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

Anna Y Georgieva [1] , **Eric R L Gordon** [Corresp., 1] , **Christiane Weirauch** [1]

[1] Department of Entomology, University of California, Riverside, Riverside, California, United States

Corresponding Author: Eric R L Gordon
Email address: egord003@ucr.edu

Background: The 152 extant species of kissing bug include important vectors of the debilitating, chronic, and often fatal Chagas disease, which affects several million people mainly 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 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 each 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 United States 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.

1   **Sylvatic host associations of Triatominae and implications for Chagas disease reservoirs: a**

2   **review and new host records based on archival specimens**

3   **Short title: Sylvatic hosts of Triatominae**

5   Anna Georgieva*[1], Eric Gordon*^[1], Christiane Weirauch[1]

7   [1]Department of Entomology, University of California Riverside, 900 University Avenue, Riverside, CA

8   92521, USA

10   * equal contribution; ^ corresponding author: egord003@ucr.edu

11   **Abstract**

12   *Background:*

13   The 152 extant species of kissing bug include important vectors of the debilitating,

14   chronic, and often fatal Chagas disease, which affects several million people mainly in Central

15   and South America. An understanding of the natural hosts of this speciose group of blood-

16   feeding insects has and will continue to aid ongoing efforts to impede the spread of Chagas

17   disease. However, information on kissing bug biology is piecemeal and scattered, developed

18   using methods with varying levels of accuracy over more than 100 years. Existing host records

19   are heavily biased towards well-studied primary vector species and are derived from primarily

20   three different types of observations, associational, immunological or DNA-based, with varying

21   reliability.

23   *Methods:*

24   We gather a comprehensive and unparalleled number of sources reporting host

25   associations via rigorous targeted searches of publication databases to review all known natural,

26   or sylvatic, host records including information on how each record was collected. We integrate

27   this information with novel host records obtained via attempted amplification and sequencing of

28   a ~160 base pair (bp) region of the vertebrate 12S mitochondrial gene from the gastrointestinal

29   tract of 64 archival specimens of Triatominae, representing 19 species collected primarily in

30   sylvatic habitats throughout the southern United States and Central and South America during

31   the past 10 years. We show the utility of this method for uncovering novel and under-studied

32   groups of Triatominae hosts, as well as detecting the presence of the Chagas disease pathogen

33   via Polymerase Chain Reaction (PCR) of a ~400 bp sequence of the trypanosome 18S gene.

34

35   *Findings:*

36   New host associations for several groups of arboreal mammals were determined

37   including sloths, New World monkeys, coatis, arboreal porcupines and, for the first time as a

38   host of any Triatominae, tayras. A thorough review of previously documented sylvatic hosts,

39   organized by triatomine species and the type of observation (associational, antibody-based, or

40   DNA-based), is presented in a phylogenetic context and highlights large gaps in our knowledge

41   of Triatominae biology.

42

43   *Conclusion:*

44   The application of DNA-based methods of host identification towards additional species

45   of Triatominae, including rarely collected species that may require use of archival specimens, is

46   the most efficient and promising way to resolve recognized shortfalls.

47

Introduction

48

49   Triatominae, or kissing bugs, are blood-feeding members of the primarily predatory

50   insect family Reduviidae (Order Hemiptera). This subfamily consists of 152 living species,

51   nearly all distributed in the Americas, though several species occur in the Oriental region and

52   *Triatoma rubrofasciata* (De Geer) is considered invasive throughout the tropics [1,2]. These

53   insects are vectors of *Trypanosoma cruzi* Chagas, the parasite responsible for Chagas disease.

54   Chagas disease is a chronic debilitating disease, prevalent in Latin America, and affecting up to

55   10 million people worldwide [3]. There is no vaccine or effective cure once the symptoms of the

56   chronic disease have manifested and the disease has been termed an emerging threat of the 21st

57   century [4,5].

58   Most kissing bug species are suspected to be oligo- or polyphagous across a broad range

59   of wild mammal and other vertebrate species [6,7]. Many Triatominae hosts are sylvatic

60   mammals, but domestic mammals such as dogs, cats, and rodents can also be fed upon and act as

61   reservoir hosts of the parasite [8]. Generalist kissing bug species will also feed on humans and

62   some generalist species, e.g., *Triatoma infestans* Klug and *Rhodnius prolixus* (Stål), can exhibit

63   infection rates with *T. cruzi* equal to or even higher than 40% [9–11] though *T. cruzi* infection

64   rates vary considerably among populations. Some triatomine species will target certain hosts if

65   available and avoid other potential blood meal sources [1]. *Cavernicola pilosa* Barber appears to

66   only feed on bats [12,13], while *Triatoma delpontei* Romaña and Abalos and *Psammolestes*

67   Bergroth species are usually found in association with various birds [12,14]. In addition, there

68   are reports of some kissing bug species feeding on other arthropods [15–17], feeding on other

69   engorged kissing bug individuals [18] and even facultative nectar feeding [19]. The extent of

70   these behaviors in a natural environment and for the great majority of kissing bug species is

71   unknown. A diet lacking blood has been shown experimentally to result in complete or increased

72   mortality in at least some species [20], or suggesting that arthropod feeding may be rare or

73   driven by the lack of more suitable hosts. Overall, existing host association data is biased

74   towards a handful of heavily studied and well-documented primary vector species and little data

75   exists for many other Triatominae, particularly in sylvatic habitats [21]. Understanding patterns

76   of host associations across Triatominae can help to identify as yet underappreciated triatomine

77   species of medical interest as well as determine populations of vertebrate hosts that have

78   significant roles in sustaining vectors and potentially serve as reservoir hosts of *Trypanosoma*

79   *cruzi*.

80   Records of Triatominae host associations are based on different types of observations that

81   we here classify as associational (i.e. visual observation of a kissing bug in presumed or actual

82   close association with a possible host), immunological, and DNA-based methods. Each of these

83   approaches possess a unique set of advantages and disadvantages. Many early studies on kissing

84   bug-host interactions depended on observations of the insects during laboratory feeding tests or,

85   more rarely, in the wild [12,22]. Despite presenting direct evidence for feeding, laboratory

86   experiments are necessarily unnatural. Laboratory tests either offer insects a single possible host

87   species and observe whether feeding occurs [23–25] or present them with a choice between two

88   or more hosts to determine preference [26]. Both approaches may drive insects to feed on

89   organisms that they would not feed upon in natural habitats. Observations of cohabitation of

90   Triatominae with vertebrates in the wild, e.g., in animal nests and burrows, while more natural,

91   are usually tentative because the association may not reflect actual feeding. There is likely also a

92   bias towards more accessible terrestrial nests and burrows compared to corresponding arboreal

93   habitats. Immunological methods to detect host associations in Triatominae were first used in the

94   1960s [27–29], seemingly overcoming problems posed by associational methods. By utilizing

95   specific, but often polyclonal, antibodies in antisera developed for a predetermined range of

96   potential host species, experimenters infer direct host associations. Precipitin tests have

97   continued to be a popular way to detect host antigens in Triatominae blood meals [30–32]. While

98   a promising technique for forensic determination of actual hosts, disadvantages of precipitin tests

99   are twofold. First, tests may suffer from non-specificity in antibody binding or irreproducibility

100   as a result of variance in antibodies [33]. Second, due to the cost of developing different sets of

101   antibodies for different groups of hosts, antibodies are typically developed to be specific only for

102   large groups of potential host vertebrates, such as rodents. This approach results in reduced

103   resolution of hosts, i.e. vertebrates are not identified to genus or species, but will also fail to

104   detect unexpected or rare host taxa for which no antibody set is available.

