

Parasitoid phorid flies of leaf-cutting ants are negatively affected by loss of forest cover

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Abstract

Habitat fragmentation can have a high impact on parasitoid–ant interactions. Phorid flies are among the most important groups of natural enemies of leaf-cutting ants. We studied the effects of loss in forest cover upon phorids of the leaf-cutting ant *Acromyrmex niger* Smith (Hymenoptera: Formicidae: Attini) in a fragmented area in the Southeastern Atlantic Forest, Brazil. We sampled 10 forest fragments, five large (>75 ha) and five small (<20 ha), as well as three areas of continuous forest (>1 000 ha). We marked 1–5 colonies of *A. niger* in the interior of each forest location. At each nest, we collected all of the phorids in interaction with the worker ants for a period of 15 min. We then collected ca. 200 worker ants, which we maintained in the laboratory for rearing phorids from them. We identified three phorid genera – *Apocephalus*, *Myrmosicarius*, and *Neodohrniphora* – which we both observed in the field and reared in the laboratory. The abundance and parasitism percentage were significantly greater in continuous forest sites than in forest fragments, whereas there were no significant differences between fragments of different sizes. These results provide further evidence for the effects of habitat size on the phorid–*Acromyrmex* system in a tropical rain forest, based on the abundance of parasitoids both as adults in the field and as reared immature phorids in the laboratory.

Introduction

The process of habitat fragmentation affects biodiversity and ecosystem functioning (Fahrig, 2003). The consequences depend upon the size and shape of the fragments, the degree of isolation, edge effects, and the type of land use of the surrounding matrix (Harris, 1984; Cerqueira et al., 2003). Fragment size is one of the most important variables to consider (Barbosa & Marquet, 2002; Fahrig, 2003; Ewers & Didham, 2006). The species–area relationship indicates that larger habitats support more diverse communities – smaller fragments can have reduced habitat heterogeneity, lower abundance of individuals (Kruess & Tscharntke, 2000), altered interspecific interactions (Sabatino & Maceira, 2010; Kaartinen &

Roslin, 2011), and increased edge effects (Murcia, 1995). Habitat fragmentation can have different effects on different organisms, with some being strongly negatively affected, whereas others are unaffected or even benefited (Fahrig, 2003).

Organisms that occupy higher trophic levels may be more severely affected by habitat fragmentation (Kruess & Tscharntke, 2000; Montoya et al., 2006); the same can occur to organisms with narrow ecological niches (Henle et al., 2004; Cagnolo et al., 2009). In ecological specialization, the degree of environmental specialization as well as specialization in interactions with other species explain the responses of organisms to perturbation (Vázquez & Simberloff, 2002; Gomes et al., 2013). An empirical study evaluating the effects of area loss on trophic (plant–herbivore–parasitoid) interactions found that organisms were indeed affected according to their degree of specialization and trophic level, with specialist secondary consumers being the most affected (Cagnolo et al., 2009).

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Herbivore populations can be regulated by the abundance of predators and parasitoids (Kruess & Tscharnke, 1994). As an example of this type of control, in disturbed areas and forest edges a reduction in the abundance and effectiveness of parasitoids (Almeida et al., 2008) and other predators (Wirth et al., 2008) favors the increase in density of leaf-cutting ant colonies. Thus, community structure, interspecific interactions, and ecological functions can be affected, and the magnitude and direction of the changes are often substantial (Tscharnke et al., 2002).

Leaf-cutting ants in the genera *Atta* and *Acromyrmex* (Hymenoptera: Formicidae: Attini) are endemic to the American continent and considered the dominant herbivores in the Neotropics (Hölldobler & Wilson, 1990). Furthermore, due to their great capacity to modify the environment, leaf-cutting ants are considered 'ecosystem engineers' (Jones et al., 1994; Leal et al., 2014). Aside from their relevance to conservation, these ants can be major agricultural pests, especially in Brazil (Della Lucia, 2003).

Among the natural enemies of leaf-cutting ants are the parasitoid flies of the family Phoridae (Diptera). Phorids are considered important as population regulators of leaf-cutting ants (Morrison & Porter, 2005), because they deposit their eggs inside the body of worker ants, after which the phorid larvae develop and feed on the tissue of the host ant, eventually killing it (Godfray, 1994). Besides the effect of host mortality, the presence of parasitoids can produce important indirect effects, such as the reduction in foraging activity, as the ants stop foraging to defend themselves against phorid attacks (Orr, 1992; Bragança et al., 1998; Elizalde & Folgarait, 2012; Guillade & Folgarait, 2015).

