

## Trophobiosis between ants and *Eurystethus microlobatus* Ruckes 1966 (Hemiptera: Heteroptera: Pentatomidae) a cryptic, gregarious and subsocial stinkbug

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In this study we describe trophobiosis between ants and *Eurystethus microlobatus* (Hemiptera: Pentatomidae) at a highland quartzite rocky outcrop in southern Espinhaço Range, southeastern Brazil. We found stinkbugs exclusively on the mistletoe *Psittacanthus robustus* (Loranthaceae). The stinkbug species is gregarious, forming dense clusters, with females guarding eggs and young nymphs. In addition, this species presents cryptic colouration and most individuals were located on roots and shoots, camouflaged within the bark. *Eurystethus microlobatus* produced honeydew by day and night, and four ant species attended aggregations: *Camponotus rufipes*, *Camponotus crassus*, *Camponotus melanoticus* and *Cephalotes pusillus*. *Camponotus rufipes* was the most frequent and aggressive, and the only species to attend the stinkbugs by day and night. This is the first documented case of trophobiosis between true bugs and ants in the New World.

**Keywords:** Formicidae; Heteroptera; camouflage; maternal care; mistletoe; mutualism; Serra do Cipó

### Introduction

Trophobiosis is a mutualistic interaction in which organisms excretes droplets of honeydew, an exudate rich in carbohydrates and amino acids, which is used as a food source by partners that provide protection to the trophobionts against their natural enemies (Buckley 1987; Styrsky and Eubanks 2007). Trophobiosis between ants and phloem-feeding hemipterans like aphids, whiteflies, mealybugs, scales, leafhoppers and treehoppers are widespread worldwide, and represent the classical example of this biotic interaction (Delabie 2001). Conversely, trophobiosis between ants and true bugs is unusual, and has been reported for only a few species in tropical forests from southeastern Asia and Africa.

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Ants interacting with true bugs were first reported for *Coptosoma* sp. (Plataspidae) in Ceylon (Green 1900) and for *Coptosomoides myrmecophylus* (Plataspidae) in Sumatra (China 1931). Maschwitz and Klinger (1974) observed an association between ants and coreid bug (Coreidae) in Malaysia. Later, Maschwitz et al. (1987) confirmed this species as *Hygia cliens*, and described two new cases of trophobiosis between ants and the coreids *Cloesmus* sp. and *Notobitus affinis*. Maschwitz et al. (1987) also described two new plataspidae bug species attended by ants, *Tropidotylus servus* and *Tropidotylus minister* (Plataspidae), also in Malaysia. Waldkircher et al. (2004) described a new plataspidae species, *Tetrisia vacca*, which produces honeydew and is tended by ants in peninsular Malaysia. More recently Blüthgen et al. (2006) reported trophobiotic interactions between ants and two coreids and one plataspidae species in the rainforest of Borneo. However, the best documented case of mutualism between ants and true bugs was studied in Cameroon, where the honeydew-producing bug *Catantopidae rugosa* (Plataspidae) is attended by ants that provide full day protection against a predator coccinellid beetle larva and a parasitoid wasp (Dejean et al. 2000, 2002; Gibernau and Dejean 2001).

Pentatomidae is one of the largest families in suborder Heteroptera, order Hemiptera, with worldwide distribution and comprising more than 4100 species popularly known as stinkbugs (Schuh and Slater 1995; Grazia and Schwertner 2008). Most stinkbugs feed on plants and carry symbiont bacteria in the gut (Schuh and Slater 1995; Prado and Almeida 2009). Selective pressure exerted by predators and parasites promoted the evolution of cryptic colouration (Schuh and Slater 1995), gregariousness (Lockwood and Story 1986), subsocial behaviour or maternal care (Tallamy and Schaefer 1997), and the production of allomone volatiles (Borges and Aldrich 1992) among Heteroptera, and are described for several stinkbug species. Nevertheless, true bug protection by ants is poorly explored and despite the great diversity of Formicidae and Heteroptera clades in the New World, mutualistic interactions involving these insects are still speculative (Hölldobler and Wilson 1990). Stahel (1954) observed *Lincus spathuliger* (Pentatomidae, Discocephalinae) feeding on the roots of coffee trees and interacting with *Pheidole* sp. in Surinam, but did not determine the nature of this interaction (Waldkircher et al. 2004).

The subfamily Discocephalinae (Hemiptera, Pentatomidae) is restricted to the Neotropical region, comprising approximately 270 phytophagous species in 70 genera (Grazia and Schwertner 2008), including *Eurystethus* Mayr with 18 poorly known species (Ruckes 1958, 1966; Becker 1966). *Eurystethus microlobatus* Ruckes 1966 is the smallest species in the genus, but there is no information except the original description based on a single male specimen from Surinam (Ruckes 1966). We found this stinkbug in a highland rocky outcrop, southeastern Brazil, associated with Neotropical mistletoe and interacting with different species of ants. In this paper we described the strategies of *E. microlobatus* to exploit host plants and cope with natural enemies, including trophobiosis with ants.

## Materials and methods

### *Study area and host plant*

We conducted this study between December 2007 and May 2008 in Reserva Particular do Patrimônio Natural (RPPN) Vellozia, Serra do Cipó, Minas Gerais, Brazil

(43°35' W, 19°17' S). The study site varies from 1230 to 1300 m above sea level. Local vegetation is characteristic of *campos rupestres*, with a mosaic of areas of rocky outcrops with small tortuous trees and bushes sparsely distributed with a developed herbaceous stratum and open fields dominated by grasses. *Campos rupestres* along the Espinhaço Range have highly diversified flora, with high levels of endemism and a large number of threatened species (Giulietti et al. 1997). These ecosystems are inserted in the Cerrado Biome (Neotropical Savanna), and related to areas higher than 1000 m with quartzite rock outcrops, where soils are shallow, acid, sandy and nutrient-poor (Ribeiro and Fernandes 2000). The woody mistletoe *Psittacanthus robustus* Mart. (Loranthaceae) is widely distributed in the Cerrado biome in central Brazil, also occurring in forests edges in Venezuela (Rizzini 1980). This hemiparasite evolved specificity for host within plants from families Vochysiaceae and Melastomataceae (Monteiro et al. 1992). In the study site these mistletoe grow on branches of four tree species: *Vochysia thyrsoidea* and *Qualea cordata* (both Vochysiaceae), and *Trembleya laniflora* and *Miconia ferruginata* (both Melastomataceae).

