Lizards as Silent Hosts of *Trypanosoma cruzi*

Carezza Botto-Mahan,[1] Juana P. Correa,[1] Raúl Araya-Donoso, Francisca Farías, Esteban San Juan, Nicol Quiroga, Ricardo Campos-Soto, Claudio Reyes-Olivares, Daniel González-Acuña

We assessed 4 lizard species in Chile for *Trypanosoma cruzi*, the causative agent of Chagas disease, and 1 species for its ability to transmit the protozoan to uninfected kissing bugs. All lizard species were infected, and the tested species was capable of transmitting the protozoan, highlighting their role as *T. cruzi* reservoirs.

Chagas disease is one of the most neglected vectorborne diseases, infecting 6–7 million persons worldwide; 70 million persons are at risk for infection (*1*), and the disease is a concern in several nonendemic countries (*2*). The etiologic agent is *Trypanosoma cruzi*, a zoonotic protozoan maintained in the Americas by wild and domestic mammals and transmitted by hematophagous triatomine vectors (kissing bugs) (*3*,*4*). Infection of mammals occurs by contamination of broken skin or mucous membranes with the protozoan in kissing bug feces, by congenital transmission, and orally when feeding on infected kissing bugs (or their feces) or other infected mammals (*3*,*4*). Kissing bugs become infected mainly when feeding on infected mammals (*3*).

More than 150 species of wild mammals in the Americas are naturally infected with *T. cruzi* protozoa; some of these hosts are relevant in the maintenance and interplay of the domestic and wild transmission cycles of Chagas disease (*3*,*4*). Although the role of mammals in *T. cruzi* transmission has been studied, less is known about the relevance of nonmammalian vertebrates (*5*). Reptiles have been reported as blood meal sources of kissing bugs, but their status as hosts for *T. cruzi* protozoa is not well documented (*6*).

Author affiliations: Universidad de Chile, Santiago, Chile (C. Botto-Mahan, F. Farías, E. San Juan, N. Quiroga); Universidad San Sebastián, Concepción, Chile (J.P. Correa); Arizona State University, Tempe, Arizona, USA (R. Araya-Donoso); Universidad Viña del Mar, Viña del Mar, Chile (R. Campos-Soto); Universidad Andrés Bello, Santiago (C. Reyes-Olivares); Universidad de Concepción Campus Chillán, Chillán, Chile (D. González-Acuña)

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Reptiles have been described as natural hosts of some Trypanosomatid species transmitted by fly species (*7*). Although studies have shown how lizards could become experimentally infected by *T. cruzi* protozoa (*8*,*9*) and one showed an association between kissing bug infection and lizard abundance (*10*), most studies have not included reptiles as potential vertebrates involved in persistence and transmission of *T. cruzi* protozoa. To determine persistence of vectorborne infections in natural systems, it is essential to describe and characterize all host species directly (i.e., naturally infected species) or indirectly (i.e., vector blood meal sources) involved and evaluate their contribution to kissing bug infection.

We examined *T. cruzi* infection in 4 lizard species from the Pacific coast of Chile coexisting with kissing bug species. We also evaluated the competence of the most abundant lizard species to transmit *T. cruzi* protozoa to kissing bugs.

The Study

We conducted capturing and processing procedures after approval was obtained from the Institutional Committee for the Care and Use of Animals, University of Chile (permit 19275-FCS-UCH), the Agricultural and Livestock Service of Chile (permits 805/2018, 334/2019, and 4944/2019), and the National Forestry Corporation of Chile (permit 66/2018). We captured lizards at 3 sites in interior valleys and 2 Pacific islands in the arid–semiarid Mediterranean ecosystem of South America, where lizards naturally occupy the same microhabitats as kissing bugs (*Mepraia* spp.).

