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Abundance, Natural Infection with Trypanosomes, and Food Source of an Endemic Species of Triatomine, *Panstrongylus howardi* (Neiva 1911), on the Ecuadorian Central Coast

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Abstract. The elimination of domestic triatomines is the foundation of Chagas disease control. Regional initiatives are eliminating introduced triatomine species. In this scenario, endemic triatomines can occupy the ecological niches left open and become a threat to long-term Chagas disease control efforts. This study determined the abundance, colonization, and *Trypanosoma cruzi* infection rate of the endemic *Panstrongylus howardi* in 10 rural communities located in Ecuador's Manabí Province. In total, 518 individuals of *P. howardi* were collected. Infestation indices of 1.4% and 6.6% were found in the domestic and peridomestic environments, respectively. We determined a *T. cruzi* infection rate of 53.2% ($N = 47$) in this species. *P. howardi* has a high capacity to adapt to different habitats, especially in the peridomicile. This implies a considerable risk of transmission because of the frequency of intradomicile invasion. Therefore, this species needs to be taken into account in Chagas control and surveillance efforts in the region.

INTRODUCTION

Chagas disease is caused by the parasite *Trypanosoma cruzi* and constitutes a complex public health problem in Latin America, where an estimated 8 million people are infected.[1,2] In Ecuador, approximately 230,000 people are infected, and 6.2 million people are at risk of infection.[1] Chagas is transmitted to humans and other mammals by Reduviidae bugs that belong to the subfamily Triatominae. This subfamily is currently composed of 140 species distributed among six tribes and 19 genera.[3] Three of the most abundant genera within the subfamily triatomine are *Panstrongylus* Berg 1879, *Triatoma* Laporte 1832, and *Rhodnius* Stal 1859; all of them are important in Chagas transmission.[4] The genus *Panstrongylus* comprises 13 species: *P. chinai* Del Ponte 1929, *P. diasi* Pinto and Lent 1946, *P. geniculatus* Latreille 1811, *P. guentheri* Berg 1879, *P. herreri* Wygodzinsky 1948, *P. howardi* Neiva 1911, *P. humeralis* Usinger 1939, *P. lenti* Galvão and Palma 1968, *P. lignarius* Walker 1873, *P. lutzi* Neiva and Pinto 1923, *P. megistus* Burmeister 1835, *P. rufotuberculatus* Champion 1899, and *P. tupynambai* Lent 1942.[3,5,6] These species are distributed from Argentina to Nicaragua[4,7–9] and widespread in sylvatic, peridomestic, and domestic habitats, especially those associated with mammals and birds.[6]

The epidemiological importance of some species of *Panstrongylus* has increased in the last few years, because species that were previously restricted to sylvatic environments have now invaded and colonized human dwellings.[6,10] In addition to that, some species of this genus have a high capacity as Chagas vectors because of their longevity, rapid response to the presence of a host, large volume of blood ingested, and frequent defecation during the feeding process.[10]

In Ecuador, 16 species of triatomines have been reported, including 6 species of *Panstrongylus* (*P. chinai*, *P. geniculatus*, *P. howardi*, *P. lignarius*, *P. herreri*, and *P. rufotuberculatus*).[3,11–13] *P. howardi* is endemic to Ecuador[9,11,14] and considered a sylvatic species, with occasional records of specimens found in human dwellings.[10] Its distribution seems to be limited to Manabí Province in the central coastal region of Ecuador.[11]

Diverse habitats, temperature, and humidity conditions, such as housing characteristics, are factors that influence the distribution of different species of triatomines. For example, traditional house construction in Manabí Province consists of walls made with bamboo cane, locally known as guadua cane (*Guadua angustifolia* [Kunth, 1822]), and roofs constructed with leaves of the *Phytelephas aequatorialis* (Spruce 1869) palm, locally known as cade or tagua palm, the nuts of which are used to manufacture handcrafts and buttons as an income source.[15] Previous studies have suggested that, although permeable to bugs, guadua cane walls do not offer good triatomine hiding places, possibly preventing colonization.[16] However, the environmental heterogeneity of the peridomestic habitat increases the probability of the presence of different species of triatomines.[17,18] The presence of sylvatic triatomines in this region[13] that can readily invade treated houses when residual effect of the insecticide declines constitutes a major obstacle for long-term control of Chagas disease.[16]

Although little is known about its ecology and habitats, *P. howardi* has been shown to have strong synanthropic tendencies and should be considered as an important secondary vector of *T. cruzi*, especially in areas where the populations of primary vectors (e.g., *T. dimidiata*) are being eliminated from domestic and peridomestic environments.

