions, singletons and Tajima's D with DnaSP v5 [31]. We did not calculate relative genetic differentiation (F_{ST}) as it has been shown to be overestimated when low nucleotide diversities are obtained [42], as in our dataset (Table 3). Instead, we calculated an absolute divergence measure (D_{XY}) and its nucleotide diversity corrected version (Da) with DnaSP v5. D_{XY} was visualized as a heatmap drawn with the R package *"fields"*. We also calculated Kimura 2 parameter distance (K2P) which has been previously used in triatomines to validate different species [43].

Genetic clustering between *R. neglectus* and *R. taquarussuensis* was validated with a discriminant analysis of principal components (DAPC) performed with both nDNA and mtDNA using the '*adegenet*' R package [44]. We did this by transforming fasta sequences into a genind object that contains individual genotypes and loading it into '*adegenet*' [44]. We performed a principal component analysis (PCA) on these data and retained the first two components (that accounted for >90% of the total variation in both mtDNA and nDNA). We then applied a discriminant analysis using the dapc function and assuming two prior groups (i.e. two species). This produced a single canonical function that summarizes the individual genetic variability, which was then visualized with a density plot. Finally, we constructed haplotype median-joining networks per locus with POPART [45].

Interspecific crosses

As a first attempt to determine the presence of reproductive isolation between *R. taquarussuensis* and *R. neglectus*, we performed interspecific (direct and reciprocal) and conspecific crosses. These were conducted in the Triatominae insectary of the School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil, following the methodology established by Costa et al. [46] and Mendonça et al. [47]. Each cross was

Table 3. Summary statistics for each locus.

Species	Gene	Pi (π)	SS	Tajima's D*	Hd	Synonymous sites	Non- synonymous sites	Singletons
R. neglectus	CYTB	0	0	0	0	0	0	0
	ND4	0.00089	1	-0.61	0.5	0	1	1
	PCB	0.0012	2	1.085	0.49	1	1	0
	TOPO	0	0	0	0	0	0	0
	URO	0.00015	1	-1.15	0.083	0	1	1
	ZNFP	0	0	0	0	0	0	0
R. taquarussuensis	CYTB	1.00E-07	1	-1.05	0.25	1	0	1
	ND4	0	0	0	0	0	0	0
	PCB	0.00074	1	1.38	0.53	0	1	0
	TOPO	0.00353	6	0.02	0.62	3	3	0
	URO	0.00143	4	-1.38	0.56	0	4	3
	ZNFP	0.00091	1	0.85	0.81	0	1	0
R. prolixus	CYTB	0.00965	13	-1.1	1	2	12	12
	ND4	0.00714	4	0	1	1	3	4
	PCB	0.00141	3	0.021	0.35	1	2	0
	TOPO	0.00028	1	-1.14	0.17	1	0	1
	URO	0.00328	4	1.39	0.77	1	3	0
	ZNFP	0.00181	2	1.031	0.53	0	2	0

*None of the Tajima's D were significant.

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replicated three times for a total of 12 matings. First, insects were sexed as 5th instar nymphs [48], and males and females were kept separately until they reached the adult stage [49]. Then, a virgin female was placed with a male inside a plastic box (5cm diameter × 10cm height) for a maximum period of 120 days and kept at room temperature. The success or failure of mating was recorded by direct observation. After seven days, we collected the eggs of each cross weekly throughout the females' oviposition period (120 days). The eggs collected were placed inside a plastic box (5cm diameter × 10 cm height) and their hatching was recorded weekly.

We calculated hatching success of the interspecific crosses as a measure of egg viability relative to conspecific crosses. A likelihood approximation was implemented in Betabino 1.1 [50] to analyze these data. Because using a binomial model alone does not account for the variation in hatching rate among families in each type of cross, Betabino fits a beta-binomial distribution to count data (in our case, number of eggs that hatched), thus solving this issue. Four alternative models that contrast the number of parameters in the data (i.e. mean and variance in the hatching rate) were tested. For details see http://www.ucl.ac.uk/~ucbhdjm/bin/betabino/betabino.pdf and the appendix section in [50].

Results

Molecular phylogenetics and species delimitation

All sequences obtained for this study were deposited in the Genbank and their accession numbers are found in Table 1. Our dataset for the CYTB gene consisted of 162 sequences corresponding to six species and confirmed the phylogenetic relationships previously shown by Monteiro et al. [11]. Briefly, the ML topology obtained with this gene (evolution model TN+F+I; BIC score 4339.957) revealed that the *prolixus* group is subdivided into two clades, one exclusively formed by *R. barreti* (Abad-Franch, Palomeque and Monteiro, 2013), and the second consisting of *R. robustus*, *R. montenegrensis*, *R. prolixus*, *R. neglectus*, *R. nasutus* (Stål,

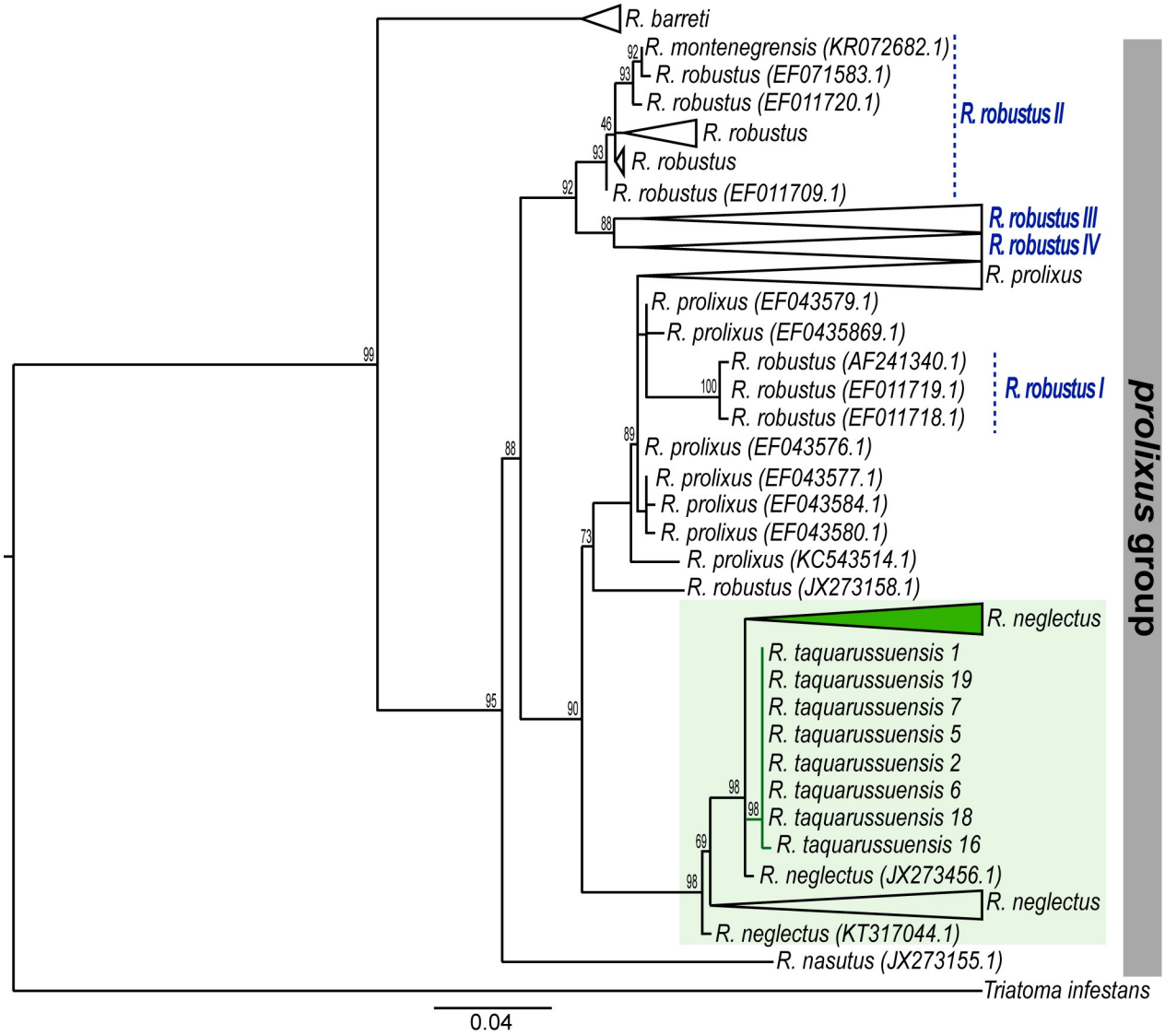

Fig 1. Maximum Likelihood tree for *Rhodnius* based on CYTB. Numbers on the nodes are bootstrap supports. The vertical bar on the right highlight the *prolixus* group. The focal species, namely, *R. taquarussuensis* and *R. neglectus*, are highlighted in the green square. Green branches and the collapsed clade (green triangle) correspond to the sequences obtained here for *R. taquarussuensis* and *R. neglectus* respectively.

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1859), and *R. taquarussuensis*. The relations within this latter clade are complicated. For example, we recovered the four groups previously described for *R. robustus* [11], where *R. robustus*-I falls inside the *R. prolixus* clade, and *R. montenegrensis* is part of *R. robustus*-II (Fig 1 and S1 Fig). Additionally, the species *R. neglectus* is recovered as sister to *R. prolixus* and contains all individuals from the newly described species *R. taquarussuensis*, which although monophyletic, has virtually no differentiation from *R. neglectus* (Fig 1 and S1 Fig).

To better explore this unexpected pattern, we constructed haplotype networks of the gene fragments studied with *R. neglectus*, *R. taquarussuensis* and *R. prolixus* (Fig 2). In the case of CYTB, we found *R. prolixus* separated from the other two species by 15 mutational steps. In contrast, *R. taquarussuensis* haplotypes were less distant to *R. neglectus* (only two mutational steps). In fact, the divergence of *R. taquarussuensis* from *R. neglectus* (H.1 and H.2) is less than the divergence between such haplotypes and others from the same species (i.e. H.3 to H.8).

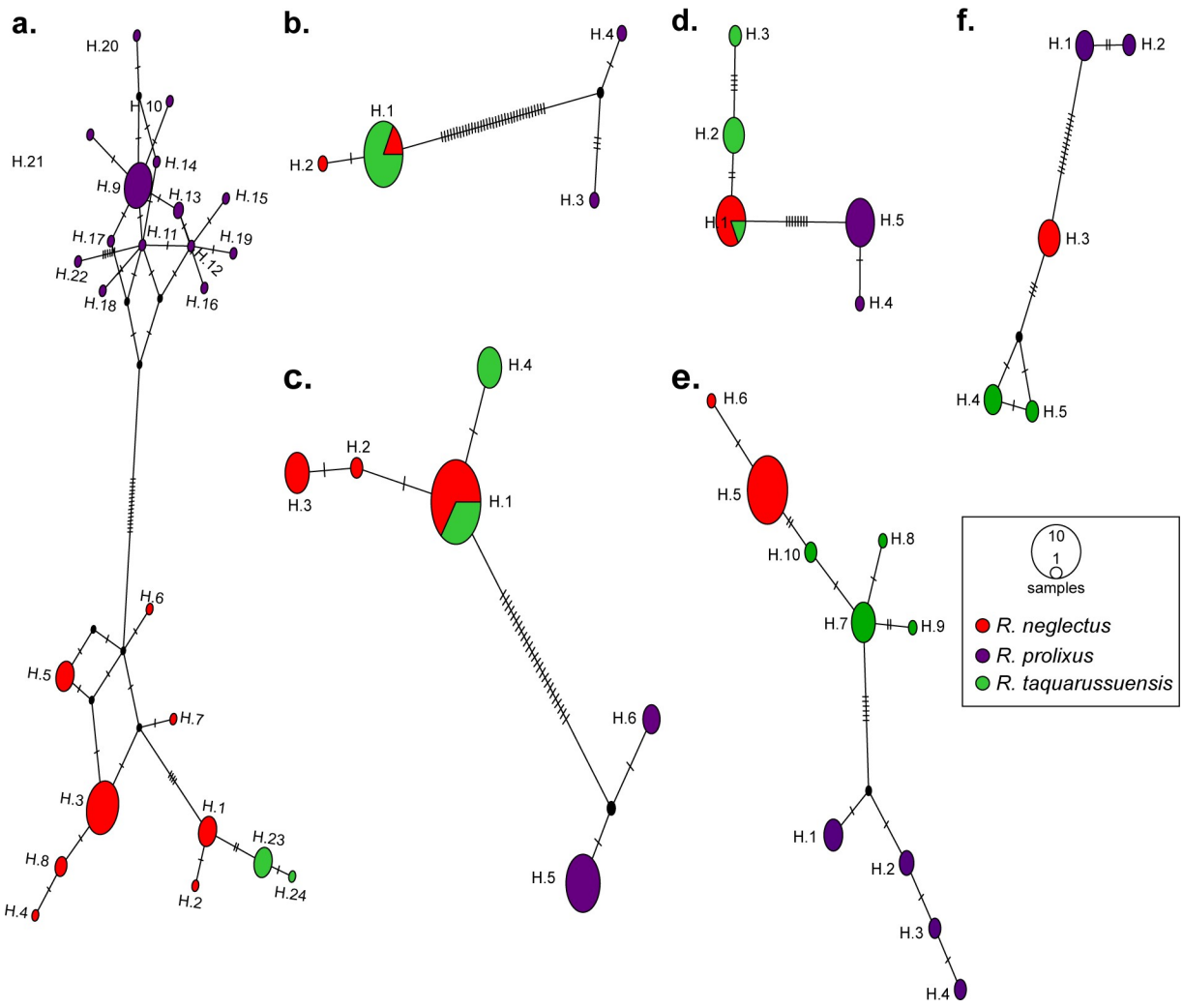

Fig 2. Haplotype networks. (a) CYTB; (b) ND4; (c) PCB; (d) TOPO; (e) URO; (f) ZNFP. Ticks on branches indicate mutational steps between haplotypes. Circle size is proportional to the number of individuals having a haplotype.

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Consistently, nucleotide diversity of *R. prolixus* and *R. neglectus* is higher than that of *R. taquarussuensis* (Table 3).

We recovered the same multilocus phylogeny for *R. prolixus*, *R. neglectus* and *R. taquarussuensis* with ML and Bayesian approaches (ML substitution models were CYTB: HKY+F+I; ND4: HKY+F; PCB: F81+I; TOPO: F81+I; URO: HKY+F; ZNFP: TPM2+F+I). The three species were monophyletic and all of them with posterior probabilities of 100 (Fig 3A) Bootstrap support values were > 90 for *R. prolixus* and *R. neglectus*, while *R. taquarussuensis* has a bootstrap support of 78. Also, the branch length of *R. taquarussuensis* is less than one in a thousand changes. The unlinked and superimposed Bayesian gene trees consistently recovered two main clades: one exclusively composed of *R. prolixus*, and the second where *R. neglectus* and *R. taquarussuensis* show incomplete coalescence (Fig 3B). Consistently, in the analysis of species delimitation (PTP), both the Maximum Likelihood and Bayesian inference found two species as the most probable partition (Fig 4). These two partitions correspond to *R. prolixus* and *R. neglectus*. All other internal nodes had probabilities lower than 0.1 (Fig 4).

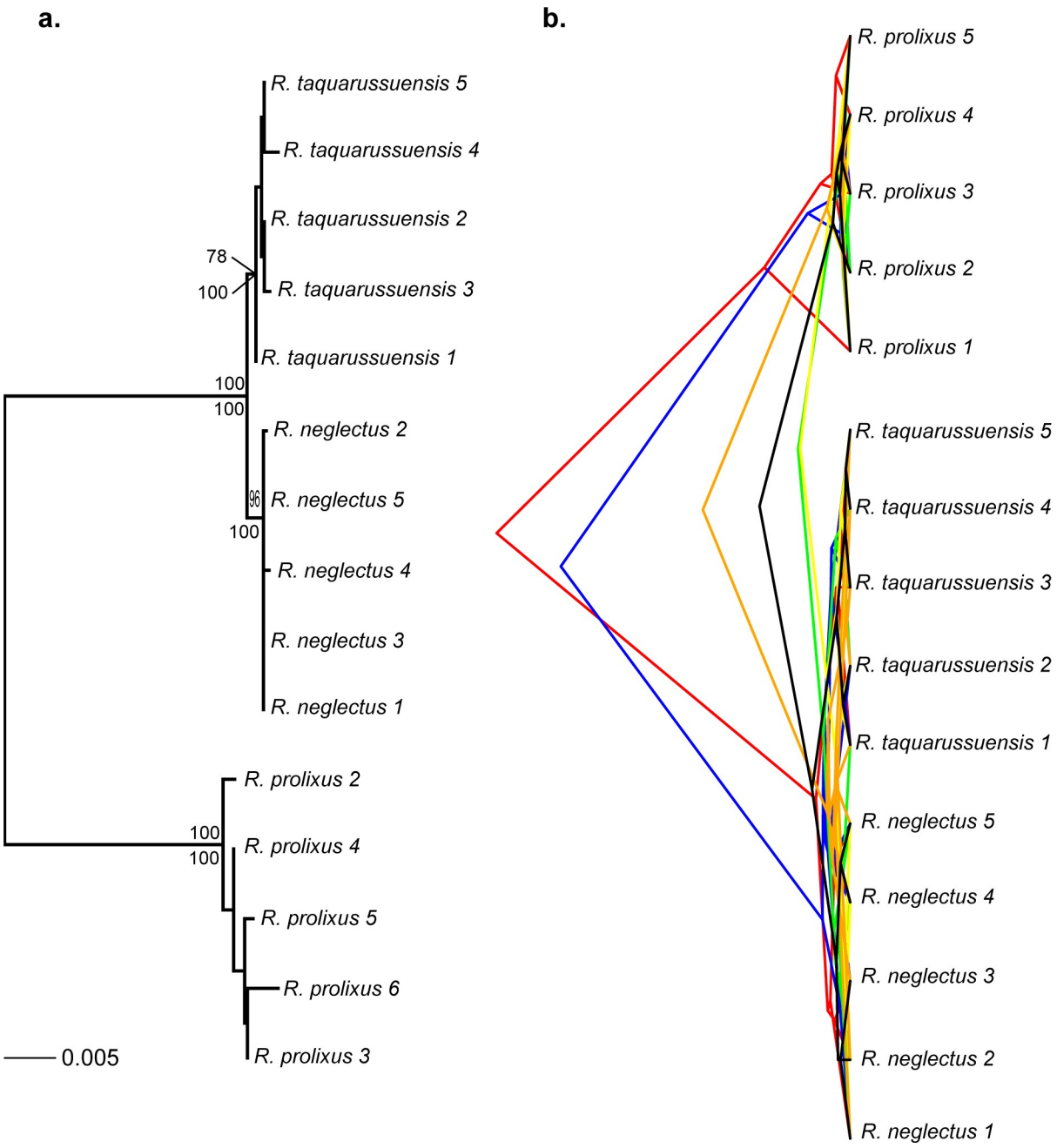

Fig 3. Phylogenetic trees for *R. prolixus*, *R. neglectus* and *R. taquarussuensis* based on all molecular markers. A. Multilocus phylogeny where node support is indicated on each branch: bootstrap (above) and posterior probability (below). B. Bayesian superimposed gene trees: red (CYTB), blue (ND4), green (TOPO), yellow (URO), orange (PCB) and black (ZNFP). The alignment consisted of 3731 bp.

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Genetic differentiation

Overall, all markers showed low genetic diversity for the three taxa, *R. prolixus*, *R. neglectus* and *R. taquarussuensis*. In particular, the loci PCB and ND4 showed the same pattern as CYTB, where *R. taquarussuensis* is less diverse than the other two species (Table 4). The remaining loci showed *R. taquarussuensis* less diverse than *R. prolixus* and the diversity of *R. neglectus* was zero. This is consistent with the low number of haplotypes observed in the

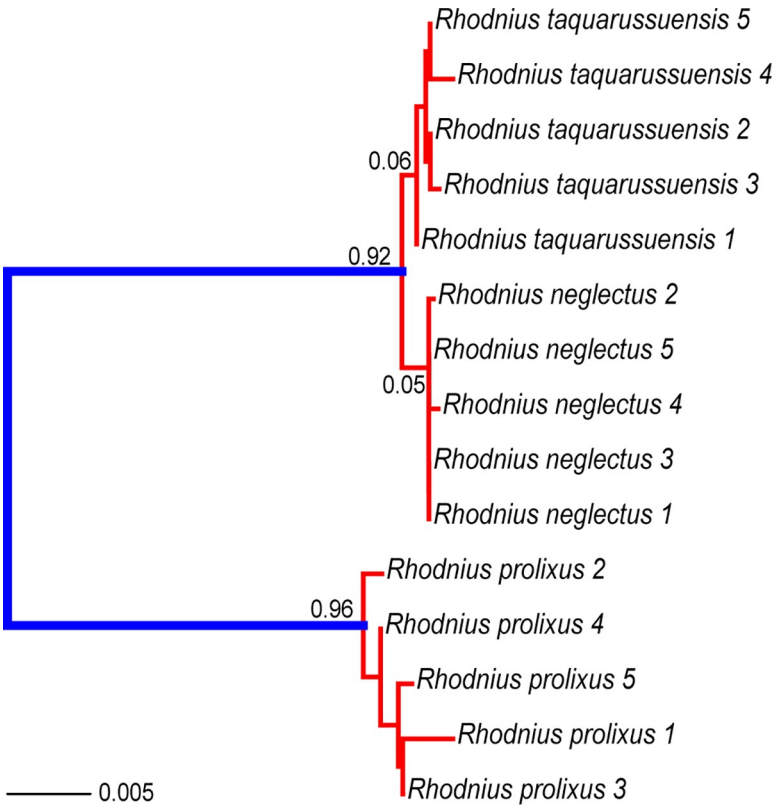

Fig 4. Species delimitation based on the Poisson Tree Process (PTP). Maximum Likelihood and Bayesian inference yielded identical results. Numbers on each node are posterior probabilities of the inner taxa forming one species. Thus, red branches indicate taxa that should be considered as part of the same lineage.

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haplotype networks (Fig 2), where *R. prolixus* has private haplotypes that clearly differentiate it from the other two species (Fig 2B–2F), while *R. taquarussuensis* and *R. neglectus* exhibit substantial haplotype sharing (Fig 2).

Consistent with these findings, D_{XY} shows *R. prolixus* highly differentiated from *R. neglectus* and *R. taquarussuensis* in all loci whilst the latter two taxa do not differentiate between them (S2 Fig). When correcting for the nucleotide diversity, the same pattern is observed (Table 4). The genetic distance (K2P) between *R. neglectus* and *R. taquarussuensis* in all loci was less than 7.5%, a value previously used to define species in triatomines using CYTB [43]. Also, the discriminant analysis = of genetic variation for both mtDNA and nDNA fails to separate the taxa *R. neglectus* and *R. taquarussuensis*, which is reflected by the overlap of their densities on the canonical function (S3 Fig).

Interspecific crosses

All interspecific matings attempted were successful (n = 6), suggesting that there are no mechanical and/or gametic mechanisms that act against hybridization between *R. neglectus* and *R. taquarussuensis*. When we tested homogeneity across categories in the hatching rate, we did not observe differences between interspecific crosses (direct or reciprocal) and controls (Table 5; $G_6 = 7.06$, $P = 0.3152$). Models that have multiple means ($G_3 = 1.243$, $P = 0.7428$) or variances ($G_3 = 2.097$, $P = 0.5525$) for the hatching rate were not supported by the data, indicating the absence of maternal or cytoplasmic effects.

Table 4. Absolute genetic divergence corrected by nucleotide diversity (Da) and Kimura 2 Parameter distance (K2P) between *R. prolixus*, *R. taquarussuensis* and *R. neglectus*.

Gene	Species pair	Da	K2P
CYTB	*R. neglectus–R. taquarussuensis*	0.003	0.003
	R. neglectus–R. prolixus	0.06639	0.082
	R. taquarussuensis–R. prolixus	0.06939	0.086
ND4	*R. neglectus–R. taquarussuensis*	0	0
	R. neglectus–R. prolixus	0.0625	0.075
	R. taquarussuensis–R. prolixus	0.0625	0.075
PCB	*R. neglectus–R. taquarussuensis*	0.00037	0.001
	R. neglectus–R. prolixus	0.0359	0.038
	R. taquarussuensis–R.prolixus	0.0359	0.039
TOPO	*R. neglectus–R. taquarussuensis*	0.00221	0.004
	R. neglectus–R. prolixus	0.01325	0.014
	R.taquarussuensis–R. prolixus	0.01545	0.018
URO	*R. neglectus–R. taquarussuensis*	0.00476	0.005
	R. neglectus–R. prolixus	0.01701	0.017
	R. taquarussuensis–R. prolixus	0.01701	0.012
ZNFP	*R. neglectus–R. taquarussuensis*	0.00635	0.007
	R. neglectus–R. prolixus	0.02234	0.024
	R. taquarussuensis–R. prolixus	0.02585	0.028

https://doi.org/10.1371/journal.pone.0211285.t004

Discussion

Rhodnius exhibits morphological traits that facilitate its identification at the genus level [18, 51], but the low morphological variation within the genus precludes an easy species identification based on morphology alone [23]. This has led to suggest the existence of cryptic species in *Rhodnius*, where multiple look-alike lineages should be considered as different species based on their genetic differentiation [11, 16, 23, 51]. However, morphological species identification in *Rhodnius* relies on intraspecifically variable traits, which can lead to over-estimate the number of species [5]. Therefore, it is necessary to validate the status of the currently described species in the genus implementing a comprehensive approach that uses morphology, genetics, and measures of reproductive isolation.

R. taquarussuensis is the most recently described species in *Rhodnius*, based on morphological, morphometric and cytogenetic evidence [22]. However, the description of this species lacked other crucial evidence. Here, we tested the species status of *R. taquarussuensis* sequencing six molecular markers and performing interspecific crosses. Our results suggest that, despite the morphological differences between *R. taquarussuensis* and *R. neglectus* [22], these taxa constitute a single species.

Firstly, the known distribution range of *R. taquarussuensis* overlaps that of *R. neglectus* (Fig 5). Thus, for them to be different species it would be necessary to evolve strong intrinsic and/

Table 5. Results for interspecific and conspecific crosses. R denotates replicate number for each cross. SE = standard error.

Type of cross		Laid eggs (hatched)				Proportion of viable eggs (SE)	Variance (SE)
		R1	R2	R3	Total		
Interspecific	*R. taquarussuensis* ♀ x *R. neglectus* ♂	230 (198)	86 (80)	230 (193)	510 (471)	0.83 (0.03)	0.0016 (0.002)
	R. neglectus ♀ x *R. taquarussuensis* ♂	300 (275)	181 (105)	256 (244)	708 (624)	0.88 (0.02)	0.0006 (0.0007)
Conspecific	*R. neglectus* ♀ x *R. neglectus* ♂	337 (308)	409 (346)	174 (155)	901 (809)	0.86 (0.02)	0.0001 (0.0016)
	R. taquarussuensis ♀ x *R. taquarussuensis* ♂	151 (127)	168 (150)	201 (156)	501 (433)	0.78 (0.14)	0.034 (0.046)

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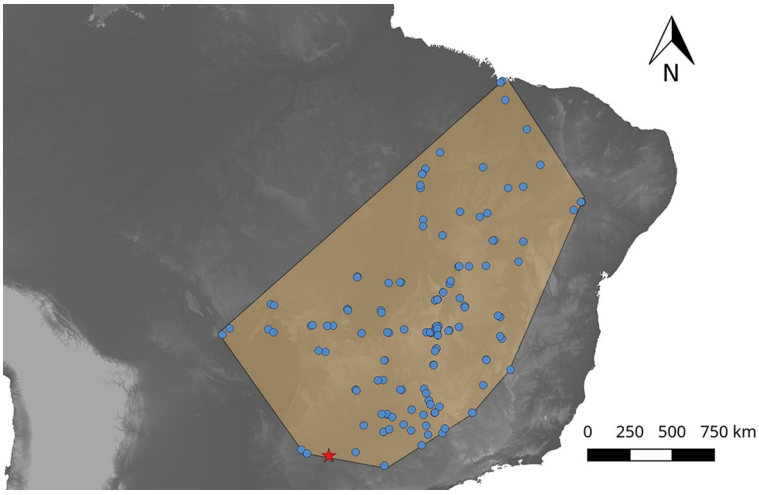

Fig 5. Geographical distribution of *R. neglectus* (blue) and *R. taquarussuensis* (red). Distribution of *R. neglectus* is based on records available on DataTri [55] whilst that of *R. taquarussuensis* is based on collections made by the authors.

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or extrinsic isolation barriers that restrict gene flow. In contrast, we found that *R. taquarussuensis* and *R. neglectus* successfully cross and there are no maternal or cytoplasmic effects that affect offspring viability, as reflected by the high hatching rates we obtained. This also suggests the absence of mechanical or gametic mechanisms acting against their hybridization. Although we did not test the fertility of the "hybrid" offspring, the egg viability observed in our crosses is higher than that reported for other interspecific crosses between different species in the subfamily Triatominae, where hybrid disfunction has been detected [47, 52–54]. However, the role of other pre-zygotic barriers such as temporal asynchrony, mate choice and/or habitat differences, among others, remains to be tested.

Secondly, our phylogenies and haplotype networks showed *R. taquarussuensis* nested within *R. neglectus*, with no differentiation from this species. Consequently, the species delimitation analysis collapsed these two taxa as a single one. Additionally, genetic differentiation measures as well as the discriminant analysis failed to show genetic structure between these lineages. Recent genomic analysis in animals have established that 'good-species' usually have a genetic divergence (Da) $> 2\%$, although there is a "grey zone" of speciation (in which taxonomy is often controversial), that spans from 0.5% to 2% of Da. However, any Da $< 0.5\%$ undoubtedly corresponds to populations of the same species [56]. Therefore, our Da values are consistent with a scenario of *R. taquarussuensis* being *R. neglectus* rather than a different species. Furthermore, our genetic distance (K2P) estimates between *R. neglectus* and *R. taquarussuensis* were lower than those between *R. neglectus* and *R. prolixus*, and between *R. taquarussuensis* and *R. prolixus*. This genetic similarity between *R. taquarussuensis* and *R. neglectus* in all our analyses contrast with the clear differentiation observed between *R. neglectus* and *R. prolixus*, which are known to be distinct yet closely related species. In agreement with these findings, recent studies have suggested that *R. milesi* (Carcavalho et al., 2001), another species described based on cytogenetic differences [57, 58], shows high genetic similarity with *R. neglectus* thus questioning its validity as a true species [11]. This further suggests that *R. neglectus* may be a species that shows important polymorphism in cytogenetic patterns, which should not be used for species diagnosis.

The original description of *R. taquarussuensis* reported differences in the constitutive heterochromatin pattern and nanocomposition of TA and CG rich DNA base pairs between *R.*

taquarussuensis and *R. neglectus*, mainly because *R. taquarussuensis* shows more heterochromatic blocks in the autosomes and the Y chromosome compared to the other *Rhodnius* species. Although gain and/or loss of constitutive heterochromatin has been previously used as evidence of species differentiation in the *R. pallescens* group [59], the *T. sordida* subcomplex [60, 61], and *T. dimidiata* (Latreille, 1811) [62], such heterochromatin differences between *R. neglectus* and *R. taquarussuensis* are likely just intraspecific polymorphism of *R. neglectus*. The presence of intraspecific heterochromatin variation with no apparent consequences on speciation is not new in Triatominae and has been observed in *T. infestans* (Klug, 1834) [63–65], *P. geniculatus* (Latreille, 1811) [66], and *R. pallescens* [67]. Therefore, although cytogenetics is a valuable methodology for taxonomic studies [68], heterochromatin variation between populations (i.e. the existence of cytotypes) is not a reliable trait to delimit species when evaluated alone. This agrees with the fact that cytogenetics is known to have a 20% failure rate in delimiting arthropods' species [69]. In conclusion, after performing a comprehensive analysis using mitochondrial and newly developed nuclear markers, as well as crosses between *R. taquarussuensis* and *R. neglectus*, we can confidently suggest that *R. taquarussuensis* is not a separate species and must be considered a synonym of *R. neglectus*. Our study highlights the importance of revising carefully the current taxonomy of *Rhodnius*, because only a confident species delimitation will permit to study the processes and mechanisms involved in their diversification, as well as to unveil vector/parasite associations with epidemiological relevance.

Supporting information

S1 Table. CYTB accession number for individuals downloaded from GenBank.
(DOCX)

S1 Fig. CYTB Maximum likelihood phylogeny.
(DOCX)

S2 Fig. Absolute genetic divergence (DXY) between *R. prolixus*, *R. neglectus* and *R. taquarussuensis*. (a) CYTB; (b) ND4; (c) PCB; (d) TOPO; (e) URO; (f) ZNFP. Note that DXY scale for all genes is not the same.
(DOCX)

S3 Fig. Discriminant analysis based on mtDNA (a) and nDNA (b). Densities for a single discriminant function are shown, with red being *R. taquarussuensis* and blue being *R. neglectus*.
(DOCX)

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References

1. Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, et al. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. 2007; 22(3):148–55. https://doi.org/10.1016/j.tree.2006.11.004.
2. Minard G, Tran Van V, Tran FH, Melaun C, Klimpel S, Koch LK, et al. Identification of sympatric cryptic species of *Aedes albopictus* subgroup in Vietnam: new perspectives in phylosymbiosis of insect vector. Parasites & Vectors. 2017; 10(1):276. https://doi.org/10.1186/s13071-017-2202-9 PMID: 28577575
3. Noireau F, Gutierrez T, Zegarra M, Flores R, Brenière F, Cardozo L, et al. Cryptic speciation in *Triatoma sordida* (Hemiptera: Reduviidae) from the Bolivian Chaco. Tropical medicine & international health: TM & IH. 1998; 3(5):364–72.
4. Skoracka A, Magalhães S, Rector BG, Kuczyński L. Cryptic speciation in the Acari: a function of species lifestyles or our ability to separate species? Experimental and Applied Acarology. 2015; 67(2):165–82. https://doi.org/10.1007/s10493-015-9954-8 PMID: 26209969
5. Zapata F, Jimenez I. Species delimitation: inferring gaps in morphology across geography. Systematic biology. 2012; 61(2):179–94. Epub 2011/08/16. https://doi.org/10.1093/sysbio/syr084 PMID: 21840841.
6. Galvão C. Vetores da doença de chagas no Brasil: SciELO-Sociedade Brasileira de Zoologia; 2014.
7. WHO. Chagas Disease (American trypanosomiasis). Fact sheet 340. 2018;http://www.who.int/mediacentre/factsheets/fs340/en/ 2018(cited 2018 October 3).
8. Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bulletin of the American museum of Natural History. 1979; 163(3):123–520.
9. Pinto C. Valor do rostro e antenas na caracterização dos gêneros de Triatomídeos. Hemiptera, Reduvidioidea. Bol Biol. 1931; 19:45–136.
10. Justi SA, Galvão C, Schrago CG. Geological changes of the Americas and their influence on the diversification of the Neotropical kissing bugs (Hemiptera: Reduviidae: Triatominae). PLoS neglected tropical diseases. 2016; 10(4):e0004527. https://doi.org/10.1371/journal.pntd.0004527 PMID: 27058599
11. Monteiro FA, Weirauch C, Felix M, Lazoski C, Abad-Franch F. Evolution, Systematics, and Biogeography of the Triatominae, Vectors of Chagas Disease. Advances in parasitology. 2018; 99:265–344. https://doi.org/10.1016/bs.apar.2017.12.002 PMID: 29530308
12. Monteiro FA, Barrett TV, Fitzpatrick S, Cordon-Rosales C, Feliciangeli D, Beard CB. Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and *R. robustus*. Molecular Ecology. 2003; 12(4):997–1006. PMID: 12753218
13. Rosa JA, Mendonça VJ, Gardim S, de Carvalho DB, de Oliveira J, Nascimento JD, et al. Study of the external female genitalia of 14 *Rhodnius* species (Hemiptera, Reduviidae, Triatominae) using scanning electron microscopy. Parasites & vectors. 2014; 7(1):17.

14. Neiva A, Pinto C. Estado actual dos conhecimentos sobre o gênero *Rhodnius* Stål, com a descrição de uma nova espécie. Bras Med. 1923; 37:20–4.

15. Pavan M, Monteiro F. A multiplex PCR assay that separates *Rhodnius prolixus* from members of the *Rhodnius robustus* cryptic species complex (Hemiptera: Reduviidae). Tropical Medicine & International Health. 2007; 12(6):751–8.

16. Pavan MG, Mesquita RD, Lawrence GG, Lazoski C, Dotson EM, Abubucker S, et al. A nuclear single-nucleotide polymorphism (SNP) potentially useful for the separation of *Rhodnius prolixus* from members of the *Rhodnius robustus* cryptic species complex (Hemiptera: Reduviidae). Infection, Genetics and Evolution. 2013; 14:426–33. https://doi.org/10.1016/j.meegid.2012.10.018. PMID: 23219914

17. Barata JMS. Aspectos morfológicos de ovos de triatominae: II-Características macroscópicas e exocoriais de dez espécies do gênero *Rhodnius* Stal, 1859 (Hemiptera-Reduviidae). Revista de Saúde pública. 1981; 15:490–542. PMID: 7048506

18. Rosa JA, Rocha CS, Gardim S, Pinto MC, Mendonca VJ, Ferreira Filho J, et al. Description of *Rhodnius montenegrensis* n. sp.(Hemiptera: Reduviidae: Triatominae) from the state of Rondônia, Brazil. Zootaxa. 2012; 3478:62–76.

19. Almeida FBd, Santos EI, Sposina G. Triatomíneos da Amazônia III.(). Acta Amazônica. 1973; 3(2):43–6.

20. Lent H, Jurberg J, Galvão C. Rhodnius stali n. sp. related to *Rhodnius pictipes* Stal, 1872 (Hemiptera, Reduviidae, Triatominae). Memórias do Instituto Oswaldo Cruz. 1993; 88(4):605–14.

21. Abad-Franch F, Monteiro FA, Jaramillo N, Gurgel-Gonçalves R, Dias FBS, Diotaiuti L. Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). Acta Tropica. 2009; 110(2–3):159–77. https://doi.org/10.1016/j.actatropica.2008.06.005 PMID: 18619938

22. Rosa JA, Justino HHG, Nascimento JD, Mendonça VJ, Rocha CS, de Carvalho DB, et al. A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae). ZooKeys. 2017;(675):1. https://doi.org/10.3897/zookeys.675.12024 PMID: 28769676

23. Justi SA, Galvão C. The evolutionary origin of diversity in Chagas disease vectors. Trends in parasitology. 2017; 33(1):42–52. https://doi.org/10.1016/j.pt.2016.11.002 PMID: 27986547

24. Hypša V, Tietz DF, Zrzavý J, Rego RO, Galvao C, Jurberg J. Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. Molecular phylogenetics and evolution. 2002; 23(3):447–57. PMID: 12099798

25. Justi SA, Russo CA, dos Santos Mallet JR, Obara MT, Galvão C. Molecular phylogeny of Triatomini (Hemiptera: Reduviidae: Triatominae). Parasites & vectors. 2014; 7(1):149.

26. Lyman DF, Monteiro FA, Escalante AA, Cordon-Rosales C, Wesson DM, Dujardin J-P, et al. Mitochondrial DNA sequence variation among triatomine vectors of Chagas' disease. The American journal of tropical medicine and hygiene. 1999; 60(3):377–86. PMID: 10466963

27. Meneguetti DUdO Soares EB, Campaner M Camargo LMA. First report of *Rhodnius montenegrensis* (Hemiptera: Reduviidae: Triatominae) infection by Trypanosoma rangeli. Revista da Sociedade Brasileira de Medicina Tropical. 2014; 47(3):374–6. PMID: 24728471

28. Ravazi A, Alevi K, Oliveira J, Rosa J, Azeredo-Oliveira M. Cytogenetic analysis in different populations of *Rhodnius prolixus* and *R. nasutus* from different countries of South America. Brazilian Journal of Biology. 2018; 78(1):183–5.

29. Díaz S, Panzera F, Jaramillo-O N, Pérez R, Fernández R, Vallejo G, et al. Genetic, cytogenetic and morphological trends in the evolution of the *Rhodnius* (Triatominae: Rhodniini) trans-Andean group. PLoS One. 2014; 9(2):e87493. https://doi.org/10.1371/journal.pone.0087493 PMID: 24498330

30. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics (Oxford, England). 2007; 23(10):1289–91. Epub 2007/03/24. https://doi.org/10.1093/bioinformatics/btm091 PMID: 17379693.

31. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinforma. 2009; 25. https://doi.org/10.1093/bioinformatics/btp187 PMID: 19346325

32. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32. https://doi.org/10.1093/nar/gkh340 PMID: 15034147

33. Kumar V, Reinartz W. Customer relationship management: Concept, strategy, and tools: Springer; 2018.

34. Maddison WPMaddison D. Mesquite: a modular system for evolutionary analysis. Version 3.04. 2015.

35. Maglott D, Ostell J, Pruitt KD, Tatusova T. Entrez Gene: gene-centered information at NCBI. Nucleic acids research. 2010; 39(suppl_1):D52–D7.

36. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular biology and evolution. 2014; 32(1):268–74. https://doi.org/10.1093/molbev/msu300 PMID: 25371430

37. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchene S, Fourment M, Gavryushkina A, et al. BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis. bioRxiv. 2018.

38. Bouckaert RR, Drummond AJ. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology. 2017; 17(1):42. https://doi.org/10.1186/s12862-017-0890-6 PMID: 28166715

39. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic biology. 2018; 67(5):901–4. https://doi.org/10.1093/sysbio/syy032 PMID: 29718447

40. Schliep KP. phangorn: phylogenetic analysis in R. Bioinformatics (Oxford, England). 2011; 27(4):592–3. Epub 2010/12/21. https://doi.org/10.1093/bioinformatics/btq706 PMID: 21169378; PubMed Central PMCID: PMCPmc3035803.

41. Zhang J, Kapli P, Pavlidis P, Stamatakis A. A general species delimitation method with applications to phylogenetic placements. Bioinformatics (Oxford, England). 2013; 29(22):2869–76.

42. Merilä J, Crnokrak P. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology. 2001; 14(6):892–903. https://doi.org/10.1046/j.1420-9101.2001.00348.x

43. Monteiro FA, Donnelly MJ, Beard CB, Costa J. Nested clade and phylogeographic analyses of the Chagas disease vector *Triatoma brasiliensis* in Northeast Brazil. Mol Phylogenet Evol. 2004; 32(1):46–56. Epub 2004/06/10. https://doi.org/10.1016/j.ympev.2003.12.011 PMID: 15186796.

44. Jombart T, Ahmed I. adegenet 1.3–1: new tools for the analysis of genome-wide SNP data. Bioinformatics (Oxford, England). 2011; 27(21):3070–1.

45. Leigh JW, Bryant D. popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution. 2015; 6(9):1110–6.

46. Costa J, Almeida CE, Dujardin JP, Beard CB. Crossing experiments detect genetic incompatibility among populations of *Triatoma brasiliensis* Neiva, 1911 (Heteroptera, Reduviidae, Triatominae). Memórias do Instituto Oswaldo Cruz. 2003; 98(5):637–9. PMID: 12973530

47. Mendonça VJ, Alevi KCC, de Oliveira Medeiros LM, Nascimento JD, de Azeredo-Oliveira MTV, da Rosa JA. Cytogenetic and morphologic approaches of hybrids from experimental crosses between *Triatoma lenti* Sherlock & Serafim, 1967 and *T. sherlocki* Papa et al., 2002 (Hemiptera: Reduviidae). Infection, Genetics and Evolution. 2014; 26:123–31. https://doi.org/10.1016/j.meegid.2014.05.015 PMID: 24861813

48. Rosa JA, Barata JMS, Barelli N, Santos JLF, Neto B, Miguel F. Sexual distinction between 5th instar nymphs of six species of Triatominae (Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz. 1992; 87(2):257–64.

49. Martínez-Ibarra J, Grant-Guillén Y, Delgadillo-Aceves I, Zumaya-Estrada F, Rocha-Chávez G, Salazar-Schettino P, et al. Biological and genetic aspects of crosses between phylogenetically close species of mexican triatomines (Hemiptera: Reduviidae). Journal of medical entomology. 2011; 48(3):705–7. PMID: 21661335

50. Jiggins CD, Linares M, Naisbit RE, Salazar C, Yang ZH, Mallet J. Sex-linked hybrid sterility in a butterfly. Evolution; international journal of organic evolution. 2001; 55(8):1631–8. Epub 2001/10/03. PMID: 11580022.

51. Abad-Franch F, Pavan MG, Jaramillo-O N, Palomeque FS, Dale C, Chaverra D, et al. *Rhodnius barretti*, a new species of Triatominae (Hemiptera: Reduviidae) from western Amazonia. Memórias do Instituto Oswaldo Cruz. 2013; 108:92–9. https://doi.org/10.1590/0074-0276130434 PMID: 24473808

52. Heitzmann-Fontenelle T. Bionomia comparativa de triatomíneos. VI-Híbridos de *Triatoma brasiliensis* Neiva, 1911 X *Triatoma lenti*, Sherlocki & Serafim, 1967 (Hemiptera, Reduviidade). Memórias do Instituto Butantan. 1984; 47:175–81.

53. Mendonca VJ, Alevi KCC, Pinotti H, Gurgel-Goncalves R, Pita S, Guerra AL, et al. Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the *T. brasiliensis* species complex. Zootaxa. 2016; 4107(2):239–54. https://doi.org/10.11646/zootaxa.4107.2.6 PMID: 27394816

54. Perez R, Hernández M, Quintero O, Scvortzoff E, Canale D, Mendez L, et al. Cytogenetic analysis of experimental hybrids in species of Triatominae (Hemiptera-Reduviidae). Genetica. 2005; 125(2–3):261–70. https://doi.org/10.1007/s10709-005-0369-z PMID: 16247698

55. Ceccarelli S, Balsalobre A, Medone P, Cano ME, Gonçalves RG, Feliciangeli D, et al. DataTri, a database of American triatomine species occurrence. Scientific data. 2018; 5.

56. Roux C, Fraisse C, Romiguier J, Anciaux Y, Galtier N, Bierne N. Shedding light on the grey zone of speciation along a continuum of genomic divergence. PLoS biology. 2016; 14(12):e2000234. https://doi.org/10.1371/journal.pbio.2000234 PMID: 28027292

57. Pita S, Panzera F, Ferrandis I, Galvão C, Gómez-Palacio A, Panzera Y. Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal location of ribosomal genes. Memórias do Instituto Oswaldo Cruz. 2013; 108(3):376–82.

58. Valente VdC, Valente SAdS, Carcavallo RU, Rocha DdS, Galvão C, Jurberg J. Considerações sobre uma nova espécie do gênero *Rhodnius* stal, do Estado do Pará, Brasil (Hemiptera, Reduviidae, Triatominae. Entomol vectores. 2001; 8(1):65–80.