### *Stinkbug distribution on host plants*

During December 2007 and January 2008 we searched for *E. microlobatus* on mistletoes located in a 20-ha plot. For each host plant we recorded if the plant was reproductive or not and its size, determined by the height of the largest branch and the diameter at the base of the haustorial connection with the host plant. There was a correlation between these variables ( $R^2 = 0.53$ ,  $p < 0.0001$ , log-transformed data) and in the following analyses we used height as a measure of plant size. For the host plants we determined presence and number of ovipositions, nymphs and adults by carefully searching the whole plant and its host. Aggregation size (hereafter) is the number of adults and nymphs found on individual mistletoe. For 70 randomly selected aggregations we determined the location of each stinkbug on hosts, classifying plant microhabitats in: haustorial root, primary shoots (those connected to haustorial root), secondary shoots (ramifying from primary shoots), leaves, and reproductive shoot (inflorescences). Counts were performed during the day when stinkbugs are mostly motionless. To visualize nymphs and areas of oviposition we lightly displaced adult stinkbugs using a stick.

### *Trophobiotic interactions*

To record the behaviour of stinkbugs and tending ants we made scattered observations on 98 aggregations from December 2007 to April 2008. Observations of each aggregation varied from 10 minutes to 4 hours for a total period of 30 hours, including daily and nocturnal observations. To evaluate variation number and visitation rates by ants (Hymenoptera, Formicidae) on a round-the-clock basis, we tagged 50 mistletoes with aggregations varying in size on 50 different plants. Besides *E. microlobatus*, the host plant is very attractive to other hemipterans and treehoppers, aphids and soft scale insects were observed together with *E. microlobatus* on the same plant being attended by ants (T.J. Guerra, unpublished results). Because different trophobiont species could compete for ants, plants simultaneously inhabited by other honeydew-producing hemipterans were excluded to avoid bias on ant counting. Between 3 and

4 May 2008 we conducted eight consecutive ant count sessions at 3-hour intervals for each plant, four counts during the day (6–7.00 h, 9–10.00 h, 12–13.00 h, 15–16.00 h) and four counts at night (18–19.00 h, 21–22.00 h, 0–1.00 h, 3–4.00 h). Four groups of aggregations were evaluated by four independent observers simultaneously through the entire day. Each observer took approximately 1 hour to inspect all aggregations in each group, with a 2-hour interval between counting sessions. Ant counts consisted of careful 1-minute snapshot inspections of aggregations. We counted only those ants that were close to or walking on aggregations and actively touching stinkbugs with their antennae and collecting honeydew droplets from stinkbug anus and plant surfaces (Del-Claro and Oliveira 1996). We performed ant counts on a single day to control for environmental factors affecting ant activity at a given period of the day on independent aggregations. The mean number of ants at each period of the day was calculated using all aggregations in which ant species were found on at least one count. We used four counts to calculate mean number of ants during the day and night for each aggregation. To determine the ratio between tending ants and stinkbugs (ant : stinkbug ratio, ASR hereafter) we also divided the mean number of ants by the number of stinkbugs on the aggregation. We recorded temperature and air humidity at approximately 10-min intervals during each ant count session.

### ***Statistical analyses***

We compared the observed and expected frequencies of aggregation distribution on *P. robustus* in six height classes (5–10 cm; 11 to 30 cm; 31 to 50 cm; 51 to 70 cm; 71 to 100 cm; > 101 cm), and between reproductive and nonreproductive plants using chi-square tests. Mean temperature through the day was inversely correlated with mean humidity ( $R^2$  0.98,  $p < 0.0001$ ,  $n = 8$ ). Therefore, to evaluate the influence of abiotic factors on mean number of each species attending aggregations, we used a simple linear regression with temperature as the independent variable. To compare number of ants attending aggregations along periods of the day, we perform repeated measures analysis of covariance. In the model, ant species was considered as the fixed factor, periods of the day as the repeated measures with aggregation size as the covariate. The number of ants was evaluated for those aggregations in which the ant species was observed attending stinkbugs on at least one period of the day. Only data of the two most common ant species were used in this analysis. To evaluate the relationship among mean number of ants for each ant species, attending aggregations, and temperature averages recorded for each period of the day, we used simple linear regressions. We also fitted best regression curves for relationships among mean number of ants, ASR and aggregation size, for most common species separately and for all species pooled.

## **Results**

### ***Stinkbug distribution, micro-habitat use and behaviour***

During 3 years working in rocky outcrops in Serra do Cipó, more than 20,000 plants of at least 300 species, including trees, shrubs and herbs, were inspected by the first author, but *E. microlobatus* was found exclusively on *P. robustus*. We found *E. microlobatus* on 19% of inspected mistletoes ( $n = 513$ ), however stinkbug aggregations were

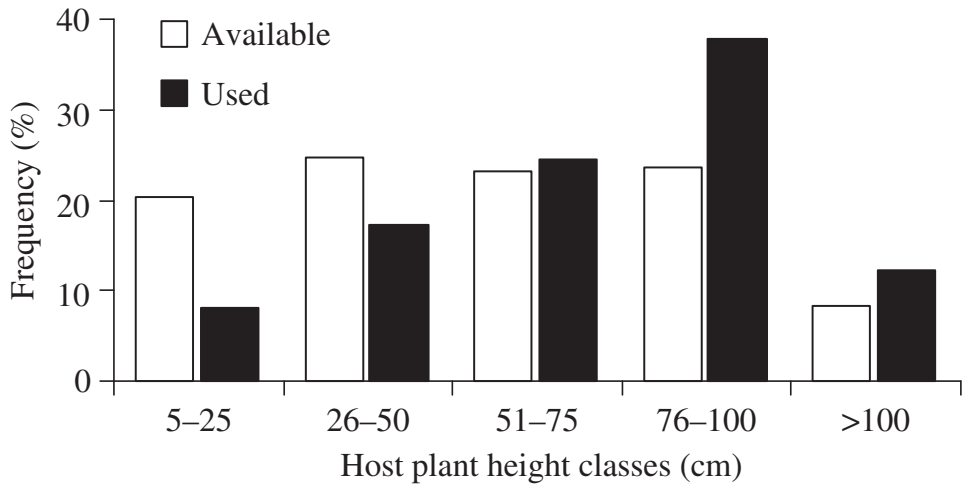


Figure 1. Observed distribution of *Eurystethus microlobatus* aggregations ( $n = 98$ ; black bars) contingent with *Psittacanthus robustus* distribution ( $n = 519$ , white bars) on five plant size classes in rocky outcrops, southeastern Brazil.

not randomly distributed. We found more aggregations on larger host plants than expected by their availability ( $\chi^2 = 17.1$ ,  $df = 5$ ,  $p = 0.004$ , Figure 1). Because larger mistletoes were often reproductive, we also found more aggregations in reproductive plants than expected by availability ( $\chi^2 = 13.1$ ,  $df = 1$ ,  $p < 0.001$ ).

