



EFFECT OF FOREST COVER ON ECTOPARASITES OF SMALL MAMMALS IN THE BRAZILIAN ATLANTIC FOREST

Norlan de Jesus Santos^{1*}, *Jacques Hubert Charles Delabie*², *Danilo Gonçalves Saraiva*^{3,5}, *Fernanda Aparecida Nieri-Bastos*³, *Thiago Fernandes Martins*³, *Marcelo Bahia Labruna*³, *Ianei de Oliveira Carneiro*¹ & *Carlos Roberto Franke*⁴

¹ Universidade Federal da Bahia, Departamento de Veterinária, Programa de Pós-Graduação em Ciência Animal nos Trópicos. Av. Adhemar de Barros, 500, Ondina, CEP 40170-110, Salvador, BA, Brazil.

² Comissão Executiva do Plano da Lavoura Cacaueira, Centro de Pesquisas do Cacau, Laboratório de Mirmecologia, AC Itabuna, CEP 45600-970, Itabuna, BA, Brazil.

³ Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, CEP 05508 270, São Paulo, SP, Brazil.

⁴ Universidade Federal da Bahia, Departamento de Medicina Veterinária e Zootecnia. Av. Adhemar de Barros, 500, Ondina, CEP 40170-110, Salvador, BA, Brazil.

⁵ *In memoriam*

E-mails: norlansantos@hotmail.com (*corresponding author); jacques.delabie@gmail.com; fenieri@usp.br; thiagodogo@hotmail.com; labruna@usp.br; ianeica@gmail.com; franke@ufba.br

Abstract: Habitat reduction is one of the greatest threats to biodiversity and one of its many expected consequences is the negative impacts on host species. In this study, we evaluated if the reduction in the forest cover positively affected the richness and abundance of ectoparasites and rates of parasitism on mammal species. The study was carried out in the Atlantic Forest biome in the state of Bahia between January to March and September to November of 2011. We sampled four areas with different amounts of forest cover (%) in the municipalities of Valença (25%), Nilo Peçanha (35%), Camamú (45%) and Jaguaripe (55%). We identified twenty species of ectoparasites (fleas, mites, ticks) on 130 small mammals from 18 species (14 rodents and four marsupials), and the most abundant ectoparasites were mites. We confirmed our main hypothesis: the greater richness and abundance of ectoparasites was associated with a reduction in forest cover, with possible epidemiological implications. The prevalence of ectoparasitism per area varied from 91% in Valença to 50% in Jaguaripe. The mean intensity of ectoparasites per host was 6.5 for Valença, 6.4 for Nilo Peçanha, 10.4 for Camamú and 3.2 for Jaguaripe. Our findings confirm that the reduction in the amount of forested is related to amplified rates of parasitism and changes in ectoparasites abundance and species richness.

Keywords: habitat reduction; parasitism; public health; vectors.

INTRODUCTION

The process of reduction and fragmentation of natural habitats is one of the main threats to biodiversity (Fahrig 2003, Prugh *et al.* 2008) because, among other effects, it results in the loss of species, isolation of populations, increase of edge effects in fragments of native vegetation and reduction of the adaptive potential due to the loss of genetic variability (Rolstad 1991). The Atlantic Forest is considered one of the global hotspots of biodiversity, due to its biological richness and high number of endemic species (Myers *et al.* 2000). However, despite its ecological importance, the Atlantic Forest has suffered progressive reduction in its total area due to anthropogenic actions, whereby less than 15% of its original extension remains (SOS Mata Atlântica & INPE 2016). This process results in a mosaic landscape made up of patches of original habitat (islands of habitat) surrounded by areas with different levels of human activity (altered matrices; Murcia 1995). One of the direct consequences of habitat reduction and fragmentation is the increase of border areas between forest fragments and altered landscapes (Gascon *et al.* 1999). This process enables the contact between wild hosts and domestic animals in the surrounding areas, which favors the exchange of parasites and the alteration of their community structures (Small & Hunter 1975, Holmes 1996, Patten *et al.* 2006).

