

Patterns of parasitism by *Eutrombicula alfreddugesi* (Oudemans) (Acari, Trombiculidae) in three species of *Tropidurus* Wied (Squamata, Tropiduridae) from Cerrado habitat of Central Brazil

André L. G. de Carvalho; Alexandre F. B. de Araújo & Hélio R. da Silva

Laboratório de Herpetologia, Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro. Caixa Postal 74524, 23851-970 Seropédica, Rio de Janeiro, Brasil.
E-mail: andreluiz.carvalho@ig.com.br; araujo@ufrj.br; helio@ufrj.br

ABSTRACT. This study concerns the patterns of parasitism by the larvae of *Eutrombicula alfreddugesi* (Oudemans, 1910) on three species of *Tropidurus* Wied, 1820 from Cerrado habitats of Central Brazil: *T. oreadicus* Rodrigues, 1987 (n = 97), *T. itambere* (n = 85) and *T. torquatus* (Wied, 1820) (two samples n = 75, n = 23). The highest prevalence value was observed in *T. itambere* (88.2%), followed by *T. oreadicus* (87.6%), and *T. torquatus* (17.3% and 65.2%). The most important sites of infestation were the mite pockets and skin folds, especially on the neck and inguinal region. *Tropidurus itambere* Rodrigues, 1987 was the most heavily parasitized species (mean intensity of infestation: 36.67 ± 41.09), followed by *T. oreadicus* (15.38 ± 21.08), and *T. torquatus* from Unai, Minas Gerais (12.13 ± 21.09). The lowest intensity of infestation was found in *T. torquatus* from urban and periurban areas of Brasília, Distrito Federal (1.97 ± 5.43). The analysis did not indicate differences in intensity of infestation between sexes for any of the species. Differences in the patterns of parasitism among the three lizard species may be related to the morphological and numerical variation of the skin folds (especially mite pockets), to the degree of conservation of the host's habitats, and to selective processes related to reduction of damage to the host's bodies, to the evolution of mechanisms of decreasing illness transmission by parasites, or even to some behavioral traits of the lizards.

KEY WORDS. Lizards; mites; ectoparasitism.

RESUMO. Padrões de parasitismo por *Eutrombicula alfreddugesi* (Oudemans) (Acari, Trombiculidae) em três espécies de *Tropidurus* Wied (Squamata, Tropiduridae) do Cerrado brasileiro. Estudaram-se os padrões de parasitismo por larvas de *Eutrombicula alfreddugesi* (Oudemans, 1910) em três espécies de *Tropidurus* Wied, 1820 do Cerrado brasileiro: *T. oreadicus* Rodrigues, 1987 (n = 97), *T. itambere* (n = 85) e *T. torquatus* (Wied, 1820) (duas amostras n = 75, n = 23). A maior prevalência foi observada em *T. itambere* Rodrigues, 1987 (88,2%), seguido por *T. oreadicus* (87,6%) e *T. torquatus* (17,3% e 65,2%). Os sítios de infestação mais importantes foram as bolsas de ácaro e dobras de pele, em especial do pescoço e região inguinal. *Tropidurus itambere* foi a espécie mais intensamente parasitada (intensidade média de infestação: $36,67 \pm 41,09$), seguido por *T. oreadicus* ($15,38 \pm 21,08$) e *T. torquatus* de Unai, Minas Gerais ($12,13 \pm 21,09$). A intensidade parasitária mais baixa foi registrada para os indivíduos de *T. torquatus* oriundos de áreas urbanas e periurbanas de Brasília, Distrito Federal ($1,97 \pm 5,43$). As análises não indicaram diferenças nas intensidades parasitárias entre os sexos para nenhuma das espécies. As diferenças no padrão de parasitismo entre as três espécies de lagartos podem estar relacionadas a variações morfológicas e numéricas das dobras de pele (em especial das bolsas de ácaro), ao grau de conservação do hábitat dos hospedeiros, a processos seletivos relacionados à redução de danos ao corpo dos hospedeiros, à evolução de mecanismos de diminuição da transmissão de doenças por parasitas, ou até mesmo a aspectos comportamentais dos lagartos.

PALAVRAS-CHAVE. Lagartos; ácaros; ectoparasitismo.

The genus *Eutrombicula* Ewing, 1938 (Trombiculidae) includes mites commonly found as ectoparasites on vertebrates (VERCAMMEN-GRANDJEAN & AUDY 1965, BRENNAN & REED 1974). In the Americas, *Eutrombicula alfreddugesi* (Oudemans, 1910) is one of the most widespread species. It commonly parasitizes rep-

tiles, mainly lizards and snakes (HYLAND 1950, VERCAMMEN-GRANDJEAN & AUDY 1965, ZIPPEL *et al.* 1996, DANIEL & STEKOL'NIKOV 2004, KLUKOWSKI 2004).

The relationship between lizards and mites seems to be so old that some lizard families independently developed skin folds

(BAUER *et al.* 1990, FROST 1992, MCGUIRE 1996, FROST *et al.* 2001), forming structures known as "mite pockets" (RODRIGUES 1987) in different parts of the body where the parasites aggregate.