Depending on the species, we obtained blood from lizards in the field by using tail clipping and releasing (*Microlophus atacamensis*, Pacific Atacama racerunner) or in the laboratory by using tissue/organ extraction (*Liolaemus platei*, Plate's lizard; *Liolaemus fuscus*, dark lizard; *Garthia gaudichaudii*, Chilean marked gecko). We kept lizards for 1 week in the

[1]These authors contributed equally to this article.

Table. Lizard species from southwestern South America tested for *Trypanosoma cruzi* infection, 2011–2019*

Lizard species	Common name	No. infected/no. tested	Infected tissue or organ†	Competence (range)
Microlophus atacamensis	Pacific Atacama racerunner	11/13	Blood	ND
Liolaemus platei	Plate's lizard	18/18	Liver, spleen, stomach, intestine, lung, heart, fat, muscle, bone, gonad, blood	96.43 (50–100)
Liolaemus fuscus	Dark lizard	3/3	Liver, spleen, stomach, intestine, lung, heart, fat, muscle, bone, gonad, blood	ND
Garthia gaudichaudii	Chilean marked gecko	10/10	Liver, stomach, intestine, lung, heart, muscle, bone	ND

*Competence was assessed by real-time PCR on xenodiagnostic triatomine nymphs in *Liolaemus platei* lizard only. ND, not done.
†Not all types of organs were obtained for all sampled lizards (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/6/22-0079-App1.pdf).

laboratory in terraria containing food, water, and light. Before processing, Plate's lizards were subjected to xenodiangoses with 3 axenic second nymph stage kissing bugs of the endemic triatomine species *Mepraia spinolai*, obtained from a laboratory colony (Faculty of Science, University of Chile, Santiago, Chile). All engorged kissing bugs were kept in vials in a climate chamber (28°C, relative humidity 75%) for 40 days to enable *T. cruzi* multiplication in instances of infection. We then froze kissing bugs for 48 hours and extracted intestines and feces. After lizards were euthanized, we extracted their tissues (blood, bone, and fat) and organs (heart, stomach, intestine, lung, liver, spleen, and gonads) when possible. We stored all samples at −20°C.

We isolated whole genomic DNA from lizard and kissing bug samples by using the DNeasy Blood and Tissue Kit (QIAGEN, https://www.qiagen.com) according to manufacturer instructions. We performed real-time PCR specific for a nuclear segment of a repetitive genomic DNA sequence of *T. cruzi* DNA by using the primers Cruzi 1 and Cruzi 2 (*11*). The reaction was performed by using the Hot FIREPol EvaGreen qPCR Mix (Solis Biodyne, https://solisbiodyne.com), 0.4 μmol/L of primers, and 5 μL of template in a final volume of 20 μL. Cycling conditions were 95°C for 15 min, followed by 50 cycles at 95°C for 15 s, 65°C for 20 s, and 72°C for 20 s, which resulted in a default melting curve. We used water as a nontemplate control and DNA from a *T. cruzi* culture (Institute of Biomedical Sciences, University of Chile, Santiago, Chile) as a positive control. Each sample was analyzed in duplicate and considered positive when ≥1 of the replicates had specific amplification and a cycle threshold (Ct) value <40.0 (*12*).

We submitted ≥1 amplicons/sampled animal that had a band visualized by electrophoresis for sequencing of both strands by Macrogen (https://www.macrogen.com). We checked quality of sequences by inspection of each chromatogram,

