Sylvatic host associations of Triatominae and implications for Chagas disease reservoirs: a comprehensive review and new host records based on archival specimens

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Background: The 148 species of kissing bug include important vectors of the debilitating, chronic, and often fatal Chagas disease, which affects several million people in Central and South America. An understanding of the natural hosts of this speciose group of blood-feeding insects has and will continue to aid ongoing efforts to impede the spread of Chagas disease. However, information on kissing bug biology is piecemeal and scattered, developed using methods with varying levels of accuracy over more than 100 years. Existing host records are heavily biased towards well-studied primary vector species and are derived from primarily three different types of observations, associational, immunological or DNA-based, with varying reliability.

Methods: We here gather a comprehensive and unparalleled number of sources reporting host associations via rigorous targeted searches of publication databases to review all known natural, or sylvatic, host records including information on how that record was collected. We integrate this information with novel host records obtained via attempted amplification and sequencing of a ~160 base pair (bp) region of the vertebrate 12S mitochondrial gene from the gastrointestinal tract of 64 archival specimens of Triatominae representing 19 species collected primarily in sylvatic habitats throughout the southern U.S. and Central and South America during the past 10 years. We show the utility of this method for uncovering novel and under-studied groups of Triatominae hosts, as well as detecting the presence of the Chagas disease pathogen via Polymerase Chain Reaction (PCR) of a ~400 bp sequence of the trypanosome 18S gene.

Results: New host associations for several groups of arboreal mammals were determined including sloths, New World monkeys, coatis, arboreal porcupines and, for the first time as a host of any Triatominae, tayras. A thorough review of previously documented sylvatic hosts, organized by triatomine species and the type of observation (associational, antibody-based, or DNA-based), is presented in a phylogenetic context and highlights large gaps in our knowledge of Triatominae biology.

Conclusion: The application of DNA-based methods of host identification towards additional species of Triatominae, including rarely collected species that may require use of archival specimens, is the most efficient and promising way to resolve recognized shortfalls.

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Short title: Sylvatic hosts of Triatominae

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Abstract

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Findings:

New host associations for several groups of arboreal mammals were determined including sloths, New World monkeys, coatis, arboreal porcupines and, for the first time as a host of any Triatominae, tayras. A thorough review of previously documented sylvatic hosts, organized by triatomine species and the type of observation (associational, antibody-based, or DNA-based), is presented in a phylogenetic context and highlights large gaps in our knowledge of Triatominae biology.

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46

47 **Introduction**

48 Triatominae, or kissing bugs, are blood-feeding members of the primarily predatory
49 insect family Reduviidae (Order Hemiptera). This subfamily consists of 148 described species,
50 nearly all distributed in the Americas, though several species occur in the Oriental region and
51 *Triatoma rubrofasciata* (De Geer) is considered invasive throughout the tropics [1,2]. These
52 insects are the sole vectors of *Trypanosoma cruzi* Chagas, the parasite responsible for Chagas
53 disease. Chagas disease is a chronic debilitating disease, prevalent in Latin America, and
54 affecting up to 10 million people worldwide [3]. There is no vaccine or effective cure once the
55 symptoms of the chronic disease have manifested and the disease has been termed an emerging
56 threat of the 21st century [4,5].

57 Most kissing bug species are suspected to be oligo- or polyphagous across a broad range
58 of wild mammal and other vertebrate species [6,7]. Many Triatominae hosts are sylvatic
59 mammals, but domestic mammals such as dogs, cats, and rodents can also be fed upon upon and
60 act as reservoir hosts of the parasite [8]. Generalist kissing bug species also tend to feed on
61 humans, e.g., *Triatoma infestans* Klug and *Rhodnius robustus* Larrousse, that can exhibit
62 infection rates with *T. cruzi* higher than 40% [9,10]. Some triatomine species will target certain
63 hosts if available and avoid other potential blood meal sources [1]. *Cavernicola pilosa* Barber
64 appears to only feed on bats [11,12], while *Triatoma delpontei* Romana and Abalos and
65 *Psammolestes* Bergroth species are usually found in association with various birds [11,13]. In
66 addition, there are reports of some kissing bug species feeding on other arthropods [14–16] or
67 exhibiting cleptohemophagy by feeding on other engorged kissing bug individuals [17]. The
68 extent of both of these behaviors in a natural environment and for the great majority of kissing

bug species is unknown and may be driven by host availability. A diet lacking blood has been shown experimentally to result in complete mortality in at least some species [18], suggesting that arthropod feeding may be rare or driven by the lack of more suitable hosts. Overall, existing host association data is biased towards a handful of heavily studied and well-documented primary vector species and little data exists for many other Triatominae, particularly in sylvatic habitats [19]. Understanding patterns of host associations across Triatominae may help to elucidate their natural history and identify as yet underappreciated species of medical interest.

Records of Triatominae host associations are based on different types of observations that we here classify as associational (i.e. visual observation of a kissing bug in presumed or actual close association with a possible host), immunological and DNA-based methods. Each of these approaches possess a unique set of advantages and disadvantages. Many early studies on kissing bug-host interactions depended on observations of the insects during laboratory feeding tests or, more rarely, in the wild [11,20]. Despite presenting direct evidence for feeding, laboratory experiments are necessarily unnatural. Laboratory tests either offer insects a single possible host species and observe whether feeding occurs [21–23] or present them with a choice between two or more hosts to determine preference [24]. Both approaches may drive insects to feed on organisms that they would not feed upon in natural habitats. Observations of cohabitation of Triatominae with vertebrates in the wild, e.g., in animal nests and burrows, while more natural, are usually tentative because the association may not reflect actual feeding. There is likely also a bias towards more accessible terrestrial nests and burrows compared to corresponding arboreal habitats. Immunological methods to detect host associations in Triatominae were first used in the 1960s [25–27], seemingly overcoming problems posed by associational methods. By utilizing specific, but often polyclonal, antibodies in antisera developed for a predetermined range of

92 potential host species, experimenters infer direct host associations. Precipitin tests have
93 continued to be a popular way to detect host antigens in Triatominae blood meals [28–30]. While
94 a promising technique for forensic determination of actual hosts, disadvantages of precipitin tests
95 are twofold. First, tests may suffer from non-specificity in antibody binding or irreproducibility
96 as a result of variance in antibodies [31]. Second, due to the cost of developing different sets of
97 antibodies for different groups of hosts, antibodies are typically developed to be specific only for
98 large groups of potential host vertebrates, such as rodents. This approach results in reduced
99 resolution of hosts, i.e. vertebrates are not identified to genus or species, but will also fail to
100 detect unexpected or rare host taxa for which no antibody set is available.
101 More recently, studies have begun to use DNA-based methods to detect host identity
102 [16,32,33]. These methods typically use PCR that targets the conserved regions of variable,
103 mitochondrial genes for amplification. When amplified sequences are compared to a known
104 database, it is usually possible to determine what species or at least genus of organism that the
105 blood originated from. These studies have documented that certain species, such as *Triatoma*
106 *rubida* (Uhler), *Triatoma protracta* (Uhler)*, and Triatoma gerstaerckeri* (Stål)*,* feed on a large
107 variety of vertebrate hosts [16,34]. PCR-based studies also have the potential to determine the
108 percentage of specimens within a given kissing bug population that have fed on humans [34].
109 While PCR is useful in detecting a wide range of hosts, given that databases such as GenBank
110 now hold a library of barcodes for most mammal species and many other vertebrates, it does
111 have a relatively high risk for human contamination [35]. Primers can also have biases in
112 amplifying DNA that closely mirrors their sequence while not amplifying other sequences or
113 also amplify the insect's own DNA, which can interfere with detecting host DNA from the blood

sample. Multiple blood meals per specimen can amplify and interfere with determining a single sequence and must be separated via cloning of the PCR product or next generation sequencing.

