Parasitoids of the endangered leafcutter ant *Atta robusta* Borgmeier in urban and natural areas

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ABSTRACT. Parasitoids of the endangered leafcutter ant *Atta robusta* Borgmeier in urban and natural areas. Hosts of parasitoids in urban areas may suffer from a double threat of habitat destruction by urbanization and parasitism pressure. Moreover, the parasitoids themselves might be at risk if they are specialists. Here, we studied whether *Atta robusta* (Hymenoptera, Formicidae), which is on the red list of Brazilian threatened species, suffers from higher parasitism pressure in an urban area compared to a natural one. In addition, we determined whether their specialist parasitoids, *Eibesfeldtphora breviloba* and *Myrmosicarius exrobusta* (Diptera, Phoridae), are in risk and evaluated whether they are influenced by habitat structure, temperature, humidity, ant traffic, and time of the day. The study was carried out in an urban park and in a natural protected area in the city of Rio de Janeiro. In each site we chose an open area and a closed area (forest) and sampled nine nests in each area. We found that parasitism pressure was similar in urban and natural areas, with the same two parasitoid species present in both areas. The main difference was related to habitat structure, since *M. exrobusta* was mainly present in open areas while *E. breviloba* was almost exclusively found in closed areas. *Myrmosicarius exrobusta* was not present during the hottest midday times, and its abundance was negatively correlated to vapor pressure deficit. These results suggest that green areas can be an important component in efforts to conserve diversity in urban areas. However, the complexity of the habitats in those areas is a fundamental issue in designing urban parks.

KEYWORDS. Biodiversity; Insecta; leafcutters; Restinga; urban ecology.

Habitat destruction has been a major cause of current species extinctions. One type of habitat destruction is urbanization, although parks and green areas can maintain a high diversity of species, especially for those which are generalists (Samways 2005). Natural enemies, particularly parasitoids, are often quite affected by urbanization (Denys & Schmidt 1998). Changes of parasitoid communities in urban areas are often associated with different environmental tolerances of species (Fenoglio *et al*. 2009). On the other hand, a high pressure of parasitism may be a threat to its host population (Lafferty & Gerber 2002), especially if the host is already compromised by habitat destruction. Moreover, the host-parasite system may be at risk of coextinction if the host supports specialist parasites (Koh *et al*. 2004).

The leafcutter ant *Atta robusta* Borgmeier (Hymenoptera, Formicidae) is endemic to the *restingas* in the Brazilian states of Rio de Janeiro and Espírito Santo (Teixeira *et al*. 2004). The *restingas* are characterized by having sandy soils covered with herbaceous and shrub-arboreal vegetation located in coastal lowlands (Rocha *et al*. 2007). The degradation of the *restinga* resulting from anthropic pressures (Rocha *et al*. 2007) led *A. robusta* to be threatened with extinction (Machado *et al*. 2008). In fact, it was recently stated that the species was extinct in the city of Rio de Janeiro (Dattilo *et al*. 2012), but it is still possible to find *A. robusta* popula-

tions in highly urbanized areas of this city (Brown *et al*. 2012). Its extinction may have important consequences for ecosystem functioning, because this species plays an important role as a seed disperser, helping the seeds of several species to germinate (Teixeira *et al*. 2008).

Flies of the family Phoridae are among the main natural enemies of leafcutter ants (Bragança 2011). The phorid flies that parasitize ants use the host's body to complete their life cycle, and their occurrence is restricted by certain conditions, such as habitat structure, temperature and relative humidity (Folgarait *et al*. 2007; Almeida *et al*. 2008; Elizalde & Folgarait 2010). In addition, parasitoid abundance may depend on host foraging activity (Tonhasca Jr. 1996), and the form of attack or oviposition site (such as nest entrance holes or foraging trails) vary among phorid species (Erthal Jr. & Tonhasca Jr. 2000; Bragança & Medeiros 2006; Gazal *et al*. 2009; Elizalde & Folgarait 2011, 2012). Recently, three species of phorid flies were described attacking workers of *A. robusta*, two of them were recorded only in Rio de Janeiro: *Eibesfeldtphora breviloba* Brown and *Myrmosicarius exrobusta* Brown (Brown *et al*. 2012). These phorid species are specialists on *A. robusta* (Brown *et al*. 2012).