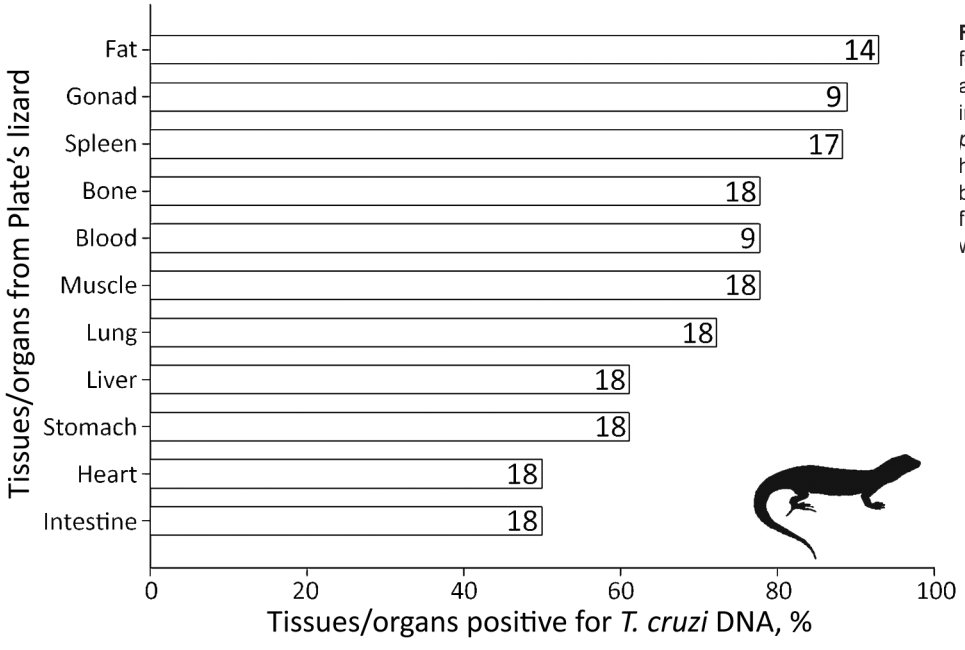

Figure 1. Tissues/organs tested for *Trypanosoma cruzi* infection and their percentages of infection in Plate's lizards (*Liolaemus platei*) in study of lizards as silent hosts of *T. cruzi*. Numbers in each bar indicate number of lizards from which a specific tissue/organ was extracted and tested.