Despite the importance of identifying reservoir hosts of Chagas disease and the biology of its vectors, most studies have focused on known primary vector species. These studies most often targeted domestic or peridomestic habitats where the transmission risk to humans is considered higher (e.g., [36,37]). In addition, most previous DNA-based studies have surveyed narrow geographic areas, with several focusing on North America, and have used only live or very recently preserved specimens. A comprehensive overview of triatomine-host associations that specifies the method through which the record was obtained and allows for assessing the reliability of the record is yet unavailable. Our study therefore has three objectives: 1) to contribute to the growing knowledge base of host associations across Triatominae, we conducted PCR of gastrointestinal contents extracted from 19 species of Triatominae from a range of localities (Bolivia to U.S.); most specimens were collected in sylvatic habitats using light traps and the sample comprises rarely encountered kissing bug species; 2) to determine the feasibility of assessing host associations and trypanosome infection for archival kissing bug specimens that have been preserved in ethanol for up to 10 years, we conducted PCR-based identification of host sequences and trypanosomes for 64 specimens; 3) to establish currently documented sylvatic host associations, we conducted a thorough literature review for all species of Triatominae while recording the method used to determine that association; host patterns and gaps in our current knowledge were visualized in phylogenetic context for both kissing bug species and vertebrate hosts.

Materials and Methods

137 *Taxon sampling:* Triatominae specimens were primarily collected in sylvatic conditions
138 via light trapping or hand collection throughout the southern U.S. and Central and South
139 America and were preserved in ethanol (concentrations either unknown or 95%) between 2005
140 and 2015. We classify the habitat that each specimen as either domestic (found in a residence),
141 peridomestic (found outside in a residential area, near a residence) or sylvatic (found in natural
142 habitats, sometimes near field research stations). It was our aim to survey as wide a variety of
143 Triatominae species as possible, but certain species (e.g., *Triatoma protracta*, *Panstrongylus*
144 *geniculatus* [Latreille]) were sampled more thoroughly due to the availability of specimens
145 present in the Weirauch lab ethanol repository. Voucher specimen data (unique specimen
146 identifier [USI], determination, sex, specimen depository, collecting locality and event) were
147 recorded using the Planetary Biodiversity Institute instance of the Arthropod Easy Capture
148 database (research.amnh.org/pbi/locality). Images of voucher specimens were taken using a
149 Leica DFC450 C Microsystems system with a Planapo 1.0x objective. Voucher data, including
150 collecting technique and images, are available online at Heteroptera Species Pages
151 (research.amnh.org/pbi/heteropteraspeciespage) and are best searched by species and then USI
152 number.
153 *DNA extraction:* DNA of the gastrointestinal contents of Triatominae specimens was
154 extracted in order to perform PCR. To avoid cross-contamination as well as the recognized threat
155 of contamination with human DNA, all equipment and work benches were sterilized (dissecting
156 petri dish, forceps, iris scissors) before and after processing each specimen using 10% bleach.
157 Cuticular surfaces of specimens were sterilized with 1% bleach for 3 minutes, to eliminate
158 possible contaminants acquired before capture in ethanol or during ethanol storage. The thorax
159 and abdomen were separated and contents of the abdomen were removed with forceps and

placed into an Eppendorf tube. While performing this procedure, the sex of the specimen and whether blood was visible in the gut or not was recorded. When a large volume of blood was present, contents were divided into separate Eppendorf tubes. Gut contents were homogenized for 2 minutes with an Eppendorf pestle and DNA was extracted with a QIAGEN DNeasy blood and tissue kit. We recorded the amount of blood in each specimen on a scale of 1 to 4 where: 1 -- no material visible in digestive tract; 2 -- small amount of dark, digested blood present; 3 -- obvious blood present; 4 -- completely engorged with blood. For seven specimens of *Triatoma protracta* and one specimen of *Eratyrus mucronatus* Stål which had been extracted previously, we were not able to record the amount of blood present in the digestive tract.

PCR: We tested seven previously developed sets of primers targeting three different mitochondrial genes (Table 1) designed for identification of vertebrate hosts from invertebrate blood meals via PCR on each of our extracts. We were not able to achieve consistent, acceptably broad or specific results for several sets of previously used primers listed in Table 1 (all those listed without asterisks). These primers amplified the corresponding DNA sequence of certain species of Triatominae or did not amplify DNA that should be present based on the results of other primer sets. We found that the "Kitano" 12S primers [38] yielded the most consistent and the greatest number of amplified bands across samples among the primer sets tested and this is the only primer set for which we present results. If samples yielded multiple bands after electrophoresis on 1% agarose gels, they were gel extracted using QIAquick Gel Extraction kit. If samples extracted from insects containing a large volume of blood did not result in bands after PCR, the extract was diluted 1 in 10 and PCR was conducted again to ensure that a PCR-inhibitor from the blood meal was not present (e.g., approach successful with sample UCR_ENT 00123869). We used primers for the 18S region to determine presence of trypanosomes in our

183 extracts (Table 1; [39]). PCR conditions consisted of an initial denaturation step of 94˚ C for 5
184 minutes, denaturation at 94˚C for 30 seconds, the annealing temperature listed in Table 1 for 30
185 seconds, extension at 72˚C for 30 seconds repeated for 35 cycles with a final extension time at
186 72˚C for 10 minutes.

187 **Table 1. Primer sequences and PCR conditions used in this study.**

DNA target	Primer set	Locus	Direction	Sequence	Annealing temperature	Reference
Vertebrate DNA	**Kitano 12S***	**12S**	**F**	**5'-CCC AAA CTG GGA TTA GAT ACC C-3'**	**57˚**	[40]
			R	**5'-GTT TGC TGA AGA TGG CGG TA-3'**		[40]
	Melton 12S	12S	F	5'-ACT GGG ATT AGA TAC CCC ACT ATG-3'	53˚	[41]
			R	5'-ATC GAT TAT AGA ACA GGC TCC TC-3'		[41]
	Vert COI	COI	M13BC-FW	5′-TGT AAA ACG ACG GCC AGT HAA YCA YAA RGA YAT YGG NAC-3′	45˚	[42]
			BCV-RV1	5′-GCY CAN AYY ATN CYY RTR TA-3′		[42]
	DC-CytB	CytB	UP	5-CRT GAG GMC AAA TAT CHT TYT-3	42.5˚	[43]
			DW	5-ART ATC ATT CWG GTT TAA TRT-3		[43]
	Avian CytB	CytB	F	5'-GAC TGT GAC AAA ATC CCN TTC CA-3'	55˚	[44]
			R	5'-GGT CTT CAT CTY HGG YTT ACA AGA C-3'		[44]
	Mammalian CytB	CytB	F	5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3'	55˚	[44]
			R	5'-TGT AGT TRT CWG GGT CHC CTA-3'		[44]
	Vert CytB	CytB	CB1-L	5'-CCC CTC AGA ATA TTT GTC CTC A-3'	57˚	[45]
			CB2-H	5'-CAT CCA ACA TCT CAG CAT GAT GAA A-3'		[45]
Trypanosome DNA	**Tcz***	**18S**	**18sf**	**5′-TTA ACG GGA ATA TCC TCA GC-3'**	**50˚**	[46]
			S829r	**5'-GCA TCA CAG ACC TGC TGT TG-3'**		[47]