59. Alevi K, Ravazi A, Franco-Bernardes M, Rosa J, Azeredo-Oliveira M. Chromosomal evolution in the pallescens group (Hemiptera, Triatominae). Genetics and Molecular Research. 2015; 14(4):12654–9. https://doi.org/10.4238/2015.October.19.9 PMID: 26505416

60. Bardella VB, Pita S, Vanzela ALL, Galvão C, Panzera F. Heterochromatin base pair composition and diversification in holocentric chromosomes of kissing bugs (Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz. 2016; 111(10):614–24. https://doi.org/10.1590/0074-02760160044 PMID: 27759763

61. Panzera F, Hornos S, Pereira J, Cestau R, Canale D, Diotaiuti L, et al. Genetic variability and geographic differentiation among three species of triatomine bugs (Hemiptera-Reduviidae). The American journal of tropical medicine and hygiene. 1997; 57(6):732–9. PMID: 9430537

62. Panzera F, Ferrandis I, Ramsey J, Ordonez R, Salazar-Schettino P, Cabrera M, et al. Chromosomal variation and genome size support existence of cryptic species of *Triatoma dimidiata* with different epidemiological importance as Chagas disease vectors. Tropical Medicine & International Health. 2006; 11(7):1092–103.

63. Panzera F, Dujardin JP, Nicolini P, Caraccio MN, Rose V, Tellez T, et al. Genomic changes of Chagas disease vector, South America. Emerging Infectious Diseases. 2004; 10(3):438. https://doi.org/10.3201/eid1003.020812 PMID: 15109410

64. Panzera F, Ferreiro MJ, Pita S, Calleros L, Pérez R, Basmadjián Y, et al. Evolutionary and dispersal history of *Triatoma infestans*, main vector of Chagas disease, by chromosomal markers. Infection, Genetics and Evolution. 2014; 27:105–13. https://doi.org/10.1016/j.meegid.2014.07.006 PMID: 25017654

65. Panzera Y, Pita S, Ferreiro M, Ferrandis I, Lages C, Pérez R, et al. High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Cytogenetic and Genome Research. 2012; 138(1):56–67. https://doi.org/10.1159/000341888 PMID: 22907389

66. Crossa RP, Hernández M, Caraccio MN, Rose V, Valente SAS, da Costa Valente V, et al. Chromosomal evolution trends of the genus *Panstrongylus* (Hemiptera, Reduviidae), vectors of Chagas disease. Infection, Genetics and Evolution. 2002; 2(1):47–56. PMID: 12798000

67. Gómez-Palacio A, Jaramillo-Ocampo N, Triana-Chávez O, Saldaña A, Calzada J, Pérez R, et al. Chromosome variability in the Chagas disease vector *Rhodnius pallescens* (Hemiptera, Reduviidae, Rhodniini). Memórias do Instituto Oswaldo Cruz. 2008; 103(2):160–4. PMID: 18425268

68. Ueshima N. Cytotaxonomy of the triatominae (Reduviidae: Hemiptera). Chromosoma. 1966; 18(1):97–122.

69. Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. Integrative taxonomy: a multisource approach to exploring biodiversity. Annual review of entomology. 2010; 55:421–38. Epub 2009/09/10. https://doi.org/10.1146/annurev-ento-112408-085432 PMID: 19737081.

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RESEARCH

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Phylogenetic relationships and evolutionary patterns of the genus *Psammolestes* Bergroth, 1911 (Hemiptera: Reduviidae: Triatominae)

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Abstract

Background: The evolutionary history of biodiversity in South America has been poorly studied in the seasonal dry tropical forest (SDTF). Species diversification in this ecosystem may have a twofold explanation. First, intermittent connections in the middle and late Pleistocene promoted species dispersal and/or genetic connectivity between lineages isolated in disjunct patches of forest. Second, allopatric speciation proceeded immediately after the formation and colonization of the SDTF in the Neogene. Here we studied the diversification of *Psammolestes*, a genus endemic of the SDTF and naturally infected with *Trypanosoma cruzi* (agent of Chagas disease), using a combination of phylogenetic, population genetics and niche model methods, and evaluated the reliability of the three morphospecies currently recognized.

Results: Our multilocus analyses recovered *P. coreodes* and *P. tertius* in a monophyletic clade sister to *P. arthuri*. Species delimitation tests recovered these lineages as different species despite the shared genetic variation observed between *P. coreodes* and *P. tertius* in five genes. Also, genetic variation of the genus clustered in three groups that were consistent with the three morphospecies. Our demographic model predicted a scenario of divergence in absence of gene flow, suggesting that mixed haplotypes may be the result of shared ancestral variation since the divergence of the subtropical-temperate species *P. coreodes* and *P. tertius*. In contrast, the tropical species *P. arthuri* was highly differentiated from the other two in all tests of genetic structure, and consistently, the Monmonier's algorithm identified a clear geographical barrier that separates this species from *P. coreodes* and *P. tertius*.

Conclusions: We found three genetically structured lineages within *Psammolestes* that diverged in absence of gene flow in the late Miocene. This result supports a scenario of species formation driven by geographical isolation rather than by divergence in the face of gene flow associated with climatic oscillations in the Pleistocene. Also, we identified the Amazon basin as a climatic barrier that separates tropical from subtropical-temperate species, thus promoting

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allopatric speciation after long range dispersion. Finally, each species of *Psammolestes* occupies different climatic niches suggesting that niche conservatism is not crucial for species differentiation. These findings influence the current vector surveillance programs of Chagas disease in the region.

Keywords: *Psammolestes*, Niche divergence, Seasonal dry tropical forest, Triatominae, Rhodniini, Phylogenetic, Population genetics

Background

The Andes uplift and the formation of the Amazon Basin promoted species diversification via vicariance and/or dispersal which may be associated with climatic oscillations. Many examples from multiple organisms show the effect of such geological events in species differentiation [1–6], but only a handful show the role of geomorphology and climatic variations in the diversification of species from the seasonal dry tropical forest (SDTF) [7–9]. In tropical Americas, this ecosystem includes disjunct patches characterized by relatively low rainfall and high climatic seasonality [8, 10].

Species diversification in SDTF may be the result of these dry forest patches being intermittently connected during cold and dry periods in the middle and late Pleistocene, thus promoting species dispersal and/or genetic connectivity between isolated lineages ([8, 11]; the Pleistocene Arc hypothesis). Alternatively, such diversification may be due to genetic differentiation in allopatry, that could either be coupled or not with occasional long distance dispersal events [12–15]. For example, the diversification of geckos of the genus *Phyllopezus* was not influenced by Pleistocene climatic oscillations, but show a high phylogenetic structure associated with Miocene geomorphology [16]. In contrast, divergence in birds of the genus *Phacellodomus* and arthropods such as *Nephila* or *Drosophila gouveai* seems to be a consequence of Pleistocene climatic variation [11, 17, 18]. Also, studies in plants suggest that a combination of both climatic and geological changes were important for their diversification [9, 19, 20]. However, studies on the matter are scarce, and more evidence is needed to understand the evolutionary history of species inhabiting STDF [8].

The genus *Psammolestes* belongs to the subfamily Triatominae that excels between the subfamilies of Reduviidae due to their hematophagous behavior, but specially for being vectors of *Trypanosoma cruzi* [21] (Kinetoplastida, Trypanosomatidae), which causes the Chagas disease [22]. As Chagas disease has no effective treatment (e.g. vaccine), vector control strategies arise as alternatives to prevent and control the spread of not only the Chagas disease, but other tropical diseases as well [23–25]. The establishing of successful vector control strategies could benefit from a deep understanding of the vector's biology, ecology, and evolution [26–28].

The genus *Psammolestes* (Reduviidae: Triatominae: Rhodniini) occurs in SDTF in apparent association with nests of Furnariidae birds [29–33]. This genus comprises three species, *P. arthuri* (Pinto, 1926), *P. tertius* (Lent & Jurberg, 1965) and *P. coreodes* (Bergroth, 1911), whose ecology and behavior remain largely unknown [31]. *Psammolestes arthuri* occurs across the eastern plains of Colombian and Venezuela, *P. tertius* is found in coastal regions near the Cerrado, Caatinga and the Mata Atlantica in Brazil, and *P. coreodes* distributes across the Chaco in Argentina, Paraguay, Bolivia, and Brazil [26, 34]. These species do not differ in karyotype [35–37], but are recognized based on morphological traits [31]. For example, *P. arthuri* is the most easily recognizable species based on a smooth and shiny cuticle in the thorax and the head, lack of cervical constriction, long hairs restricted to the apex of the second and third segments of the stylet, an anterolateral pronotal margin distinctly extended, and male genitalia with basal plate struts completely fused [31]. In addition, *P. tertius* and *P. coreodes* are recognized based on male genitalia morphology, anteocular distance, and post-ocular distance. Specifically, *P. tertius* has basal plate struts broadly S-shaped, while those of *P. coreodes* are hook shaped. Also, the anteocular distance in *P. tertius* is at least 2× higher than its post-ocular distance, while that of *P. coreodes* is always less than 2× [31, 38]. Additionally, recent evidence reported the existence of hybrid inviability in controlled crosses between *P. tertius* and *P. coreodes* [38].

Species of *Psammolestes* were initially grouped into the tribe Psammolestini and separated from Rhodniini [26, 39], but later they were placed back within Rhodniini because they occur in arboreal habitats and have protuberances behind the eyes [31]. Nonetheless, *Psammolestes* and *Rhodnius* were kept as separate genera as the femur and head of *Psammolestes* are wider and shorter than those of *Rhodnius* [31]. These taxonomic classifications have been tested at the molecular level, and it is well known that *Rhodnius* is paraphyletic compared to *Psammolestes* [28, 40–44]. However, only one molecular study on the phylogenetic relationships in the Triatominae subfamily included the three species of *Psammolestes*, and found *P. arthuri* sister to *P. tertius* and this clade sister to *P. coreodes* [45]. These findings

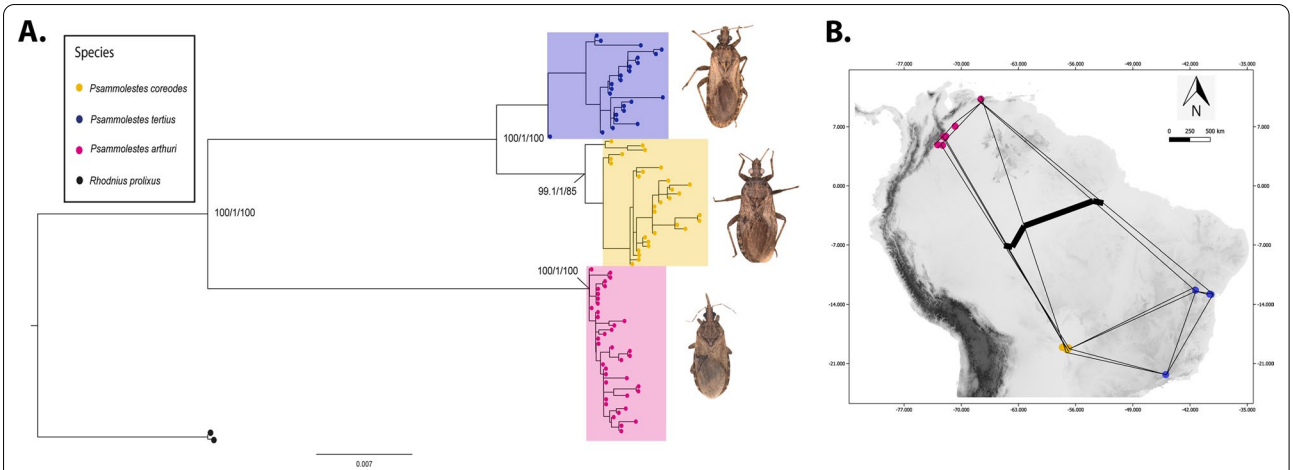


Fig. 1 Maximum Likelihood phylogenetic reconstruction and geographical barrier test based on the seven molecular loci used in this study. **A** Phylogenetic reconstruction with the ML algorithm based on the seven molecular loci used in this study. Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown. **B** Geographical barrier test (Monmonier's algorithm) with the thick black line representing the main geographical barrier, and thin lines being the Voronoi tessellation and Delaunay triangulation

suggest that *Psammolestes* is a monophyletic clade within the *prolixus* group [45].

Additionally, multiple studies have revealed a major role of niche conservatism in the diversification of the subfamily Triatominae [46–49]. For example, at the macroevolutionary scale, Ceccarelli et al. [47] found that tropical species of Triatominae share the same niche despite their phylogenetic differences, while niche conservatism in temperate species is due to shared evolutionary history. Nevertheless, the effect of niche conservatism in the diversification of species of *Psammolestes* remains to be tested. This is especially relevant as *P. arthuri* is a tropical species but *P. tertius* and *P. coreodes* have temperate distributions.

In this study, we used phylogenetic, population genetics analyses, and niche modeling to test the existence of discrete lineages within *Psammolestes* and investigate the role of the niche in maintaining these species. Our hypothesis was that the Amazon basin acts as a dispersion barrier that separates tropical and subtropical-temperate species thus suggesting a major role of geomorphology events in the divergence of *Psammolestes*. This scenario predicts that: (i) *P. coreodes* and *P. tertius* are most closely related to each other than they are to *P. arthuri,* and (ii) species differentiation proceeds despite niche conservatism. The understanding of the biotic and abiotic processes that shape vector species diversity of tropical diseases, as well as, the factors involved in their speciation process are essential for the settlement of adequate strategies for disease transmission control [23].

Results

Molecular phylogenetics

The resulting ML gene topologies were not concordant. The CYTB and PJH topologies (see Additional files 1 and 2) recovered *P. coreodes* and *P. tertius* as sister monophyletic clades, while 28S, CISP, LSM, TRNA and UPCA topologies (see Additional files 3, 4, 5, 6 and 7) did not recover them as reciprocally monophyletic. However, all the seven gene topologies showed *P. arthuri* as a well-supported monophyletic clade. Topological discordance is likely due to differences in coalescence times between loci, where the process of lineage sorting occurred faster in genes with small population size [50]. Alternatively, gene flow could explain allele sharing (see below: "Assessment of different demographic models").

Our concatenated ML phylogenetic reconstruction recovered *P. coreodes* and *P. tertius* as sister species, and this clade was sister to *P. arthuri*. Overall, the three *Psammolestes* species were monophyletic with strong node supports (Fig. 1). Also, a multilocus Bayesian species coalescent (MSC) analysis revealed a species tree with the same topology than the ML tree with posterior probabilities > 0.96 (Additional file 8).

Finally, the mtDNA tree estimated by Bayesian inference also recovered the same relationships between the *Psammolestes* species with high posterior probabilities (see Additional file 9). Our dated phylogeny suggests that *P. arturi* diverged from the ancestor of *P. coreodes* and *P. tertius* 4.84 Mya (95% HPD interval = 1.32–10.38 Mya; see Additional file 9). We also found that the subtropical tempered species diverged 3.75 Mya (95% HPD interval = 0.92–8.15 Mya; see Additional file 9).

Species delimitation tests

Three out of four models tested in BPP with nDNA loci recovered the known *Psammolestes* species. The only exception was the model 'deep divergence and large population size', which delimited two species: (i) *P. arthuri*, and (ii) *P. coreodes* + *P. tertius* (Table 1). Also, mtDNA delimited three species in the four models tested (Table 1). Consistently, mPTP strongly supported (ASV = 0.87) the same three independent lineages (see Additional file 10).

Population genetics analyses

Population substitution rate (θ) and nucleotide diversity (π) values were similar among the three *Psammolestes* species in each of the seven loci (Table 2). The three species showed signatures of population expansion in some loci, but this pattern was stronger and more consistent in *P. arthuri*. Consistently, haplotype networks displayed the typical star-like pattern where central haplotypes are coupled with multiple haplotypes with singletons (Fig. 2). In agreement with the haplotype networks, we detected stronger genetic differentiation between *P. arthuri* and both *P. coreodes* and *P. tertius* (see Additional files 11, 12, 13, 14, 15, 16 and 17), whereas genetic differences were weaker between *P. coreodes* and *P. tertius.* The structure algorithm recovered three clusters that were concordant with the three *Psammolestes* species (Fig. 3, see Additional files 10 and 18), although some *P. tertius* individuals showed shared ancestry with *P. coreodes*. Additionally, we found that isolation by distance contributed to the genetic structure observed in our data (Fig. 1B, Additional files 19, 20). This is mainly due to the geographical distance of *P. arthuri* compared to the other two species. Consequently, Monmonier's algorithm [51] supports a geographical break that coincides with the Amazon basin (see Additional files 1, 2, 3, 4, 5, 6 and 7) splitting tropical species (*P. arthuri*) from temperate species (*P. coreodes* and *P. tertius*). This geographic break was recovered in all genes, suggesting that the tropical *P. arthuri* diverged from the other two temperate species in allopatry (Additional files 1, 2, 3, 4, 5, 6 and 7).

Assessment of different demographic models

Our results suggest that the demographic model of 'divergence without gene flow' fitted our data better than other models with unidirectional or bidirectional gene flow. However, this scenario shows some uncertainty (wAIC = 0.30) as AIC values were not considerable different between models (see Additional files 18 and 21).

Environmental niche modeling

We found that the ensemble model fitted better than each independent algorithm (ROC > 0.95). This model showed different non-overlapping suitable areas for each species of *Psammolestes* (Fig. 4). Overall, areas with higher occurrence probability for the three species were restricted to dry environments such as tropical savannas and the amazon basin showed the lowest suitability values. Moreover, we discovered that the distribution of each *Psammolestes* species was determined by different environmental variables: annual precipitation for *P. tertius,* annual range of temperature for *P. coreodes*, and isothermality for *P. arthuri* (Fig. 4). Consistently, the niche equivalence tests indicate that climatic niches of these species have diverged (Table 3).

Discussion

We recovered three well supported lineages that are concordant with the previously described morphospecies and experimental crosses: *P. coreodes, P. tertius* and *P. arthuri* [38]. Both phylogenetic and population genetics analyses indicate that *P. coreodes* and *P. tertius* are genetically more similar than they are to *P. arthuri*. The species distribution analyses suggest that these species are restricted to tropical savannas and have a low probability of occurrence in humid areas. These findings support a role for the Amazon basin as an absolute barrier for the dispersal of species of *Psammolestes*.

Our phylogenetic reconstruction contrasts with a previous study where *P. tertius* and *P. arthuri* were recovered as sister species (bootstrap support = 66%), and this clade sister to *P. coreodes* (bootstrap support = 87%) [45]. However, here we obtained higher support values in our ML tree (Fig. 1A) and the species tree (Additional file 9A) for

Table 1 Species delimitation by Bayesian phylogenetics and phylogeography program

Model	nDNA loci			mtDNA loci		
	Posterior	Species	Species delimited	Posterior	Species	Species delimited
Deep large	0.9950	2	*P. arthuri* *P. terius/P. coreodes*	1	3	*P. arthuri* *P. terius* *P. coreodes*
Deep small	1	3	*P. arthuri* *P. terius* *P. coreodes*	1	3	
Shallow large	1	3		0.8043	3	
Shallow small	1	3		1	3	

Table 2 Population genetics summary statistics for each species per locus

Statics	28S			CISP			CYTB			LSM			PJH			TRNA			UPCA		
	P.art	*P.cor*	*Pter*	*P.art*	*P.cor*	*Pter*	*P.art*	*P.cor*	*Pter*	*P.art*	*P.cor*	*Pter*	*P.art*	*P.cor*	*Pter*	*P.art*	*P.cor*	*Pter*	*P.art*	*P.cor*	*Pter*
n	35	16	4	20	28	25	13	21	7	36	26	23	37	28	30	50	39	22	40	29	16
h	7	6	3	7	19	9	6	6	7	8	9	7	5	8	8	15	10	7	14	11	3
S	8	6	5	6	19	11	11	27	27	29	6	7	14	17	5	38	9	7	43	12	2
θ	0.0035	0.0032	0.0049	0.0027	0.0080	0.0048	0.0070	0.0128	0.0222	0.0099	0.0022	0.0027	0.0051	0.0067	0.0019	0.0132	0.0033	0.0030	0.0167	0.0050	0.0010
π	0.0012	0.0020	0.0045	0.0012	0.0058	0.0045	0.0057	0.0082	0.0206	0.0051	0.0020	0.0018	0.0013	0.0034	0.0018	0.0051	0.0024	0.0020	0.0052	0.0056	0.0005
D_T	− 1.92*	− 1.27*	− 0.79*	− 1.71*	− 0.97*	− 0.21	− 0.76	− 1.39	− 0.40	− 1.68*	− 0.31	− 0.96	− 2.38*	− 1.71*	− 0.15	− 2.06*	− 0.74	− 0.97	− 2.42*	0.36	− 1.03
R2	0.07	0.10	0.25	0.07*	0.08*	0.12	0.12	0.08*	0.17	0.05*	0.11	0.09	0.12	0.12	0.11	0.04*	0.08	0.09	0.13	0.13	0.13
Fu and Li's F	− 2.82*	− 1.53	− 0.75	− 1.85	− 1.10	0.71	− 0.59	− 0.67	− 0.66	− 0.05	− 0.33	− 0.86	− 3.93^	− 3.00*	0.16	− 1.78	− 0.68	− 1.38	− 4.74^	0.19	− 0.73
Fu and Li's D	− 2.63*	− 1.36	− 0.79	− 1.55	− 0.93	0.96	− 0.43	− 0.23	− 0.65	0.78	− 0.27	− 0.66	− 3.79^	− 2.94*	0.26	− 1.17	− 0.50	− 1.27	− 4.82^	0.07	− 0.50

n: number of samples, h: number of haplotypes, S: number of segregating sites, θ: population mutation rate, π: average pairwise distance, D_T: Tajima's D. R2: Ramos-Onsins & Rozas' R2, Fu and Li's F, and Fu and Li's D

"*" symbolizes $p < 0.05$ and "^" symbolizes $p < 0.02$

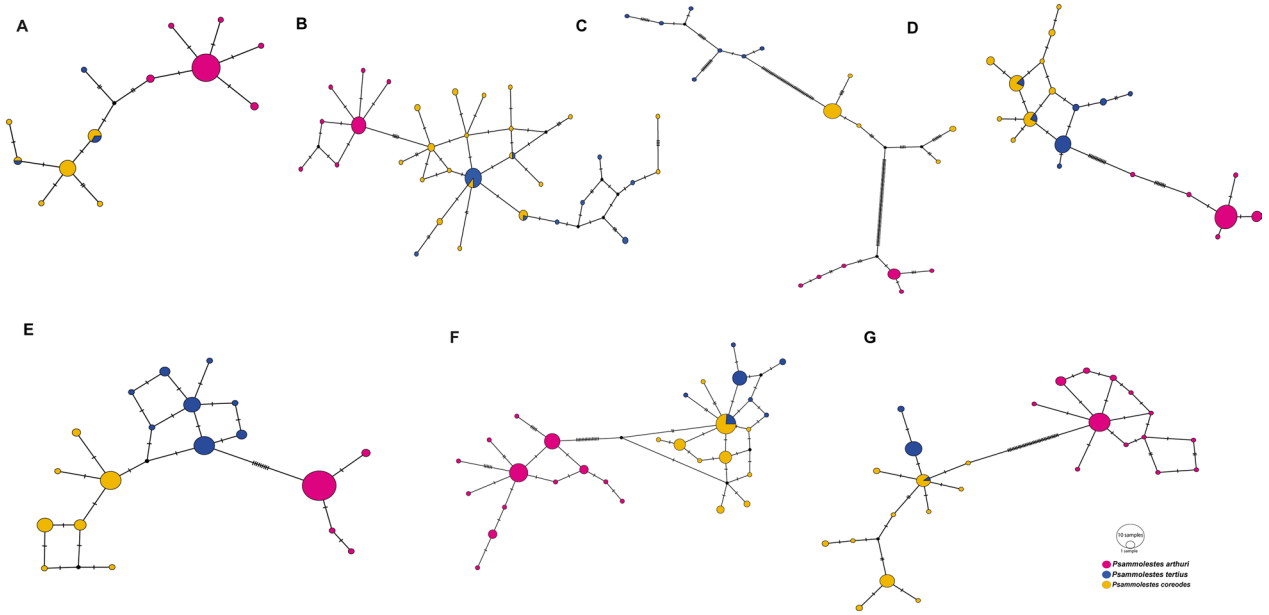


Fig. 2 Haplotype networks obtained from the molecular data of the markers. **A** 28S, **B** CISP, **C** CYTB, **D** LSM, **E** PJH, **F** TRNA, **G** UPCA

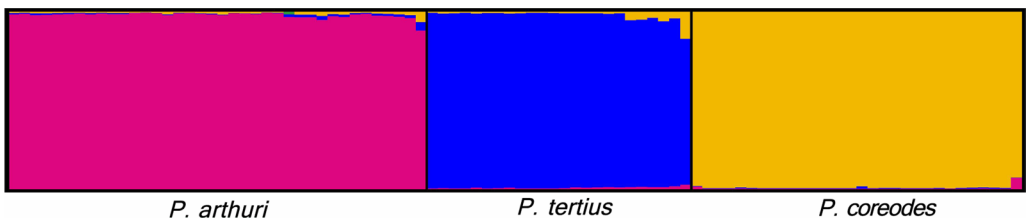
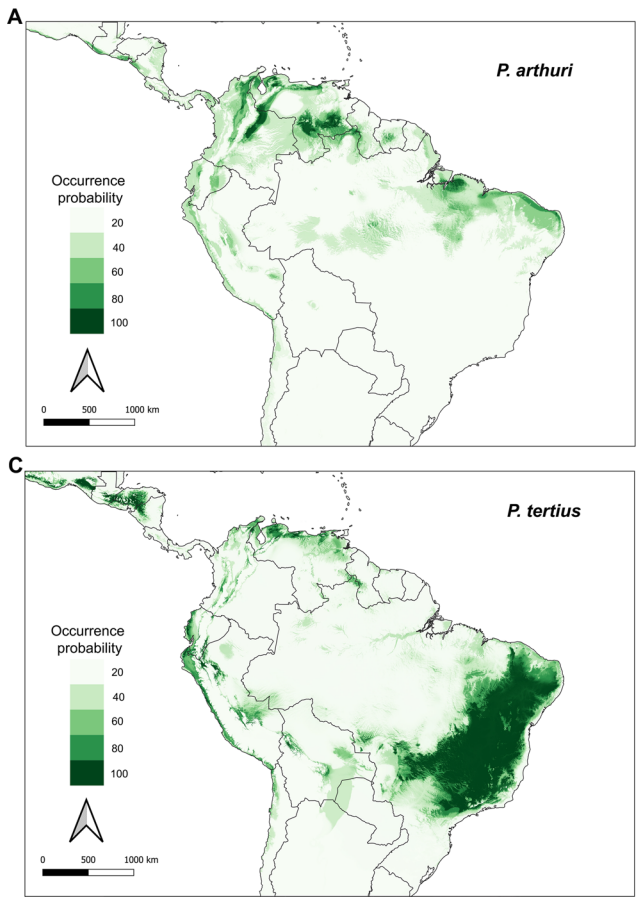


Fig. 3 Population structure analysis plot values using STRUCTURE. Graphical output from the *distruct* software plotted using K = 3 and the matrix of aligned Q values from populations and individuals obtained from CLUMPP. Input sequences were organized from left to right in the following order: *P. arthuri*, *P. tertius*, and *P. coreodes*. Each bar represents an individual, and the color of the bar represents the likelihood of that individual of belonging to a population. Pink color represents the likelihood of belonging to *P. arthuri*, blue to *P. tertius*, and yellow to *P. coreodes*

the monophyly of the clade composed by *P. tertius* and *P. coreodes,* sister to *P. arthuri*. This result is consistent both with the geographic and genetic distance between these taxa. Despite of the contentious systematics of the genus [28, 40–44], all our analyses validate the existence of three lineages of *Psammolestes* thus supporting the original species description based on morphological traits [31, 35, 38].

Our mtDNA divergence times estimation, the strong genetic structure we observed, and the absence of gene flow between species suggest that the diversification of *Psammolestes* is not explained by recent dispersal events across corridors in the forested Amazon basin nor by the Pleistocene arc hypothesis [7]. In contrast, our results agree with a scenario of allopatric differentiation via long distance dispersal event(s) across the Amazon in the late Miocene, followed by recent local geographic expansion as suggested by the Tajimas' D value [52–55]. However, we cannot rule out that the current disjunct distribution of the different species of *Psammolestes* is the result of extinction in the Amazon basin. Interestingly, the diversification times of *Psammolestes* do not mirror those of *Phacellodomus rufifrons* (Furnariidae), a bird whose nests are commonly invaded by these kissing bugs [11] and whose diversification occurred in the presence of gene flow in the Pleistocene [11]. Therefore, the historical dispersion patterns of Furnariidae birds do not explain the diversification of *Psammolestes*. Nevertheless, future studies are needed to understand the evolutionary importance of this peculiar association with Furnariidae birds,



D

Variable	*P. tertius*	*P. coreodes*	*P. arthuri*
Altitude	0.193	0.023	0.081
Mean of the annual temperature	0.113	0.159	0.074
Isothermality	0.110	0.240	0.410
Annual range of temperature	0.109	0.513	0.110
Annual precipitation	0.433	0.059	0.084
Precipitation seasonality	0.020	0.040	0.075

Fig. 4 Environmental niche modeling test. **A** *P. arthuri* **B** *P. coreodes* **C** *P. tertius*. Highlighted areas in dark green correspond to the areas where it is more probable to find individuals of each one of the three species. **D** Variables measured on the models. Highlighted in red there is the variable that influences the most of the distribution of each one of the species

Table 3 Niche overlap test (NOT) and Niche Divergence test (NDT) results for each one of the combinations between *Psammolestes* species

Species 1	Species 2	Niche overlap test (NOT)				Niche divergence test (NDT)				Interpretation
		Equivalency test		Background test		Equivalency test		Background test		
		D	p value	D	p value	D	p value	D	p value	
P. arthuri	*P. tertius*	0.03309	0.00099	0.16666	0.375	0.03309	0.00099	0.14285	0.1666	Strong evidence niches have diverged
P. arthuri	*P. coreodes*	0.00189	0.00099	0.1578	0.768	0.00189	0.00099	0.456	0.9809	Strong evidence niches have diverged
P. coreodes	*P. tertius*	0.00351	0.00099	0.2	0.05882	0.00351	0.00099	0.00099	0.0625	Strong evidence niches have diverged

which seems to be exclusive to these Triatominae species.

Our niche modeling results suggest that, although all species of *Psammolestes* occur in the SDTF, they have divergent niches shaped by different climatic predictors, indicating that niche conservatism does not play a role in the diversification of these triatomines. This finding agrees with previous studies that documented nonoverlapping niches for *P. coreodes* and *P. tertius* [30, 32]. Such an scenario of niche divergence agrees with the absence of gene flow between the three species and the inviability reported in experimental crosses between *P. tertius* and *P. coreodes* [38]. However, the relevance of other factors in species divergence, such as biotic interactions need to be investigated.

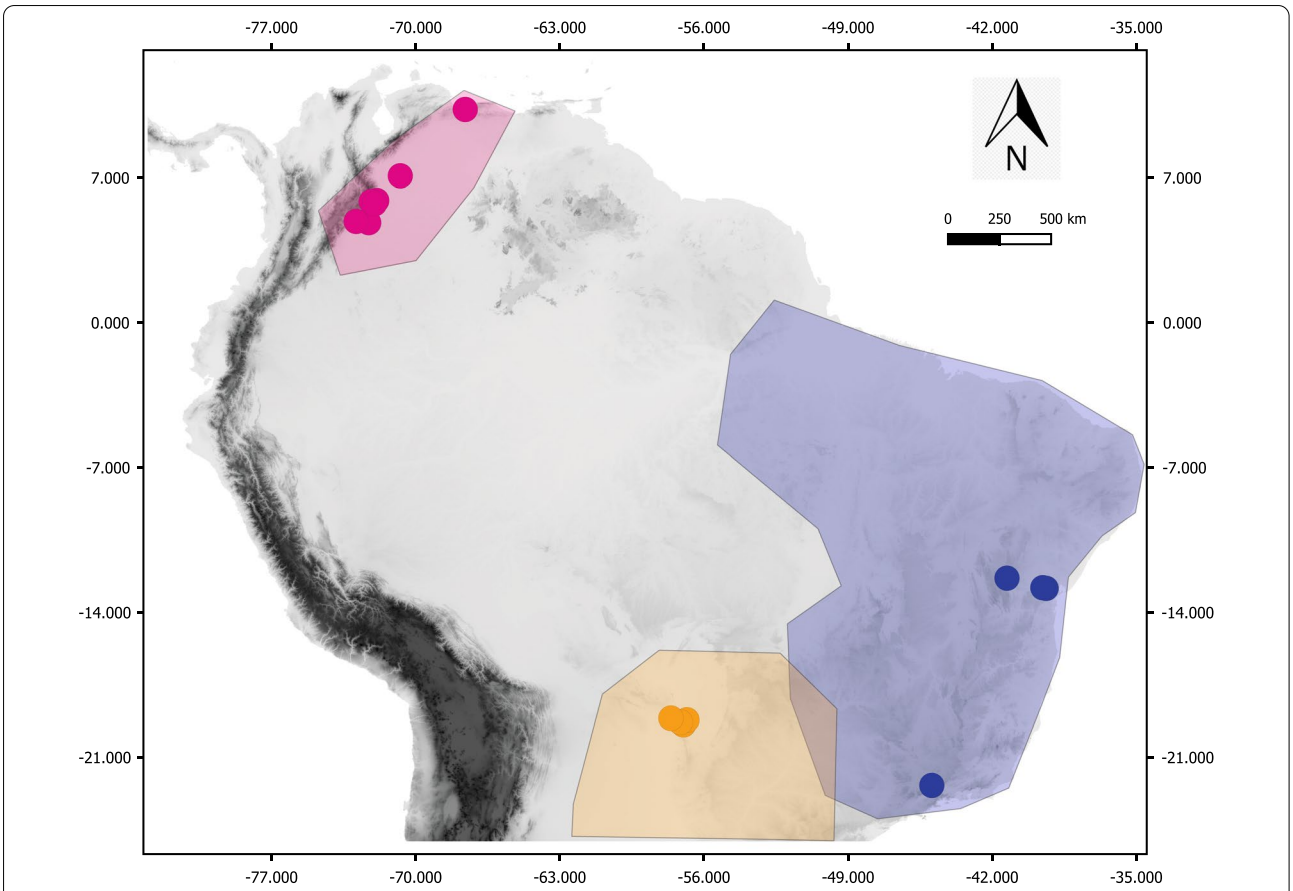


Fig. 5 Distribution of the three *Psammolestes* species sampled in this study. Dots represent the sampling sites of this work while polygons symbolize previously reported sampling sites by Ceccarelli et al. [34], where the *Psammolestes* species have been found. *P. arthuri* is represented by the pink color, *P. coreodes* with yellow, and *P. tertius* with blue

In summary, *Psammolestes* has three genetically structured species that also differ in their climate niches and morphology. They diverged in allopatry without gene flow, and their differentiation involved long distance dispersal event(s) across the Amazon basin (which is a current barrier for their dispersal). Further investigation is needed to elucidate the behavior and ecology of each species as well as the reproductive barriers maintaining their integrity. These findings are relevant in terms of understanding the transmission dynamics of Chagas disease and future improvement of vector control strategies in endemic countries.

Materials and methods

Sampling

We collected a total of 92 individuals of the three *Psammolestes* species, from 12 localities in Venezuela, Colombia, and Brazil (Fig. 5; Additional file 22). We also sampled *Rhodnius prolixus* to use as an outgroup in our phylogenetic inferences (see below). Outgroup selection was based on previous phylogenetic reconstructions, where *Psammolestes* was shown to be sister taxa to some of the *prolixus* group species (*Rhodnius* seems to be paraphyletic with respect to *Psammolestes*), including *R. prolixus* [45]. The samples obtained were preserved in absolute ethanol and stored at − 20 °C until needed. All collections were done under the permit 63257-2014 awarded to Universidad del Rosario by the ANLA (Autoridad Nacional de Licencias ambientales).

Ethical statement

This study was submitted and approved by the ethics committee of Universidad del Rosario entitled "*Genómica, evolución y biogeografía de especies del género Rhodnius: vectores de la enfermedad de Chagas*" act number 007/2016.

Extraction, amplification, and alignment of DNA data

We extracted DNA from leg tissue, using the DNeasy® Blood & Tissue kit, with modifications in the original protocol suggested by the manufacturer for extractions

in insects [56]. We amplified and sequenced seven loci to explore phylogenetic relations among *Psammolestes*: Four new nuclear loci, tRNA Guanine (37) -N (1) methyltransferase (TRNA), Putative juvenile hormone inducible protein (PJH), Probable cytosolic iron sulfur protein assembly protein Ciao 1 (CISP), Lipoyl synthase, mitochondrial (LSM), along with the previously reported Uncharacterized Protein for Cell Adhesion (UPCA) [57, 58] and two loci previously used in Rhodniini tribe phylogenetic analyses, 28S rRNA (28S) [43] and Cytochrome b (CYTB) [28, 59] (see Additional file 23). Amplicons were visualized on a 1.5% agarose gel and the products amplified were purified using the PCR kit ExoSAP-IT Product Cleanup (Affymetrix, Santa Clara, CA, USA) and bidirectionally sequenced by the Sanger method. Contigs were assembled, checked, and edited in CLC Main Workbench 20.0 (https://digitalinsights.qiagen.com). Sequence alignment per locus was performed using MAFFT [60] and the results were visually inspected and manually corrected if necessary, using Mesquite [61]. We ran PHASE algorithm with 1000 iterations per simulation implemented in DnaSP v6.12.03 [62] to resolve alignment ambiguities. Finally, we generated a concatenated alignment with the seven loci in Mesquite (nuclear and mitochondrial: 4.342 bp) [61]. Sequences from this study were submitted in GenBank and numbers can be visualized in Additional file 24.

Molecular phylogenetic analysis

We reconstructed phylogenetic relationships among the three *Psammolestes* species for each locus and the concatenated alignment (one partition per locus) using maximum likelihood (ML) inference in IQ-Tree 2 [63]. We selected the best substitution model for each case using the IQ-Tree 2 tool ModelFinder [64] based on the Bayesian Information Criterion (BIC; Schwarz, 1978). The substitution model selected for each locus was: HKY + F for 28S rRNA (28S) and RNA Guanine (37) -N (1) methyltransferase (TRNA), F81 + F + I for Lipoyl synthase, mitochondrial (LSM), F81 + F for Probable cytosolic iron sulfur protein assembly protein Ciao 1 (CISP), HKY + F + I for Putative juvenile hormone inducible protein (PJH), K2P for Uncharacterized Protein for Cell Adhesion (UPCA), and HKY + F + G4 for Cytochrome b (CYTB). Node support was assessed with UltraFast Bootstrap [66], aBayes [67] and SH-aLRT [68] with 10,000 pseudoreplicates in all cases. For the partitioned analysis, node supports were calculated by resampling both the partitions and the sites within the resampled partitions [69].

We also estimated the *Psammolestes* species tree using multilocus coalescence species approach in BEAST2 v.2.6.6 with genes included in this study [70, 71]. We executed three independent runs of 50 million generations, sampling every 1000 generations with burn-in of 15.000 chains. We determined the appropriate molecular clock in MEGA 10.0 [72] and used relaxed uncorrelated lognormal clocks for all partitions. We selected Yule model for speciation process and used the best models of substitutions estimated in IQ-tree [63]. The LogCombiner v.1.10.4 [73] tool was used to combine independent log files and species trees files obtained in each run (three). Trees were visualized in DensiTree v.2.1. The convergence of the chains in the model was examined by confirming the trace files in Tracer v.1.7.1 [74], obtaining an effective sample size of > 200 for all parameters. Lastly, maximum credibility tree was produced in Tree Annotator with burn in of 10% and visualized in Figtree [74].

Finally, we estimated divergence times using the mitochondrial locus CYTB in BEASTv.2.6.6 [70]. We only used this locus, because is the only one with a reported substitution rate, which is 0.012–0.018 substitution/site/million years, and has been used for node dating in previous works [75, 76]. We used a Yule model with two independent runs of 80 million generations, sampled every 1000 generations. We examined the convergence of the chains in Tracer [74] to confirm that the effective sample sizes of the parameters were > 200. We combined the independent runs in Logcombiner [73, 77] and selected the maximum credibility tree in tree annotator, discarding the 10% of the trees as burn-in .

Species delimitation tests

We established the number of *Psammolestes* lineages with two delimitation methods: The Bayesian Phylogenetics and Phylogeography method (BPP; [78]) and the multi-rate Poisson Tree Processes method (mPTP; [79]). For the BPP analysis, we analysed the mtDNA and nDNA independently as recommended elsewhere [78]. We performed a species tree estimation and joint species delimitation for both datasets, assigning individuals to a "species" based on the results of the phylogenetic trees previously constructed [80]. We implemented four combinations of priors, for divergence times (t) and population size parameters (q), allowing to test different evolutionary scenarios: large population sizes ($\theta = G$ (1, 10)), shallow population sizes ($\theta = G$ (2, 2000)), deep divergence times (t = G (1, 10)) and shallow divergence times (t = G(2, 2000)). Each analysis used 100,000 iterations per run, sampling every 2 iterations, and using 10% of the iterations in the chain as burn-in.

We used the best ML concatenated tree for the mPTP method. The first step on this method is to calculate the minimum branch length of the tree, correcting the potential error when similar sequences are present. Then, we ran 10 MCMC replicates of 100,000,000 steps, sampling

every 1000 steps, of which 10% were used as burn-in. Lineage congruence between both methods were considered as putative species following Carstens et al. [81].

Population genetics analyses

We calculated the haplotype diversity (*h*), number of segregating sites (*S*), population substitution rate (θ), and nucleotide diversity (π) to characterize the genetic variability of each *Psammolestes* species in DNASP v6.12.03 [62]. Moreover, we determined the genetic structure among the three species of *Psammolestes* with a relative measure (F_{ST}) and two absolute ones (D_a, D_{xy}). To evaluate deviations from panmixia, we implemented the Hudson permutation test [82] with 1000 replicates. We also computed three neutrality tests: Ramos-Onsins and Rozas R_2 (R_2; Ramos-Onsins and Rozas [83]), Tajima's D (D; Tajima [84]) and Fu & Li's F and D statistics (FF, FD; Fu and Li [85]), in order to examine possible signatures of population expansion or contraction. We constructed TCS haplotype networks [86] for each locus using PopArt v1.7 [87].

We explored the geographical diversification of *Psammolestes* testing for isolation by distance implementing a Mantel test in the R package *vegan* [88] and a linear regression between the genetic distance ($1/1 - F_{ST}$) and the geographical distances calculated in the package *geosphere* [89]. Additionally, we employed the Monmonier's algorithm [90] in the R package *adegenet* [91] using a Delaunay triangulation to detect possible boundaries associated with geographic barriers.

Lastly, STRUCTURE v2.3.4 [92] was implemented to determine the number of genetic clusters (K) present in our data. We ran the analysis with the admixture model with uncorrelated alleles using 100,000 MCMC iterations, sampling K values from 1 to 10, and 5 iterations per K, along with a burn-in length of 100,000. The best K value was selected following Evanno et al. [93] and plotting the mean likelihood L(k) and variance per K using the STRUCTURE HARVESTER (Earl and vonHoldt [94]; http://taylor0.biology.ucla.edu/structureHarvester/; Evanno et al. [95]). The results of the best identified values of k were summarized in clump [95] and plotted using *distruct* [96].

Environmental niche modelling

Species distribution modelling

Models were constructed using BIOMOD2 package [97] for each *Psammolestes* species using four algorithms: Artificial Neural Networks (ANN; [98]), Generalized Linear Models (GLM; [99], Generalized Boosting Models (GBM; [100]), and Maximum Entropy Models (MAXENT [101]). We obtained *Psammolestes* species occurrence records from DataTri [34]. As we do not have an absence record for these species, we generated a pseudoabsences database limited to areas in south America where: (i) other Triatominae species were recorded, but *Psammolestes* was absent, and (ii) environmental conditions are not suitable for these taxa [102, 103]. An equal weighting for presences and pseudo-absences (prevalence weights = 0.5) was applied for modeling as recommended [104]. Five environmental variables were used (annual mean temperature, isothermality, annual range of temperature, annual precipitation and precipitation seasonality) at spatial resolution of 1 km. These variables were chosen from the 19 CHELSA layers [105] because they exhibited correlation values < 0.5 among them. Additionally, we used a topographic variable (altitude) obtained from Reuter, Nelson and Jarvis [106]. Algorithms were calibrated using 80% of the occurrence points and evaluated the accuracy of the models with the remaining 20%. This procedure (cross-validation) was repeated three times. Three different ensemble models were generated for the three *Psammolestes* species based on the combination of the four models produced by the previously mentioned algorithms. Two metrics were used to choose the model that best predicts the distribution of the taxa: The True Skill Statistic (TSS) and the area under the curve (AUC) of the receiver-operating characteristic (ROC) [107]. Variable importance to the model was calculated based on the Pearson correlation coefficient between the model with all variables and model where each variable was omitted in turn, using BIOMOD2 package [97].

Environmental niche of the parental species

We estimated the environmental niche equivalence between all pairs of *Psammolestes* species using R package *humboldt* [108]. To do this, the overlap *Schoener's D* statistic was calculated. This statistic goes from 0 to 1, meaning no overlap and full overlap respectively [109]. D statistical significance was obtained comparing the realized niche overlap against a null distribution of 1000 randomly generated overlaps from the reshuffled occurrence dataset and tested whether niche background and niche equivalency were different from the expectations by chance at α = 0.05 [108]. This was done using the entire species distribution under comparison (niche overlap test = NOT) and using only the area where they overlap (niche divergence test = NDT) [110]. We interpreted the NOT and NDT results following Table 2 from Brown and Carnaval [110].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-022-01987-x.

Additional file 1. CYTB Phylogenetic reconstruction and Barrier test. (A) Phylogenetic reconstruction with the ML algorithm based on the mitochondrial marker CYTB (B) Barrier test algorithm based on molecular and geographical arrays. Bootstrap values on the internal nodes are shown

in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 2. PJH Phylogenetic reconstruction and Barrier test (A) Phylogenetic reconstruction with the ML algorithm based on the nuclear marker PJH (B) Barrier test algorithm based on molecular and geographical arrays. Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 3. 28S Phylogenetic reconstruction and Barrier test (A) Phylogenetic reconstruction with the ML algorithm based on the nuclear marker 28S (B) Barrier test algorithm based on molecular and geographical arrays (B). Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 4. CISP Phylogenetic reconstruction and Barrier test (A) Phylogenetic reconstruction with the ML algorithm based on the nuclear marker CISP (B) Barrier test algorithm based on molecular and geographical arrays. Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 5. LSM Phylogenetic reconstruction and Barrier test. (A) Phylogenetic reconstruction wit the ML algorithm based on the nuclear marker LSM (B) Barrier test algorithm based on molecular and geographical arrays. Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 6. TRNA Phylogenetic reconstruction and Barrier test. (A) Phylogenetic reconstruction with the ML algorithm based on the nuclear marker TRNA (B) Barrier test algorithm based on molecular and geographical arrays. Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 7. UPCA Phylogenetic reconstruction and Barrier test. (A) Phylogenetic reconstruction with the ML algorithm based on the nuclear marker UPCA (B) Barrier test algorithm based on molecular and geographical arrays. Bootstrap values on the internal nodes are shown in the following order: SH-aLRT/aBayes/ultrafast bootstrap support. Only nodes with bootstrap values higher than 60 are shown.