105   More recently, studies have begun to use DNA-based methods to detect host identity

106   [17,34,35]. These methods typically use PCR that targets the conserved regions of variable,

107   mitochondrial genes for amplification. When amplified sequences are compared to a known

108   database, it is usually possible to determine which species or at least genus of organism the blood

109   originated from. These studies have documented that certain species, such as *Triatoma rubida*

110   (Uhler), *Triatoma protracta* (Uhler)*, and Triatoma gerstaeckeri* (Stål)*,* feed on a large variety of

111   vertebrate hosts [17,36]. PCR-based studies also have the potential to determine the percentage

112   of specimens within a given kissing bug population that have fed on humans [36]. While PCR is

113   useful in detecting a wide range of hosts, given that databases such as GenBank now hold a

114   library of barcodes for most mammal species and many other vertebrates, it does have a

115   relatively high risk for human contamination [37]. Primers can also have biases in amplifying

116 DNA that closely mirrors their sequence while not amplifying other sequences or also amplify

117 the insect's own DNA, which can interfere with detecting host DNA from the blood sample.

118 Multiple blood meals per specimen can amplify and interfere with determining a single sequence

119 and must be separated via cloning of the PCR product or next generation sequencing.

120 While Chagas disease incidence has trended downward in the past thirty years due to

121 screening of blood donations for *T. cruzi* as well as successful vector control applications of

122 insecticides targeting domiciliated Triatominae species [38], significant challenges remain for

123 further reducing the spread of the disease. One remaining hurdle relates to sylvatic species of

124 Triatominae that continue to transmit Chagas disease to humans primarily in rural areas (e.g.,

125 areas of the Amazon [39]). Studies focusing on the host associations of such species could help

126 to inform policies to limit their impact, for example, by identifying primary vertebrate hosts of

127 sylvatic species. However, most existing studies have focused on known primary vector species

128 and often targeted only domestic or peridomestic habitats where the transmission risk to humans

129 is considered higher (*e.g.,* [40,41]). In addition, most previous DNA-based studies have surveyed

130 narrow geographic areas, with several focusing on North America, and have used only living or

131 very recently preserved specimens. While there are some aggregations of known hosts of

132 Triatominae (*e.g.,* [21]) as well as one analysis of host association patterns using combined

133 precipitin data from 61 studies [42] there exists no comprehensive review of reported

134 Triatominae hosts. In particular, a synthesis of known triatomine-host associations that specifies

135 the method through which the record was obtained and allows for assessing the reliability of the

136 record is yet unavailable. Our study therefore has three objectives: 1) to contribute to the

137 growing knowledge base of host associations across Triatominae, we conducted PCR of

138 gastrointestinal contents extracted from 19 species of Triatominae from a range of localities

139   (Bolivia to United States); most specimens were collected in sylvatic habitats using light traps

140   and the sample comprises rarely encountered kissing bug species; 2) to determine the feasibility

141   of assessing host associations and trypanosome infection for archival kissing bug specimens that

142   have been preserved in ethanol for up to 10 years, we conducted PCR-based identification of

143   host sequences and trypanosomes for 64 specimens; 3) to establish currently documented

144   sylvatic host associations, we conducted a thorough literature review for all species of

145   Triatominae while recording the method used to determine that association; host patterns and

146   gaps in our current knowledge were visualized in phylogenetic context for both kissing bug

147   species and vertebrate hosts.

148   **Materials and Methods**

149   *Taxon sampling:* Triatominae specimens were primarily collected in sylvatic conditions

150   via light trapping or hand collection throughout the southern United States and Central and South

151   America and were preserved in ethanol (concentrations either unknown or 95%) between 2005

152   and 2015. We classify the habitat that each specimen as either domestic (found in a residence),

153   peridomestic (found outside in a residential area, near a residence) or sylvatic (found in natural

154   habitats, sometimes near field research stations). It was our aim to survey as wide a variety of

155   Triatominae species as possible, but certain species (e.g., *Triatoma protracta*, *Panstrongylus*

156   *geniculatus* [Latreille]) were sampled more thoroughly due to the availability of specimens

157   present in the Weirauch lab ethanol repository. One specimen of *Opisthacidius parkoi* (Lent and

158   Wygodzinsky), a close predatory relative of Triatominae, was also sampled and served as a

159   control. Voucher specimen data (unique specimen identifier [USI], determination, sex, specimen

160   depository, collecting locality and event) were recorded using the Planetary Biodiversity Institute

161   instance of the Arthropod Easy Capture database (research.amnh.org/pbi/locality). Images of

162   voucher specimens were taken using a Leica DFC450 C Microsystems system with a Planapo

163   1.0x objective. Voucher data, including collecting technique and images, are available online at

164   Heteroptera Species Pages (research.amnh.org/pbi/heteropteraspeciespage) and are best searched

165   by species and then USI number.

166   *DNA extraction:* DNA of the gastrointestinal contents of Triatominae specimens was

167   extracted in order to perform PCR. To avoid cross-contamination as well as the recognized threat

168   of contamination with human DNA, all equipment and work benches were sterilized (dissecting

169   petri dish, forceps, iris scissors) before and after processing each specimen using 10% bleach.

170   Cuticular surfaces of specimens were sterilized with 1% bleach for 3 minutes, to eliminate

171   possible contaminants acquired before capture in ethanol or during ethanol storage. The thorax

172   and abdomen were separated and contents of the abdomen were removed with forceps and

173   placed into an Eppendorf tube. While performing this procedure, the sex of the specimen and

174   whether blood was visible in the gut or not was recorded. When a large volume of blood was

175   present, contents were divided into separate Eppendorf tubes. Gut contents were homogenized

176   for 2 minutes with an Eppendorf pestle and DNA was extracted with a QIAGEN DNeasy blood

177   and tissue kit. We recorded the amount of blood in each specimen on a scale of 1 to 4 where: 1 --

178   no material visible in digestive tract; 2 -- small amount of dark, digested blood present; 3 --

179   obvious blood present; 4 -- completely engorged with blood. For seven specimens of *Triatoma*

180   *protracta* and one specimen of *Eratyrus mucronatus* Stål which had been extracted previously,

181   we were not able to record the amount of blood present in the digestive tract.

182   *PCR:* We tested seven previously developed sets of primers targeting three different

183   mitochondrial genes (Table 1) designed for identification of vertebrate hosts from invertebrate

184   blood meals via PCR on each of our extracts. We were not able to achieve consistent, acceptably

185 broad or specific results for several sets of previously used primers listed in Table 1 (all those

186 listed without asterisks). These primers amplified the corresponding DNA sequence of certain

187 species of Triatominae or did not amplify DNA that should be present based on the results of

188 other primer sets. We found that the "Kitano" 12S primers [43] yielded the most consistent and

189 the greatest number of amplified bands across samples among the primer sets tested and this is

190 the only primer set for which we present results. If samples yielded multiple bands after

191 electrophoresis on 1% agarose gels, they were gel extracted using QIAquick Gel Extraction kit.

192 If samples extracted from insects containing a large volume of blood did not result in bands after

193 PCR, the extract was diluted 1 in 10 and PCR was conducted again to ensure that a PCR-

194 inhibitor from the blood meal was not present (e.g., approach successful with sample UCR_ENT

195 00123869). We used primers for the 18S region to determine presence of trypanosomes in our

196 extracts (Table 1; [44]). PCR conditions consisted of an initial denaturation step of 94˚ C for 5

197 minutes, denaturation at 94˚C for 30 seconds, the annealing temperature listed in Table 1 for 30

198 seconds, extension at 72˚C for 30 seconds repeated for 35 cycles with a final extension time at

199 72˚C for 10 minutes. For each set of PCRs conducted, we also included a blank PCR control that

200 did not result in amplification.