188 *Purification, sequencing and analyzing:* All PCR products were cleaned using SureClean
189 (Bioline) before sequencing using the Macrogen EZ-Seq service. Once sequences were obtained,
190 the program Sequencher was used to process chromatographs. Blastn was then used to compare
191 sequences to the GenBank database. Sequences of the 12S gene were considered to be derived
192 from the same species as the closest match represented in GenBank only if they were 100%
193 identical and most or all other members of that genus were also represented and differed in
194 sequence (e.g. sequences KX779919, 100% to *Choloepus didactylus,* and KX779929, 100% to

195 *Neotoma lepida*) otherwise we classified it only to genus (e.g., sequence KX779923, 100% to
196 *Lagothrix lagotricha*) or to an even higher level (e.g., sequence KX779938, 100% to *Mustela*
197 *kathiah* and classified as Mustelidae sp.). When multiple members of a genus were represented
198 in GenBank, but none matched 100%, we classified our sequence to that genus if it was more
199 than 98% identical to one member of the genus and closer to other members of that genus than to
200 any other genus (most sequences, e.g. sequences KX779920 98.6% to *Dasyprocta leporina* and
201 KX779934 98.7% to *Saguinus oedipus*). Trypanosome-derived PCR products of the 18S rRNA
202 gene were sequenced and the sequence compared to the GenBank database for identification.
203 *Phylogeny construction:* We gathered all available data for Triatominae species and
204 closely related reduviids (Stenopodainae, *Zelurus* spp. *Opisthacidius* spp.) on GenBank totaling
205 9,343 bp from 120 taxa as aligned using the MAFFT EINS-i algorithm [48] comprising the loci
206 from the nuclear rRNA operon and the mitochondrial genome (18S rRNA, ITS 1 5.8 rRNA and
207 ITS 2, D2, D3-D5 regions of the 28S rRNA; 16S rRNA COI, COII, Cytb) and constructed a
208 phylogeny using a partitioned RaxML analysis (Fig. S1, sampled species in red) partitioned by
209 the best scheme as determined by PartitionFinder (Phylip, Data S1; 9 partitions, Data S2). We
210 excluded *Belminus herreri* Lent & Wygodzinsky from our final analysis due to the
211 reconstruction of this taxon as sister to *T. rubrofasciata* with low support and a long terminal
212 branch; this result may be due to this taxon only being represented by the 18S gene that has low
213 phylogenetic utility for relatively recent divergences and the lack of that gene in the only other
214 representative of the tribe Bolboderini in our dataset, *Microtriatoma trinidadensis* (Lent) (rRNA
215 28S D2 region only). To visualize all species of Triatominae in a single figure, we placed all taxa
216 for which molecular data are currently unavailable to this backbone phylogeny using information
217 on morphological similarities from [6].

Literature review: We used a Web of Science search (all databases) to query the species name of all the 148 currently recognized Triatominae species and surveyed results for literature records of host associations. For all matches, we assessed titles and abstracts for the inclusion of information on host associations and if we determined that they may include relevant information, we scrutinized the publication for the record of the host association and the type of method with which it was achieved. For a few widely studied organisms with an extremely high number of matching publications (e.g., *Triatoma infestans*, *Rhodnius prolixus*), we limited our searches with additional target words such as "host" or "association" to increase the feasibility of surveying all relevant results. Every attempt was made to find the primary source of a host record but occasionally, we resorted to using a source that also included a review of other sources and for which the source of the record was unclear and may be either primary or secondary. We limited the inclusion of host records to evolutionarily relevant host species by excluding laboratory results or host associations of exclusively domestic animals such as dogs, chickens or other farmed animals due to the unnatural presence of these hosts. An exception was made for domestic rodents, including mice and rats, that also occur in natural environments. Host taxa were divided into major groups with all arthropods, amphibians, birds and reptiles each comprising a single group and the remaining mammals split primarily by order with some exceptions (suborder level for xenarthrans, superfamily level for primates and rodents, family level for carnivores)

Results and Discussion

Of 64 total specimens tested (38 males and 26 females), we were able to determine a host association for 24 specimens (37.5%) with a maximum of a single host determined per specimen. Of hosts with observable blood (28 out of 56 with observations recorded; quantity of blood

241 categories 2-4), 18 or 64.3% gave positive host results, compared to 2 or 7.1% of those observed
242 without visible blood (category 1) in the digestive tract for which we obtained a host sequence.
243 Specimens with observable blood but without amplifiable host DNA may represent samples with
244 DNA degraded beyond allowing for amplification with primers targeting the ~160 bp region of
245 the 12S gene. Alternatively, it is possible that the Kitano 12S primers do not amplify 12S
246 sequences from certain hosts, though they were designed based on sequences from divergent
247 vertebrate sequences from sharks to fish to reptiles to amphibians and mammals (Kitano 2007).
248 Of the 24 specimens with host determinations, 17 or 70.8% were male, a heavily skewed ratio.
249 Similarly, of the 28 specimens with visible blood in the digestive tract, 21 or 75% were male. We
250 speculate that blood-fed males may be more predisposed to dispersing in flight while searching
251 for mates and thus be more susceptible to light traps. In contrast, females may be stationary after
252 feeding, attempting to find a suitable place for oviposition, possibly near or in the same location
253 as her blood meal. The infection rate of all specimens with *T. cruzi* across sampled Triatominae
254 was 31.3% or 20 specimens with one specimen of *Rhodnius pictipes* producing a band that, after
255 sequenced, matched that of *Trypanosoma rangeli* Tejera. Of the 20 specimens testing positive
256 for *T. cruzi*, 50% also possessed detectable host DNA and 50% did not. *Panstrongylus*
257 *geniculatus* was the species with the highest number of *T. cruzi* positive individuals (5/11)
258 followed by *Triatoma protracta* (4/17) and all three individuals tested of *T. dimidiata* which was
259 the species with the highest positive percentage rate (100%; 3/3) along with *T. dispar* (100%;
260 1/1) and *T. recurva* (100%; 1/1). Specimens of seven additional species also tested positive for *T.*
261 *cruzi* (Table 2), all of which were previously known to be capable of hosting the parasite [7].
262

263 **Table 2. Specimen data including data obtained by vertebrate and trypanosome**

264 **DNA targeted PCR**.