The number of stinkbugs per mistletoe varied from 1 to 617 (mean  $\pm$  SD,  $89.1 \pm 126.8$ ,  $n = 98$ ). Aggregation size was positively weakly correlated with mistletoe height ( $R^2 = 0.06$ ,  $p = 0.01$ ,  $n = 98$ ). The number of adults varied from 1 to 147 ( $17.6 \pm 24.2$ ). The number of nymphs varied from 1 to 575 ( $71 \pm 105.6$ ) and was positively correlated with number of adults present on aggregations ( $R^2 = 0.73$ ,  $p < 0.0001$ ,  $n = 98$ ). The number of ovipositions per aggregation varied from 1 to 20 ( $3.1 \pm 4.0$ ) and was also positively correlated with number of adults ( $R^2 = 0.53$ ,  $p < 0.0001$ ). Only six out of 98 plants had a single adult and three were females guarding eggs. Nearly 55% of plants had aggregations smaller than 51 individuals, 30% between 51 and 200 and only 13% had more than 200 individuals (Figure 2). Although most aggregations were small or intermediate in number of individuals, most individuals in the population were found in those few but very large aggregations (Figure 3), indicating a highly clumped distribution on hosts.

Micro-habitat use on hosts was contingent with aggregation size (Figure 3), being intimately related to stinkbug cryptic colouration. *Eurystethus microlobatus* have flavescent ground colour with discontinuous dark brown, grey and ferruginous punctures. On small aggregations stinkbugs were often located on roots and primary shoots (Figure 3), where they are camouflaged within host bark (Figure 4A). They also used secondary shoots, that vary greatly in degree of bark thickness and colour, but stinkbugs were often clumped on brownish twigs where they were better camouflaged. However, in larger aggregations stinkbugs also occupied green leaves and yellowish inflorescences (Figure 3), where the aggregations became more conspicuous (Figure 4B).

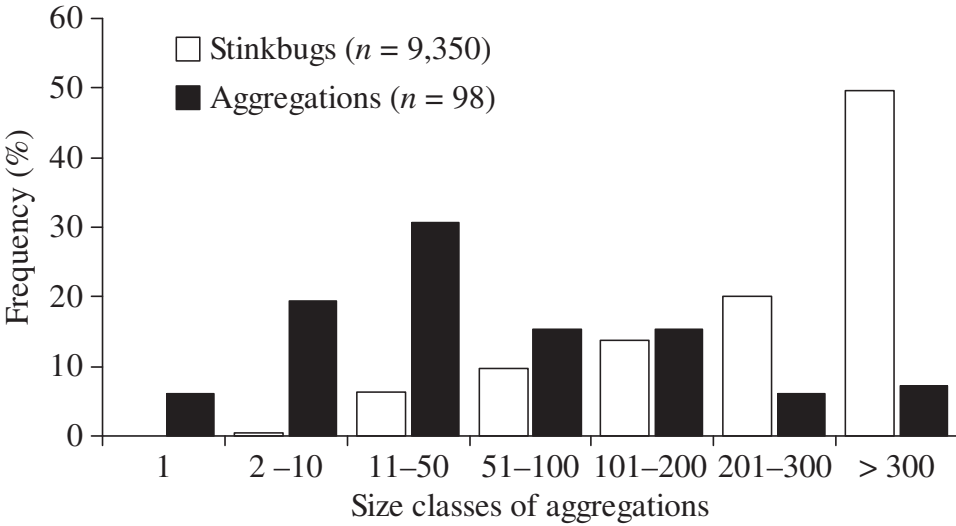


Figure 2. Distribution of *Eurystethus microlobatus* aggregations ( $n = 98$ ) and population ( $n = 9350$ ) on seven aggregation size classes in rocky outcrops, southeastern Brazil.

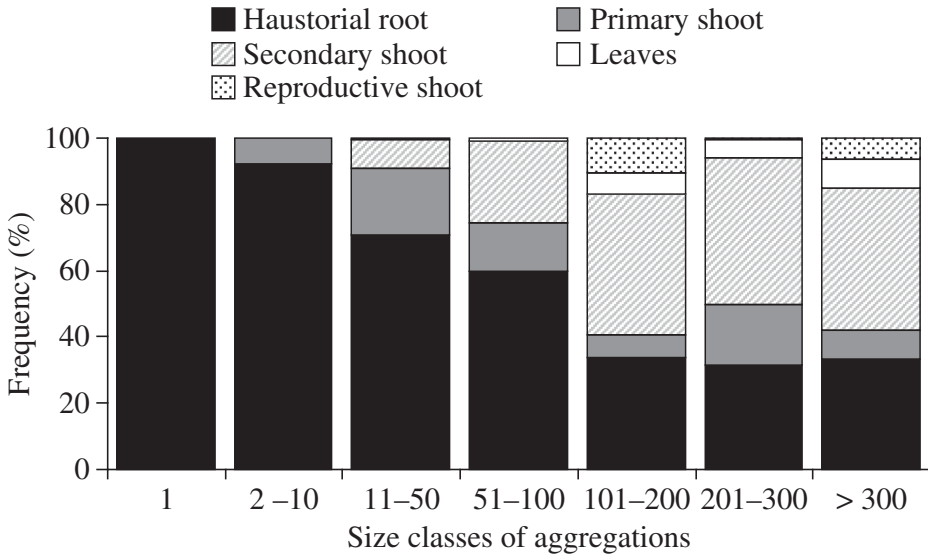


Figure 3. Frequency of adults plus nymphs, found on five plant microhabitats contingent with aggregation size, found on 70 randomly selected aggregations in rocky outcrops, southeastern Brazil. Total number of individuals per aggregation class: 1,  $n = 6$ ; 2 to 10,  $n = 39$ ; 11 to 50,  $n = 590$ ; 51 to 100,  $n = 906$ ; 101 to 200,  $n = 1293$ ; 201 to 300,  $n = 1874$ ; > 301,  $n = 4646$ ).