Parasites perform important functions within the ecosystem by, for example, affecting biodiversity (Stanko *et al.* 2002), reducing competition (Moore 2002), mediating interactions (Dunn *et al.* 2012), and causing impacts on reproduction and survival of their host species (Daszak *et al.* 2001). The invasion of parasites can result in the introduction of new diseases, endangering the survival of host species that are not yet adapted to new parasite species (Hudson *et al.* 2002). Concerning ectoparasites of mammals, the majority belongs to the classes Insecta and Arachnida, with the latter group represented mainly by the orders Astigmata, Gamasida, and Ixodida. In particular, ticks, hematophagous mites and fleas constitute groups of interest to Conservation Biology and Veterinary Medicine (Ribeiro *et al.* 1997, Labruna *et al.* 2000), since they may act as vectors of emerging diseases in Brazil,

such as Brazilian spotted fever and ehrlichiosis (Labruna *et al.* 2004), which could infect wildlife, domestic animals and man (Fonseca *et al.* 2005).

Small mammals (marsupials and rodents) are hosts of several species of ectoparasites (Brooks & Hoberg 2000, Saraiva *et al.* 2012). In Brazil, small mammals encompass more than 200 species and are considered the most diverse group among neotropical mammals (Paglia *et al.* 2012). They perform key ecological roles in the dynamic of the forests, such as predation and dispersion of seeds, and can be used as indicators of anthropogenic alterations (Brewer & Rejmánek 1999). The density of these hosts seems to influence the distribution and abundance of ectoparasites, since the intensification of contact between the animals in a restricted area increases the probability of transmission (Arneberg *et al.* 1998, Morand & Poulin 1998). Studies on parasite transmission evaluating the role of habitat reduction and increase in contact between hosts showed different results; Gillespie *et al.* (2005) and Mbora & McPeck (2009) showed that the increase in contact between hosts was an important variable for the parasite transmission, abundance and, consequently, for the prevalence of endoparasites in non-human primates in Africa. On the other hand, neither Stanko *et al.* (2002) in mountains and forested plains in Slovakia, nor Püttker *et al.* (2007) in areas of the Atlantic Forest in Brazil, have identified any association between the parasite load of small mammals and the state of vegetation cover.

In this study, we evaluated the prevalence, intensity and changes in ectoparasite community (richness and abundance) collected in small mammals in relation to the amount of forest cover in four areas in the Atlantic Forest, Brazil. In addition, we analyzed if the community structure of parasites differed between forested areas and the matrix. We expected an increase in the richness and abundance of ectoparasites in small mammals in areas with reduced forest cover.

MATERIAL AND METHODS

Sample design

The Atlantic Forest in the state of Bahia, Brazil, is considered one of the centers of regional ende-

mism in the biome (Silva & Casteleti 2003). It is currently dominated by secondary forest surrounded by a matrix of pasture intermixed with a variety of tree crops, including cocoa, rubber, bananas, palm oil, eucalyptus, and coffee, mainly in privately-owned land (Saatchi *et al.* 2001). The study region comprised the coastal strip of Atlantic Forest south to “Todos os Santos” Bay (13°00′–14°50′S, 39°00′–39°30′W). We sampled four areas of 6 x 6 km (3,600 ha) varying in the amount of remaining forest, in all of which altitude was less than 300 m. Forest remnants were in mid to advanced stages of regeneration and the matrix was dominated by open areas.

We calculated forest cover, the size of the largest forest patch (Largest Patch Index; Mcgarigal & Marks 1995) and the percentage of the matrix that is non-forested and non-urban based on recent satellite images (2005–2008) from the “Atlas of forest remnants of the Atlantic Forest” (*Atlas dos remanescentes florestais da Mata Atlântica*”; www.sosma.org.br and www.inpe.br). We controlled for both forest cover and the size of the largest forest patch in the surrounding, larger areas, since both could act as source areas, thus considering only the 6 x 6 km landscapes where forest cover and the size of the largest forest patches were larger than the observed in the surroundings (18 x 18 km). We also controlled for the permeability of the matrix by considering only landscapes where at least 80% of the matrix presented low permeability, with vegetation height less than 2 m. Thus, we not compute urban areas, tree plantations, such as cacao, pines, eucalyptus, and rubber trees, and young secondary forests. We then chose the sampled landscapes by randomizing their spatial distribution to avoid correlation between geographical position and percentage of forest cover (Figure 1). The four selected areas sampled in this study were located in the following municipalities: Valença (25% of forest cover), Nilo Peçanha (35%), Camamú (45%), and Jaguaripe (55%) (Figure 1).