USI	Species	COUNTRY: Primary subdivision	Ecotype	Collecting date (year)	EtOH concentration	Sex	Quantity of blood (1-4)	Host 12S sequence	Top BLAST hit of host 12S sequence	Inferred host	Trypanosome sequence	Top trypanosome BLAST hit
UCR_ENT 00012958	*Eratyrus mucronatus*	PERU; Loreto	Sylvatic	2007	?	M	NA					
UCR_ENT 00119043	*Panstrongylus geniculatus*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	M	4	KX779934	98.7% to *Saguinus oedipus*	*Saguinus* sp. (Tamarin)		
UCR_ENT 00119044	*Panstrongylus geniculatus*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	M	3	KX779919	100% to *Choloepus didactylus*	*Choloepus didactylus* (Southern two-toed sloth)		
UCR_ENT 00119045	*Panstrongylus geniculatus*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	M	1					
UCR_ENT 00119046	*Panstrongylus geniculatus*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	F	1	KX779920	98.6% to *Dasyprocta leporina*	*Dasyprocta* sp. (Agouti)	KX779898	100% to *Trypanosoma cruzi*
UCR_ENT 00119047	*Panstrongylus geniculatus*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	F	1					
UCR_ENT 00012932	*Panstrongylus geniculatus*	PERU; Loreto	Sylvatic	2007	?	M	4	KX779923	100% to *Lagothrix lagotricha*	*Lagothrix* sp. (Wooly monkey)	KX779901	100% to *Trypanosoma cruzi*
UCR_ENT 00012959	*Panstrongylus geniculatus*	PERU; Loreto	Sylvatic	2007	?	M	3	KX779927	99.3% to *Eira barbara*	*Eira 13imidia* (Tayra)		
UCR_ENT 00063360	*Panstrongylus geniculatus*	NICARAGUA; Rio San Juan	Sylvatic	2010	95%	M	3	KX779922	97.1% to Dasyprocta punctata	*Dasyprocta* sp. (Agouti)	KX779905	100% to *Trypanosoma cruzi*
UCR_ENT 00063367	*Panstrongylus geniculatus*	BOLIVIA; Santa Cruz	Sylvatic	2009	95%	M	2					
UCR_ENT 00119054	*Panstrongylus geniculatus*	COSTA RICA; Heredia	Sylvatic	2013	95%	M	2	KX779938	100% to *Mustela kathiah*	Mustelidae sp. (Weasels, badgers, otters and allies)	KX779914	100% to *Trypanosoma cruzi*
UCR_ENT 00123859	*Panstrongylus geniculatus*	PANAMA; Colon	Sylvatic	2008	?	F	1				KX779915	100% to *Trypanosoma cruzi*
AMNH_PBI 00021872	*Panstrongylus lignarius*	FRENCH GUIANA; Montsinery	Sylvatic	2004	?	M	2				KX779910	100% to *Trypanosoma cruzi*
UCR_ENT 00012933	*Panstrongylus rufotuberculatus*	PERU; Loreto	Sylvatic	2007	?	M	3	KX779933	99.4% to Ateles belzebuth	Ateles sp. (Spider monkey)		
UCR_ENT 00063350	*Panstrongylus rufotuberculatus*	BOLIVIA; Santa Cruz	Sylvatic	2009	95%	M	1	KX779925	100% to *Homo sapiens*	*Homo sapiens* (Human)		
UCR_ENT 00012961	*Panstrongylus rufotuberculatus*	PERU; Loreto	Sylvatic	2007	?	M	2					
UCR_ENT 00063364	*Panstrongylus rufotuberculatus*	NICARAGUA; Rio San Juan	Sylvatic	2010	95%	M	3	KX779940	98.5% to *Coendou prehensilis*	*Coendou* sp. (Prehensile-tailed porcupine)	KX779907	100% to *Trypanosoma cruzi*
UCR_ENT 00123860	*Paratriatoma hirsuta*	MEXICO; Baja California Norte	Sylvatic	2009	95%	M	2	KX779929	100% to *Neotoma lepida*	*Neotoma lepida* (Desert woodrat)		
UCR_ENT 00003254	*Paratriatoma hirsuta*	USA; California	Peridomestic	2009	95%	M	4					
UCR_ENT 00063365	*Rhodnius pallescens*	NICARAGUA; Rio San Juan	Sylvatic	2010	95%	M	1					
UCR_ENT 00002736	*Rhodnius pictipes*	ECUADOR; Orellana	Sylvatic	2009	95%	F	1	KX779936	100% to *Homo sapiens*	*Homo sapiens* (Human)		
UCR_ENT 00119039	*Rhodnius pictipes*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	F	1					
UCR_ENT 00119040	*Rhodnius pictipes*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	F	1					
UCR_ENT 00119041	*Rhodnius pictipes*	FRENCH GUIANA; Cayenne	Sylvatic	2010	95%	M	4				KX779911	100% to *Trypanosoma rangeli*
UCR_ENT 00063354	*Rhodnius prolixus*	BOLIVIA; Santa Cruz	Sylvatic	2009	95%	F	4	KX779932	99.4% to *Nasua nasua*	*Nasua* sp. (Coati)	KX779904	100% to *Trypanosoma cruzi*
UCR_ENT 00012924	*Rhodnius robustus*	PERU; Loreto	Sylvatic	2007	?	F	1	KX779937	99.3% to *Saimiri sciureus*	*Saimiri* sp. 3 (Squirrel monkey)		
UCR_ENT 00012925	*Rhodnius robustus*	PERU; Loreto	Sylvatic	2007	?	M	1					
UCR_ENT 00012929	*Rhodnius robustus*	PERU; Loreto	Sylvatic	2007	?	M	4	KX779918	100% to *Saimiri sciureus*	*Saimiri* sp. 1 (Squirrel monkey)		
UCR_ENT 00012928	*Rhodnius robustus*	PERU; Loreto	Sylvatic	2007	?	F	1					
UCR_ENT 00002734	*Rhodnius robustus*	ECUADOR; Orellana	Sylvatic	2009	95%	M	3	KX779921	99.4% to *Saimiri sciureus*	*Saimiri* sp. 2 (Squirrel monkey)	KX779912	100% to *Trypanosoma cruzi*
UCR_ENT 00002735	*Rhodnius robustus*	ECUADOR; Orellana	Sylvatic	2009	95%	F	1				KX779900	100% to *Trypanosoma cruzi*
UCR_ENT 00119053	*Rhodnius robustus*	PERU; Madre de Dios	Sylvatic	2005	95%	F	4	KX779924	99.4% to various *Cebus* spp.	*Cebus* sp. 2 (Gracile capuchin		

										monkey)		
UCR_ENT 00123871	*Triatoma dimidiata*	COSTA RICA; Heredia	Sylvatic	2010	95%	M	4				KX779899	100% to *Trypanosoma cruzi*
UCR_ENT 00123870	*Triatoma dimidiata*	COSTA RICA; Heredia	Sylvatic	2010	95%	M	1				KX779897	100% to *Trypanosoma cruzi*
UCR_ENT 00063362	*Triatoma dimidiata*	NICARAGUA; Rio San Juan	Sylvatic	2010	95%	M	4	KX779917	100% to various *Cebus* spp.	*Cebus* sp. 1 (Gracile capuchin monkey)	KX779913	100% to *Trypanosoma cruzi*
UCR_ENT 00052210	*Triatoma dispar*	COSTA RICA; Alajuela	Sylvatic	2008	95%	M	2	KX779931	98.2% to *Potos flavus*	Procyonidae sp. (Raccoons, kinkajous, coatis, olingos and allies)	KX779902	100% to *Trypanosoma cruzi*
UCR_ENT 00119051	*Triatoma lecticularia*	USA; Texas	Sylvatic	2009	95%	F	3	KX779935	100% to *Cathartes aura*	*Cathartes aura* (Turkey vulture)	KX779908	100% to *Trypanosoma cruzi*
UCR_ENT 00119052	*Triatoma lecticularia*	USA; Texas	Sylvatic	2009	95%	M	1					
UCR_ENT 00123526	*Triatoma mexicana*	MEXICO: Guerrero	Domestic	2011	?	F	4					
UCR_ENT 00119055	*Triatoma mexicana*	MEXICO: Guerrero	Domestic	2011	?	F	4					
UCR_ENT 00123869	*Triatoma pallidipennis*	MEXICO: Guerrero	Domestic	2011	?	M	4	KX779926	99.4% to *Peromyscus maniculatus*	*Peromyscus* sp. (Deer mouse)		
UCR_ENT 00005163	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	F	NA				KX779909	100% to *Trypanosoma cruzi*
UCR_ENT 00005165	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	NA	KX779930	100% to *Canis lupus*	*Canis lupus* (Dog)		
UCR_ENT 00005167	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	F	NA				KX779906	100% to *Trypanosoma cruzi*
UCR_ENT 00005184	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	NA					
UCR_ENT 00005185	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	F	NA				KX779896	100% to *Trypanosoma cruzi*
UCR_ENT 00005186	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	NA				KX779903	100% to *Trypanosoma cruzi*
UCR_ENT 00003031	*Triatoma protracta*	USA; California	Peridomestic	2009	95%	F	1					
AMNH_PBI 00218748	*Triatoma protracta*	USA; California	Peridomestic	2007	95%	F	1					
AMNH_PBI 00218756	*Triatoma protracta*	USA; California	Peridomestic	2007	95%	M	1					
AMNH_PBI 00218755	*Triatoma protracta*	USA; California	Peridomestic	2007	95%	F	NA	KX779928	100% to *Canis lupus*	*Canis lupus* (Dog)		
UCR_ENT 00123868	*Triatoma protracta*	USA; California	Peridomestic	2014	95%	M	2					
UCR_ENT 00123866	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	1					
UCR_ENT 00123867	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	3	KX779939	100% to *Canis lupus*	*Canis lupus* (Dog)		
UCR_ENT 00123865	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	2					
UCR_ENT 00123864	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	F	1					
UCR_ENT 00123863	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	M	1					
UCR_ENT 00123862	*Triatoma protracta*	USA; California	Peridomestic	2008	95%	F	1					
UCR_ENT 00119056	*Triatoma recurva*	USA; Arizona	Sylvatic	2014	95%	F	1				KX779916	100% to *Trypanosoma cruzi*
UCR_ENT 00119038	*Triatoma rubida*	USA; Arizona	Sylvatic	2008	?	F	1					
UCR_ENT 00119048	*Triatoma rubida*	USA; Arizona	Sylvatic	2006	?	F	1					
UCR_ENT 00123861	*Triatoma ryckmani*	GUATEMALA; Peten	Sylvatic	2015	95%	F	1					
UCR_ENT 00119049	*Triatoma sanguisuga*	USA; Florida	Sylvatic	2011	95%	M	1					
UCR_ENT 00119050	*Triatoma sanguisuga*	USA; Florida	Sylvatic	2011	95%	M	1					