Stinkbugs showed gregarious behaviour, forming multiple clusters, usually with individuals maintaining contact with each other (Figure 4C). During the day, they remained static most of the time, being more active during the night. At this period, adults and nymphs walked on plants, some adults performed a shaking behaviour,

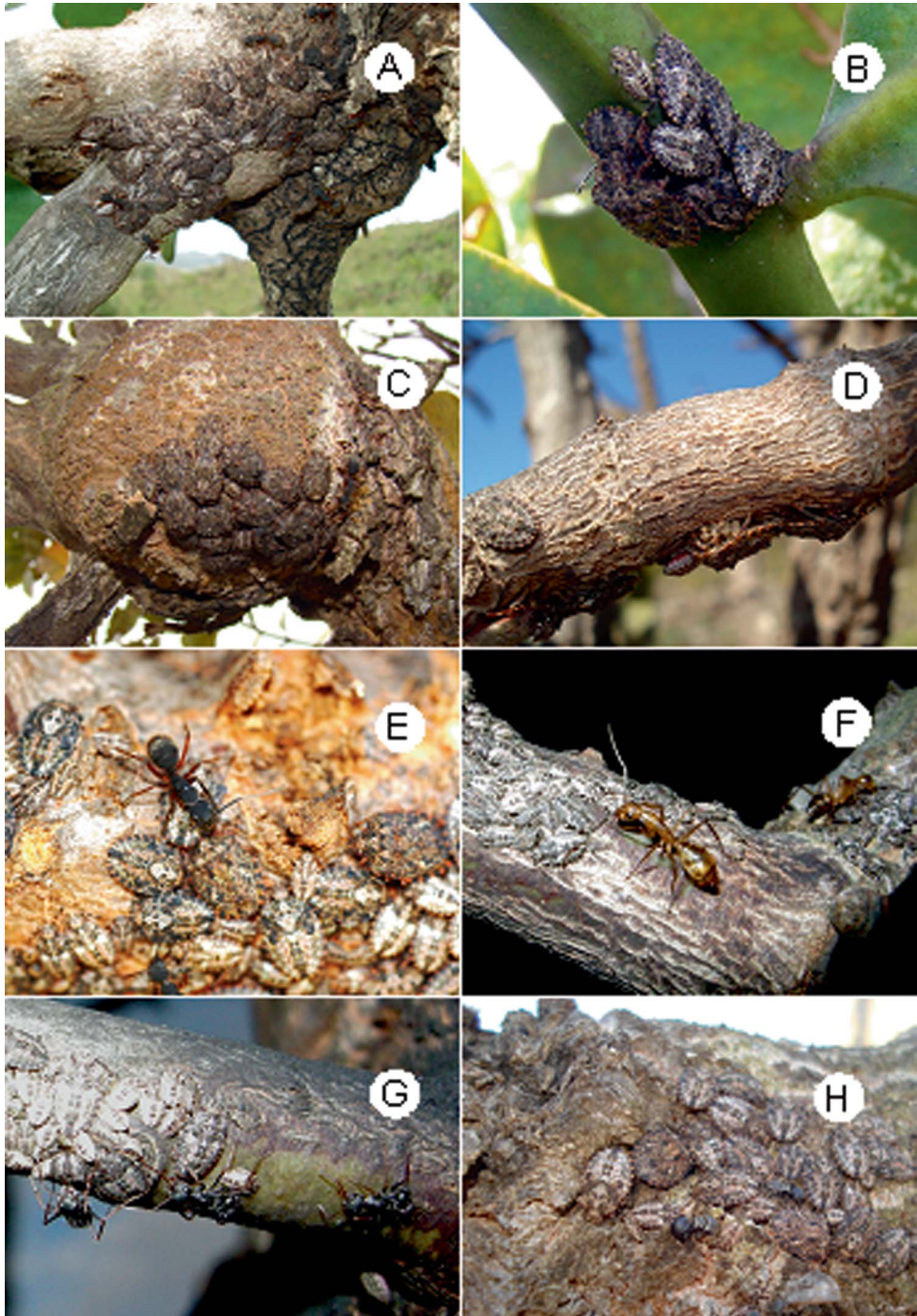


Figure 4. The stinkbug *Eurystethus microlobatus* on its host plant *Psittacanthus robustus* in campos rupestres, southeastern Brazil. (A) Stinkbug aggregation on haustorial root highly camouflaged within host bark. (B) Stinkbug clump on secondary shoot. (C) Gregarious adults copulating and forming a shield over younger instars. (D) Female stinkbug guarding oviposition on primary shoot. Ants attending stinkbugs: (E) *Camponotus rufipes*, (F) *Camponotus melanoticus*, (G) *Camponotus crassus*, (H) *Cephalotes pusillus*.

probably some kind of vibrational communication. However, even at night most individuals were observed motionless, feeding on plants using their long piercing stylets. We recorded 303 ovipositions placed exclusively on bark, 31% on haustorial roots, and 21% and 48% on primary and secondary shoots, respectively, never on leaves or inflorescences. The number of eggs per oviposition varied from 11 to 16 eggs ( $14.0 \pm 1.2$ , mode = 14,  $n = 76$ ). We determined the sex of 36 individuals guarding eggs, all were females. They protected ovipositions simply by shielding them with their bodies until they hatched (Figure 4D), also protecting first and second instars. We found couples copulating in most aggregations. They attach to each other through the extremities of the abdomen facing in opposite directions. During counts we disturbed stinkbugs to visualize nymphs and eggs covered by other individuals. This procedure often induced bugs to expel allomone volatiles with their characteristic unpleasant odour. Both adults and nymphs produced droplets of honeydew day and night, raising their abdomens during droplet exudation.

### ***Trophobiosis with ants***

We recorded four species of ants attending *E. microlobatus* at the study site during the period of this study: *Camponotus rufipes* Fabricius 1775; *Camponotus melanoticus* Emery 1894; *Camponotus crassus* Mayr 1862 (Formicinae) and *Cephalotes pusillus* Klug 1824 (Myrmicinae). All ant species performed similar behaviour: touching stinkbugs' dorsum and anus using antennae and collecting honeydew droplets with their mouthparts directly from stinkbug anus or from plant surfaces. The most frequent ant, *C. rufipes* was the only species observed attending stinkbugs during both day and night (Figure 4E). It was the largest species recorded and behaved aggressively toward the other species. It usually excluded other ants from stinkbug aggregations, and encounters between *C. rufipes* and the other species invariably resulted in dropping-off by the other ants. We observed workers attending stinkbugs, but soldiers were also observed walking on plants, we recorded trophallaxis between workers and soldiers. We found three *C. rufipes* satellite nests located in holes on mistletoe root, with individuals moving in and out, feeding continuously on the stinkbug aggregations located in the same plant.