The aims of this study are to (1) describe the abundance and colonization of *P. howardi* in domestic and peridomestic habitats in the coastal Ecuadorian province of Manabí, (2) determine the infection by trypanosomes in the bugs and identify the blood source used for feeding, and (3) determine the habitat overlap with other epidemiologically important triatomine species—such as *T. dimidiata* and *R. ecuadoriensis*—that are currently being targeted by active vector control campaigns and its potential to replace them.

MATERIALS AND METHODS

Study area. This study was carried out in 10 rural communities in Portoviejo County, Manabí Province, Ecuador (Figure 1 and Table 1) during 2004, 2007, and 2008. Manabí Province is located along the central coast of Ecuador and receives an average annual rainfall of 563 mm per year.[19] The main economic activity is agriculture, predominantly sugar cane, oranges, bananas, yucca, corn (or maize), and rice. In addition,

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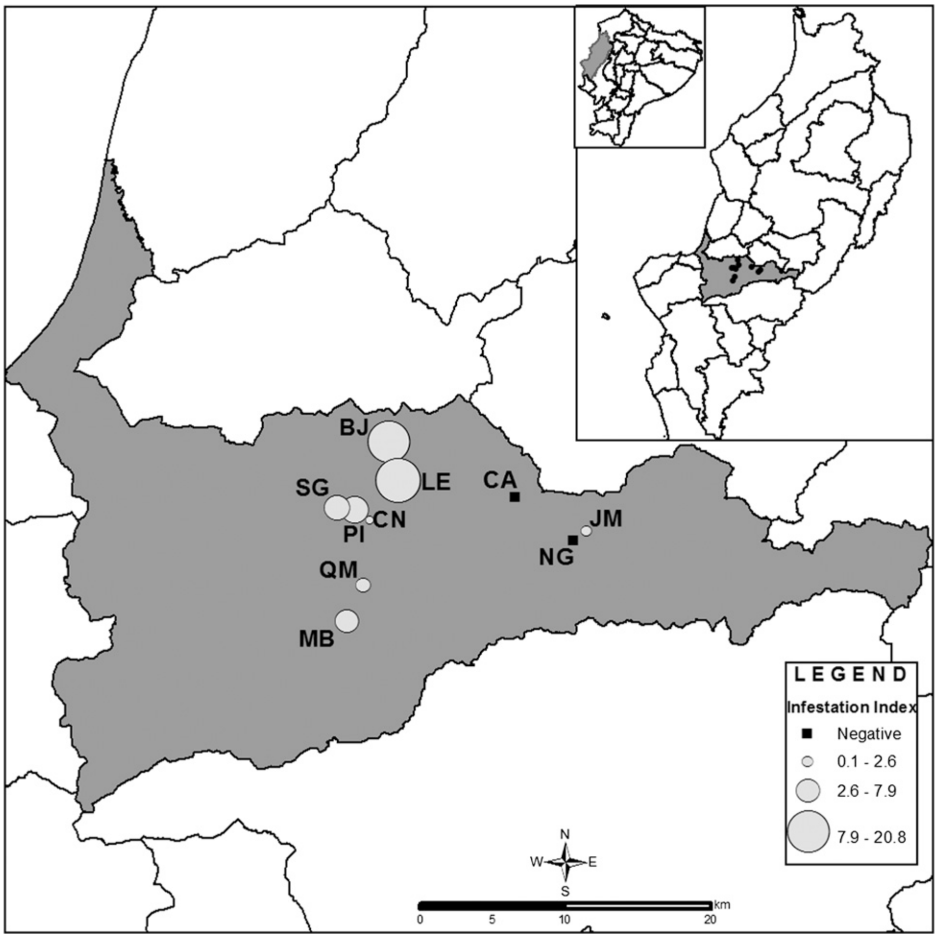

FIGURE 1. Map of Portoviejo County, Manabí Province indicating the location and *P. howardi* infestation rates of 10 rural communities studied on the central coast of Ecuador. Lines indicate county boundaries. Inset shows Ecuador's political division and study area. BJ, Bejuco; CA, Cruz Alta de Miguelillo; CN, La Ciénega; JM, Jesús María; LE, La Encantada; MB, Maconta Abajo; NG, San Gregorio; PI, Pimpiguasí; QM, Quebrada de Maconta; SG, San Gabriel.

some palms, such as cade or tagua palm (*P. aequatorialis*) and coconut (*Cocos nucifera* L.),[15,20] are cultivated between 0 and 1,500 m in this region.