201 **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˚**	[45]
			R	**5'-GTT TGC TGA AGA TGG CGG TA-3'**		[45]
	Melton 12S	12S	F	5'-ACT GGG ATT AGA TAC CCC ACT ATG-3'	53˚	[44]
			R	5'-ATC GAT TAT AGA ACA GGC TCC TC-3'		[44]
	Vert COI	COI	M13BC-FW	5'-TGT AAA ACG ACG GCC AGT HAA YCA YAA RGA YAT YGG NAC-3'	45˚	[43]
			BCV-RV1	5'-GCY CAN AYY ATN CYY RTR TA-3'		[43]
	DC-CytB	CytB	UP	5-CRT GAG GMC AAA TAT CHT TYT-3	42.5˚	[46]
			DW	5-ART ATC ATT CWG GTT TAA TRT-3		[46]
	Avian CytB	CytB	F	5'-GAC TGT GAC AAA ATC CCN TTC CA-3'	55˚	[47]

			R	5'-GGT CTT CAT CTY HGG YTT ACA AGA C-3'		[47]
	Mammalian CytB	CytB	F	5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3'	55°	[47]
			R	5'-TGT AGT TRT CWG GGT CHC CTA-3'		[47]
	Vert CytB	CytB	CB1-L	5'-CCC CTC AGA ATA TTT GTC CTC A-3'	57°	[34]
			CB2-H	5'-CAT CCA ACA TCT CAG CAT GAT GAA A-3'		[34]
Trypanosome DNA	**Tcz***	**18S**	**18sf**	**5′-TTA ACG GGA ATA TCC TCA GC-3'**	**50°**	[48]
			S829r	**5'-GCA TCA CAG ACC TGC TGT TG-3'**		[49]

202   *Purification, sequencing and analyzing:* All PCR products were cleaned using SureClean

203   (Bioline) before sequencing using the Macrogen EZ-Seq service. Once sequences were obtained,

204   the program Sequencher was used to process chromatographs. A nucleotide similarity BLAST

205   search was then used to compare sequences to the GenBank database. Sequences of the 12S gene

206   were considered to be derived from the same species as the closest match represented in

207   GenBank only if they were 100% identical and most or all other members of that genus were

208   also represented and differed in sequence (e.g. sequences KX779919, 100% to *Choloepus*

209   *didactylus* [Linnaeus], and KX779929, 100% to *Neotoma lepida* Thomas) otherwise we

210   classified it only to genus (e.g., sequence KX779923, 100% to *Lagothrix lagotricha* [Humboldt])

211   or to an even higher level (e.g., sequence KX779938, 100% to *Mustela kathiah* Hodgson and

212   classified as Mustelidae sp.). When multiple members of a genus were represented in GenBank,

213   but none matched 100%, we classified our sequence to that genus if it was more than 98%

214   identical to one member of the genus and closer to other members of that genus than to any other

215   genus (most sequences, e.g. sequences KX779920 98.6% to *Dasyprocta leporina* [Linnaeus] and

216   KX779934 98.7% to *Saguinus oedipus* [Linnaeus]). Trypanosome-derived PCR products of the

217   18S rRNA gene were sequenced and the sequence compared to the GenBank database for

218   identification.

219   *Phylogeny construction:* We gathered all available data for Triatominae species and

220   closely related reduviids (Stenopodainae, *Zelurus* spp. *Opisthacidius* spp.) on GenBank totaling

221   9,343 bp from 122 taxa as aligned using the MAFFT EINS-i algorithm [50] comprising the loci

222   from the nuclear rRNA operon and the mitochondrial genome (18S rRNA, ITS 1 5.8 rRNA and

223   ITS 2, D2, D3-D5 regions of the 28S rRNA; 16S rRNA COI, COII, Cytb) and constructed a

224   phylogeny using a partitioned RaxML analysis (Fig. S1, sampled species in red) partitioned by

225   the best scheme as determined by PartitionFinder (Phylip, Data S1; 9 partitions, Data S2). We

226   excluded *Belminus herreri* Lent & Wygodzinsky from our final analysis due to the

227   reconstruction of this taxon as sister to *T. rubrofasciata* with low support and a long terminal

228   branch; this result may be due to this taxon only being represented by the 18S gene that has low

229   phylogenetic utility for relatively recent divergences and the lack of that gene in the only other

230   representative of the tribe Bolboderini in our dataset, *Microtriatoma trinidadensis* (Lent) (rRNA

231   28S D2 region only). We also excluded two mitochondrial loci: the cytochrome b sequence from

232   *Panstrongylus rufotuberculatus* (Champion) that has been hypothesized to be introgressed from

233   *Panstongylus chinai* (Del Ponte) [51] as well as a purported cytochrome oxidase II from *T.*

234   *carrioni* Larrouse that shares a high similarity to members of the *T. infestans* clade. To visualize

235   all species of Triatominae in a single figure, we placed all taxa for which reliable molecular data

236   are currently unavailable to this backbone phylogeny using information on morphological

237   similarities from [6].

238   *Literature review*: We used a Web of Science search (all databases) to query the species

239   name of all the 152 currently recognized extant Triatominae species and surveyed results for

240   literature records of host associations. Briefly, we assessed titles and abstracts for the inclusion

241   of information on host associations for all matches and if we determined that they may include

242   relevant information, we scrutinized the publication for the record of the host association and the

243   type of method with which it was achieved. For a few widely studied triatomine species with an

244   extremely high number of matching publications (e.g., *Triatoma infestans*, *Rhodnius prolixus*),

245   we limited our searches with additional target words such as "gut contents", "blood meal" or

246   "host feed*" to increase the feasibility of surveying all relevant results. Every attempt was made

247   to find the primary source of a host record, but occasionally we resorted to citing reviews for

248   which the source of the record was unclear (and may have been first reported by the authors of

249   that review). We limited the inclusion of host records to evolutionarily relevant host species by

250   excluding laboratory results or host associations of exclusively domestic animals such as dogs,

251   chickens or other farmed animals due to the unnatural and recent modern presence of these hosts.

252   An exception was made for domestic or peridomestic rodents, including mice, rats and guinea

253   pigs that may also occur in natural environments. A detailed protocol for our literature is

254   included as a supplementary document (Article S1). Host taxa were divided into major groups

255   with all arthropods, amphibians, birds and reptiles each comprising a single group and the

256   remaining mammals split primarily by order with some exceptions (suborder level for

257   xenarthrans, superfamily level for primates and rodents, family level for carnivores). A record of

258   *Mepraia parapatrica* Frías-Lasserre feeding on a sea lion (*Otaria flavescens* Péron) was not

259   visualized on the figure. While our literature review was as exhaustive as reasonably possible, it

260   is possible that some relevant studies have not been recorded, particularly references in the grey

261   literature (e.g., otherwise unpublished data from theses or technical reports) and others not

262   referenced by Web of Science.

263   **Results and Discussion**

264   Of 64 total Triatominae specimens tested (38 males and 26 females), we were able to

265   determine a host association for 24 specimens (37.5%) with a maximum of a single host

266   determined per specimen. Of hosts with observable blood (28 out of 56 with observations

267   recorded; quantity of blood categories 2-4), 18 or 64.3% gave positive host results, compared to

268   2 or 7.1% of those observed without visible blood (category 1) in the digestive tract for which we

269   obtained a host sequence. Specimens with observable blood but without amplifiable host DNA

270   may represent samples with DNA degraded beyond allowing for amplification with primers

271   targeting the ~160 bp region of the 12S gene. Alternatively, it is possible that the Kitano 12S

272   primers do not amplify 12S sequences from certain hosts, though they were designed based on

273   sequences from divergent vertebrates from sharks to fish, reptiles, amphibians and mammals

274   (Kitano 2007). Of the 24 specimens with host determinations, 17 or 70.8% were male, a heavily

275   skewed ratio. Similarly, of the 28 specimens with visible blood in the digestive tract, 21 or 75%

276   were male. We speculate that blood-fed males may be more predisposed to dispersing in flight

277   while searching for mates and thus be more susceptible to light traps. In contrast, females may be

278   stationary after feeding, attempting to find a suitable place for oviposition, possibly near or in the

279   same location as the source of a blood meal. The infection rate of all specimens with *T. cruzi*

280   across sampled Triatominae was 31.3% or 20 specimens with one specimen of *Rhodnius pictipes*

281   Stål producing a band that, after sequenced, matched that of *Trypanosoma rangeli* Tejera. Of the

282   20 specimens testing positive for *T. cruzi*, 50% also possessed detectable host DNA and 50% did

283   not. *Panstrongylus geniculatus* was the species with the highest number of *T. cruzi* positive

284   individuals (5/11) followed by *Triatoma protracta* (4/17) and all three individuals tested of *T.*

285   *dimidiata* (Latrielle) which was the species with the highest positive percentage rate (100%; 3/3)

286   along with the single positive specimens of *T. dispar* Lent and *T. recurva* Stål. Specimens of

287   seven additional species also tested positive for *T. cruzi* (Table 2), all of which were previously

288   known to be capable of hosting the parasite [7].