*Camponotus melanoticus* (Figure 4F) was mainly nocturnal with few individuals recorded early in the morning and late in the afternoon. It was found feeding on aggregations also attended by *C. rufipes*, but exploring different stinkbug clusters during distinct periods of the day. It was also observed using holes on mistletoes as a nest. We observed replacement between *C. melanoticus* and *C. crassus* (Figure 4G) during the day in some aggregations. The later was also observed using one hole on a mistletoe trunk as a nest, feeding directly on stinkbug honeydew. It is interesting to note that we observed a single agonistic encounter between these species. *Camponotus crassus*, which are active during the day, drove back one *C. melanoticus* from a stinkbug cluster that it was attending, but the latter just moved to another cluster without leaving the plant. In fact, in the same aggregations at dusk and dawn both species were observed attending stinkbugs pacifically. *Cephalotes pusillus* (Figure 4H) was observed attending stinkbugs only during the day and in aggregations also attended by *C. rufipes* and *C. melanoticus*. *Cephalotes pusillus* individuals were not aggressive, and in contrast to other species, they simply left aggregations when disturbed. This species was also observed using holes on mistletoes as nests and, although not frequent in attendance, *Cephalotes pusillus* was commonly found on the canopy of *P. robustus* and its hosts.



### Round-the-clock ant attendance

All aggregations observed were attended by ants for at least one period of the day. Thirty-one of 50 aggregations were attended during four periods of the day, but we found no relationship between aggregation size and continuous attendance by ants through the day. However, during the night all aggregations with more than 15 stinkbugs ( $n = 45$ ) were attended at all four periods of observation.

*Camponotus rufipes* was found on 45 aggregations (90%), with 37 (74%) being attended exclusively by this ant species. The mean number of *C. rufipes* attending aggregations increased during the night (Figure 5) and was inversely correlated with temperature through the day ( $r^2 = 0.59$ ,  $p = 0.02$ ,  $n = 8$ ). *Camponotus melanoticus* was observed on 16 aggregations (24%), but only one was attended exclusively by this ant species. In 10% of the aggregations we observed *C. melanoticus* and *C. rufipes* attending stinkbugs, but always in larger aggregations with individuals attending stinkbugs in different parts of host plant. Mean number of *C. melanoticus* increased significantly at night, during the day few individuals were observed at 6 h, and they were absent from all aggregations from 9.00 h until dusk (Figure 5). There was also a negative relationship between mean number of *C. melanoticus* and mean temperature through the day ( $r^2 = 0.52$ ,  $p = 0.04$ ,  $n = 8$ ). We observed *C. crassus* attending ants in four aggregations (8%), exclusively during the day being replaced by *C. melanoticus* at night. Mean number of ants was positively correlated with temperature through the day ( $r^2 = 0.62$ ,  $p = 0.01$ ,  $n = 8$ ). *Cephalotes pusillus* attended stinkbugs in only three aggregations (6%) during the day, being replaced by *C. melanoticus* at night in two aggregations. We also observed this species at one large aggregation dominated by *C. rufipes*, three individuals attended a small cluster of stinkbugs for a few hours during the day, but by afternoon they were excluded from the plant as the number of *C. rufipes* increased. Mean number of *Cephalotes pusillus* on aggregations was positively correlated with temperature through the day ( $r^2 = 0.96$ ,  $p < 0.001$ ,  $n = 8$ ).

Although *C. rufipes* was the most frequent ant attending stinkbugs at the study site, the number of ants attending aggregations was variable and differences between this species and *C. melanoticus* were not statistically significant, being better explained by aggregation size (Figure 5; Table 1). However, differences on number of ants at different periods of the day were also statistically significant, which could be explained by differences among species in their daily activity rhythms. During the night, mean number of ants were similar between *C. rufipes* and *C. melanoticus* with slightly higher means for *C. rufipes* because of its dominance on larger aggregations. However, the number of *C. melanoticus* attending a large aggregation can reach 35 individuals, being as high as the number of *C. rufipes* (40 individuals) attending larger aggregations at night.

For those aggregations attended exclusively by *C. rufipes*, mean number of ants was positively correlated with aggregation size during the day ( $r^2 = 0.45$ ,  $p < 0.0001$ ,  $n = 37$ ) and night ( $r^2 = 0.44$ ,  $p < 0.0001$ ,  $n = 37$ ). However, ASR for *C. rufipes* decreased rapidly as the aggregation size increased and this relationship seemed to be better fitted by an inverse exponential curve during the day ( $r^2 = 0.25$ ,  $p < 0.001$ ,  $n = 37$ ) and night ( $r^2 = 0.50$ ,  $p < 0.0001$ ,  $n = 37$ ). The mean number of *C. melanoticus* during the night was also positively correlated with aggregation size ( $r^2 = 0.87$ ,  $p = 0.007$ ,  $n = 8$ ), but ASR was also negatively correlated with aggregation size with the relationship fitting an inverse geometric curve with marginally non-significant