Each of the four areas was divided into grids of 100 numbered plots of 600 x 600 m for convenience and classified as either forest or matrix plots. We then randomly chose eight plots of each type (16 per area) to locate our sampling

sites, which were checked in the field to guarantee that forests corresponded to secondary forest in mid to advanced stages of regeneration and the matrix to non-urban: open areas with vegetation height less than 2 m. We ensured that in forest plots were settled in a minimum distance of 30 m to forest edge to reduce changes in forest cover as a result of the edge effect (Murcia 1995).

Sampling of small mammal species

We sampled small non-volant mammals (rodents and marsupials) in all 64 plots (eight forest and eight matrix sites in each of the four areas) using both pitfall traps (plastic buckets of 60-liter) not baited and medium-sized Sherman® (10 x 10 x 30 cm) and Tomahawk® (15 x 17 x 45 cm) live traps. In each sampling site, we established ten equidistant pitfall traps connected by a 50 cm high plastic drift fence and 20 equidistant live traps on the ground (ten of each type) 5 m apart from each other. We baited the traps with a mixture of peanut butter, sunflowers seeds, oat grains, palm oil and sardines. The traps were active for eight days and inspected daily. The captured specimens were duly registered, following bioethical procedures and using license 12,023-4 from the Chico Mendes Institute for the Conservation of Biodiversity – ICMBio. All sites and landscapes were sampled between January to March and September to November of 2011. The 16 sites in a landscape were sampled simultaneously and thus the sampling of each landscape took around ten days. We identified small mammals to species level using the external morphology, consulting the literature and specialists. Voucher specimens (of all species) were hosted at the Natural History Museum of the Federal University of Bahia.

Collection and identification of ectoparasites

Collected small mammals were placed in individual cotton bags and had their ectoparasites collected through direct inspection of the corporal surface and brushing. The instruments used in the collection of the ectoparasites were sanitized between each collection. The parasites were put in microtubes containing ethanol and identified concerning the location of collection and the host.

