Lizards as Silent Hosts of Trypanosoma cruzi

Carezza Botto-Mahan,1 Juana P. Correa,1 Raúl Araya-Donoso, Francisca Farias, 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 zoontic 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).

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

Author affiliations: Universidad de Chile, Santiago, Chile (C. Botto-Mahan, F. Farias, 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)

DOI: https://doi.org/10.3201/eid2806.220079

1These authors contributed equally to this article.
laboratory in terraria containing food, water, and light. Before processing, Plate’s lizards were subjected to xenodiagnoses 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.

### 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).

![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.](image-url)
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.

**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.
Lizards as Silent Hosts of *Trypanosoma cruzi*

**Acknowledgments**

We thank Patricio Arroyo, Sandra Brito, Nora Peña, and Andrea Yáñez-Meza for providing field and laboratory support.

Daniel González-Acuña is deceased.

This study was supported by the Vicerrectoría de Investigación y Desarrollo of the Universidad de Chile (grant ENL01/21); grants ANID-FONDECYT 1221045 (C.B.-M. and J.P.C.) and 11181182 (J.P.C.); ANID Programa Becas - Doctorado Becas Chile 72200094 (R.A.-D.); and Universidad Viña del Mar Project FIIUVM-CTC-2211 (R.C.-S.).

**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.

**References**

1. World Health Organization. Descriptive note. Chagas disease (American trypanosomiasis). 2020 [cited 2022 Mar 28]. http://www.who.int/es/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)

2. Lidani KC, Andrade FA, Bavia L, Damasceno FS, Beltrame MH, Messias-Reason IJ, et al. Chagas disease: from discovery to a worldwide health problem. Front Public Health. 2019;7:166. https://doi.org/10.3389/fpubh.2019.00166

3. Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas’ disease in the United States. Clin Microbiol Rev. 2011;24:655–81. https://doi.org/10.1128/CMR.00005-11

4. Telleria J, Tibayrenc M, editors. American trypanosomiasis Chagas disease. One hundred years of Research. 2nd ed. Amsterdam: Elsevier; 2017.

5. Martínez-Hernández F, Oria-Martínez B, Rendón-Franco E, Villalobos G, Muñoz-García CI. *Trypanosoma cruzi*, beyond the dogma of non-infection in birds. Infect Genet Evol. 2022; 99:105239. https://doi.org/10.1016/j.meegid.2022.105239

6. Georgieva AY, Gordon ER, Weirauch C. Sylvatic host associations of Triatominae and implications for Chagas disease reservoirs: a review and new host records based on archival specimens. PeerJ. 2017;5:e3826. https://doi.org/10.7717/peerj.3826

7. Mendoza-Roldan JA, Modry D, Otranto D. Zoonotic parasites of reptiles: a crawling threat. Trends Parasitol. 2020;36:677–87. 10.1016/j.pt.2020.04.014 https://doi.org/10.1016/j.pt.2020.04.014

8. Ryckman RE. Epizootiology of *Trypanosoma cruzi* in southwestern North America. Part IV. lizards, laboratory hosts for *Trypanosoma cruzi* and Triatominae (Kinetocephalida: Trypanosomidae) (Hemiptera: Squama: Sauria: Gekkonidae, Iguanidae, Teiidae, Anguidae). J Med Entomol. 1965;2:93–5.

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

10. San Juan E, Araya-Donoso R, Sandoval-Rodríguez A, Yáñez-Meza A, Quiroga N, Botto-Mahan C. Lizards and rabbits may increase Chagas infection risk in the Mediterranean-type ecosystem of South America. Sci Rep. 2020;10:1853. https://doi.org/10.1038/s41598-020-59054-8

11. Piron M, Fissa R, Casamitjana N, López-Chejade P, Puig L, Vergés M, et al. Development of a real-time PCR assay for *Trypanosoma cruzi* detection in blood samples. Acta Trop. 2007;103:195–200. https://doi.org/10.1016/j.actatropica.2007.05.019

12. Yefi-Quinteros E, Muñoz-San Martín C, Bacigalupo A, Correa JP, Cattan PE. *Trypanosoma cruzi* load in synanthropic rodents from rural areas in Chile. Parasit Vectors. 2018;11:171. https://doi.org/10.1186/s13071-018-2771-2

13. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.