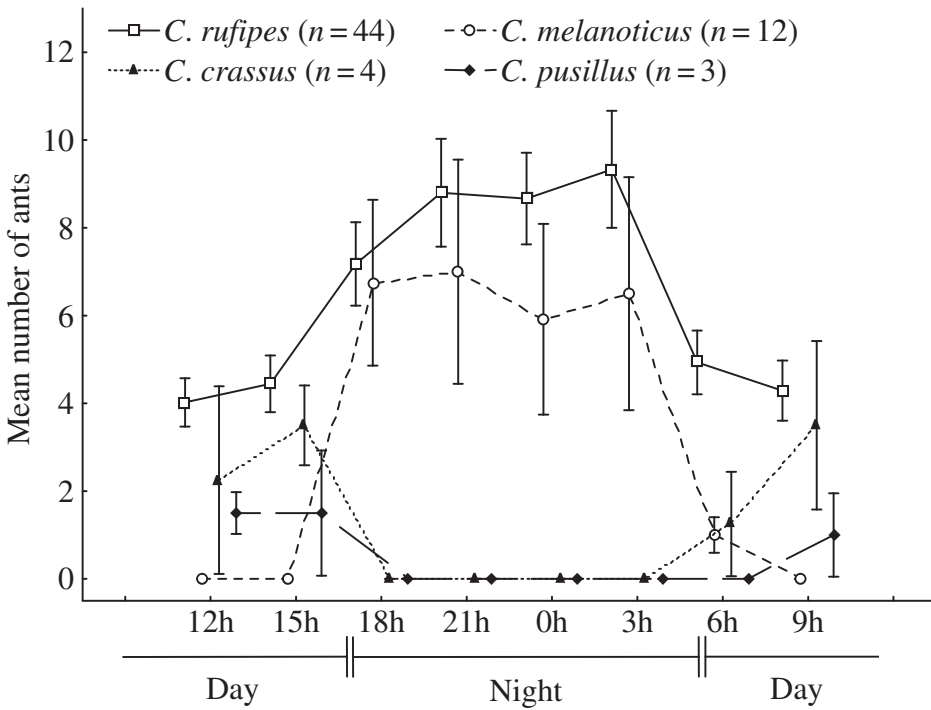


Figure 5. Daily variation on ant species recruitment to aggregations in rocky outcrops, south-eastern Brazil. Number of aggregations with ant species present on at least one count in parenthesis, points represents mean and whiskers represent standard error.

Table 1. Repeated measures analysis of covariance results evaluating differences on mean number of *Camponotus rufipes* and *Camponotus melanoticus* at eight periods of the day and its relationship with *Eurystetus microlobatus* aggregation size.

Source of variation	DF	MS	F	P	G-G
Ant species	1	71.80	0.41	0.52	
Aggregation size	1	105.2	18.8	< 0.001	
Ant species × Aggregation size	1	3.231	18.82	< 0.001	
Error	54	171.6			
Time of day	7	287.2	18.78	< 0.001	0.008
Time × Ant species	7	45.22	2.95	0.004	0.11
Time × Ant species × Aggregation size	7	43.40	2.83	0.006	0.23
Error	378	15.28			

Note: Analyses used square-root-transformed data.

results ( $r^2 = 45, p = 0.06, n = 8$ ). The same trends were found when analysed for all species pooled (Figure 6). Mean number of ants attending an aggregation during the day was positively correlated with aggregation size ( $r^2 = 0.31, p < 0.0001, n = 50$ ), and this relationship was even more marked for mean number of ants attending aggregations during the night ( $r^2 = 57, p < 0.0001, n = 50$ ). Conversely, ASR decreased

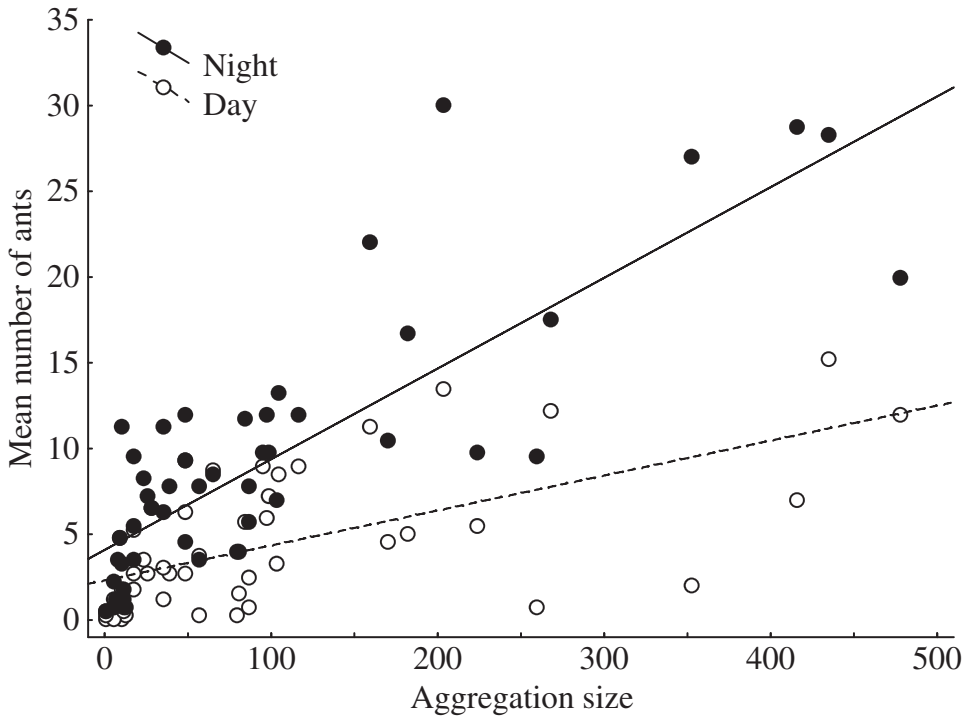


Figure 6. Relationship among mean number of ants recruited and stinkbug aggregation size in rocky outcrops, southeastern Brazil.

rapidly as the aggregation size increased and these relationships were non-linear, and better fitted by inverse geometric curves (Figure 7), for day ( $r^2 = 0.24$ ,  $p = 0.0004$ ,  $n = 48$ ) and night ( $r^2 = 0.50$ ,  $p < 0.0001$ ,  $n = 50$ ) datasets.

## Discussion

Regardless of the great diversity of plants on areas of *campos rupestres* in Serra do Cipó (Giulietti et al. 1997), we found *E. microlobatus* exclusively on *P. robustus*. In fact, other heteropteran trophobionts are also specialized phytophages, living on few host species locally, even in diversified tropical communities from Asia and Africa. Maschwitz et al. (1987) found *Cloresmus* sp. (Hemiptera, Coreidae) on the giant bamboo *Gigantochloa scortechini* (Poaceae) in Malaysia. Blüthgen et al. (2006) also found two unidentified coreids exclusively on the climbing bamboo *Dinochloa trichogona* in Borneo. Dejean et al. (2000) found *Caternautiella rugosa* (Hemiptera, Plataspidae) exclusively on two *Bridelia* trees (Euphorbiaceae) in Cameroon, and Waldkircher et al. (2004) found another plataspidid *Tetrisia vacca* (Hemiptera, Plataspidae) on *Macaranga gigantea* (Euphorbiaceae) trees in Peninsular Malaysia. Although stinkbugs in the subfamily Discocephalinae are considered to be phloem feeders, there is no study addressing host specificity in this group. Among these Neotropical stinkbugs we can find generalist species in relation to host plants, like *Antiteuchus tripterus* found on 15 tree species from 11 families, including native and exotic trees in