Additional file 8. Bayesian inference of species tree based on multilocus data (A) Maximum clade credibility tree based on Bayesian inference of the seven genes used in this study. The values observed represent posterior probabilities. (B) Bayesian species tree from multilocus data.

Additional file 9. Bayesian inference phylogenetics tree for the locus CYTB obtained in *BEAST. Horizontal purple bars illustrate the 95% HPD for the nodes' divergence time. Branch with a posterior probability above 0.95 are show

Additional file 10. Posterior probabilities on nodes, calculated by the mPTP algorithm.

Additional file 11. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the nuclear marker 28S.

Additional file 12. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the nuclear marker CISP.

Additional file 13. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the mitochondrial marker CYTB.

Additional file 14. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the nuclear marker LSM.

Additional file 15. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the nuclear marker PJH.

Additional file 16. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the nuclear marker TRNA.

Additional file 17. Heatmaps calculated for three different statistics: A) Fst, B) Dxy and C) Da for three species based on the molecular data obtained from the nuclear marker UPCA.

Additional file 18. Demographic models created with Phylogeographic Inference Using Approximate Likelihoods (PHRAPL) to test the evolution of *Psammolestes*. (A) Divergence with no migration (B) Divergence with bidirectional migration between *P. coreodes* and *P. tertius*. (C) Divergence with bidirectional migration between *P. tertius* and *P. arthuri*. (D) divergence with bidirectional migration between *P. tertius* with *P. coreodes*, and *P. tertius* with *P. arthuri*. (E) Divergence with bidirectional migration between *P. coreodes* and *P. arthuri*. (F) Divergence with bidirectional migration between *P. tertius* with *P. coreodes*, and *P. coreodes* with *P. arthuri*. (G) Divergence with bidirectional migration between *P. tertius* with *P. arthuri*, and *P. arthuri* with *P. coreodes* (H) Divergence with bidirectional migration between the three *Psammolestes* species. Starting from this point, all of the demographic models include bidirectional migration between *P. arthuri* and the MRCA (most recent common ancestor) of *P. tertius* and *P. coreodes*. (I) Divergence with bidirectional migration between *P. arthuri* and the MRCA of *P. tertius* and *P. coreodes*. (J) Divergence with bidirectional migration between *P. coreodes* and *P. tertius*. (K) Divergence with bidirectional migration between *P. tertius* and *P. arthuri*. (L) divergence with bidirectional migration between *P. tertius* with *P. coreodes* and *P. arthuri*. (M) Divergence with bidirectional migration between *P. coreodes* and *P. arthuri*. (N) divergence with bidirectional migration between *P. coreodes* with *P. tertius* and *P. arthuri*. (O) Divergence with bidirectional migration between *P. arthuri* with *P. coreodes* and *P. tertius*. (P) Divergence with bidirectional migration between the three *Psammolestes* species. Support values for the demographic scenarios are shown under each figure.

Additional file 19. Linear correlations of Isolation by distance (IBD) test. (A) 28S (B) CISP (C) CYTB (D) LSM (E) TRNA (F) UPCA (G) PJH.

Additional file 20. Mantel's test for isolation by distance (IBD) and linear correlation results. The result of the Mantel's test is shown in the two first columns of the table, and the results of the Pearson's correlation test correspond to the third column. The last two columns show the results of the linear correlation tested between geographical and genetic distances.

Additional file 21. Fit of the demographic models tested in.

Additional file 22. Individuals of *Psammolestes* species collected in this study.

Additional file 23. Genes included in this study, the primers used to obtain their corresponding sequence, and the length of each one of them. "*" symbolizes a new marker used for the delimitation of the Psammolestes species.

Additional file 24. GenBank accession numbers of seven loci analyzed in this study and samples origin included in this study.

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Authors' contributions
Conceptualization: JDR, CS and CH. Data curation: CH, MA NB, JDR. Formal analysis: CH, MA, FCSR, CS and JDR. Funding acquisition: JDR, CS, CH. Methodology: MA, FCSR, CS, CH, NB, NR, JO, KCCA JAR, JDR, PU. Project administration: JDR, CS. Resources: JDR, PU. Software: CH, MA, FCSR and JDR. Supervision: CS and JDR. Validation: FCSR, CH, CS and JDR. Writing—original draft: CH, MA, FCSR, CS. Writing—review and editing: CH, MA, FCSR, PU, CS and JDR. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its additional information files]. The sequences obtained in this study are available under the GenBank accession numbers OM256834-OM256940, OM256942-OM257062.

Declarations

Ethics and consent to participate

This study was submitted and approved by the ethics committee of Universidad del Rosario entitled "***Genómica, evolución y biogeografía de especies del género Rhodnius: vectores de la enfermedad de Chagas"*** act number 007/2016.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Bedoya AM, Leaché AD, Olmstead RG. Andean uplift, drainage basin formation, and the evolution of plants living in fast-flowing aquatic ecosystems in northern South America. New Phytol. 2021;232:2175–90. https://doi.org/10.1111/nph.17649.
2. Salgado-Roa FC, Gamez A, Sanchez-Herrera M, Pardo-Díaz C, Salazar C. Divergence promoted by the northern Andes in the giant fishing spider *Ancylometes bogotensis* (Araneae: Ctenidae). Biol J Linn Soc. 2021;132:495–508. https://doi.org/10.1093/biolinnean/blaa220.
3. Quintero E, Ribas CC, Cracraft J. The Andean *Hapalopsittaca* parrots (Psittacidae, Aves): an example of montane-tropical lowland vicariance: biogeography of *Hapalopsittaca* parrots. Zool Scr. 2013;42:28–43. https://doi.org/10.1111/j.1463-6409.2012.00567.x.
4. Rosser N, Shirai LT, Dasmahapatra KK, Mallet J, Freitas AVL. The Amazon river is a suture zone for a polyphyletic group of co-mimetic heliconiine butterflies. Ecography. 2021;44:177–87. https://doi.org/10.1111/ecog.05282.
5. Menezes RST, Lloyd MW, Brady SG. Phylogenomics indicates Amazonia as the major source of Neotropical swarm-founding social wasp diversity. Proc R Soc B Biol Sci. 2020;287:20200480. https://doi.org/10.1098/rspb.2020.0480.
6. Oberdorff T, Dias MS, Jézéquel C, Albert JS, Arantes CC, Bigorne R, et al. Unexpected fish diversity gradients in the Amazon basin. Sci Adv. 2019;5:eaav8681. https://doi.org/10.1126/sciadv.aav8681.
7. Norambuena HV, Van Els P. A general scenario to evaluate evolution of grassland birds in the Neotropics. Ibis. 2021;163:722–7. https://doi.org/10.1111/ibi.12905.
8. Prado DE, Gibbs PE. Patterns of species distributions in the dry seasonal forests of South America. Ann Mo Bot Gard. 1993;80:902. https://doi.org/10.2307/2399937.
9. Collevatti RG, Terribile LC, Lima-Ribeiro MS, Nabout JC, de Oliveira G, Rangel TF, et al. A coupled phylogeographical and species distribution modelling approach recovers the demographical history of a Neotropical seasonally dry forest tree species. Mol Ecol. 2012;21:5845–63. https://doi.org/10.1111/mec.12071.
10. Pennington RT, Lewis GP, Ratter JA, editors. Neotropical savannas and seasonally dry forests: plant diversity, biogeography, and conservation. 1st ed. Boca Raton: CRC Press; 2006. https://doi.org/10.1201/9781420004496.
11. Corbett EC, Bravo GA, Schunck F, Naka LN, Silveira LF, Edwards SV. Evidence for the pleistocene arc hypothesis from genome-wide SNPs in a Neotropical dry forest specialist, the Rufous-fronted Thornbird (Furnariidae: *Phacellodomus rufifrons*). Mol Ecol. 2020;29:4457–72. https://doi.org/10.1111/mec.15640.
12. Hazzi NA, Moreno JS, Ortiz-Movliav C, Palacio RD. Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. Proc Natl Acad Sci. 2018;115:7985–90. https://doi.org/10.1073/pnas.1803908115.
13. Frankiewicz KE, Banasiak Ł, Oskolski A, Reduron J-P, Reyes-Betancort JA, Alsarraf M, et al. Long-distance dispersal events rather than growth habit and life-history traits affect diversification rate in tribe Apieae (Apiaceae). Bot J Linn Soc. 2021. https://doi.org/10.1093/botlinnean/boab032.
14. Cain ML, Milligan BG, Strand AE. Long-distance seed dispersal in plant populations. Am J Bot. 2000;87:1217–27.
15. Bohrer G, Nathan R, Volis S. Effects of long-distance dispersal for metapopulation survival and genetic structure at ecological time and spatial scales. J Ecol. 2005;93:1029–40. https://doi.org/10.1111/j.1365-2745.2005.01048.x.
16. Werneck FP, Gamble T, Colli GR, Rodrigues MT, Sites JW Jr. Deep diversification and long-term persistence in the south american 'dry diagonal': integrating continent-wide phylogeography and distribution modeling of geckos: deep divergence of south american 'dry diagonal' biomes. Evolution. 2012;66:3014–34. https://doi.org/10.1111/j.1558-5646.2012.01682.x.
17. Moraes EM, Yotoko KSC, Manfrin MH, Solferini VN, Sene FM. Phylogeography of the cactophilic species *Drosophila gouveai*: demographic events and divergence timing in dry vegetation enclaves in eastern Brazil. J Biogeogr. 2009;36:2136–47. https://doi.org/10.1111/j.1365-2699.2009.02145.x.
18. Bartoleti LFdM, Peres EA, Sobral-Souza T, Fontes FvHM, Silva MJd, Solferini VN. Phylogeography of the dry vegetation endemic species *Nephila sexpunctata* (Araneae: Araneidae) suggests recent expansion of the neotropical dry diagonal. J Biogeogr. 2017;44:2007–20. https://doi.org/10.1111/jbi.12998.
19. Ramos ACS, Lemos-Filho JP, Ribeiro RA, Santos FR, Lovato MB. Phylogeography of the Tree *Hymenaea stigonocarpa* (Fabaceae: Caesalpinioideae) and the influence of quaternary climate changes in the Brazilian Cerrado. Ann Bot. 2007;100:1219–28. https://doi.org/10.1093/aob/mcm221.
20. Caetano S, Prado D, Pennington RT, Beck S, Oliveira-Filho A, Spichiger R, et al. The history of seasonally dry tropical forests in eastern South America: inferences from the genetic structure of the tree *Astronium urundeuva* (Anacardiaceae). Mol Ecol. 2008;17:3147–59. https://doi.org/10.1111/j.1365-294X.2008.03817.x.
21. Chagas C. Nova tripanozomiase humana. The new human tripanosomiase. Studies about morphology and life cycle of *Schizotrypanum cruzi* n. gen., n. sp., etiologic agent of the new human morbidity. Mem Inst Oswaldo Cruz. 1909;1:159–2118.
22. Hwang WS, Weirauch C. Evolutionary history of Assassin Bugs (Insecta: Hemiptera: Reduviidae): insights from divergence dating and ancestral state reconstruction. PLoS ONE. 2012;7:e45523. https://doi.org/10.1371/journal.pone.0045523.
23. Dias JCP, Silveira AC, Schofield CJ. The impact of Chagas disease control in Latin America: a review. Mem Inst Oswaldo Cruz. 2002;97:603–12. https://doi.org/10.1590/S0074-02762002000500002.
24. Dujardin J-P, Beard CB, Ryckman R. The relevance of wing geometry in entomological surveillance of Triatominae, vectors of Chagas disease.

Infect Genet Evol. 2007;7:161–7. https://doi.org/10.1016/j.meegid.2006.07.005.
25. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, et al. The importance of vector control for the control and elimination of vector-borne diseases. PLoS Negl Trop Dis. 2020;14:e0007831. https://doi.org/10.1371/journal.pntd.0007831.
26. Hernández C, AristeudaRosa J, Vallejo GA, Guhl F, Ramírez JD. Taxonomy, evolution, and biogeography of the rhodniini tribe (Hemiptera: Reduviidae). Diversity. 2020;12:97. https://doi.org/10.3390/d12030097.
27. Justi SA, Galvão C. The evolutionary origin of diversity in chagas disease vectors. Trends Parasitol. 2017;33:42–52. https://doi.org/10.1016/j.pt.2016.11.002.
28. Monteiro FA, Weirauch C, Felix M, Lazoski C, Abad-Franch F. Evolution, systematics, and biogeography of the triatominae, vectors of Chagas disease. Adv Parasitol. 2018;99:265–344. https://doi.org/10.1016/bs.apar.2017.12.002.
29. Abad-Franch F, Monteiro FA, Jaramillo ON, Gurgel-Gonçalves R, Dias FBS, Diotaiuti L. Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). Acta Trop. 2009;110:159–77. https://doi.org/10.1016/j.actatropica.2008.06.005.
30. Gurgel-Gonçalves R, Silva RB. Analysis of the geographical distribution of Psammolestes Bergroth (Hemiptera: Heteroptera: Reduviidae: Triatominae) in South America, with new records of Psammolestes tertius Lent & Jurberg. Zootaxa. 2009;2033:41–8. https://doi.org/10.11646/zootaxa.2033.1.4.
31. Lent H. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. New York: American Museum of Natural History; 1979.
32. Gurgel-Gonçalves R, Cuba CAC. Infestation of thornbird nests (Passeriformes: Furnariidae) by *Psammolestes tertius* (Hemiptera: Reduviidae) across Brazilian Cerrado and Caatinga ecoregions. Zool Curitiba Impresso. 2011;28:411–4. https://doi.org/10.1590/S1984-46702011000300017.
33. Marti GA, Echeverria MG, Waleckx E, Susevich ML, Balsalobre A, Gorla DE. Triatominae in furnariid nests of the Argentine Gran Chaco. J Vector Ecol. 2014;39:66–71. https://doi.org/10.1111/j.1948-7134.2014.12071.x.
34. Ceccarelli S, Balsalobre A, Medone P, Cano ME, Gurgel Gonçalves R, Feliciangeli D, et al. DataTri, a database of American triatomine species occurrence. Sci Data. 2018;5:180071. https://doi.org/10.1038/sdata.2018.71.
35. Oliveira J, Alevi KCC, Ravazi A, Herrera HM, Santos FM, Azeredo-Oliveira MTV, et al. New evidence of the monophyletic relationship of the genus. Am J Trop Med Hyg. 2018;99:1485–8. https://doi.org/10.4269/ajtmh.18-0109.
36. Oliveira J, Alevi KC, Fonseca EO, Souza OM, Santos CG, Azeredo-Oliveira MT, et al. New record and cytogenetic analysis of *Psammolestes tertius* Lent & Jurberg, 1965 (Hemiptera, Reduviidae, Triatominae) from Bahia State, Brazil. Genet Mol Res. 2016. https://doi.org/10.4238/gmr.15028004.
37. Panzera Y, Pita S, Ferreiro MJ, Ferrandis I, Lages C, Pérez R, et al. High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Cytogenet Genome Res. 2012;138:56–67. https://doi.org/10.1159/000341888.
38. Ravazi A, de Oliveira J, Campos FF, Madeira FF, dos Reis YV, de Oliveira ABB, et al. Trends in evolution of the Rhodniini tribe (Hemiptera, Triatominae): experimental crosses between *Psammolestes tertius* Lent & Jurberg, 1965 and *P. coreodes* Bergroth, 1911 and analysis of the reproductive isolating mechanisms. Parasit Vectors. 2021;14:350. https://doi.org/10.1186/s13071-021-04854-8.
39. Carvalheiro J, Barretto M. Estudos sobre reservatórios e vectores silvestres do *Trypanosoma cruzi*. LX – Tentativas de cruzamento de *Rhodnius prolixus* Stål, 1859 com *Rhodnius neglectus* Lent, 1954 (Hemiptera, Reduviidae). Rev Inst Med Trop São Paulo. 1976;18:17–23.
40. Monteiro FA, Wesson DM, Dotson EM, Schofield CJ, Beard CB. Phylogeny and molecular taxonomy of the Rhodniini derived from mitochondrial and nuclear DNA sequences. Am J Trop Med Hyg. 2000;62:460–5.
41. de Paula AS, Diotaiuti L, Schofield CJ. Testing the sister-group relationship of the Rhodniini and Triatomini (Insecta: Hemiptera: Reduviidae: Triatominae). Mol Phylogenet Evol. 2005;35:712–8. https://doi.org/10.1016/j.ympev.2005.03.003.
42. Hypsa V, Tietz DF, Zrzavý J, Rego RO, Galvao C, Jurberg J. Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. Mol Phylogenet Evol. 2002;23:447–57.
43. Justi SA, Galvão C, Schrago CG. Geological changes of the Americas and their influence on the diversification of the Neotropical Kissing Bugs (Hemiptera: Reduviidae: Triatominae). PLoS Negl Trop Dis. 2016;10:1–22. https://doi.org/10.1371/journal.pntd.0004527.
44. Patterson JS, Gaunt MW. Phylogenetic multi-locus codon models and molecular clocks reveal the monophyly of haematophagous reduviid bugs and their evolution at the formation of South America. Mol Phylogenet Evol. 2010;56:608–21. https://doi.org/10.1016/j.ympev.2010.04.038.
45. Kieran TJ, Gordon ERL, Zaldívar-Riverón A, Ibarra-Cerdeña CN, Glenn TC, Weirauch C. Ultraconserved elements reconstruct the evolution of Chagas disease-vectoring kissing bugs (Reduviidae: Triatominae). Syst Entomol. 2021;46:725–40. https://doi.org/10.1111/syen.12485.
46. Garrido R, Bacigalupo A, Peña-Gómez F, Bustamante RO, Cattan PE, Gorla DE, et al. Potential impact of climate change on the geographical distribution of two wild vectors of Chagas disease in Chile: *Mepraia spinolai* and *Mepraia gajardoi*. Parasit Vectors. 2019;12:478. https://doi.org/10.1186/s13071-019-3744-9.
47. Ceccarelli S, Justi SA, Rabinovich JE, Diniz Filho JAF, Villalobos F. Phylogenetic structure of geographical co-occurrence among New World Triatominae species, vectors of Chagas disease. J Biogeogr. 2020;47:1218–31. https://doi.org/10.1111/jbi.13810.
48. Ibarra-Cerdeña CN, Zaldívar-Riverón A, Peterson AT, Sánchez-Cordero V, Ramsey JM. Phylogeny and niche conservatism in North and Central American triatomine bugs (Hemiptera: Reduviidae: Triatominae), vectors of Chagas' disease. PLoS Negl Trop Dis. 2014;8:e3266. https://doi.org/10.1371/journal.pntd.0003266.
49. de Paula AS, Barreto C, Telmo MCM, Diotaiuti L, Galvão C. Historical biogeography and the evolution of hematophagy in Rhodniini (Heteroptera: Reduviidae: Triatominae). Front Ecol Evol. 2021;9:660151. https://doi.org/10.3389/fevo.2021.660151.
50. Toews DPL, Brelsford A. The biogeography of mitochondrial and nuclear discordance in animals: BIOGEOGRAPHY OF MITO-NUCLEAR DISCORDANCE. Mol Ecol. 2012;21:3907–30. https://doi.org/10.1111/j.1365-294X.2012.05664.x.
51. Patten MA, Smith-Patten BD. Biogeographical boundaries and Monmonier's algorithm: a case study in the northern Neotropics. J Biogeogr. 2008;35:407–16. https://doi.org/10.1111/j.1365-2699.2007.01831.x.
52. Méndez-Camacho K, Leon-Alvarado O, Miranda-Esquivel DR. Biogeographic evidence supports the Old Amazon hypothesis for the formation of the Amazon fluvial system. PeerJ. 2021;9:e12533. https://doi.org/10.7717/peerj.12533.
53. Carneiro L, Bravo GA, Aristizábal N, Cuervo AM, Aleixo A. Molecular systematics and biogeography of lowland antpittas (Aves, Grallariidae): the role of vicariance and dispersal in the diversification of a widespread Neotropical lineage. Mol Phylogenet Evol. 2018;120:375–89. https://doi.org/10.1016/j.ympev.2017.11.019.
54. Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC. Amazonian amphibian diversity is primarily derived from Late Miocene Andean Lineages. PLoS Biol. 2009;7:e1000056. https://doi.org/10.1371/journal.pbio.1000056.
55. Maciel NM, Collevatti RG, Colli GR, Schwartz EF. Late Miocene diversification and phylogenetic relationships of the huge toads in the Rhinella marina (Linnaeus, 1758) species group (Anura: Bufonidae). Mol Phylogenet Evol. 2010;57:787–97. https://doi.org/10.1016/j.ympev.2010.08.025.
56. Quiagen. https://www.qiagen.com/us/resources/resourcedetail?id=cabd47a4-cb5a-4327-b10d-d90b8542421e&lang=en.
57. Nascimento JD, Da Rosa JA, Salgado-Roa FC, Hernández C, Pardo-Diaz C, Alevi KCC, et al. Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae). PLoS ONE. 2019;14:1–17. https://doi.org/10.1371/journal.pone.0211285.
58. Caicedo-Garzón V, Salgado-Roa FC, Sánchez-Herrera M, Hernández C, Arias-Giraldo LM, García L, et al. Genetic diversification of *Panstrongylus geniculatus* (Reduviidae: Triatominae) in northern South America. PLoS ONE. 2019;14:1–18. https://doi.org/10.1371/journal.pone.0223963.

59. Lyman DF, Monteiro FA, Escalante AA, Cordon-Rosales C, Wesson DM, Dujardin JP, et al. Mitochondrial DNA sequence variation among triatomine vectors of Chagas' disease. Am J Trop Med Hyg. 1999;60:377–86. https://doi.org/10.4269/ajtmh.1999.60.377.
60. Katoh K. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30:3059–66. https://doi.org/10.1093/nar/gkf436.
61. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 3.51. 2018. 2019.
62. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, et al. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 2017;34:3299–302. https://doi.org/10.1093/molbev/msx248.
63. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–4. https://doi.org/10.1093/molbev/msaa015.
64. Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017;14:587–9. https://doi.org/10.1038/nmeth.4285.
65. Schwarz G. Estimating the dimension of a model. Ann Stat. 1978;6:461–4. https://doi.org/10.1214/aos/1176344136.
66. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 2018;35:518–22. https://doi.org/10.1093/molbev/msx281.
67. Anisimova M, Gil M, Dufayard J-F, Dessimoz C, Gascuel O. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Syst Biol. 2011;60:685–99. https://doi.org/10.1093/sysbio/syr041.
68. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59:307–21. https://doi.org/10.1093/sysbio/syq010.
69. Gadagkar SR, Rosenberg MS, Kumar S. Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. J Exp Zool B Mol Dev Evol. 2005;304B:64–74. https://doi.org/10.1002/jez.b.21026.
70. Ogilvie HA, Bouckaert RR, Drummond AJ. StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. Mol Biol Evol. 2017;34:2101–14. https://doi.org/10.1093/molbev/msx126.
71. Heled J, Drummond AJ. Bayesian inference of species trees from multilocus data. Mol Biol Evol. 2010;27:570–80. https://doi.org/10.1093/molbev/msp274.
72. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9. https://doi.org/10.1093/molbev/msy096.
73. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 2018. https://doi.org/10.1093/ve/vey016.
74. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian Phylogenetics Using Tracer 1.7. Syst Biol. 2018;67:901–4. https://doi.org/10.1093/sysbio/syy032.
75. Peretolchina T, Pavan MG, Corrêa-Antônio J, Gurgel-Gonçalves R, Lima MM, Monteiro FA. Phylogeography and demographic history of the Chagas disease vector *Rhodnius nasutus* (Hemiptera: Reduviidae) in the Brazilian Caatinga biome. PLoS Negl Trop Dis. 2018;12:e0006731. https://doi.org/10.1371/journal.pntd.0006731.
76. Pfeiler E, Bitler BG, Ramsey JM, Palacios-Cardiel C, Markow TA. Genetic variation, population structure, and phylogenetic relationships of *Triatoma rubida* and *T. recurva* (Hemiptera: Reduviidae: Triatominae) from the Sonoran Desert, insect vectors of the Chagas' disease parasite *Trypanosoma cruzi*. Mol Phylogenet Evol. 2006;41:209–21. https://doi.org/10.1016/j.ympev.2006.07.001.
77. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. BEAST 25: an advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol. 2019;15:e1006650. https://doi.org/10.1371/journal.pcbi.1006650.
78. Yang Z. The BPP program for species tree estimation and species delimitation. Curr Zool. 2015;61:854–65. https://doi.org/10.1093/czoolo/61.5.854.
79. Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, et al. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics. 2017;33:1630–8. https://doi.org/10.1093/bioinformatics/btx025.
80. Yang Z, Rannala B. Unguided species delimitation using DNA sequence data from multiple loci. Mol Biol Evol. 2014;31:3125–35. https://doi.org/10.1093/molbev/msu279.
81. Carstens BC, Pelletier TA, Reid NM, Satler JD. How to fail at species delimitation. Mol Ecol. 2013;22:4369–83. https://doi.org/10.1111/mec.12413.
82. Hudson RR, Boos DD, Kaplan NL. A statistical test for detecting geographic subdivision. Mol Biol Evol. 1992;9:138–51. https://doi.org/10.1093/oxfordjournals.molbev.a040703.
83. Ramos-Onsins SE, Rozas J. Statistical properties of new neutrality tests against population growth. Mol Biol Evol. 2002;19:2092–100. https://doi.org/10.1093/oxfordjournals.molbev.a004034.
84. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989;123:585–95.
85. Fu YX, Li WH. Statistical tests of neutrality of mutations. Genetics. 1993;133:693–709.
86. Clement M, Snell Q, Walker P, Posada D, Crandall K. TCS: estimating gene genealogies. 7.
87. Leigh JW, Bryant D. popart: full-feature software for haplotype network construction. Methods Ecol Evol. 2015;6:1110–6. https://doi.org/10.1111/2041-210X.12410.
88. Dixon P. VEGAN, a package of R functions for community ecology. J Veg Sci. 2003;14:927–30. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.
89. Hijmans RJ, Williams E, Vennes C, Hijmans MRJ. Package 'geosphere.' Spherical Trigonometry. 2017;1:7.
90. Manni F, Rard EG, Heyer E. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. 18.
91. Jombart T, Ahmed I. adegenet 1.3–1: new tools for the analysis of genome-wide SNP data. Bioinformatics. 2011;27:3070–1. https://doi.org/10.1093/bioinformatics/btr521.
92. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59.
93. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol. 2005;14:2611–20. https://doi.org/10.1111/j.1365-294X.2005.02553.x.
94. Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012;4:359–61. https://doi.org/10.1007/s12686-011-9548-7.
95. Jakobsson M, Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 2007;23:1801–6. https://doi.org/10.1093/bioinformatics/btm233.
96. Rosenberg NA. Distruct: a program for the graphical display of population structure. Mol Ecol Notes. 2004;4:137–8. https://doi.org/10.1046/j.1471-8286.2003.00566.x.
97. Thuiller W, Lafourcade B, Engler R, Araújo MB. BIOMOD: a platform for ensemble forecasting of species distributions. Ecography. 2009;32:369–73.
98. Ripley BD, Naylor P. Pattern recognition and neural networks. Nature. 1996;381:206–206.
99. McCullagh P, Nelder JA. Generalized linear models. London: Chapman; 1989.
100. Friedman JH. Recent advances in predictive (machine) learning. J Classif. 2006;23:175–97.
101. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecol Model. 2006;190:231–59. https://doi.org/10.1016/j.ecolmodel.2005.03.026.
102. Chefaoui RM, Lobo JM. Assessing the effects of pseudo-absences on predictive distribution model performance. Ecol Model. 2008;210:478–86. https://doi.org/10.1016/j.ecolmodel.2007.08.010.
103. Wisz MS, Guisan A. Do pseudo-absence selection strategies influence species distribution models and their predictions? An information-theoretic approach based on simulated data. BMC Ecol. 2009;9:8. https://doi.org/10.1186/1472-6785-9-8.

104. Barbet-Massin M, Jiguet F, Albert CH, Thuiller W. Selecting pseudo-absences for species distribution models: how, where and how many? Methods Ecol Evol. 2012;3:327–38. https://doi.org/10.1111/j.2041-210X.2011.00172.x.
105. Karger DN, Conrad O, Böhner J, Kawohl T, Kreft H, Soria-Auza RW, et al. Climatologies at high resolution for the earth's land surface areas. Sci Data. 2017;4:170122. https://doi.org/10.1038/sdata.2017.122.
106. Reuter HI, Nelson A, Jarvis A. An evaluation of void-filling interpolation methods for SRTM data. Int J Geogr Inf Sci. 2007;21:983–1008. https://doi.org/10.1080/13658810601169899.
107. Thuiller W, Araújo MB, Lavorel S. Generalized models vs. classification tree analysis: predicting spatial distributions of plant species at different scales. J Veg Sci. 2003;14:669–80. https://doi.org/10.1111/j.1654-1103.2003.tb02199.x.
108. Brown JL, Carnaval AC. A tale of two niches: methods, concepts, and evolution. Front Biogeogr. 2019;1:1.
109. Rödder D, Engler JO. Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks. Glob Ecol Biogeogr. 2011;20:915–27. https://doi.org/10.1111/j.1466-8238.2011.00659.x.
110. Brown JL, Carnaval AC. A tale of two niches: methods, concepts, and evolution. Front Biogeogr. 2019. https://doi.org/10.21425/F5FBG44158.

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Capítulo 3. Utilidad de enfoque multilocus en la resolución de conflictos filogenéticos y evolutivos de la tribu Rhodniini

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Review

Taxonomy, Evolution, and Biogeography of the Rhodniini Tribe (Hemiptera: Reduviidae)

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Abstract: The Triatominae subfamily includes 151 extant and three fossil species. Several species can transmit the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, significantly impacting public health in Latin American countries. The Triatominae can be classified into five tribes, of which the Rhodniini is very important because of its large vector capacity and wide geographical distribution. The Rhodniini tribe comprises 23 (without *R. taquarussuensis*) species and although several studies have addressed their taxonomy using morphological, morphometric, cytogenetic, and molecular techniques, their evolutionary relationships remain unclear, resulting in inconsistencies at the classification level. Conflicting hypotheses have been proposed regarding the origin, diversification, and identification of these species in Latin America, muddying our understanding of their dispersion and current geographic distribution. Clarifying these factors can help for the design of vector control strategies. The aim of this review is to depict the different approaches used for taxonomy of the Rhodniini and to shed light on their evolution and biogeography.

Keywords: Triatominae; Chagas Disease; *Rhodnius*; *Psammolestes*; evolution; taxonomy; Rhodniini; biogeography

1. Introduction

Chagas disease is caused by the protozoan parasite and hemoflagellate *Trypanosoma cruzi*. This pathology mainly affects Latin American countries, where there are an estimated 6–8 million people infected and causes approximately 50,000 deaths per year [1]. The parasite can be transmitted by insect vectors, blood transfusion, vertical transmission, organ transplantation, laboratory accidents, and oral route [1,2]. The major transmission mechanism is via contact between humans and the feces of infected insects of the subfamily Triatominae (Hemiptera: Reduviidae) [1–4]. Human infection occurs accidentally during urbanization and environmental imbalances (deforestation and habitat loss) which cause triatomine insects infected with the parasite to invade human dwellings [3–7].

In the Triatominae subfamily, 151 extant and three fossil species have been reported and classified into five tribes according to their morphological, biological, and ecological characteristics [8,9]. Most subfamily diversity is restricted to Latin America, where 135 species have been described [10]. The

Rhodniini tribe is one of the most diverse and has 23 described species (20 of the genus *Rhodnius* and three of *Psammolestes*). Within the genus *Rhodnius*, some species are important vectors of *T. cruzi* [3,8,11,12], and some have been found to have the capacity to produce metacyclic trypomastigotes of *T. rangeli* in their salivary glands [13–20].

Rhodnius prolixus is one of the main vectors of Chagas disease. Its wide geographic distribution (extending from Central America through the Andean countries and the Amazon basin), its capacity for domiciliation, its high dispersion, and its strong vector capacity are a threat to the future of vector control programs [21–23]. In addition to *R. prolixus*, three species within the Rhodniini tribe have been found domiciled: *Rhodnius ecuadoriensis* in the northern zone of Peru and Ecuador, *Rhodnius stali* in Bolivia, and *Rhodnius pallescens* in Panama. *Rhodnius prolixus*, *R. ecuadoriensis*, *R. pallescens*, and *R. stali* are infected with *T. cruzi* at prevalence rates of 12.0–82.0% [24–26], 10.0–42.0% [15,27,28], 42.0–87.4% [24,29–33], and 7.7% [34], respectively. Additionally, *R. neglectus* and *R. nasutus* are epidemiologically relevant species in Brazil, where they often invade and colonize human environments [7,35–37].

Although the taxonomy of the genus *Rhodnius* has been widely studied, there are controversies regarding the number of species, the classification of these species into groups, and the phylogenetic relationships and monophyletic status of these groups. These have arisen from the conflicting results of morphometric analyses, cytogenetic analyses, and analyses of isoenzyme markers and molecular markers [8,12,38]. There is also controversy regarding the paraphyly of two genera belonging to the Rhodniini tribe (*Rhodnius* and *Psammolestes*): although morphological differences are present, other techniques used for taxonomic analysis group *Psammolestes* with *Rhodnius* [8,38].

The objective of this review is to describe the current state of knowledge of the phylogenetic and biogeographical relationships among the species of the Rhodniini tribe and to highlight the need for new studies to develop a more comprehensive understanding of the systematics of these species.

2. The Rhodninii Tribe: the Current Taxonomy

The Rhodniini tribe is composed of two genera: the genus *Rhodnius* consisting of 20 species and the genus *Psammolestes* consisting of three species (Table 1). Initially, it was proposed that the three species belonging to the genus *Psammolestes* should be grouped into a tribe called Psammolestini given their marked morphological differences compared with the genus *Rhodnius* [39]. Later the two genera were grouped into the Rhodniini tribe, based mostly on the presence of tuberosities posterior to the eyes and bearing in mind that they represented mostly arboreal species with the exception of some of the *Rhodnius* [8,40,41]. The Rhodniini tribe has been extensively studied due to its epidemiological importance and wide geographical distribution. Additionally, members of the genus *Rhodnius* have been classified into three groups—*pictipes*, *pallescens*, and *prolixus*—based on geographical distribution, biogeography, and morphology. Initially these groups were called lineages; however, since their monophyletic origin has been questioned, the term group is currently preferred [8].

Table 1. Genera and species of the Rhodniini tribe.

Genus	Group	Species
Rhodnius	*pictipes*	*R. amazonicus, R. brethesi, R. paraensis, R. pictipes, R. stali, R. zeledoni*
	pallescens	*R. colombiensis, R. ecuadoriensis, R. pallescens*
	prolixus	*R. barretti, R. dalessandroi, R. domesticus, R. milesi, R. marabaensis, R. montenegrensis, R. nasutus, R. neglectus, R. neivai, R. prolixus, R. robustus, R. taquarussuensis* *
Psammolestes		*P. arthuri, P. coreodes, P. tertius*

* *R. taquarussuensis* is currently considered a phenotypic form of *R. neglectus*.

3. Geographical Distribution of Members of the Rhodniini Tribe

The Rhodniini tribe has a wide geographical distribution ranging from Central America to the Southern Cone (Figure 1A). The distribution of species richness within the tribe is unimodal, with a greater number of species identified towards the northern hemisphere at low latitudes and some species identified in the southern hemisphere, reaching latitudes of 30° south [10].

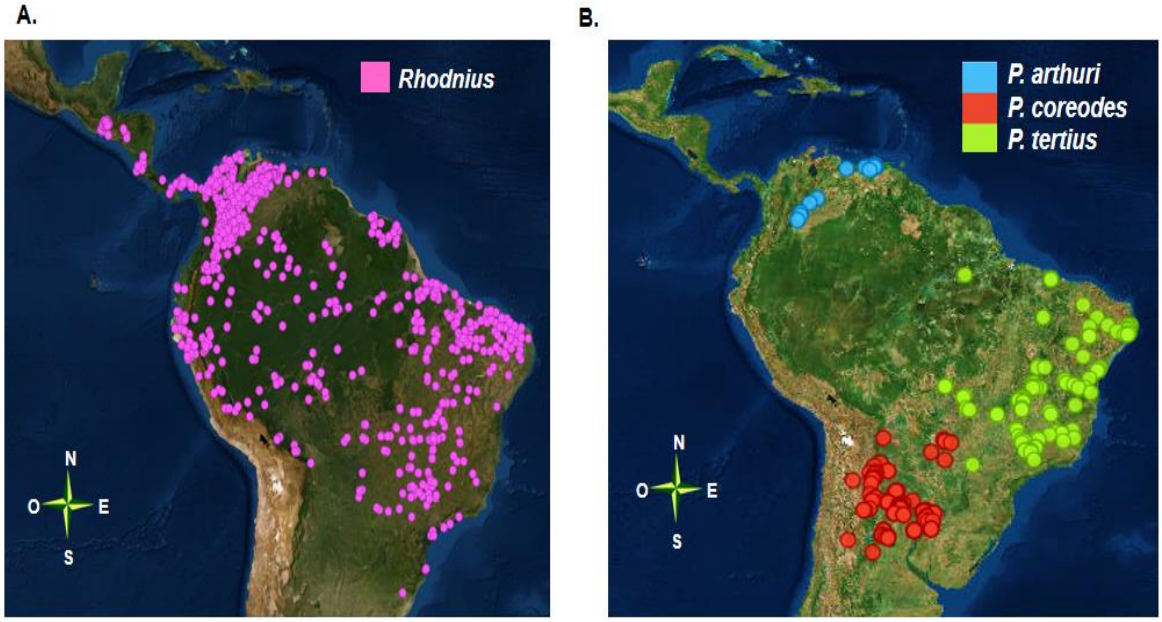

Figure 1. Geographical distribution of the Rhodniini tribe (**A**) Geographical distribution of the genus *Rhodnius* (**B**) Geographical distribution of species within the genus *Psammolestes*. Each point corresponds to geographical locations where individuals of each species were identified, these points were reported in several studies and compilated in a database by Ceccarelli and colleagues. This database was used to reconstruct the maps in Orange software (v.3.24.1) [42].

The genus *Psammolestes* has an interesting geographical distribution, and each of the three species with this genus has a distinct distribution (Figure 1B). Members of this genus are found mainly in bird nests (Furnariidae and Psittacidae), although they have also been identified in palm trees [43,44]. *Psammolestes arthuri* is distributed in Colombia and Venezuela. In Colombia, it has been described in the departments of the Orinoco region and in Venezuela it has been described in 15 different states, mainly in the plains region [42,45]. The range of *P. tertius* extends widely in Brazil, mainly in the Caatinga and much of the Cerrado [44]. *Psammolestes coreodes* is distributed in the Gran Chaco, and is widely distributed throughout 11 provinces in Argentina as well as in Paraguay, Bolivia, and the state of Mato Grosso do Sul, in the Brazilian Cerrado [46].

The geographical distribution of the genus *Rhodnius* is wider, covering part of Central America and a large part of South America. Geographical distribution is one of the criteria used for classification of the previously mentioned groups: species of the group *pallescens* (trans-Andean) are distributed to the west of the Andes mountain range (Figure 2A), while species of the groups *prolixus* and *pictipes* (cis-Andean) are distributed east of the Andes and the Amazon region (Figure 2B,C) [8].

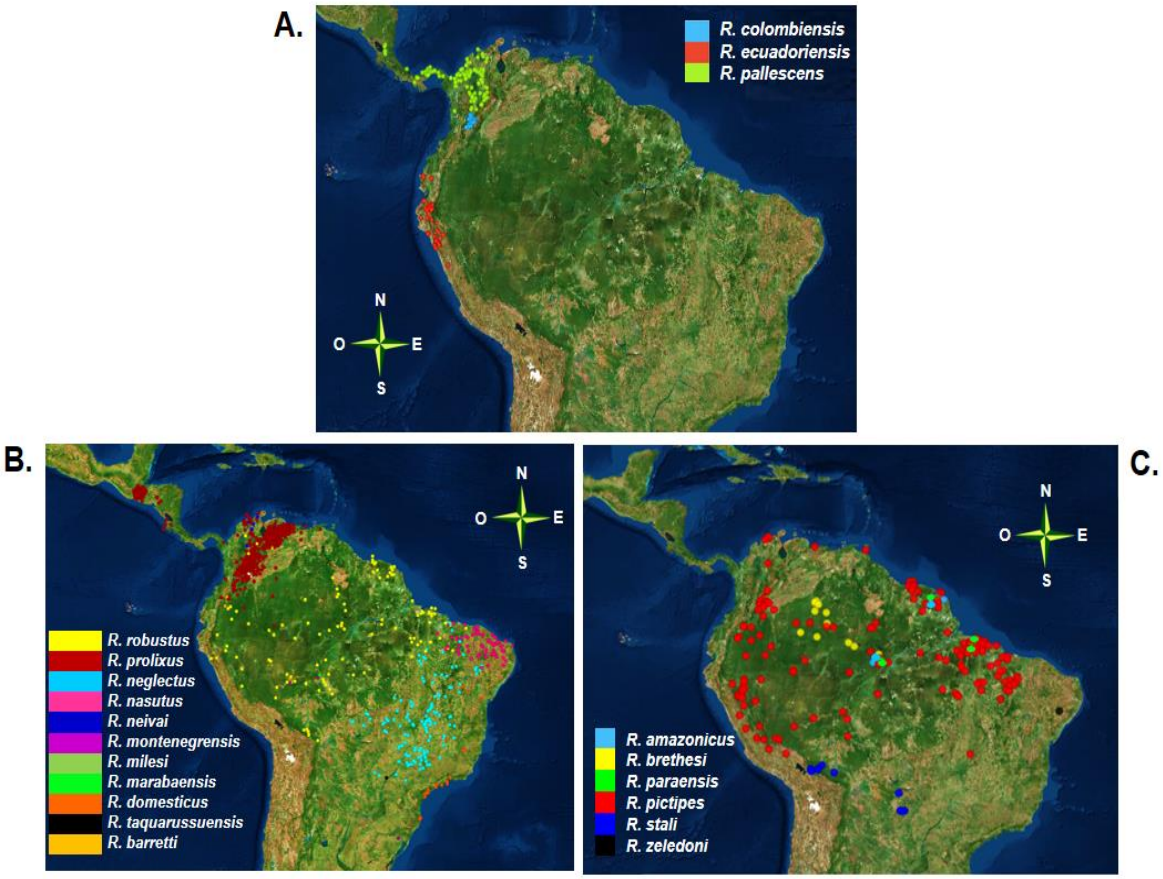

Figure 2. Distribution of species within the genus Rhodnius (**A**) Geographical distribution of species of the trans-*pallescens* group (**B**) Geographical distribution of species of the cis-*prolixus* group (**C**) Geographical distribution of species of the cis-*pictipes* group. Each point corresponds to geographical locations where individuals of each species were identified, these points were reported in several studies and compiled in a database by Ceccarelli and colleagues. This database was used to reconstruct the maps in Orange software (v.3.24.1) [42].

In the trans-*pallescens* group, all species are associated mainly with palms. The species with the widest geographical distribution in this group is *R. pallescens*, whose range extends into western Colombia, Panama, Costa Rica, Belize, and Nicaragua. *R. pallescens* is mainly associated with wine palms (*Attalea butyracea*) and oil (*Elaeis oleífera*) and has also been found in human dwellings, mainly in Panama [47,48]. *R. ecuadoriensis* is distributed in Ecuador and northern Peru and is strongly associated with human dwellings and with the palm *Phytelephas aequatorialis* in northern Ecuador, although it has also been identified in squirrel nests [28,47,49]. Finally, *Rhodnius colombiensis* is found in Colombia in the departments of Cundinamarca and Tolima, where it is also associated with the wine palm *A. butyracea* [10,46,47,50,51].

The cis-*prolixus* group has the largest number of species and the largest geographical distribution of the three groups. Among the species of this group, *R. prolixus* is the best studied given its epidemiological importance and has been reported in countries of Central America as well as in Colombia and Venezuela [12,43,46,52]. As a major vector, *R. prolixus* is mainly found in domestic habitats, although jungle populations associated with palms have also been identified in Colombia and Venezuela [22,52]. Control programs in Central America have achieved both the interruption of *T. cruzi* transmission and the direct elimination of *R. prolixus*, drastically decreasing its distribution in Mesoamerican countries [40,53–56]. However, the presence of *R. prolixus* has been reported again in Mexico, in 2019 [57]. Additionally, some reports of *R. prolixus* in Bolivia, Brazil, Ecuador, Guyana,

French Guiana, Panama, and Suriname were erroneous, probably due to confusion with *R. robustus* and in central Brazil with *R. neglectus* [10,58–62].

Another species within the *prolixus* group with a wide geographical distribution is *R. robustus*, whose range extends through Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, Venezuela, and Suriname [10,46,60,61]. Five cryptic species (I–V) have been reported within this species: *R. robustus* I is found in Venezuela, while *R. robustus* II, III, and IV are distributed in the Amazon region spanned by the previously mentioned countries [61]. There are reports of species of the *prolixus* group outside of Brazil with more restricted ranges such as *R. barretti* and *R. dalessandroi*. *R. barretti* has been identified from different palms in the Napo ecoregion in the western Amazon, the region that encompasses the lowlands of eastern Ecuador and the adjacent areas of southern Colombia (south of the Caguán River), and the north from Peru [63]. *Rhodnius dalessandroi* has only been reported once in the department of Meta, Colombia [46,56].

The other species of the *prolixus* group are exclusively Brazilian. *R. neglectus*, *R. nasutus*, and *R. domesticus* are associated with the Brazilian ecoregions, *R. neglectus* is dispersed in the Cerrado and São Paulo state, *R. nasutus* in the arid regions of the Caatinga, and *R. domesticus* in the Atlantic forest. The first two species are found in palm trees and bird nests, while *R. domesticus* has been identified in bromeliads [7,40,46,56,64]. *Rhodnius montegrensis* has been found in two states of Brazil (Acre and Rondonia), while the ranges of *R. milesi* and *R. marabaensis* are limited to the state of Pará [65–67]. *Rhodnius taquarussuensis* n.sp. was initially proposed as a new species in Taquarussu (Mato Grosso do Sul), but is currently considered a phenotypic form of *R. neglectus* [68,69].

Finally, the cis-*pictipes* group also has a wide geographic distribution. Within this group, *R. pictipes* has the widest geographical distribution of the entire Rhodniini tribe, extending throughout the Amazon basin (north and northwest of South America) in association with different palms. There have been reports of this species in Belize, Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guiana, Peru, Suriname, Trinidad, and Venezuela (Figure 2C) [10,40,46,60]. The species with the next-widest distribution is *R. brethesi*, which is characterized by its association with the palm *Leopoldina piassaba*. The geographical distribution of this species extends to the Amazon basin in Colombia and Venezuela and the states of Para and Amazonas in Brazil [10,40,46,47,52,56,70].