In this study, we quantified the occurrence of *E. breviloba* and *M. exrobusta* in natural and urban environments of the city of Rio de Janeiro to evaluate whether parasitoid pres-

sure differs in both environments. In addition, we analyzed the influence of habitat complexity structure (open *vs*. closed), to test whether a more complex and protected habitat supported more phorids. Then, we evaluated the effect of temperature, humidity, and ant traffic on the foraging activity of these phorid species. Lastly, we described some natural history aspects of these parasitoids, such as the site where parasitoids oviposit and phorid species co-occurrence in nests, because there is scarce information on phorids that attack *A. robusta* (Brown *et al.* 2012).

MATERIAL AND METHODS

Study areas. The study was carried out, between 2009 and 2010, in two sites: *Parque Natural Municipal Marapendi* (PNMM) and in *Restinga da Marambaia* (RM). These sites are 15 km apart, both located in the city of Rio de Janeiro. PNMM (23°1'4"S, 43°26'54"W) has a total area of 195 ha and is located in a region of high population density, relatively isolated from other habitat fragments. RM (23°2'47"S, 43°35'24"W) is well preserved, has a total area of 5,000 ha, and is located in a region protected by a unit of the Brazilian Army. Hence, we will consider here PNMM an urban area and RM a natural habitat.

Experimental design and data collection. In each site we chose an open, less complex and less protected area (with predominance of grasses and few trees) and a closed area (forest) and sampled nine nests of *A. robusta* in each area, for a total of 36 nests in the two sites. Observation and data collection over each nest began at 9:00h, and ended after eight intervals of 45 min. At each interval we collected all phorid flies attacking ants in nest entrance holes and trails, and labeled them accordingly. Although the sampling implied parasitoid removal, we found parasitoids at the beginning and at the end of sampling over nests. We later identified them under a binocular microscope. We also recorded temperature and air humidity using a wet and dry bulb hygrometer. We measured ant traffic by counting the number of ants passing a point of a foraging trail for 15 minutes.

Statistical analysis. To evaluate whether ant nests in urban areas suffered higher parasitism, we tested for an effect of urbanization on pooled phorid abundance, *i.e.* each nest represented a replicate, using Kruskal-Wallis ANOVA, because data were not normal. We also evaluated the effect of urbanization on the abundance of each phorid species with a Kruskal-Wallis ANOVA. Then, we tested for the effect of habitat structure, i.e. open or closed, on overall phorid abundance over nests, and for each parasitoid species using Kruskal-Wallis ANOVAs.

To determine if foraging phorid flies of each species were influenced by the effect of environmental variables (temperature and relative humidity), traffic of worker ants, or showed a daily pattern of activity, we used logistic mixed models (Zuur *et al.* 2009). Because of our sampling design, we used nest identity as a random variable (to consider the repeated measurements over time on each nest) nested within each

habitat (because nests in each area are closer to each other). We first evaluated with GAMM (generalized additive mixed models) whether the relationships with the variables were non-linear. Otherwise we used GLMM (generalized linear mixed models). We used the presence of phorids, applying logistic models, instead of abundance because most of the 45 minute periods had only 1 phorid (see Results). Interactions between variables were not significant.

We tested for an effect of these environmental variables on the abundance of parasitoids. Due to the low abundance of phorids in each sampling period (see Results), we added phorid abundance by phorid species for each sampling time for all nests sampled. We averaged temperature, humidity and traffic for all ant nests in each period. However, because average temperature and average humidity were highly correlated ($r = -0.98$, $df = 6$, $P < 0.001$), and to reduce the number of variables in a model with only 8 points, we obtained average vapor pressure deficit, a variable that combines information from temperature and humidity, and is an important ecological variable (Anderson 1936). We evaluated the relationship between the abundance of each phorid species and these variables using multiple linear regressions. By averaging the values between different nests, the effect of repeated sampling on each nest is reduced, as well as the wide variability in the independent variables.

We examined where parasitoid species attacked their host in different microsites, i.e. nest entrances or foraging trails by comparing their abundance with chi-squared tests. We used R environment to perform statistical analyses (R development Core Team 2009), with "mgcv" package for the logistic additive mixed models (Wood 2012) and "lme4" package to run the logistic linear mixed models (Bates *et al*. 2012).