Triatomine searches and entomological indices. Teams of two trained field workers from the National Chagas Disease Control Program from the Ecuadorian Ministry of Health searched for triatomines in each domestic unit (DU), which included domestic and peridomestic habitats. Pontifical Catholic University of Ecuador and Ohio University personnel closely monitored all field activities. The teams conducted manual searches using the 1 man-hour method as previously described.[12] Signed informed consent was obtained from the head of each DU according to a protocol approved by the Ohio University and the Catholic University of Ecuador Institutional Review Boards (IRBs).

The following entomological indices for domestic and peridomestic infestations were calculated: infestation index (100 × number of houses infested/number of houses searched), density (number of triatomines captured/number of houses searched), crowding (number of triatomines captured/number

TABLE 1
Entomological indices of *P. howardi* in rural communities in the central coastal region of Ecuador

Communities	Altitude (m)	DUs	DUs searched	Number of bugs collected	Infestation index (%)	Density	Crowding	Colonization index (%)
Pimpiguasí	24–70	117	91	93	8.8	1.0	11.6	25.0
La Encantada	19–290	124	98	109	21.4	1.1	5.2	23.8
Cruz Alta de Miguelillo	105–150	132	102	–	–	–	–	–
San Gabriel	56	113	94	126	8.5	1.3	15.8	50.0
La Ciénega	52	84	84	99	1.2	1.2	99.0	100.0
Quebrada de Maconta	92	162	123	30	4.1	0.2	6.0	60.0
Bejuco	65–400	106	72	50	20.8	0.7	3.3	60.0
Maconta Abajo	68–144	53	38	4	7.9	0.1	1.3	–
Jesús María	65–400	50	39	7	2.6	0.2	7.0	100.0
San Gregorio	74–220	50	34	–	–	–	–	–
Total		991	775	518	8.0	0.7	8.4	40.3

TABLE 2
Peridomestic and domestic entomological indices of *P. howardi* in rural communities in central coastal Ecuador

Communities	Infestation index (%)		Density		Crowding		Colonization index (%)	
	P	D	P	D	P	D	P	D
Bejuco	18.1	2.8	0.7	0.04	3.6	1.5	61.5	50.0
Pimpiguasí	7.7	1.0	1.0	0.01	13.1	1.0	28.6	–
Cruz Alta	–	–	–	–	–	–	–	–
San Gabriel	7.5	1.1	1.3	0.01	17.9	1.0	42.9	100.0
La Encantada	15.3	6.1	1.0.	0.2	6.3	2.5	20.0	33.3
Macota Abajo	7.9	–	0.1	–	1.3	–	–	–
Jesús María	2.6	–	0.2	–	7.0	–	100.0	–
San Gregorio	–	–	–	–	–	–	–	–
La Ciénega	1.2	–	1.2	–	99.0	–	100.0	–
Quebrada de Maconta	3.3	0.8	0.2	0.01	7.3	1.0	75.0	–
Total	6.6	1.4	0.6	0.03	9.8	1.9	41.2	36.4

D = domestic; P = peridomestic.

of houses infested), and colonization index (100 × number of houses with nymphs/number of houses infested).[21]

Natural infection with trypanosomes and meal source analysis. We determined natural infection with trypanosomes by direct microscopy. Differential identification of *T. cruzi* and *T. rangeli* infection was carried out by polymerase chain reaction (PCR) of the kinetoplast DNA from intestinal contents samples with the S35/S36 primer set as previously described.[22] One band of 330 bp was expected for samples infected with *T. cruzi*, and a band of 740 bp, together with a series of bands among 300–400 pb, was expected for *T. rangeli* infections. A mixed infection can be identified when bands of 330 and 740 bp are present. All of the samples determined as *T. rangeli* or mixed infection were analyzed with an additional pair of primers that targets the D7a divergent domain of the large subunit ribosomal RNA gene (D75/D76),[23] which differentiates the species of *Trypanosoma* because of the expected band size of 250 or 265 bp for *T. cruzi* and 210 bp for *T. rangeli*. The infection index (100 × number of triatomines with *Trypanosoma* spp./number of studied triatomines)[21] was calculated for infection with *T. cruzi*, *T. rangeli*, and mixed infections.