Elizalde & Folgarait (2010, 2011) argue that the phorid parasitoid-leafcutter ant system is highly specialized, and the species that attack leaf-cutting ants are, as far as we know, exclusive to them. Additionally, the species of phorid parasitoids that attack one genus of leaf-cutting ants do not attack other genera (Elizalde & Folgarait, 2011); therefore, our understanding of the phorid-*Atta* system cannot be extrapolated to the phorid-*Acromyrmex* system and vice versa. Evidence suggests that these parasitoids may be more negatively affected by habitat fragmentation than the ants (Almeida et al., 2008; Elizalde & Queiroz, 2013). Given the strong specialization in this system, it is very likely that any effects that fragmentation has upon phorid parasitoids could end up indirectly affecting the ants and thus the equilibrium of the entire forest community (Almeida et al., 2008). Thus, increasing our knowledge of how these interactions are affected by habitat fragmentation is highly relevant to forest conservation and restoration.

The leaf-cutting ant *Acromyrmex niger* Smith is highly abundant in Brazil and is considered a very harmful species for crop plants (Gonçalves, 1961; Elizalde & Queiroz, 2013). Evaluating the interactions between *A. niger* and parasitoid phorids is especially relevant for the development of management techniques for conservation biological control (Barbosa, 1998). In this study, we evaluated the effects of forest cover loss on the phorid parasitoids of *A. niger* in a fragmented landscape in Southeastern Brazil. Specifically, we measured the variation in parasitoid abundance and parasitism rates of forest fragments of different sizes. This was done through observing and collecting parasitoids attacking ants in the field and by maintaining samples of live worker ants in the laboratory under conditions that allowed for the development and emergence of immature phorids that had already been deposited in the worker ants' bodies. This dual sampling approach allowed us to gain a better understanding of the true abundance and parasitism rates in the system.

Materials and methods

Study site and species

We conducted this study in the Ecological Reserve of Guapiaçu (REGUA, www.regua.org) and neighboring areas. REGUA is located 100 km northeast of the city of Rio de Janeiro (22°22'16"S, 42°44'19"W). This region contains remnants of the Atlantic Forest in various stages of succession, the majority of which are situated on hilltops and embedded within a matrix of agricultural land use, including pastures and annual and perennial crops. The forest is especially well-preserved above 400 m altitude, lower lying areas are comprised of a mosaic of secondary forest surrounded by agricultural landscape (Cabral et al., 2007). The forest type of this region can be characterized as lowland, submontane, and montane ombrophilous forest (Oliveira-Filho & Fontes, 2000); the mean annual temperature is 20 °C and the annual precipitation is 2 010 mm (Massera da Hora & Gonçalves Costa, 2010).

Acromyrmex niger is abundantly distributed throughout the southern and southeastern regions of Brazil (Delabie et al., 2011), found frequently in the mountains of the states of Rio de Janeiro, São Paulo, and Espírito Santo (Gonçalves, 1961). The nests are difficult to locate because of the underground chambers and long galleries with very discrete entrances (simple openings in the soil). Workers can be seen foraging on distinct trails, carrying leaves to feed the symbiotic fungal culture they tend within the nest (Elizalde & Queiroz, 2013).

Study design

We selected a total of 13 study sites: 10 fragments (five large: mean \pm SE = 279 \pm 119 ha; five small: 12 \pm 1.8 ha) and 3 areas of continuous forest (>1 000 ha) (Figure 1; Appendix S1). The selected locations were distributed across an area of 300 km². Sites were chosen between the months of August and December 2014, using Google Earth to select fragments of different sizes that were more or less regular in shape (e.g., not long and thin because of stronger edge effects). Only sites where we were able to locate *A. niger* nests were included in the study, which was about one out of five small fragments we searched and about one out of two large fragments. We found *A. niger* in all of the continuous forest sites we explored.

In each site, we conducted sampling in the interior of the fragment starting from a minimum distance of 300 m from the edge whenever possible. We marked a minimum of one and a maximum of five nests per site; in total there were 51 nests: 15 in continuous forest sites, 24 in large fragments, and 12 in small fragments. We flagged all of the selected nests with marking tape and recorded their coordinates with a GPS so they could be located again whenever necessary.

Sampling of phorid parasitoids

We returned to the marked nests to conduct the sampling of phorid parasitoids – in cases when the nest was no longer active due to colony death or migration, we used neighboring colonies (Elizalde & Folgarait, 2011). During