Rhodnius stali is distributed in Brazil (Matto Grosso and Acre) and in several provinces of Bolivia, where it is associated with *A. phalerata* and is also found in human dwellings, primarily in Alto Bení (Bolivia) [40,43,44,46,71]. *Rhodnius neivai* is found on fallen tree trunks and in the crowns of the llanera palm (*Copernicia tectorium*) in Colombia and Venezuela [47,56]. *Rhodnius amazonicus* and *R. paraensis* are distributed in French Guiana and Brazil, where *R. paraensis* has been found in nests of arboreal rodents of the genus *Echimys* [11,56,58]. Finally, *R. zeledoni* was identified in a single report in the northeast of Brazil in the state of Sergipe [72].

4. Taxonomic and Phylogenetic Studies of the Rhodniini Tribe

The taxonomy and systematics of the Rhodniini tribe are complex. Efforts to classify the species of this tribe date back to the middle of the 18th century, at which time *R. prolixus*, *R. pictipes*, and *R. nasutus* were first described. The most studied species is *R. prolixus*. Since its life cycle is relatively short compared with other triatomines, it has been used as a biological model for studies of the physiology and biology of the Triatominae [40]. In addition to morphological studies, cytogenetic, isoenzymatic, and molecular studies have all been applied to members of the Rhodniini tribe, providing different levels of resolution in the study of their specific relationships [40]. However, it should be noted that many of these studies have focused only on the differentiation or analysis of selected species and few attempted to clarify the relationships among all members of the tribe.

4.1. Morphological Studies

Taxonomic classification of the Rhodniini tribe was initially based on morphological similarities and differences between species and included biogeographical aspects. However, one of the main limitations,

especially for the Rhodniini tribe, was low morphological variability between species [8,41,73]. The characters that have been classically used for classification of the Rhodniini tribe were generally size, color, or patterns of coloration in some parts of the insect, and aspects of the cuticle. Additionally, various characters are used at the level of the head, thorax, abdomen, and legs [41,74]. New characters have been proposed for identification of triatomines and have been applied to the Rhodniini tribe. These include the spermatheca, geometric morphometry of the wings, morphology of abdominal segments IX and X, coloration of the salivary glands, and morphology of the genitalia. Use of some of these new characters has enabled differentiation at the level of the tribe presence of nitrophorins that confer red coloration to the salivary glands of the Rhodniini tribe and the morphometry of the wings as well as at the intra-specific level of the genus *Rhodnius* (Form of the female genitalia) [74–78].

The inclusion of the genera *Rhodnius* and *Psammolestes* within the Rhodniini tribe was based on their mainly arboreal behavior and the presence of post-ocular tuberosities in members of both genera. The latter feature is exclusive to both genera [41]. Between the two genera, differences can be observed in the morphology of the head and the shape of the femurs. However, these characters were insufficient to accurately reconstruct a cladogram and investigate relationships between the genera (Figure 3) [8,40,41].

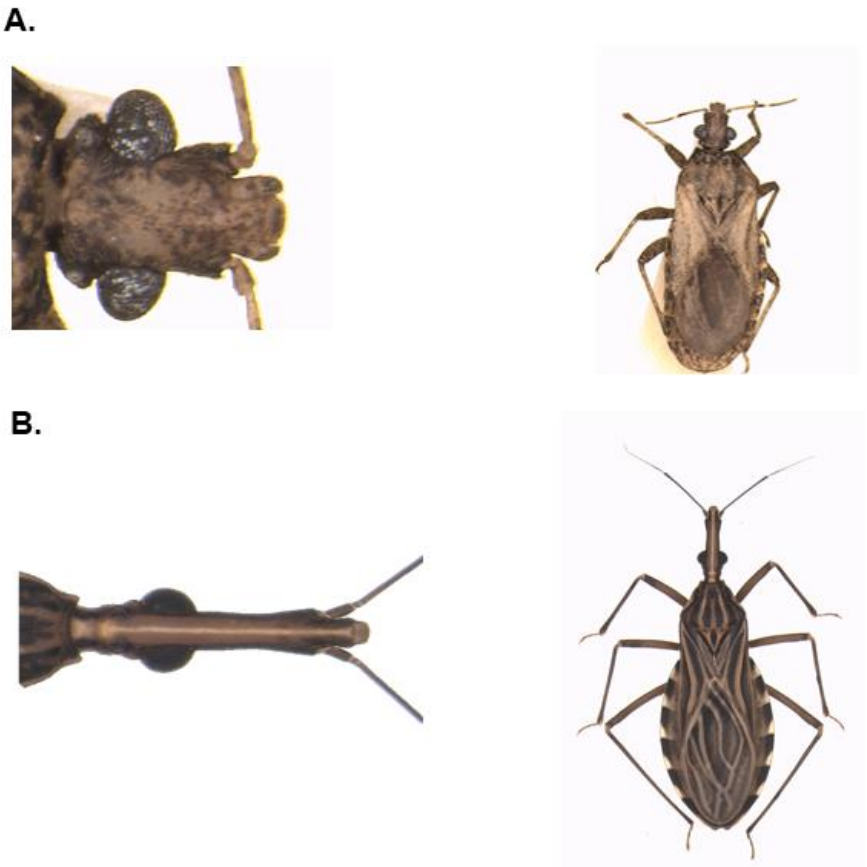

Figure 3. Morphology of the head and complete body of genera of the Rhodniini tribe (**A**) Morphology of the genus *Psammolestes* (*P. tertius*) (**B**) Morphology of the genus *Rhodnius (R. prolixus*). The images were supplied by João Aristeu da Rosa (Co-author of this manuscript).

In the genus *Psammolestes*, the morphology of the head, antennae, pronotum, genitalia of the males, and their phallosoma represent characters enabling identification of the three described species [24]. In the case of the genus *Rhodnius*, morphological characters enabling species differentiation are very few and can be affected by phenotypic variability. These characters also vary in association

with environmental changes yielding minor changes, which have been postulated to contribute to misclassification of species [12,38,40,46].

The cis-*prolixus* group presents very little morphological variability (Figure 4A). Thus, the feasibility of species identification using morphological characters has been questioned. There have even been mistakes in distinguishing *R. prolixus* and *R. robustus*, because the most useful difference between them for classification lies in the coloration of the hind tibiae of nymphs IV and V [41,61,79,80]. Additionally, the identity of some species within the *prolixus* group has been questioned: (i) *R. milesi* and *R. taquarussuensis* due to their similarity with *R. neglectus;* (ii) *R. montenegrensis* and *R. marabaensis* due to similarities with the cryptic species *R. robustus* II and III, respectively and (iii) *R. dalessandroi* due to morphological similarities with *R. brethesi* (group *pictipes*) and *R. robustus* [38,41,56,68,69]. With respect to coloration patterns, *R. neivai* differs from other species in the group by its dark coloration, and a dark black morphotype of *R. nasutus* has been identified with size variations in accordance with the environment and colonized palm trees [41,81,82].

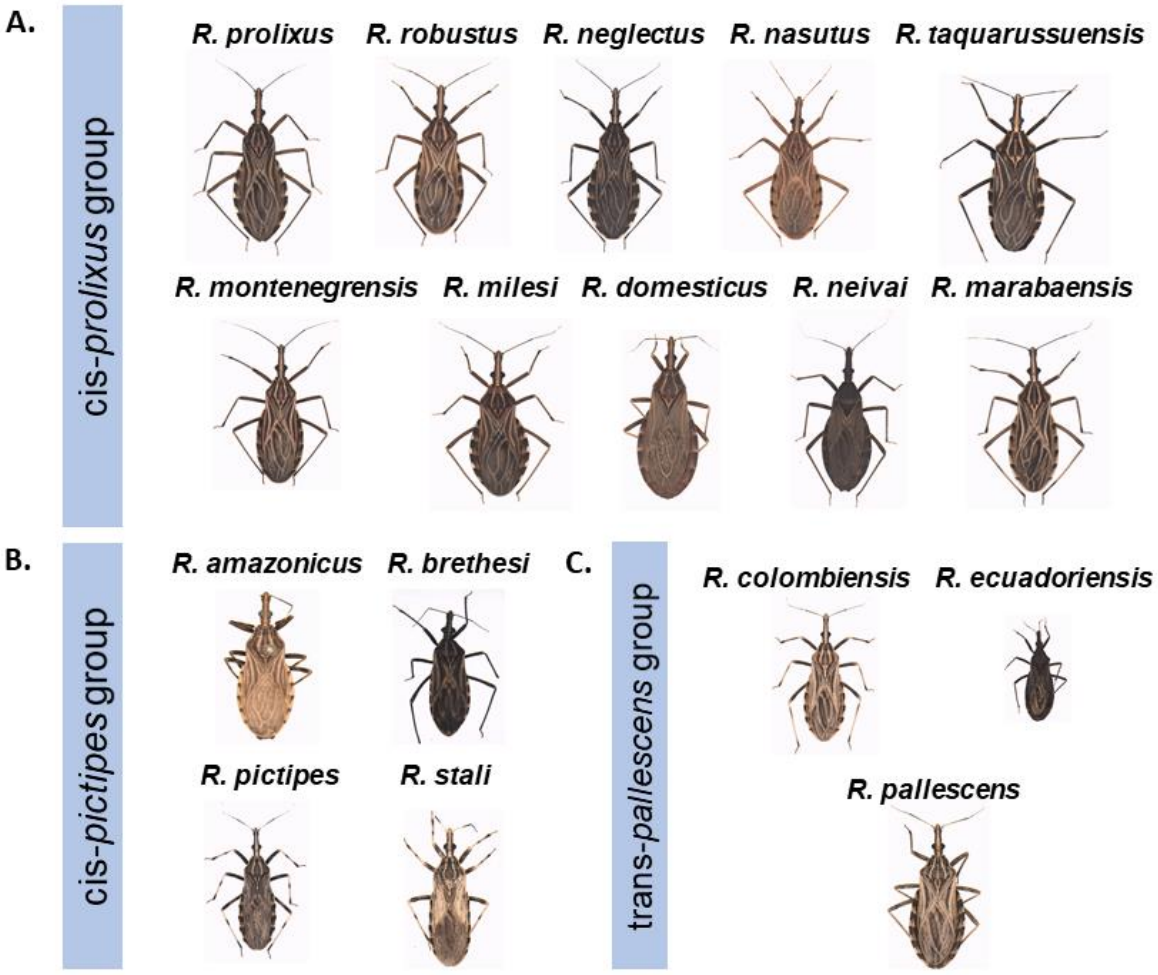

Figure 4. Morphology of species within the genus *Rhodnius*. (**A**) cis-*prolixus* group species, *R. taquarussuensis* is currently considered a phenotypic form of *R. neglectus*, (*) indicates that *R. nasutus* has two isoforms (**B**) cis-*pictipes* group species (**C**) trans-*pallescens* group species. The images were obtained by João Aristeu da Rosa. The morphology of *R. barreti* can be observed in Abad-Franch et al., 2013 [63] and *R. dalessandroi*, *R. paraensis* and *R. zeledoni* can be found in Jurberg et al., 2014 [83].

More morphological differences are observed between members of the group *pictipes* (Figure 4B). The validity of the species *R. amazonicus* has been questioned because it was documented in only a single original report and differences with respect to *R. pictipes* were not evident [41]. However,

subsequent reports in French Guiana and Breves, Pará, Brazil reaffirmed the characteristics observed in the original report [11,58,84]. *Rhodnius zeledoni* was also described in only a single report and is thought to be more similar morphologically to *R. domesticus* (group *prolixus*) than to members of the group *pictipes* and correspond to a poorly-preserved adult male therefore, currently it is difficult to validate its identity as a species [8,72]. *Rhodnius brethesi* differs from the remainder of the tribe by the size of the second antenna segment, which is longer than the third and the red or orange spots on the connexivum [41]. Finally, the *pallescens* group is composed of three morphologically very similar species. However, *R. colombiensis* was initially confused with *R. prolixus* of wild origin due to morphological similarities [56]. *Rhodnius ecuadoriensis* is characterized by its smaller size (Figure 4C). However, it has been proposed that this species should be grouped with *R. pictipes* due to the similarities of their antennal sensilla [56,85].

4.2. Cytogenetic Studies

In the Rhodniini tribe, the number of chromosomes is homogeneous in all of the 17 species analyzed so far (14 species of *Rhodnius* and three of *Psammolestes*), which were representative of the two genera and the three groups of the genus *Rhodnius*. Thus, the diploid chromosome number of the Rhodniini tribe is made up of 20 autosomes and the XY sex chromosomes; variations are observed between the Triatomini and Bolboderini tribes in the number of autosomes and sex chromosomes present in species and complexes [40,86–89]. Nucleolar persistence has also been observed during meiosis, as reflected by the presence of nucleoli or nucleolar corpuscles during the meiotic metaphase in 15 members of the tribe (*P. tertius*, *R. brethesi*, *R. colombiensis*, *R. domesticus*, *R. ecuadoriensis*, *R. milesi*, *R. montenegrensis*, *R. nasutus*, *R. neglectus*, *R. neivai*, *R. pallescens*, *R. pictipes*, *R. prolixus*, *R. robustus*, and *R. stali*) [90,91]. Flow cytometry measurements of genome size have been performed in four species of the tribe (*R. ecuadoriensis*, *R. colombiensis*, *R. pallescens*, and *R. prolixus*) and indicated sizes of 0.72, 0.58, 0.73, and 0.75 picograms of haploid DNA, respectively, smaller than all other triatomines [87,92,93].

Studies at the genus level in *Psammolestes* have shown that in all three species, heterochromatin and AT repeats are present in the Y chromosome. The chromocenter is formed by the sex chromosomes and autosomal heterochromatin is absent [40,44,87]. Studies of the genus *Rhodnius* have demonstrated the presence of autosomal heterochromatin in *R. pallescens*, *R. colombiensis*, *R. domesticus*, *R. nasutus*, *R. taquarussuensis*, and its absence in *R. brethesi*, *R. ecuadoriensis*, *R. pictipes*, *R. neglectus*, *R. prolixus*, *R. robustus*, and *R. montenegrensis*. In species that have constitutive heterochromatin, usually very tiny C-bands are observed [40,87,89,92,94]. However, in spite of the homogeneity of cytogenetic characteristics observed in the tribe, intra- and inter-specific differences exist in the chromosomal location of ribosomal genes. Two patterns of location have been observed: one on both sex chromosomes X and Y (*P. tertius*, *R. domesticus*, *R. neglectus*, *R. neivai*, *R. milesi*, *R. pictipes*, *R. pallescens*, and *R. stali*) and another only on the X chromosome (*R. nasutus*, *R. prolixus*, *R. robustus*, *R. colombiensis*, and *R. ecuadoriensis*). In *R. ecuadoriensis*, both patterns were observed in populations from Peru and Ecuador. Analysis of ribosomal genes using cytogenetic techniques can be a useful marker of recent divergence of species or populations and allowed the formulation of two hypotheses regarding the evolution of ribosomal gene patterns, which may be due to: (i) loss of the loci in the Y chromosome, with the XY pattern representing the ancestral state; or (ii) partial transfer of genes from the X to the Y chromosome through mechanisms of transposition or ectopic recombination between sex chromosomes [95,96].

There is clear utility in analyses of heterochromatin C at the intraspecific level (e.g., for *Triatoma infestans* and *Panstrongylus geniculatus*) [40,97,98]. Cytogenetic analyses have been applied to different specimens of *R. pallescens* obtained from different geographical locations in Colombia and Panama, and revealed intraspecific variability: two cytotypes were described differing in terms of size, number and distribution of heterochromatin C during mitosis and meiosis. The frequencies of cytotypes varied in relation to the ecological and geographical characteristics of collection sites. Additionally, the cytotypes were consistent with morphological differences in size, morphometry of the wings and characters of the head [92,99]. Finally, cytogenetic characteristics (number and size of chromosomes, autosomal

heterochromatin, and sex chromosomes) have also been used to evaluate intraspecific variability of different populations of *R. prolixus* and *R. neglectus* and no variation in cytotypes was observed with respect to the ecological and geographical characteristics of the populations analyzed [100].

4.3. Isoenzymatic Studies

Isoenzyme analyses have also been applied to species of the Rhodniini tribe, although few cross-sectional studies have examined all species at once. The first phylogenetic study addressing in several species of the Rhodniini tribe (*R. brethesi*, *R. ecuadoriensis*, *R. nasutus*, *R. neglectus*, *R. pallescens*, *R. prolixus*, *R. pictipes*, and *R. stali*) analyzed 12 enzymes, and the results of the analysis of distances and genetic variability allowed the grouping of species into the three groups currently known, in agreement with their geographical distribution [101]. Differently, one study proposed that *Rhodnius* species should be classified into only two groups: *prolixus* and *pictipes*, with the *pictipes* group including members of the group *pallescens* [80]. In addition, other isoenzymatic studies also showed that: (i) *R. stali* and *R. pictipes* despite their morphological similarities and geographical proximity are different species (ii) *R. nasutus* and *R. neglectus* are very closely related, indicating that their speciation probably occurred very recently, and that (ii) wild specimens erroneously identified as *R. prolixus* in Tolima (Colombia) were placed within the group *pallescens* and not with *R. prolixus*, and that they therefore could represent a new species that is known today as *R. colombiensis* [101,102]. Subsequently, two isoenzyme studies were published and included the species *P. coreodes* [103] and *P. tertius* [80] along with species of the three groups of the genus *Rhodnius*. In these studies, it was not possible to define the species status of *Psamolestes* in the tribe, because these species showed paraphyly with Rhodnius [80,104]. Another isoenzyme studies, addressing in species of the genus *Rhodnius*, showed that *R. prolixus* and *R. robustus* had identical electrophoretic patterns and were not reproductively isolated, however by using salivary, heme proteins was possible to differentiate these species [40,80,104–106]. The species status of *R. neglectus* was confirmed by genetic distance analysis and reproductive isolation although only one locus allowed its differentiation with *R. nasutus* [104,106].

4.4. Random Amplification of Polymorphic DNA (RAPD)

With the advent of molecular techniques based on DNA, the use of high resolution markers, such as random amplification of polymorphic DNA (RAPD), became possible. This technique was used mainly for differentiating species of the tribe where morphological and isoenzyme differentiation was not possible (e.g., *R. prolixus* vs. *R. robustus*, *R. ecuadoriensis* vs. *R. pictipes*, and *R. nasutus* vs. *R. neglectus*). The electrophoretic patterns by RAPD allowed differentiation of all six species [107]. Additionally, RAPD was used to compare populations of domestic *R. prolixus* from Central America (Honduras) and South America (Colombia). RAPD electrophoretic patterns differed according to geographic location and showed that genetic variability was greater in specimens obtained from Colombia. Thus, it can be deduced that the Central American specimens were derived from those in South America; this conclusion was reached not only from the results of RAPD but also based on differences in morphology, because the specimens from Honduras were smaller. Finally, an isoenzymatic analysis showed no differences between the two types of specimens, suggesting a common origin [108]. However, RAPD studies also showed that *R. prolixus* (domestic) and *R. colombiensis* (wild) have different electrophoretic patterns, suggesting no genetic flow and that the effective migration rate between the species is insufficient to maintain genetic homogeneity in the two species [109]. RAPD has also been used to evaluate intraspecific genetic variability of populations of *P. tertius* of differing geographic origins in Brazil. One study found differences in electrophoretic patterns in association with geographic origin, and these results were supported by differences in isoenzyme patterns and morphological features [110].

4.5. Microsatellites

Microsatellite markers have also been used in the Rhodniini tribe, mainly with the aim of analyzing intraspecific variation given the high resolution of these markers. A scheme of 10 microsatellite loci was designed based on DNA sequences of *R. prolixus* and its amplification was tested in 10 species of the genus *Rhodnius*. Amplification of all loci was successful in *R. robustus* and in 6–9 of the species of the *prolixus* group, while in species of the groups *pallescens* and *pictipes*, amplification of more than three loci was not achieved [111]. Subsequent studies have used microsatellites to analyze intraspecific genetic structure in different populations of *R. pallescens* [111,112], *R. nasutus* [36], *R. ecuadoriensis* [113], and *R. prolixus* [22]. In populations of *R. pallescens*, field and laboratory specimens have been compared, and laboratory colonies of *R. pallescens* showed a different genetic structure than their wild relatives [112]. Studies of microsatellite markers in *R. nasutus* and *R. ecuadoriensis* showed that populations of these species from different geographic locations had genetic differences. For *R. nasutus*, four differentiated groups were revealed in eight geographic localities of the Brazilian Caatinga [36], and for *R. ecuadoriensis*, specimens in two biogeographically distinct localities of Ecuador had distinct genetic structures in association with other phenotypic differences [113]. Given its epidemiological importance, microsatellite markers were analyzed in wild and domestic populations of *R. prolixus* from Venezuela. No genetic differences were found, reflecting a high risk of wild populations easily invading human habitations [22].

4.6. Sequencing of Mitochondrial and Nuclear Markers

Improvements in sequencing technologies have enabled the analysis of mitochondrial and nuclear DNA markers. Analysis of these markers has been applied in several species of the tribe, both for intra- and inter-species analysis and together with other species of the Triatominae subfamily. Some of these studies have been carried out in combination with other molecular markers [8,38]. Nuclear and mitochondrial marker sequencing studies (26 conducted to date) have explored the phylogenetic relationships among the species of the tribe. Twelve studies explored species of the Rhodniini tribe and other tribes. The following mitochondrial markers have been evaluated: 16S rRNA (16S ribosomal RNA), 12S rRNA (12S ribosomal RNA), ND4 (4 subunit NADH dehydrogenase), Cytb (Cytochrome b), COI (Cytochrome oxidase I), and COII (Cytochrome Oxidase II). The following nuclear markers have been evaluated: 18S (18S ribosomal RNA), ITS-2 (internal transcribed spacer of ribosomal DNA 2), EF-1α (elongation factor 1 alpha), Wg (Wingless), and 28S rRNA (RNA ribosomal 28S) [8,38,40]. The sequences of the Cytb gene were searched in Genbank using the following Entrez line: "esearch -db nucleotide -query "<organism> CYTB"|efetch -format fasta" that yielded 226 records corresponding to 13 species of *Rhodnius* (Table S1) and *Triatoma infestans* sequence was used as outgroup. The alignment was made using MUSCLE, correct by hand and the sequence were translated to proteins in order to verify for stop codons in Mesquite 3.04. A maximum likelihood topology was obtained with the evolution model HKY + F + I + G4 (BIC score: 6711.067753) in IQ-TREE and node support was calculated with 1000 ultrafast bootstrap replicates. (Figure 5). In relation to the other tribes (mostly the Triatomini tribe), the Rhodniini tribe is of monophyletic origin [114–119]. In studies in which specimens of the Cavernicolini and Borbodelini tribes were included, these tribes were grouped with the Rhodniini and not with the Triatomini, although all tribes preserved their monophyletic status. However, it should be noted that some of these studies only included one or two species of the Rhodniini tribe, given that their focus was not on the Rhodniini tribe but on the subfamily Triatominae and other Reduviidae [120–125].

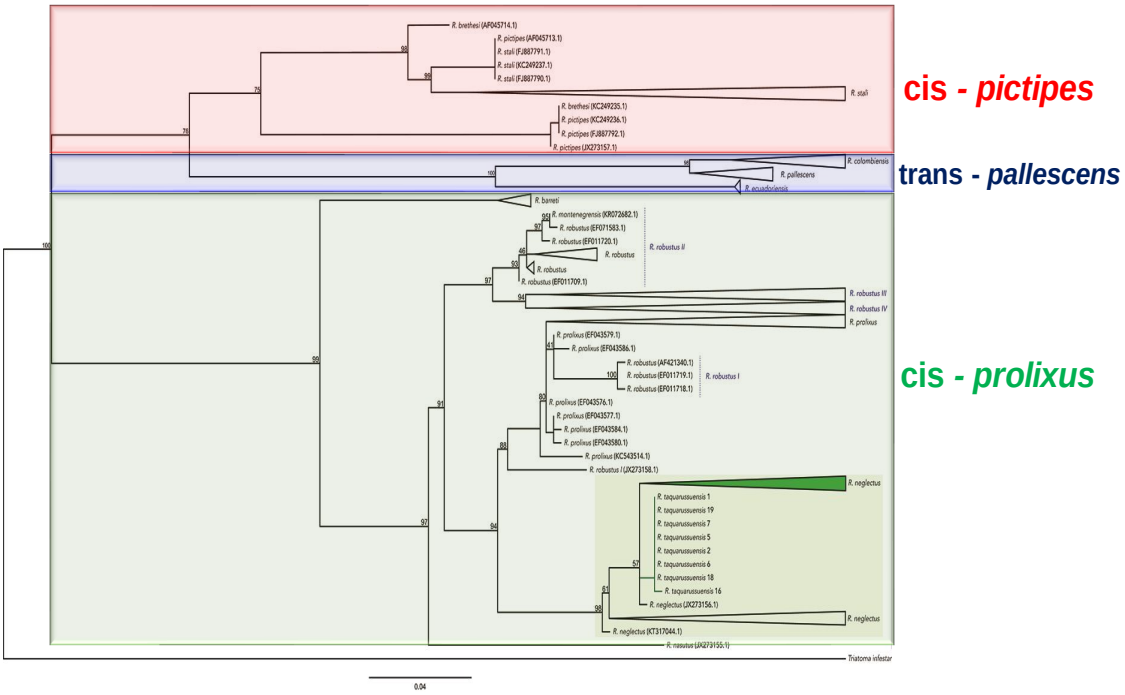

Figure 5. Maximum likelihood tree for *Rhodnius* based on Cytb sequences. The tree was reconstructed using sequences of *Rhodnius* species from GenBank.

The presence of two groupings within the *pictipes* group (Table 2) has led some authors to propose that the genus *Rhodnius* should be composed of clades or lineages. Some authors have proposed that the lineage *prolixus* be formed only by species of the group *prolixus* (named *robustus* lineage for other authors) and that another lineage should be formed by the species of the *pallescens* and *pictipes* groups called lineage *pictipes* [7,79,80,126]. Other authors proposed that the *prolixus* clade should be formed by species of the group *prolixus* and the group *pictipes* [122].

The remaining 14 studies focused on evaluating the phylogenetic relationships of Rhodniini species only and the grouping of species in particular. In these studies, the number of markers was limited to five: two mitochondrial (Cytb and ND4) and three nuclear (28S, AMPg, and ITS-2) [8]. Regarding phylogenetic relationships within the tribe, different issues have been identified that have been controversial with respect to previous studies: (i) paraphyly of the two genera of the tribe and grouping of the genus *Psammolestes* with members of the group *prolixus* were observed; (ii) the grouping of lineages within the genus *Rhodnius* was incongruent as well as the assignment of some species within groups; and (iii) the validity of some species was questionable due to inconsistencies in some phylogenies. These controversial aspects arose from the fact that many studies did not address all species of the tribe: most studies were intraspecific and used a limited number of markers. The objectives of most studies were limited to describing lineages but did not attempt the accurate delineation of all species nor the understanding the evolutionary processes that led to their formation [8,38].

Table 2. Characteristics of phylogenetic studies that have evaluated the relationships between groups of the genus *Rhodnius*.

Grouping	Nuclear Marker	Mitochondrial Marker	Taxa	Reconstruction Method	Reference
pictipes + *pallescens*		16S	4	MP	Stothard et al., 1998
		16S, Cyt b	8	NJ	Lyman et al., 1999
		16S, Cyt b	15		Schofield and Dujardin, 1999 *±
	28S	16S, Cyt b	13	MP, NJ	Monteiro et al., 2000
		Cyt b	18	NJ	Monteiro et al., 2018 ±
	28S	Cyt b	9	NJ	Marquez et al., 2011
		Cyt b	12	MP	Maia da Silva et al., 2007
		Cyt b	5	ML, BI	Da Rosa et al., 2012
pictipes + *prolixus*		16S, 12S	14	MP, NJ	Hypsa et al., 2002
		16S	14	MP, ML	De Paula et al., 2007 ±
		16S	14	MP, ML	De Paula et al., 2005
	18S, 28S	16S, Cyt b, COI, COII	10	ML, BI	Justi et al., 2014
	18S, 28S, Wg	16S	11	ML, BI	Justi et al., 2016

* In this study, several markers were consolidated: RAPD, isoenzymes, morphometry and marker sequencing. NJ: Neighbor-Joining, MP: Maximum parsimony, ML: Maximum likelihood, BI: Bayesian inference. ± In this study, the sequences were collected from previous studies.

Several primarily morphological studies established that the Rhodniini tribe is made up of two monophyletic genera [41,46,56]. However, in nine studies both nuclear [ITS-2, 28S] and mitochondrial (Cyt b, 18S, 12S, and 16S) molecular markers have indicated the paraphyly of *Psammolestes* and *Rhodnius* [38,79,114,115,117,118,122,123,127]. Some studies showed that *P. coreodes* [79,114,115,117, 122,127], *P. tertius* [79,114,115,117,122,127] and *P. arthuri* [123] were grouped with the species of the *prolixus* group of the genus *Rhodnius*, while a neighbor-joining analysis of Cytb sequences indicated the paraphyly of *Psammolestes* but not its grouping with the *prolixus* group [38,123].

Sequencing of molecular markers has detected groupings within the genus that have been postulated using other markers. However, sequencing did not confirm that the three groups were monophyletic nor the expected basal location of the *pictipes* group; these results have been controversial [40,117]. Some studies have found that the *pictipes* group is more closely related to the *pallescens* group [38,56,79,118,119,126,128], while other studies described topologies in which the *pictipes* group is more closely associated with the *prolixus* group [117,121,122,127]. These differences in the associations between groups may be due to variability in study characteristics (Table 2): the number of taxa may be an important aspect that provides greater precision to phylogenetic reconstructions [121,129].

Within the genus *Rhodnius*, there are also controversies regarding groupings of species. One example consists of the associations among species of the group *pallescens*, since after the identification of *R. colombiensis* it was grouped in some studies with *R. ecuadoriensis* [79,122] and in others with *R. pallescens* [38,117,127,130]. The position of *R. neivai* within the group *prolixus* has also been questioned, because some studies have proposed that it should belong to the group *pictipes* [56] and others that it should belong to the *prolixus* group [79,117,127]. In other cases, a clear location was not identified in any of the groups [38,80]. The validity of the species *R. robustus* has been strongly questioned and for that reason phylogenetic studies have been carried out to explore its associations with *R. prolixus*, mainly due to the important epidemiological implications of confusing the two species due to their morphological similarity. Monteiro et al., 2000, conducted a first study using specimens

of both species and sequenced 28S, 16S, and Cytb. These analyses showed discordances between morphological identification and phylogenetic grouping of individuals of both species [79].

These confusions in classification of these species led to a second study in which 26 different specimens of the two species from seven countries of Central and South America were used. Mitochondrial Cytb sequences were analyzed and the results showed that all specimens of *R. prolixus*—both from Central and South America—were grouped into a monophyletic and homogeneous cluster. By contrast, *R. robustus* showed a paraphyletic assembly composed of four clades (I–IV) from two different geographic regions: specimens of *R. robustus* clade I came from Venezuela and clades II, III, and IV came from French Guyana and different subregions of the Brazilian Amazon. Phylogenetic and distance analyses showed two main groups, one formed by *R. prolixus* and *R. robustus* I and another formed by the three remaining clades of *R. robustus*. This drew attention to the genetic distance between *R. robustus* clade I and *R. robustus* clades II, III, and IV, and to the distance between *R. robustus* I and *R. prolixus* [61]. These groupings and the topology of the two species were replicated in a study that included 551 specimens from Orinoco and Amazonia [22]. Subsequently, *R. robustus* clade V was discovered in the central-north zone of the Amazon and grouped with *R. robustus* clade I and *R. prolixus* [38,131]. Subsequent studies used the term cryptic species for *R. robustus* instead of clades or genotypes I–IV and confirmed the paraphyly of this species with *R. prolixus*. In these studies, two techniques were used to differentiate the two species: band size of PCR amplicons derived from Cytb [62] or sequencing the nuclear marker AMPg (a region located in the fourth intron of transmembrane protein 165) [132]. Other arguments such as loss of fertility (number of eggs per female) in interspecific crosses favored the differentiation of the two species; however, no overall loss of fertility was observed (number of females that lay eggs) in these crosses [133,134].

Another study revealed that specimens similar to *R. robustus* (identified by classical morphometry) collected in Puerto Asis, Colombia, corresponded according to sequence analysis of Cytb and 28S a new cryptic species of *R. robustus*, previously reported in Ecuador (Abad-Franch 2005,111). These specimens did not group with any other cryptic species of *R. robustus* and were located as a basal clade of the *prolixus* group [126]. However, more recent analyses of Cytb and morphometry indicated that the previously reported *R. robustus* is located as a basal clade only of *R. robustus* clade I and *R. prolixus*, and that these specimens do not correspond to *R. robustus* but rather represent a new species called *R. barretti* that is basal to the *prolixus* group [38,63].

The analysis of Cytb and ITS-2 markers performed by Monteiro et al. has been applied in some species whose validity has been questioned. However, in some species the number of specimens analyzed was very limited. The first was *R. amazonicus*, which was questioned as a potential variant of *R. pictipes*; the study showed that this species had an independent origin and did not group with specimens of *R. pictipes*. Other species questioned by marker sequencing were *R. milesi* (because of its monophyly with specimens of *R. neglectus*) and *R. montenegrensis* (because of its location within *R. robustus* clade II). However, another study using Cytb sequences identified *R. montenegrensis* as an independent species of *R. robustus*, supported by morphological characters and PCR restriction fragment length polymorphism analysis of ITS-2 [135]. Several species have also been questioned due to a lack of information regarding molecular markers, including *R. marabaensis*, *R. taquarusuensis*, *R. zeledoni*, and *R. dalessandroi*. Sequencing of Cytb has also enabled identification of species such as *R. stali* in *A. phalerata* palms in Alto Beni, Bolivia, and *R. prolixus* in *A. butyracea* palms in Casanare, Colombia [38].

Finally, Cytb sequencing has also been used to assess intraspecific variability of several species of the tribe including *R. prolixus*. The results showed very low genetic diversity and that wild and domestic specimens shared 7/18 haplotypes found in all analyzed specimens [22]. In 157 specimens of *R. nasutus* collected in eight localities of the Brazilian Caatinga, 16 haplotypes of Cytb were detected, of which two major haplotypes were shared by specimens from five localities with low levels of diversity observed [36]. In the case of *R. ecuadoriensis*, 174 specimens from two provinces of Ecuador (Loja and Manabí) were analyzed and 34 haplotypes were identified, of which only three were shared between the

two provinces. High haplotype diversity was observed and the model of isolation by distance applied to the two populations, showing that the populations of the two provinces were highly differentiated at the genetic level [113]. In *R. pallescens*, analysis of the Cytb, ND4 and 28S markers in populations from Panama and Colombia indicated the presence of two evolutionary lineages with genetic and morphological differences associated with their biogeographical and ecological distribution. It was even possible to observe genetic variation within lineages. In this manner, the Colombian lineage *R. pallescens* clade I could be subdivided into two populations, one from the north and one from southern Colombia and the Central American lineage *R. pallescens* clade II, which is composed of populations collected in western Colombia and different areas of Panama [92,130].

4.7. The Advent of Genomic Data: Genomics and Transcriptomics in the Rhodniniі

Recently, additional sequencing tools have been applied to some members of the Triatominae subfamily and specifically to the Rhodniini tribe to obtain genome and transcriptome data. So far, the only member of the subfamily whose genome has been sequenced is *R. prolixus*; its genome was sequenced with 8× coverage using Sanger and 454 technologies and assembled using the CABOG program [120]. The genome was composed of 16,537 scaffolds with an N50 of 1.08 Mb, had an estimated size of 733 Mb and achieved an assembly that was 95% complete, corresponding to 706 Mb without chromosome mapping. The most updated version (October 2017) includes annotation of 15,738 genes and 15,752 transcripts (Rpro version C3.3 in VectorBase) (https://www.vectorbase.org/organisms/rhodnius-prolixus/cdc/rproc33).

This important approach to the genome of *R. prolixus* showed that 5.6% of the genome corresponded to transposable elements and demonstrated the presence of transcriptionally active genes transferred horizontally from *Wolbachia*. Exploration of the *R. prolixus* genome and knockdown experiments confirmed the presence of genes from different immune pathways (Toll and Imd) and the expansion of defensins, which function to control the intestinal microbiota but are unrelated to *T. cruzi* infection. Finally, comparative analysis of proteins revealed tandem expansions of genes families related to chemoreception, feeding, and digestion that potentially contributed to the evolution of a blood-feeding lifestyle [136].

The genome of *R. prolixus* has been used to search for satellite DNA sequences previously described in *T. infestans* using BLAST. The two species share four families of satellite DNA sequences of the 42 that are present in *T. infestans*, suggesting that their genomes are highly differentiated. Through hybridization experiments, it was demonstrated that these shared sequences were found at the autosomal level and the X chromosome in both species. By contrast, these sequences were absent in the Y chromosome in *T. infestans* and present in *R. prolixus*, suggesting a possible origin and evolution of the independent Y chromosome [137].

Recently, mitochondrial genomes of three triatomine species (*R. pictipes*, *Triatoma migrans*, and *Panstrongylus rufotuberculatus*) were sequenced and compared with the mitochondrial genomes of *Triatoma rubrofasciata*, *T. infestans*, and *Triatoma dimidiata* to explore evolutionary relationships with the other Reduviidae species and between the Rhodniini and Triatomini tribes. The results showed that mitochondrial genes had different rates of molecular evolution and six genes (ND1, ND2, ND4L, ND5, ND6, and ATP8) had higher rates than other protein-coding genes (PCGs) in all species. *R. pictipes* showed differences in the start codons of three PCGs (ND4L, ND6, and ND1) and in the stop codons of two PCGs (ATP6 and ND1) compared with other *Triatoma* species. Additionally, the sister relationship between Stenopodainae and Triatominae subfamilies was strongly supported, and the Triatomini species formed a sister group to *R. pictipes* [138].

Several transcriptomes of *R. prolixus* have been analyzed. The first was derived from ovarian follicle tissue in order to explore the role of gene expression in insect reproductive processes. This study showed expression of some genes that promote oogenesis and development of the embryo, suggesting that they may be important in insect control processes [139]. The transcriptome of the digestive system has also been described, which enabled exploration and differentiation of transcripts present in the three

segments of the intestine. The results showed that increased levels of some transcripts were related to the processes of digestion, detoxification, and transport of proteins through the digestive tract [140]. The transcriptome of the antennas has been described in all larval stages in order to characterize the expression of genes involved with the sensory functions of the insect. The results showed increased expression of genes related to chemoreceptors mainly in adult stages, as well as high expression of odorant binding proteins and chemosensory proteins in all stages [141]. Transcriptomes of *T. dimidiata*, *T. infestans*, and *T. pallidipennis* and the genome of *R. prolixus* have also been compared to evaluate the expression of gene families related to resistance to insecticides. In the case of *R. prolixus*, these studies revealed the expansion of two families, CYP4 (cytochrome P450-4) and CCE (carboxyl-Cholinesterases), related to pyrethroid resistance, odor processing, and degradation of hormones and pheromones [142].

A recent study analyzed and compared the transcriptomes of the head and salivary glands of *R. robustus* and *R. montenegrensis*; the validity of the latter species has been questioned due to its limited morphological differences compared with the former. The authors used RNA-Seq and detected 3055 single nucleotide polymorphisms (SNPs) distinguishing the two species and 216 transcripts with high levels of divergence. Several SNPs were detected in the same contig, suggesting that the two species were highly differentiated with possibly an extended time of divergence. In addition, the authors suggested that some of the genes studied could be subsequently tested for use in the identification and differentiation of these two species [143]. Finally, the most recent study combined the analysis of the previously reported transcriptomes of *R. robustus* and *R. montenegrensis* with the analysis of three molecular markers: Cytb, 28S, and ITS-2. In this study, it was concluded that *R. montenegrensis* and *R. robustus* clade II are in all likelihood the same species [127]. However, the repeatome and proteomic analyses detected high differentiation between *R. prolixus*, *R. montenegrensis*, and *R. marabaensis*, showing they are different species [144,145].

5. Biogeographical Hypotheses Pertaining to the Rhodninii Tribe

Studies of biogeography in the Rhodniini tribe are limited in number but revealed high complexity, including theories of possible vicariance, duplications (sympatry), dispersion, and extinction events. These are related to several geological events such as: (i) the elevation of the Central Andes during the Miocene; (ii) the branching of the Andes into three separate mountain ranges (eastern, central, and western) during the Plio-Pleistocene; (iii) the formation of a land corridor connecting South and North America during the Pliocene; and (iv) the elevation of the Serra do Mar and the mountain systems of the Serra da Mantiqueira between the Oligocene and the Pleistocene [56,127]. Because morphometric, cytogenetic, and molecular markers have yielded contradictory results in several systematic and phylogenetic studies of the Rhodniini tribe, there are several contradictory hypotheses regarding the origin and diversification of the species within this tribe.

5.1. Monophyletic Groups Hypothesis

The first hypothesis was formulated by Schofield and Dujardin in 1999. It assumes that the ancestor of the Rhodniini was closest to *R. pictipes* species and originated during the Quaternary Period in arboreal habitats of the Amazon-Orinoco and tropical forests dispersed towards the south of the Brazilian Amazon and the northeast of Bolivia. In turn, the ancestor gave rise to *R. stali* in palms and invaded homes. This ancestor underwent other more specific dispersions, giving rise to the remaining species of the *pictipes* group: (i) towards the north of Colombia and central Venezuela, to *R. neivai* which is found in *Copernicia tectorum* palms; (ii) towards the border area between Brazil, Colombia and Venezuela, to *R. brethesi* which is found in palms of *Leopoldinia piassaba*; (iii) towards the Colombian Orinoquia, to *R. dalessandroi* which is morphologically very similar to *R. brethesi* but is found in other palms; and (iv) towards the state of Pará, Brazil, to *R. paraenesis* which is found in nests of *Echymis crysurus* (Figure 6A). The ancestor *pictipes* also gave rise to the group *pallescens*. In this case, the ancestor *R. pictipes* was dispersed towards the northwest of Colombia and through a bottleneck in the Sierra Nevada de Santa Marta and the northern tip of the Cordillera de los Andes, gave rise to *R. pallescens*,

which is associated with *Attalea butyracea* palms and whose range extends to Central America and northwestern Colombia. The ancestor then dispersed through the Magdalena valley giving rise to *R. colombiensis* and into eastern Ecuador and Peru, giving rise to *R. ecuadoriensis* in Ecuador and northern Peru, possibly due to adaptation to the palm *Phytolepas aequatorialis* (Figure 6B) [56].

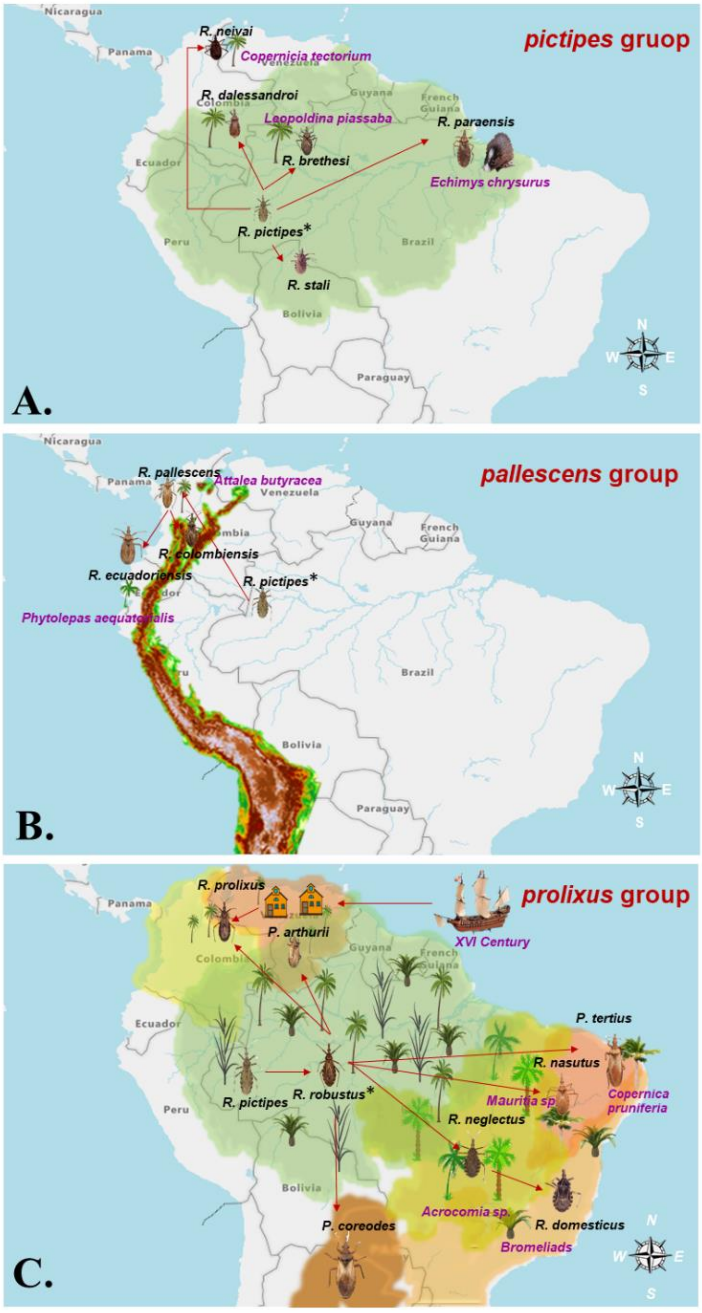

Figure 6. Monophyletic groups biogeographic hypothesis explaining speciation within the Rhodninii tribe. (**A**). Speciation and dispersion of *pictipes* group species (**B**). Speciation and dispersion of *pallescens* group species (**C**). Speciation and dispersion of *prolixus* group species. The asterisk (*) indicates the species closest to the most recent common ancestor (MRCA) of Rhodniini tribe by each hypothesis.

The ancestor *R. pictipes* gave rise to *R. robustus* which was scattered throughout the Amazon in two forms: (i) a first form dispersed to the north reaching Venezuela where it gives rise to wild *R. prolixus* and *P. arthuri*; and (ii) a second form dispersed towards the south reaching the Brazilian Cerrado, where

it gave rise to *R. neglectus* (associated with *Mauritia* and *Acrocomia* palms) and *P. tertius*. The latter and the southern form of *R. robustus* are dispersed towards the Caatinga where *R. robustus* gave rise to *R. nasutus* associated with the palm *Copernicia prunifera*. Additionally, *R. neglectus* is dispersed towards the Atlantic Forest where it gave rise to *R. domesticus* associated with Bromelia. Finally, this hypothesis proposes that the Spanish colonization of Venezuela triggered the domiciliation of wild *R. prolixus* and that the presence of *R. prolixus* domiciled in Central American countries may be due to the transport of some collections from Venezuela to El Salvador followed by their accidental release in rural homes. Subsequently, they dispersed and adapted to homes of several Central American countries. This last hypothesis is supported by morphometric analysis, isoenzyme analysis, and RAPD (Figure 6C) [56,108].

5.2. Lineages Hypothesis

Conflicting with these hypotheses, analysis of molecular markers did not support the idea that *R. pictipes* was the species closest to the ancestor of the three groups of *Rhodnius*. Instead, two topologies were generated, one in which *R. pictipes* was a sister group to the *pallescens* group and another in which it was a sister group to the *prolixus* group (Table 2). In addition, some studies grouped *R. colombiensis* as a sister species of *R. pallescens* and others as a sister speices of *R. ecuadoriensis*. Thus, two different hypotheses supported the two types of topologies and groupings observed [7,122,127].