We did not see evidence of double peaks in our chromatographs and direct sequencing always resulted in a single, uncontroversial host sequence, similar to results from other studies conducted using similar primers [49], but in contrast to other studies where cloning of the PCR product was performed and as many as four hosts were detected from a single specimen [50]. The archival nature of our specimens may have contributed towards the lack of detection of blood meals other than the one with most abundant DNA available. The 24 hosts detected

represented mainly animals present in a sylvatic environment with the exception of three records of the domestic dog from *T. protracta* (n = 17) specimens collected in Southern California in residential areas, one record of a deer mouse detected from *T. pallidipennis* (n= 1) at a residence in Mexico and two records of humans from sylvatic areas in Ecuador and Bolivia from *Rhodnius pictipes* (n=4) and *Panstrongylus rufotuberculatus* (n=3), respectively. These results represent fewer human records than some similar DNA-based studies (48.8% in [50]; 38% in [34]), which may reflect the sylvatic nature of most of our collecting sites and, potentially, the rigorous sterilization protocol we utilized. Because we did not obtain any sequences from marsupials or armadillos, it is possible that the Kitano 12S primers do not amplify 12S sequences from these hosts. While the primers match the corresponding sequence from armadillos at all but two mismatches at beginning of the reverse primer, the sequence in opossums appears to have several mismatches with the reverse primer that may have prevented binding and amplification. Of the sylvatic hosts detected, many are arboreal mammals and a surprising number reflect new host species, or new records for the larger groups of vertebrates they belong to (Fig. 1, dark red rectangles) as defined in Figure 1. Even after an extensive literature review, we were unable to find any record of these new groups of animals being recorded for hosts of these species. The new hosts detected for *Panstrongylus geniculatus* (n=11) include sloths (*Choloepus didactylus* Linnaeus), the arboreal weasel-like animal known as a tayra (*Eira barbara* [Linnaeus]), an unidentified member of Mustelidae, New World monkeys (*Lagothrix* sp. and *Saguinus* sp.) and agoutis (*Dasyprocta* sp.), all but the last, representing the first record for that group of vertebrate for that species. The tayra record represents the first record of any Triatominae species feeding on this species. We recovered several new host records for New World monkeys, apart from the two species identified for *P. geniculatus,* including *Cebus* sp. And *Saimiri* sp., *Ateles* sp., and

295 *Cebus* sp. For *Rhodnius robustus* (n=7), *Panstrongylus rufotuberculatus* (n=3) and *T. dimidiata*
296 (n=3), respectively. These all represent the first New World monkey hosts recorded for those
297 species except for *T. dimidiata* which has been previously noted as possessing antigens reacting
298 to antibodies developed for detecting New World monkey specific proteins. Our new host
299 records also included the arboreal porcupine genus *Coendou* for *P. rufotuberculatus*, coati
300 (*Nasua* sp.) for *R. prolixus* (n=1) and a sequence with the closest match to a kinkajou (*Potos sp.*)
301 but only determined to the level of Procyonidae for *Triatoma dispar* Lent (n=1), all new animal
302 groups for those species. The prevalence of arboreal mammals among our new host records may
303 reflect the inaccessibility of these habitats for gathering associational observations or absence of
304 antigens targeting these taxa in previous studies. Potentially, these under recognized groups of
305 hosts play a more prominent role in sylvatic cycle of *T. cruzi* than has been previously
306 understood.
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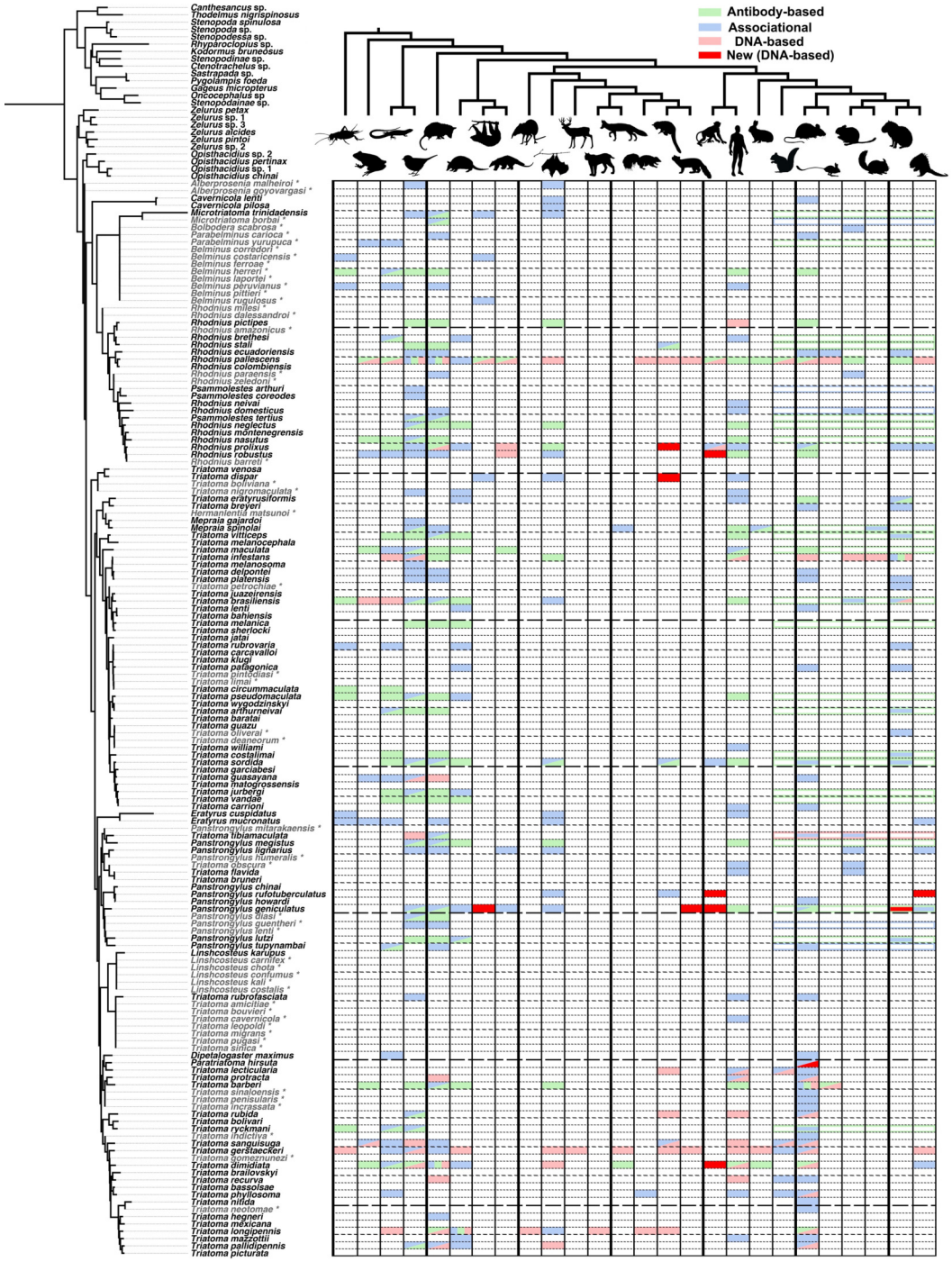