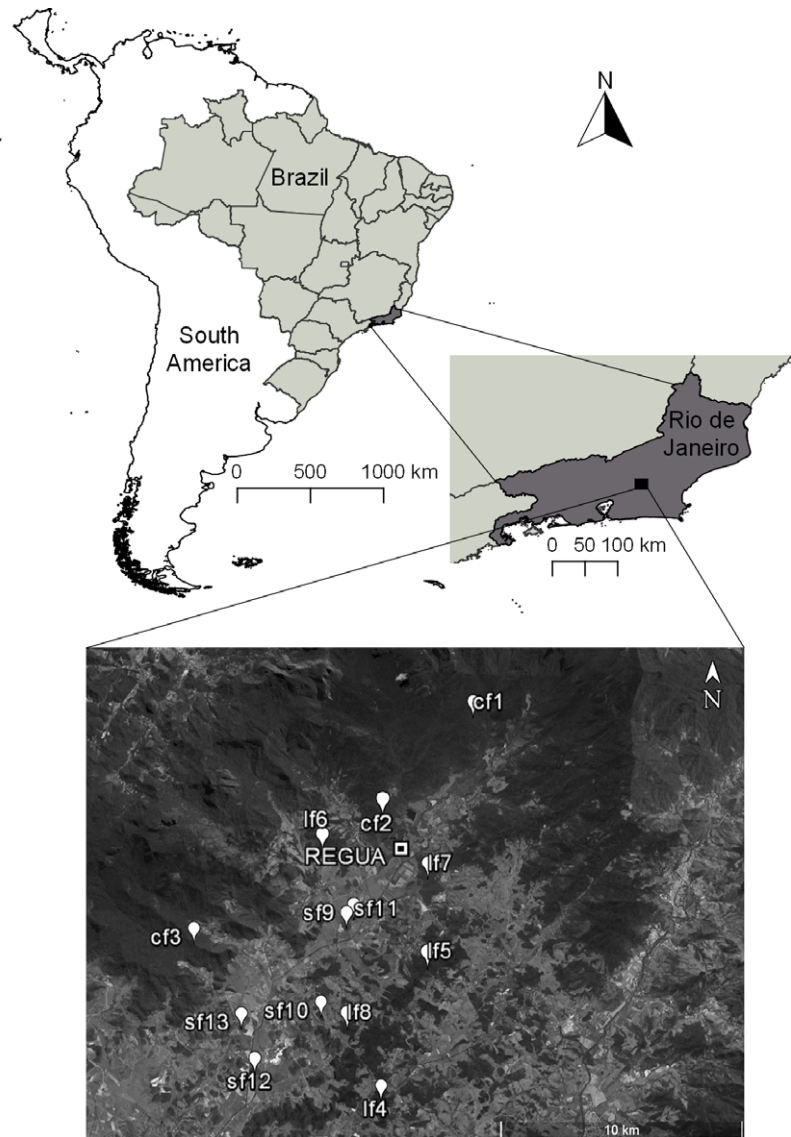


Figure 1 Study area location and sites selected in the Guapiassu Ecological Reserve (REGUA) and surrounding areas, Rio de Janeiro State, Brazil. ‘REGUA’ indicates the REGUA headquarters, cf = continuous forest site, lf = large forest fragment, sf = small forest fragment. Numbers indicate the labels given to each site. See Appendix S1 for a description of site details (latitude, longitude, altitude, and area).

a 15-min period, we used an aspirator to collect all the detected phorid parasitoids interacting with the foraging worker ants. This sampling was conducted at the entrance and along ca. 2 m of the main foraging trails (Elizalde & Folgarait, 2012). Sampling was done in the daytime, between 10:00 and 16:00 hours, during periods when the ants were found actively foraging and, therefore, when the parasitoids would be the most active (Orr, 1992; Bragança et al., 2008; Elizalde & Queiroz, 2013). After collection, parasitoids were preserved in alcohol and later identified in the laboratory using a 90× dissecting microscope and with the available identification keys (Brown, 1997; Disney et al., 2006, 2008, 2009; Brown et al., 2010).

We also collected a ‘Larval Parasitoid Collection’ (LPC) of phorids from each nest as outlined in Elizalde & Folgarait (2011). This sampling involved collecting worker ants over a fixed point on the trail for 15 min or until we collected ca. 200 individuals, from medium to large body size, to minimize the possible influence of worker size on parasitism rate (Orr, 1992; but see also Folgarait, 2013). Nevertheless, we measured head width of 10 randomly selected workers of each nest in the laboratory afterwards. Head width is considered a precise standard measurement of body size (Hölldobler & Wilson, 1990). The LPC allowed us to assess the parasitism rate of ants over a larger period of time, as ants containing fly larvae may have been attacked any time in the previous several weeks. Workers were then transported to the laboratory where they were maintained in a BOD (bio-oxygen demand) chamber. Temperature and relative humidity were kept at 28 °C and 80%, as these were the conditions that Guillade & Folgarait (2014) found to produce the shortest developmental period.

As the ants died, the dead ants were separated from the live ones and kept in a separate container. Dead ants were examined under a dissecting microscope to look for signs that larvae had entered the pupal stage, which were detectable as one or more oval masses in the ant head or as pupae protruding from the thorax. When pupae were found, we separated each of these into its own individual container. Dead ants were checked for pupae for 10 days before discarding and ants containing pupae were maintained for up to 60 days or until adults emerged. When adult flies emerged, they were captured with an aspirator and placed in alcohol for later identification (Elizalde & Folgarait, 2011).

Collections were made on a rotational basis by habitat type during four consecutive months from January to April 2015. This period corresponds to the summer/fall season in this region (Dereczynski et al., 2009), and we assumed that the environmental conditions were similar throughout the geographical area encompassed by our

study and throughout the sampling period. We decided to conduct the sampling during this time of the year given the higher abundance of phorids seen by other researchers when the temperature and humidity are higher (Morrison et al., 2000; Wuellner & Saunders, 2003).