The second hypothesis, based on morphometric studies, mitochondrial markers and isoenzyme analysis, was proposed by Abad-Franch et al., 2009 and Díaz et al., 2016. Under this hypothesis, the genus *Rhodnius* is classified into two lineages (*pictipes* and *robustus*). The first gave rise to the transandean (*pallescens*) group and the Amazonian species (*pictipes*), while the second diversified into the Amazon (group *robustus*) and underwent radiation in nearby ecoregions (Orinoco, Chaco, Caatinga, Cerrado, and Mata Atlântica) allowing the formation of the cis-*prolixus*. Under this hypothesis it is assumed that *R. colombiensis* and *R. pallescens* are sister species. Thus, the ancestor of the *pictipes* lineage was dispersed to the northern part of the then low eastern mountain range of Colombia in the late Miocene (11–6 Ma) [7,131] or the mid-Miocene (16–11 Ma) [116]. Thus, the diversification of the *pallescens* and *pictipes* could be explained by a vicariance, either caused by the formation of the Pebas system [130] or by the elevation of the Andes mountain range during the Pliocene (5 Ma) [7,131]. Regarding the diversification of the ancestral *pallescens*, two hypotheses have been proposed: (i) the divergence of the ancestor of *R. pallescens*/*R. colombiensis* and *R. ecuadoriensis* could have occurred during the late Miocene, a period during which the Northern Andes had not reached more than half of its modern elevation (11–7 Ma), and the diversification of the ancestor of *R. pallescens*/*R. colombiensis* occurred with the elevation of the northern Andes and the separation of the *R. pallescens* lineages with the formation of the isthmus of Panama during the early Pliocene [130]; and (ii) diversification of the ancestral group *pallescens* occurred during the Pliocene with the elevation of the then-low eastern mountain range of Colombia, which divided the population into two main clades, a northern group comprising the ancestral forms of *R. pallescens* and *R. colombiensis*, and an isolated southern group which adapted to the new ecotopes and eventually gave rise to *R. ecuadoriensis* [7,131]. The radiation of the ancestor of *pictipes* in the Amazon is postulated to have occurred during the Pliocene and Pleistocene, according to the specific adaptations described under the first hypothesis, with the exception of *R. neivai* that was included in the *robustus* lineage [7].

However, the radiation of the taxa of the *robustus* lineage (*R. domesticus*, *R. neivai*, *R. robustus*, and *R. nasutus*) in the ecoregions mentioned above can be attributed to another possible vicariance event at the end of the Miocene and subsequent radiation during the Pliocene. The division of the *robustus-neglectus-prolixus* group can be explained by more recent cladogenic events during the Pliocene and Pleistocene. Additionally, it was proposed that the lineages of *R. robustus* were potentially generated by parapatric speciation during the Pleistocene, and *R. robustus* in Venezuela does not appear to be a product of gene flow between *R. prolixus* and *R. robustus* from Amazon basin, but rather *R. prolixus* and *R. robustus* I seem to share a (relatively young) MRCA (most recent common ancestor) [7,61].

5.3. Clades Hypothesis

Finally, the third hypothesis proposed by Justi et al., 2016 using nuclear and mitochondrial markers proposes the formation of the *prolixus* clade (cis-andean) and locates the *pictipes* and *prolixus* groups as siblings, suggesting that the separation of the *prolixus* (cis-andean) group from the *pictipes* group (cis-andean) occurred by means of a vicariance during the formation of the Pebas system (23–11 Ma). Thus, the ancestor of the *prolixus* group was isolated in subregions of Brazil and that of the *pictipes* in the Chaco subregion and then the Amazon. Subsequently, the formation of the Acre system (10–7 Ma) allowed the ancestor of *R. neivai*/*R. domesticus* to diversify towards the north, giving rise to *R. neivai*, and towards the south, giving rise to *R. domesticus*. With respect to the group *pallescens*, this hypothesis proposes that divergence from the clade *prolixus* occurred during the late Miocene via the formation of the Pebas and that the separation of the three species occurred during the Pliocene. However unlike the second hypothesis, it proposes that separation occurred between *R. pallescens* and an ancestor *R. colombiensis*/*R. ecuadoriensis*, probably induced by the expansion of *R. pallescens* in Central America [122].

6. Future Perspectives

In the Andean countries, the main triatomines responsible for vector-mediated transmission of *T. cruzi* to people are those of the genus *Rhodnius*; thus, control measures are directed to species of this genus. Therefore, further studies of the evolution, phylogeny, biogeography, ecology, physiology, and behavior of *Rhodnius* species are needed to help improve existing Chagas disease control programs [8,10,38]. One of the primary steps in any control program is the accurate identification of vector species and detailed understanding of their genetic and population structures.

Therefore, future studies should be based on integrative taxonomy approaches, taking into account the delimitations of species, the synthesis of morphological characteristics with information obtained from molecular genetic studies, biogeography, phylogeography, behavior, ecology, and development [146]. The incongruities shown so far between morphological and genetic studies are due to several intrinsic limitations of several techniques. Studies to date have focused on describing groupings of species within the tribe but have not delimited these groups within the tribe, which has generated systematic problems.

One of the most important limitations of current studies lies in the limited morphological variability between different species of the tribe; therefore, there are few synapomorphic characters. Added to this, identification of some species of the tribe (*R. prolixus* vs. *R. robustus*) relies on few characters with complex interpretation, resulting in inconsistent results between morphological studies and errors in morphological identification. These problems arose because few cladistic studies or studies based on morphological characters of all species of the Rhodniini tribe have been conducted [8,74,147].

Some characters used for identification of species are affected by phenotypic variability and morphological plasticity in association with environmental changes. The latter generates minor morphological changes between populations of the same species and can therefore lead to erroneous identification, such as the presence of color morphotypes in *R. nasutus*. These changes can occur before genetic barriers are present between species and therefore will generate inconsistencies between morphological and genetic studies. Phenotypic variability and morphological plasticity can also lead to morphological convergence between species that are genetically distinct but are adapted to the same ecological niche, as is the case for species of the *prolixus* group [38,40]. Due to these complicating factors, future studies should focus on identifying morphological synapomorphic characters that facilitate differentiation and identification of appropriate species, and are complemented by genetic, biogeographic, behavioral, and ecological analyses. This would help in laying out a rational approach to the systematics of the tribe.

Complexities in delineation of species of the Rhodniini tribe could also be addressed through crosses in the laboratory: these would allow evaluation of reproductive isolation and the potential presence of hybrids between species. Although several studies have been carried out in the Triatomini tribe, few reports have documented a cross between members of the Rhodniini tribe (*R. prolixus*–*R.*

robustus and *R. prolixus–R. nasutus*) [134,148]. This reflects the complex morphological identification of species of the tribe and the difficulties associated with identification of hybrids, because these usually correspond to morphological intermediates between the parental species. Thus, it is important that future studies carry out interspecific crosses to verify if members of the tribe meet the biological definition of species. These crosses should be evaluated not only at the level of morphological characters, but also using genetic characters such as molecular markers. Analyses of nuclear DNA and mitochondrial DNA can both be used for appropriate identification of species involved in crosses as well as for analysis of the progeny of crosses: the nuclear and mitochondrial DNA sequences allow identification of introgression and the latter allows identification of hybridization processes [149].

Finally, genetic studies in the tribe have been limited, and the majority of them have focused on studying relationships between the Rhodniini tribe and other members of the Triatominae or Reduviidae. Additionally, studies that have exclusively examined species of the tribe have focused on analysis of epidemiologically important species or been focused at the intra-specific level. In addition, different studies have examined different species and are thus not directly comparable. There is only one study in which Cytb sequences from most of the tribe's species were included [38]. Therefore, it is necessary to carry out studies that include a large number of species of the Rhodniini tribe to attempt relevant delineation of tribe members.

In *Anopheles* and several species of the Triatomini tribe, nuclear ribosomal sequences present intragenomic variability due to their high copy number. Thus, data obtained from these specimens should be analyzed with caution because errors of interpretation may occur. Multilocus approaches will enable better resolution in phylogenetic analyses and also allow identification of introgression and potential hybridization events. Therefore, attempts to design molecular markers for multilocus studies are required; these markers need to be sequenced in a representative number of species to elucidate their phylogenetic relationships and to provide useful tools for integrative taxonomy [149].

Finally, genomic and transcriptomic resources in the tribe are scarce. To date, only the mutation rates of Cytb sequences in species of the genus *Triatoma* and of mitochondrial loci in *R. pictipes* have been examined. This information may be used for coalescence analyses and phylogeographic reconstructions in future studies. However, the genomic mutation rate of at least one representative species of the Rhodniini tribe remains to be determined. The genome of *R. prolixus* has low coverage and has not yet been assembled at a chromosomal level. Therefore, future studies could improve genomic resources, especially because the resolution of phylogenies drastically improves when genomic data are used, as previously documented in plants and in a recent phylogenomic analysis of the Hemiptera [150,151].

7. Conclusions

In spite of the valuable contributions of studies carried out to date on species of the Rhodniini tribe, limitations in our understanding have led to inconsistencies between and within morphological and phylogenetic studies. In turn, this has created three problems in the systematics of the Rhodniini tribe: (i) paraphyly of two genera in the tribe, (ii) different types of classification and grouping of the species of the genus *Rhodnius*, and (iii) difficulties in identification and adequate delineation of some species. These issues have resulted in generations of conflicting hypotheses regarding the origin, evolution, and dispersion of the species of the Rhodniini tribe. This information is of great importance because of the biologic role of several species of the tribe in transmission of *T. cruzi*. Thus, it is necessary to conduct further genetic, ecological, morphological, and biogeographical studies of the tribe to provide an integrative approach that can address the current systematic and taxonomic inconsistencies; in turn, these studies will provide the information necessary to develop improved vector control strategies for Chagas disease.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/3/97/s1, Table S1: Table S1_Cytb_Rhodnius_Sequences.

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References

1. Lidani, K.C.F.; Andrade, F.A.; Bavia, L.; Damasceno, F.S.; Beltrame, M.H.; Messias-Reason, I.J.; Sandri, T.L. Chagas Disease: From Discovery to a Worldwide Health Problem. *Front. Public Health* **2019**, *7*, 166. [CrossRef]
2. WHO. Chagas disease in Latin America: An epidemiological update based on 2010 estimates. *Wkly. Epidemiol. Rec.* **2015**, *90*, 33–43.
3. Waleckx, E.; Gourbière, S.; Dumonteil, E. Intrusive versus domiciliated triatomines and the challenge of adapting vector control practices against Chagas disease. *Mem. Inst. Oswaldo Cruz* **2015**, *110*, 324–338. [CrossRef] [PubMed]
4. Bern, C. Chagas' Disease. *N. Engl. J. Med.* **2015**, *373*, 456–466. [CrossRef] [PubMed]
5. De Noya, B.A.; González, O.N. An ecological overview on the factors that drives to Trypanosoma cruzi oral transmission. *Acta Trop.* **2015**, *151*, 94–102. [CrossRef]
6. Flores-Ferrer, A.; Marcou, O.; Waleckx, E.; Dumonteil, E.; Gourbière, S. Evolutionary ecology of Chagas disease; what do we know and what do we need? *Evol. Appl.* **2018**, *11*, 470–487. [CrossRef]
7. Abad-Franch, F.; Monteiro, F.A.; Jaramillo-O., N.; Gurgel-Gonçalves, R.; Dias, F.B.S.; Diotaiuti, L. Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: A multi-scale appraisal of the tribe Rhodniini (*Triatominae*). *Acta Trop.* **2009**, *110*, 159–177. [CrossRef]
8. Justi, S.A.; Galvão, C. The Evolutionary Origin of Diversity in Chagas Disease Vectors. *Trends Parasitol.* **2017**, *33*, 42–52. [CrossRef]
9. Poinar, G. A primitive triatomine bug, Paleotriatoma metaxytaxa gen. et sp. nov. (*Hemiptera*: Reduviidae: *Triatominae*), in mid-Cretaceous amber from northern Myanmar. *Cretac. Res.* **2019**, *93*, 90–97. [CrossRef]
10. Gorla, D.; Noireau, F. Geographic Distribution of Triatominae Vectors in America. In *American Trypanosomiasis Chagas Disease*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 197–221. ISBN 978-0-12-801029-7.
11. Da Rosa, J.A.; Souza, E.S.; da Costa Teixeira, A.; Barbosa, R.R.; de Souza, A.J.; Belintani, T.; Nascimento, J.D.; Gil-Santana, H.R.; de Oliveira, J. Third record of Rhodnius amazonicus and comparative study with R. pictipes (*Hemiptera*, *Reduviidae*, *Triatominae*). *Acta Trop.* **2017**, *176*, 364–372. [CrossRef]
12. Schofield, C.J.; Galvão, C. Classification, evolution, and species groups within the Triatominae. *Acta Trop.* **2009**, *110*, 88–100. [CrossRef] [PubMed]
13. Bilheiro, A.B.; da Rosa, J.A.; de Oliveira, J.; Belintani, T.; Fontes, G.; Medeiros, J.F.; de Meneguetti, D.U.O.; Camargo, L.M.A. First Report of Natural Infection with Trypanosoma cruzi in Rhodnius montenegrensis (*Hemiptera*, *Reduviidae*, *Triatominae*) in Western Amazon, Brazil. *Vector Borne Zoonotic Dis.* **2018**, *18*, 605–610. [CrossRef] [PubMed]
14. Guhl, F.; Vallejo, G.A. Trypanosoma (Herpetosoma) rangeli Tejera, 1920: An updated review. *Memórias do Instituto Oswaldo Cruz* **2003**, *98*, 435–442. [CrossRef] [PubMed]
15. Ocaña-Mayorga, S.; Aguirre-Villacis, F.; Pinto, C.M.; Vallejo, G.A.; Grijalva, M.J. Prevalence, Genetic Characterization, and 18S Small Subunit Ribosomal RNA Diversity of *Trypanosoma rangeli* in Triatomine and Mammal Hosts in Endemic Areas for Chagas Disease in Ecuador. *Vector-Borne Zoonotic Dis.* **2015**, *15*, 732–742. [CrossRef] [PubMed]

16. Salazar-Antón, F.; Urrea, D.A.; Guhl, F.; Arévalo, C.; Azofeifa, G.; Urbina, A.; Blandón-Naranjo, M.; Sousa, O.E.; Zeledón, R.; Vallejo, G.A. Trypanosoma rangeli genotypes association with Rhodnius prolixus and R. pallescens allopatric distribution in Central America. *Infect. Genet. Evol.* **2009**, *9*, 1306–1310. [CrossRef]
17. Saldaña, A.; Santamaría, A.M.; Pineda, V.; Vásquez, V.; Gottdenker, N.L.; Calzada, J.E. A darker chromatic variation of Rhodnius pallescens infected by specific genetic groups of Trypanosoma rangeli and Trypanosoma cruzi from Panama. *Parasit. Vectors* **2018**, *11*, 423. [CrossRef]
18. Urrea, D.A.; Carranza, J.C.; Cuba, C.A.C.; Gurgel-Gonçalves, R.; Guhl, F.; Schofield, C.J.; Triana, O.; Vallejo, G.A. Molecular characterisation of Trypanosoma rangeli strains isolated from Rhodnius ecuadoriensis in Peru, R. colombiensis in Colombia and R. pallescens in Panama, supports a co-evolutionary association between parasites and vectors. *Infect. Genet. Evol.* **2005**, *5*, 123–129. [CrossRef]
19. Urrea, D.A.; Guhl, F.; Herrera, C.P.; Falla, A.; Carranza, J.C.; Cuba-Cuba, C.; Triana-Chávez, O.; Grisard, E.C.; Vallejo, G.A. Sequence analysis of the spliced-leader intergenic region (SL-IR) and random amplified polymorphic DNA (RAPD) of Trypanosoma rangeli strains isolated from Rhodnius ecuadoriensis, R. colombiensis, R. pallescens and R. prolixus suggests a degree of co-evolution between parasites and vectors. *Acta Trop.* **2011**, *120*, 59–66.
20. Vallejo, G.A.; Guhl, F.; Schaub, G.A. *Triatominae*–Trypanosoma cruzi/T. rangeli: Vector–parasite interactions. *Acta Trop.* **2009**, *110*, 137–147. [CrossRef]
21. Angulo, V.M.; Esteban, L.; Luna, K.P. Attalea butyracea próximas a las viviendas como posible fuente de infestación domiciliaria por Rhodnius prolixus (*Hemiptera*: *Reduviidae*) en los Llanos Orientales de Colombia. *Biomedica* **2012**, *32*, 277–285. [CrossRef]
22. Fitzpatrick, S.; Feliciangeli, M.D.; Sanchez-Martin, M.J.; Monteiro, F.A.; Miles, M.A. Molecular Genetics Reveal That Silvatic Rhodnius prolixus Do Colonise Rural Houses. *PLoS Negl. Trop. Dis.* **2008**, *2*, e210. [CrossRef] [PubMed]
23. Fitzpatrick, S.; Watts, P.C.; Feliciangeli, M.D.; Miles, M.A.; Kemp, S.J. A panel of ten microsatellite loci for the Chagas disease vector Rhodnius prolixus (*Hemiptera*: *Reduviidae*). *Infect. Genet. Evol.* **2009**, *9*, 206–209. [CrossRef]
24. Hernández, C.; Salazar, C.; Brochero, H.; Teherán, A.; Buitrago, L.S.; Vera, M.; Soto, H.; Florez-Rivadeneira, Z.; Ardila, S.; Parra-Henao, G.; et al. Untangling the transmission dynamics of primary and secondary vectors of Trypanosoma cruzi in Colombia: Parasite infection, feeding sources and discrete typing units. *Parasit. Vectors* **2016**, *9*, 620. [CrossRef] [PubMed]
25. Mejía-Jaramillo, A.; Agudelo-Uribe, L.; Dib, J.; Ortiz, S.; Solari, A.; Triana-Chávez, O. Genotyping of Trypanosoma cruzi in a hyper-endemic area of Colombia reveals an overlap among domestic and sylvatic cycles of Chagas disease. *Parasit. Vectors* **2014**, *7*, 108. [CrossRef] [PubMed]
26. Monroy, C.; Rodas, A.; Mejía, M.; Rosales, R.; Tabaru, Y. Epidemiology of Chagas disease in Guatemala: Infection rate of Triatoma dimidiata, Triatoma nitida and Rhodnius prolixus (*Hemiptera*, *Reduviidae*) with Trypanosoma cruzi and Trypanosoma rangeli (Kinetoplastida, Trypanosomatidae). *Mem. Inst. Oswaldo Cruz* **2003**, *98*, 305–310. [CrossRef] [PubMed]
27. Cuba Cuba, C.A.; Abad-Franch, F.; Roldán Rodríguez, J.; Vargas Vásquez, F.; Pollack Velásquez, L.; Miles, M.A. The Triatomines of Northern Peru, with Emphasis on the Ecology and Infection by Trypanosomes of Rhodnius ecuadoriensis (*Triatominae*). *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 175–183. [CrossRef]
28. Grijalva, M.J.; Villacís, A.G.; Moncayo, A.L.; Ocaña-Mayorga, S.; Yumiseva, C.A.; Baus, E.G. Distribution of triatomine species in domestic and peridomestic environments in central coastal Ecuador. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005970. [CrossRef]
29. Calzada, J.E.; Pineda, V.; Montalvo, E.; Alvarez, D.; Santamaría, A.M.; Samudio, F.; Bayard, V.; Cáceres, L.; Saldaña, A. Human trypanosome infection and the presence of intradomicile Rhodnius pallescens in the western border of the Panama Canal, Panama. *Am. J. Trop. Med. Hyg.* **2006**, *74*, 762–765. [CrossRef]
30. Gottdenker, N.L.; Chaves, L.F.; Calzada, J.E.; Saldaña, A.; Carroll, C.R. Host Life History Strategy, Species Diversity, and Habitat Influence Trypanosoma cruzi Vector Infection in Changing Landscapes. *PLoS Negl. Trop. Dis.* **2012**, *6*, e1884. [CrossRef]
31. Pineda, V.; Montalvo, E.; Alvarez, D.; Santamaría, A.M.; Calzada, J.E.; Saldaña, A. Feeding sources and trypanosome infection index of Rhodnius pallescens in a Chagas disease endemic area of Amador County, Panama. *Rev. Inst. Med. Trop. Sao Paulo* **2008**, *50*, 113–116. [CrossRef]

32. de Vasquez, A.M.; Samudio, F.E.; Saldaña, A.; Paz, H.M.; Calzada, J.E. Eco-epidemiological aspects of Trypanosoma cruzi, Trypanosoma rangeli and their vector (Rhodnius pallescens) in Panama. *Rev. Inst. Med. Trop. Sao Paulo* **2004**, *46*, 217–222. [CrossRef] [PubMed]
33. Zeledón, R.; Marín, F.; Calvo, N.; Lugo, E.; Valle, S. Distribution and ecological aspects of Rhodnius pallescens in Costa Rica and Nicaragua and their epidemiological implications. *Mem. Inst. Oswaldo Cruz* **2006**, *101*, 75–79. [CrossRef] [PubMed]
34. Justi, S.A.; Noireau, F.; Cortez, M.R.; Monteiro, F.A. Infestation of peridomestic *Attalea phalerata* palms by *Rhodnius stali*, a vector of *Trypanosoma cruzi* in the Alto Beni, Bolivia: Infestation of peridomestic palms by a vector of *Trypanosoma cruzi* in Bolivia. *Trop. Med. Int. Health* **2010**, *15*, 727–732. [CrossRef] [PubMed]
35. Gurgel-Gonçalves, R.; Galvão, C.; Costa, J.; Peterson, A.T. Geographic Distribution of Chagas Disease Vectors in Brazil Based on Ecological Niche Modeling. *J. Trop. Med.* **2012**, *2012*, 1–15. [CrossRef]
36. Peretolchina, T.; Pavan, M.G.; Corrêa-Antônio, J.; Gurgel-Gonçalves, R.; Lima, M.M.; Monteiro, F.A. Phylogeography and demographic history of the Chagas disease vector Rhodnius nasutus (*Hemiptera*: *Reduviidae*) in the Brazilian Caatinga biome. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006731. [CrossRef]
37. Ramos, L.J.; de Castro, G.V.S.; de Souza, J.L.; de Oliveira, J.; da Rosa, J.A.; Camargo, L.M.A.; da Cunha, R.M.; de Meneguetti, D.U.O. First report of Rhodnius neglectus (*Hemiptera*, *Reduviidae*, *Triatominae*) from the State of Acre, Brazil, and the Brazilian Western Amazon Region. *Rev. Soc. Bras. Med. Trop.* **2018**, *51*, 212–214. [CrossRef]
38. Monteiro, F.A.; Weirauch, C.; Felix, M.; Lazoski, C.; Abad-Franch, F. Evolution, Systematics, and Biogeography of the *Triatominae*, Vectors of Chagas Disease. *Adv. Parasitol.* **2018**, *99*, 265–344.
39. Carcavallo, R. Aspects of the epidemiology of Chagas disease in Venezuela and Argentina. American Trypanosomiasis Research. *PAHO Sci. Publ.* **1976**, *318*, 347–358.
40. Dujardin, J.; Schofield, C.; Panzera, F. *Les vecteurs de la maladie de Chagas. Brussels: Académie Royale des Sciences*; Académie Royale des Sciences d'Outre-Mer: Brussels, Belgium, 2002; ISBN 90-75652-27-5.
41. Lent, H.; Wygodzinsky, P. Revision of the *Triatominae* [*Hemiptera*, *Reduviidae*], and their significance as vectors of Chagas' disease. *Bull. Am. Mus. Nat. Hist.* **1979**, *163*, 123–520.
42. Ceccarelli, S.; Balsalobre, A.; Medone, P.; Cano, M.E.; Gurgel Gonçalves, R.; Feliciangeli, D.; Vezzani, D.; Wisnivesky-Colli, C.; Gorla, D.E.; Marti, G.A.; et al. DataTri, a database of American triatomine species occurrence. *Sci. Data* **2018**, *5*, 180071. [CrossRef]
43. Abad-Franch, F.; Lima, M.M.; Sarquis, O.; Gurgel-Gonçalves, R.; Sánchez-Martín, M.; Calzada, J.; Saldaña, A.; Monteiro, F.A.; Palomeque, F.S.; Santos, W.S.; et al. On palms, bugs, and Chagas disease in the Americas. *Acta Trop.* **2015**, *151*, 126–141. [CrossRef] [PubMed]
44. Oliveira, J.; Alevi, K.C.C.; Fonseca, E.O.L.; Souza, O.M.F.; Santos, C.G.S.; Azeredo-Oliveira, M.T.V.; da Rosa, J.A. New record and cytogenetic analysis of Psammolestes tertius Lent & Jurberg, 1965 (*Hemiptera*, *Reduviidae*, *Triatominae*) from Bahia State, Brazil. *Genet. Mol. Res.* **2016**, *15*, 1–6.
45. Velásquez-Ortiz, N.; Hernández, C.; Herrera, G.; Cruz-Saavedra, L.; Higuera, A.; Arias-Giraldo, L.M.; Urbano, P.; Cuervo, A.; Teherán, A.; Ramírez, J.D. Trypanosoma cruzi infection, discrete typing units and feeding sources among Psammolestes arthuri (*Reduviidae*: *Triatominae*) collected in eastern Colombia. *Parasit. Vectors* **2019**, *12*, 157. [CrossRef] [PubMed]
46. Galvão, C.; Carcavallo, R.; Rocha, D.D.S.; Jurberg, J. A checklist of the current valid species of the subfamily *Triatominae* Jeannel, 1919 (*Hemiptera*, *Reduviidae*) and their geographical distribution, with nomenclatural and taxonomic notes. *Zootaxa* **2003**, *202*, 1. [CrossRef]
47. Abad-Franch, F.; Palomeque, F.S.; Aguilar, H.M.; Miles, M.A. Field ecology of sylvatic Rhodnius populations (*Heteroptera*, *Triatominae*): Risk factors for palm tree infestation in western Ecuador: Rhodnius and palm trees in Ecuador. *Trop. Med. Int. Health* **2005**, *10*, 1258–1266. [CrossRef]
48. Arboleda, S.; Gorla, D.E.; Porcasi, X.; Saldaña, A.; Calzada, J.; Jaramillo-O., N. Development of a geographical distribution model of Rhodnius pallescens Barber, 1932 using environmental data recorded by remote sensing. *Infect. Genet. Evol.* **2009**, *9*, 441–448. [CrossRef]
49. Abad-Franch, F.; Paucar, A.; Carpio, C.; Cuba, C.A.; Aguilar, H.M.; Miles, M.A. Biogeography of *Triatominae* (*Hemiptera*: *Reduviidae*) in Ecuador: Implications for the design of control strategies. *Mem. Inst. Oswaldo Cruz* **2001**, *96*, 611–620. [CrossRef]

50. Arévalo, A.; Carranza, J.C.; Guhl, F.; Clavijo, J.A.; Vallejo, G.A. Comparación del ciclo de vida de Rhodnius colombiensis Moreno, Jurberg & Galvão, 1999 y Rhodnius prolixus Stal, 1872(*Hemiptera*, *Reduviidae*, *Triatominae*) en condiciones de laboratorio. *Biomedica* **2007**, *27*, 119.
51. Moreno Mejia, J.; Galvao, C.; Jurberg, J. Rhodnius colombiensis sp N. da Colombia com quadros comparativos entre estruturas fálicas do gênero Rhodnius Stal, 1859. *Entomologia y Vectores* **1999**, *6*, 601–617.
52. Guhl, F.; Aguilera, G.; Pinto, N.; Vergara, D. Actualización de la distribución geográfica y ecoepidemiología de la fauna de triatominos (*Reduviidae*: *Triatominae*) en Colombia. *Biomedica* **2007**, *27*, 143. [CrossRef]
53. Cedillos, R.A.; Romero, J.E.; Sasagawa, E. Elimination of Rhodnius prolixus in El Salvador, Central America. *Mem. Inst. Oswaldo Cruz* **2012**, *107*, 1068–1069. [CrossRef] [PubMed]
54. Hashimoto, K.; Schofield, C.J. Elimination of Rhodnius prolixus in Central America. *Parasites Vectors* **2012**, *5*, 45. [CrossRef] [PubMed]
55. Nakagawa, J.; Juárez, J.; Nakatsuji, K.; Akiyama, T.; Hernandez, G.; Macal, R.; Flores, C.; Ortiz, M.; Marroquín, L.; Bamba, T.; et al. Geographical characterization of the triatomine infestations in north-central Guatemala. *Ann. Trop. Med. Parasitol.* **2005**, *99*, 307–315. [CrossRef] [PubMed]
56. Schofield, C.; Dujardin, J.-P. Theories on the Evolution of Rhodnius. *Actu. Biol.* **1999**, *21*, 183–197.
57. Antonio-Campos, A.; Nicolás-Cruz, A.; Girón-Arias, J.I.; Rivas, N.; Alejandre-Aguilar, R. Presence of *Rhodnius prolixus* Stäl, 1859 (*Hemiptera*: *Reduviidae*) in Oaxaca, Mexico, ten years after the certification of its elimination. *J. Vector Ecol.* **2019**, *44*, 293–295. [CrossRef]
58. Bérenger, J.-M.; Pluot-Sigwalt, D.; Pagès, F.; Blanchet, D.; Aznar, C. The triatominae species of French Guiana (*Heteroptera*: *Reduviidae*). *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 1111–1116. [CrossRef]
59. Gurgel-Gonçalves, R.; Abad-Franch, F.; Ferreira, J.B.C.; Santana, D.B.; Cuba, C.A.C. Is Rhodnius prolixus (*Triatominae*) invading houses in central Brazil? *Acta Trop.* **2008**, *107*, 90–98. [CrossRef]
60. Hiwat, H. *Triatominae* species of Suriname (*Heteroptera*: *Reduviidae*) and their role as vectors of Chagas disease. *Mem. Inst. Oswaldo Cruz* **2014**, *109*, 452–458. [CrossRef]
61. Monteiro, F.A.; Barrett, T.V.; Fitzpatrick, S.; Cordon-Rosales, C.; Feliciangeli, D.; Beard, C.B. Molecular phylogeography of the Amazonian Chagas disease vectors Rhodnius prolixus and R. robustus. *Mol. Ecol.* **2003**, *12*, 997–1006. [CrossRef]
62. Pavan, M.G.; Monteiro, F.A. A multiplex PCR assay that separates Rhodnius prolixus from members of the Rhodnius robustus cryptic species complex (*Hemiptera*: *Reduviidae*). *Trop. Med. Int. Health* **2007**, *12*, 751–758. [CrossRef]
63. Abad-Franch, F.; Pavan, M.G.; Jaramillo-O., N.; Palomeque, F.S.; Dale, C.; Chaverra, D.; Monteiro, F.A. Rhodnius barretti, a new species of *Triatominae* (*Hemiptera*: *Reduviidae*) from western Amazonia. *Mem. Inst. Oswaldo Cruz* **2013**, *108*, 92–99. [CrossRef] [PubMed]
64. Carvalho, D.B.; Almeida, C.E.; Rocha, C.S.; Gardim, S.; Mendonça, V.J.; Ribeiro, A.R.; Alves, Z.C.P.V.T.; Ruellas, K.T.; Vedoveli, A.; da Rosa, J.A. A novel association between Rhodnius neglectus and the Livistona australis palm tree in an urban center foreshadowing the risk of Chagas disease transmission by vectorial invasions in Monte Alto City, São Paulo, Brazil. *Acta Trop.* **2014**, *130*, 35–38. [CrossRef] [PubMed]
65. de Meneguetti, D.U.O.; Soares, E.B.; Campaner, M.; Camargo, L.M.A. First report of Rhodnius montenegrensis (*Hemiptera*: *Reduviidae*: *Triatominae*) infection by Trypanosoma rangeli. *Rev. Soc. Bras. Med. Trop.* **2014**, *47*, 374–376. [CrossRef] [PubMed]
66. Souza, E.D.S.; Von Atzingen, N.C.B.; Furtado, M.B.; de Oliveira, J.; Nascimento, J.D.; Vendrami, D.P.; Gardim, S.; da Rosa, J.A. Description of Rhodnius marabaensis sp. n. (*Hemiptera*, *Reduviidae*, *Triatominae*) from Pará State, Brazil. *Zookeys* **2016**, 45–62.
67. Valente, V.; Valente, S.; Ru, C.; Rocha, D.; Galvão, C.; Jurberg, J. Considerações Sobre Uma Nova Espécie Do Gênero Rhodnius Stâl, Do Estado Do Pará, Brasil [*Hemiptera*, *Reduviidae*, *Triatominae*]. *Entomología y Vectores* **2001**, *8*, 65–80.
68. da Rosa, J.A.; Justino, H.H.G.; Nascimento, J.D.; Mendonça, V.J.; Rocha, C.S.; de Carvalho, D.B.; Falcone, R.; de Azeredo-Oliveira, M.T.V.; Alevi, K.C.C.; de Oliveira, J. A new species of Rhodnius from Brazil (*Hemiptera*, *Reduviidae*, *Triatominae*). *ZooKeys* **2017**, *675*, 1–25. [CrossRef]
69. Nascimento, J.D.; da Rosa, J.A.; Salgado-Roa, F.C.; Hernández, C.; Pardo-Diaz, C.; Alevi, K.C.C.; Ravazi, A.; de Oliveira, J.; de Azeredo Oliveira, M.T.V.; Salazar, C.; et al. Taxonomical over splitting in the Rhodnius prolixus (Insecta: *Hemiptera*: *Reduviidae*) clade: Are, R. taquarussuensis (da Rosa et al., 2017) and R. neglectus (Lent, 1954) the same species? *PLoS ONE* **2019**, *14*, e0211285. [CrossRef]

70. Cazorla, D. Revision of the vectors of Chagas disease in Venezuela [*Hemiptera-Heteroptera*, *Reduviidae*, *Triatominae*]. *Saber* **2016**, *28*, 387–470.
71. Matias, A.; de la Riva, J.; Martinez, E.; Torrez, M.; Dujardin, J.P. Domiciliation process of Rhodnius stali (*Hemiptera*: *Reduviidae*) in Alto Beni, La Paz, Bolivia. *Trop. Med. Int. Health* **2003**, *8*, 264–268. [CrossRef]
72. Jurberg, J.; da Rocha, D.S.; Galvão, C. *Rhodnius zeledoni* sp. nov. afim de Rhodnius paraensis Sherlock, Guitton & Miles, 1977 (*Hemiptera*, *Reduviidae*, *Triatominae*). *Biota Neotrop.* **2009**, *9*, 123–128.
73. Neiva, A.; Pinto, C. Estado actual dos conhecimentos sobre o gênero Rhodnius Stal, com a descrição de uma nova espécie. *Brasil-Médico* **1923**, *37*, 20–24.
74. da Rosa, J.A.; Mendonça, V.J.; Gardim, S.; de Carvalho, D.B.; de Oliveira, J.; Nascimento, J.D.; Pinotti, H.; Pinto, M.C.; Cilense, M.; Galvão, C.; et al. Study of the external female genitalia of 14 Rhodnius species (*Hemiptera*, *Reduviidae*, *Triatominae*) using scanning electron microscopy. *Parasit Vectors* **2014**, *7*, 17. [CrossRef] [PubMed]
75. Nascimento, J.D.; Ribeiro, A.R.; Almeida, L.A.; de Oliveira, J.; Mendonça, V.J.; Cilense, M.; da Rosa, J.A. Morphology of the spermathecae of twelve species of *Triatominae* (*Hemiptera*, *Reduviidae*) vectors of Chagas disease. *Acta Trop.* **2017**, *176*, 440–445. [CrossRef] [PubMed]
76. dos Rodrigues, J.M.S.; da Rosa, J.A.; Moreira, F.F.F.; Galvão, C. Morphology of the terminal abdominal segments in females of *Triatominae* (Insecta: *Hemiptera*: *Reduviidae*). *Acta Trop.* **2018**, *185*, 86–97. [CrossRef] [PubMed]
77. Soto-Vivas, A.; Liria, J.; Luna, E. Geometric morphometrics and phylogeny in Rhodniini (*Hemiptera*, *Reduviidae*) of Venezuela. *Acta Zool. Mex.* **2011**, *27*, 87–102.
78. Ravazi, A.; Oliveira, J.; Rosa, J.A.; Azeredo-Oliveira, M.T.V.; Alevi, K.C.C. Spermiotaxonomy of the tribe Rhodniini (*Hemiptera*, *Triatominae*). *Genet. Mol. Res.* **2016**, *15*. [CrossRef] [PubMed]
79. Monteiro, F.A.; Wesson, D.M.; Dotson, E.M.; Schofield, C.J.; Beard, C.B. Phylogeny and molecular taxonomy of the Rhodniini derived from mitochondrial and nuclear DNA sequences. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 460–465. [CrossRef]
80. Monteiro, F.A.; Lazoski, C.; Noireau, F.; Solé-Cava, A.M. Allozyme relationships among ten species of Rhodniini, showing paraphyly of Rhodnius including Psammolestes. *Med. Vet. Entomol.* **2002**, *16*, 83–90. [CrossRef]
81. Dias, F.B.S.; de Paula, A.S.; Belisário, C.J.; Lorenzo, M.G.; Bezerra, C.M.; Harry, M.; Diotaiuti, L. Influence of the palm tree species on the variability of Rhodnius nasutus Stål, 1859 (*Hemiptera*, *Reduviidae*, *Triatominae*). *Infect. Genet. Evol.* **2011**, *11*, 869–877. [CrossRef]
82. Dias, F.B.S.; Jaramillo-O., N.; Diotaiuti, L. Description and characterization of the melanic morphotype of Rhodnius nasutus Stål, 1859 (*Hemiptera*: *Reduviidae*: *Triatominae*). *Rev. Soc. Bras. Med. Trop.* **2014**, *47*, 637–641. [CrossRef]
83. Jurberg, J.; Rodrigues, J.M.S.; Moreira, F.F.F.; Dale, C.; Cordeiro, I.R.S.; Valdir, D.L., Jr.; Galvão, C.; Rocha, D.S. *Atlas Iconográfico Dos Triatomíneos Do Brasil (Vetores Da Doença De Chagas)*; Instituto Oswaldo Cruz: Rio de Janeiro, Brasil, 2014.
84. Bérenger, J.; Pluot-Sigwalt, D. Rhodnius amazonicus Almeida, Santos & Sposina, 1973, bona species, close to R. pictipes Stål, 1872 [*Heteroptera*, *Reduviidae*, *Triatominae*]. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 73–77. [PubMed]
85. Catalá, S.; Schofield, C. Antennal sensilla of *Rhodnius*: Antennal Sensilla of *Rhodnius*. *J. Morphol.* **1994**, *219*, 193–203. [CrossRef] [PubMed]
86. Panzera, F.; Pérez, R.; Hornos, S.; Panzera, Y.; Cestau, R.; Delgado, V.; Nicolini, P. Chromosome numbers in the *Triatominae* (*Hemiptera-Reduviidae*): A review. *Mem. Inst. Oswaldo Cruz* **1996**, *91*, 515–518. [CrossRef] [PubMed]
87. Panzera, F.; Pérez, R.; Panzera, Y.; Ferrandis, I.; Ferreiro, M.J.; Calleros, L. Cytogenetics and genome evolution in the subfamily *Triatominae* (*Hemiptera*, *Reduviidae*). *Cytogenet. Genome Res.* **2010**, *128*, 77–87. [CrossRef]
88. *Genetics and Evolution of Infectious Diseases*, 2nd ed.; Tibayrenc, M. (Ed.) Elsevier: Amsterdam, The Netherlands, 2017; ISBN 978-0-12-799942-5.
89. Alevi, K.C.C.; Ravazi, A.; Mendonça, V.J.; Rosa, J.A.; Azeredo-Oliveira, M.T.V. Karyotype of Rhodnius montenegrensis (*Hemiptera*, *Triatominae*). *Genet. Mol. Res.* **2015**, *14*, 222–226. [CrossRef]
90. Alevi, K.C.C.; da Costa Castro, N.F.; Lima, A.C.C.; Ravazi, A.; Morielle-Souza, A.; de Oliveira, J.; da Rosa, J.A.; de Azeredo Oliveira, M.T.V. Nucleolar persistence during spermatogenesis of the genus Rhodnius (*Hemiptera*, *Triatominae*). *Cell. Biol. Int.* **2014**, *38*, 977–980. [CrossRef]

91. Madeira, F.F.; Borsatto, K.C.; Lima, A.C.C.; Ravazi, A.; de Oliveira, J.; da Rosa, J.A.; de Azeredo-Oliveira, M.T.V.; Alevi, K.C.C. Nucleolar Persistence: Peculiar Characteristic of Spermatogenesis of the Vectors of Chagas Disease (*Hemiptera*, *Triatominae*). *Am. J. Trop. Med. Hyg.* **2016**, *95*, 1118–1120. [CrossRef]
92. Gómez-Palacio, A.; Jaramillo-O., N.; Caro-Riaño, H.; Diaz, S.; Monteiro, F.A.; Pérez, R.; Panzera, F.; Triana, O. Morphometric and molecular evidence of intraspecific biogeographical differentiation of Rhodnius pallescens (*Hemiptera*: *Reduviidae*: Rhodniini) from Colombia and Panama. *Infect. Genet. Evol.* **2012**, *12*, 1975–1983. [CrossRef]
93. Panzera, F.; Ferrandis, I.; Ramsey, J.; Salazar-Schettino, P.M.; Cabrera, M.; Monroy, C.; Bargues, M.D.; Mas-Coma, S.; O'Connor, J.E.; Angulo, V.M.; et al. Genome size determination in chagas disease transmitting bugs (hemiptera-triatominae) by flow cytometry. *Am. J. Trop. Med. Hyg.* **2007**, *76*, 516–521. [CrossRef]
94. Pérez, R.; Panzera, F.; Page, J.; Suja, J.A.; Rufas, J.S. Meiotic behaviour of holocentric chromosomes: Orientation and segregation of autosomes in Triatoma infestans (*Heteroptera*). *Chromosome Res.* **1997**, *5*, 47–56. [CrossRef]
95. Panzera, Y.; Pita, S.; Ferreiro, M.J.; Ferrandis, I.; Lages, C.; Pérez, R.; Silva, A.E.; Guerra, M.; Panzera, F. High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (*Triatominae*, *Heteroptera*). *Cytogenet. Genome Res.* **2012**, *138*, 56–67. [CrossRef] [PubMed]
96. Pita, S.; Panzera, F.; Ferrandis, I.; Galvao, C.; Gomez-Palacio, A.; Panzera, Y. Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal location of ribosomal genes. *Mem. Inst. Oswaldo Cruz* **2013**, *108*, 376–382. [CrossRef]
97. Panzera, F.; Dujardin, J.P.; Nicolini, P.; Caraccio, M.N.; Rose, V.; Tellez, T.; Bermúdez, H.; Bargues, M.D.; Mas-Coma, S.; O'Connor, J.E.; et al. Genomic changes of Chagas disease vector, South America. *Emerg. Infect. Dis.* **2004**, *10*, 438–446. [CrossRef] [PubMed]
98. Pérez, R.; Hernández, M.; Quintero, O.; Scvortzoff, E.; Canale, D.; Méndez, L.; Cohanoff, C.; Martino, M.; Panzera, F. Cytogenetic analysis of experimental hybrids in species of *Triatominae* (*Hemiptera-Reduviidae*). *Genetica* **2005**, *125*, 261–270. [CrossRef] [PubMed]
99. Oliveira, J.; Alevi, K.C.C.; Ravazi, A.; Herrera, H.M.; Santos, F.M.; de Azeredo-Oliveira, M.T.V.; da Rosa, J.A. New Evidence of the Monophyletic Relationship of the Genus Psammolestes Bergroth, 1911 (*Hemiptera*, *Reduviidae*, *Triatominae*). *Am. J. Trop. Med. Hyg.* **2018**, *99*, 1485–1488. [CrossRef] [PubMed]
100. Ravazi, A.; Alevi, K.C.C.; Oliveira, J.; Rosa, J.A.; Azeredo-Oliveira, M.T.V. Cytogenetic analysis in different populations of Rhodnius prolixus and R. nasutus from different countries of South America. *Braz. J. Biol.* **2018**, *78*, 183–185. [CrossRef] [PubMed]
101. Chavez, T.; Moreno, J.; Dujardin, J.P. Isoenzyme electrophoresis of Rhodnius species: A phenetic approach to relationships within the genus. *Ann. Trop. Med. Parasitol.* **1999**, *93*, 299–307. [CrossRef] [PubMed]
102. Lopez, G.; Moreno, J. Genetic variability and differentiation between populations of Rhodnius prolixus and R. pallescens, vectors of Chagas' Disease in Colombia. *Mem. Inst. Oswaldo Cruz* **1995**, *90*, 353–357. [CrossRef]
103. Dujardin, J.P.; Chavez, T.; Moreno, J.M.; Machane, M.; Noireau, F.; Schofield, C.J. Comparison of isoenzyme electrophoresis and morphometric analysis for phylogenetic reconstruction of the Rhodniini (*Hemiptera*: *Reduviidae*: *Triatominae*). *J. Med. Entomol.* **1999**, *36*, 653–659. [CrossRef]
104. Soares, R.P.; Sant'Anna, M.R.; Gontijo, N.F.; Romanha, A.J.; Diotaiuti, L.; Pereira, M.H. Identification of morphologically similar Rhodnius species (*Hemiptera*: *Reduviidae*: *Triatominae*) by electrophoresis of salivary heme proteins. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 157–161. [CrossRef]
105. Harry, M. Isozymic data question the specific status of some blood-sucking bugs of the genus Rhodnius, vectors of Chagas disease. *Trans. R. Soc. Trop. Med. Hyg.* **1993**, *87*, 492. [CrossRef]
106. Solano, P.; Dujardin, J.; Schofield, C.; Romana, C.; Tibayrenc, M. Isoenzymes as a tool for the identification of Rhodnius species [*Hemiptera*:*Reduviidae*:*Triatominae*]. *Res. Rev. Parasitol.* **1996**, 41–47.
107. Garcia, A.L.; Carrasco, H.J.; Schofield, C.J.; Stothard, J.R.; Frame, I.A.; Valente, S.A.; Miles, M.A. Random amplification of polymorphic DNA as a tool for taxonomic studies of triatomine bugs (*Hemiptera*: *Reduviidae*). *J. Med. Entomol.* **1998**, *35*, 38–45. [CrossRef] [PubMed]
108. Dujardin, J.P.; Muñoz, M.; Chavez, T.; Ponce, C.; Moreno, J.; Schofield, C.J. The origin of Rhodnius prolixus in Central America. *Med. Vet. Entomol.* **1998**, *12*, 113–115. [CrossRef]
109. Jaramillo, C.; Montaña, M.F.; Castro, L.R.; Vallejo, G.A.; Guhl, F. Differentiation and genetic analysis of Rhodnius prolixus and Rhodnius colombiensis by rDNA and RAPD amplification. *Mem. Inst. Oswaldo Cruz* **2001**, *96*, 1043–1048. [CrossRef]

