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Short communication

Trypanosoma cruzi infection in the sylvatic kissing bug *Mepraia gajardoi* from the Chilean Southern Pacific Ocean coast

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Abstract

The Southern Pacific Ocean coast has been traditionally considered a non-active transmission area for Chagas disease. In this report, we show evidence of *Trypanosoma cruzi* infection in the sylvatic kissing bug *Mepraia gajardoi* from the northern Chilean coast.

Keywords: Chagas disease; Insect vector; Reduviidae; Triatominae

Chagas disease is a serious human parasitic disease in America that is caused by the flagellate protozoan *Trypanosoma cruzi*, and transmitted by blood-sucking insects of the subfamily Triatominae (Hemiptera: Reduviidae) (Lent and Wygodzinsky, 1979). Detection of *T. cruzi* can be performed through different methodologies, but PCR-based detection from feces/urine of reduviid bugs and mammal blood samples is one of the most efficient techniques (Moser et al., 1989; Breniere et al., 1992; Botto-Mahan et al., 2005a). In Chile, it has been reported that the vectors *Triatoma infestans* and *Mepraia spinolai* propagate *T. cruzi* in the domestic/peridomestic and sylvatic/peridomestic habitats, respectively (Lent and Wygodzinsky, 1979; Lent et al., 1994). Currently, *T. infestans* has been reported as controlled (Lorca et al., 2001), however, *M. spinolai* is frequently found in corrals of domestic animals, stony hills and rock crevices of arid and semiarid zones of northern Chile (Botto-Mahan et al., 2002, 2005a,b).

Mepraia gajardoi is a triatomine species recently described as a separate species from *M. spinolai*, primarily on the basis of karyotype differences (Frías et al., 1998; Galvao et al., 2003; Pérez et al., 2004). It is distributed on the northern coast of Chile between 18° and 26°S (Frías et al., 1998). According to Frías et al. (1998), females are micropterous and males brachypterous which overall color is black. This coastal species is found associated to nests of seabirds and caves of reptiles (Flores et al., 1977; Frías et al., 1998). Sagua et al. (2000) reported that an insular population of *M. spinolai* from the Southern Pacific Ocean coast fed mainly on seabirds, marine mammals and reptiles, but no *T. cruzi* infection was detected. On the other hand, there is no published information about the alimentary profile and status of *T. cruzi* infection in *M. gajardoi*. Assessment of *T. cruzi* infection in wild triatomines is relevant when examining the epidemiological importance of a potential vector species, because it may have consequences for disease control. In this study, we examine the status of *T. cruzi* infection on *M. gajardoi* individuals by PCR-based detection, and genotyping to describe the *T. cruzi* lineage(s) harbored by infected insects.

The taxon *T. cruzi* is divided into two groups, TCI and TCII. *T. cruzi* I corresponds to zymodeme Z1, and *T. cruzi* II has been proposed to be subdivided in five sublineages, one corresponding to Z2 (TCIIb), another to Z3 (TCIIa), and the hybrids TCIIc, TCIId and TCIIe (Brisse et al., 2001). TCI and TCIIb represent the ancestral *T. cruzi* lineages, and genetic recombination of these two entities it is thought to have generated the hybrid and homozygous TCIIa and TCIIc sublineages. A more recent TCIIc/TCIIb fusion gave rise to the largely heterozygous TCIId and TCIIe sublineages (Westenberger et al., 2006). In Chile, the TCI and TCIIb are distributed singly and combined among

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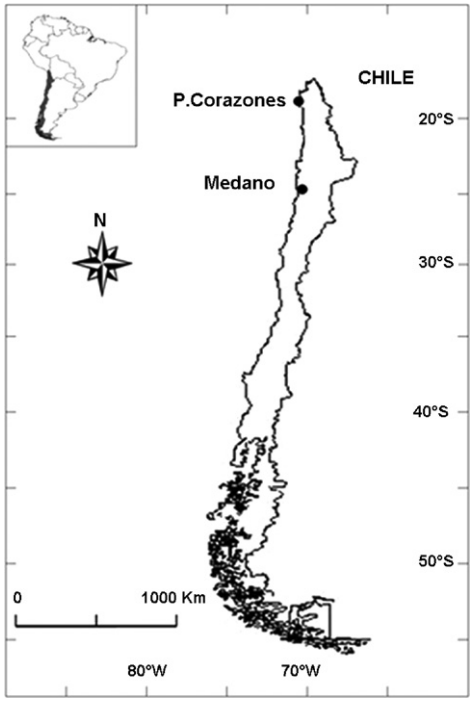