RESULTS

Phorid parasitism pressure, measured by parasitoid abundance, was the same for nests of *Atta robusta* in the urban or natural area (Kruskal-Wallis $\chi^2_1 = 0.045$, $P = 0.83$, $N = 36$), and most nests located in natural or urban habitat were attacked by parasitoids (89 and 83%, respectively). Each of the two parasitoid species collected attacking this ant, *Myrmosicarius exrobusta* and *Eibesfeldtphora breviloba*, also did not show different abundance in natural or urban nests (Kruskal-Wallis $\chi^2_1 = 1.22$, $P = 0.27$, $N = 36$; Kruskal-Wallis $\chi^2_1 = 0.90$, $P = 0.34$, $N = 36$; respectively). Phorid abundance was higher in nests located in closed areas (Fig. 1; Kruskal-Wallis $\chi^2_1 = 7.62$, $P = 0.006$, $N = 36$), due to the higher overall abundance of *E. breviloba* in closed areas (Fig. 1; Kruskal-Wallis $\chi^2_1 = 22.75$, $P < 0001$, $N = 36$), while *M. exrobusta* showed higher abundance on nests located in open areas (Fig. 1; Kruskal-Wallis $\chi^2_1 = 12.07$, $P < 0.001$, $N = 36$). In fact, only 4% of *E. breviloba* attacked ants in open habitats, and 15% of *M. exrobusta* attacked ants in the closed habitat. In addition, some nests located in the open habitat did not have any phorid attack (28%), while all nests in the closed habitat had parasitoids.
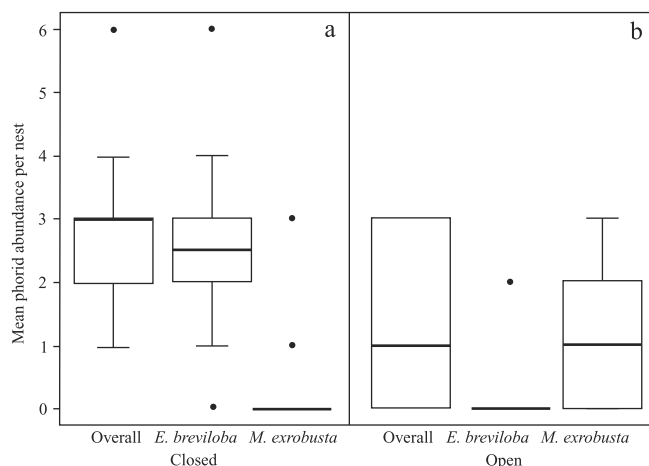
Fig. 1. Overall phorid abundance per nest and abundance by species attacking workers of *Atta robusta* in closed or open habitat in the city of Rio de Janeiro.

Nests of *Atta robusta* located in closed habitats were exposed to higher relative humidity than in open habitats (Kruskal-Wallis χ^2_1 = 57.83, P < 0.001, N = 144, 67.5 vs. 59.0%, median for closed vs. open habitats) but similar average temperature (Kruskal-Wallis χ^2_1 = 2.26, P = 0. 13, N = 144, 28 vs. 27ºC, median for closed vs. open habitats). However, variation in daily temperature was stronger in open habitats (Fig. 2a), and relative humidity varied similarly in both habitats along sampling times (Fig. 2b).

Because of the low occurrence or complete absence of *M. exrobusta* and *E. breviloba* in closed and open habitats respectively, phorid presence was modeled with the dataset from the habitat were each species was frequently found. Thus, the presence of *M. exrobusta* in open habitats had a curvilinear relationship with the sampling time (Time: F = 6.47, degrees of freedom = 1.89, P = 0.003), with the lowest probability of presence during midday (Fig. 3), the other variables being not significant. None of the variables measured were important in explaining the presence of *E. breviloba* in closed habitats (P > 0.30 for all variables). No interactions were recorded between variables (all P > 0.1). In both models, almost no random variation was due to habitat, and variation among nests was very low (logistic additive mixed model for the presence of *Myrmosicarius exrobusta*: variance of random effects: Nest = 0.0002, Habitat = 0, AIC = 741.2, N = 140; logistic linear model for the presence of *Eibesfeldtphora breviloba*: variance of random effects: Nest = 0.00, Habitat = 0.00, AIC = 172.1, N = 144).