110. Soares, R.P.P.; Barbosa, S.E.; Borges, E.C.; Melo Júnior, T.A.; Romanha, A.J.; Dujardin, J.P.; Schofield, C.J.; Diotaiuti, L. Genetic Studies of Psammolestes tertius (*Hemiptera*: *Reduviidae*: *Triatominae*) Using Male Genital Morphology, Morphometry, Isoenzymes, and Random Amplified Polymorphic DNA. *Biochem. Genet.* **2001**, *39*, 1–13. [CrossRef]
111. Harry, M.; Roose, C.L.; Vautrin, D.; Noireau, F.; Romaña, C.A.; Solignac, M. Microsatellite markers from the Chagas disease vector, Rhodnius prolixus (*Hemiptera*, *Reduviidae*), and their applicability to Rhodnius species. *Infect. Genet. Evol.* **2008**, *8*, 381–385. [CrossRef]
112. Gómez-Sucerquia, L.J.; Triana-Chávez, O.; Jaramillo-Ocampo, N. Quantification of the genetic change in the transition of Rhodnius pallescens Barber, 1932 (*Hemiptera*: *Reduviidae*) from field to laboratory. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 871–877. [CrossRef]
113. Villacís, A.G.; Marcet, P.L.; Yumiseva, C.A.; Dotson, E.M.; Tibayrenc, M.; Brenière, S.F.; Grijalva, M.J. Pioneer study of population genetics of Rhodnius ecuadoriensis (*Hemiptera*: *Reduviidae*) from the central coastand southern Andean regions of Ecuador. *Infect. Genet. Evol.* **2017**, *53*, 116–127. [CrossRef]
114. Bargues, M.D.; Marcilla, A.; Dujardin, J.P.; Mas-Coma, S. Triatomine vectors of Trypanosoma cruzi: A molecular perspective based on nuclear ribosomal DNA markers. *Trans. R. Soc. Trop. Med. Hyg.* **2002**, *96* (Suppl. S1), S159–S164. [CrossRef]
115. de Paula, A.S.; Diotaiuti, L.; Schofield, C.J. Testing the sister-group relationship of the Rhodniini and Triatomini (Insecta: *Hemiptera*: *Reduviidae*: *Triatominae*). *Mol. Phylogenet. Evol.* **2005**, *35*, 712–718. [CrossRef] [PubMed]
116. Díaz, S.; Triana-Chávez, O.; Gómez-Palacio, A. The nuclear elongation factor-1α gene: A promising marker for phylogenetic studies of *Triatominae* (*Hemiptera*: *Reduviidae*). *Infect. Genet. Evol.* **2016**, *43*, 274–280. [CrossRef] [PubMed]
117. Hypsa, V.; Tietz, D.F.; Zrzavý, J.; Rego, R.O.M.; Galvao, C.; Jurberg, J. Phylogeny and biogeography of *Triatominae* (*Hemiptera*: *Reduviidae*): Molecular evidence of a New World origin of the Asiatic clade. *Mol. Phylogenet. Evol.* **2002**, *23*, 447–457. [CrossRef]
118. Lyman, D.F.; Monteiro, F.A.; Escalante, A.A.; Cordon-Rosales, C.; Wesson, D.M.; Dujardin, J.P.; Beard, C.B. Mitochondrial DNA sequence variation among triatomine vectors of Chagas' disease. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 377–386. [CrossRef] [PubMed]
119. Stothard, J.R.; Yamamoto, Y.; Cherchi, A.; Garcia, A.L.; Valente, S.A.S.; Schofield, C.J.; Miles, M.A. A preliminary survey of mitochondrial sequence variation in *Triatominae* (*Hemiptera*: *Reduviidae*) using polymerase chain reaction-based single strand conformational polymorphism (SSCP) analysis and direct sequencing. *Bull. Entomol. Res.* **1998**, *88*, 553–560. [CrossRef]
120. Hwang, W.S.; Weirauch, C. Evolutionary history of assassin bugs (insecta: *Hemiptera*: *Reduviidae*): Insights from divergence dating and ancestral state reconstruction. *PLoS ONE* **2012**, *7*, e45523. [CrossRef] [PubMed]
121. Justi, S.; Russo, C.A.M.; Mallet, J.; Obara, M.; Galvão, C. Molecular phylogeny of Triatomini (*Hemiptera*: *Reduviidae*: *Triatominae*). *Parasit. Vectors* **2014**, *7*, 149. [CrossRef]
122. Justi, S.A.; Galvão, C.; Schrago, C.G. Geological Changes of the Americas and their Influence on the Diversification of the Neotropical Kissing Bugs (*Hemiptera*: *Reduviidae*: *Triatominae*). *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004527. [CrossRef]
123. Patterson, J.S.; Gaunt, M.W. Phylogenetic multi-locus codon models and molecular clocks reveal the monophyly of haematophagous reduviid bugs and their evolution at the formation of South America. *Mol. Phyl. Evol.* **2010**, *56*, 608–621. [CrossRef]
124. Weirauch, C.; Munro, J.B. Molecular phylogeny of the assassin bugs (*Hemiptera*: *Reduviidae*), based on mitochondrial and nuclear ribosomal genes. *Mol. Phyl. Evol.* **2009**, *53*, 287–299. [CrossRef]
125. Zhang, J.; Gordon, E.R.L.; Forthman, M.; Hwang, W.S.; Walden, K.; Swanson, D.R.; Johnson, K.P.; Meier, R.; Weirauch, C. Evolution of the assassin's arms: Insights from a phylogeny of combined transcriptomic and ribosomal DNA data (*Heteroptera*: *Reduvioidea*). *Sci. Rep.* **2016**, *6*, 22177. [CrossRef] [PubMed]
126. Márquez, E.; Jaramillo-O., N.; Gómez-Palacio, A.; Dujardin, J.-P. Morphometric and molecular differentiation of a Rhodnius robustus-like form from R. robustus Larousse, 1927 and R. prolixus Stal, 1859 (*Hemiptera*, *Reduviidae*). *Acta Trop.* **2011**, *120*, 103–109. [CrossRef] [PubMed]
127. de Paula, A.S.; Diotaiuti, L.; Galvão, C. Systematics and biogeography of Rhodniini (Reduvioidea: *Reduviidae*: *Triatominae*) based on 16S mitochondrial rDNA sequences. *J. Biogeogr.* **2007**, *34*, 699–712. [CrossRef]

128. Maia Da Silva, F.; Junqueira, A.C.V.; Campaner, M.; Rodrigues, A.C.; Crisante, G.; Ramirez, L.E.; Caballero, Z.C.E.; Monteiro, F.A.; Coura, J.R.; Añez, N.; et al. Comparative phylogeography of Trypanosoma rangeli and Rhodnius (*Hemiptera*: *Reduviidae*) supports a long coexistence of parasite lineages and their sympatric vectors. *Mol. Ecol.* **2007**, *16*, 3361–3373. [CrossRef]
129. Wiens, J.J.; Tiu, J. Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. *PLoS ONE* **2012**, *7*, e42925. [CrossRef]
130. Díaz, S.; Panzera, F.; Jaramillo-O., N.; Pérez, R.; Fernández, R.; Vallejo, G.; Saldaña, A.; Calzada, J.E.; Triana, O.; Gómez-Palacio, A. Genetic, Cytogenetic and Morphological Trends in the Evolution of the Rhodnius (*Triatominae*: Rhodniini) Trans-Andean Group. *PLoS ONE* **2014**, *9*, e87493. [CrossRef]
131. Abad-Franch, F.; Monteiro, F.A. Biogeography and evolution of Amazonian triatomines (*Heteroptera*: *Reduviidae*): Implications for Chagas disease surveillance in humid forest ecoregions. *Mem. Inst. Oswaldo Cruz* **2007**, *102* (Suppl. S1), S57–S70. [CrossRef]
132. Pavan, M.G.; Mesquita, R.D.; Lawrence, G.G.; Lazoski, C.; Dotson, E.M.; Abubucker, S.; Mitreva, M.; Randall-Maher, J.; Monteiro, F.A. A nuclear single-nucleotide polymorphism (SNP) potentially useful for the separation of Rhodnius prolixus from members of the Rhodnius robustus cryptic species complex (*Hemiptera*: *Reduviidae*). *Infect. Genet. Evol.* **2013**, *14*, 426–433. [CrossRef]
133. Barret, T. Species interfertility and crossing experiments in triatomine systematics. In Proceedings of the International Workshop on Population Genetics and Control of Triatominae, Santo Domingo de los Colorados, Ecuador, 24–28 September 1995; pp. 72–77.
134. Monteiro, F.A.; Escalante, A.A.; Beard, C.B. Molecular tools and triatomine systematics: A public health perspective. *Trends Parasitol.* **2001**, *17*, 344–347. [CrossRef]
135. Da Rosa, J.; Solano, C.; Gardim, S.; Pinto, M.; Mendoca, V.; Rente, J. Description of Rhodnius montenegrensis n. sp. [*Hemiptera*: *Reduviidae*: *Triatominae*] from the state of Rondônia, Brazil. *Zootaxa* **2012**, *3478*, 62–76. [CrossRef]
136. Mesquita, R.D.; Vionette-Amaral, R.J.; Lowenberger, C.; Rivera-Pomar, R.; Monteiro, F.A.; Minx, P.; Spieth, J.; Carvalho, A.B.; Panzera, F.; Lawson, D.; et al. Genome of Rhodnius prolixus, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 14936–14941. [CrossRef] [PubMed]
137. Pita, S.; Mora, P.; Vela, J.; Palomeque, T.; Sánchez, A.; Panzera, F.; Lorite, P. Comparative Analysis of Repetitive DNA between the Main Vectors of Chagas Disease: Triatoma infestans and Rhodnius prolixus. *Int. J. Mol. Sci.* **2018**, *19*, 1277. [CrossRef] [PubMed]
138. Zhao, Y.; Jiang, M.; Wu, Y.; Song, F.; Cai, W.; Li, H. Mitochondrial genomes of three kissing bugs (*Reduviidae*: Triatominae) and their phylogenetic implications. *Int. J. Biol. Macromol.* **2019**, *134*, 36–42. [CrossRef] [PubMed]
139. Medeiros, M.N.; Logullo, R.; Ramos, I.B.; Sorgine, M.H.F.; Paiva-Silva, G.O.; Mesquita, R.D.; Machado, E.A.; Coutinho, M.A.; Masuda, H.; Capurro, M.L.; et al. Transcriptome and gene expression profile of ovarian follicle tissue of the triatomine bug Rhodnius prolixus. *Insect Biochem. Mol. Biol.* **2011**, *41*, 823–831. [CrossRef]
140. Ribeiro, J.M.C.; Genta, F.A.; Sorgine, M.H.F.; Logullo, R.; Mesquita, R.D.; Paiva-Silva, G.O.; Majerowicz, D.; Medeiros, M.; Koerich, L.; Terra, W.R.; et al. An insight into the transcriptome of the digestive tract of the bloodsucking bug, Rhodnius prolixus. *PLoS Negl. Trop. Dis.* **2014**, *8*, e2594. [CrossRef]
141. Latorre-Estivalis, J.M.; Robertson, H.M.; Walden, K.K.O.; Ruiz, J.; Gonçalves, L.O.; Guarneri, A.A.; Lorenzo, M.G. The molecular sensory machinery of a Chagas disease vector: Expression changes through imaginal moult and sexually dimorphic features. *Sci. Rep.* **2017**, *7*, 40049. [CrossRef]
142. Traverso, L.; Lavore, A.; Sierra, I.; Palacio, V.; Martinez-Barnetche, J.; Latorre-Estivalis, J.M.; Mougabure-Cueto, G.; Francini, F.; Lorenzo, M.G.; Rodríguez, M.H.; et al. Comparative and functional triatomine genomics reveals reductions and expansions in insecticide resistance-related gene families. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005313. [CrossRef]
143. de Carvalho, D.B.; Congrains, C.; Chahad-Ehlers, S.; Pinotti, H.; de Brito, R.A.; da Rosa, J.A. Differential transcriptome analysis supports Rhodnius montenegrensis and Rhodnius robustus (*Hemiptera*, *Reduviidae*, *Triatominae*) as distinct species. *PLoS ONE* **2017**, *12*, e0174997. [CrossRef]
144. Castro, M.R.J.; Goubert, C.; Monteiro, F.A.; Vieira, C.; Cararetto, C.M.A. Homology-Free Detection of Transposable Elements Unveils Their Dynamics in Three Ecologically Distinct Rhodnius Species. *Genes* **2020**, *11*, 170. [CrossRef]

145. Dos Santos Souza, É.; Fernandes, R.P.; Guedes, W.N.; Dos Santos, F.N.; Eberlin, M.N.; Lopes, N.P.; Padovani, V.D.; da Rosa, J.A. *Rhodnius* spp. are differentiated based on the peptide/protein profile by matrix-assisted laser desorption/ionization mass spectrometry and chemometric tools. *Anal. Bioanal. Chem.* **2020**, *412*, 1431–1439. [CrossRef]
146. Dayrat, B. Towards integrative taxonomy. *Biol. J. Linn. Soc.* **2005**, *85*, 407–415. [CrossRef]
147. Weirauch, C. Cladistic analysis of *Reduviidae* (*Heteroptera*: *Cimicomorpha*) based on morphological characters. *Syst. Entomol.* **2008**, *33*, 229–274. [CrossRef]
148. Perlowagora-Szumlewicz, A. Induction of male sterility through manipulation of genetic mechanisms present in vector species of Chagas disease. (Remarks on integrating sterile-male release with insecticidal control measures against vectors of Chagas disease). *Rev. Inst. Med. Trop. Sao Paulo* **1972**, *14*, 360–371. [PubMed]
149. Mas-Coma, S.; Bargues, M.D. Populations, hybrids and the systematic concepts of species and subspecies in Chagas disease triatomine vectors inferred from nuclear ribosomal and mitochondrial DNA. *Acta Trop.* **2009**, *110*, 112–136. [CrossRef] [PubMed]
150. Herrando-Moraira, S.; Calleja, J.A.; Galbany-Casals, M.; Garcia-Jacas, N.; Liu, J.-Q.; López-Alvarado, J.; López-Pujol, J.; Mandel, J.R.; Massó, S.; Montes-Moreno, N.; et al. Nuclear and plastid DNA phylogeny of tribe Cardueae (Compositae) with Hyb-Seq data: A new subtribal classification and a temporal diversification framework. *Mol. Phyl. Evol.* **2019**, *137*, 313–332. [CrossRef]
151. Johnson, K.P.; Dietrich, C.H.; Friedrich, F.; Beutel, R.G.; Wipfler, B.; Peters, R.S.; Allen, J.M.; Petersen, M.; Donath, A.; Walden, K.K.O.; et al. Phylogenomics and the evolution of hemipteroid insects. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 12775–12780. [CrossRef]

Enfoque multilocus en la resolución de conflictos filogenéticos y evolutivos de la tribu Rhodniini

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Resumen

La tribu Rhodniini comprende 24 especies reportadas en numerosos estudios de su taxonomía. El panorama evolutivo de la tribu es confuso por incongruencias filogenéticas que persisten, ya que los estudios realizados no incluyen muestreo representativo de especies, de su distribución geográfica y/o enfoques multilocus, reconstrucciones según el tipo de marcador y según los métodos y algoritmos de reconstrucción. El objetivo de este trabajo fue determinar la estructura filogenética y evolutiva de la tribu Rhodniini. Se secuenciaron 8 marcadores moleculares (7 nucleares/Citocromo b) en 504 ejemplares (18 especies) colectadas en 7 países de Latinoamérica. Los alineamientos por locus y concatenados se realizaron MAFFT y Mesquite. Se realizaron reconstrucciones filogenéticas mediante Máxima Verosimilitud en IQ-Tree, FastTree y PhyML e Inferencia Bayesiana utilizando BEAST2, MRBAYES y ASTRAL. La credibilidad de los clados y topologías se evaluó en IQ-Tree. Se calcularon estadísticos de diversidad genética y Structure. Se estimaron tiempos de divergencia en BEAST2. La topología que evidenció mayor credibilidad recupero las especies del género *Psammolestes* en un clado monofilético (Bootstrap >95.0%). En el género *Rhodnius*, se recuperaron dos clados: el primero compuesto por dos clados monofiléticos que corresponden a los grupos *pallescens* y *pictipes*; el segundo compuesto por dos clados uno con *R. neivai* y otro que agrupa dos clados internos con las especies del grupo *prolixus*. La estructura genética mostro agrupación de las especies en 6 clúster. La tribu Rhodniini diverge hace 5.26 millones de años (95%HPD: 2:49-6.86) y los géneros Psammolestes y Rhodnius divergen hace 3.86 (95%HPD: 2:49-6.86) y 4.59 (95%HPD: 3.59-5.77) millones de años, respectivamente. A la fecha está es la primera evidencia filogenética de la clasificación de la tribu en dos géneros, el estatus de algunas especies del grupo *prolixus* y las relaciones interespecíficas del grupo *pallescens*.

1. Introducción

La enfermedad de Chagas es una enfermedad tropical desatendida causada por el parásito protozoario hemoflagelado *Trypanosoma cruzi*. Está enfermedad afecta cerca de 6 a 8 millones de personas en el mundo y es endémica en 21 países de América Latina en los cuales genera alrededor de 12.000 muertes por año (1). Existen diferentes mecanismos de transmisión de la enfermedad, entre ellos se encuentran: la transmisión vectorial, transfusional, congénita, mediante transplante de órganos, accidental en el laboratorio y la transmisión oral (2).

El principal mecanismo de transmisión es el vectorial que se produce por el contacto de humanos con heces de insectos infectados, de la subfamilia Triatominae (Hemiptera: Reduviidae) y aproximadamente 70 millones de personas están en riesgo de adquirir la infección de *T. cruzi* por este mecanismo de transmisión (1). La subfamilia Triatominae está conformada por 158 especies (155 existentes y 3 fósiles) las cuales han sido agrupadas en 5 tribus y 18 géneros (3,4). La mayoría de las especies (145/155) de la subfamilia han sido descritas mediante taxonomía clásica (morfología descriptiva, morfología comparativa y/o morfometría). Se han realizado cerca de 190 sinonimizaciones en la subfamilia Triatominae, reflejando su complejidad taxonómica, siendo está ultima de gran importancia en las tribus Rhodniini y Triatominii, ya que es donde se encuentran concentras las especies involucradas en la transmisión de *Trypanosoma cruzi* (5)

La tribu Rhodniini comprende 24 especies, de las cuales tres pertenecen al género *Psammmolestes* y 21 al género *Rhodnius,* estás ultimas han sido clasificadas en tres grupos (*prolixus*, *pictipes* y *pallescens*) según su distribución geográfica, biología y ecología (6). La tribu Rhodniini se destaca en la subfamilia por su elevada diversidad de especies, amplia distribución geográfica y por su importancia epidemiológica en enfermedad de Chagas. Se han descrito cuatro especies de la tribu que son vectores domiciliados de la enfermedad de Chagas (*R. prolixus, R. stali, R. ecuadoriensis y R. pallescens*) *y* objeto de programas regionales de control de la transmisión vectorial en países endémicos (6–9). Adicionalmente, se han descrito varias especies de la tribu (*R.robustus*, *R. neglectus*, *R. neivai*, *R.nasutus*, *R.brethesi*, *R. pictipes, R. colombiensis* y *P. arthuri*) infectadas con *T. cruzi* y sus diferentes variantes genéticas invadiendo ecotopos domiciliados y resaltando su relevancia en la transmisión vectorial (10–13).

Se han realizado varios esfuerzos por esclarecer el panorama evolutivo y taxonómico de la tribu Rhodniini, usando diferentes enfoques de la taxonomía clásica y de la taxonomía molecular (citogenética, isoenzimas, marcadores moleculares, transcriptomas y genomas) que han permitido solucionar algunos conflictos taxonómicos de manera exitosa (5,6,14,15). Sin embargo, persisten incongruencias entre las relaciones taxonómicas recuperadas mediante taxonomía clásica y la taxonomía molecular, siendo las más relevantes: (*i*) la parafilia del género *Rhodnius* con respecto a *Psammolestes*, (*ii*) diferencias en las agrupaciones de especies del género *Rhodnius* y (*iii*) estatus taxonómico de algunas especies del grupo *prolixus* en el género *Rhodnius* (6,15,16). Por lo anterior, las hipótesis acerca del origen, evolución y dispersión de las especies de la tribu Rhodniini también han sido contradictorias. Recientemente se ha hecho una importante aproximación filogenómica con una amplia contribución de datos provenientes de 36 ejemplares (17 especies) para describir las relaciones taxonómicas de la tribu. Sin embargo, se observó persistencia de los problemas taxonómicos descritos previamente e incongruencias entre marcadores mitocondriales y nucleares (15).

Por todo lo anterior, en este estudio nuestro objetivo fue realizar análisis filogenéticos y una aproximación filogeográfica mediante enfoque multilocus de secuencias obtenidas a partir de 8 marcadores moleculares (7 nucleares y 1 mitocondrial) en 506 ejemplares que corresponden a los dos géneros de la tribu, así como a los grupos descritos del género *Rhodnius*, utilizando diferentes enfoques de reconstrucción filogenética y aplicándolos en diferentes tipos de marcador. Lo anterior con el fin de describir, comprender y solucionar en un amplio set de datos, mediante diferentes métodos de reconstrucción y tipos de marcadores, los conflictos taxonómicos descritos en la tribu Rhodniini disminuyendo los sesgos que hasta el momento puedan haber generado hipótesis contradictorias acerca de sus características filogenéticas, evolutivas y biogeográficas.

2. Materiales y métodos

2.1. Recolección de las muestras

Se recolectaron 503 ejemplares de la tribu Rhodniini que corresponden a 17 especies, las cuales fueron colectados e 7 países de América Latina **(Figura 1, Apéndice S1.**) Se añadieron como grupos externos 5 ejemplares de *Panstrongylus geniculatus* y 2 de *Triatoma dimidiata*. Las muestras fueron colectadas en etanol y almacenadas en refrigeración hasta la extracción de ácidos nucleicos.

Figura 1. Distribución de muestras de insectos de la tribu Rhodniini colectadas en este estudio. Los puntos representan los ejemplares colectados en este estudio. A. Genero *Psammolestes* B. Genero *Rhodnius*: Especies del grupo *pallescens* C. Genero *Rhodnius*: Especies del grupo *prolixus* D. Genero Rhodnius: Especies del grupo *pictipes*

2.2. Extracción, amplificación y construcción de alineamientos

Se realizo extracción de los insectos utilizando el kit comercial DNeasy ® Blood & Tissue, con algunas modificaciones (11,17). Se realizó amplificación de 8 loci que fueron previamente utilizados para realizar reconstrucciones filogenéticas de los géneros *Rhodnius* (18) y *Psammolestes* (17) de la tribu Rhodniini (Tabla 1). La amplificación por PCR se verifico en geles de agarosa al 1,5 % y los productos amplificados se purificaron con ExoSAP-IT Product Cleanup (Affymetrix, Santa Clara, CA, EE. UU.). Se realizó se secuenciaron bidireccionalmente por el método de Sanger. Las secuencias directas y reversas se ensamblaron, verificaron y editaron en CLC Main Workbench 20.0 (https://www.qiagenbioinformatics.com/products/clc-main-workbench/).

Los alineamientos por locus se realizaron utilizando MAFFT (https://mafft.cbrc.jp/alignment/software/), posteriormente se inspeccionaron visualmente y se corrigieron manualmente utilizando Mesquite (https://www.mesquiteproject.org/). Con el fin resolver ambigüedades se implementó el algoritmo PHASE con 10000 iteraciones por simulación en DnaSP v6.12.03 (http://www.ub.edu/dnasp/). Finalmente, se construyó un concatenado de los siete loci nucleares (3.944 pb) y de todos los loci secuenciados en Mesquite (nuclear y mitocondrial: 4.441pb). Se construyeron adicionalmente alineamientos de los marcadores CYTB y 28S (Tabla 1) de las muestras amplificadas en esté estudio y las secuencias reportadas previamente en la Genbank para la tribu Rhodniini.

2.3. Análisis filogenéticos

Se realizaron reconstrucciones filogenéticas mediante Máxima Verosimilitud (MV) en IQ-Tree 2 (http://www.iqtree.org), FastTree (https://bio.tools/fasttree) y PhyML (https://bio.tools/phyml). Las reconstrucciones se realizaron para cada locus y los concatenados construidos (en los concatenados se realizaron particiones por locus). Los modelos de sustitución fueron seleccionados utilizando el criterio de información bayesiano (BIC) de ModelFinder (http://www.iqtree.org/ModelFinder/). Los nodos se evaluaron utilizando bootstrap tradicional (1000 réplicas), Ultrafast Bootstrap (10000 réplicas), abayes (19) y SH-aLRT (19) (Tabla S2).

Adicionalmente, se realizaron reconstrucciones filogenéticas mediante Inferencia Bayesiana (IB) para cada locus y los concatenados construidos en MrBayes 3.2.6 (https://bioweb.pasteur.fr/packages/pack@mrbayes@3.2.6/), para lo cual se utilizaron particiones, se establecieron los modelos de sustitución de nucleótidos con MrModeltest (https://github.com/nylander/MrModeltest2) y se corrieron dos corridas de 15 millones de generaciones muestreadas ca 1000 generaciones. Se confirmó la convergencia de las corridas y valores de tamaño de muestra efectivo ESS>200 para todos los parámetros mediante Tracer v 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). Los archivos de las dos corridas fueron combinados con LogCombiner (20). Mediante Tree Annotator (20) se descartó el 10% de árboles como precalentamiento y se generaron árboles consenso con mayor credibilidad y distribuciones de densidad de probabilidad al 95% (HPDs) por nodo. También se realizó reconstrucción mediante inferencia bayesiana de los concatenados utilizando ASTRAL (21). Todas las reconstrucciones filogenéticas fueron graficadas utilizando FigTree (http://tree.bio.ed.ac.uk/software/figtree/) e iToll

(https://itol.embl.de/personal_page.cgi). La credibilidad de los clados y topologías obtenidas se evaluaron mediante el Test de topologías en IQ-Tree (22).

2.4. Estimación de árbol de especies y tiempos de divergencia

Se usaron particiones por locus mediante el programa PartitionFinder (23). Las particiones fueron cargadas a *BEAST v 2.3.2 (https://www.beast2.org/) implementando modelos de árboles vinculados y no vinculados. Se realizó la inferencia del modelo de sustitución de nucleótidos con el paquete bModelTest (https://github.com/BEAST2-Dev/bModelTest). Se testeo el tipo de reloj molecular mediante la prueba de máxima verosimilitud en MEGA X (https://www.megasoftware.net/) y se corrió con un modelo de especiación de Yule en el programa *BEAST2 (https://www.beast2.org/).

La calibración se realizó utilizando el fósil de *Panstrongylus hispaniolae* (PaleoDatabase), y se usó una distribución previa uniforme con el siguiente rango límite superior e inferior (20.44–13.82 Ma) (https://paleobiodb.org). Se realizaron cuatro corridas independientes con 10.000.000 generaciones, muestreadas cada 1000 generaciones. Se confirmo la convergencia de las corridas y valores de tamaño de muestra efectivo ESS>200 para todos los parámetros mediante Tracer v 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). Los archivos de las cuatro corridas fueron combinados con LogCombiner (20). Mediante Tree Annotator (20) se descartó el 10% de árboles como precalentamiento y se generó un árbol consenso por locus con mayor credibilidad y distribuciones de densidad de probabilidad al 95% (HPDs) por nodo. Los intervalos del 95% de confianza para las fechas estimadas serán calculados a partir de la distribución posterior de las edades de cada uno de los clados en TreeAnnotator (20).

2.5. Análisis de genética de poblaciones

Para todos los loci se determinó la diversidad haplotipica (*h*), el número de sitios segregantes (*S*), la tasa de sustitución de la población (θ), la diversidad nucleotídica (π), D de Tajima (D; Tajima y las estadísticas F y D de Fu & Li (FF, FD; Fu y Li) en DNASP v6.12.03 (http://www.ub.edu/dnasp/). Se determinó la estructura genética entre todas las especies y grupos de la tribu Rhodniini, mediante la estimación del índice de fijación F_{ST} y dos medidas absolutas (D_a, D_{xy}).

Adicionalmente, en el conjunto de datos nucleares se implementó STRUCTURE v2.3 (https://web.stanford.edu/group/pritchardlab/structure.html) para determinar el número de grupos genéticos (K) en la tribu Rhodniini. Se llevo a cabo el modelo 100. 000 generaciones de MCMC, muestreando valores de K de 1 a 10 y 5 iteraciones por K. El mejor valor de K se seleccionó siguiendo a Evanno et al. 2005 (24) y graficando la probabilidad media L(k) y la varianza por K utilizando STRUCTURE HARVESTER (https://taylor0.biology.ucla.edu/structureHarvester/). El grafico correspondiente al mejor valor de K se graficó usando pophelper (http://pophelper.com/).

3. Resultados

3.1. Análisis filogenéticos

Se realizaron 53 reconstrucciones filogenéticas a partir de los alineamientos obtenidos según lo descrito en la Tabla S2 (Tabla S2.). En todas las reconstrucciones por loci individual (Archivo S4 y S5) y concatenados de genes nucleares (Archivo S6) y con el marcador mitocondrial (Archivo S7) obtenidas de manera independiente mediante MV e IB se observó parafilia del género *Rhodnius*

(todos los grupos) con respecto a *Psammolestes,* y diferentes agrupaciones entre los grupos del género *Rhodnius* (*prolixus, pictipes, pallescens*) similar a lo descrito previamente (6,15,16,25).

3.1.1. Relaciones filogenéticas de los géneros de la tribu Rhodniini

Posterior a la observación de todas las reconstrucciones de MV e IB, fueron las obtenidas a partir del concatenado de todos los marcadores utilizados las que evidenciaron mejores soportes de Bootstrap, se detectaron 9 topologías probables de la tribu Rhodniini (Archivo S8). Las topologías que mayor credibilidad mostraron (Tabla S3) fueron las reconstruidas en PHYML y específicamente la reconstruida con Bootstrap clásico (Figura 2).

En está topología se observan dos grupos monofiléticos en la tribu Rhodniini, uno en el que se encuentra las tres especies del género *Psammolestes* y otro en el que se pueden detectar los tres grupos del género *Rhodnius.* Las tres especies del género *Psammolestes* se recuperan en tres clados monofiléticos. Para el género *Rhodnius*, en está reconstrucción se ubican como clados hermanos los grupos *pallescens* y *pictipes* al igual que en todas las reconstrucciones obtenidas mediante los diferentes métodos de MV (Figura 2 y Archivos S4-S8). Adicionalmente, se obtienen tres agrupaciones dentro del grupo *prolixus*, un clúster externo con *R. neivai*, la segunda está conformada por las especies *R. nasutus, R. neglectus* y *R. milesi*, en la que se observa parafilia de *R. neglectus* y la tercera que está conformada por las especies *R. prolixus, R. robustus, R. marabaensis* y *R. montenegrensis* y en esta última se observó adicionalmente la parafilia de *R. robustus* con *R. prolixus y R. montenegrensis* (Figura 2).

Figura 2. Reconstrucción filogenética de máxima credibilidad mediante MV de la tribu Rhodniini. Reconstrucción basada en los 8 loci utilizados en este estudio realizada con PHYML y 1000 repeticiones de Bootstrap clásico. Se evidencian todos los niveles taxonómicos.

Con respecto a los géneros de la tribu, en las topologías restantes se lograron reconstruir topologías similares a las reportadas previamente en la literatura encontrando la parafilia del género *Rhodnius*-grupo *prolixus* en las reconstrucciones obtenidas con el marcador mitocondrial (Archivo S7) y nuevas topologías donde la parafilia es de los grupos *pictipes*/*pallescens* y algunos miembros del grupo *prolixus* (Archivo S8) obtenidas con los concatenados de marcadores nucleares/ribosomal y algunos loci individuales (Archivos S4-S6).

3.1.1.1. Relaciones filogenéticas del género *Psammolestes*

En las reconstrucciones de concatenados de marcadores nucleares/ribosomal y concatenado de todos los loci, se logró recuperar las tres especies de *Psammolestes* como un grupo monofilético, (Figura 2 y Archivos S6 y S8) en concordancia con reportes previos que usan ejemplares de las tres especies (6,16). Sin embargo, se observó que en algunas topologías no se logran recuperar las especies *P. coreodes* y *P. tertius* en clados monofiléticos (Archivos S6 y S8).

Contrario a lo anterior, el marcador Cytb, ubica a *P. arthuri* formando un clado monofilético con el grupo *prolixus* del género *Rhodnius* que es hermano de otro clado que agrupa *P. tertius* y *P. coreodes* como clados monofiléticos hermanos (Archivo S7). Siendo está la primera reconstrucción obtenida de todas las especies de *Psammolestes* y especies representativas del género *Rhodnius* usando un marcador mitocondrial.

3.1.1.2. Relaciones filogenéticas del género *Rhodnius*

En este estudio se logró una reconstrucción filogenética de máxima credibilidad con altos valores de soporte de bootstrap que recupero los tres grupos monofiléticos con valores de bootstrap clásico y SH-aLRT superiores al 95.0%, se observó la agrupación *R. pallescens*/*pictipes* como clado hermano de *prolixus*. De manera consistente en todas las reconstrucciones se observó la agrupación *pallescens*/*pictipes* con cambios en las agrupaciones de las especies del grupo *prolixus* en varias topologías que reflejan diferencias que corresponden a tipos de de marcadores y tipos de reconstrucción filogenética utilizados:

(i) Reconstrucciones de MV (IQtree-Fastree) de todos los loci y de marcadores nucleares/ribosomales: Se obtuvo un clado formado los miembros de *pictipes*/*pallescens*, hermano de *R. neivai* formando otro clado hermano de *Psammolestes* y esté último un clado hermano con un clado del grupo *prolixus* formado por las especies *R.neglectus, R. nasutus y R. milesi* (Archivos S6 y S8).

(ii) Todas las reconstrucciones del marcador mitocondrial *Cytb* y las reconstrucciones de IB del concatenado de nucleares/ribosomal: mostraron la agrupación entre *pallescens*/*pictipes* formando un clado hermano del grupo *prolixus* (monofilético) que incluye las especies de *Psammolestes*. Específicamente las del marcador mitocondrial no recuperan el grupo *pictipes* monofilético ya que algunos ejemplares de pictipes se agrupan con las especies del grupo *pallescens* (Archivos S6 y S7).

(iii) Métodos de IB como MrBayes y Astral: Todos los grupos politómicos usando todos los marcadores lograron recuperar los grupos monofiléticos pero politómicos, así probablemente en este caso si sea necesario un conjunto de datos de marcadores que proporcioné mayor número de sinapomorfias (Archivo S8).

(iv) Árbol de especies multicoalescente realizado en BEAST2: se observó que el clado hermano se formó entre especies del grupo *prolixus* y *pictipes*, específicamente la agrupación de ejemplares de *R. brethesi* con especies del grupo *prolixus*¸ al observar los árboles de genes se detectó que específicamente esta agrupación ocurrió en 2 marcadores nucleares: UPMETAL y UPCA. Adicionalmente, el marcador CISP ubico a *R. brethesi* como grupo externo.

3.1.1.3. Relaciones filogenéticas de los grupos del género *Rhodnius*

3.1.1.3.1. Grupo *pallescens*:

En este estudio obtuvimos en todas las reconstrucciones de concatenados nucleares/ribosomal y mitocondrial, la agrupación de las especies *R. colombiensis* y *R. pallescens* en un clado hermano de *R. ecuadoriensis* con valores de bootstrap clásicos y SH-aLRT superiores al 95.0% (Figura 2) y probabilidad posterior > 0.96 (Figura 2 y Apéndices S4-S9). Las topologías construidas con todos los loci y el marcador mitocondrial mostraron consistencia con los reportes previos en relación con los dos genotipos descritos de *R. pallescens* mediante diferentes marcadores moleculares y morfología (26–28). Sin embargo, no se observó la discriminación de los genotipos con el concatenado de

marcadores nucleares, esta discrepancia mito-nuclear probablemente se relacione principalmente con la tasa de evolución de los marcadores y se observó previamente usando el marcador 28S(27).

3.1.1.3.2. Grupo *pictipes*:

La topología de mayor credibilidad mostro la agrupación de las especies *R. stali* y *R. pictipes* formando un clado hermano de *R. brethesi* con valores de bootstrap clásicos y SH-aLRT superiores al 95.0%. Esta topología se observó en todas las reconstrucciones de MV usando marcadores nucleares y concatenado de todos los loci (Apéndice S3) y mediante IB utilizando MRbayes y ASTRAL. Se observó una marcada diferencia entre las topologías reconstruidas con el marcador mitocondrial *Cytb* y en el análisis de multiespecies coalescente con Beast2 (Apéndice S3 y Figura 3). En la reconstrucción mitocondrial se obtiene una agrupación que integra como clados hermanos la especie *R. pictipes* y las especies del grupo *pallescens,* mientras en la reconstrucción por Beast2 se obtiene la agrupación de *R. pictipes* y *R.stali* , como clado hermano de *R. brethesi* y el grupo *prolixus*. Adicionalmente, en todas las topologías se observan dos clados para la especie *R. pictipes* uno que agrupa los ejemplares obtenidos en la Orinoquía colombiana y otros los colectados en Brasil (Figura 2 y Apéndice S6-S8).

3.1.1.3.3. Grupo *prolixus:*

Las especies del grupo *prolixus* se agruparon en varios clados, el primero formado por un clado monofilético *R. neivai,* el segundo formado por las especies *R. nasutus, R. neglectus* y *R. milesi y* el tercero formado por las especies *R. robustus, R. prolixus, R. montenegrensis* y *R. marabaensis*. Estás agrupaciones fueron obtenidas mediante la topología de máxima credibilidad y las topologías reconstruidas con el concatenado de todos los genes (Figura 2 y Apéndice S8). Contrario a estás topologías, las topologías reconstruidas con el marcador mitocondrial (Apéndice S7) y todas las secuencias reportadas en Genbank agrupan las especies *R. nasutus, R. neglectus* y *R. milesi* con *R. prolixus* y con algunos ejemplares de *R. robustus* (I) en un clado y otro clado integrado por dos grupos monofiléticos uno formado por *R. marabaensis* y ejemplares de *R. robustus* (III), y otro formado a su vez por dos grupos monofiléticos uno con *R. montengrensis* y *R. robustus* IV y otro con *R. robustus* II. Sumado a esto se añadieron secuencias de *Cytb* de *R. barreti* de Genbank, que formaron un clado hermano externo como se ha reportado previamente (14,15).

- En el clado de *R. neivai* en todas las reconstrucciones se recuperaron dos ejemplares provenientes de Cubará-Boyacá (Colombia) que fueron identificados morfológicamente como *R. prolixus* en el mismo clado que los ejemplares de *R. neivai* obtenidos en Valencia-Venezuela (Apéndices S4-S8) al igual que se evidencia el mismo patrón de ancestría en el análisis de structure (Figura 4), esté resultado puede deberse a un error en la identificación morfológica de los ejemplares provenientes de Boyacá, esto dado que Cubará es el único municipio de Boyacá en contacto con la frontera Colombo-Venezolana y aunque esté sería el primer reporte en el departamento de Boyacá, se ha documentado la presencia de esta especie en otros departamentos fronterizos con Venezuela, tales como Cesar y Guajira (29).

- En el clado formado por las especies *R. nasutus*, *R. neglectus* y *R. milesi* se evidenciaron varias topologías, algunas similares a las previamente reportadas (14) y otras nuevas, cuyas diferencias son atribuibles a la incongruencia entre marcadores mitocondriales y nucleares previamente reportados (15). Los ejemplares de estas especies corresponden a 7 grupos diferentes según su clasificación morfológica *R. neglectus* (*i*) 85-96 (*ii*) 353-361-375 (*iii*) 403-412 (Antes *R. taquarusuensis*) y (*iv)* 363-372; *R. nasutus* (*v*) 110-118 y *R. milesi* (*vi*) 294-306 (*vii*) 460-468 (Apéndice S1). En la topología de máxima credibilidad se recuperó los ejemplares de los grupos *R. neglectus* (*i, iii, iv*) en un clado hermano de los ejemplares de *R. milesi* y a su vez estos hermanos de *R. nasutus* y el grupo (ii) *R. neglectus*. Lo que indica parafilia de *R. neglectus* con respecto a *R. milesi* y *R. nasutus* (Figura 2 y Apéndice S4-S8). Estás mismas agrupación se recuperó en todas las reconstrucciones realizadas con el concatenado de todos los marcadores a excepción del árbol de especies en el cual se agrupan *R. neglectus* y *R. nasutus* en un clado hermano de *R. milesi* (Figura 2 y 3). Realizamos la comparación de las topologías obtenidas por tipo de marcador, en las reconstrucciones del marcador mitocondrial (Apéndice S7) se observaron las especies *R. nasutus* y *R.milesi* agrupadas, formando un clado hermano de *R. neglectus* (*i, iii, iv*) y el grupo (*ii*) de *R. neglectus* como clado basal y hermano de las demás especies del grupo *prolixu*s. Al observar las reconstrucciones del marcador mitocondrial incluyendo todas las secuencias previas de *R. nasutus* y *R. neglectus* reportadas en Genbank todas las reconstrucciones mostraron: los ejemplares el grupo (*ii*) 353-361-375 *R. neglectus* en el mismo clado que las secuencias de *R. nasutus* reportadas por Peretolchina et al.,2018 (30) y los ejemplares del grupo *(v*) 110-118 *R. nasutus* en el mismo clado con secuencias de *R. neglectus* previamente reportadas en Genbank (14) y con los demás ejemplares de *R. neglectus* (*i-iv*). Contrario a esto en las topologías nucleares/ribosomal se observan un clado formado por todas las especies de *R. neglectus* en dos grupos uno formado por *R. neglectus* (*i, iii, iv*) hermano de *R. milesi* (en algunas topologías se observa en un grupo monofilético) y como basal el grupo (*ii*) *R. neglectus* que en conjunto forma un clado hermano de *R. nasutus* (Apéndice S6).

- En el clado *R. montenegrensis*, *R. marabaensis*, *R. prolixus y R. robustus,* la topología obtenida en la reconstrucción filogenética de máxima credibilidad recupero dos clados: un clado basal que agrupa los ejemplares de *R. montenegrensis* y algunos de *R. robustus* de Perú (108) y de Brasil (318, 311, 307,308) y otro clado en el que se encuentran varios subclados en los que se encuentran *R. robustus*, *R. prolixus* y *R. marabaensis*, los ejemplares de esta ultima especie se recuperan en un clado monofilético hermano de otro formado por *R. robustus* de Brasil (Apéndice S8). Los ejemplares de *R. prolixus* forman un clado con ejemplares de *R. robustus* de Venezuela (No. 529-543) que forman un clado interno monofilético y con otros *R. robustus* de Ecuador (No. 561), Perú (No.102) y Brasil (No. 289, 282, 291, 312, 314, 317, 346) (Figura 2 y Apéndice S8). Se observa parafilia de *R. robustus* con respecto a *R. marabaensis* y *R. prolixus*.

Las demás reconstrucciones de MV construidas con el concatenado de todos los loci (IqTree y Fastree) presentaron las mismas agrupaciones, pero a diferencia de la topología de máxima

credibilidad, evidencian a *R. marabaensis* como un clado basal hermano de las demás especies (Figura 2 y Apéndice S8).

Las reconstrucciones de IB y MV realizadas con los marcadores nucleares, recuperan ejemplares de las 4 especies mezcladas en diferentes clados que integran el grupo, pero no se logran identificar claramente. Posiblemente esto se debe a que los marcadores nucleares/ribosomal no cuentan con la resolución necesaria para resolver las relaciones intra-grupo cuya diversificación parece ser la más reciente según el análisis de tiempos de divergencia y otros estudios previos (15,25,31). Por lo anterior, se evaluaron las reconstrucciones del marcador mitocondrial Cytb realizadas tanto con IB como con MV con o sin las secuencias de Genbank previamente reportadas. A diferencia del concatenado de todos los loci, no recupera las cuatro especies en un mismo clado, por el contrario, recupera las especies de *R. prolixus* y *R. robustus* de Venezuela como clado hermano de las especies *R. nasutus-R.neglectus-R.milesi,* formando un clado hermano de las especies *R. montegrensis, R. marabaensis* y *R. robustus*, esta topología en concordancia con las realizadas en estudios previos con *Cytb* (14,15).

Finalmente, se detectaron varias incongruencias entre las topologías obtenidas por los métodos de MV del concatenado de todos los loci por y las obtenidas con Cytb incluyendo con las secuencias previamente reportadas en Genbank, que son atribuibles al tipo de marcador:

(i) Usando *Cytb* todos los ejemplares de *R. prolixus* provenientes de campo en Colombia y Venezuela de este estudio se agrupan con secuencias de *R. prolixus* previamente reportadas en Genbank, en algunas reconstrucciones forman un clado hermano de las especies de *R. robustus* provenientes de Venezuela, algunas de ellas provenientes de campo que también se agrupan con las secuencias de *R. robustus* de Venezuela tipo I reportadas en Genbank, en concordancia con lo reportado previamente (32). En discordancia con lo anterior, las reconstrucciones con todos los loci recuperan los ejemplares de *R. robustus* de Venezuela en un clúster con ejemplares de *R. robustus* de Ecuador y a su vez agrupados con ejemplares de *R. prolixus* y estos últimos con algunos ejemplares de Perú, Brasil y Ecuador.

(ii) Los ejemplares de *R. robustus* 312 y 314 de Caipuru Brasil, siempre se obtienen agrupados con *R. prolixus* en todas las reconstrucciones tanto mitocondriales como nucleares, por lo que probablemente su clasificación errónea como *R. robustu*s pueda deberse a errores en la identificación morfológica, la cual es muy compleja entre las dos especies sumado a la controversia generada acerca de la presencia de *R. prolixus* en Brasil.

(iii) Usando *Cytb* se forma varios clados en el que se agrupan ejemplares de este estudio que corresponden a *R. montenegrensis* y *R. robustus* (Perú 99-108/Brasil 307-319) con secuencias reportadas en Genbank de *R. robustus* provenientes de Ecuador, Brasil y Bolivia, que son clasificadas como *R. robustus* II. En contraste en las reconstrucciones de todos los loci se obtiene la agrupación de *R. montenegrensis* y solo algunos ejemplares de *R. robustus* (Perú 108/Brasil 307, 308,311,318).

(iv) Usando *Cytb* se obtiene una agrupación de *R. marabaensis* en un clado monofilético agrupado con secuencias de Genbank que son identificadas como *R. robustus* III y esté clado hermano de otro clado que está integrado por secuencias de Genbank provenientes de Guayana y Brasil (clasificadas con *R. robustus IV*) y secuencias de ejemplares de este estudio provenientes de Ceará y Rio de Janeiro (Clasificados en su descripción original como *R. prolixus*). En contraste, en las reconstrucciones de todos los loci *R. marabaensis* forma un clado hermano con las secuencias de ejemplares de este estudio provenientes de Ceará y Rio de Janeiro

(Clasificados en su descripción original como *R. prolixus*) y este hermano de otro clado que está formado por los ejemplares de *R. robustus* provenientes de Perú (99-107), Venezuela (534) y Brasil (310).

3.2 Estimación de tiempos de divergencia

La tribu Rhodniini diverge hace 5.26 millones de años (95%HPD: 2:49-6.86) y los géneros *Psammolestes* y *Rhodnius* divergen hace 3.86 (95%HPD: 2:49-6.86) y 4.59 (95%HPD: 3.59-5.77) millones de años, respectivamente. A su vez los grupos del género *Rhodnius* divergen alrededor hace 2.7 y 3.6 millones de años (Figura 3).