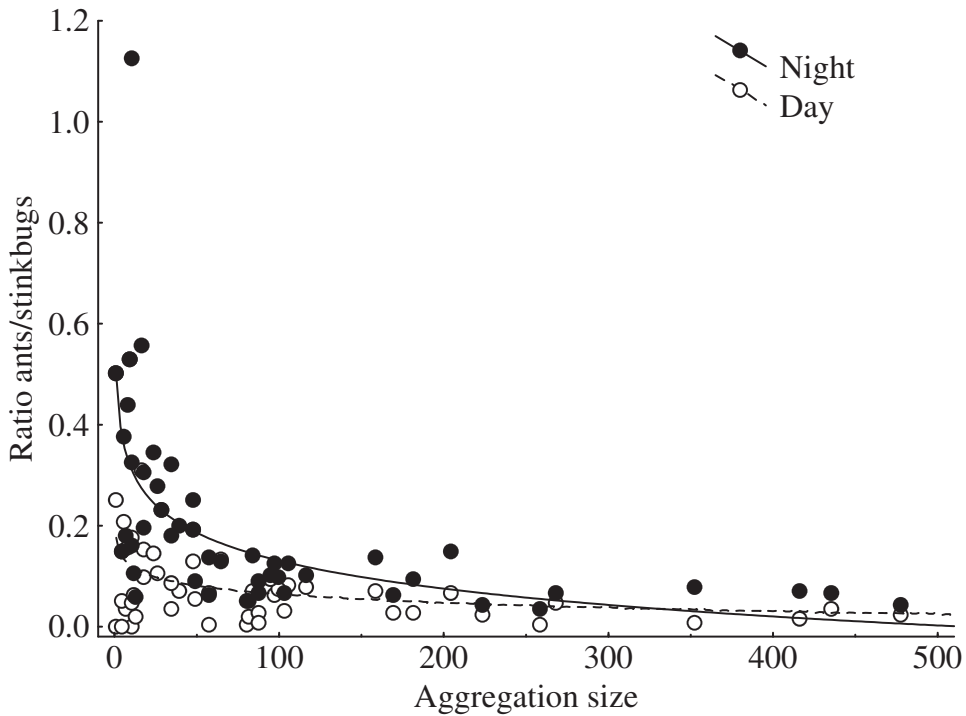


Figure 7. Relationship among ant:stinkbug ratio and aggregation size in rocky outcrops, southeastern Brazil.

urban areas of Cali, Colombia (Eberhard 1975). But, there are also reports of more specialized discocephaline species, as *Lincus spurcus* and *Lincus malevolus*, which are exclusively related to native *Astrocaryum* palms (Arecaceae) in Peruvian Amazonia (Llosa et al. 1990).

We found *E. microlobatus* eggs, nymphs and adults on *P. robustus*, suggesting that stinkbugs have their complete life cycle on this mistletoe. Stinkbug aggregations were found preferentially in larger and reproductive mistletoes, an indication of host size selection during colonization of new host plants. Llosa et al. (1990) also found *Lincus spurcus* and *Lincus malevolus* preferentially on larger and reproductive palms. Nonetheless, these stinkbugs use reproductive shoots for feeding, which explains the preference for older fertile palms. Indeed, sugar content on phloem could be higher on reproductive plants, providing better resources for sap feeders (Quental et al. 2005). However, wider host selection by *E. microlobatus* could also be related to its micro-habitat use and cryptic colouration. These stinkbugs often use haustorial roots and shoots for feeding, where the individuals receive better protection being camouflaged on the bark. They seem to spread to leaves and reproductive shoots only when aggregations become crowded, and space on camouflaging micro-habitats becomes limited. In fact, mature plants have more developed roots and shoots, providing wider bark background for camouflage during aggregation proliferation than seedlings and saplings. Therefore, host plant selection on habitat and micro-habitat selection on host plants by this stinkbug might be related to its cryptic colouration.

Camouflage is one the commonest predator avoidance strategies among phytophagous species, especially among true bugs (Schuh and Slater 1995). The main advantage of cryptic colouration is related to a reduction of predation rates by visually oriented predators, especially birds (Exnerová et al. 2003). For *E. microlobatus*, camouflage on mistletoe bark must be very important because the host plant is ornithophilous and ornithocorous, being visited throughout the year by pollinating and seed-dispersing birds, including generalist species that also include insects in their diets (T.J. Guerra, personal observation). Indeed, camouflage must be crucial for *E. microlobatus* because of their clumped distribution and gregarious behaviour, which may increase detectability by predators (Riipi et al. 2001; Pitt and Ritchie 2002). In effect, gregarious behaviour seems to enhance stinkbug camouflage, making it more difficult to visualize individual boundaries when in clumps.

Gregarious behaviour has been reported for other related stinkbugs (i.e. Eberhard 1975; Llosa et al. 1990). However, adaptive significance is still poorly explored for stinkbugs. Lockwood and Story (1986) found that the *Nezara viridula* nymphs developed more rapidly and suffered reduced predation by ants when in aggregations compared with when isolated. Protection against predators through dilution effects have been proposed as one main advantage for individuals living in groups (Foster and Treherne 1981). However, gregariousness could also represent a strategy to explore host plants, by increasing water uptake (Lockwood and Story 1986), feeding efficiency (Lawrence 1990) and thermoregulation (Seymour 1974). In addition, together many stinkbugs could improve allomones nauseating effects (Aldrich and Blum 1978).

Females of *E. microlobatus* provided physical protection to ovipositions, and first and second instar nymphs, simply by standing over them. Ovipositions of other stinkbug species have been found exclusively on leaves and reproductive shoots (Eberhard 1975; Llosa et al. 1990; Santos and Albuquerque 2001). Although *E. microlobatus* use leaves and inflorescence as micro-habitat, females guarding ovipositions were always located on roots and shoots. One cost of egg guarding is increased exposure of adults to natural enemies (Tallamy and Schaefer 1997). Hence, females guarding eggs on better camouflaging micro-habitats should suffer reduced predation and parasitism, leading to site selection for oviposition. Subsocial behaviour was reported for 5% of true bugs, but there is evidence supporting maternal care as a plesiomorphy lost during Hemiptera clade evolution (Tallamy and Schaefer 1995). This behaviour has been reported for other Discocephalinae stinkbugs, with clear advantages related to protection against predators and parasitoids, but high ecophysiological costs (Eberhard 1971; Santos and Albuquerque 2002). However, for this gregarious stinkbug study, maternal care could be not so costly, once adults and nymphs are mostly sedentary and usually keep intimate contact. Besides, maternal care may be crucial against predation by ant partners of this trophobiont stinkbug.