Fig. 1. Map of Chile indicating the location of *Mepraia gajardoi* collecting sites on the Pacific Ocean coast (Playa Corazones and Médano).

wild mammals, a finding that is consistent with the observation that these two sublineages are the most pure and ancestral ones (Rozas et al., 2007). Molecular epidemiology of chronically infected human patients from the same geographic area (29°02′–32°16′S, inland localities) showed that the most common sublineages in blood are TCI and TCIIb also (Coronado et al., 2006).

Individuals of *M. gajardoi* were collected during the austral spring of 2004 and 2005 from two coastal zones of northern Chile: Playa Corazones (18°31′37″S, 70°19′18″W) and Médano (24°37′16″S, 70°33′34″W) (Fig. 1). The study sites are under a coastal desert climatic regime and they receive less than 2 mm of rain per year (di Castri and Hajek, 1976). They present low plant cover and correspond to beaches with a mixture of rocks (with heights from 0.2 to 3.0 m), pebbles, cobblestones, and sand (Vidal et al., 2002). Lizards (*Microlophus atacamensis* and *M. quadrivittatus*), seabirds (e.g., *Larus dominicanus*, *Larus modestus*, *Cathartes aura*) and wild rodents (e.g., *Phyllotis* sp., *Mus* sp.) inhabit the collecting sites (Donoso-Barros, 1966; Ortíz, 1980; Acuña and Sepúlveda, personal observation). In addition, a colony of South American sea lions (*Otaria flavescens*) is established at Playa Corazones. Insects were collected manually during 3 consecutives days from 12:00 to 16:00 h by one and four people in Playa Corazones and Médano, respectively. Captured bugs were carried to the laboratory and kept separately inside a climate chamber at 27 °C, 70% RH and 14-h light:10-h dark photocycle. Species diagnosis was performed following Frías et al. (1998) description.

For molecular analyses, we obtained intestinal content from the entire intestine of bugs through abdominal extrusion and examined in a light microscope (Schenone, 1999). Intestinal contents were mixed with 200 μl of PBS buffer, centrifuged at 10,000 × *g*, and frozen at −20 °C for PCR assay. A sample of 1–5 μl of this crude 10 min-preboiled extract was used as DNA template. Intestinal contents were free of fresh blood, therefore, no DNA extraction was required. PCR was performed as previously reported, using primers 121 and 122 directed to amplify the variable region of minicircle kinetoplast DNA (Wincker et al., 1994; Campos et al., 2007). Each run included positive and negative controls. Samples were tested in triplicate, and a bug was considered positive when at least two out of the three assays turned out positives. A 330 base pairs (bp) product represents a positive assay. For confirmation, Southern analyses were performed using 10 μl of each PCR assay. PCR products were electrophoresed, transferred onto Hybond N+ nylon membranes (Amersham), and cross-linked by ultraviolet light for DNA fixation. After transferring PCR products, membranes were pre-hybridized for at least 2 h at 55 °C, and finally hybridized with total *T. cruzi* kinetoplast DNA labeled with P^{32} (1×10^6 cpm/membrane) as probe. After hybridization, membranes were washed three times for 30 min each with 2× SSC, 0.1% SDS at 55 °C, and later exposed in the Molecular Imager FX (Bio-Rad). For *T. cruzi* genotyping, four different *T. cruzi* clones (sp 104cl1, CBBcl3, NRcl3, and v195cl1) corresponding to TCI, TCIIb, TCIId, and TCIIe, respectively, were used to generate specific probes. Construction of minicircles probes was performed as previously described (Veas et al., 1991), and radiolabeled as described for total kinetoplast DNA.

The two collecting sites presented low population densities. Overall, 17 *M. gajardoi* individuals (mainly III and IV nymphal stages) were captured (Playa Corazones: $N = 10$; Médano: $N = 7$). Light microscopy and PCR analyses detected two *T. cruzi*-positive insects from Médano. This figure represents 28.5% of infection in Médano population and 11.8% in all of the analyzed insects. Results from genotyping indicate that *M. gajardoi* is singly infected with TCIIb and one unidentified *T. cruzi* genotype (Fig. 2). In Fig. 2, we also show some *M. spinolai* samples infected with TCI and TCIIb as positive controls.