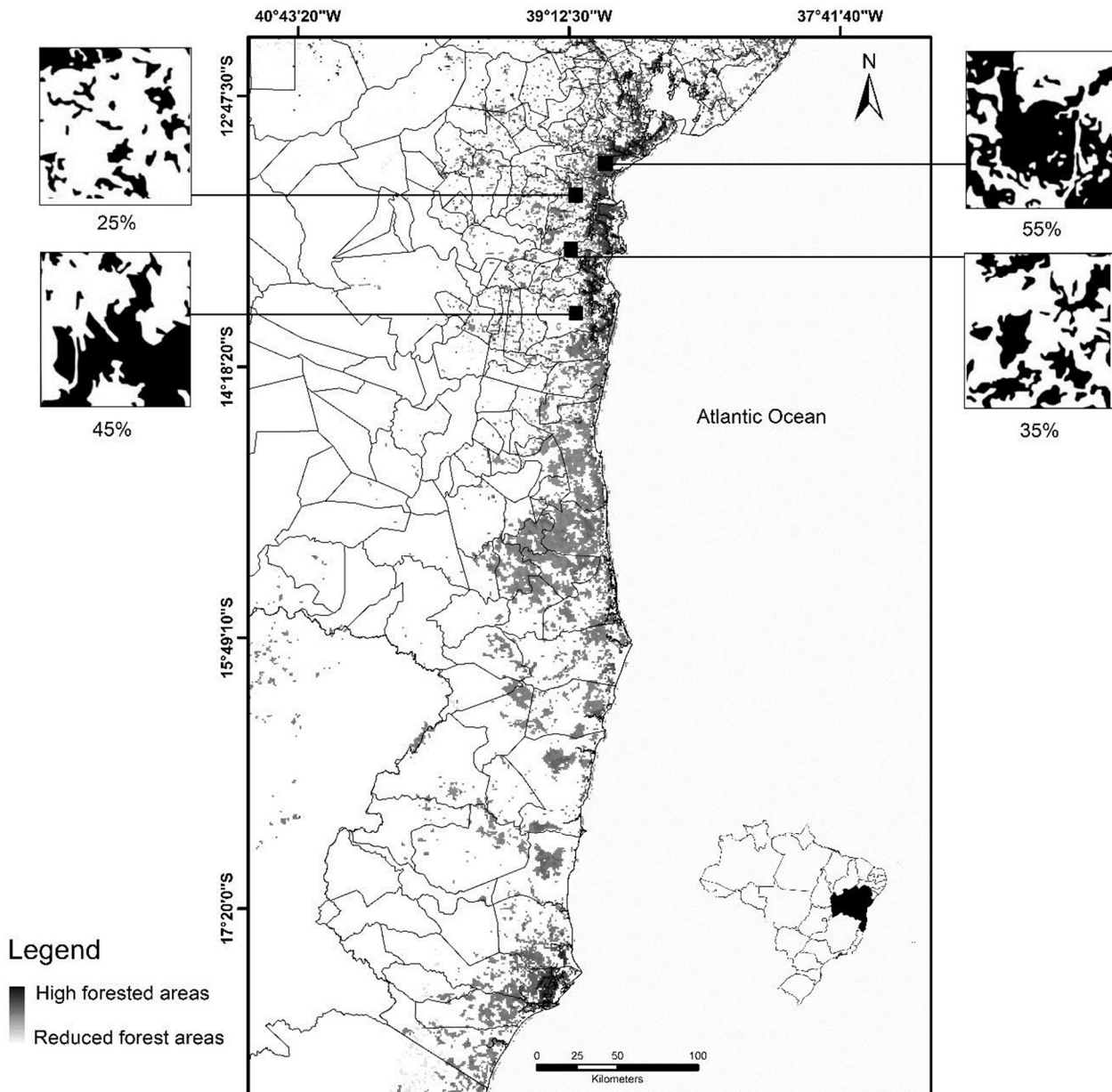


Figure 1. Map showing the position of the state of Bahia, Brazil, and the location where four sampled areas (back squares) were established with the respective percentages of forest cover.

At the end of each sample week, the contents in the microtubes were sent to the Laboratory of Parasitic Diseases at the Department of Veterinary Medicine and Animal Health of the University of São Paulo (*Laboratório de Doenças Parasitárias do Departamento de Medicina Veterinária e Saúde Animal da Universidade de São Paulo*), where they were identified. The identification of the ectoparasites was undertaken with stereo microscope according to Fonseca (1936, 1939) for mites, Linardi & Guimarães (2000) for fleas, and Onofrio *et al.* (2009) and Martins *et al.* (2010) for ticks.

Data analysis

The analyses were carried out with the IBM SPSS Statistics 21 program and the significance level adopted for all the tests was 5% ($\alpha = 0.05$). Normality was evaluated using a Kolmogorov-Smirnov test. We applied Mann-Whitney U test to evaluate possible differences in the richness and abundance of the parasite specimens between the forest and the matrix. Additionally, a Kruskal-Wallis test was performed in order to compare parasite richness and abundance using the percentage of forest cover as group variable. The

host abundance was compared using One-Way ANOVA to evaluate significant differences using the percentage of forest cover as a factor. Additionally, the Spearman correlation was performed between the abundance and richness of host and abundance and richness of ectoparasites.

The prevalence of ectoparasitism per area was estimated as the percentage of the number of infested individuals over the total of individuals analysed in the study. Intensity of the infestation was estimated as the rate between the number of ectoparasites collected and the number of infested hosts in each area.