Figura 3. Árboles filogenéticos de IB obtenidos para concatenado de los 8 loci utilizados en este estudio. A. Árbol de especies B. Estimación de tiempos de divergencia. Las barras moradas horizontales ilustran el HPD del 95 % para el tiempo de divergencia de los nodos.

3.2. Estructura genética

Los valores de la tasa de sustitución de la población (θ) y la diversidad nucleotídica (π) fueron similares entre los dos géneros de la tribu, a su vez entre las tres especies de *Psammolestes* y las especies del género *Rhodnius* en cada uno de los siete loci (Apéndice S9). A su vez los índices de diferenciación genética (Fst, Da y D_{xy}) son elevados entre los géneros de la tribu y los grupos del género *Rhodnius* (Apéndice S10), en concordancia con las agrupaciones observadas en el análisis de Structure y las observadas mediante aproximación filogenética (Figura 4).

Figura 4. Análisis de estructura poblacional usando STRUCTURE. Gráfico obtenido mediante software pophelper con K = 6.

4. Discusión

4.1 Relaciones filogenéticas de los géneros *Rhodnius* y *Psammolestes*

En este estudio reportamos por primera vez evidencia filogenética de la clasificación de la tribu Rhodniini en dos grupos monofiléticos con altos valores de soporte en dos reconstrucciones que fueron realizadas utilizando el concatenado de todos los genes incluidos en este estudio y obtenidas por MV (SH-aLRT / Bootstrap = 100.0% / 82.0%) e IB del árbol de especies basado en coalescencia de datos multilocus (probabilidad posterior = 1.0) (Figuras 2 y 3), soportando el estatus taxonómico de los géneros en la tribu Rhodniini y de esta manera resolviendo una de las incongruencias taxonómicas más importantes de la tribu. Como soporte adicional a nuestros hallazgos recientemente se ha reportado mediante cruces de especies de *Psammolestes* (*P. coreodes* y *P. tertius*) y del grupo *prolixus* del género *Rhodnius* (*R. neglectus*) que existe aislamiento reproductivo y ausencia de formación de híbridos entre los dos géneros soportando su estatus genérico (33). Lo anterior deja en evidencia la utilidad que tiene el uso de datos obtenidos de múltiples genes en las reconstrucciones filogenéticas mediante enfoques de concatenación o de coalescencia, sumado a ello al análisis de set de datos de diferentes tipos de marcadores y en combinación con el análisis de genes individuales, todos estos enfoques que ha mostrado ser de gran utilidad en resolución de conflictos taxonómicos (34–41)

La incongruencia observada entre las filogenias de los géneros de la tribu Rhodniini puede deberse a limitaciones en cuanto al muestreo de taxones representativos de los dos géneros (42,43), el número y tipo de marcadores (mitocondrial, ribosomal y nuclear) y los tipos de reconstrucción filogenética (IB y/o MV) (15,31). Algunas de las topologías encontradas en este estudio para el género *Psammolestes* no se habían reportado previamente por que ningún estudio enfocado en la tribu Rhodniini, había incluido secuencias provenientes de las tres especies de *Psammolestes,* de los principales representantes de grupos del género *Rhodnius,* de los tres tipos de marcadores y concatenaciones (nucleares, ribosomal y mitocondrial) y usando diferentes enfoques de reconstrucción filogenéticas, lo cual se ha documentado previamente influye drásticamente en la evaluación de estatus taxonómicos (31,42,43).

Las topologías obtenidas de acuerdo con el tipo de marcador pueden deberse a las diferencias entre tasas de evolución de los marcadores nucleares y mitocondrial, introgresión, transferencia horizontal de genes, hibridación y por clasificación de linaje incompleto. En concordancia, nuestros resultados muestran ancestría compartida en el análisis de Structure entre las especies brasileras de *Psammolestes* y especies del género *Rhodnius*. Las discordancias entre las topologías obtenidas a partir de diferentes marcadores o entre marcadores mitocondriales y nucleares han sido ampliamente documentadas en diferentes estudios taxonómicos, en insectos, triatominos e incluso en la tribu Rhodniini y género *Psammolestes* (15,17,27,31,44). Estas discordancias pueden obedecer a clasificación de linaje incompleto, que ocurre más rápidamente en aquellos genes con un tamaño de población pequeño o a fenómenos de introgresión, especiación hibrida y/o flujo genético (31,45). Cabe resaltar que adicionalmente las medidas de diferenciación genética (Fst, Dxy y Da) dan soporte a la clasificación de los dos géneros de la tribu especialmente en los marcadores nucleares (Apéndice S9).

La parafilia de Rhodnius-grupo *prolixus* con respecto a *Psammolestes* en esté estudio corresponden a las reconstrucciones realizadas con el marcador mitocondrial, cabe resaltar que esté tipo de marcadores en insectos evolucionan a tasas de 2 a 9 veces más altas que los genes que codifican proteínas nucleares (31,46). En esté marcador los sitios variables pueden haber acumulado múltiples sustituciones (especialmente transiciones en las posiciones del tercer codón) y en consecuencia, saturarse causando homoplasias y reduciendo las señales filogenéticas en niveles taxonómicos altos como es el caso de los géneros de la tribu y es por esto que varios estudios sugieren que los resultados obtenidos con genes mitocondriales sean interpretados con precaución en niveles taxonómicos altos o aquellos cuya divergencia corresponda a más de 5 millones de años, lo cual está en concordancia con la diferenciación de los dos géneros de la tribu según nuestros resultados de estimación de tiempos de divergencia (47,48). Por todo lo anterior, se considera que, a diferencia de lo sugerido en estudios previos, no se deben incluir las especies del género *Psammolestes* en el género *Rhodnius* en concordancia con sus características biológicas (49,50), eco-epidemiológicas (10,51), morfológicas (49), citogenéticas (52,53) y moleculares (17).

4.2. Relaciones filogenéticas del género *Psammolestes*

En todas las reconstrucciones obtenidas mediante concatenados de todos los genes (Nucleares/ribosomal y mitocondrial) y el marcador mitocondrial se logró recuperar las especies de

P. coreodes y *P. tertius* como grupos monofiléticos con soportes de bootstrap > 95.0% y probabilidad posterior >0.95, a diferencia de las obtenidas en algunos genes nucleares de manera independiente, esto indica la utilidad del enfoque multilocus y concatenado de genes en las reconstrucciones filogenéticas y resolución de este tipo de conflictos (34–38) . En concordancia con lo anterior, el análisis de structure recupero en un único clúster a *P. arthuri* y para el caso de *P. coreodes* y *P. tertius* patrones de ancestría similares entre sí, pero compartidos con algunas especies del género *Rhodnius* diferentes, también los valores de estadísticos de estructura genética que fueron menores entre las especies brasileras en comparación con *P. arthuri* (Figure 4 y Apéndice S3 y S4). En concordancia con estos hallazgos, se han logrado cruces exitosos entre *P. coreodes* y *P. tertius* con producción de híbridos F1, pero que presentan una alta tasa de mortalidad >90.0% sugiriendo la presencia de barreras postcigoticas e inviabilidad hibrida (33).

Es probable que la discordancia entre topologías sea debido a las diferencias en los tiempos de coalescencia entre los loci y aún más teniendo que el marcador mitocondrial puede tener una tasa de evolución más alta que los genes nucleares, lo que se considera es una ventaja para el análisis de taxones estrechamente relacionados que han divergido recientemente como es el caso de *P. coreodes* y *P. tertius* (31,46,54,55). También podría ser atribuible a flujo de genes, aunque en estudios previos de estas especies se ha evidenciado la ausencia de este último través de modelamiento demográfico (17). Por todo lo anterior, en esté estudio ratificamos el estatus de las tres especies previamente descritas en el género *Psammolestes* en concordancia con análisis previos a nivel biológico, ecológico y molecular (17,33,52).

4.3. Relaciones filogenéticas de los grupos del género *Rhodnius*

En este estudio en todas las reconstrucciones se recuperaron los grupos descritos para el género *Rhodnius* como grupos monofiléticos con la agrupación de *pallescens/pictipes* como clado hermano de *prolixus.* En concordancia con esta topología, algunos estudios han descrito está misma relación, incluso soportándola mediante detección en genomas de patrones InDel (15). Todas nuestras reconstrucciones de Cytb (MV-IB) también evidenciaron la agrupación en un clado de los grupos *pictipes* y *pallescens*. En discordancia, la reconstrucción de *Cytb* realizada con los ejemplares usados para genomas con MV mostro los grupos *prolixus* y *pictipes* como clados hermanos no monofiléticos (15), atribuyendo este resultado como erróneo por eventos de presión selectiva e introgresión, esto dado que la reconstrucción con los genomas mitocondriales si evidenció la agrupación de *pictipes/ pallescens* (15). Sin embargo, debido a nuestros resultados consideramos que esta discordancia observada previamente y en otros estudios (Apéndice 10) se debe a sesgos causados por el muestreo, ya que el aumento de taxones realizados en este estudio genero un resultado consistente con las reconstrucciones obtenidas con genomas mitocondriales agrupando *pictipes*/*pallescens* como clado hermano de *prolixus*. Se ha descrito en insectos que se pueden generar hipótesis filogenéticas sesgadas con marcadores mitocondriales en casos de muestreos limitados en especies estrechamente relacionadas (40). Será de gran importancia generar análisis de genomas mitocondriales en un muestreo más representativo de la tribu para evitar sesgos. Dado que las reconstrucciones de todos los marcadores usando IB mostraron los grupos del género *Rhodnius* como monofiléticos, pero no evidencian relaciones intragrupos será necesario usar un mejor muestreo ejemplares con estudios de

genómica para proporcionar mayor cantidad de datos y lograr la resolución que permita establecer las relaciones en este nivel taxonómico con este tipo de análisis.

4.3.1. Relaciones filogenéticas de especies del grupo *pallescens*

En este estudio obtuvimos en todas las reconstrucciones de concatenados nucleares/ribosomal y mitocondrial, la agrupación de las especies *R. colombiensis* y *R. pallescens* en un clado hermano de *R. ecuadoriensis* con valores de bootstrap clásicos y SH-aLRT superiores al 95.0% (Figura 2) y probabilidad posterior > 0.96(Figura 4). Estudios previos no lograron esclarecer las relaciones intragrupo, resaltando la necesidad de incluir un mayor número de taxa y especialmente de *R. pallescens* (15). Las reconstrucciones realizadas en esté estudio incluyeron 59 ejemplares de *R. pallescens* representativos de su distribución geográfica en Colombia y Panamá, así como de diferentes regiones de Ecuador para el caso de 29 ejemplares incluidos de *R. ecuadoriensis.* La adición de más ejemplares en el muestreo pudo eliminar el sesgo que ha generado discordancias previas y por ende en esté estudio se logró recuperar la misma agrupación en todas nuestras reconstrucciones (42,56,57), que además es concordante con la distribución geográfica actual y con reportes previos en la literatura que usan concatenados de diferentes tipos de marcadores y análisis de espectrometría de masas(27,58,59). En este último *R. colombiensis* muestra un patrón diferencial al de otras especies del género *Rhodnius* y en concordancia en nuestro análisis de structure muestra un patrón de ancestría similar al de *P. tertius* (59).

Los resultados discordantes entre estudio y los previamente reportados (*R. pallescens* hermano *R. ecuadoriensis*) se deben a que los resultados previos se realizaron teniendo en cuenta marcadores ribosomales únicamente (28s rRNA), por lo que probablemente se deben a falta de resolución de esté marcador a esté nivel taxonómico (27,31,60). En concordancia con lo anterior, en esté estudio las medidas de diferenciación genética para el marcador ribosomal fueron menores entre *R. ecuadoriensis* y *R. pallescens* que entre *R. colombiensis* y *R. pallescens,* contrario a lo observado en los genes nucleares y mitocondrial que están en concordancia con la agrupación reconstruida en las topologías de este estudio. Adicionalmente, se observó ancestría compartida de *R. pallescens* y *R. ecuadoriensis*, y de este último con *R. colombiensis* y especies del grupo *prolixus* y del género *Psammolestes* (Figura 4). Sin embargo, la consistencia de las relaciones filogenéticas entre especies del grupo *pallescens* en las diferentes topologías reportadas en esté en esté estudio, dejan en evidencia la importancia del enfoque multilocus bien sea por concatenación y/o coalescencia, análisis de varios tipos de marcadores y muestreo representativo para reducir sesgos en las reconstrucciones filogenéticas (34–41).

Sumado esto que a que se lograron recuperar los dos genotipos previamente para *R. pallescens* con la reconstrucción del marcador mitocondrial y el concatenado de todos los loci, lo cual refleja la utilidad del marcador mitocondrial para evidenciar relaciones de taxones con divergencia reciente (31), La discordancia mito-nuclear puede deberse a la resolución del marcador. Sin embargo, también se ha descrito la asociación con la infección por *Wolbachia* la cual se ha documentado que es altamente frecuente en *R. pallescens* colectados en Panamá en las zonas de muestreo a las que corresponde esté estudio (45,61). Finalmente, llama la atención el patrón de ancestría que se observa

para *R. ecuadoriensis* en el análisis de structure que podría estar en concordancia con el patrón citogenético de distribución de cromatina particular de esta especie en cromosomas sexuales (62,63).

4.3.2. Relaciones filogenéticas de especies del grupo *pictipes*

Aunque se observó en la mayoría de las reconstrucciones una agrupación específica para el grupo *pictipes* con altos valores de soporte (Figura 2), se observaron dos incongruencias de agrupación con el marcador *Cytb* y en el análisis de multiespecies coalescente con Beast2, que ubican especies de este grupo con los dos restantes.

Adicionalmente, en el análisis de structure las especies del grupo pictipes muestran un patrón de ancestría compartida similar entre ellas y a su vez con *R. ecuadoriensis* (*pallescens)*, *R. nasutus*, *R. neglectus* y *R. neivai* (prolixus) (Figura 4), esto también está en concordancia con la consideración de que las especies del grupo pictipes son las más ancestrales [81] y de igual forma se puede observar en los tiempos de divergencia calculados en esté estudio (Figura 3). Para el caso del marcador mitocondrial, puede deberse a saturación del marcador, homoplasias y perdida de señal filogenética, sesgos por AT, procesos de selección que se han descrito pueden generar señales sesgadas en niveles taxonómicos altos inclusive en la tribu Rhodniini (15,31,44,47,48,64,65). La discordancia con el árbol de especies puede deberse a clasificación de linaje incompleto en concordancia con las topologías observadas en loci individuales, también se han asociado esté tipo de discordancias con retención de polimorfismos ancestrales lo que está en concordancia con los perfiles de ancestría observados (35,38). Consideramos que los grupos son monofiléticos teniendo en cuenta las demás topologías observadas con el concatenado de genes nucleares, concatenado de todos los loci y los resultados de la prueba de topología. Análisis estadísticos y simulaciones de las topologías reconstruidas con enfoques de multigenes (coalescencia y concatenaciones) han dejado en evidencia que las reconstrucciones con concatenados son más precisas y robustas para solucionar incongruencias y formular hipótesis filogenéticas, incluso cuando estás corresponden a loci no vinculados (mitocondrial, ribosomal y nuclear) (31,37,40,41).

4.3.2. Relaciones filogenéticas de especies del grupo *prolixus*

Las discrepancias mito-nucleares observadas para el grupo *prolixus* pueden deberse a las diferentes razones que se han expuesto previamente. Se espera este tipo de discordancia porque el genoma mitocondrial es haploide y se hereda uniparentalmente en la mayoría de los animales y, por lo tanto, tiene un tamaño de población efectivo cuatro veces menor. Esto significa que el ADN mitocondrial completará el proceso de clasificación de linaje, donde los polimorfismos ancestrales se pierden con el tiempo, más rápido que el ADN nuclear, ya que esta tasa es inversamente proporcional al tamaño efectivo de la población (31,45). Por ende, los datos obtenidos con el marcador mitocondrial se consideran útiles en el estudio de especies que han sufrido divergencia reciente dada su tasa de evolución más rápida (45,61).En este orden, consideramos que las diferencias observadas en este estudio con respecto a los 3 clados del grupo *prolixus* podrían obedecer a saturación del marcador mitocondrial, dado que es un nivel taxonómico mayor que a nivel de especies y que el concatenado de todos los genes guarda consistencia con el concatenado de marcadores nucleares/ribosomal (31,45,48). En concordancia con estos hallazgos cruces entre *R. neglectus* y *R. prolixus* han generado híbridos que no logran llegar a la adultez, evidenciando barreras postcigoticas (66).

Relaciones filogenéticas clado *R. nasutus, R. neglectus* y *R. milesi*

Las incongruencias de agrupación cruzada entre el marcador mitocondrial y los marcadores nucleares/ribosomal observadas entre *R. neglectus* y *R. nasutus,* presentan concordancia con patrones de hibridación e introgresión entre especies (15,45), lo cual también se ve reflejado en las medidas de estructura genética (Fst, Dxy y Da) (Apéndice S9). Sumado a esto existen reportes de simpatría de las dos especies ya que coexisten en las ecorregiones brasileras del cerrado y la catinga (51,67).

Por otro lado, queremos resaltar que los ejemplares de *R. milesi* en la mayoría de las reconstrucciones se obtuvieron en un clado monofilético y fueron agrupadas en un único clúster en el análisis de structure (Figura 4). Las medidas de distancia genéticas observadas entres las tres especies fueron bajas, sin embargo, en todos los marcadores se observó una mayor distancia entre *R. milesi* respecto a *R. neglectus* y *R. nasutus*, que entre estas dos últimas, por lo cual sugerimos que su estatus taxonómico si puede ser el de una especie del grupo *prolixus*, en concordancia con análisis de espectrometría de masas en que se mostró un espectro diferente a *R. neglectus* y *R. nasutus* (59).

Nuestros hallazgos con respecto a este grupo de especies son discordantes con un estudio previo en los que las relaciones entre estas especies solo habían sido exploradas con el marcador mitocondrial *Cytb* (14) y presenta algunas diferencias con un estudio de genomas en el que solo incluyo 6 ejemplares de este grupo de especies, el reducido número de ejemplares puede explicar que algunas relaciones filogenéticas sean diferentes (15). Por tanto, consideramos que mediante nuestras aproximaciones logramos resolver el estatus taxonómico de este grupo de especies y detectamos la razón de la parafilia de *R. neglectus* dado el aumento de muestreo y representatividad biogeográfica en comparación con estudios previos (14,15).

Relaciones filogenéticas clado *R. montenegrensis, R. marabaensis, R. prolixus y R. robustus.*

Esté es el primer estudio en el que se usa un enfoque multilocus en ejemplares de *R. robustus* I, II y IV que fueron clasificados por el marcador mitocondrial *Cytb*, por lo que pudimos verificar que la parafilia de *R. robustus* en este subgrupo, relacionada con todas incongruencias detectadas que a su vez corresponden a discordancias mitonucleares, en concordancia con la inferencia de ancestría observada en el análisis de Structure y a través de las distancias genéticas entre las especies de esté subgrupo. Teniendo en cuenta nuestros resultados en conjunto con las distancias genéticas y ancestría en structure, sugerimos que *R. marabaensis* corresponde a una especie y puede ser sinonimizada con *R. robustus* III, en concordancia con lo observado por repeatomas y análisis de espectrometría de masas (59,68).

Para el caso de *R. montenegrensis*, en el marcador mitocondrial se observa su agrupación con todos los ejemplares de *R. robustus* II. Sin embargo, sugerimos que solo algunos ejemplares de *R. robustus* II sean sinonimizados como *R. montenegrensis*: los provenientes de Caipuru-Brasil (307, 308, 311, 318) y uno de los de Perú (108) de *R. robustus* II, esto en concordancia con que también son parafiléticos en todas las filogenias de marcadores nucleares y de todos los loci y en el análisis de structure se evidencia que estos ejemplares pertenecen principalmente al clúster formado por *R. montenegrensis* y *R. marabaensis* y en combinación pero con menor proporción al cluster formado por *R. prolixus* de Colombia y Venezuela. Así en conjunto con los análisis de distancias genéticas, también sugerimos el estatus taxonómico de especie para *R. montenegrensis*, en concordancia con análisis de repeatomas, transcriptomas y análisis de espectrometría de masas (59,68,69).

Los ejemplares restantes de *R. robustus* II de Perú (99-107 y 440-449) identificados con el marcador mitocondrial son parafiléticos a *R. prolixus* en los marcadores nucleares y reconstrucción de todos los loci, al igual que pertenecen al mismo clúster *R. prolixus* de Colombia y Venezuela en el análisis de structure. Estás discordancias mito-nucleares observadas muestran concordancia con procesos de hibridación e introgresión y/o retención de polimorfismo ancestral con *R. prolixus* de Colombia y Venezuela, concordantes con la distribución biogeográfica y tiempos de divergencia de este grupo de ejemplares (15,45).

Los ejemplares identificados como *R. robustus* IV clasificados en este estudio mediante *cytb*, incluyen dos grupos brasileros entre ellos el grupo identificados morfológicamente como *R. prolixus* que proviene de Rio de Janeiro que ha sido muy controversial debido a que se cuestiona la presencia de esta especie en Brasil y que evidencio diferencia mito-nuclear (14,32,44). Sin embargo, teniendo en cuenta que pertenecen al mismo clúster de *R. prolixus* de Colombia y Venezuela en la inferencia de ancestría, la parafilia observada en todas las reconstrucciones nucleares y de todos los loci y las medidas de distancia genética, sugerimos que estos ejemplares sean sinonimizados como *R. prolixus*, en concordancia con su descripción morfológica y filogenética en conjunto, al igual que los ejemplares 312 y 314 brasileros del grupo II que filogenéticamente y mediante análisis de ancestría se evidencian como *R. prolixus* confirmando su presencia en Brasil y simpatría con *R. robustus* en esté país y por se da cabida a la introgresión-hibridación entre estos individuos.

Los ejemplares de *R. robustus* de Venezuela incluidos en esté estudio se agrupan con ejemplares de *R. robustus* I, sin embargo, su posición en las filogenias mitocondriales cambia según el tipo de reconstrucción (MV e IB), mostrando discordancia mito-nuclear, que a diferencia de los demás especies cripticas de *R. robustus* no mostro el mismo patrón único de ancestría de *R. prolixus*, sino una mezcla de *R. prolixus*, *R. marabaensis* y en muy baja proporción *R. milesi*, que además forma un clado parafilético con *R. prolixus*, lo cual puede ser evidencia de patrones introgresión/hibridación y/o retención de polimorfismos ancestrales y explica el comportamiento de los cruces entre ejemplares de estos grupos que se han visto exitosos parcialmente en una dirección con supervivencia de híbridos hasta estadio V mencionándose (70).

Finalmente, en este grupo la mayoría de discordancias ocurren por el marcador mitocondrial *cytb*, por ende aunque este marcador es muy informativo ya que da luces acerca de la complejidad filogenética y evolutiva, no debe ser la única fuente de información filogenética y/o biogeográfica dado que se ha descrito que su acelerada tasa de evolución y más en insectos puede generar que las mutaciones que producen haplogrupos de una especie sean eliminadas mediante selección antes de su fijación entre los haplogrupos, lo cual ya se ha descrito para la tribu Rhodniini y en concordancia con nuestros análisis de selección dando lugar a distintos linajes intraespecíficos de mtDNA que se comportan más como linajes entre especies como puede ser el caso de *R. robustus* (15,71,72).

Las discordancias mitonucleares pueden darse por otros aspectos que afectan marcadores nucleares, ribosomales y mitocondriales. Por ejemplo la adaptación a determinadas condiciones climáticas y

ambientales, incluso las que pueden darse en procesos de domiciliación o por intervenciones humanas que pueden estar involucradas en procesos de selección o cambios en las tasas evolutivas que causan la subdivisión de una especie, aumento de variabilidad genética y da como resultado la agrupación de diferentes especies que existen, o existieron, en condiciones climáticas similares o adaptación a nuevas como se ha descrito en *T. infestans* por su domiciliación (60). Las especies de la tribu Rhodniini dada su amplia extensión geográfica se encuentran expuestas a una elevada variación climática y ambiental en distribuciones simpátricas, dicha variación puede estar relacionada con especiación, selección, introgresión y aumento de la variabilidad como puede ser el casos de las especies del grupo *prolixus*, así como los procesos de domiciliación en el caso de las especies de la tribu domiciliadas y como se describió para *R. prolixus* en américa central (25,73). También la infección con simbiontes como Wolbachia en insectos, se ha documentado influye en discrepancias entre las filogenias basadas en genes nucleares y mitocondriales, incluso sin presentarse infección directamente y mediante herencia de la línea materna, así la reconstrucción mitocondrial puede evidenciar la propagación del simbionte en lugar de la relación de las poblaciones según lo evaluado por el ADN nuclear, esto último de gran importancia debido a que se ha descrito transferencia horizontal de genes de esté simbionte a *R. prolixus* mediante análisis de su genoma de referencia y recientemente se ha descrito en otras especies del género *Rhodnius* (15,45,74,75)

5. **Conclusión**

En conclusión, la taxonomía de la tribu Rhodniini es altamente compleja lo que se refleja en las incongruencias reportadas a la fecha. Estás ultimas obedecen a discordancias mito-nucleares, métodos de inferencia filogenética y sesgos generados por falta de muestreo y/o datos que al ser descritas de manera independiente han generado hipótesis conflictivas acerca de aspectos biológicos, filogenéticos, biogeográficos y evolutivos de la tribu. Este estudio es el primero en utilizar un muestreo altamente representativo de los dos géneros y grupos reportados en la tribu, sumado a ello exploramos todos los posibles escenarios generados por el uso de diferentes métodos filogenéticos y diferentes marcadores moleculares, con el fin de disminuir los sesgos que pueden generarse por estás variables y proporcionamos una solución a las incongruencias reportadas para la tribu Rhodniini mediante el uso y análisis de los marcadores nucleares y mitocondriales de manera independiente y concatenados, siendo esté ultimo enfoque el que proporcionó la información necesaria para comprender las incongruencias. Esto último, probablemente se logró debido a que dichas incongruencias corresponden a diferentes niveles taxonómicos y por tanto el uso de varios marcadores con diferentes tasas de evolución en un concatenado, diferentes métodos de reconstrucción sumados a un muestreo representativo proporcionan la información necesaria para resolver los conflictos taxonómicos que ocurren a diferentes niveles en la tribu Rhodniini.

Será de gran utilidad establecer un genoma de referencia para la tribu Rhodniini y realizar análisis filogenómicos en un conjunto de ejemplares que sea representativos de la tribu de tal forma que está aproximación proporcione la cantidad de datos necesaria para eliminar sesgos y esclarecer no solo aspectos acerca de la taxonomía, sino también el panorama evolutivo, biogeográfico y la información genética de la tribu en relación con las características que favorecen el comportamiento vectorial de algunas especies de la tribu Rhodniini.

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Bibliografía

1. WHO. https://www.who.int/campaigns/world-chagas-disease-day/2022#:~:text=In%202022%20we%20are%20shining,case%20to%20defeat%20Chagas%20disease. [Internet]. 2022. Disponible en: https://www.who.int/campaigns/world-chagas-disease-day/2022#:~:text=In%202022%20we%20are%20shining,case%20to%20defeat%20Chagas%20disease.

2. Bern C. Chagas' Disease. Vol. 373, N Engl J Med. 2015. p. 1882.

3. Ceccarelli S, Justi SA, Rabinovich JE, Diniz Filho JAF, Villalobos F. Phylogenetic structure of geographical co-occurrence among New World Triatominae species, vectors of Chagas disease. J Biogeogr. junio de 2020;47(6):1218-31.

4. Zhao Y, Galvão C, Cai W. Rhodnius micki, a new species of Triatominae (Hemiptera, Reduviidae) from Bolivia. Zookeys. 2021;1012:71-93.

5. Alevi KCC, de Oliveira J, da Silva Rocha D, Galvão C. Trends in Taxonomy of Chagas Disease Vectors (Hemiptera, Reduviidae, Triatominae): From Linnaean to Integrative Taxonomy. Pathogens. 15 de diciembre de 2021;10(12):1627.

6. Hernández C, Aristeu da Rosa J, Vallejo GA, Guhl F, Ramírez JD. Taxonomy, Evolution, and Biogeography of the Rhodniini Tribe (Hemiptera: Reduviidae). Diversity. 11 de marzo de 2020;12(3):97.

7. Flores-Ferrer A, Marcou O, Waleckx E, Dumonteil E, Gourbière S. Evolutionary ecology of Chagas disease; what do we know and what do we need? Evol Appl. abril de 2018;11(4):470-87.

8. Gourbière S, Dorn P, Tripet F, Dumonteil E. Genetics and evolution of triatomines: from phylogeny to vector control. Heredity. marzo de 2012;108(3):190-202.

9. Gürtler RE, Cecere MC. Chagas Disease Vector Control. En: Guarneri A, Lorenzo M, editores. Triatominae - The Biology of Chagas Disease Vectors [Internet]. Cham: Springer International Publishing; 2021 [citado 17 de octubre de 2022]. p. 491-535. (Entomology in Focus; vol. 5). Disponible en: https://link.springer.com/10.1007/978-3-030-64548-9_18

10. Velásquez-Ortiz N, Hernández C, Herrera G, Cruz-Saavedra L, Higuera A, Arias-Giraldo LM, et al. Trypanosoma cruzi infection, discrete typing units and feeding sources among Psammolestes arthuri (Reduviidae: Triatominae) collected in eastern Colombia. Parasit Vectors. 8 de abril de 2019;12(1):157.

11. Hernández C, Salazar C, Brochero H, Teherán A, Buitrago LS, Vera M, et al. Untangling the transmission dynamics of primary and secondary vectors of Trypanosoma cruzi in Colombia: parasite infection, feeding sources and discrete typing units. Parasites & Vectors. 1 de diciembre de 2016;9(1):620.

12. Gürtler RE, del Pilar Fernández M, Cardinal MV. Eco-Epidemiology of Vector-Borne Transmission of Trypanosoma cruzi in Domestic Habitats. En: Guarneri A, Lorenzo M, editores. Triatominae - The Biology of Chagas Disease Vectors [Internet]. Cham: Springer International Publishing; 2021 [citado 17 de octubre de 2022]. p. 447-89. (Entomology in Focus; vol. 5). Disponible en: https://link.springer.com/10.1007/978-3-030-64548-9_17

13. Velásquez-Ortiz N, Herrera G, Hernández C, Muñoz M, Ramírez JD. Discrete typing units of Trypanosoma cruzi: Geographical and biological distribution in the Americas. Sci Data. diciembre de 2022;9(1):360.

14. Monteiro FA, Weirauch C, Felix M, Lazoski C, Abad-Franch F. Evolution, Systematics, and Biogeography of the Triatominae, Vectors of Chagas Disease. Vol. 99, Adv Parasitol. 2018. p. 265-344.

15. Filée J, Merle M, Bastide H, Mougel F, Bérenger JM, Folly-Ramos E, et al. Phylogenomics for Chagas Disease Vectors of the Rhodnius Genus (Hemiptera, Triatominae): What We Learn From Mito-Nuclear Conflicts and Recommendations. Front Ecol Evol. 12 de enero de 2022;9:750317.

16. Kieran TJ, Gordon ERL, Zaldívar-Riverón A, Ibarra-Cerdeña CN, Glenn TC, Weirauch C. Ultraconserved elements reconstruct the evolution of Chagas disease-vectoring kissing bugs (Reduviidae: Triatominae). Syst Entomol. julio de 2021;46(3):725-40.

17. Hernández C, Alvarado M, Salgado-Roa FC, Ballesteros N, Rueda-M N, Oliveira J, et al. Phylogenetic relationships and evolutionary patterns of the genus Psammolestes Bergroth, 1911 (Hemiptera: Reduviidae: Triatominae). BMC Ecol Evo. diciembre de 2022;22(1):30.

18. Nascimento JD, da Rosa JA, Salgado-Roa FC, Hernández C, Pardo-Diaz C, Alevi KCC, et al. Taxonomical over splitting in the Rhodnius prolixus (Insecta: Hemiptera: Reduviidae) clade: Are R. taquarussuensis (da Rosa et al., 2017) and R. neglectus (Lent, 1954) the same species? Vol. 14, PLoS One. 2019. p. e0211285.

19. Anisimova M, Gil M, Dufayard JF, Dessimoz C, Gascuel O. Survey of Branch Support Methods Demonstrates Accuracy, Power, and Robustness of Fast Likelihood-based Approximation Schemes. Systematic Biology. 1 de octubre de 2011;60(5):685-99.

20. Heled J, Drummond AJ. Bayesian inference of species trees from multilocus data. Vol. 27, Mol Biol Evol. 2010. p. 570-80.

21. Mirarab S, Reaz R, Bayzid MdS, Zimmermann T, Swenson MS, Warnow T. ASTRAL: genome-scale coalescent-based species tree estimation. Bioinformatics. 1 de septiembre de 2014;30(17):i541-8.

22. Shimodaira H. An Approximately Unbiased Test of Phylogenetic Tree Selection. Goldman N, editor. Systematic Biology. 1 de mayo de 2002;51(3):492-508.

23. Lanfear R, Calcott B, Ho SY, Guindon S. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Vol. 29, Mol Biol Evol. 2012. p. 1695-701.

24. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol. julio de 2005;14(8):2611-20.

25. de Paula AS, Barreto C, Telmo MCM, Diotaiuti L, Galvão C. Historical Biogeography and the Evolution of Hematophagy in Rhodniini (Heteroptera: Reduviidae: Triatominae). Front Ecol Evol. 10 de mayo de 2021;9:660151.

26. Gomez-Palacio A, Jaramillo-Ocampo N, Triana-Chavez O, Saldana A, Calzada J, Perez R, et al. Chromosome variability in the Chagas disease vector Rhodnius pallescens (Hemiptera, Reduviidae, Rhodniini). Vol. 103, Mem Inst Oswaldo Cruz. 2008. p. 160-4.

27. Díaz S, Panzera F, Jaramillo-O N, Pérez R, Fernández R, Vallejo G, et al. Genetic, cytogenetic and morphological trends in the evolution of the Rhodnius (Triatominae: Rhodniini) trans-Andean group. Vol. 9, PLoS One. 2014. p. e87493.

28. Gómez-Palacio A, Jaramillo-O N, Caro-Riaño H, Diaz S, Monteiro FA, Pérez R, et al. Morphometric and molecular evidence of intraspecific biogeographical differentiation of Rhodnius pallescens (HEMIPTERA: REDUVIIDAE: RHODNIINI) from Colombia and Panama. Vol. 12, Infect Genet Evol. 2012. p. 1975-83.

29. Guhl F, Aguilera G, Pinto N, Vergara D. Actualización de la distribución geográfica y ecoepidemiología de la fauna de triatominos (Reduviidae: Triatominae) en Colombia,. Vol. 27, Biomedica. 2007. p. 143-62.

30. Peretolchina T, Pavan MG, Corrêa-Antônio J, Gurgel-Gonçalves R, Lima MM, Monteiro FA. Phylogeography and demographic history of the Chagas disease vector Rhodnius nasutus (Hemiptera: Reduviidae) in the Brazilian Caatinga biome. Vol. 12, PLoS Negl Trop Dis. 2018. p. e0006731.

31. Mas-Coma S, Bargues MD. Populations, hybrids and the systematic concepts of species and subspecies in Chagas disease triatomine vectors inferred from nuclear ribosomal and mitochondrial DNA. Acta Tropica. mayo de 2009;110(2-3):112-36.

32. Monteiro FA, Barrett TV, Fitzpatrick S, Cordon-Rosales C, Feliciangeli D, Beard CB. Molecular phylogeography of the Amazonian Chagas disease vectors Rhodnius prolixus and R. robustus. Vol. 12, Mol Ecol. 2003. p. 997-1006.

33. Ravazi A, de Oliveira J, Campos FF, Madeira FF, dos Reis YV, de Oliveira ABB, et al. Trends in evolution of the Rhodniini tribe (Hemiptera, Triatominae): experimental crosses between Psammolestes tertius Lent & Jurberg, 1965 and P. coreodes Bergroth, 1911 and analysis of the reproductive isolating mechanisms. Parasites Vectors. diciembre de 2021;14(1):350.

34. Herrando-Moraira S, Calleja JA, Galbany-Casals M, Garcia-Jacas N, Liu JQ, López-Alvarado J, et al. Nuclear and plastid DNA phylogeny of tribe Cardueae (Compositae) with Hyb-Seq data: A new subtribal classification and a temporal diversification framework. Molecular Phylogenetics and Evolution. agosto de 2019;137:313-32.

35. Gadagkar SR, Rosenberg MS, Kumar S. Inferring species phylogenies from multiple genes: Concatenated sequence tree versus consensus gene tree. J Exp Zool. 15 de enero de 2005;304B(1):64-74.

36. Tonini J, Moore A, Stern D, Shcheglovitova M, Ortí G. Concatenation and Species Tree Methods Exhibit Statistically Indistinguishable Accuracy under a Range of Simulated conditions. PLoS Curr [Internet]. 2015 [citado 19 de octubre de 2022]; Disponible en: https://currents.plos.org/treeoflife/article/concatenation-and-species-tree-methods-exhibit-statistically-indistinguishable-accuracy-under-a-range-of-simulated-conditions/

37. Kubatko LS, Degnan JH. Inconsistency of Phylogenetic Estimates from Concatenated Data under Coalescence. Collins T, editor. Systematic Biology. 1 de febrero de 2007;56(1):17-24.

38. Thiergart T, Landan G, Martin WF. Concatenated alignments and the case of the disappearing tree. BMC Evol Biol. diciembre de 2014;14(1):266.

39. Slowinski JB, Page RDM. How Should Species Phylogenies Be Inferred from Sequence Data? Olmstead R, editor. Systematic Biology. 1 de octubre de 1999;48(4):814-25.

40. Ballard JWO. When One Is Not Enough: Introgression of Mitochondrial DNA in Drosophila. Molecular Biology and Evolution. 1 de julio de 2000;17(7):1126-30.

41. Bull JJ, Huelsenbeck JP, Cunningham CW, Swofford DL, Waddell PJ. Partitioning and Combining Data in Phylogenetic Analysis. Systematic Biology. 1 de septiembre de 1993;42(3):384-97.

42. Lecointre G, Philippe H, Lê HLV, Le Guyader H. Species Sampling Has a Major Impact on Phylogenetic Inference. Molecular Phylogenetics and Evolution. septiembre de 1993;2(3):205-24.

43. Nabhan AR, Sarkar IN. The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. Briefings in Bioinformatics. 1 de enero de 2012;13(1):122-34.

44. Monteiro FA, Wesson DM, Dotson EM, Schofield CJ, Beard CB. Phylogeny and molecular taxonomy of the Rhodniini derived from mitochondrial and nuclear DNA sequences. Vol. 62, Am J Trop Med Hyg. 2000. p. 460-5.

45. Toews DPL, Brelsford A. The biogeography of mitochondrial and nuclear discordance in animals: BIOGEOGRAPHY OF MITO-NUCLEAR DISCORDANCE. Molecular Ecology. agosto de 2012;21(16):3907-30.

46. Monteiro A, Pierce NE. Phylogeny of Bicyclus (Lepidoptera: Nymphalidae) Inferred from COI, COII, and EF-1α Gene Sequences. Molecular Phylogenetics and Evolution. febrero de 2001;18(2):264-81.

47. Bullerwell CE, Gray MW. Evolution of the mitochondrial genome: protist connections to animals, fungi and plants. Current Opinion in Microbiology. octubre de 2004;7(5):528-34.

48. Ballard JWO, Rand DM. The Population Biology of Mitochondrial DNA and Its Phylogenetic Implications. Annu Rev Ecol Evol Syst. 1 de diciembre de 2005;36(1):621-42.

49. Lent H. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. New York: American Museum of Natural History; 1979. (Bulletin of the American Museum of Natural History,).

50. Ravazi A, Oliveira J de, Madeira FF, Reis YV dos, Oliveira ABB de, Galvão C, et al. Trends in Taxonomy of the Rhodniini Tribe (Hemiptera, Triatominae): Reproductive Incompatibility between Rhodnius neglectus Lent, 1954 and Psammolestes spp. Confirms the Generic Status of Psammolestes Bergroth, 1911. Diversity. 15 de septiembre de 2022;14(9):761.

51. Abad-Franch F, Lima MM, Sarquis O, Gurgel-Gonçalves R, Sánchez-Martín M, Calzada J, et al. On palms, bugs, and Chagas disease in the Americas. Vol. 151, Acta Trop. 2015. p. 126-41.

52. Oliveira J, Alevi KCC, Ravazi A, Herrera HM, Santos FM, Azeredo-Oliveira MTV, et al. New Evidence of the Monophyletic Relationship of the Genus. Vol. 99, Am J Trop Med Hyg. 2018. p. 1485-8.

53. Panzera F, Pérez R, Panzera Y, Ferrandis I, Ferreiro MJ, Calleros L. Cytogenetics and genome evolution in the subfamily Triatominae (Hemiptera, Reduviidae). Vol. 128, Cytogenet Genome Res. 2010. p. 77-87.

54. DeSalle R, Freedman T, Prager EM, Wilson AC. Tempo and mode of sequence evolution in mitochondrial DNA of HawaiianDrosophila. J Mol Evol. noviembre de 1987;26(1-2):157-64.

55. Moriyama EN, Powell JR. Synonymous substitution rates in Drosophila: Mitochondrial versus nuclear genes. J Mol Evol. octubre de 1997;45(4):378-91.

56. Hillis DM. Inferring complex phytogenies. Nature. septiembre de 1996;383(6596):130-1.

57. Poe S, Swofford DL. Taxon sampling revisited. Nature. marzo de 1999;398(6725):299-300.

58. Justi SA, Galvão C, Schrago CG. Geological Changes of the Americas and their Influence on the Diversification of the Neotropical Kissing Bugs (Hemiptera: Reduviidae: Triatominae). Vol. 10, PLoS Negl Trop Dis. 2016. p. e0004527.

59. Dos Santos Souza É, Fernandes RP, Guedes WN, Dos Santos FN, Eberlin MN, Lopes NP, et al. Rhodnius spp. are differentiated based on the peptide/protein profile by matrix-assisted laser desorption/ionization mass spectrometry and chemometric tools. Anal Bioanal Chem. febrero de 2020;412(6):1431-9.

60. Bargues MD, Marcilla A, Dujardin JP, Mas-Coma S. Triatomine vectors of Trypanosoma cruzi: a molecular perspective based on nuclear ribosomal DNA markers. Vol. 96 Suppl 1, Trans R Soc Trop Med Hyg. 2002. p. S159-64.

61. Kieran TJ, Arnold KMH, Thomas JC, Varian CP, Saldaña A, Calzada JE, et al. Regional biogeography of microbiota composition in the Chagas disease vector Rhodnius pallescens. Parasites Vectors. diciembre de 2019;12(1):504.

62. Panzera Y, Pita S, Ferreiro MJ, Ferrandis I, Lages C, Pérez R, et al. High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Vol. 138, Cytogenet Genome Res. 2012. p. 56-67.

63. Pita S, Panzera F, Ferrandis I, Galvão C, Gómez-Palacio A, Panzera Y. Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal location of ribosomal genes [Internet]. Vol. 108, Mem Inst Oswaldo Cruz. 2013. Disponible en: https://www.ncbi.nlm.nih.gov/pubmed/23778665

64. Lyman DF, Monteiro FA, Escalante AA, Cordon-Rosales C, Wesson DM, Dujardin JP, et al. Mitochondrial DNA sequence variation among triatomine vectors of Chagas' disease. Vol. 60, Am J Trop Med Hyg. 1999. p. 377-86.

65. Maia Da Silva F, Junqueira AC, Campaner M, Rodrigues AC, Crisante G, Ramirez LE, et al. Comparative phylogeography of Trypanosoma rangeli and Rhodnius (Hemiptera: Reduviidae) supports a long coexistence of parasite lineages and their sympatric vectors. Vol. 16, Mol Ecol. 2007. p. 3361-73.

66. Carvalheiro J, Barretto M. Estudos sobre reservatórios e vectores silvestres do Trypanosoma cruzi. LX – Tentativas de cruzamento de Rhodnius prolixus Stål, 1859 com Rhodnius neglectus Lent, 1954 (Hemiptera, Reduviidae). Rev Inst Med Trop São Paulo. 1976;18:17-23.

67. Ceccarelli S, Balsalobre A, Vicente ME, Curtis-Robles R, Hamer SA, Ayala Landa JM, et al. American triatomine species occurrences: updates and novelties in the DataTri database. Gigabyte. 31 de mayo de 2022;2022:1-8.

68. Castro MRJ, Goubert C, Monteiro FA, Vieira C, Carareto CMA. Homology-Free Detection of Transposable Elements Unveils Their Dynamics in Three Ecologically Distinct Rhodnius Species. Genes (Basel). 6 de febrero de 2020;11(2).

69. de Carvalho DB, Congrains C, Chahad-Ehlers S, Pinotti H, Brito RA, da Rosa JA. Differential transcriptome analysis supports Rhodnius montenegrensis and Rhodnius robustus (Hemiptera, Reduviidae, Triatominae) as distinct species. Vol. 12, PLoS One. 2017. p. e0174997.

70. Superlano Y, Lizano E, Galindez I, Aldana E. Aislamiento reproductivo postcigótico entre Rhodnius prolixus Stal 1859 y R. robustus Larrousse 1927 (Heteroptera, Triatominae). Parasitol Latinoam. 61:23-31.

71. Dean MD, Ballard JWO. High divergence among Drosophila simulans mitochondrial haplogroups arose in midst of long term purifying selection. Molecular Phylogenetics and Evolution. agosto de 2005;36(2):328-37.

72. Edmands S. Phylogeography of the intertidal copepod Tigriopus californicus reveals substantially reduced population differentiation at northern latitudes: PHYLOGEOGRAPHY OF Tigriopus CALIFORNICUS. Molecular Ecology. 21 de diciembre de 2001;10(7):1743-50.

73. Dujardin JP, Munoz M, Chavez T, Ponce C, Moreno J, Schofield CJ. The origin of Rhodnius prolixus in Central America. Vol. 12, Med Vet Entomol. 1998. p. 113-5.

74. Dean MD, Ballard JWO. Linking phylogenetics with population genetics to reconstruct the geographic origin of a species. Molecular Phylogenetics and Evolution. septiembre de 2004;32(3):998-1009.

75. Mesquita RD, Vionette-Amaral RJ, Lowenberger C, Rivera-Pomar R, Monteiro FA, Minx P, et al. Genome of Rhodnius prolixus, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. Vol. 112, Proc Natl Acad Sci U S A. 2015. p. 14936-41.