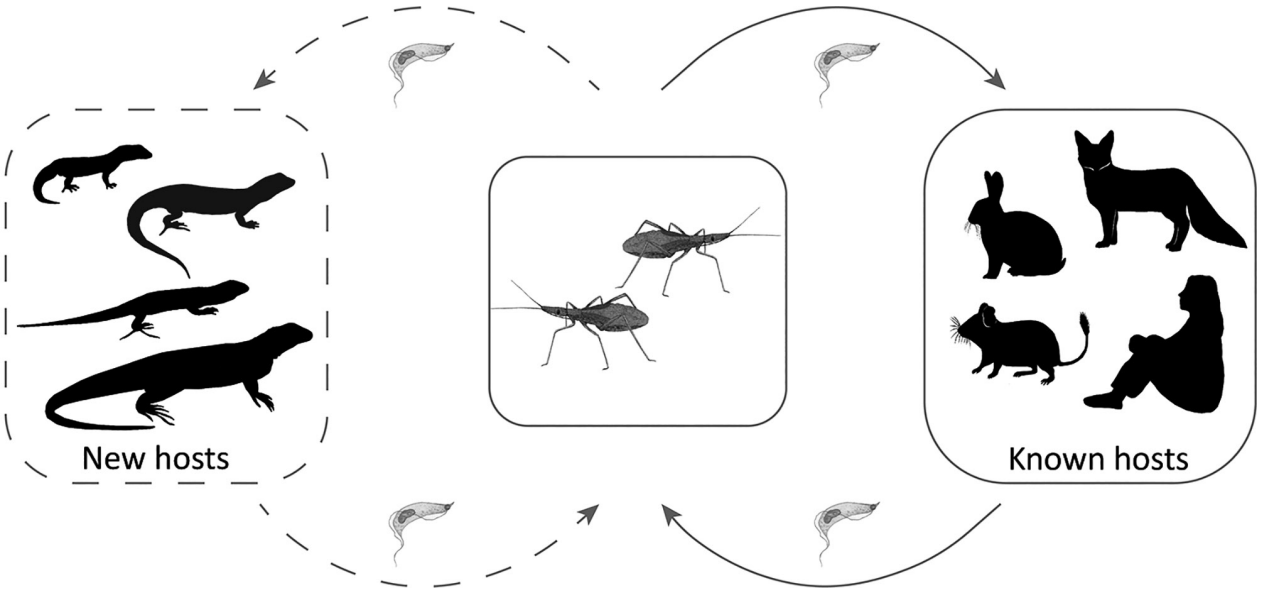

Figure 2. Transmission cycle of *Trypanosoma cruzi* parasites in the arid–semiarid Mediterranean ecosystem of South America. Solid lines indicate known *T. cruzi* transmission between mammal hosts and kissing bugs, and dashed lines indicate transmission between lizards (newly described hosts) and kissing bugs.

obtained the consensus sequence by using Bioedit 7.0.4.1 (*13*), and compared sequences with those available in GenBank. To assess if sequences were more similar to other trypanosomatid species, we compared sequences against a custom database that included other trypanosomatids with a full reference genome available (*T. brucei*, *T. conorhini*, *T. grayi*, *T. rangeli*, and *T. theileri*), excluding *T. cruzi*.

We detected *T. cruzi* infection in nearly all lizard species analyzed (Table), but not in all tested tissues or organs of *L. platei* lizards (Figure 1) and individual lizards of the other species (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/6/22-0079-App1.pdf). We detected *T. cruzi* DNA in 11/13 blood samples from the Pacific Atacama racerunner (mean ± SD Ct 36.84 ± 1.47). All 18 Plate's lizards had *T. cruzi* DNA (Ct 34.28 ± 2.57) in blood or heart when blood was not available). All 3 Dark lizards had a *T. cruzi* infection (Ct 32.42 ± 0.90) in blood or heart when blood was not available. All 10 Chilean marked geckos had *T. cruzi* DNA (Ct 32.30 ± 1.97) in heart.

Results from standard sequencing showed all samples matched *T. cruzi* sequences (98.66% mean identity and 99.29% mean query cover). We detected no match between the samples and available reference genomes from other trypanosomatids. We submitted sequences to GenBank (access nos. OM730035–75) and compiled complete BLAST analysis results (i.e., score, query cover, percentage of identity, and GenBank access number) (Appendix Table 2).

We tested Plate's lizard competence (i.e., mean percentage of kissing bugs becoming infected after feeding on infected lizards) for 14 lizards. Nearly all (27/28) triatomine nymphs (Table) that fed on *L. platei* lizards were infected (mean ± SD Ct 33.97 ± 1.00; Appendix Table 3).

Conclusions

We show that some lizard species from southern South America can be infected by *T. cruzi*; ≥1 species is a competent host for transmitting the protozoan to kissing bugs. This reptile group is part of the transmission cycle of Chagas disease (Figure 2), highlighting the role reptiles might have in other neglected vectorborne diseases, such as leishmaniasis and African trypanosomiasis (*7*). However, it is not clear whether lizards are infected with *T. cruzi* by kissing bug consumption, vectorborne transmission, or both.

It is crucial to assess the contribution of lizards to *T. cruzi* transmission in the sylvatic and domestic cycles of Chagas disease. Lizards might not only be competent hosts transmitting the protozoan to kissing bugs but can also be part of the diet of domestic carnivores (e.g., cats and dogs) (*14*), implying that transmission could be maintained by the presence of this new group of hosts being prey for domestic animals (*15*). Determining the threats associated with new host species and vulnerability of persons living in rural areas or in low-income countries will help evaluate transmission risk to humans and generate adequate control strategies.

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About the Author

Dr. Botto-Mahan is a professor at the University of Chile, Santiago, Chile. Her major research interests are the ecology of host–vector–parasite interactions and the ecology of *T. cruzi* of the semiarid-Mediterranean ecosystem of South America.