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

290   **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 00012937	*Opisthacidius parkoi*	PERU; Loreto	Sylvatic	2007	?	F	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 barbara* (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 00012924	*Rhodnius barretti*	PERU; Loreto	Sylvatic	2007	?	F	1	KX779937	99.3% to *Saimiri sciureus*	*Saimiri* sp. 3 (Squirrel monkey)		
UCR_ENT 00012925	*Rhodnius barretti*	PERU; Loreto	Sylvatic	2007	?	M	1					
UCR_ENT 00012929	*Rhodnius barretti*	PERU; Loreto	Sylvatic	2007	?	M	4	KX779918	100% to *Saimiri sciureus*	*Saimiri* sp. 1 (Squirrel monkey)		
UCR_ENT 00012928	*Rhodnius barretti*	PERU; Loreto	Sylvatic	2007	?	F	1					
UCR_ENT 00002734	*Rhodnius barretti*	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 barretti*	ECUADOR; Orellana	Sylvatic	2009	95%	F	1				KX779900	100% to *Trypanosoma cruzi*
UCR_ENT 00119053	*Rhodnius barretti*	PERU; Madre de Dios	Sylvatic	2005	95%	F	4	KX779924	99.4% to various *Cebus* spp.	*Cebus* sp. 2 (Gracile capuchin monkey)		
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 robustus*	BOLIVIA; Santa Cruz	Sylvatic	2009	95%	F	4	KX779932	99.4% to *Nasua nasua*	*Nasua* sp. (Coati)	KX779904	100% to *Trypanosoma cruzi*
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					

291

292   We did not see evidence of double peaks in our chromatographs and direct sequencing

293 always resulted in a single, uncontroversial host sequence, similar to results from other studies

294 conducted using similar primers [52], but in contrast to other studies where cloning of the PCR

295 product was performed and as many as four hosts were detected from a single specimen [53].

296 The archival nature of our specimens may have contributed towards the lack of detection of

297 blood meals other than the one with most abundant DNA available. The 24 hosts detected

298 represented mainly animals present in a sylvatic environment with the exception of three records

299 of the domestic dog from *T. protracta* (n = 17) specimens collected in Southern California in

300   residential areas, one record of a deer mouse detected from *T. pallidipennis* Stål (n= 1) at a

301   residence in Mexico and two records of humans from sylvatic areas in Ecuador and Bolivia from

302   *Rhodnius pictipes* (n=4) and *Panstrongylus rufotuberculatus* (n=3), respectively. These results

303   represent fewer human records than some similar DNA-based studies (48.8% in [53]; 38% in

304   [36]), which may reflect the sylvatic nature of most of our collecting sites and, potentially, the

305   rigorous sterilization protocol we utilized. Because we did not obtain any sequences from

306   marsupials or armadillos, it is possible that the Kitano 12S primers do not amplify 12S sequences

307   from these hosts. While the primers match the corresponding sequence from armadillos at all but

308   two mismatches at beginning of the reverse primer, the sequence in opossums appears to have

309   several mismatches with the reverse primer that may have prevented binding and amplification.

310   Of the sylvatic hosts detected, many are arboreal mammals and a surprising number reflect new

311   host species, or new records for the larger groups of vertebrates they belong to (Fig. 1, dark red

312   rectangles) as defined in Figure 1. Even after an extensive literature review, we were unable to

313   find any record of these new groups of animals being recorded for hosts of these species. The

314   new hosts detected for *Panstrongylus geniculatus* (n=11) include sloths (*Choloepus didactylus*

315   Linnaeus), the arboreal weasel-like animal known as a tayra (*Eira barbara* [Linnaeus]), an

316   unidentified member of Mustelidae, New World monkeys (*Lagothrix* sp. and *Saguinus* sp.) and

317   agoutis (*Dasyprocta* sp.), all but the last, representing the first record for that group of vertebrate

318   for that species. The tayra record represents the first record of any Triatominae species feeding

319   on this species though it has been previously known to be infected with *T. cruzi* [54]. We

320   recovered several new host records for New World monkeys, apart from the two species

321   identified for *P. geniculatus,* including *Saimiri* sp., *Ateles* sp., and *Cebus* sp. For *Rhodnius*

322   *barretti* (n=7), *Panstrongylus rufotuberculatus* (n=3) and *T. dimidiata* (n=3)*, respectively. These

323   all represent the first New World monkey hosts recorded for those species except for *T.*

324   *dimidiata* which has been previously noted as possessing antigens reacting to antibodies

325   developed for detecting New World monkey specific proteins. Our new host records also

326   included the arboreal porcupine genus *Coendou* for *P. rufotuberculatus*, coati (*Nasua* sp.) for *R.*

327   *robustus* (n=1) and a sequence with the closest match to a kinkajou (*Potos sp.*) but only

328   determined to the level of Procyonidae for *Triatoma dispar* (n=1), all new animal groups for

329   those species. The prevalence of arboreal mammals among our new host records may reflect the

330   inaccessibility of these habitats for gathering associational observations or absence of antigens

331   targeting these taxa in previous studies. For example, while various populations of non-human