Statistical analysis

We obtained the genus abundance of parasitoids from the identification of the phorids collected in the field. Later, upon deeper analysis of the specimens, we were able to identify the phorids to the species level; however, we did not consider this in the data analysis because the number of individuals was very low (Appendix S2). Nevertheless, we consider the genus-level analysis valid because it is known that phorid species within the same genus have similar natural histories (Elizalde & Folgarait, 2011, 2012).

To estimate the percentage of parasitized ants (i.e., parasitism rate), we counted the collected worker ants hosting parasitoid larvae and divided this number by the total number of ants collected per nest. The parasitism rates obtained from the laboratory rearing allowed for comparison with the adult parasitoids observed in the field. We performed the analysis at the genus level as the phorids in the larval stage were identifiable to the genus level but not the species level – the morphology of the pupae is genus-specific (Elizalde & Folgarait, 2011).

To evaluate the effects of forest cover loss upon abundance and parasitism rate of the phorids, we conducted linear mixed-effects models with habitat type (i.e., continuous forest, large and small fragments) as fixed variable. In addition, we included the average worker size for each nest as a covariate. The random part of the model grouped the nests that belong to the same forest or fragment. In total, we built six models, all with the same structure for fixed and random variables, but the dependent variables were the total abundance of adult phorids in the field and the parasitism rate (data were arcsine \sqrt{x} transformed) for all phorids together, as well as for two genera separately (the other phorid genus abundance was very low).

To analyze the effects of forest cover loss upon the phorid occurrence interacting with the workers, we used mixed-effects logistic models with the same fixed and random structure as above and the presence (represented in the model as 1) or absence (represented as 0) of the parasitoids as the dependent variable. We considered phorids to be present at a nest when we observed them in the field and/or in the laboratory rearing and we considered them absent when they were not present in either the field or the laboratory.

The independent effect of each explanatory variable in multiple correlations can be assessed partially if variables are not correlated. Because most our dependent variables

were positively correlated with elevation (Appendix S3), probably due to the fact that the better-preserved forests lie at higher elevation, as explained above, we also built six other models with the elevation of each nest (obtained from a GPS) as covariate.

We used log-likelihood tests to evaluate the statistical significance of all the independent variables, and continuous variables were z-score transformed to reduce the effect of the various scale measurements in model estimation. All analyses ($\alpha = 0.05$) were carried out with the statistical software R v.3.3.1 (R Development Core Team, 2016).

Results

After a combined 13 h of observation above the nests (15 min over each of 51 nests), we found adult phorids to be present in 27.4% of the total nests sampled. We observed 22 adult phorids attacking worker ants; 11 of the genus *Myrmosciarius*, seven of the genus *Apocephalus*, three of the genus *Neodohrniphora*, and one of an unidentified genus. Adult phorids were observed attacking ants in eight of the 15 nests (53%) in continuous forest, 17% of the nests in large fragments, and 8% of the nests in small fragments. Of the nests with parasitoids, 64.3% had only one phorid, whereas 21.4% had two, and 7.1% had three or four. Only nests in the continuous forest had more than one genus of phorids attacking at the same time, with *Myrmosciarius* and *Apocephalus* in two cases and *Neodohrniphora* and *Apocephalus* in one other case. We did not find *Neodohrniphora* interacting with ants in the small fragments.

Through LPC, we obtained a total of 85 pupae (0.82% of the total ants collected) distributed among the genera *Apocephalus*, *Myrmosciarius*, and *Neodohrniphora*. Parasitized workers were observed in 10 of the 15 nests (67%) in continuous forest, 29% of large fragments, and 16% of small fragments. The highest parasitism rate (7.5%) was recorded in a nest from the continuous forest. Of all the pupae, only 30 adults were able to emerge, all of which were of the genus *Apocephalus*, except for one of the genus *Myrmosciarius*. The observation of more than one genus of phorid in parasitized ants from the same nest only occurred in continuous forest samples, with all three genera present in three nests and *Apocephalus* and *Myrmosciarius* in another two (Appendix S4).

We found that forest loss has a negative effect both on parasitoid abundance and parasitism rate (Table 1), and that the effect was due to differences in the fragments (large vs. small), compared to the continuous forest (Table 1, Figure 2A,B). In the case of phorids of the genus *Apocephalus*, their abundance of adults attacking ants indicated significant differences between continuous forest

sites and fragments (Table 1, Figure 2C). On the other hand, the parasitism rate for this genus was not significantly different for any of the habitat types (Table 1, Figure 2D). For phorids of the genus *Myrmosciarius*, the opposite pattern was observed; there was no significant difference between habitat types for abundance of adults (Table 1, Figure 2E), but there was for parasitism rate (Table 1, Figure 2F). However, the occurrence of all parasitoids grouped and of the genus *Myrmosciarius* alone was not affected by habitat type (Table 2); phorids of the genus *Apocephalus* occurred more in continuous forests than in fragments (Table 2).