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Address for correspondence: Carezza Botto-Mahan, Laboratorio de Ecología de Interacciones, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, PO Box 653, Santiago, Chile; email: cbotto@uchile.cl

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Appendix

Appendix Table 1. Cycle threshold values of real-time PCR performed in tissues/organs tested for *Trypanosoma cruzi* infection in lizard species (*Microlophus atacamensis*, *Liolaemus platei*, *L. fuscus*, and *Garthia gaudichaudii*)*

Code	Species	Blood	Heart	Stomach	Intestine	Lung	Liver	Muscle	Bone	Spleen	Fat	Gonad
M1	*M. atacamensis*	35.192†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M2	*M. atacamensis*	–	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M3	*M. atacamensis*	35.406†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M4	*M. atacamensis*	38.868†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M5	*M. atacamensis*	34.719†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M6	*M. atacamensis*	36.797†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M7	*M. atacamensis*	–	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M8	*M. atacamensis*	38.623†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M9	*M. atacamensis*	36.889†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M10	*M. atacamensis*	37.966†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M11	*M. atacamensis*	38.469†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M12	*M. atacamensis*	35.955†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
M13	*M. atacamensis*	36.299†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
R1	*L. platei*	34.030	–	33.192	–	–	–	35.948	–	34.686	NT	NT
R2	*L. platei*	NT	–	31.976†	–	33.653†	33.937	30.339†	33.496	33.250	28.718†	NT
R3	*L. platei*	32.920	34.256	36.766	37.355	33.028	37.943	35.111	37.468	32.876	37.228	–
R4	*L. platei*	–	32.585	35.808	35.139	26.094†	33.012	32.445†	32.844	–	35.073	32.032†
R5	*L. platei*	NT	38.915	–	–	39.497	37.643	35.828	–	38.913	35.648	38.923
R6	*L. platei*	35.863	–	35.303†	–	34.250	39.268	38.099	33.482	34.438	36.690	34.974
R7	*L. platei*	32.171	37.234	35.180	37.264†	32.794	36.594	35.838	34.718	34.017	34.897	NT
R8	*L. platei*	–	–	36.194	38.747	–	36.194	36.616	31.577†	35.471	34.747	39.461
R9	*L. platei*	30.653	30.756†	–	34.985	37.226	35.923	32.127†	32.763	31.655	32.620	30.559†
R10	*L. platei*	36.440	34.424	35.054	35.988	32.990	–	32.909	31.727†	35.231	33.073	32.507†
R11	*L. platei*	NT	33.698	37.164	–	35.187	37.457	–	36.683	36.032	36.062	NT
R12	*L. platei*	NT	38.300	–	–	36.232	37.834	–	38.486	NT	NT	NT
R13	*L. platei*	32.342	–	34.846	37.441	–	37.703	36.323	35.463	35.988	33.904	33.869
R14	*L. platei*	NT	–	–	–	37.995	–	–	–	35.431	35.607	NT
R15	*L. platei*	NT	–	–	38.563	–	–	–	–	35.946	NT	NT
R16	*L. platei*	NT	33.492	–	–	–	–	36.515	34.635	35.765	33.609	37.233
R17	*L. platei*	NT	–	36.939	37.152	37.096	–	36.288	37.286	35.560	NT	NT
R18	*L. platei*	NT	–	–	–	36.200	–	38.389	36.760	–	–	NT
R19	*L. fuscus*	NT	31.851†	32.941	32.447	34.583	31.017	33.666	32.269	32.070	33.393	NT
R20	*L. fuscus*	33.463	30.064	30.531	30.640†*	29.592	30.392	30.153	30.728	30.078	29.709	32.191
R21	*L. fuscus*	31.958	30.822	32.965	34.295	31.455†	31.775	29.609	29.129	31.426	NT	30.34
R22	*G. gaudichaudii*	NT	30.357	31.382	30.738*	32.026	33.285	34.627	33.534	NT	NT	NT
R23	*G. gaudichaudii*	NT	32.436†	32.908	34.930	33.456	33.223	33.863	30.226	NT	NT	31.867
R24	*G. gaudichaudii*	NT	29.942†	31.970	34.882	31.090	33.232	30.424	30.128	NT	NT	NT
R25	*G. gaudichaudii*	NT	34.307	30.295	33.322	31.559†	31.038	33.171	30.996	NT	NT	NT
R26	*G. gaudichaudii*	NT	33.552	33.635	33.689	32.148†	32.284	32.577	31.736	NT	NT	NT
R27	*G. gaudichaudii*	NT	31.130	34.587	31.309†	31.458	30.303	29.250	28.874	NT	NT	NT
R28	*G. gaudichaudii*	NT	29.524	28.178	30.323†	29.536	30.577	31.402	30.749	NT	NT	NT