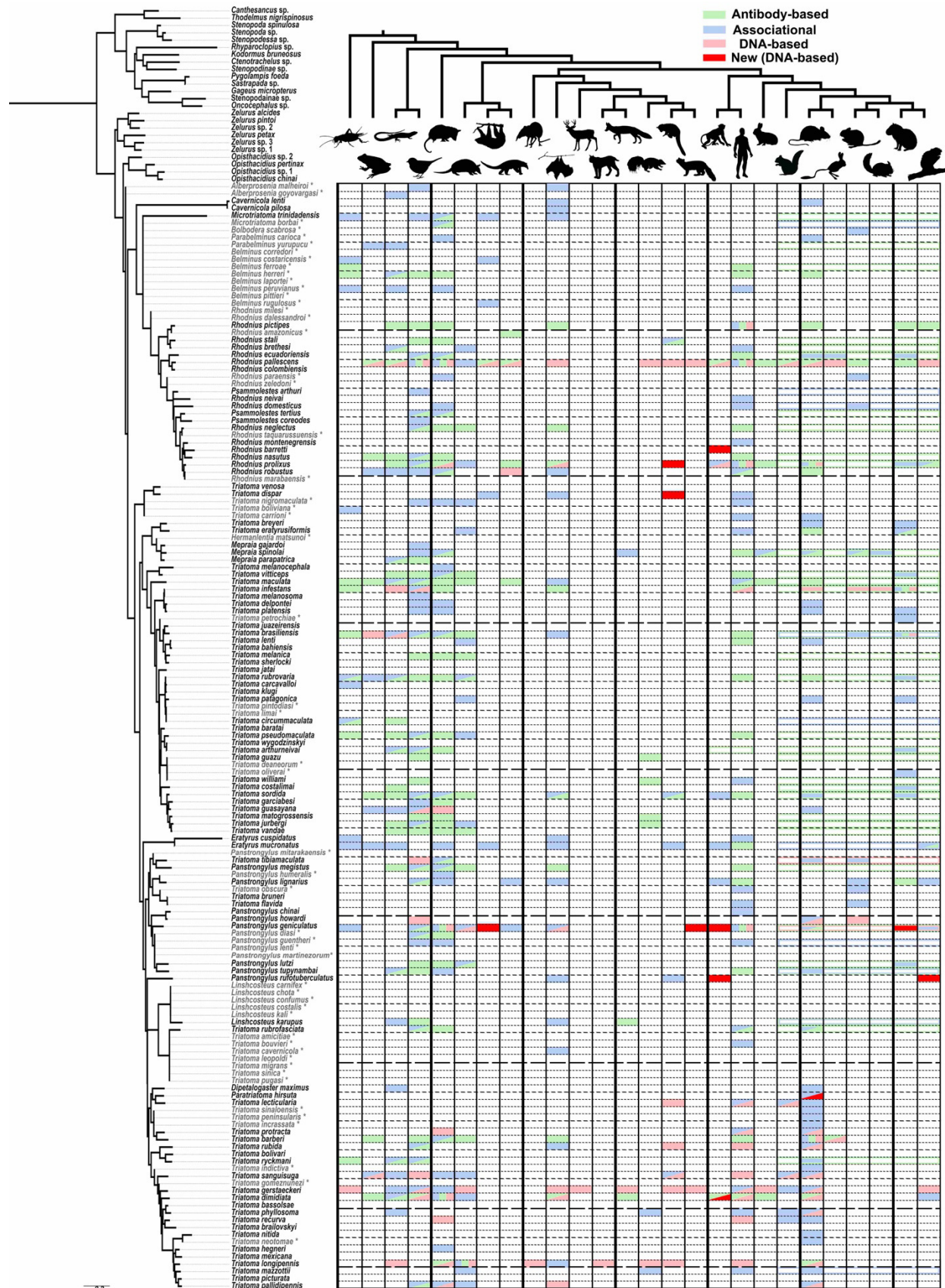
332   primates are known to have high infection rates with *T. cruzi [55]* and were even one of the first

333   mammals found to be infected with this parasite [8], only six species of Triatominae had

334   previously been recorded in association with New World monkeys with four new records (and

335   three new species) in this study alone. Potentially, these under recognized groups of hosts play a

336   more prominent role in the sylvatic cycle of *T. cruzi* than has been previously understood.

337

338
339 **Figure 1. A visualization of the known sylvatic hosts of Triatominae and the type of record(s) supporting that association.** The animals at
340 the top of the figure represent the following groups from left to right, alternating from top to bottom: arthropod (Arthropoda), amphibian
341 (Amphibia), lizards (Lepidosauromorpha), bird (Aves), opossums (Didelphimorphia), armadillo (Cingulata), sloth (Folivora), anteater
342 (Vermilingua), shrew (Eulipotyphla), bat (Chiroptera), even-toed ungulate (Artiodactyls), feline (Felidae), canine (Canidae), musteloid
343 (Mephitidae; Skunk), musteloid (Procyonidae; Racoons and relatives), musteloid (Mustelidae; Weasel), platyrrhine monkey (Ceboidea), human
344 (Hominidae), rabbits (Lagomorpha), rodent (Sciuromorpha), rodent (Muroidea), rodent (Geomyoidea), rodent (Octodontoidea), rodent
345 (Chinchilloidea), rodent (Cavioidea), rodent (Erethizontoidea). Relationships among mammals were simplified from [56] for Carnivora, [57] for
346 deep level relationships and [58] for rodents. Triatominae taxa in light grey and indicated with an asterisk were added to the phylogeny based on
347 morphological similarities indicated in the literature. Filled in matrix rectangle indicate observations with green indicating antibody-based
348 observations; blue, associational; and red, DNA-based with bright red indicating newly obtained DNA-based host records for that species in our
349 study. Colored outlines of rectangles across all represented rodent superfamilies represent observations specified only to a monophyletic higher
350 level, *e.g.*, the order Rodentia or Primates.

351   As a result of our literature review, we were able to detect some feeding patterns that

352 have not been previously widely recognized. However, it should be noted that host preferences

353 cannot be determined with this kind of data nor does the lack of evidence for a particular host

354 suggest that this host association may not be uncovered in the future. All of our aggregated data

355 is reported in Table S1 with full references in Article S2. The number of species with

356 associations with amphibians (14), reptiles (35) and birds (55, or 36% of all Triatominae species)

357 is higher than we expected as these species tend to be thought of as minor hosts for Triatominae

358 or are primarily associated only with certain species [42]. This discrepancy may reflect that these

359 hosts are not known to be able to harbor the *T. cruzi* pathogen [59,60] and deliberate attention

360 has been paid to mammalian reservoir hosts. The most common group of known hosts among all

361 species of Triatominae are marsupials (55 species), birds (55 species), humans (51) and

362 Muroidea including rats and mice (47 species). Some of the rarest groups of known sylvatic

363 hosts are shrews, artiodactyls, felines and sea lions, each known only from a single host record,

364 although this excludes records for domestic species classified in these groups such as the

365 domestic cat and the domestic pig. There are 40 kissing bug species for which we were unable to

366 find any records for sylvatic hosts. The largest group of Triatominae with scarce host data are the

367 Old World species including the genus *Linshcosteus* and the *T. rubrofasciata* species group.

368 Species which have been studied using DNA-based methods tend to have a broader range of

369 known hosts than species only investigated using associational and immunological approaches.

370   For example, *Rhodnius pallescens* Barber and *T. gerstaeckeri* have the broadest ranges of

371   recorded host groups (20 and 15 respectively, out of 26 groups recorded across all species)

372   primarily because of single studies focused on each of those species [52,61]. Some species

373   thought to have narrow host ranges show some evidence, although mostly associational and

374   rarely antibody-based, of also feeding on additional hosts such as rodents for *Cavernicola*

375   (thought to associate with bats) and both rodents and marsupials for *Psammolestes* (thought to

376   associate with birds). There is no obvious host specificity of Triatominae clades and, overall, our

377   review points towards the generalist bent of the great majority Triatominae species, perhaps

378   influenced more by hosts present in a given habitat and microhabitat than innate preference

379   towards certain groups of hosts.

380   A comprehensive understanding of the ecology of *Trypanosoma cruzi* and relevant

381   vectors, including primarily sylvatic Triatominae species, will be important in further controlling

382   Chagas disease. One important consideration in any eventual attempt to eradicate Chagas disease

383   is that oral transmission in the sylvatic cycle may be the primary mechanism of parasite spread

384   [62] and some insectivorous mammals often have a higher prevalence of the disease [63]. It has

385   been reported that even *T. cruzi*-infected vertebrates eaten as prey can be infective [64] and it

386   appears that Chagas disease can "bioaccumulate" in predators including highly carnivorous top

387   predators (e.g., ocelots), that are nocturnal without permanent dens and are thus unlikely to be

388   exposed to feeding Triatominae [65]. Predators that probably are frequently exposed to

389   Triatominae, for example nest-building arboreal species like coatis and tayras, play an important

390   role in the maintenance and spread of *T. cruzi*. For that reason, it has been predicted that if there

391   was development of a successful vaccine against *T. cruzi* in the future, targeting such predators

392   for vaccines among other highly-infected host species could theoretically be an effective strategy

393   in limiting the disease [66]. Thus, the confirmation of Triatominae feeding on certain carnivores

394   near the top of the food web can be of major importance in understanding and limiting the

395   sylvatic cycle of *T. cruzi*.

396   New approaches for modeling networks of vector host interactions based on the co-

397   occurrence of mammal and Triatominae species offer a promising method for understanding

398   Triatominae ecology [67,68]. Combining such studies with reviews of *T. cruzi*-infected

399   mammals [69] as well as documented host associations of individual species such as provided in

400   our review offers a route for advancing our understanding of the spread of *Trypanosoma cruzi*.