Figure 1. A visualization of the known sylvatic hosts of Triatominae and the type of record(s) supporting that association. The animals at the top of the figure represent the following groups from left to right, alternating from top to bottom: arthropod (Arthropoda), amphibian (Amphibia), lizards (Lepidosauromorpha), bird (Aves), opossums (Didelphimorphia), armadillo (Cingulata), sloth (Folivora), anteater (Vermilingua), shrew (Eulipotyphla), bat (Chiroptera), even-toed ungulate (Artiodactyls), feline (Felidae), canine (Canidae), musteloid (Mephitidae; Skunk), musteloid (Procyonidae; Racoons and relatives), musteloid (Mustelidae; Weasel), platyrrhine monkey (Ceboidea), human (Hominidae), rabbits (Lagomorpha), rodent (Sciuromorpha), rodent (Muroidea), rodent (Geomyoidea), rodent (Octodontoidea), rodent (Chinchilloidea), rodent (Cavioidea), rodent (Erethizontoidea). Relationships among mammals were simplified from [51] for Carnivora, [52] for deep level relationships and [53] for rodents. Triatominae taxa in light grey and indicated with an asterisk were added to the phylogeny based on morphological similarities indicated in the literature. Filled in matrix rectangle indicate observations with green indicating antibody-based observations; blue, associational; and red, DNA-based with bright red indicating newly obtained DNA-based host records for that species in our study. Colored outlines of rectangles across all represented rodent superfamilies represent observations specified only to the order Rodentia.

As a result of our literature review, we were able to detect some patterns that have not been previously widely recognized though it should be noted that host preferences cannot be determined with this data and a lack of evidence of a particular record does not indicate that such a host association may not be uncovered in the future. All of our aggregated data is reported in Table S1 with full references in Article S1. The number of species with associations with amphibians (11), reptiles (30) and birds (46, or 32% of all Triatominae species) is higher than we would have expected as these species tend to be thought of as minor hosts for Triatominae or only primarily associated with certain species [54]. This discrepancy may reflect that these hosts are not known to be able to harbor the *T. cruzi* pathogen [55,56] and deliberate attention has been paid to mammalian reservoir hosts. The most common group of known hosts among all species of Triatominae are marsupials (49 species), birds (46 species) and Muroidea including rats and mice (45 species). Humans rank as the fourth most commonly associated group with species of Triatominae (40 species) although it is possible that some human records have evaded our attention. Some of the rarest groups of known sylvatic hosts are shrews, artiodactyls and felines known only from a single host record, although this excludes records associated only with domestic species of those groups such as the domestic cat and the domestic pig. The rodent group Chinchilloidea which includes chinchillas had surprisingly few records (only for *Mepraia spinola* [Porter] and *Triatoma infestans,* both associated with viscachas in the genus *Lagidium*). There are 51 kissing bug species for which we were unable to find any records for sylvatic hosts. The largest group with scarce host data are the Old World species including the genus *Linshcosteus,* where associations with rats, birds, and humans have been recorded for *T. rubrofasciata* and only humans for *Triatoma cavernicola* Else & Cheong. Species which have been studied using DNA-based methods tend to have a broader range of known hosts than

398 species only investigated using associational and immunological approaches. For example,
399 *Rhodnius pallescens* Barber and *T. gerstaeckeri* have the broadest ranges of recorded host groups
400 (20 and 15 respectively, out of 26 groups recorded across all species) primarily because of single
401 studies focused on each of those species [49,57]. Some species thought to have narrow host
402 ranges show some evidence, although mostly associational and rarely antibody-based, of also
403 feeding on additional hosts such as rodents for *Cavernicola*, normally thought to associate only
404 with bats, and both rodents and marsupials for *Psammolestes*, normally associated with birds.
405 There is no obvious host specificity of Triatominae clades and, overall, our review points
406 towards the generalist bent of all Triatominae species, perhaps influenced more by hosts present
407 in a habitat than innate preference towards certain groups of hosts.

408 **Conclusion**

409 We believe that DNA-based methods for determining host associations of blood-feeding
410 species offers the best route forward in understanding the biology and epidemiological
411 importance of these speciose group of vectors. While there may be bias in representation of
412 known vertebrates on public databases, these databases continue to encompass data for an ever
413 growing range of species. We recommend the deposition of sequences acquired from vertebrate
414 hosts of vectors into public databases even if their identity is not known at the time, not yet
415 routinely done, as the influx of known sequences may later shed light on their identity. While
416 DNA-based studies can suffer from primer specificity biases, rigorous testing of primers sets for
417 universality and specificity can minimize this possibility. We have shown that this method can
418 be used on archival specimens and we recommend future efforts using this relatively cheap,
419 efficient and effective method in order to better understand the habits of all species of these
420 vectors, particularly those that are less well studied. We found that 95% ethanol seemed to

421 preserve DNA well enough for amplification of the 150 bp chunk of 12S rRNA using our
422 preferred set of primers (38). While we did achieve some results for specimens without
423 observable blood in the digestive tract, we had a much higher success rate for those with
424 observable blood and time and energy could be saved by not extracting DNA from the former
425 group of specimens.
426
427 Acknowledgements
428 We thank Jason Cryan, Andrew Ernst, Dimitri Forero, Sarah Frankenberg, Jeremy Huff,
429 Warren Macdonald, Michael E. Irwin, J.J. Ramirez, Gavin Svenson, Julie Urban, and Michael
430 Whiting for donations of specimens.
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434 1. Otálora-Luna F, Pérez-Sánchez AJ, Sandoval C, Aldana E. Evolution of hematophagous habit in
435 Triatominae (Heteroptera: Reduviidae). Rev Chil Hist Nat. 2015;88: 4. doi:10.1186/s40693-014-
436 0032-0

437 2. Justi SA, Russo CAM, Mallet JR dos S, Obara MT, Galvão C. Molecular phylogeny of Triatomini
438 (Hemiptera: Reduviidae: Triatominae). Parasit Vectors. 2014;7: 149. doi:10.1186/1756-3305-7-149

439 3. Pereira PCM, Navarro EC. Challenges and perspectives of Chagas disease: a review. J Venom Anim
440 Toxins Incl Trop Dis. 2013;19: 34. doi:10.1186/1678-9199-19-34

441 4. Bonney KM. Chagas disease in the 21st century: a public health success or an emerging threat?
442 Parasite. 2014;21: 11. doi:10.1051/parasite/2014012

443 5. Viotti R, Alarcón de Noya B, Araujo-Jorge T, Grijalva MJ, Guhl F, López MC, et al. Towards a
444 paradigm shift in the treatment of chronic Chagas disease. Antimicrob Agents Chemother. 2014;58:
445 635–639. doi:10.1128/AAC.01662-13

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

448 7. Galvão C, Justi SA. An overview on the ecology of Triatominae (Hemiptera:Reduviidae). Acta Trop.
449 2015;151: 116–125. doi:10.1016/j.actatropica.2015.06.006

450 8. Deane LM. Animal reservoirs of *Trypanosoma cruzi* in Brazil. Rev Bras Malariol Doencas Trop.
451 1964;16: 27–48.