Code	Species	Blood	Heart	Stomach	Intestine	Lung	Liver	Muscle	Bone	Spleen	Fat	Gonad
R29	*G. gaudichaudii*	NT	32.881	32.939	32.913	30.878†	34.206	31.447	34.032	NT	NT	NT
R30	*G. gaudichaudii*	NT	35.271	37.863	36.830	34.066†	–	35.291	35.055	NT	NT	NT
R31	*G. gaudichaudii*	NT	33.612†	32.676	35.089	33.657	35.333	32.727	36.036	NT	NT	NT

*Samples were considered positive when at least one of the replicates had specific amplification with a cycle threshold value <40.0. NT, nontested organs; –, absence of infection.
†Sequenced samples.

Appendix Table 2. BLAST analysis results to identify *Trypanosoma cruzi* infection in tissues and organs of 4 lizard species (*Microlophus atacamensis*, *Liolaemus platei*, *L. fuscus*, and *Garthia gaudichaudii*)

Code	Sampled species	Tissue or organ	Sequence length, bp	GenBank access number	Score	Query cover, %	Identity, %	Species
M1	*M. atacamensis*	Blood	164	OM730035	281	100	97.56	*Trypanosoma cruzi*
M3	*M. atacamensis*	Blood	130	OM730036	230	100	98.47	*Trypanosoma cruzi*
M4	*M. atacamensis*	Blood	164	OM730037	281	100	97.58	*Trypanosoma cruzi*
M5	*M. atacamensis*	Blood	164	OM730038	303	100	100.0	*Trypanosoma cruzi*
M6	*M. atacamensis*	Blood	164	OM730039	298	100	99.39	*Trypanosoma cruzi*
M8	*M. atacamensis*	Blood	164	OM730040	298	100	99.39	*Trypanosoma cruzi*
M9	*M. atacamensis*	Blood	164	OM730041	298	100	99.39	*Trypanosoma cruzi*
M10	*M. atacamensis*	Blood	164	OM730042	298	100	99.39	*Trypanosoma cruzi*
M11	*M. atacamensis*	Blood	164	OM730043	292	100	98.78	*Trypanosoma cruzi*
M12	*M. atacamensis*	Blood	164	OM730044	292	100	98.78	*Trypanosoma cruzi*
M13	*M. atacamensis*	Blood	164	OM730045	276	100	96.99	*Trypanosoma cruzi*
R2C	*L. platei*	Stomach	171	OM730046	298	95	99.33	*Trypanosoma cruzi*
R2E	*L. platei*	Lung	161	OM730047	281	100	98.14	*Trypanosoma cruzi*
R2G	*L. platei*	Fat	168	OM730048	303	99	99.40	*Trypanosoma cruzi*
R2H	*L. platei*	Muscle	168	OM730049	302	98	99.40	*Trypanosoma cruzi*
R4E	*L. platei*	Lung	166	OM730050	307	100	100.0	*Trypanosoma cruzi*
R4H	*L. platei*	Muscle	166	OM730051	296	98	99.39	*Trypanosoma cruzi*
R4J	*L. platei*	Gonads	168	OM730052	307	98	100.0	*Trypanosoma cruzi*
R6C	*L. platei*	Stomach	166	OM730053	307	100	100.0	*Trypanosoma cruzi*
R7D	*L. platei*	Intestine	167	OM730054	307	99	100.0	*Trypanosoma cruzi*
R8I	*L. platei*	Bone	166	OM730055	279	98	97.55	*Trypanosoma cruzi*