When the mixed models included elevation as covariate, there was a near significant effect of habitat on phorid abundance (Table 1), and a significant effect on abundance of *Apocephalus* adults: fewer *Apocephalus* adults occurred in small forest fragments than in continuous forest or in large fragments (Table 1). For the parasitism rate, we found only a near significant effect of habitat for *Myrmosciarius* (Table 1). The effect of habitat on phorid occurrence was not statistically significant in any case (Table 2). Ant worker size was not a significant factor in any model.

Discussion

The Atlantic Forest was once one of the largest rainforests in the world, but today less than 16% remains as fragments, about 80% of which are <50 ha, separated by anthropogenic land uses (Ribeiro et al., 2009). Still, this biome remains a hotspot of biodiversity, and understanding how forest fragmentation affects species and their interactions is key to the preservation of this biodiversity and any ecosystem services (such as biological pest control) provided by natural habitats (Barbosa, 1998). The results presented in this study indicate that parasitoid phorids of the leaf-cutting ant *A. niger* are affected by forest fragmentation in the Atlantic Forest. These results corroborate previous findings of the negative effect of fragmentation upon phorid parasitoids of leaf-cutting ants in the genera *Atta* (Rao, 2000; Almeida et al., 2008) and *Acromyrmex* (Elizalde & Queiroz, 2013).

In general, our results suggest that colonies of *A. niger* in smaller forest fragments are exposed to a decrease in both abundance of phorids and parasitism rate. Thus, the present study offers more evidence for the effects of forest area loss on phorid parasitoids of the leaf-cutting ant genus *Acromyrmex* in a tropical rainforest, adding to the few reports of an impact of forest fragments in comparison to continuous forest (Rao, 2000; Elizalde & Queiroz, 2013) and of edge effects (Almeida et al., 2008) on leaf-cutting ant parasitoids. In addition, this is the first evaluation of

Table 1 Statistics and P-values for mixed-effects linear models of phorid abundance and % parasitism in *Acromyrmex niger* ant nests in continuous forest and large and small forest fragments, for all parasitoids combined, and for *Apocephalus* spp. and *Myrmosciarius* spp. separately. Models were run without and with elevation of ant nests as covariate, and in all cases the sampling site was included as random effect. All continuous variables were transformed using the z-score

	Phorid abundance						% parasitism									
	Without elevation			With elevation			Without elevation			With elevation						
	Estimate	Statistic ¹	d.f.	P	Estimate	Statistic ¹	d.f.	P	Estimate	Statistic ¹	d.f.	P				
All parasitoids																
Habitat		9.86	2	0.007		4.86	2	0.09		9.25	2	0.01		3.82	2	0.15
Continuous vs. large fragment	-0.74	-2.79		0.02						-0.62	-2.91		0.01			
Continuous vs. small fragment	-0.91	-2.96		0.01						-0.65	-2.81		0.02			
Large vs. small fragment	-0.17	-0.6		0.56						-0.65	-0.16		0.87			
Worker size	0.07	0.41	1	0.52	0.05	0.23	1	0.63	0.01	0.14	1	0.71	-0.01	0.01	1	91
Elevation					0.08	0.19	1	0.66					0.28	7.91	1	0.005
Model		AIC = 135.3; residual d.f. = 46							AIC = 81.9; residual d.f. = 46							
<i>Apocephalus</i> spp.																
Habitat		6.81	2	0.03		7.32	2	0.03		4.98	2	0.08		2.95	2	0.23
Continuous vs. large fragment	-0.36	-2.34		0.04		-0.48		0.03								
Continuous vs. small fragment	-0.41	-2.12		0.05		-0.6		0.04								
Large vs. small fragment	-0.04	-0.24		0.81		-0.12		0.51								
Worker size	-0.04	0.31	1	0.58	-0.01	0.04	1	0.85	0.04	0.58	1	0.44	0.02	0.24	1	0.64
Elevation					-0.11	1.34	1	0.24					0.29	7.93	1	0.005
Model		AIC = 87.2; residual d.f. = 46							AIC = 90.8; residual d.f. = 45							
<i>Myrmosciarius</i> spp.																
Habitat		3.37	2	0.18		0.75	2	0.69		7.51	2	0.02		4.05	2	0.13
Continuous vs. large fragment										-0.12	-2.23		0.05			
Continuous vs. small fragment										-0.17	-2.56		0.03			
Large vs. small fragment										-0.04	-0.72		0.48			
Worker size	0.08	1.32	1	0.25	0.04	0.28	1	0.59	-0.03	1.81	1	0.18	-0.03	1.64	1	0.2
Elevation					0.24	5.63	1	0.02					-0.001	0.6	1	0.99
Model		AIC = 95.8; residual d.f. = 46							AIC = 96.1; residual d.f. = 45							

¹For 'habitat' the χ^2 value for the log-likelihood test is shown, for continuous variables it is the slope.