401   While each individual study does not manage to sample a completely representative population

402   of vectors and is limited in their recovery of host identity by methodology-specific biases, a

403   synthetic record of host associations overcomes many shortcomings.

404   **Conclusion**

405   We believe that DNA-based methods for determining host associations of blood-feeding

406   species offer the best route forward in understanding the biology and epidemiological importance

407   of this speciose group of vectors. While there may be bias in representation of known vertebrates

408   on public databases, these databases continue to encompass data for an ever-growing range of

409   species. We recommend the deposition of sequences acquired from vertebrate hosts of vectors

410   into public databases even if their identity is not known at the time, a practice not yet routinely

411   conducted, as the influx of DNA data may later shed light on their identity. While DNA-based

412   studies of blood meals can suffer from primer specificity biases, rigorous testing of primers sets

413   for universality and specificity can minimize this possibility. We have shown that this method

414   can be used on archival specimens and we recommend future efforts using this relatively cheap,

415   efficient and effective method in order to better understand the habits of all species of these

416   vectors, particularly those that are less well studied or rarely collected. PCR on archival

417   specimens may sometimes give false negative results or only allow sequencing of the most

418   recent host, but this is preferable to over reactivity of antibody based tests [70] and can be

419   overcome given a sufficiently large enough sample size. We found that 95% ethanol seemed to

420   preserve DNA well enough for amplification of the 150 bp chunk of 12S rRNA using our

421   preferred set of primers [43]. While we did achieve some results for specimens without

422   observable blood in the digestive tract, we had a much higher success rate for those with

423   observable blood and time and energy could be saved by not extracting DNA from the former

424   group of specimens. We hope that the application of this approach in combination with extensive

425   sampling of additional Triatominae, especially in sylvatic environments, can offer critical and

426   robust data on potential vectors and help to impede the spread of Chagas disease.

427

428   Acknowledgements

429   We thank Jason Cryan, Andrew Ernst, Dimitri Forero, Sarah Frankenberg, Jeremy Huff,

430   Warren Macdonald, Michael E. Irwin, J.J. Ramirez, Gavin Svenson, Julie Urban, and Michael

431   Whiting for donations of specimens; and two reviewers for valuable suggestions that greatly

432   improved this manuscript.

433

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, Ramsey JM, Ribeiro

444   I, Schijman AG, Sosa-Estani S, Torrico F, Gascon J. Towards a paradigm shift in the treatment of

445   chronic Chagas disease. Antimicrob Agents Chemother. 2014;58: 635–639. doi:10.1128/AAC.01662-

446   13

447   6.   Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance

448   as vectors of Chagas' disease. Bull Am Mus Nat Hist. 1979;163: 123–520.

449   7.   Galvão C, Justi SA. An overview on the ecology of Triatominae (Hemiptera:Reduviidae). Acta Trop.

450   2015;151: 116–125. doi:10.1016/j.actatropica.2015.06.006

451   8.   Deane LM. Animal reservoirs of *Trypanosoma cruzi* in Brazil. Rev Bras Malariol Doencas Trop.

452   1964;16: 27–48.

453   9.   Pizarro JC, Stevens L. A new method for forensic DNA analysis of the blood meal in Chagas disease

454   vectors demonstrated using *Triatoma infestans* from Chuquisaca, Bolivia. PLoS One.

455   journals.plos.org; 2008;3: e3585. doi:10.1371/journal.pone.0003585

456   10.   Feliciangeli MD, Dujardin J-P, Bastrenta B, Mazzarri M, Villegas J, Flores M, Muñoz M. Is

457   *Rhodnius robustus* (Hemiptera: Reduviidae) responsible for Chagas disease transmission in Western

458   Venezuela? Trop Med Int Health. 2002;7: 280–287.

459   11.   Guhl F, Pinto N, Aguilera G. Sylvatic triatominae: a new challenge in vector control transmission.

460   Mem Inst Oswaldo Cruz. 2009;104 Suppl 1: 71–75.

461   12.   Usinger RL. The Triatominae of North and Central America and the West Indies and their public

462   health significance. Public Health Bulletin 288, Washington: US Public Health Service; 1944.

463   13.   Oliveira MA, Ferreira RL, Carneiro MA, Diotaiuti L. Ecology of *Cavernicola pilosa* Barber, 1937

464   (Hemiptera: Reduviidae: Triatominae) in the Boa Esperança Cave, Tocantins, Brazil. Ecotropica.

465   2008;14: 63–68.

466   14. Salvatella AR, Basmadjian Y, Rosa R, Puime A. *Triatoma delpontei* Romana & Abalos, 1947

467   (Hemiptera, Tratominae) in the Brazilian State of Rio Grande do Sul. Rev Inst Med Trop Sao Paulo.

468   1992;35: 73–76.

469   15. Garrouste R. La première observation in natura de l'entomophagie de *Panstrongylus geniculatus*

470   (Latreille 1811) hématophage vecteur de la maladie de Chagas (Hemiptera: Reduviidae). Ann Soc

471   Entomol Fr. 2009;45: 302–304. doi:10.1080/00379271.2009.10697614

472   16. Sandoval CM, Ortiz N, Jaimes D, Lorosa E, Galvão C, Rodriguez O, Scorza JV, Gutiérrez R. Feeding

473   behaviour of *Belminus ferroae* (Hemiptera: Reduviidae), a predaceous Triatominae colonizing rural

474   houses in Norte de Santander, Colombia. Med Vet Entomol. 2010;24: 124–131. doi:10.1111/j.1365-

475   2915.2010.00868.x

476   17. Kjos SA, Marcet PL, Yabsley MJ, Kitron U, Snowden KF, Logan KS, Barnes JC, Dotson EM.

477   Identification of bloodmeal sources and *Trypanosoma cruzi* infection in triatomine bugs (Hemiptera:

478   Reduviidae) from residential settings in Texas, the United States. J Med Entomol. 2013;50: 1126–

479   1139. doi:10.1603/ME12242

480   18. Sandoval CM, Duarte R, Gutíerrez R, Rocha D da S, Angulo VM, Esteban L, Reyes M, Jurberg J,

481   Galvão C. Feeding sources and natural infection of *Belminus herreri* (Hemiptera, Reduviidae,

482   Triatominae) from dwellings in Cesar, Colombia. Mem Inst Oswaldo Cruz. 2004;99: 137–140.

483   doi:10.1590/S0074-02762004000200004

484   19. Díaz-Albiter HM, Ferreira TN, Costa SG, Rivas GB, Gumiel M, Cavalcante DR, Pavan MG,

485   Gonzalez MS, de Mello CB, Dillon VM, Bruno RV, Garcia E de S, Lima MM, de Castro DP, Dillon

486   RJ, de Azambuja P, Genta FA. Everybody loves sugar: first report of plant feeding in triatomines.

487   Parasit Vectors. 2016;9: 114. doi:10.1186/s13071-016-1401-0

488   20. Durán P, Siñani E, Depickère S. On triatomines, cockroaches and haemolymphagy under laboratory

489   conditions: new discoveries. Mem Inst Oswaldo Cruz. 2016;111: 605–613. doi:10.1590/0074-

490   02760160027

491   21. Carcavallo RU, da Silva Rocha D, Galindez Giron I. Feeding sources and patterns. In: Carcavallo

492   RU, Galindez Giron I, Jurberg J, editors. Atlas of Chagas' disease vectors in the Americas Vol 2.

493   1998. pp. 537–560.

494   22. Packchanian A. Experimental transmission of *Trypanosoma cruzi* infection in animals by *Triatoma*

495   *sanguisuga ambigua*. Public Health Rep. Association of Schools of Public Health; 1940;55: 1526–

496   1532. doi:10.2307/4583416

497   23. Sant'Anna MRV, Diotaiuti L, de Figueiredo Gontijo A, de Figueiredo Gontijo N, Pereira MH.