RESULTS

A total of 130 small mammals of 18 different species were captured (14 rodents and four marsupials) (Table 1). The most abundant species were the rodents *Akodon cursor* (Rodentia, Cricetidae; N = 30) and *Necomys lasiurus* (Rodentia, Cricetidae; N = 22) and the marsupial *Metachirus nudicaudatus* (Didelphimorphia, Didelphidae; N = 13), with the exception of Camamú municipality where *M. nudicaudatus* was not found (Table 1). Twenty species of ectoparasites were identified. The following species of mites were the most abundant in the set of the four areas: *Androlaelaps rotundus* (Acari, Laelapidae; N = 328), *Gigantolaelaps oudemansi* (Acari, Laelapidae; N = 78) and *Gigantolaelaps mattogrossensis* (Acari, Laelapidae; N = 64). The most abundant species of ticks were *Amblyomma* sp. (Acari, Laelapidae; N = 26 larvae), *Ixodes* sp. (Acari, Laelapidae; N = 18 larvae) and *Amblyomma ovale* (Acari, Ixodidae; N = 17 nymphs). The most abundant flea genus in the landscapes was *Polygenis* sp. (Siphonaptera, N = 28).

The highest number of ectoparasites was found in the area with less percentage of forest cover (Figure 2). With a reduction in forest cover, there was an increase in the abundance of ectoparasites with the following differences between the areas: 25% and 35% of forest cover ($p = 0.01$), 25% and 55% of forest cover ($p < 0.001$), and 45% and 55% of forest cover ($p < 0.001$). The abundance of hosts showed no difference between the four areas sampled (ANOVA, $F_{3, 130} = 0.851$, $p = 0.473$; Table 1). No correlation was observed either between

ectoparasite and host species richness ($R_s = -0.046$; $p = 0.599$) or between ectoparasite and host abundance ($R_s = -0.085$; $p = 0.334$).

There was no difference between the set of ectoparasites collected in the forest and in the matrix, both in terms of richness ($U = 1785.0$; $p = 0.086$) and abundance ($U = 1991.5$; $p = 0.719$). However, the richness of ectoparasites differed between the plots located in the study areas ($\chi^2_3 = 25.16$; $p \leq 0.001$) (Figure 3).

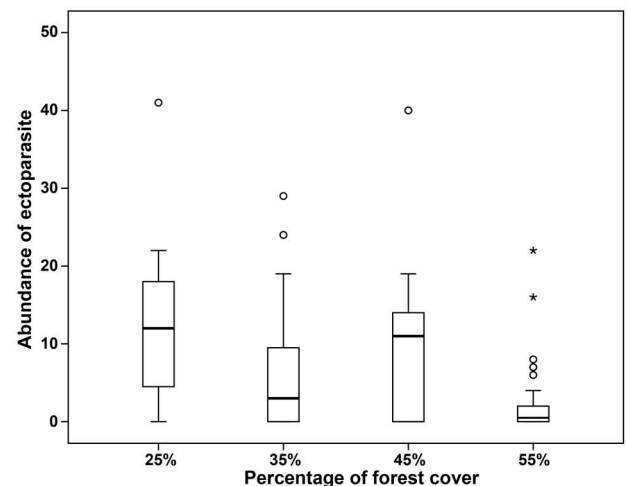


Figure 2. Box plot (median, interquartile and outliers-white dot) presenting the abundance of ectoparasites in relation to the percentage of forested cover.

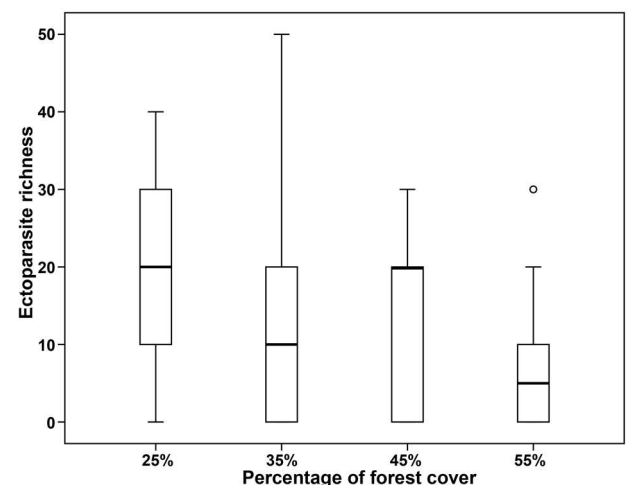


Figure 3. Box plot (median, interquartile and outliers-white dot) presenting the richness of ectoparasites in relation to the percentage of forested cover.