The triatomines' intestinal contents were analyzed using primers for cytochrome b as described previously[24] to determine the origin of their blood meal (mammal and/or avian). Additional analysis with cytochrome b of amphibians was carried out in a sample isolated from a triatomine found in association with frogs using the protocol as previously described.[25] Sequencing of cytochrome b of seven intestinal content samples from triatomines was carried out as previously described.[26] Sequences were visualized and edited using BioEdit software (Tom Hall Ibis Biosciences, Carlsbad, CA), and the identification of the blood meal source was done comparing the homology of the sequences obtained with the sequences in the nucleotide BLAST database (http://blast.ncbi.nlm.nih.gov/).

RESULTS

Entomological indices. In total, 775 DUs were enrolled in 2004, 2007, and 2008. Sixty-two houses (infestation index = 8%) in 8 of 10 communities visited were found to be infested with *P. howardi*. The infestation index was higher in La Encantada (21.4%) and El Bejuco (20.8%). In total, 518 bugs were collected (density = 0.7 bugs/DUs searched, crowding = 8.4 bugs/infested DUs, colonization index = 40.3% DUs with nymphs/infested DUs) (Figure 1 and Table 1).

Domiciliary infestation. In total, 21 bugs were collected inside 11 DUs (infestation index = 1.4%, density = 0.03, crowding = 1.9). All of the individuals were found in bedrooms under the beds. Only 4 of 11 houses harbored nymphs inside of the dwelling (colonization index = 36.4%) (Table 2).

Peridomiciliary infestation. In total, 474 bugs were collected in the peridomestic habitats in 50 DUs (infestation index = 6.6%, density = 0.6, crowding = 9.8). Colonization was found in the peridomicile of 41.2% of the infested DUs (Table 2).

Microhabitat preferences in the peridomicile. In total, specimens of *P. howardi* were found in 50 infested DUs occupying a variety of microhabitats that included rodent nests under bricks (71.1% of bugs collected), wood (11.8%), rock piles (1.7%), and within leafs of the thorny piñuela plant (*Aechmea magdalenae* [André ex Baker], Bromeliaceae; 15.4%). Of those 474 specimens, 13 individuals (7 individuals in bricks, 5 individuals in wood, and 1 individual within piñuelas) were found sharing microhabitats with *R. ecuadoriensis*. In El Bejuco, nine individuals (one nymph I [NI], four NII, three NIII, and one male adult) of *P. howardi* were found near two bullfrogs (cf. *Rana catesbeiana*) that were hiding under a pile of bricks.

Natural infection with trypanosomes and meal source analysis. In total, 47 individuals of *P. howardi* from 5 (La Ciénega, El Bejuco, La Encantada, Pimpiguasí, and San Gabriel) of 10 communities (entomological searches) were assessed for infection by trypanosomes. *Trypanosoma*-like parasites were detected in 29.8% of the samples analyzed by microscopy. By PCR, infection was detected in 61.7% of the samples; from these samples, 57.5% were infected only with *T. cruzi*, and 8.6% were infected with *T. rangeli*, including mixed infections (Table 3). Triatomines infected with

TABLE 3
Infection index with *T. cruzi* and/or *T. rangeli* in intestinal content samples from *P. howardi* collected in the peridomestic environment from five communities in central coastal Ecuador

Community (*n*)*	*T. cruzi* (%)	*T. rangeli* (%)	Mixed infection *T. cruzi*/ *T. rangeli* (%)
El Bejuco (14)	78.6	–†	7.1
La Ciénega (15)	66.7	6.7	–†
La Encantada (6)	–†	–†	–†
Pimpiguasí (1)	–†	–†	–†
San Gabriel (11)	36.4	9.1	9.1
Total (47)	53.2	4.3	4.3

*Number of triatomines analyzed from each community.
†Represents 0.00% of infection of trypanosomes.

T. cruzi were collected in the peridomestic environment, brick and rock piles, leaves of piñuelas, and association with rat and opossum nests. One infected triatomine was collected under a pile of bricks near cf. *R. catesbiana*. All of the intestinal content samples were also analyzed to identify the source of blood meals. Mammalian blood was found in 14 samples (29.8%), and avian blood was found in 3 samples (6.4%). PCR of the remaining 30 triatomine samples did not produce any product. The sample from the triatomine found associated with frogs did not amplify for amphibian cytochrome b. Specific determination of source blood meal conducted by DNA sequencing of the cytochrome b was possible in three samples. The blood meal sources included pigeon (Columbidae), a spiny rat of the genus *Proechimys*, and black rat (*Rattus rattus*). It was not possible to get analyzable sequences from the other samples.