498   Feeding behaviour of morphologically similar Rhodnius species: influence of mechanical

499   characteristics and salivary function. J Insect Physiol. 2001;47: 1459–1465.

500   24. Bodin A, Vinauger C, Lazzari CR. Behavioural and physiological state dependency of host seeking in

501   the blood-sucking insect *Rhodnius prolixus*. J Exp Biol. 2009;212: 2386–2393.

502   doi:10.1242/jeb.030668

503   25. Martínez-Ibarra JA, Alejandre-Aguilar R, Paredes-González E, Martínez-Silva MA, Solorio-Cibrián

504   M, Nogueda-Torres B, Trujillo-Contreras F, Novelo-López M. Biology of three species of North

505   American Triatominae (Hemiptera: Reduviidae: Triatominae) fed on rabbits. Mem Inst Oswaldo

506   Cruz. 2007;102: 925–930.

507   26. Gürtler RE, Ceballos LA, Ordóñez-Krasnowski P, Lanati LA, Stariolo R, Kitron U. Strong host-

508   feeding preferences of the vector *Triatoma infestans* modified by vector density: implications for the

509   epidemiology of Chagas disease. PLoS Negl Trop Dis. 2009;3: e447.

510   doi:10.1371/journal.pntd.0000447

511   27. Barretto MP. Estudos sobre reservatorios e vectores silvestres do *Trypanosoma cruzi*. 17.

512   Contribuiçao para o estudo dos focos naturais da tripanossomose americana, con especial referência a

513   regiao nordeste do Estado de Sao Paulo, Brasil. Rev Soc Bras Med Trop. 1967;1: 23–35.

514   28. Freitas J, Siqueira AF, Ferreira OA. Investigações epidemiológicas sobre triatomíneos de hábitos

515   domésticos e silvestres com auxílio da reação de precipitina. Rev Inst Med Trop Sao Paulo. 1960;2:

516   90–99.

517   29. Pipkin AC. Reduviid bugs from central Panama and incidence of infection with hemoflagellates. J

518   Parasitol. 1962;48: 103–111.

519   30. Christensen HA, de Vasquez AM. Host feeding profiles of *Rhodnius pallescens* (Hemiptera:

520   Reduviidae) in rural villages of Central Panama. Am J Trop Med Hyg. 1981;30: 278–283.

521   31. Villela MM, Rodrigues VLCC, Casanova C, Dias JCP. Analysis on the food source of *Panstrongylus*

522   *megistus* (Hemiptera, Reduviidae, Triatominae) and its present importance as a vector for

523   *Trypanosoma cruzi*, in the State of Minas Gerais. Rev Soc Bras Med Trop. 2010;43: 125–128.

524   32. Lorosa ES, de Andrade RE, Pujol-Luz JR, Jurberg J, Carcavallo RU. Determinação das fontes

525   alimentares e da infecção natural do *Triatoma jurbergi* (Carcavallo, Galvão & Lent, 1998) e *Triatoma*

526   *vandae* Carcavallo, Jurberg, Rocha, Galvão, Noireau & Lent, 2001 capturados no estado do Mato

527   Grosso, Brasil. Zoociências. 2003;5: 243–252.

528   33. Baker M. Reproducibility crisis: Blame it on the antibodies. Nature. 2015;521: 274–276.

529   doi:10.1038/521274a

530   34. Mota J, Chacón JC, Gutiérrez-Cabrera AE, Sánchez-Cordero V, Wirtz RA, Ordoñez R, Panzera F,

531   Ramsey JM. Identification of blood meal source and infection with *Trypanosoma cruzi* of Chagas

532   disease vectors using a multiplex cytochrome b polymerase chain reaction assay. Vector Borne

533   Zoonotic Dis. 2007;7: 617–627. doi:10.1089/vbz.2007.0106

534   35. Dias FBS, Quartier M, Romaña CA, Diotaiuti L, Harry M. *Tamandua tetradactyla* Linnaeus, 1758

535   (Myrmecophagidae) and *Rhodnius robustus* Larrousse, 1927 (Triatominae) infection focus by

536   *Trypanosoma rangeli* Tejera, 1920 (Trypanosomatidae) in *Attalea phalerata* Mart. ex Spreng

537   (Arecaceae) palm tree in the Brazilian Amazon. Infect Genet Evol. 2010;10: 1278–1281.

538   doi:10.1016/j.meegid.2010.06.020

539   36. Stevens L, Dorn PL, Hobson J, de la Rua NM, Lucero DE, Klotz JH, Schmidt JO, Klotz SA. Vector

540   blood meals and Chagas disease transmission potential, United States. Emerg Infect Dis. 2012;18:

541   646–649. doi:10.3201/eid1804.111396

542   37. Lucero DE, Ribera W, Pizarro JC, Plaza C, Gordon LW, Peña R Jr, et al. Sources of blood meals of

543   sylvatic *Triatoma guasayana* near Zurima, Bolivia, assayed with qPCR and 12S cloning. PLoS Negl

544   Trop Dis. 2014;8: e3365. doi:10.1371/journal.pntd.0003365

545   38. Moncayo A, Silveira AC. Current epidemiological trends for Chagas disease in Latin America and

546   future challenges in epidemiology, surveillance and health policy. Mem Inst Oswaldo Cruz. 2009;104

547   Suppl 1: 17–30.

548   39. Quinde-Calderón L, Rios-Quituizaca P, Solorzano L, Dumonteil E. Ten years (2004–2014) of Chagas

549   disease surveillance and vector control in Ecuador: successes and challenges. Trop Med Int Health.

550   2016;21: 84–92. doi:10.1111/tmi.12620

551   40. Cecere MC, Leporace M, Fernández MP, Zárate JE, Moreno C, Gürtler RE, Cardinal MV. Host-

552   feeding sources and infection with *Trypanosoma cruzi* of *Triatoma infestans* and *Triatoma*

553   *eratyrusiformis* (Hemiptera: Reduviidae) from the Calchaqui valleys in Northwestern Argentina. J

554   Med Entomol. 2016; doi:10.1093/jme/tjw002

555   41. Cantillo-Barraza O, Garcés E, Gómez-Palacio A, Cortés LA, Pereira A, Marcet PL, Jansen AM,

556   Triana-Chávez O. Eco-epidemiological study of an endemic Chagas disease region in northern

557   Colombia reveals the importance of *Triatoma maculata* (Hemiptera: Reduviidae), dogs and *Didelphis*

558   *marsupialis* in *Trypanosoma cruzi* maintenance. Parasit Vectors. 2015;8: 482. doi:10.1186/s13071-

559   015-1100-2

560   42. Rabinovich JE, Kitron UD, Obed Y, Yoshioka M, Gottdenker N, Chaves LF. Ecological patterns of

561   blood-feeding by kissing-bugs (Hemiptera: Reduviidae: Triatominae). Mem Inst Oswaldo Cruz.

562   Fundação Oswaldo Cruz; 2011;106: 479–494. doi:10.1590/S0074-02762011000400016

563   43. Kitano T, Umetsu K, Tian W, Osawa M. Two universal primer sets for species identification among

564   vertebrates. Int J Legal Med. 2007;121: 423–427. doi:10.1007/s00414-006-0113-y

565   44. Hwang WS, Zhang G, Maslov D, Weirauch C. Infection rates of *Triatoma protracta* (Uhler) with

566   *Trypanosoma cruzi* in Southern California and molecular identification of trypanosomes. Am J Trop

567   Med Hyg. 2010;83: 1020–1022. doi:10.4269/ajtmh.2010.10-0167

568   45. Alcaide M, Rico C, Ruiz S, Soriguer R, Muñoz J, Figuerola J. Disentangling vector-borne

569   transmission networks: a universal DNA barcoding method to identify vertebrate hosts from

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

571 46. Maslov DA, Lukeš J, Jirku M, Simpson L. Phylogeny of trypanosomes as inferred from the small and

572 large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa.