Tabla 1. Loci amplificados, iniciadores utilizados y tamaños del producto de PCR

Sigla*	**Nombre del gen***	**Secuencia de los iniciadores**	**Tamaño de amplicon (pb)**
TRNA	*tRNA (Guanina (37) -N (1) metiltransferasa*	Directo: GGGCCACGTTTCTAACAAAA	842
		Reverso: CAATTGGAATGCTGCTGAAA	
PJH	*Putative juvenile hormone inducible protein*	Directo: CCCTTTTAGCAAAATGTTCCA	720
		Reverso: TGCCATTATTGCAAGCAGAA	
CISP	*Probable cytosolic iron sulfur protein assembly protein Ciao 1*	Directo: TTATCTGCGCAAGCAGTAGC	706
		Reverso: TAAGACTTTGGGGGAAGCAA	
LSM	*Lipoyl synthase, mitocondrial*	Directo: AAAAAGCCCATTTCGTTTCC	768
		Reverso: AATGGGCCCACATTATTCAA	
UPCA	*Uncharacterized protein (Cell adhesion)*	Directo: TGAAAGGGATCGTACCTTGG	795
		Reverso: CCTCCAGACTGATGGCTTGT	
UPMETAL	*Uncharacterized protein- metal ion binding*	Directo:TAGGCGGCGATGTA	725
		Reverso: GGGCAATTCTTGTCC	
CYTB	*Cytochrome b*	Directo: GGACG(AT)GG(AT)ATTTATTATGG ATC	840
		Reverso: GC(AT)CCAATTCA(AG)GTTA(AG)T AA	
28S	28S rRNA	Directo: GCGAGTCGTGTTGCTTGATAG TGCAG	696
		Reverso: TTGGTCCGTGTTTCAAGACGG	

*Siglas enunciadas por sus nombres en inglés

Figura 1. Distribución de muestras de insectos de la tribu Rhodniini colectadas en este estudio. Los puntos representan los ejemplares colectados en este estudio. A. Genero *Psammolestes* B. Genero *Rhodnius*: Especies del grupo *pictipes* C. Genero *Rhodnius*: Especies del grupo *pallescens* D. Genero Rhodnius: Especies del grupo *prolixus*.

A.

Guatemala
Nicaragua
Panama
Venezuela
Guyana
Colombia
Ecuador
Peru
Brazil
Bolivia
Paraguay
Uruguay
Argentina
Chile
Falkland Islands (Islas Malvinas)
Scotia

Psammolestes

- *P.arthuri*
- *P.coreodes*
- *P.tertius*

B.

Guatemala
Nicaragua
Venezuela
Guyana
Colombia
Ecuador
Peru
Brazil
Bolivia
Paraguay
Uruguay
Argentina
Chile
Falkland Islands (Islas Malvinas)
Scotia

pallescens

- *R.colombiensis*
- *R.ecuadoriensis*
- *R.pallescens*

C.

Guatemala
Nicaragua
Panama
Venezuela
Guyana
Colombia
Ecuador
Peru
Brazil
Bolivia
Paraguay
Uruguay
Argentina
Chile
Falkland Islands (Islas Malvinas)
Scotia

prolixus

- *R.nasutus*
- *R.neglectus*
- *R.milesi*
- *R.montenegrensis*
- *R.marabaensis*
- *R.prolixus*
- *R.robustus*
- *R.neivai*

D.

Guatemala
Nicaragua
Panama
Venezuela
Guyana
Colombia
Ecuador
Peru
Brazil
Bolivia
Paraguay
Uruguay
Argentina
Chile
Falkland Islands (Islas Malvinas)
Scotia

pictipes

- *R.brethesi*
- *R.pictipes*
- *R.stali*

Figura 2. Figura 2. Reconstrucción filogenética de máxima credibilidad mediante MV de la tribu Rhodniini. Reconstrucción basada en los 8 loci utilizados en este estudio realizada con PHYML y 1000 repeticiones de Bootstrap clásico. Se evidencian todos los niveles taxonómicos.

Géneros y especies

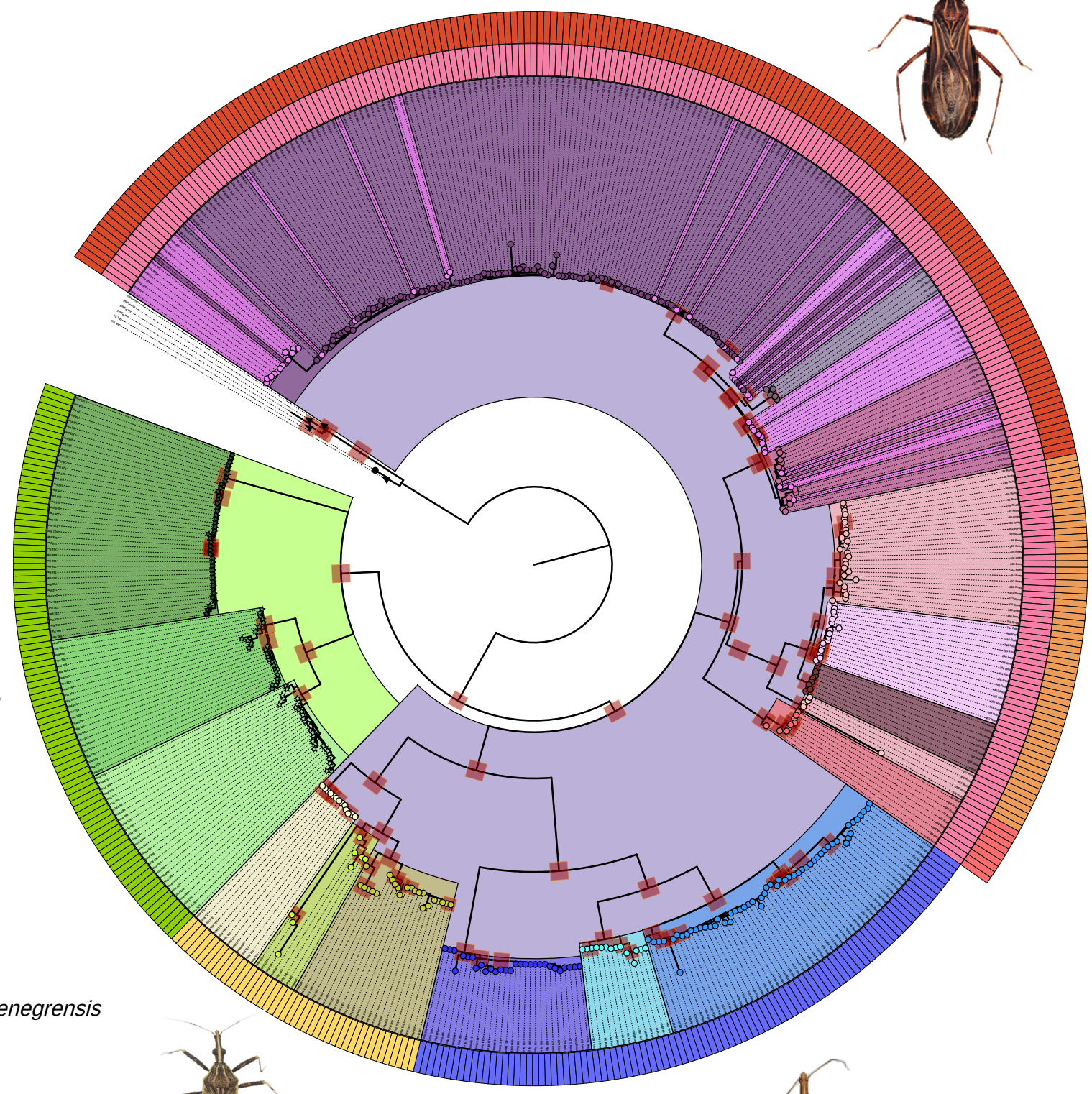
- ***Psammolestes***
- *P. arthuri*
- *P. tertius*
- *P. coreodes*
- ***Rhodnius***
- *R. pallescens*
- *R. brethesi*
- *R. montenegrensis*
- *R. robustus*
- *R. pictipes*
- *R. ecuadoriensis*
- *R. marabaensis*
- *R. colombiensis*
- *R. milesi*
- *R. stali*
- *R. nasutus*
- *R. neivai*
- *R. prolixus*
- *R. neglectus*

Grupo

- *prolixus*
- *pallescens*
- *pictipes*
- *psammolestes*

Subgrupos prolixus

- *R.prolixis/R.robustus/R.marabaensis/R.montenegrensis*
- *R.neglectus/R.nasutus/R.milesi*
- *R. neivai*



bootstrap

- 0.75
- 0.81
- 0.88
- 0.94
- 1

Tree scale: 0.01

Figura 3. Árbol filogenético de IB obtenido para concatenado de los 8 loci utilizados en este estudio. Las barras moradas horizontales ilustran el HPD del 95 % para el tiempo de divergencia de los nodos.

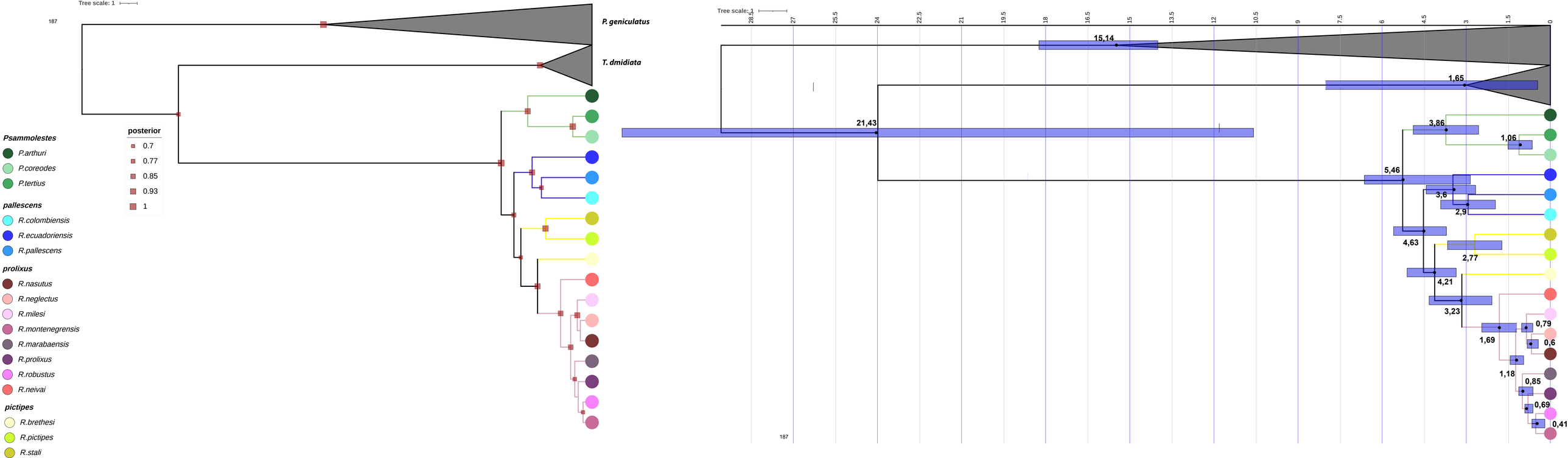
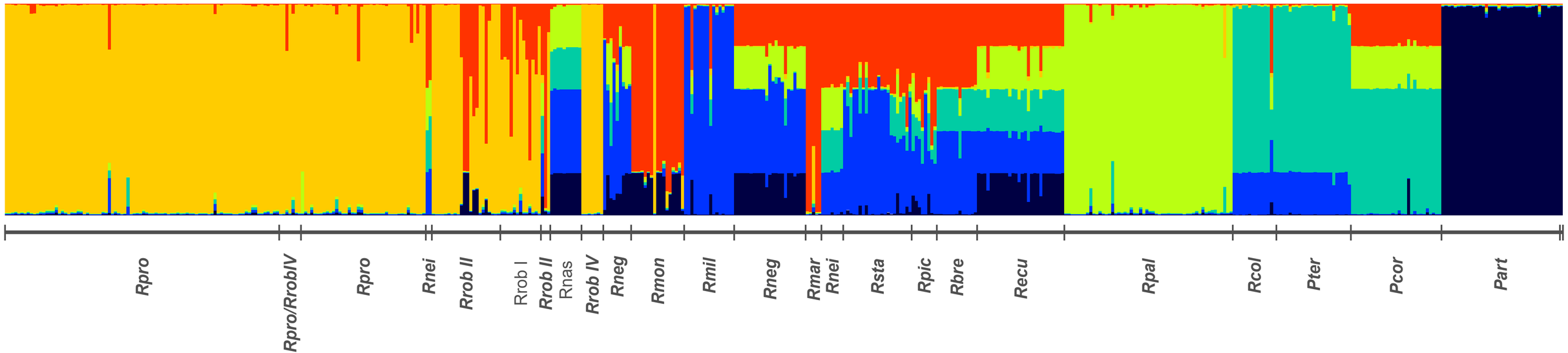

Tree scale: 1
187
P. geniculatus
T. dmidiata
posterior
0.7
0.77
0.85
0.93
1
Psammolestes
P.arthuri
P.coreodes
P.tertius
pallescens
R.colombiensis
R.ecuadoriensis
R.pallescens
prolixus
R.nasutus
R.neglectus
R.milesi
R.montenegrensis
R.marabaensis
R.prolixus
R.robustus
R.neivai
pictipes
R.brethesi
R.pictipes
R.stali
Tree scale: 1
28.5
27
25.5
24
22.5
21
19.5
18
16.5
15
13.5
12
10.5
9
7.5
6
4.5
3
1.5
0
15,14
1,65
21,43
3,86
1,06
5,46
3,6
2,9
4,63
2,77
4,21
3,23
0,79
1,69
0,6
1,18
0,85
0,69
0,41
187

Figura 4. Análisis de estructura poblacional usando STRUCTURE. Gráfico obtenido mediante software pophelper con K = 6.



G1. R. prolixus y R. robustus *G2. R. pallescens* *G3. P. arthuri* *G4. R. milesi* *G5. R. montenegrensis y R. marabaensis*

G6. P. coreodes, P. tertius, R. colombiensis

G7. R. nasutus, R. neglectus, R. neivai, R.sta, R.pictipes, R.brethesis , R. ecuadoriensis (Mezcla)

9. CONCLUSIONES

- Se detectaron tres especies representativas de cada uno de los grupos del género *Rhodnius* (*R. pictipes, R. prolixus* y *R. pallescens*) infectadas con *T. cruzi,* distribuidas en diferentes ciclos epidemiológicos de transmisión y se analizaron las preferencias alimenticias simultáneamente, reafirmando su rol en la transmisión del parásito en el marco de la enfermedad de Chagas en Colombia, dada la detección de infección simultanea con Sangre humana y el parásito.
- Se encontró infección en las especies *R. pictipes, R. pallescens* y *P. arthuri* con *T. cruzi* (en la mayoría de los casos con la DTU TcI), y fuentes alimenticias principalmente asociadas con ciclos epidemiológicos silvestres y con sangre humana, dejando en evidencia que, si bien no son vectores primarios, pueden generar intrusiones en ciclos domésticos y por ende pueden ser responsables de transmisión del parasito al humano.
- Nuestros resultados confirman el rol de la especie *R. prolixus* en Colombia como vector primario de *T. cruzi,* ya que se encontraron altas tasas de infección en ejemplares recolectados en diferentes zonas del país, elevada variabilidad de fuentes alimenticias especialmente asociadas con ciclos domésticos de transmisión, alta frecuencia de infección con sangre humana e infección con el parásito de forma simultánea, corroborando la importancia de esta especie como vector.
- Se analizó el comportamiento de la especie *R. prolixus* en ambientes domésticos, caracterizándose su presencia en las viviendas en una de las zonas endémicas más afectadas del país (departamento de Casanare), encontrándose variación temporal durante el año asociada con la variación en los periodos de lluvia, sumada a frecuencias de infección superiores al 50%, infección con la DTU TcI superior al 90% y alta frecuencia de alimentación con sangre humana al igual que variabilidad de alimentación con fuentes alimenticias asociadas a ecotopos domesticos y silvestres.
- El género *Psammolestes* tiene tres especies genéticamente estructuradas que también difieren en sus nichos climáticos y morfología. Divergieron en alopatría sin flujo de genes, y su diferenciación involucró eventos de dispersión a larga distancia a través de la cuenca del Amazonas (que es una barrera actual para su dispersión).

- Los cruces interespecíficos, análisis de divergencia y delimitación de especies evidencian que *R. taquarussuensis* es una forma fenotípica de *R. neglectus* mas no una especie del grupo prolixus. Lo anterior, resalta la inminente necesidad de diferentes abordajes (morfológicos/citogenéticos/moleculares/biológicos), para delimitar especies de Triatominos, lo cual es de gran importancia dado su rol como vectores de Enfermedad de Chagas.
- El enfoque multilocus usando el concatenado de todos los genes en un set amplio de muestras representativo de las especies de la tribu Rhodniini y mediante comparación de marcadores nucleares, ribosomales y mitocondrial permitió dar solución a tres problemas importantes de la taxonomía de la tribu: Se confirmo el estatus genérico de los géneros de la tribu, se confirma que la agrupación de *pallescens/pictipes* como clado hermano del grupo *prolixus*, se sugiere el status de especies del grupo prolixus cuestionadas: *R. montenegresis, R. marabaensis* y *R. milesi.*
- Se detectan patrones filogenéticos compatibles con introgresión entre las especies del grupo *prolixus* que sumado a su divergencia reciente explica los conflictos mito-nucleares observados, por lo cual las filogenias inferidas con el marcador mitocondrial deben ser analizadas a la luz de los resultados obtenidos con marcadores nucleares y ribosomales en conjunto, esto con el fin de comprender las discordancias. Sin embargo, se resalta que el enfoque multilocus mediante concatenación de todos los marcadores resuelve las incongruencias.

10. PERSPECTIVAS

- Será de gran utilidad evaluar la variabilidad genética del parásito *T. cruzi* en las diferentes especies de la tribu Rhodniini, en conjunto con información acerca de las cargas parasitarias en las especies de la tribu Rhodniini en conjunto con preferencias alimenticias, esto para profundizar en el impacto que pueden tener estás especies en términos de transmisión vectorial del parasito en los diferentes escenarios epidemiológicos.
- Se requiere un genoma de referencia de la tribu Rhodniini de tal forma que se puedan realizar análisis filogenómicos en un conjunto de ejemplares que sea representativo de la tribu, de tal forma que está aproximación proporcione la cantidad de datos necesaria para eliminar sesgos y esclarecer no solo aspectos acerca de la taxonomía, sino también el panorama evolutivo, biogeográfico y la información genética de la tribu en relación con las características que favorecen el comportamiento vectorial de algunas especies de la tribu Rhodniini.
- Se requiere el análisis de microbiomas, en conjunto con análisis de genomas de las en las diferentes especies de la tribu de tal forma que se pueda evaluar el impacto de la transferencia horizontal de genes desde los simbiontes a los insectos y sus implicaciones en reconstrucciones filogenéticas, especialmente las de genomas mitocondriales uniparentales.

10. PRODUCTOS DE LA TESIS

10.1. Cursos

- **2019.** Workshop Evolutionary Genomics Trainning on the Caribbean Coast of Colombia.
- **2017.** "Introducción a los Métodos Filogenéticos Comparativos en R". Cali, Colombia. VI Simposio Colombiano de Biología Evolutiva.
- **2016.** 10th Arthropod Genomics Symposium (AGS). Universidad de Notre Dame. Indiana, USA.

10.2. Reconocimientos

- **2020. Beca Pasantía estudiantes doctorales.** Universidad del Rosario
- **2019.** Beca para asistencia Workshop Evolutionary Genomics Trainning on the Caribbean Coast of Colombia. Universidad de los Andes, Sede Caribe.
- **2017.** Beca para asistencia a curso "Introducción a los Métodos Filogenéticos Comparativos en R". Cali, Colombia. VI Simposio Colombiano de Biología Evolutiva
- **2016** Beca para asistencia a 10th Arthropod Genomics Symposium (AGS). Universidad de Notre Dame. Indiana, USA.
- **2016.** Beca Estudiante de doctorado. Minciencias.

10.3Presentaciones en congresos

- **Carolina Hernandez,** Fabian C. Salgado-Roa, Mateo Alvarado, Nathalia Ballesteros, Nicol Rueda, Jader Oliveira, Kaio Cesar Chaboli Alevi, Joao Aristeu Da Rosa, Plutarco Urbano, Camilo Salazar, Juan David Ramirez-Gonzalez. British Society for Parasitology Spring Meeting en York 2022: "Phylogenetic relationships and evolutionary patterns of the genus Psammolestes Bergroth, 1911 (Hemiptera: Reduviidae:Triatominae): tools for vector control of Chagas disease". 15th International Congress of Parasitology Copenhagen, Denmark. August 21-26, 2022
- **Carolina Hernandez,** Antonella Bacigalupo, Nathalia Ballesteros, Claudia M. Muñoz, Luz Stella Buitrago, Marina Stella González, Bachar Cheaib, Martin Llewellyn, Juan David Ramirez-Gonzalez. "Stepping towards a whole genome for *Panstrongylus geniculatus* (Hemiptera: Reduviidae), the triatomine with the largest

geographic distribution in Latin America. 15th International Congress of Parasitology Copenhagen, Denmark. August 21-26, 2022

- **Carolina Hernandez,** Fabian C. Salgado-Roa, Mateo Alvarado, Nathalia Ballesteros, Nicol Rueda, Jader Oliveira, Kaio Cesar Chaboli Alevi, Joao Aristeu Da Rosa, Plutarco Urbano, Camilo Salazar, Juan David Ramirez-Gonzalez. "Phylogenetic relationships and evolutionary patterns of the genus Psammolestes Bergroth, 1911 (Hemiptera: Reduviidae:Triatominae): tools for vector control of Chagas disease". Marzo 2022.
- Juliana Damieli Nascimento, João Aristeu da Rosa, Fabián C. Salgado-Roa, **Carolina Hernández**, Carolina Pardo-Díaz, Kaio César Chaboli-Alevi, Amanda Ravazi, Jader de Oliveira, Maria Tercília Vilela de Azeredo Oliveira, Camilo Salazar, Juan David Ramírez. Delimitación de especies dentro del género Rhodnius: ¿es R. taquarussuensis una nueva especie?. Presentación en poster. XVII Congreso Colombiano de Parasitología y Medicina Tropical. Cali, Colombia diciembre de 2019.
- **Carolina Hernández**, Valentina Caicedo-Garzón, Fabián C. Salgado-Roa, Melissa Sánchez-Herrera, Luisa María Arias-Giraldo, Lineth García, Gustavo Vallejo, Omar Cantillo, Catalina Tovar, Joao Aristeu da Rosa, Hernán Carrasco, Maikell Segovia, Camilo Salazar, Juan David Ramírez. Diversificación geográfica de *Panstrongylus geniculatus* (Reduviidae: Triatominae) en Colombia. Presentación en poster. XVII Congreso Colombiano de Parasitología y Medicina Tropical. Cali, Colombia diciembre de 2019.
- Natalia Velásquez-Ortiz, **Carolina Hernández**, Giovanny Herrera, Lissa Cruz-Saavedra, Adriana Higuera, Luisa M. Arias-Giraldo, Plutarco Urbano, Andrés Cuervo, Aníbal A. Teherán, Juan David Ramírez. Detección de *Trypanosoma cruzi*, unidades de tipificación discretas y preferencias alimenticias en *Psammolestes arthuri* Presentación en poster. XVII Congreso Colombiano de Parasitología y Medicina Tropical.
- **Diana Carolina Hernández Castro**, Valentina Caicedo Garzon, Fabian C. Salgado-Ro,Melissa Sánchez-Herrera, Luisa María Arias-Giraldo, Lineth García, Gustavo Vallejo, Omar Cantillo, Catalina Tovar, Joao Aristeu Da Rosa, Hernan

Carrasco, Maikell Segovia, Camilo Salazar, Juan David Ramirez Gonzalez, Diversificación geográfica de Panstrongylus geniculatus (Reduviidae: Triatominae) en Colombia. Presentación en poster. XXV Congreso de la Federación Latinoamericana de Parasitología. XVII Congreso y XXII Jornada Científica de Estudiantes de Medicina.

- **Carolina Hernández**, Claudia M Sandoval-Ramírez, Anibal A. Teherán Valderrama, Reinaldo Gutierrez-Marin, Ruth A. Martínez-Vega, Duvan Morales, Astrid Araque-Mogoll Araque-Mogollon, Juan David Ramirez Gonzalez, Dinámica de transmisión en un foco de Leishmaniasis cutánea en Norte de Santander:Patrones de diversidad, especies de Leishmania y preferencia alimenticias de flebotominos". Presentación en poster. XXV Congreso de la Federación Latinoamericana de Parasitología. XVII Congreso y XXII Jornada Científica de Estudiantes de Medicina.
- **Diana Carolina Hernández Castro**, Natalia Velásquez-Ortiz, Giovanny Herrera, Lissa Cruz-Saavedra, Adriana Higuera, Luisa María Arias-Giraldo, Plutarco Urbano, Andres Cuervo, Andres Cuervo, Anibal Alfonso Teheran Valderrama, Juan David Ramirez Gonzalez. Detección de Trypanosoma cruzi, unidades de tipificación discretas y preferencias alimenticias en Psammolestes arthuri". Presentación en poster. XXV Congreso de la Federación Latinoamericana de Parasitología. XVII Congreso y XXII Jornada Científica de Estudiantes de Medicina.
- **Hernández C**, Salazar C, Brochero H, Teherán A, Buitrago LS, Vera M, et al. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units. 2016. 10th Arthropod Genomics Symposium (AGS). Presentación en poster.
- **Hernández C**, Brochero H, Vera M, Parra-Henao GJ, Buitrago LE, Teherán A, et al., Identificación de fuentes alimenticias e infección por Trypanosoma cruzi en diferentes especies de triatominos vectores de enfermedad de Chagas. . XVI Congreso Colombiano de Parasitología y Medicina Tropical. Octubre 21 a 23 de

2015.Memorias Biomédica. Biomédica 2015;35(Supl.4) p:138,139. Presentación Oral.https://www.revistabiomedica.org/index.php/biomedica/article/view/3101

10.4. Pasantía

- Capacitación en genómica, ensamblaje y análisis de genomas de Triatominos. Institute of Biodiversity Animal Health and Comparative Medicine. Glasgow University. Duración: 6 meses. Director: Martyn Llewellyn.

11. BIBLIOGRAFIA

1. WHO. https://www.who.int/campaigns/world-chagas-disease-day/2022#:~:text=In%202022%20we%20are%20shining,case%20to%20defeat%20Chagas%20disease. 2022. Available: https://www.who.int/campaigns/world-chagas-disease-day/2022#:~:text=In%202022%20we%20are%20shining,case%20to%20defeat%20Chagas%20disease.

2. WHO. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec. 2015. pp. 33–43.

3. Bern C. Chagas' Disease. N Engl J Med. 2015. p. 1882. doi:10.1056/NEJMc1510996

4. Ceccarelli S, Justi SA, Rabinovich JE, Diniz Filho JAF, Villalobos F. Phylogenetic structure of geographical co-occurrence among New World Triatominae species, vectors of Chagas disease. J Biogeogr. 2020;47: 1218–1231. doi:10.1111/jbi.13810

5. Ceccarelli S, Balsalobre A, Vicente ME, Curtis-Robles R, Hamer SA, Ayala Landa JM, et al. American triatomine species occurrences: updates and novelties in the DataTri database. Gigabyte. 2022;2022: 1–8. doi:10.46471/gigabyte.62

6. Gil-Santana HR, Chavez T, Pita S, Panzera F, Galvão C. Panstrongylus noireaui, a remarkable new species of Triatominae (Hemiptera, Reduviidae) from Bolivia. ZK. 2022;1104: 203–225. doi:10.3897/zookeys.1104.81879

7. Costa J, Dale C, Galvão C, Almeida CE, Dujardin JP. Do the new triatomine species pose new challenges or strategies for monitoring Chagas disease? An overview from 1979-2021. Mem Inst Oswaldo Cruz. 2021;116: e210015. doi:10.1590/0074-02760210015

8. Jurberg J, Galvão C, Weirauch C, Moreira FFF. Hematophagous Bugs (Reduviidae, Triatominae). In: Panizzi AR, Grazia J, editors. True Bugs (Heteroptera) of the Neotropics. Dordrecht: Springer Netherlands; 2015. pp. 353–393. doi:10.1007/978-94-017-9861-7_13

9. Lent H. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. New York: American Museum of Natural History; 1979.

10. Justi SA, Galvão C. The Evolutionary Origin of Diversity in Chagas Disease Vectors. Trends Parasitol. 2017. pp. 42–52. doi:10.1016/j.pt.2016.11.002

11. Galvão C. Taxonomy. In: Guarneri A, Lorenzo M, editors. Triatominae - The Biology of Chagas Disease Vectors. Cham: Springer International Publishing; 2021. pp. 15–38. doi:10.1007/978-3-030-64548-9_2

12. Alevi KCC, de Oliveira J, da Silva Rocha D, Galvão C. Trends in Taxonomy of Chagas Disease Vectors (Hemiptera, Reduviidae, Triatominae): From Linnaean to Integrative Taxonomy. Pathogens. 2021;10: 1627. doi:10.3390/pathogens10121627

13. Schofield CJ, Galvão C. Classification, evolution, and species groups within the Triatominae. Acta Trop. 2009. pp. 88–100.

14. Schofield C, Dujardin J. THEORIES ON THE EVOLUTION OF RHODNIUS. Actulidades biologicas. 1999. pp. 183–197.

15. Gürtler RE, Cecere MC. Chagas Disease Vector Control. In: Guarneri A, Lorenzo M, editors. Triatominae - The Biology of Chagas Disease Vectors. Cham: Springer International Publishing; 2021. pp. 491–535. doi:10.1007/978-3-030-64548-9_18

16. Gürtler RE, del Pilar Fernández M, Cardinal MV. Eco-Epidemiology of Vector-Borne Transmission of Trypanosoma cruzi in Domestic Habitats. In: Guarneri A, Lorenzo M, editors. Triatominae - The Biology of Chagas Disease Vectors. Cham: Springer International Publishing; 2021. pp. 447–489. doi:10.1007/978-3-030-64548-9_17

17. Flores-Ferrer A, Marcou O, Waleckx E, Dumonteil E, Gourbière S. Evolutionary ecology of Chagas disease; what do we know and what do we need? Evol Appl. 2018;11: 470–487. doi:10.1111/eva.12582

18. Weirauch C. Origin and Evolution of Triatominae. In: Guarneri A, Lorenzo M, editors. Triatominae - The Biology of Chagas Disease Vectors. Cham: Springer International Publishing; 2021. pp. 1–13. doi:10.1007/978-3-030-64548-9_1

19. Monteiro FA, Weirauch C, Felix M, Lazoski C, Abad-Franch F. Evolution, Systematics, and Biogeography of the Triatominae, Vectors of Chagas Disease. Adv Parasitol. 2018. pp. 265–344. doi:10.1016/bs.apar.2017.12.002

20. Hernández C, Aristeu da Rosa J, Vallejo GA, Guhl F, Ramírez JD. Taxonomy, Evolution, and Biogeography of the Rhodniini Tribe (Hemiptera: Reduviidae). Diversity. 2020;12: 97. doi:10.3390/d12030097

21. Kieran TJ, Gordon ERL, Zaldívar-Riverón A, Ibarra-Cerdeña CN, Glenn TC, Weirauch C. Ultraconserved elements reconstruct the evolution of Chagas

disease-vectoring kissing bugs (Reduviidae: Triatominae). Syst Entomol. 2021;46: 725–740. doi:10.1111/syen.12485

22. CARCAVALLO R. Aspects of the epidemiology of Chagas disease in Venezuela and Argentina. American Trvpanosomiasis Research, PAHO Scientific Publication. 1976. pp. 347–358.

23. Hernández C, Alvarado M, Salgado-Roa FC, Ballesteros N, Rueda-M N, Oliveira J, et al. Phylogenetic relationships and evolutionary patterns of the genus Psammolestes Bergroth, 1911 (Hemiptera: Reduviidae: Triatominae). BMC Ecol Evo. 2022;22: 30. doi:10.1186/s12862-022-01987-x

24. Mejía-Jaramillo AM, Agudelo-Uribe LA, Dib JC, Ortiz S, Solari A, Triana-Chávez O. Genotyping of Trypanosoma cruzi in a hyper-endemic area of Colombia reveals an overlap among domestic and sylvatic cycles of Chagas disease. Parasit Vectors. 2014. p. 108. doi:10.1186/1756-3305-7-108

25. Hernández C, Vera MJ, Cucunubá Z, Flórez C, Cantillo O, Buitrago LS, et al. High-Resolution Molecular Typing of Trypanosoma cruzi in 2 Large Outbreaks of Acute Chagas Disease in Colombia. J Infect Dis. 2016;214: 1252–1255. doi:10.1093/infdis/jiw360

26. Rincón-Galvis HJ, Urbano P, Hernández C, Ramírez JD. Temporal Variation of the Presence of Rhodnius prolixus (Hemiptera: Reduviidae) Into Rural Dwellings in the Department of Casanare, Eastern Colombia. Florin D, editor. Journal of Medical Entomology. 2020;57: 173–180. doi:10.1093/jme/tjz162

27. Monroy C, Rodas A, Mejía M, Rosales R, Tabaru Y. Epidemiology of Chagas disease in Guatemala: infection rate of Triatoma dimidiata, Triatoma nitida and Rhodnius prolixus (Hemiptera, Reduviidae) with Trypanosoma cruzi and Trypanosoma rangeli (Kinetoplastida, Trypanosomatidae). Mem Inst Oswaldo Cruz. 2003. pp. 305–10.

28. Grijalva MJ, Villacís AG, Moncayo AL, Ocaña-Mayorga S, Yumiseva CA, Baus EG. Distribution of triatomine species in domestic and peridomestic environments in central coastal Ecuador. PLoS Negl Trop Dis. 2017. p. e0005970. doi:10.1371/journal.pntd.0005970

29. Cuba CA, Abad-Franch F, Roldan Rodriguez J, Vargas Vasquez F, Pollack Velasquez L, Miles MA. The triatomines of northern Peru, with emphasis on the ecology

and infection by trypanosomes of Rhodnius ecuadoriensis (Triatominae). Mem Inst Oswaldo Cruz. 2002. pp. 175–83.

30. Ocaña-Mayorga S, Aguirre-Villacis F, Pinto CM, Vallejo GA, Grijalva MJ. Prevalence, Genetic Characterization, and 18S Small Subunit Ribosomal RNA Diversity of Trypanosoma rangeli in Triatomine and Mammal Hosts in Endemic Areas for Chagas Disease in Ecuador. Vector Borne Zoonotic Dis. 2015. pp. 732–42. doi:10.1089/vbz.2015.1794

31. Pineda V, Montalvo E, Alvarez D, Santamaría AM, Calzada JE, Saldaña A. Feeding sources and trypanosome infection index of Rhodnius pallescens in a Chagas disease endemic area of Amador County, Panama. Rev Inst Med Trop Sao Paulo. 2008. pp. 113–6.

32. Gottdenker NL, Chaves LF, Calzada JE, Saldaña A, Carroll CR. Host life history strategy, species diversity, and habitat influence Trypanosoma cruzi vector infection in Changing landscapes. PLoS Negl Trop Dis. 2012. p. e1884. doi:10.1371/journal.pntd.0001884

33. Calzada JE, Pineda V, Montalvo E, Alvarez D, Santamaría AM, Samudio F, et al. Human trypanosome infection and the presence of intradomicile Rhodnius pallescens in the western border of the Panama Canal, Panama. Am J Trop Med Hyg. 2006. pp. 762–5.

34. Vasquez AM, Samudio FE, Saldaña A, Paz HM, Calzada JE. Eco-epidemiological aspects of Trypanosoma cruzi, Trypanosoma rangeli and their vector (Rhodnius pallescens) in Panama. Rev Inst Med Trop Sao Paulo. 2004. pp. 217–22. doi:/S0036-46652004000400008

35. Zeledón R, Marín F, Calvo N, Lugo E, Valle S. Distribution and ecological aspects of Rhodnius pallescens in Costa Rica and Nicaragua and their epidemiological implications. Mem Inst Oswaldo Cruz. 2006. pp. 75–9. doi:/S0074-02762006000100014

36. Justi SA, Noireau F, Cortez MR, Monteiro FA. Infestation of peridomestic Attalea phalerata palms by Rhodnius stali, a vector of Trypanosoma cruzi in the Alto Beni, Bolivia. Trop Med Int Health. 2010. pp. 727–32. doi:10.1111/j.1365-3156.2010.02527.x

37. Velásquez-Ortiz N, Herrera G, Hernández C, Muñoz M, Ramírez JD. Discrete typing units of Trypanosoma cruzi: Geographical and biological distribution in the Americas. Sci Data. 2022;9: 360. doi:10.1038/s41597-022-01452-w

38. Velásquez-Ortiz N, Hernández C, Herrera G, Cruz-Saavedra L, Higuera A, Arias-Giraldo LM, et al. Trypanosoma cruzi infection, discrete typing units and feeding sources among Psammolestes arthuri (Reduviidae: Triatominae) collected in eastern Colombia. Parasit Vectors. 2019;12: 157. doi:10.1186/s13071-019-3422-y

39. Zhao Y, Galvão C, Cai W. Rhodnius micki, a new species of Triatominae (Hemiptera, Reduviidae) from Bolivia. Zookeys. 2021;1012: 71–93. doi:10.3897/zookeys.1012.54779

40. Nascimento JD, da Rosa JA, Salgado-Roa FC, Hernández C, Pardo-Diaz C, Alevi KCC, et al. Taxonomical over splitting in the Rhodnius prolixus (Insecta: Hemiptera: Reduviidae) clade: Are R. taquarussuensis (da Rosa et al., 2017) and R. neglectus (Lent, 1954) the same species? PLoS One. 2019. p. e0211285. doi:10.1371/journal.pone.0211285

41. Abad-Franch F, Lima MM, Sarquis O, Gurgel-Gonçalves R, Sánchez-Martín M, Calzada J, et al. On palms, bugs, and Chagas disease in the Americas. Acta Trop. 2015. pp. 126–41. doi:10.1016/j.actatropica.2015.07.005

42. Oliveira J, Alevi KCC, Ravazi A, Herrera HM, Santos FM, Azeredo-Oliveira MTV, et al. New Evidence of the Monophyletic Relationship of the Genus. Am J Trop Med Hyg. 2018. pp. 1485–1488. doi:10.4269/ajtmh.18-0109

43. Nascimento JD, Ribeiro AR, Almeida LA, de Oliveira J, Mendonça VJ, Cilense M, et al. Morphology of the spermathecae of twelve species of Triatominae (Hemiptera, Reduviidae) vectors of Chagas disease. Acta Trop. 2017. pp. 440–445. doi:10.1016/j.actatropica.2017.08.024

44. Rodrigues JMDS, da Rosa JA, Moreira FFF, Galvão C. Morphology of the terminal abdominal segments in females of Triatominae (Insecta: Hemiptera: Reduviidae). Acta Trop. 2018. pp. 86–97. doi:10.1016/j.actatropica.2018.04.021

45. da Rosa JA, Justino HHG, Nascimento JD, Mendonca VJ, Rocha CS, de Carvalho DB, et al. A new species of Rhodnius from Brazil (Hemiptera, Reduviidae, Triatominae). Zookeys. 2017. pp. 1–25. doi:10.3897/zookeys.675.12024

46. Soto-Vivas A, Liria J, Luna E. Geometric morphometrics and phylogeny in Rhodniini (Hemiptera, Reduviidae) of Venezuela . Acta zoológica mexicana. 2011. pp. 87–102.

47. Oliveira J, Alevi KCC, Ravazi A, Herrera HM, Santos FM, Azeredo-Oliveira MTV, et al. New Evidence of the Monophyletic Relationship of the Genus. Am J Trop Med Hyg. 2018. pp. 1485–1488. doi:10.4269/ajtmh.18-0109

48. Ravazi A, Oliveira J de, Madeira FF, Reis YV dos, Oliveira ABB de, Galvão C, et al. Trends in Taxonomy of the Rhodniini Tribe (Hemiptera, Triatominae): Reproductive Incompatibility between Rhodnius neglectus Lent, 1954 and Psammolestes spp. Confirms the Generic Status of Psammolestes Bergroth, 1911. Diversity. 2022;14: 761. doi:10.3390/d14090761

49. Filée J, Merle M, Bastide H, Mougel F, Bérenger J-M, Folly-Ramos E, et al. Phylogenomics for Chagas Disease Vectors of the Rhodnius Genus (Hemiptera, Triatominae): What We Learn From Mito-Nuclear Conflicts and Recommendations. Front Ecol Evol. 2022;9: 750317. doi:10.3389/fevo.2021.750317

50. Panzera F, Ferrandis I, Ramsey J, Salazar-Schettino PM, Cabrera M, Monroy C, et al. Genome size determination in chagas disease transmitting bugs (hemiptera-triatominae) by flow cytometry. Am J Trop Med Hyg. 2007. pp. 516–21.

51. Panzera F, Pérez R, Panzera Y, Ferrandis I, Ferreiro MJ, Calleros L. Cytogenetics and genome evolution in the subfamily Triatominae (Hemiptera, Reduviidae). Cytogenet Genome Res. 2010. pp. 77–87. doi:10.1159/000298824

52. Panzera F, Perez R, Panzera Y, Alvarez F, Scvortzoff E, Salvatella R. Karyotype evolution in holocentric chromosomes of three related species of triatomines (Hemiptera-Reduviidae). Chromosome Res. 1995. pp. 143–50.

53. Panzera F, Dujardin JP, Nicolini P, Caraccio MN, Rose V, Tellez T, et al. Genomic changes of Chagas disease vector, South America. Emerg Infect Dis. 2004. pp. 438–46. doi:10.3201/eid1003.020812

54. Panzera F, Pérez R, Hornos S, Panzera Y, Cestau R, Delgado V, et al. Chromosome numbers in the Triatominae (Hemiptera-Reduviidae): a review. Mem Inst Oswaldo Cruz. 1996. pp. 515–8.

55. Panzera F, Pita S, Lorite P. Chromosome Structure and Evolution of Triatominae: A Review. In: Guarneri A, Lorenzo M, editors. Triatominae - The Biology of Chagas Disease Vectors. Cham: Springer International Publishing; 2021. pp. 65–99. doi:10.1007/978-3-030-64548-9_4

56. DUJARDIN J-P, SCHOFIELD CJ, PANZERA F. Les vecteurs de la maladie de Chagas. http://www.kaowarsom.be/documents/MEMOIRES_VERHANDELINGEN/Sciences_naturelles_medicales/Nat.Sc.(NS)_T.24,5_DUJARDIN,%20J.-P.-SCHOFIELD,%20C.J.-PANZERA,%20F._Les%20vecteurs%20de%20la%20maladie%20de%20Chagas.%20Recherches%20taxonom.,%20bio%20&%20g%C3%A9n%C3%A9tiques_2000.PDF; 2000.

57. Alevi KC, da Costa Castro NF, Lima AC, Ravazi A, Morielle-Souza A, de Oliveira J, et al. Nucleolar persistence during spermatogenesis of the genus Rhodnius (Hemiptera, Triatominae). Cell Biol Int. 2014. pp. 977–80. doi:10.1002/cbin.10297

58. Madeira FF, Borsatto KC, Lima AC, Ravazi A, de Oliveira J, da Rosa JA, et al. Nucleolar Persistence: Peculiar Characteristic of Spermatogenesis of the Vectors of Chagas Disease (Hemiptera, Triatominae). Am J Trop Med Hyg. 2016. pp. 1118–1120. doi:10.4269/ajtmh.16-0149

59. Gomez-Palacio A, Jaramillo-Ocampo N, Triana-Chavez O, Saldana A, Calzada J, Perez R, et al. Chromosome variability in the Chagas disease vector Rhodnius pallescens (Hemiptera, Reduviidae, Rhodniini). Mem Inst Oswaldo Cruz. 2008. pp. 160–4.

60. Oliveira J, Alevi KC, Fonseca EO, Souza OM, Santos CG, Azeredo-Oliveira MT, et al. New record and cytogenetic analysis of Psammolestes tertius Lent & Jurberg, 1965 (Hemiptera, Reduviidae, Triatominae) from Bahia State, Brazil. Genet Mol Res. 2016. doi:10.4238/gmr.15028004

61. Panzera Y, Pita S, Ferreiro MJ, Ferrandis I, Lages C, Pérez R, et al. High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Cytogenet Genome Res. 2012. pp. 56–67. doi:10.1159/000341888

62. Alevi KC, Ravazi A, Mendonça VJ, Rosa JA, Azeredo-Oliveira MT. Karyotype of Rhodnius montenegrensis (Hemiptera, Triatominae). Genet Mol Res. 2015. pp. 222–6. doi:10.4238/2015.January.16.5

63. Pita S, Panzera F, Ferrandis I, Galvão C, Gómez-Palacio A, Panzera Y. Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal location of ribosomal genes. Mem Inst Oswaldo Cruz. 2013. doi:10.1590/S0074-02762013000300017

64. Gómez-Palacio A, Jaramillo-O N, Caro-Riaño H, Diaz S, Monteiro FA, Pérez R, et al. Morphometric and molecular evidence of intraspecific biogeographical differentiation of Rhodnius pallescens (HEMIPTERA: REDUVIIDAE: RHODNIINI) from Colombia and Panama. Infect Genet Evol. 2012. pp. 1975–83. doi:10.1016/j.meegid.2012.04.003

65. Ravazi A, Alevi KCC, Oliveira J, Rosa JA, Azeredo-Oliveira MTV. Cytogenetic analysis in different populations of Rhodnius prolixus and R. nasutus from different countries of South America. Braz J Biol. 2018. pp. 183–185. doi:10.1590/1519-6984.167196

66. de Paula AS, Barreto C, Telmo MCM, Diotaiuti L, Galvão C. Historical Biogeography and the Evolution of Hematophagy in Rhodniini (Heteroptera: Reduviidae: Triatominae). Front Ecol Evol. 2021;9: 660151. doi:10.3389/fevo.2021.660151

67. Ravazi A, de Oliveira J, Campos FF, Madeira FF, dos Reis YV, de Oliveira ABB, et al. Trends in evolution of the Rhodniini tribe (Hemiptera, Triatominae): experimental crosses between Psammolestes tertius Lent & Jurberg, 1965 and P. coreodes Bergroth, 1911 and analysis of the reproductive isolating mechanisms. Parasites Vectors. 2021;14: 350. doi:10.1186/s13071-021-04854-8

68. Cucunubá ZM, Okuwoga O, Basáñez M-G, Nouvellet P. Increased mortality attributed to Chagas disease: a systematic review and meta-analysis. Parasit Vectors. 2016;9: 42. doi:10.1186/s13071-016-1315-x

69. Hernández C, Salazar C, Brochero H, Teherán A, Buitrago LS, Vera M, et al. Untangling the transmission dynamics of primary and secondary vectors of Trypanosoma cruzi in Colombia: parasite infection, feeding sources and discrete typing units. Parasites & Vectors. 2016;9: 620. doi:10.1186/s13071-016-1907-5

70. Arias-Giraldo LM, Muñoz M, Hernández C, Herrera G, Velásquez-Ortiz N, Cantillo-Barraza O, et al. Identification of blood-feeding sources in Panstrongylus, Psammolestes, Rhodnius and Triatoma using amplicon-based next-generation sequencing. Parasites Vectors. 2020;13: 434. doi:10.1186/s13071-020-04310-z

71. Gourbière S, Dorn P, Tripet F, Dumonteil E. Genetics and evolution of triatomines: from phylogeny to vector control. Heredity. 2012;108: 190–202. doi:10.1038/hdy.2011.71