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SHORT COMMUNICATION

Bloodmeal-stealing in wild-caught *Mepraia spinolai* (Hemiptera: Reduviidae), a sylvatic vector of *Trypanosoma cruzi*

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Abstract. 1. Blood-feeding bugs in the Triatominae are the vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. *Mepraia* is a sylvatic genus endemic to Chile that transmits *T. cruzi* in the wild cycle.

2. Bloodmeal-stealing (or 'cleptohaematophagy') is the stealing of a bloodmeal from one bug's gut by another, usually conspecific bug. Bloodmeal-stealing can result in horizontal transmission of *T. cruzi* between triatomines; so far, it has been reported only in laboratory-reared bugs.

3. We performed short laboratory experiments to test whether bloodmeal-stealing occurs between wild-caught *Mepraia spinolai* nymphs. Successful bloodmeal-stealing was observed in one out of 17 trials (2/102 bugs). Even though bloodmeal-stealing was not frequent in wild-caught *M. spinolai*, this behaviour might contribute to explaining the maintenance of wild *T. cruzi* cycles.

Key words. Chile, cleptohaematophagy, *Mepraia*, Triatomine behavior, *Trypanosoma cruzi*.

Introduction

Triatomines, also known as kissing bugs, are the main vectors of *Trypanosoma cruzi*, a protozoan parasite causative of Chagas disease. Bloodmeal-stealing and coprophagy have proven to be effective for direct *T. cruzi* transmission under laboratory conditions (Schaub, 1988). Bloodmeal-stealing (or cleptohaematophagy) denotes a particular feeding behaviour observed in laboratory colonies of triatomines, which consists of the stealing of the blood meal between conspecifics by piercing and draining of the intestinal content of a recently engorged triatomine (Ryckman, 1951). This behaviour has been reported in many species within the subfamily Triatominae, including the genera *Triatoma*, *Rhodnius* and *Belminus* (Sandoval *et al.*, 2000; Otálora-Luna *et al.*, 2015). If blood ingested with trypomastigotes is sucked immediately by a bloodmeal-stealer, trypanosomes could follow the same infection route described for the donor bug (Schaub, 1988; Kollien & Schaub, 1997); however, this would depend on mammal host parasitemia. When a triatomine steals from another, it could be stealing from the Malpighian tubules, anterior gut, midgut or hindgut; therefore, bloodmeal-stealers could ingest a significant amount of trypanosomes. The main way triatomines track their hosts is by their acute ability to sense heat sources. Through thermoreceptors localised at the antennae, they can precisely locate even a single blood vessel beneath its host skin to pierce (Ferreira *et al.*, 2007). Under certain laboratory conditions, it is possible to observe triatomines attempting to pierce almost any warm object within their detection range, making it clear that they are quite responsive to heat and that this stimulus is enough to trigger a bite (Guerenstein & Lazzari, 2009). Thus bloodmeal-stealing is probably just an accidental phenomenon when the access to suitable spots to feed on hosts is prevented by space restriction. Although bloodmeal-stealing has been described for several laboratory-reared triatomine bugs, this behaviour has not been reported for wild-caught bugs.

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Mepraia is a triatomine genus endemic to Chile that inhabits arid and semiarid-Mediterranean climatic conditions in mainland, coastal, and insular zones, where *Mepraia* species transmit *T. cruzi* in the wild cycle (Campos-Soto *et al.*, 2016, 2020; San Juan *et al.*, 2020). *Mepraia spinolai* is a diurnal species whose habitats include rock crevices, rocky outcrops, caves, terrestrial bromeliads associated with burrows and nests of native rodents, on which it preferentially feeds, but it usually exhibits an opportunistic feeding behaviour, preying on several mammals, reptiles, and even birds (Canals *et al.*, 2001; Chacón *et al.*, 2016). The aim of this study is to test whether bloodmeal-stealing also occurs between wild-caught *M. spinolai* nymphs.

Materials and methods

One hundred and sixty two *M. spinolai* nymphs were manually collected from two field sites located in the semiarid-Mediterranean ecosystem of Chile, 100 nymphs from Las Chilcas Hill (LCH hereafter, Valparaiso Region; −32.8536 S, −70.8706 W) and 62 nymphs from Las Chinchillas National Reserve (LCNR hereafter, Coquimbo Region; −31.5083 S, −71.1055 W). Nymphs were transported to the laboratory and kept under optimal conditions inside a climate chamber at 26–27 °C, 65–70% relative humidity, 14 h:10 h light: dark cycle. All captured nymphs showed evidence of starvation by the shape of their abdomens, i.e. they were flat or slightly concave (Estay-Olea *et al.*, 2020). Two days after capture, six fourth and fifth stage nymphs were allowed to feed on an anesthetised laboratory mouse (*Mus musculus*) placed in a rectangular glass container (19 × 10 cm) for 30 min. The mouse (weight ≥ 20 g) was anesthetised with an intraperitoneal injection of sodium thiopental (30–40 mg kg^{-1}). Nymph feeding behaviours were individually recorded by direct observation. This experiment was replicated 17 times (seven trials for LCH-nymphs and 10 trials for LCNR-nymphs), each time with a different mouse and its own set of nymphs to avoid pseudo-replication. Experiments were performed between 11:00 and 16:00 in a laboratory with artificial light and at room temperature between 25 and 28 °C. Successful bloodmeal-stealing (or cleptohaematophagy) was recorded when a nymph managed to pierce the abdominal integument another recently fed nymph, with a visually detectable increase in the abdominal volume of the blood-stealer bug and a decrease in the abdomen volume of the donor bug. The experimental procedure followed the international recommendations for laboratory mammals (Schapiro & Everitt, 2006), and the experimental protocol for the use of mice was reviewed and approved by the Ethics Committee of the Universidad de Chile (No. 17074 FCS-UCH).