The abundance of *M. exrobusta* in open habitats showed a negative relationship with vapor pressure deficit (partial multiple linear regression coefficient = -20.26, P = 0.04, model R^2 = 0.71), but no relationship was found with ant traffic (P = 0.27). The abundance of *E. breviloba* in closed habitats did not show any relationship with these variables.

No differences were found for oviposition sites, i.e. trails or nest holes, for either species (*M. exrobusta*: χ^2_1 = 0.92; P > 0.05; *E. breviloba*: χ^2_1 = 0.87; P > 0.05). The presence of
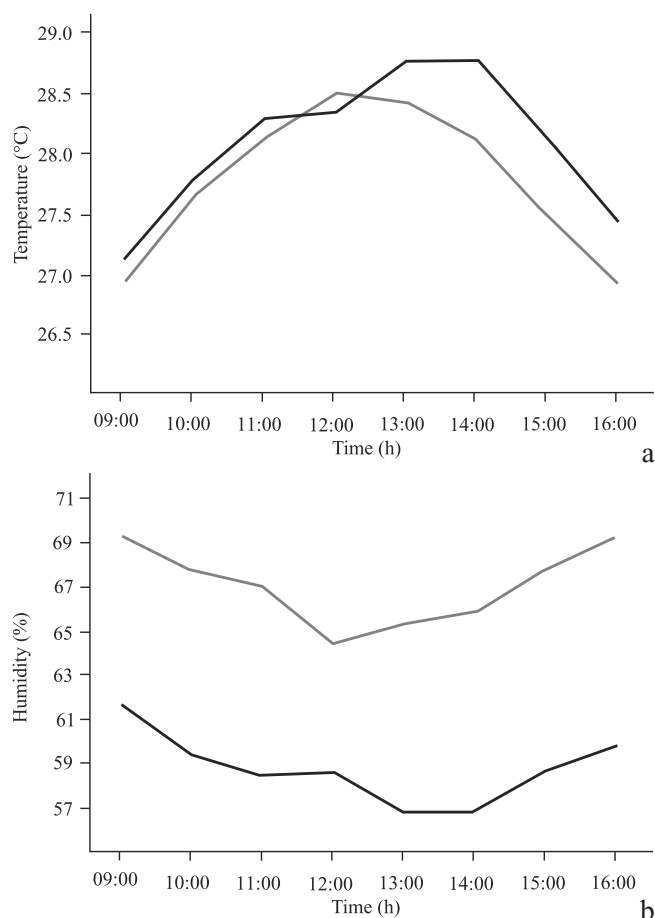

Fig. 2. Average temperature (a) and relative humidity (b) across time, for closed (grey line) and open (black line) habitats.
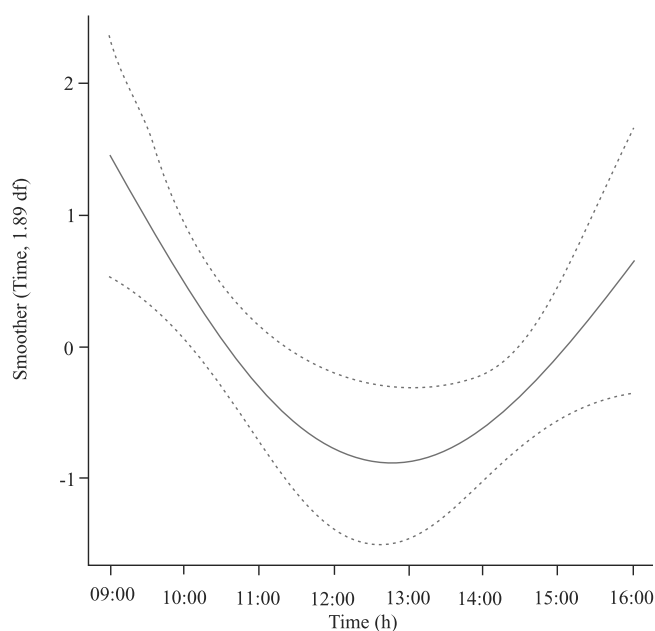

Fig. 3. Estimated smoothing curve for the effect of time of collection on the probability of presence of *Myrmosicarius exrobusta*. The y-axis shows the contribution of the time smoother to the fitted values (solid line; dotted lines are 95% confidence bands).

phorid flies near the nest holes often led *A. robusta* workers to abandon the plant fragment they were carrying and return quickly into the nest. In addition, we observed that some *M. exrobusta* flies started chasing ants on the trail but only attacked them near the nest hole.