452 9. Pizarro JC, Stevens L. A new method for forensic DNA analysis of the blood meal in chagas disease
453 vectors demonstrated using *Triatoma infestans* from Chuquisaca, Bolivia. PLoS One.
454 journals.plos.org; 2008;3: e3585. doi:10.1371/journal.pone.0003585

455 10. Feliciangeli MD, Dujardin J-P, Bastrenta B, Mazzarri M, Villegas J, Flores M, et al. Is *Rhodnius*
456 *robustus* (Hemiptera: Reduviidae) responsible for Chagas disease transmission in Western
457 Venezuela? Trop Med Int Health. 2002;7: 280–287.

458 11. Usinger RL. The Triatominae of North and Central America and the West Indies and their public
459 health significance. Public Health Bulletin 288, Washington: US Public Health Service; 1944.

460 12. Oliveira MA, Ferreira RL, Carneiro MA, Diotaiuti L. Ecology of *Cavernicola pilosa* Barber, 1937
461 (Hemiptera: Reduviidae: Triatominae) in the Boa Esperança Cave, Tocantins, Brazil. Ecotropica.
462 2008;14: 63–68.

463 13. Salvatella AR, Basmadjian Y, Rosa R, Puime A. *Triatoma delpontei* Romana & Abalos, 1947
464 (Hemiptera, Tratominae) in the Brazilian State of Rio Grande do Sul. Rev Inst Med Trop Sao Paulo.

1992;35: 73–76.

14. Garrouste R. La première observation in natura de l'entomophagie de *Panstrongylus geniculatus* (Latreille 1811) hématophage vecteur de la maladie de Chagas (Hemiptera: Reduviidae). Ann Soc Entomol Fr. 2009;45: 302–304. doi:10.1080/00379271.2009.10697614

15. Sandoval CM, Ortiz N, Jaimes D, Lorosa E, Galvão C, Rodriguez O, et al. Feeding behaviour of *Belminus ferroae* (Hemiptera: Reduviidae), a predaceous Triatominae colonizing rural houses in Norte de Santander, Colombia. Med Vet Entomol. Wiley Online Library; 2010;24: 124–131. doi:10.1111/j.1365-2915.2010.00868.x

16. Kjos SA, Marcet PL, Yabsley MJ, Kitron U, Snowden KF, Logan KS, et al. Identification of bloodmeal sources and *Trypanosoma cruzi* infection in triatomine bugs (Hemiptera: Reduviidae) from residential settings in Texas, the United States. J Med Entomol. 2013;50: 1126–1139. doi:10.1603/ME12242

17. Sandoval CM, Duarte R, Gutíerrez R, Rocha D da S, Angulo VM, Esteban L, et al. Feeding sources and natural infection of *Belminus herreri* (Hemiptera, Reduviidae, Triatominae) from dwellings in Cesar, Colombia. Mem Inst Oswaldo Cruz. 2004;99: 137–140. doi:10.1590/S0074-02762004000200004

18. Durán P, Siñani E, Depickère S. On triatomines, cockroaches and haemolymphagy under laboratory conditions: new discoveries. Mem Inst Oswaldo Cruz. 2016;111: 605–613. doi:10.1590/0074-02760160027

19. Carcavallo RU, da Silva Rocha D, Galindez Giron I. Feeding sources and patterns. In: Carcavallo RU, Galindez Giron I, Jurberg J, editors. Atlas of Chagas' disease vectors in the Americas Vol 2. 1998. pp. 537–560.

20. Packchanian A. Experimental transmission of *Trypanosoma cruzi* infection in animals by *Triatoma sanguisuga ambigua*. Public Health Rep. Association of Schools of Public Health; 1940;55: 1526–1532. doi:10.2307/4583416

21. Sant'Anna MRV, Diotaiuti L, de Figueiredo Gontijo A, de Figueiredo Gontijo N, Pereira MH. Feeding behaviour of morphologically similar *Rhodnius* species: influence of mechanical characteristics and salivary function. J Insect Physiol. 2001;47: 1459–1465.

22. Bodin A, Vinauger C, Lazzari CR. Behavioural and physiological state dependency of host seeking in the blood-sucking insect *Rhodnius prolixus*. J Exp Biol. The Company of Biologists Ltd; 2009;212: 2386–2393. doi:10.1242/jeb.030668

23. Martínez-Ibarra JA, Alejandre-Aguilar R, Paredes-González E, Martínez-Silva MA, Solorio-Cibrián M, Nogueda-Torres B, et al. Biology of three species of North American Triatominae (Hemiptera: Reduviidae: Triatominae) fed on rabbits. Mem Inst Oswaldo Cruz. SciELO Brasil; 2007;102: 925–930.

24. Gürtler RE, Ceballos LA, Ordóñez-Krasnowski P, Lanati LA, Stariolo R, Kitron U. Strong host-feeding preferences of the vector *Triatoma infestans* modified by vector density: implications for the epidemiology of Chagas disease. PLoS Negl Trop Dis. 2009;3: e447. doi:10.1371/journal.pntd.0000447

25. Barretto MP. Estudos sobre reservatorios e vectores silvestres do *Trypanosoma cruzi*. 17. Contribuiçao para o estudo dos focos naturais da tripanossomose americana, con especial referenda a regiao nordeste do Estado de Sao Paulo, Brasil. Rev Soc Bras Med Trop. 1967;1: 23–35.

26. Freitas J, Siqueira AF, Ferreira OA. Investigações epidemiológicas sobre triatomíneos de hábitos domésticos e silvestres com auxílio da reação de precipitina. Rev Inst Med Trop Sao Paulo. 1960;2:

509 90–99.

510 27. Pipkin AC. Reduviid bugs from central Panama and incidence of infection with hemoflagellates. J
511 Parasitol. 1962;48: 103–111.

512 28. Christensen HA, de Vasquez AM. Host feeding profiles of *Rhodnius pallescens* (Hemiptera:
513 Reduviidae) in rural villages of Central Panama. Am J Trop Med Hyg. 1981;30: 278–283.

514 29. Villela MM, Rodrigues VLCC, Casanova C, Dias JCP. Analysis on the food source of
515 *Panstrongylus megistus* (Hemiptera, Reduviidae, Triatominae) and its present importance as a vector
516 for Trypanosoma cruzi, in the State of Minas Gerais. Rev Soc Bras Med Trop. 2010;43: 125–128.

517 30. Lorosa ES, de Andrade RE, Pujol-Luz JR, Jurberg J, Carcavallo RU. Determinação das fontes
518 alimentares e da infecção natural do *Triatoma jurbergi* (Carcavallo, Galvão & Lent, 1998) e
519 *Triatoma vandae* Carcavallo, Jurberg, Rocha, Galvão, Noireau & Lent, 2001 capturados no estado
520 do Mato Grosso, Brasil. Zoociências. 2003;5: 243–252.