14. Doherty TS, Glen AS, Nimmo DG, Ritchie EG, Dickman CR. Invasive predators and global biodiversity loss. Proc Natl Acad Sci U S A. 2016;113:11261–5. https://doi.org/10.1073/pnas.1602480113

15. Gürtler RE, Cardinal MV. Reservoir host competence and the role of domestic and commensal hosts in the transmission of *Trypanosoma cruzi*. Acta Trop. 2015;151:32–50. https://doi.org/10.1016/j.actatropica.2015.05.029

Address for correspondence: Carezza Botto-Mahan, Laboratorio de Ecología de Interacciones, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Nuñoa, PO Box 653, Santiago, Chile; email: cbotto@uchile.cl
## Lizards as Silent Hosts of *Trypanosoma cruzi*

### 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 |
|------|--------------------------|-------|-------|---------|-----------|------|-------|--------|------|--------|-----|-------|
| R1   | *L. platei*              | 34.030| –     | 33.192  | –         | –    | –     | –      | –    | –      | –   | –     |
| R2   | *L. platei*              | NT    | 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| 39.288| 38.099  | 33.482| 34.438  | 34.690| 34.974|
| R7   | *L. platei*              | 32.171| 37.234| 35.160  | 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| 30.305  | 36.062| NT    |
| R12  | *L. platei*              | NT    | 38.300| –       | –         | 36.232| 37.834| –       | 38.486| NT      | NT   | NT    |
| R13  | *L. platei*              | 32.942| –     | 34.846  | 37.441    | 37.703| 36.323| 35.463  | 35.988| 33.904  | 33.869| –     |
| R14  | *L. platei*              | NT    | –     | 37.995  | –         | –     | –     | –       | –     | –       | –   | –     |
| R15  | *L. platei*              | NT    | –     | 38.563  | –         | –     | –     | –       | –     | –       | 35.946| 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| –       | –     | –       | –   | –     |
| 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  | 30.34| NT    |
| 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      | 31.867| NT    |
| 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    | 31.599†| 31.038| 33.171  | 30.996| NT      | NT   | NT    |
| R26  | *G. gaudichaudii*        | NT    | 33.552| 33.635  | 33.689    | 32.149†| 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 | 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   | 96             | 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. gaudichaudi*| Intestine      | 164                 | OM730066             | 281   | 100            | 97.56       | *Trypanosoma cruzi* |
| R23F | *G. gaudichaudi*| Heart          | 164                 | OM730067             | 303   | 100            | 100.0       | *Trypanosoma cruzi* |
| R24F | *G. gaudichaudi*| Heart          | 164                 | OM730068             | 270   | 100            | 96.34       | *Trypanosoma cruzi* |
| R25E | *G. gaudichaudi*| Lung           | 164                 | OM730069             | 289   | 100            | 97.77       | *Trypanosoma cruzi* |
| R26E | *G. gaudichaudi*| Lung           | 164                 | OM730070             | 298   | 100            | 99.39       | *Trypanosoma cruzi* |
| R27D | *G. gaudichaudi*| Intestine      | 164                 | OM730071             | 270   | 98             | 96.89       | *Trypanosoma cruzi* |
| R28D | *G. gaudichaudi*| Intestine      | 164                 | OM730072             | 298   | 100            | 99.39       | *Trypanosoma cruzi* |
| R29E | *G. gaudichaudi*| Lung           | 164                 | OM730073             | 287   | 100            | 98.17       | *Trypanosoma cruzi* |
| R30E | *G. gaudichaudi*| Lung           | 164                 | OM730074             | 250   | 98             | 94.44       | *Trypanosoma cruzi* |
| R31F | *G. gaudichaudi*| Heart          | 164                 | OM730075             | 281   | 100            | 97.56       | *Trypanosoma cruzi* |

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 gaudichaudi*)

*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 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*)

| Lizard code | Xenodiagnostic triatomines | Competence, % |
|-------------|----------------------------|--------------|
|             | Nymph 1                    | Nymph 2 | Nymph 3 |                  |
| 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         | –                          | 33.925   | 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.*