DISCUSSION

In this study we found that parasitism pressure on *Atta robusta* did not differ between a natural area and an urban park. In fact, the two parasitoid species were found attacking their host both in natural and urban areas. This suggests, as other recent studies showed (see Samways (2005) for a review), that both green areas and parks can be important in conserving diversity in urban areas. However, our results show that it is important to consider the complexity of the habitat, because *E. breviloba* attacked ants almost exclusively in closed habitats while *M. exrobusta* was found attacking its host in open habitats.

Habitat structure seems to be an important factor for the understanding of the spatial distribution of ant parasitoids (Folgarait *et al.* 2007; Elizalde & Folgarait 2010; but see Reese & Philpott 2012). For example, phorid fly species that attack *Solenopsis invicta* in agroecosystems are associated with annual open crops or closed perennial crops (Almeida & Queiroz 2009). Habitat structure affects microclimatic conditions and resource availability for the species, which may result in differences in parasitism rates by both dipteran and hymenopteran parasitoids (De La Mora & Philpott 2010; Vasquez-Ordonez *et al.* 2012; Pardee & Philpott 2011). Although the association of *E. breviloba* with closed areas and of *M. exrobusta* with open areas is probably related to environmental conditions, we did not find a relationship between phorid presence and either temperature or humidity. Only in relation to the abundance of *M. exrobusta* there was a negative relationship with vapor pressure deficit. Because this species is smaller than *E. breviloba* (Brown *et al.* 2012) and is associated with open areas, it is more exposed to extreme climatic conditions, which limited its foraging opportunities during the hottest times of the day. However, *E. breviloba* may attack *A. robusta* at any time of the day (the time variable was not significant in the models), possibly because the forest protects the individuals of this species against adverse conditions.

However, we could not exclude habitat segregation mediated by competition between these phorid species as a mechanism to explain the higher abundance of each species in different habitats. The two species were collected attacking *A. robusta* at the same time in only one nest, suggesting that competitive exclusion could be accounting for the pattern found here. The open habitat seems to be a harsher habitat than the closed one, due to the higher temperature and low relative humidity, and because all nests without parasitoids were located in this habitat. Thus, it might be possible that *E. breviloba* excludes *M. exrobusta* from closed forest sites, forcing it to attack in harsher conditions. Two studies

demonstrate that the parasitoid *Pseudacteon curvatus*, attacking invasive *Solenopsis* ants, behaviorally displaces *P. tricuspis* from the sites were it prefers to oviposit (LeBrun *et al.* 2009; Porter & Calcaterra 2013). To determine conclusively which mechanism is involved, experimental assays are necessary in this system.

Although some studies evidenced the preference of phorid flies for specific attack sites (Tonhasca Jr. *et al.* 2001; Bragança *et al.* 2003; Elizalde & Folgarait 2012), in the present study there was no significant difference in the number of parasitoids captured between trails and nest holes. Different oviposition sites could be important in segregating host resource by parasitoids. However, because the same ant forager goes through the same entrance hole and foraging trail (Elizalde & Farji-Brener 2012), this might not be a form of host segregation, as are other microsites such as external refuse piles or cutting sites (Elizalde & Foglarait 2012).

The threats to the few remaining forests in the area of occurrence of *A. robusta*, i.e. the coast from Rio de Janeiro to Espírito Santo, in Brazil, may endanger the survival of *E. breviloba*. The urban parks are an important component for biodiversity conservation of the remaining *restingas* in the highly urbanized areas in the city of Rio de Janeiro. However, special emphasis should be placed on the design of those urban green areas, in order to include an array of habitats, for example, that both open and closed areas are available for species with different requirements.

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