521 31. Baker M. Reproducibility crisis: Blame it on the antibodies. Nature. 2015;521: 274–276.
522 doi:10.1038/521274a

523 32. Mota J, Chacon JC, Gutiérrez-Cabrera AE, Sánchez-Cordero V, Wirtz RA, Ordoñez R, et al.
524 Identification of blood meal source and infection with *Trypanosoma cruzi* of Chagas disease vectors
525 using a multiplex cytochrome b polymerase chain reaction assay. Vector Borne Zoonotic Dis.
526 2007;7: 617–627. doi:10.1089/vbz.2007.0106

527 33. Dias FBS, Quartier M, Romaña CA, Diotaiuti L, Harry M. *Tamandua tetradactyla* Linnaeus, 1758
528 (Myrmecophagidae) and *Rhodnius robustus* Larrousse, 1927 (Triatominae) infection focus by
529 *Trypanosoma rangeli* Tejera, 1920 (Trypanosomatidae) in *Attalea phalerata* Mart. ex Spreng
530 (Arecaceae) palm tree in the Brazilian Amazon. Infect Genet Evol. 2010;10: 1278–1281.

doi:10.1016/j.meegid.2010.06.020

34. Stevens L, Dorn PL, Hobson J, de la Rua NM, Lucero DE, Klotz JH, et al. Vector blood meals and Chagas disease transmission potential, United States. Emerg Infect Dis. 2012;18: 646–649. doi:10.3201/eid1804.111396

35. 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. doi:10.1371/journal.pntd.0003365

36. Cecere MC, Leporace M, Fernández MP, Zárate JE, Moreno C, Gürtler RE, et al. Host-Feeding Sources and Infection With *Trypanosoma cruzi* of *Triatoma infestans* and *Triatoma eratyrusiformis* (Hemiptera: Reduviidae) From the Calchaqui Valleys in Northwestern Argentina. J Med Entomol. 2016; doi:10.1093/jme/tjw002

37. 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: 482. doi:10.1186/s13071-015-1100-2

38. Kitano T, Umetsu K, Tian W, Osawa M. Two universal primer sets for species identification among vertebrates. Int J Legal Med. 2007;121: 423–427. doi:10.1007/s00414-006-0113-y

39. Hwang WS, Zhang G, Maslov D, Weirauch C. Infection rates of *Triatoma protracta* (Uhler) with *Trypanosoma cruzi* in Southern California and molecular identification of trypanosomes. Am J Trop Med Hyg. 2010;83: 1020–1022. doi:10.4269/ajtmh.2010.10-0167

40. Alcaide M, Rico C, Ruiz S, Soriguer R, Muñoz J, Figuerola J. Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from

arthropod bloodmeals. PLoS One. 2009;4: e7092. doi:10.1371/journal.pone.0007092

41. Hwang WS, Zhang G, Maslov D, Weirauch C. Infection rates of *Triatoma protracta* (Uhler) with *Trypanosoma cruzi* in Southern California and molecular identification of trypanosomes. Am J Trop Med Hyg. 2010;83: 1020–1022. doi:10.4269/ajtmh.2010.10-0167

42. Kitano T, Umetsu K, Tian W, Osawa M. Two universal primer sets for species identification among vertebrates. Int J Legal Med. 2007;121: 423–427. doi:10.1007/s00414-006-0113-y

43. Maslov DA, Lukeš J, Jirku M, Simpson L. Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. Mol Biochem Parasitol. 1996;75: 197–205. doi:10.1016/0166-6851(95)02526-X

44. Melton T, Holland C. Routine forensic use of the mitochondrial 12S ribosomal RNA gene for species identification. J Forensic Sci. 2007;52: 1305–1307. doi:10.1111/j.1556-4029.2007.00553.x

45. Mota J, Chacon JC, Gutiérrez-Cabrera AE, Sánchez-Cordero V, Wirtz RA, Ordoñez R, et al. Identification of blood meal source and infection with *Trypanosoma cruzi* of Chagas disease vectors using a multiplex cytochrome b polymerase chain reaction assay. Vector Borne Zoonotic Dis. 2007;7: 617–627. doi:10.1089/vbz.2007.0106

46. Ngo KA, Kramer LD. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. J Med Entomol. 2003;40: 215–222.

47. Palumbi SR, Martin A, Romano S, McMillian WO, Stine L, Grabowski G. Simple fool's guide to PCR, Version 2. University of Hawaii, Zoology Department, Honolulu; 1991;

48. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30: 772–780. doi:10.1093/molbev/mst010

574 49. Gottdenker NL, Chaves LF, Calzada JE, Saldaña A, Carroll CR. Host life history strategy, species
575 diversity, and habitat influence *Trypanosoma cruzi* vector infection in Changing landscapes. PLoS
576 Negl Trop Dis. 2012;6: e1884. doi:10.1371/journal.pntd.0001884

577 50. Waleckx E, Suarez J, Richards B, Dorn PL. *Triatoma sanguisuga* Blood Meals and Potential for
578 Chagas Disease, Louisiana, USA. Emerging Infectious Disease journal. 2014;20: 2141.
579 doi:10.3201/eid2012.131576

580 51. Nyakatura K, Bininda-Emonds ORP. Updating the evolutionary history of Carnivora (Mammalia): a
581 new species-level supertree complete with divergence time estimates. BMC Biol. 2012;10: 12.
582 doi:10.1186/1741-7007-10-12

583 52. Foley NM, Springer MS, Teeling EC. Mammal madness: is the mammal tree of life not yet
584 resolved? Philos Trans R Soc Lond B Biol Sci. 2016;371. doi:10.1098/rstb.2015.0140

585 53. Fabre P-H, Hautier L, Dimitrov D, Douzery EJP. A glimpse on the pattern of rodent diversification:
586 a phylogenetic approach. BMC Evol Biol. 2012;12: 88. doi:10.1186/1471-2148-12-88

587 54. Rabinovich JE, Kitron UD, Obed Y, Yoshioka M, Gottdenker N, Chaves LF. Ecological patterns of
588 blood-feeding by kissing-bugs (Hemiptera: Reduviidae: Triatominae). Mem Inst Oswaldo Cruz.
589 2011;106: 479–494.

590 55. Kierszenbaum F, Gottlieb CA, Budzko DB. Antibody-independent, natural resistance of birds to
591 *Trypanosoma cruzi* infection. J Parasitol. 1981;67: 656–660. doi:10.2307/3280439

592 56. Urdaneta-Morales S, McLure I. Experimental infections in Venezuelan lizards by *Trypanosoma*
593 *cruzi*. Acta Trop. 1981;38: 99–105.

594 57. Gorchakov R, Trosclair LP, Wozniak EJ, Feria PT, Garcia MN, Gunter SM, et al. *Trypanosoma*
595 *cruzi* infection prevalence and bloodmeal analysis in triatomine vectors of Chagas disease from rural

peridomestic locations in Texas, 2013–2014. J Med Entomol. 2016;53: 911–918. doi:10.1093/jme/tjw040

Supplemental documents

Fig. S1. RaxML tree of Triatominae specie with molecular data available (excluding *Belminus herreri*) with bootstrap support values shown. Kissing bug species in red are those for which gut extracts were assayed with primers for vertebrate host and trypanosome DNA.

Table S1. Table of sylvatic host records of all Triatominae species.

Article S1. References for Table S1

Data S1. Phylip file of alignment

Data S2. Partitioning scheme as determined by PartitionFinder.