In this study, we show molecular evidence indicating that the Chilean endemic kissing bug *M. gajardoi* is naturally infected with *T. cruzi*. In addition, we determine that TCIIb (CBB c13) and one still unidentified genotype different from the clones used here are circulating in some areas of the Southern Pacific Ocean coast of northern Chile. It is worth to point out that in this study we use direct characterization method by PCR and Southern analysis. This allows detection and genotyping without parasite isolation and cultivation, procedures that could select some *T. cruzi* clones from a mixture (Campos et al., 2007).

Overall, the percentage of *M. gajardoi* nymphs infected was relatively low (11.8%) if we compare with *M. spinolai*, in which *T. cruzi* infection can reach up to 46.2% in some areas of central Chile (Botto-Mahan et al., 2005a). According to our results, infection would not be widely spread because only bugs from the southern collecting site were positive to the protozoan parasite. One explanation to this finding could be that blood donor vertebrates present in coastal areas may be refractory or dead-end hosts to maintain and reproduce *T. cruzi* parasites, due to their immune control systems (Teixeira et al., 2006). In both collect-

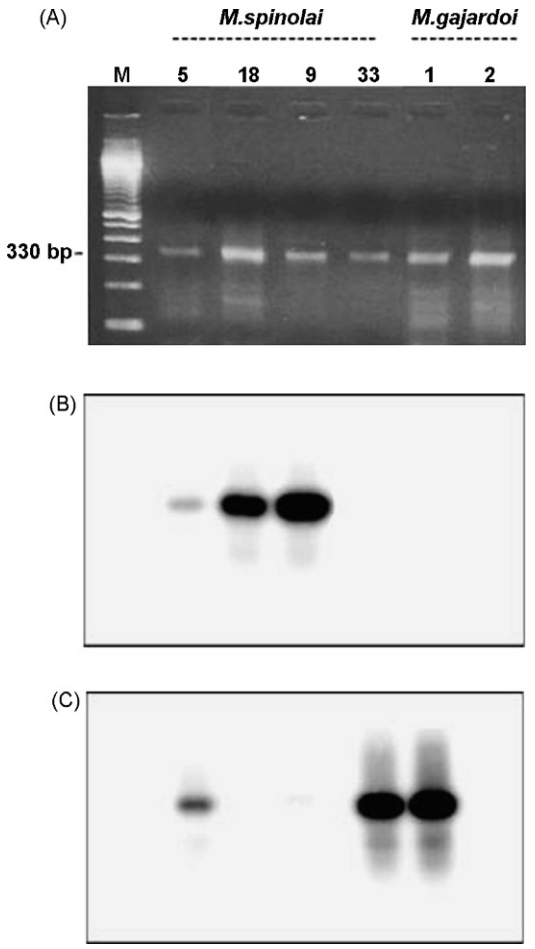

Fig. 2. (A) *Trypanosoma cruzi* amplicons stained with ethidium bromide. Hybridization profiles obtained with genotype specific probes corresponding to (B) TCI (sp104cl1), and (C) TCIIb (CBBc13). M: marker, *Mepraia spinolai* samples (5, 9, 18, and 33) represent positive controls, and *Mepraia gajardoi* samples (1 and 2) are the only PCR positives. A 330 base pairs (bp) product represents a positive assay.

ing sites, reptiles and marine birds are among the most frequent vertebrates found (Donoso-Barros, 1966; Acuña and Sepúlveda, personal observation). It is well known that all birds present natural resistance to *T. cruzi* infection (Kierszenbaum et al., 1976). As for the reptiles, Neves and de Castro (1970) reported that *Tropidurus* lizards infected with *T. cruzi* through ingestion of triatomines, intraperitoneal inoculation of infected mouse blood and feces of infected triatomines showed no evidence of infection by repeated blood examination and xenodiagnostic (but see Ryckman, 1965; Lugo-Hernández, 1973).

No previous studies had examined the status of *T. cruzi* infection in *M. gajardoi*, therefore, this study shows the first evidence of infection in the endemic Chilean triatomine *M. gajardoi*.

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