DISCUSSION

We found a high infestation of *P. howardi* in DUs located in the Portoviejo River Valley, especially in peridomestic habitats. The collection of individuals of this species in the domestic environment in addition to reports from community members describing adults of *P. howardi* invading DUs when attracted to lights suggest the potential of this species and the genus[10,11] to invade the domicile environment, with the increased chance of feeding from synanthropic animals and human beings. The results of this study indicate that *P. howardi* can colonize multiple microhabitats in the peridomicile. The main peridomestic microhabitats were associated with rodent nests located between brick piles. It is possible that the color of the bricks, which blends well with the orange dorsal coloration of *P. howardi*, provides camouflage that protects them from predators living or roaming in the spaces between the bricks. La Encantada and Bejuco were the communities with the highest infestation index, but more individuals were found in San Gabriel. The characteristics of all of the study communities are relatively similar. However, our data indicate that *P. howardi* has a preference for rat nests found within bricks piles, which could suggest that the presence of leftover bricks from house improvement projects could attract this species to colonize the peridomicile. This hypothesis should be further explored. Although less abundant, colonies of this species were also found in wood piles and piñuelas and in association with nesting places of rodents and marsupials. Moreover, it is important to consider that an increased risk of invasion from the peridomicile to the domicile has been pointed out in previous reports of the presence of adults and nymphs of *P. howardi* in the peridomestic habitats of the houses nearby sylvatic environment[13] and the stable presence of peridomestic populations, despite periodic insecticide-based control.[16] However, to date, only one individual of this endemic species has been found in a sylvatic habitat associated with mouse nests in a palm of *Aiphanes eggersi*.[13]

An important aspect to discuss is the overlapping distribution of *P. howardi* and *T. dimidiata*. The latter species has been considered for years the primary vector of Chagas disease in the coastal region of Ecuador.[11] Moreover, the local Malaria Control Service (SNEM) has multiple records of *T. dimidiata* found in Manabí Province, including the studied area (Peñaherrera J and SNEM, personal communication). Interestingly, the extensive searches conducted by our group since 2001 have not found any *T. dimidiata* in rural areas of Manabí. This raises two possible scenarios. First, it is possible that the remarkable dorsal chromatic convergence shared by *P. howardi* and *T. dimidiata*[3,9] and the lack of close examination of the antenniferous tube and pronotum could have caused misidentification of *P. howardi* as *T. dimidiata* (Figure 2) by field personnel from SNEM. Unfortunately, no specimens have been maintained by SNEM that would allow for taxonomical confirmation. Second, earlier spraying efforts to control mosquitoes and triatomine populations (especially for *T. dimidiata* in Ecuador as one of the main goals within the Andean Countries Initiative) might have decreased or eliminated *T. dimidiata* from this area, leaving an open ecological niche that could be occupied by *P. howardi*, which was previously suggested.[27]