From the four ant species observed interacting with *E. microlobatus*, three have been previously reported attending another trophobiont hemipteran in Neotropical savanna in Brazil. *Camponotus rufipes*, *C. crassus* and *Cephalotes pusillus* were also observed using similar behaviour during honeydew collection on *Guayaquila xiphias* (Hemiptera, Membracidae) living on *Dydimopanax vinosum* (Araliaceae) (Dansa and Rocha 1992; Del-Claro and Oliveira 1999; Quental et al. 2005). *Camponotus rufipes* and *C. crassus* were also observed attending another treehopper, *Enchenopa brasiliensis* (Membracidae), which lives in *Solanum lycocarpum* (Solanaceae) (Moreira and Del-Claro 2005). Ant species richness associated with stinkbugs in our study was

relatively low when compared with *Guayaquila xiphias* and *E. brasiliensis*, which are attended by 21 and 10 ant species, respectively (Del-Claro and Oliveira 1999; Moreira and Del-Claro 2005). However, most heteropteran trophobionts studied until now were also attended by only a few ant species. Earlier naturalistic observations usually reported one or two ant species, including interactions with coreids and plataspidid trophobionts (Green 1900; China 1931; Maschwitz and Klinger 1974; Maschwitz et al. 1987). Dejean et al. (2000) found that the coreid *Caternautiella rugosa* was attended by two ant species, *Camponotus brutus* and *Myrmicaria opaciventris*, whereas Waldkircher et al. (2004) found only *Technomyrmex* sp. attending the plataspidid *Tetrisia vacca*. Blüthgen et al. (2006) observed a single ant species attending an unidentified heteropteran, but they also observed that two coreids living on climbing bamboos could be attended by up to 16 ant species, including five *Camponotus* species. Our data reinforce the importance of *Camponotus* species in the worldwide attendance of trophobiont insects, especially in Neotropical savannas as pointed out by Del-Claro and Oliveira (1999).

*Camponotus rufipes* was the most frequent and aggressive ant attending stinkbugs, dominating aggregations, being active during the whole day, but increasing in number during the night. The same daily activity pattern of attendance was reported for this species during trophobiosis with the treehopper *Guayaquila xiphias* (Dansa and Rocha 1992; Del-Claro and Oliveira 1999). *Camponotus crassus* and *Cephalotes pusillus* attended *E. microlobatus* only during the day, being replaced by the nocturnal *C. melanoticus*. The latter species was not reported attending *G. xiphias*. However, in a similar way, during interaction with *G. xiphias*, *C. crassus* was replaced by nocturnal ants, *Camponotus renggeri* or *Camponotus abdominalis* (Dansa and Rocha 1992; Del-Claro and Oliveira 1999; Quental et al. 2005). The number of ants attending stinkbugs varied among species, but was significantly related to temperature through the day. For *C. rufipes*, mean number of individuals on each period of the day was inversely related to temperature. The same relationship was observed for nocturnal *C. melanoticus*, whereas two diurnal species had mean number positively correlated with temperature. Differences on activity schedule allowed the coexistence of two species on the same aggregation, as previously reported (Dansa and Rocha 1992; Del-Claro and Oliveira 1999; Quental et al. 2005). Indeed, temporal resource partitioning among ant species observed in this system may be related to specific differences in environmental tolerances (Lessard et al. 2009).

Number of ants attending aggregations increased significantly with number of honeydew-producing stinkbugs. From the ant colony perspective, more stinkbugs per plant provide larger amounts of honeydew, affecting ant recruitment rate positively. In fact, positive relationships between number of hemipteran trophobionts per aggregation and absolute number of tending ants have been observed in other ant-trophobiont systems (Dansa and Rocha 1992; Morales 2000; Blüthgen et al. 2006). From the stinkbug perspective, being on larger aggregations seems to guarantee the constant presence of ants, especially during the night. Conversely, the number of ants per stinkbug decreased as aggregation increased, suggesting reduced per capita attendance by ants on larger aggregations. Blüthgen and Fiedler (2002) also observed an inverse relationship between aggregation size and ant visitation rates for different trophobiont species. In effect, Cushman and Whitham (1991) found that the outcome of mutualistic interaction between ants and a membracid is density-dependent because of intra-specific competition for ant partners. Morales (2000) showed that the

treehopper *Publilia concava* (Hemiptera, Membracidae) received more benefits from *Formica obscuriventris* on smaller aggregations because of higher per capita attendance, indicating that density-dependent benefits of trophobiotic interactions can be related to recruitment rates of ant partners. Nevertheless, the role of ants in stinkbug protection against natural enemies and its relationship with density of aggregations awaits further investigations.

Here we describe trophobiotic interactions among heteropterans and ants for the first time in the New World. Like other trophobiont hemipterans, *E. microlobatus* is a sedentary, gregarious sap feeder. As pointed out by Delabie (2001) this kind of life history could be very important for the evolution of trophobiosis among hemipterans because it renders hemipterans more susceptible to natural enemies, but also makes honeydew a predictable food source for ants. Maschwitz et al. (1987) suggested that trophobiosis involving ants and true bugs could be more common than previously thought, but few cases have been described since then (Dejean et al. 2000; Waldkircher et al. 2004). More research on the natural history of true bugs is needed to determine the rarity or commonness of trophobiosis with ants. Since the first report by Green (1900), trophobiosis between ants and true bugs has been confirmed for only seven plataspids, four coreids (Green 1900; China 1931; Maschwitz et al. 1987; Dejean et al. 2000; Waldkircher et al. 2004; Blüthgen et al. 2006), and now one pentatomid. The evolution of trophobiosis among true bugs is still unexplored. Future studies on the ecology and phylogeny of these groups will help us to understand if adaptations related to trophobiosis with ants evolved independently among living heteropterans, or if they represent an ancient condition shared with ancestral Sternorrhyncha and Auchenorrhyncha, but lost in the majority of true bug species as Tallamy and Shaeffer (1995) proposed for maternal care.

In short, we conclude that the behaviour of these mistletoe sap-feeding stinkbugs combines as many strategies as possible. *Eurystethus microlobatus* is protected by punctual ant bodyguards, lives in cohered groups, avoids too much exposure, takes care of the brood, and uses its chemical weapons if necessary. In effect, these behavioural, morphological and physiological adaptations seem to function in synergy.

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