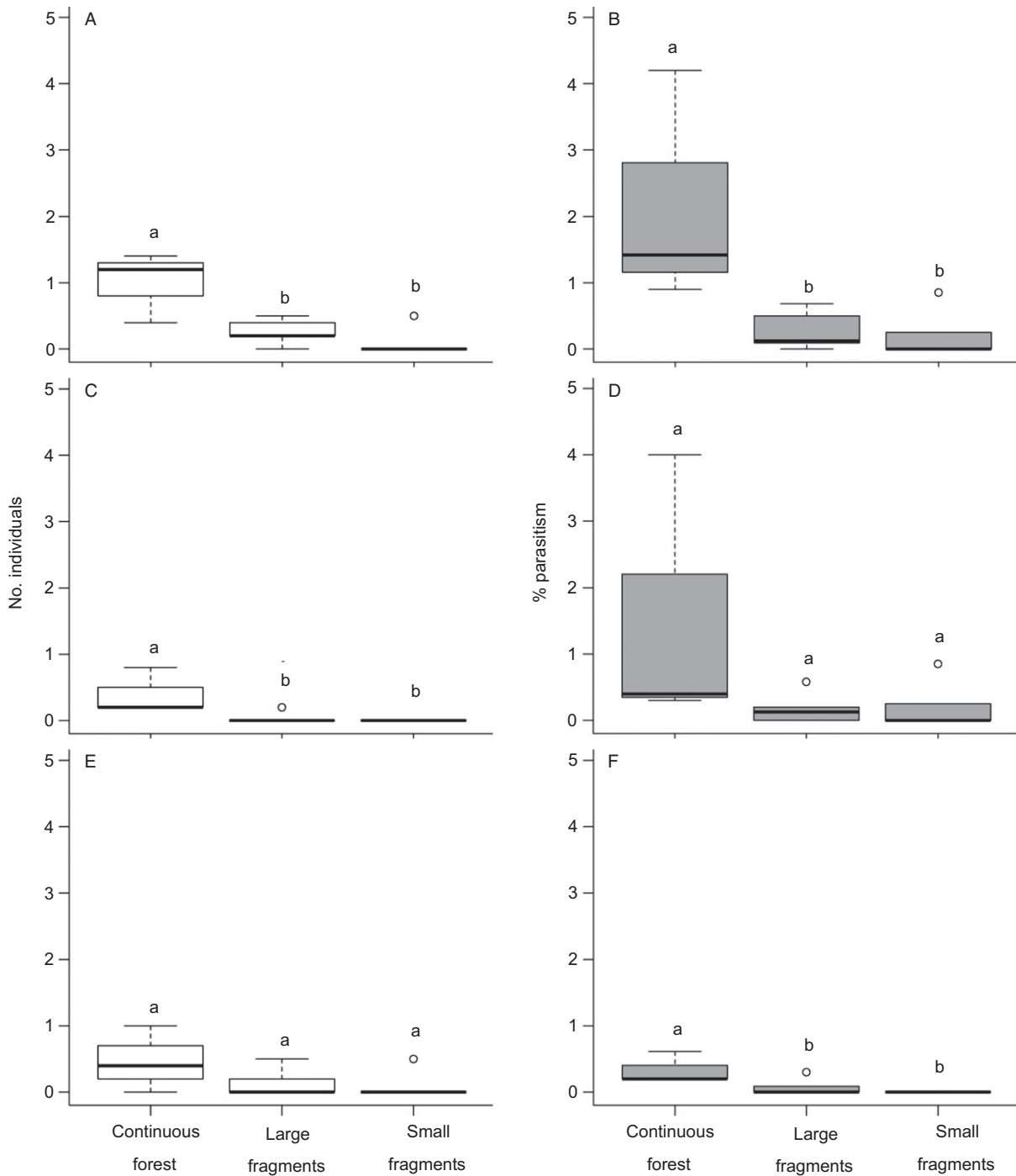


Figure 2 Boxplots of (A, C, and E) abundance of adult phorids attacking or flying over foraging *Acromyrmex niger* workers and (B, D, and F) total parasitism rate in continuous forest and large and small forest fragments. (A and B) Total phorids, (C and D) *Apocephalus* spp., and (E and F) *Myrmomicarius* spp. Solid horizontal lines in the boxes represent median values, the whiskers represent the smallest and largest non-outlier observations, dots represent outlying data points. We plotted medians because we have mixed data, some being normally distributed and others not. Different letters capping boxes within a panel indicate significant differences between means (A, B, and E) or medians (C, D, and F) based on linear mixed-effects models without elevation ($P < 0.05$).