The post-hoc tests identified differences between the area with 25% of forest cover (Valença) and the areas with 35% ($p = 0.01$) and 55% ($p < 0.01$) of forest cover (Jaguaripe). The prevalence of ectoparasitism per area was 91% in the area with 25% of forest cover, 64% in the area

Table 1. Prevalence and intensity (median) of ectoparasitism in small wild mammals in four areas with 25% (a), 35% (b), 45% (c) and 55% (d) of Atlantic Forest cover in the state of Bahia, Brazil.

| Hosts | N | Infested | Intensity |
|--------------------------------|----|----------|-----------|
| a) 25% forest cover | | | |
| <i>Akodon cursor</i> | 7 | 6 | 68 |
| <i>Cavia aperea</i> | 1 | 0 | 0 |
| <i>Cerradomys vivoi</i> | 3 | 3 | 42 |
| <i>Euryoryzomys russatus</i> | 1 | 1 | 15 |
| <i>Holochilus brasiliensis</i> | 1 | 1 | 19 |
| <i>Metachirus nudicaudatus</i> | 2 | 2 | 40 |
| <i>Necomys lasiurus</i> | 5 | 5 | 40 |
| <i>Oligoryzomys</i> sp. | 1 | 1 | 15 |
| <i>Pseudoryzomys simplex</i> | 1 | 1 | 41 |
| <i>Trinomys</i> sp. | 2 | 2 | 21 |
| b) 35% forest cover | | | |
| <i>Akodon cursor</i> | 7 | 6 | 70 |
| <i>Cerradomys vivoi</i> | 3 | 3 | 21 |
| <i>Cryptonanus</i> sp. | 1 | 1 | 5 |
| <i>Hylaeamys laticeps</i> | 4 | 3 | 22 |
| <i>Marmosops incanus</i> | 6 | 4 | 45 |
| <i>Marmosa murina</i> | 3 | 2 | 4 |
| <i>Metachirus nudicaudatus</i> | 6 | 3 | 15 |
| <i>Micoureus demerarae</i> | 1 | 0 | - |
| <i>Necomys lasiurus</i> | 10 | 6 | 61 |
| <i>Pseudoryzomys simplex</i> | 2 | 1 | 10 |
| <i>Rattus</i> sp. | 1 | 1 | 1 |
| <i>Rhipidomys mastacalis</i> | 1 | 0 | - |
| <i>Trinomys</i> sp. | 3 | 2 | 22 |
| c) 45% forest cover | | | |
| <i>Akodon cursor</i> | 5 | 4 | 67 |
| <i>Cerradomys vivoi</i> | 1 | 1 | 11 |
| <i>Didelphis aurita</i> | 1 | 0 | 14 |
| <i>Euryoryzomys russatus</i> | 3 | 2 | 16 |
| <i>Hylaeamys laticeps</i> | 8 | 6 | 99 |
| <i>Monodelphis americana</i> | 2 | 1 | 8 |
| <i>Necomys lasiurus</i> | 2 | 0 | - |
| <i>Pseudoryzomys simplex</i> | 3 | 3 | 31 |
| d) 55% forest cover | | | |
| <i>Akodon cursor</i> | 10 | 4 | 18 |
| <i>Cerradomys vivoi</i> | 3 | 3 | 23 |
| <i>Didelphis aurita</i> | 3 | 1 | 4 |
| <i>Marmosops incanus</i> | 6 | 0 | - |
| <i>Metachirus nudicaudatus</i> | 5 | 4 | 32 |
| <i>Necomys lasiurus</i> | 5 | 5 | 6 |
| <i>Rattus</i> sp. | 1 | 0 | - |

with 35% of forest cover, 68% in the area with 45% of forest cover, and 50% in the area with 55% of forest cover. The intensity of ectoparasitism (number of ectoparasites per host) was 13.6 in Valença, 8.9 in Nilo Peçanha, 13.6 in Camamú, and 4.8 in Jaguaripe (Table 2).