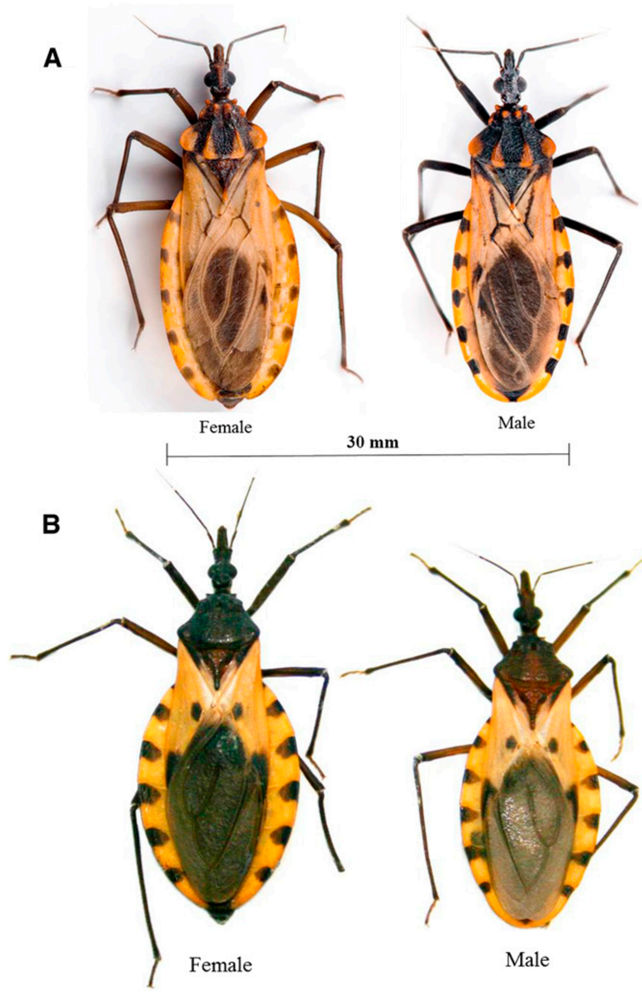

FIGURE 2. (**A**) Female and male *P. howardi* (endemic species from Manabí Province, Ecuador). (**B**) Female and male *T. dimidiata* (introduced species in Manabí and Guayas Provinces in Ecuador). Notice the similarities on dorsal coloration patterns, which could lead to misidentification.

To address a likely issue of misidentification of species, since 2004, the Center for Infectious Disease Research at the Catholic University of Ecuador has provided periodic training to field personnel from SNEM and the National Chagas Disease Control program. This training has included taxonomical identification and technical support to conduct entomological surveillance. However, to our knowledge, no systematic triatomine control work has been done during the last 5 years in these communities. Recent province-wide entomological surveys indicate that the infestation levels with *P. howardi* in this area remain unchanged (Grijalva MJ, unpublished data).

In relation to the presence of *Trypanosoma* parasite populations in the study area, previous studies in reservoirs from Manabí Province have determined the presence of *T. cruzi* and *T. rangeli* circulating in the peridomestic environment among rodents and opossums,[28] which confirms a continuous flow of parasite populations among mammalian hosts. However, the high *T. cruzi* infection rate of *P. howardi* collected in peridomestic habitats suggests the existence of active transmission between this vector species and its associated vertebrate hosts. The circulation of *Trypanosoma* populations in peridomestic hosts constitutes an important risk factor for transmission of Chagas disease to humans. Moreover, the fact that adult *P. howardi* reach houses when attracted by lights increases the potential risk for *T. cruzi* transmission.

In general, we have found a low infection rate with *T. rangeli* in triatomines collected in domestic/peridomestic habitats (Grijalva MJ, unpublished data). Furthermore, *T. rangeli* has been associated mainly with bugs from the genus *Rhodnius*.[29] The finding of this parasite in intestinal contents of *P. howardi* opens an interesting topic for research on the vectorial capacity of these bugs and their role in the dispersal of *T. rangeli* in the study area, and additional studies that include the analysis of salivary glands need to be carried out.

The meal source of the triatomines could not be assessed in most of the cases; however, the results showed presence of DNA from vertebrate hosts (mammals and birds) in *P. howardi* collected in the peridomestic environment. Interestingly, finding species such as *Proechymis* spp. and *R. rattus*, which are considered synanthropic species because of their capacity to circulate in the sylvatic environment as well as the peridomestic environment, is an important piece of information for a better understanding of the flow of *T. cruzi* among the sylvatic, peridomestic, and sylvatic transmission cycles. These synanthopic species can be the link for the parasite populations from sylvatic to domestic/peridomestic areas, which implies a source of infection for humans. These results, together with previous studies in Ecuador,[28] remark on the importance of considering the reservoirs when triatomine control strategies are designed. Additionally, the association of triatomines with other vertebrates, such as amphibians, needs to be further addressed. Although we found several *P. howardi* individuals in close association with bullfrogs, our results did not provide any evidence that the triatomines were feeding on them. However, amphibian–triatomine association has been previously reported in palm trees in the Amazon.[30]

Additional research on *P. howardi* is needed to describe its feeding and defecation habits, lifecycle, geographical distribution, and phylogenetic associations with related species. Nevertheless, the ecological preferences of *P. howardi*, the high infestation index, the high levels of *T. cruzi* infection, the efficient prandial and post-prandial defecation habits (Villacis AG, unpublished data), and the high adaptive ability observed all contribute to the importance of *P. howardi* as an emerging vector for Chagas disease transmission in the central coastal region of Ecuador and should be considered in the implementation of effective short- and long-term control strategies.

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