Table 2 Statistics and P-values for logistic models of phorid occurrence in *Acromyrmex niger* ant nests in continuous forest and large and small forest fragments, for all parasitoids combined, and for *Apocephalus* spp. and *Myrmosicarius* spp. separately. Models were run without and with elevation of ant nests as covariate, and in all cases the sampling site was included as random effect. All continuous variables were transformed using the z-score

	Without elevation				With elevation			
	Estimate	Statistic ¹	d.f.	P	Estimate	Statistic ¹	d.f.	P
All parasitoids								
Habitat		4.19	2	0.12		0.32	2	0.85
Worker size	0.37	1.15	1	0.28	0.2771	0.54	1	0.46
Elevation					1.3661	3.84	1	0.05
Model	AIC = 70.9; residual d.f. = 46				AIC = 69.1; residual d.f. = 45			
<i>Apocephalus</i> spp.								
Habitat		7.09	2	0.03		2.61	2	0.27
Continuous vs. large fragment	-2.46	-2.51		0.01				
Continuous vs. small fragment	-2.43	-2.15		0.03				
Large vs. small fragment	-0.04	0.03		0.97				
Worker size	0.15	0.15	1	0.69	0.006	0.002	1	0.99
Elevation					0.96	2.34	1	0.13
Model	AIC = 60.9; residual d.f. = 46				AIC = 60.6; residual d.f. = 45			
<i>Myrmosicarius</i> spp.								
Habitat		5.24	2	0.07		0.02	2	0.99
Continuous vs. large fragment								
Continuous vs. small fragment								
Large vs. small fragment								
Worker size	-0.07	0.04	1	0.83	-0.37	0.99	1	0.32
Elevation					1.14	5.55	1	0.02
Model	AIC = 62.1; residual d.f. = 46				AIC = 58.6; residual d.f. = 45			

¹For 'habitat' the χ^2 value for the log-likelihood test is shown, for continuous variables it is the slope.

adult phorids attacking workers in the field, complemented with the determination of their parasitism rate through the larval phorid collection and rearing in the laboratory (Elizalde & Folgarait, 2011). Previous studies of *Atta* and *Acromyrmex* parasitoids evaluated only their abundance and attack rates in the field (Rao, 2000; Almeida et al., 2008; Elizalde & Queiroz, 2013).

Of the three phorid genera that we found, the adults were previously observed attacking *A. niger* workers in the field, but their juvenile stages had not been reared in the laboratory. This method allowed us to confirm previous observations that mentioned these genera as parasitoids of *A. niger* (Borgmeier, 1931; Bragança, 2011; Elizalde & Queiroz, 2013). The small number of adults collected in the field and emerging from hosts in the laboratory allowed us to only analyze statistically the abundance of phorids and the parasitism rate to the genus level. Probably the phorids' life cycle was interrupted during rearing primarily by the presence of fungi on the dead ants. The negative effect of fungus proliferation on insect larvae is known for other species (Queiroz & Oliveira, 2001). However, because all samples stayed under the same conditions

in the laboratory, the comparisons among habitats are unlikely affected by the incidence of fungi on dead workers. Additionally, the choice of temperature and humidity levels that correspond to the shortest development time may have reduced the survival of immature phorids (Guilade & Folgarait, 2014). Previous studies with other genera of phorids demonstrated that the larval and pupal stages and the transition between them are especially sensitive to these abiotic variables (Morrison et al., 1997, 2000). Therefore, adjustments to the protocol for maintaining the hosts in the laboratory could improve the emergence rate of the adult phorids and allow comparisons at the species level.

By using two techniques to record phorids in ant nests, more nests with phorids were found: there was an increase of 75% in the number of nest with phorids compared with using only observations of adults in the field, and compared with LPC, the increase was 21%. Hence, the observation of adult phorids in the field alone, as was done in previous studies (Rao, 2000; Almeida et al., 2008; Elizalde & Queiroz, 2013), does not provide a complete picture of the phorid-ant interactions. The absence of adult

parasitoids in field observations is not necessarily evidence for a negative effect upon the phorids' parasitism rate. Thus, we suggest that the rearing of larval parasitoids and the evaluation of percentages of parasitism be incorporated into future studies that evaluate the environmental impacts upon phorid parasitoid–host interactions.

The highest parasitism rate measured in this study was 8%, which is slightly lower than the maximum of 12% measured for *Acromyrmex* in another region (Elizalde & Folgarait, 2011). The parasitism rate decreased significantly with the loss of forest cover, but these values were relatively low (the highest mean value by habitat type was 2.2% in the continuous forest). Nonetheless, these values are within the range of natural average parasitism rate observed in *Acromyrmex* in Argentina (0.9–2.2%).

The highest phorid abundance and parasitism rate in the continuous forest sites provide further evidence that this habitat type is the best for the presence and reproductive success of phorids. *Apocephalus* was the genus with the highest total abundance (summing adults and immatures) and the only genus parasitizing ants in all the habitat types; however, it was also the genus whose parasitism rate decreased the most with the decrease in forest cover. In a previous study, adult *Apocephalus* were only observed flying over colonies of *A. niger* in the continuous forest, but not in fragments (Elizalde & Queiroz, 2013).