DISCUSSION

Our results confirm that the reduction in the amount of forested cover acts as a factor intensifying the rate of parasitism and affects the abundance and species richness of ectoparasites. As expected, host species that were in areas with less amount of forested cover (25% of forest cover) had higher infestation rates when compared to the other plots, resulting in a greater abundance of ectoparasites in these areas. The differences in ectoparasite richness and abundance between areas with reduced forest cover can be the result of the influence of the matrix areas surrounding the

fragments. This influence was identified for others animal groups in forest fragments with different levels of human activity in the Amazon region (Gascon *et al.* 1999). The increase in the abundance of ectoparasites in the plots located in landscapes with the lowest percentage of forest cover (25%) could be partially explained by the greater tolerance of the habitat generalists hosts for degraded areas.

The infestation rates with ectoparasites in small mammals are within the range recorded by other authors in studies with small mammals (33.3% in Yoshizawa *et al.* 1996, 69.1% in Perez *et al.* 2008). Of the 20 species of ectoparasites identified, the highest amount of infestation was caused by Laelapidae mites (Nieri-Bastos *et al.* 2004, Reis *et al.* 2008). In the current study, Ixodidae ticks also infested both rodents and marsupials (Barros & Baggio 1992, Nieri-Bastos *et al.* 2011). We encourage more studies assessing the specificity of these parasites in the choice of their host in the natural environment. On the other hand, the ticks

Table 2. Ectoparasites in small mammals of four areas with 25%, 35%, 45% and 55% of Atlantic Forest cover in the state of Bahia, Brazil. *Amblyomma* sp. and *Ixodes* sp. were all larvae, *A. dubitatum* and *A. ovale* were nymphs, and *I. loricatus* were adult ticks.

| Taxa | 25% | 35% | 45% | 55% |
|---|-----|-----|-----|-----|
| Ticks | | | | |
| <i>Amblyomma</i> sp. | X | X | X | X |
| <i>Amblyomma dubitatum</i> | | X | | |
| <i>Amblyomma ovale</i> | X | | X | X |
| <i>Ixodes loricatus</i> | X | | | X |
| Mites | | | | |
| <i>Androlaelaps</i> sp. | X | | X | |
| <i>Androlaelaps cuicensis</i> | | | X | |
| <i>Androlaelaps fahrenheitzi</i> | X | X | X | |
| <i>Androlaelaps marmosops</i> | X | | | |
| <i>Androlaelaps rotundus</i> | X | X | X | X |
| <i>Gigantolaelaps gilmorei</i> | X | | X | |
| <i>Gigantolaelaps matogrossensis</i> | X | | | |
| <i>Gigantolaelaps</i> cf. <i>matogrossensis</i> | | | X | |
| <i>Gigantolaelaps oudemansi</i> | X | X | X | |
| <i>Gigantolaelaps vitzthumi</i> | X | X | X | |
| <i>Gigantolaelaps wolffsohni</i> | X | | | |
| <i>Laelaps</i> sp. | X | X | X | |
| <i>Laelaps manguinhos</i> | | | | |
| <i>Laelaps paulistanensis</i> | X | | | |
| <i>Ornythonyssus</i> sp. | | X | X | |
| <i>Tur turki</i> | | X | X | |
| Fleas | | | | |
| <i>Poligenys</i> sp. | X | X | X | X |

A. dubitatum and *A. ovale* infesting small rodents corroborate observations of Szabó *et al.* (2013) and Debárborá *et al.* (2014). In this study, fleas from the genus *Polygenis* (Siphonaptera) were found both on Echimyidae and Cricetidae rodents, as well as on the Didelphidae marsupials. This result is similar to Linardi (1985), which discussed the fact that the Rhopalopsyllidae did not demonstrate specificity as to their choice of host.