Results and Discussion

Successful bloodmeal-stealing (or cleptohaematophagy) was observed only once, corresponding to 5.9% of the trials. This behaviour occurred in three nymphs; one was feeding directly on the mouse and the other two were pricking its abdomen (Fig. 1a). In that specific trial, all the nymphs were crowded towards the mouse's head in comparison to the other trials in which nymphs were distributed around the mouse's body (Fig. 1b). Successful bloodmeal-stealing events can be seen in the audio-visual material at Data Availability.

While bloodmeal-stealing has been reported in three genera (*Triatoma*, *Rhodnius*, *Belminus*, and *Dipetalogaster*) of the subfamily Triatominae, and described as an effective mechanism of *T. cruzi* transmission (Schaub, 1988), it had not been described in wild-caught sylvatic triatomines such as *M. spinolai*. In this study, we showed evidence of bloodmeal-stealing during the feeding of *M. spinolai* nymphs brought from the field. In most of the trials when nymphs were feeding, they were distributed around the entire body of the mouse (Fig. 1b), and bloodmeal-stealing was observed when nymphs were crowded.

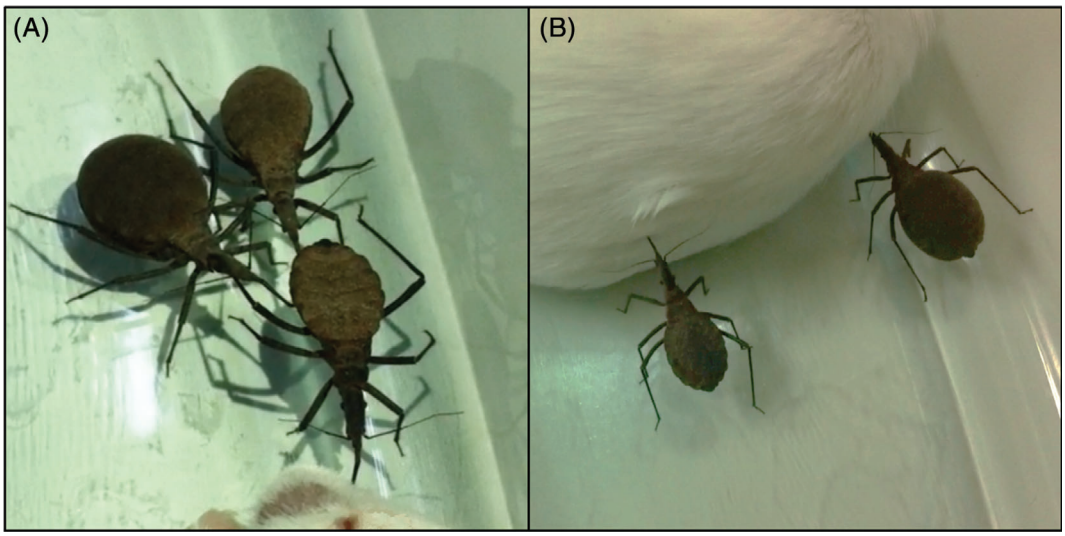

Fig. 1. (a) Bloodmeal-stealing in a trial where nymphs were crowded towards the mouse's head. (b) Trial where nymphs were distributed around the mouse's body. Photographic credit: Rubén Garrido. [Colour figure can be viewed at wileyonlinelibrary.com].

This observation might suggest that bloodmeal-stealing can be induced by nymph crowding in confined spaces (e.g. rodent burrows under natural conditions). Lack of space to reach the blood source might increase the probability of bloodmeal-stealing. This would be more relevant when the hosts are small at high triatomine densities. It has been shown that specimens of *Rhodnius prolixus* can cool recently ingested blood and thus prevent bloodmeal-stealing (Lazzari *et al.*, 2018). This cooling system has not been described in *Mepraia*, but if that were the case, the bloodmeal-stealing observed in *Mepraia* could be explained by the heat emitted by the host when the access to suitable spots to feed on hosts is prevented by space restriction. Future studies should include temperature control to establish the underlying mechanism in bloodmeal-stealing by *Mepraia* species.

In conclusion, in this study we report the occurrence and frequency of bloodmeal-stealing among wild-caught *M. spinolai*, a sylvatic vector of *T. cruzi*.

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Author contributions

R. Garrido, R. Campos-Soto and C. Botto-Mahan designed the study; R. Garrido and N. Quiroga collected data; R. Garrido, R. Campos-Soto and C. Botto-Mahan wrote the paper; and all authors read and approved the manuscript.

Data availability statement

The audio-visual data that support the findings of this study are openly available in Mendeley Data at https://doi.org/10.17632/nwwwwdyjr6.1 (Garrido *et al.*, 2020).

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