In the fragmented landscape of Guapiaçu basin, where the sampled sites are located, the forest fragments of different sizes are embedded in a matrix of pastures and small croplands. With our experimental design we are unable to disentangle the several effects that the forest fragmentation may have on biodiversity (Fahrig, 2003). In our study area, the sampled sites in continuous forest were, on average, more elevated than the small fragments, with large fragments in an intermediate position between the two others. In fact, when we included elevation as a covariate in the mixed-effects models, it proved an important variable. Moreover, the variable measuring the fragmentation of the habitat was not significant for total parasitoid abundance and % parasitism for all phorids if elevation was included in the models. Although we cannot discard the possible influence of elevation on *Myrmosicarius* parasitoids, for *Apocephalus*, which was more abundant in the system, the effect of forest area loss was significant, even after including altitudinal elevation in the models.

Microclimatic conditions may be important to explain differences in ant–parasitoid interactions in continuous and fragmented forests because of edge effects. Small fragments have a higher proportion of edge habitat, where temperature and wind flux increase, and consequently humidity decreases (Laurance et al., 2007) – these conditions are unfavorable to phorids, affecting

their survival and reproductive success and resulting in a decrease in their abundance (Morrison et al., 2000; Folgarait et al., 2007; Almeida et al., 2008). Furthermore, the increase in wind turbulence in areas of less forest cover could influence the ability of phorids to find their hosts, as the location of hosts is done primarily through olfactory signals (Orr et al., 1997). In addition to just having a higher proportion of edge habitat, biotic and abiotic processes in edges can cause greater variability in ecological processes in the interior of the fragment (Ewers & Didham, 2006).

Another possible explanation for the observed difference in parasitoid abundance and parasitism rate is connected to population dynamics. Could the negative effects of habitat fragmentation upon phorids be due to a negative effect upon their host? The reduction in abundance of specific hosts could also reduce the abundance of parasitoids (Folgarait, 2013). The little evidence that leafcutter ants respond to changes in forest cover comes from studies with *Atta* species (Jaffe & Vilela, 1989; Rao, 2000). For *Acromyrmex*, Elizalde & Queiroz (2013) found almost the same nest density of *A. niger* in continuous or fragmented forest. However, as far as we know, *A. niger* is more frequent in forest habitats (e.g., Forti et al., 2006). Thus, forest fragments in the region would have small populations of *A. niger* because of the area-size per se (Fahrig, 2003). The surrounding matrix, composed mainly of pastures, would have unfavorable conditions for populations of *A. niger*. The species of phorids that were found associated with *A. niger* were not observed attacking other *Acromyrmex* species, i.e., they are very specific (Elizalde & Queiroz, 2013). As the parasitism rates of phorid flies is usually low and because the *A. niger* phorids are very host-specific, phorid species in forest fragments would have small populations due to recruitment limitation. The small body size and behavior of phorid parasitoids would limit active dispersion to isolated forest fragments (Etienne & Olff, 2004; Elizalde & Queiroz, 2013). Small populations may suffer the negative consequences of low genetic diversity (Frankham, 1996), and they are more vulnerable to stochastic extinction (Pimm et al., 1988), thus explaining the low occurrence of phorid parasitoids in the forest fragments. We suggest that future studies should evaluate population genetic diversity of parasitoid species in continuous and fragmented forests.

The reduction in the number of adult phorids and the percentage of parasitized ants suggests a relaxing of the 'top down' forces that control ant populations in forest fragments. Our study demonstrates that the loss in forest area negatively affects phorid parasitoids of *A. niger*. Almeida et al. (2008) indicated that habitat fragmentation and especially the creation of edges contribute to an

increase in the abundance of leaf-cutting ants as a consequence of decrease in the abundance of parasitoids. Phorid parasitoids are natural enemies of these ants and may be affected by habitat fragmentation more than their hosts; this kind of disturbance may decrease parasitoid biodiversity as well as the parasitism rate, a variable that is found to be directly linked to the success of biological control (Kruess & Tscharrntke, 1994). The loss of existing biological interactions could cause an even larger loss of forest biodiversity in fragmented habitats (Galetti et al., 2006) and therefore the studies of ant–parasitoid population dynamics and the generation of possible management strategies for their conservation are very important.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Description of sites selected in the Guapiaçu Ecological Reserve (REGUA) and surrounding areas, Rio de Janeiro State, Brazil. Mean (\pm SE) elevation (m above sea level) was calculated from the elevation of each sampled colony.

Appendix S2. Number of adults of the various phorid parasitoid species found in the three forest types based on sampling in the field and the number of adults that emerged in the laboratory rearing.

Appendix S3. Pearson correlation coefficient and P-values for the dependent variables used and the altitudinal elevation of the nests.

Appendix S4. Records of adult and immature phorids of three genera for the three habitat types, sampled in the field and emerged in the laboratory rearing.