573 Mol Biochem Parasitol. 1996;75: 197–205. doi:10.1016/0166-6851(95)02526-X

574 47. Melton T, Holland C. Routine forensic use of the mitochondrial 12S ribosomal RNA gene for species

575 identification. J Forensic Sci. 2007;52: 1305–1307. doi:10.1111/j.1556-4029.2007.00553.x

576 48. Ngo KA, Kramer LD. Identification of mosquito bloodmeals using polymerase chain reaction (PCR)

577 with order-specific primers. J Med Entomol. 2003;40: 215–222.

578 49. Palumbi SR, Martin A, Romano S, McMillian WO, Stine L, Grabowski G. Simple fool's guide to

579 PCR, Version 2. University of Hawaii, Zoology Department, Honolulu; 1991;

580 50. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in

581 performance and usability. Mol Biol Evol. 2013;30: 772–780. doi:10.1093/molbev/mst010

582 51. Sempertegui-Sosa C. Phylogenetic and phylogeographic studies of *Panstrongylus* sp., vectors of

583 Chagas Disease in Loja and Manabí Provinces, Ecuador. Ohio University. 2012. Available:

584 http://rave.ohiolink.edu/etdc/view?acc_num=ohiou1343799373

585 52. Gottdenker NL, Chaves LF, Calzada JE, Saldaña A, Carroll CR. Host life history strategy, species

586 diversity, and habitat influence *Trypanosoma cruzi* vector infection in Changing landscapes. PLoS

587 Negl Trop Dis. 2012;6: e1884. doi:10.1371/journal.pntd.0001884

588 53. Waleckx E, Suarez J, Richards B, Dorn PL. *Triatoma sanguisuga* blood meals and potential for

589 Chagas disease, Louisiana, USA. Emerging Infectious Diseases. 2014;20: 2141.

590 doi:10.3201/eid2012.131576

591 54. Ferreira LC, Deane LM. Nôvo depositário silvestre do *Schizotrypanum cruzi* (Chagas, 1909): a Irára,

592 *Tayra barbara*. Brasil Med. 1938;52: 1159–1161.

593 55. Lisboa CV, Monteiro RV, Martins AF, Xavier SC das C, Lima VDS, Jansen AM. Infection with

594 *Trypanosoma cruzi* TcII and TcI in free-ranging population of lion tamarins (*Leontopithecus* spp): an

595 11-year follow-up. Mem Inst Oswaldo Cruz. SciELO Brasil; 2015;110: 394–402. doi:10.1590/0074-

596   02760140400

597   56. Nyakatura K, Bininda-Emonds ORP. Updating the evolutionary history of Carnivora (Mammalia): a

598   new species-level supertree complete with divergence time estimates. BMC Biol. 2012;10: 12.

599   doi:10.1186/1741-7007-10-12

600   57. Foley NM, Springer MS, Teeling EC. Mammal madness: is the mammal tree of life not yet resolved?

601   Philos Trans R Soc Lond B Biol Sci. 2016;371. doi:10.1098/rstb.2015.0140

602   58. Fabre P-H, Hautier L, Dimitrov D, Douzery EJP. A glimpse on the pattern of rodent diversification: a

603   phylogenetic approach. BMC Evol Biol. 2012;12: 88. doi:10.1186/1471-2148-12-88

604   59. Kierszenbaum F, Gottlieb CA, Budzko DB. Antibody-independent, natural resistance of birds to

605   *Trypanosoma cruzi* infection. J Parasitol. 1981;67: 656–660. doi:10.2307/3280439

606   60. Urdaneta-Morales S, McLure I. Experimental infections in Venezuelan lizards by *Trypanosoma cruzi*.

607   Acta Trop. 1981;38: 99–105.

608   61. Gorchakov R, Trosclair LP, Wozniak EJ, Feria PT, Garcia MN, Gunter SM, Murray KO. 2016.

609   *Trypanosoma cruzi* infection prevalence and bloodmeal analysis in triatomine vectors of Chagas

610   disease from rural peridomestic locations in Texas, 2013–2014. J Med Entomol. 2016;53: 911–918.

611   doi:10.1093/jme/tjw040

612   62. Roque ALR, Xavier SCC, da Rocha MG, Duarte ACM, D'Andrea PS, Jansen AM. *Trypanosoma*

613   *cruzi* transmission cycle among wild and domestic mammals in three areas of orally transmitted

614   Chagas disease outbreaks. Am J Trop Med Hyg. 2008;79: 742–749.

615   63. Hodo CL, Hamer SA. Toward an ecological framework for assessing reservoirs of vector-borne

616   pathogens: Wildlife reservoirs of *Trypanosoma cruzi* across the southern United States. ILAR J.

617   2017; 1–14. doi:10.1093/ilar/ilx020

618   64. Thomas ME, Rasweiler JJ IV, D'Alessandro A. Experimental transmission of the parasitic flagellates

619   *Trypanosoma cruzi* and *Trypanosoma rangeli* between triatomine bugs or mice and captive

620   neotropical bats. Mem Inst Oswaldo Cruz. Fundação Oswaldo Cruz; 2007;102: 559–565.

621   doi:10.1590/S0074-02762007005000068

622   65. Rocha FL, Roque ALR, de Lima JS, Cheida CC, Lemos FG, de Azevedo FC, Arrais RC, Bilac D,

623   Herrera HM, Mourão G, Jansen AM. *Trypanosoma cruzi* infection in neotropical wild carnivores

624   (Mammalia: Carnivora): at the top of the *T. cruzi* transmission chain. PLoS One. 2013;8: e67463.

625   doi:10.1371/journal.pone.0067463

626   66. Stella M, Selakovic S, Antonioni A, Andreazzi CS. Community interactions determine role of species

627   in parasite spread amplification: the ecomultiplex network model. arXiv. 2017. Available:

628   http://arxiv.org/abs/1706.05121

629   67. Ibarra-Cerdeña CN, Valiente-Banuet L, Sánchez-Cordero V, Stephens CR, Ramsey JM.

630   *Trypanosoma cruzi* reservoir-triatomine vector co-occurrence networks reveal meta-community

631   effects by synanthropic mammals on geographic dispersal. PeerJ. 2017;5: e3152.

632   doi:10.7717/peerj.3152

633   68. Rengifo-Correa L, Stephens CR, Morrone JJ, Téllez-Rendón JL, González-Salazar C. Understanding

634   transmissibility patterns of Chagas disease through complex vector-host networks. Parasitology.

635   2017;144: 760–772. doi:10.1017/S0031182016002468

636   69. Browne AJ, Guerra CA, Alves RV, da Costa VM, Wilson AL, Pigott DM, Hay SI, Lindsay SW,

637   Golding N, Moyes CL. The contemporary distribution of *Trypanosoma cruzi* infection in humans,

638   alternative hosts and vectors. Sci Data. 2017;4: 170050. doi:10.1038/sdata.2017.50

639   70. Barrett TV. Advances in triatomine bug ecology in relation to Chagas' disease. Advances in disease

640   vector research. 1991. pp. 143–176.

641

642   Supplemental documents

643   Fig. S1. RaxML tree of Triatominae species with molecular data available (excluding 18S

644   sequence of *Belminus herreri* and putatively introgressed mitochondrial CytB sequence from

645   *Panstrongylus rufotuberculatus*) with bootstrap support values shown. Kissing bug species in red

646   are those for which gut extracts were assayed with primers for vertebrate host and trypanosome

647   DNA.

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

649   Article S1. Standard protocol for literature review.

650   Article S2. References for Table S1.

651   Data S1. Phylip file of alignment.

652   Data S2. Partitioning scheme as determined by PartitionFinder.

653