Several species of ticks related to the transmission of emerging zoonosis in Brazil were identified, such as *A. ovale*, which is involved in the transmission of *Rickettsia parkeri* (Rickettsiales, Rickettsiaceae), responsible for mild rickettsial infection in areas of the Atlantic Forest biome (Sabatini *et al.* 2010, Szabó *et al.* 2013). Additionally, Macronyssidae mites are considered hematophagous parasites and result in additional risk to human health, since some species are etiological agents of dermatitis (Ribeiro *et al.* 1997). In Brazil, the majority of fleas (Siphonaptera) belongs to the genus *Polygenis* (Rhopanopsyllidae; Dobbin *et al.* 1969, Linardi *et al.* 1987) and can act as vectors of the bubonic plague, whose etiological agent is the bacteria *Yersinia pestis* (Enterobacteriales, Yersiniaceae), a factor which elevates the importance of our scientific findings (Ruiz 2001).

The lack of difference in richness and abundance of ectoparasites between the forest and the matrix suggests that ectoparasites have no difficulties in transiting across the landscape, which probably occurs due to the movement of wild and domestic animals between the forest and the matrix (Gascon *et al.* 1999). This result was observed in a study that evaluated the influence of the characteristics of different matrix and tropical forest fragments in the Central Amazonia in relation to, among other aspects, the distribution of small mammal species (Gascon *et al.* 1999). Several species of vertebrates in forest fragments use the surrounding matrix for movement and reproduction, as observed in small mammals of semi-deciduous forest fragments (Santos-Filho & Sanaiotti 2008).

The increase in the number of species of ectoparasites with the decrease in forest cover found in this study could be explained, at least in part, by the increase in contact between the hosts

with distinct habitats. It is important to consider that animals in areas with lower amount of forested cover may explore better the surrounding areas (matrix) enabling parasites exchange (Gillespie *et al.* 2005, Püttker *et al.* 2007, Mbora & McPeck 2009).

Both the prevalence and the intensity of infestation in small mammals by ectoparasites presented higher levels in the areas with less forest cover (91% prevalence and a 6.5 infestation intensity), and lower levels in the areas with high forest cover (50% prevalence and 3.2 infestation intensity). Although it was necessary to test statistically the mean values of prevalence and intensity of infestation by ectoparasites in the four areas suggest that a reduction in forest covered habitat could, through parasitism, play an important role in the dynamic and health of small mammals in the Atlantic Forest. These results suggest a positive relationship between the reduction in the forested area and the increase in ectoparasites of small mammals, which may lead to an increase in the risk of infestation and, consequently, compromise the health of wild and domestic animals and humans.

The host species found in this study were similar with the ones found in surveys performed in areas of the Atlantic Forest in the states of Bahia (Moura 2003) and Espírito Santo (Pinto *et al.* 2009). Among the 18 small mammals identified, only three (*A. cursor*, *Marmosops incanus* [Didelphimorphia, Didelphidae] and *N. lasiurus*) were common to all the units, and the first two were also found by Moura (2003) and Pinto *et al.* (2009). Although there is a need for larger and more complex studies about the distribution of host species, the identification of similar ectoparasite species in different areas of the Atlantic Forest shows that they are widely distributed. The abundance of hosts collected showed no significant variation among areas, corroborating other comparative studies of abundance of small mammals in different areas of the Atlantic Forest in the state of São Paulo (Pardini *et al.* 2009). Such results can suggest a resilience of habitat generalists host species to modifications of the forested landscapes. Furthermore, similarities in the abundance of host species among areas reduce the possible influence

of the quantity of small mammals on the rates of parasitism.

The habitat reduction process appears to play a key role in changes in ectoparasitism of small mammals in the Atlantic Forest. It is necessary to investigate the negative impacts of these changes to the population of hosts. Additionally, we emphasize the importance of undertaking molecular evaluations of the ectoparasites to investigate the presence of potential pathogens which cause zoonosis in Brazil. It is important to emphasize that many parasites only occur in preserved areas. Preserved areas may have more species of parasites because they may have greater diversity of host species, and many parasites are important for the stability of interactions in ecosystems. Not all parasites are pathogenic to human being and most are species-specific. Preserved areas must be maintained to sustain the biodiversity and are important to dilute the prevalence of a pathogenic parasite that causes a zoonosis. Our results can be used as a warning for a possible problem of natural areas because they reinforce the importance of maintaining the forest cover as a way of balance the rates of parasitism, conservation of populations of small mammals, and the reduction of risks to public health.

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