R9B	*L. platei*	Spleen	167	OM730056	292	100	98.20	*Trypanosoma cruzi*
R9F	*L. platei*	Heart	166	OM730057	291	98	98.77	*Trypanosoma cruzi*
R9H	*L. platei*	Muscle	167	OM730058	276	98	96.95	*Trypanosoma cruzi*
R9J	*L. platei*	Gonad	169	OM730059	294	97	98.79	*Trypanosoma cruzi*
R9S	*L. platei*	Blood	166	OM730060	298	98	99.39	*Trypanosoma cruzi*
R10I	*L. platei*	Bone	167	OM730061	307	99	100.0	*Trypanosoma cruzi*
R10J	*L. platei*	Gonad	166	OM730062	307	100	100.0	*Trypanosoma cruzi*
R19F	*L. fuscus*	Heart	164	OM730063	287	100	98.17	*Trypanosoma cruzi*
R20D	*L. fuscus*	Intestine	164	OM730064	303	100	100.0	*Trypanosoma cruzi*
R21E	*L. fuscus*	Lung	164	OM730065	287	100	98.17	*Trypanosoma cruzi*
R22D	*G. gaudichaudii*	Intestine	164	OM730066	281	100	97.56	*Trypanosoma cruzi*
R23F	*G. gaudichaudii*	Heart	164	OM730067	303	100	100.0	*Trypanosoma cruzi*
R24F	*G. gaudichaudii*	Heart	164	OM730068	270	100	96.34	*Trypanosoma cruzi*
R25E	*G. gaudichaudii*	Lung	164	OM730069	289	100	97.77	*Trypanosoma cruzi*
R26E	*G. gaudichaudii*	Lung	164	OM730070	298	100	99.39	*Trypanosoma cruzi*
R27D	*G. gaudichaudii*	Intestine	164	OM730071	270	98	96.89	*Trypanosoma cruzi*
R28D	*G. gaudichaudii*	Intestine	164	OM730072	298	100	99.39	*Trypanosoma cruzi*
R29E	*G. gaudichaudii*	Lung	164	OM730073	287	100	98.17	*Trypanosoma cruzi*
R30E	*G. gaudichaudii*	Lung	164	OM730074	250	98	94.44	*Trypanosoma cruzi*
R31F	*G. gaudichaudii*	Heart	164	OM730075	281	100	97.56	*Trypanosoma cruzi*

Appendix Table 3. Cycle threshold values of real-time PCR performed in second-stage *Mepraia spinolai* nymphs used in xenodiagnosis assays to test for *Trypanosoma cruzi* infection in Plate's lizard (*Liolaemus platei*)*

	Xenodiagnostic triatomines			
Lizard code	Nymph 1	Nymph 2	Nymph 3	Competence, %
R1	34.939	33.791	34.770	100
R2	34.499	34.140	33.825	100
R3	32.569	35.288	31.803	100
R4	34.834	NF	NF	100
R5	33.756	33.110	NF	100
R6	34.051	34.922	NF	100
R9	35.792	NF	NF	100
R10	35.307	NF	NF	100
R11	35.015	33.829	NF	100
R14	33.705	33.613	NF	100
R15	32.526	NF	NF	100
R16	–	33.935	NF	50
R17	33.696	31.912	NF	100
R18	32.454	32.810	32.416	100

*NF, nymph not feeding on the lizard specimen or eaten by the lizard; –, absence of infection. Competence is shown as percentage of nymphs that became infected after feeding on an infected lizard specimen.