The presence of mite pockets and the existence of patterns of parasitism in lizards were discussed in several earlier studies (ARNOLD 1986, BENTON 1987, BAUER *et al.* 1990, 1993). However, the main hypothesis proposed by ARNOLD (1986) to explain this subject remains insufficiently tested. That author suggested that mite pockets are adaptive and their presence would guarantee damage reduction caused by the parasites, by limiting the distribution area over the hosts' bodies. These structures seem to form microhabitats perfectly suited for occupation by mites. However, BAUER *et al.* (1990, 1993) disagree with this idea, considering the appearance of mite pockets as independent phylogenetic events without adaptive value.

Although morphological transformations and speciation events may directly influence the relationships between host and parasites, few workers have attempted to elucidate these co-evolutionary processes (ARNOLD 1986, BAUER *et al.* 1990, 1993, BENTON 1987). Most papers on the subject have dealt with the description of new parasite taxa or the intensity and prevalence of parasitism (VERCAMMEN-GRANDJEAN & AUDY 1965, CUNHA-BARROS & ROCHA 2000, DURDEN *et al.* 2002, CUNHA-BARROS *et al.* 2003, GARCÍA-DE-LA-PEÑA *et al.* 2004, KLUKOWSKI 2004). Mite pockets are also useful in the studies of lizard taxonomy (RODRIGUES 1987, FROST 1992, MCGUIRE 1996, FROST *et al.* 2001) and the presence and morphology of these structures are used as characters in analyses that intend to understand the evolutionary history and the relationship of studied taxa.

The relationship between social life (aggregation) of lizards and damage caused by parasites has also been explored. SORCI *et al.* (1997) conducted experiments with *Lacerta vivipara* Jaquin, 1787 (Lacertidae) parasitized by mites of the genus *Ophionyssus* Mégnin, 1883 (Mesostigmata). Their results suggest that ectoparasites do not represent a negative factor to denser populations, which would dilute the intensity of infestation across the aggregation.

In southeastern Brazil, patterns of infestations by mites have been investigated in lizard species inhabiting restinga (coastal sand dune) habitats. CUNHA-BARROS & ROCHA (2000) studied five lizard species, of three families, at the Restinga da Barra de Maricá, Rio de Janeiro. Their main goal was to test the existence of possible differences in the pattern of parasitism related to seasonal environmental variations, although their hypothesis has not been confirmed. CUNHA-BARROS *et al.* (2003) compared the pattern of parasitism in four lizard species, of three families, at the Restinga de Jurubatiba, also in the State of Rio de Janeiro, and found some differences when compared their results to those of the previous study (CUNHA-BARROS & ROCHA 2000).

Here we discuss the patterns of parasitism by larvae of *Eutrombicula alfreddugesi* on three species of *Tropidurus* (Wied, 1820) from Cerrado habitat of Central Brazil, evaluating parameters such as the intensity and prevalence of parasitism,

and parasite distribution over the hosts' bodies. We further analyze the variations in the patterns of parasitism and relate them to ecological aspects of lizard species.

MATERIAL AND METHODS

Recording of Data

To study the distribution of the larvae of *Eutrombicula alfreddugesi* in three species of *Tropidurus* from Cerrado habitat of Central Brazil, we examined 280 preserved adult specimens in the Herpetological Collection of Universidade de Brasília (CHUNB). Samples of *Tropidurus oreadicus* Rodrigues, 1987 (n = 97) and *T. itambere* Rodrigues, 1987 (n = 85) are from Parque Estadual da Serra dos Pirineus, Pirinópolis, State of Goiás; *T. torquatus* (Wied, 1820) (n = 75) are from urban and periurban areas (fragments of Cerrado located at the town's edge) of Brasília, Distrito Federal, and from Cerrado areas (n = 23) located next to the city of Unaí, State of Minas Gerais. We searched for ectoparasites in the skin folds and on the body of each lizard. The number and position of mites in the lizards' bodies were recorded. In order to evaluate possible site fidelity, the body of the lizard was divided in 15 sites of parasite infestation (Fig. 1).

The snout-vent length (SLV) of each specimen was measured using a digital caliper (to the nearest 0.1 mm) and were used to test the relationship between host body size and intensity of infestation. Sexual variation in intensity of infestation and distribution on the body was also investigated. Sex was determined by observation of color pattern – males have dark marks under the thighs, abdomen, and precloacal area, and females have reddish-orange venters (RODRIGUES 1987) – and by direct observation of the gonads for the animals with no evident secondary sexual characters.

Samples of *T. torquatus* are from two different areas of Cerrado. One from relatively undisturbed habitats (Unaí) and the other from impacted and heavily altered (e.g., burned) habitats with patchy vegetation (Brasília). These samples allowed us to assess the effect of Cerrado degradation on the patterns of ectoparasitism in *Tropidurus* species.

Statistical Analyses

The hypothesis of random distribution of mites on the lizards was analyzed using the index of dispersion expressed as variance/mean of the number of mites per body region (adapted from KREBS 1998). After the index was determined for each individual, the average of the index of dispersion for each species was calculated and used to determine the pattern of distribution of mites, by goodness-of-fit test to the Poisson distribution (KREBS 1998).

We used the nonparametric Mann-Whitney test to verify the hypothesis of independence of infestation sites on the lizard (between left and right sides). Differences in the prevalence of parasitism among host species were tested using chi-square test, followed by a Z-test, performed for pairs of species.

To investigate variations in the intensity of infestation

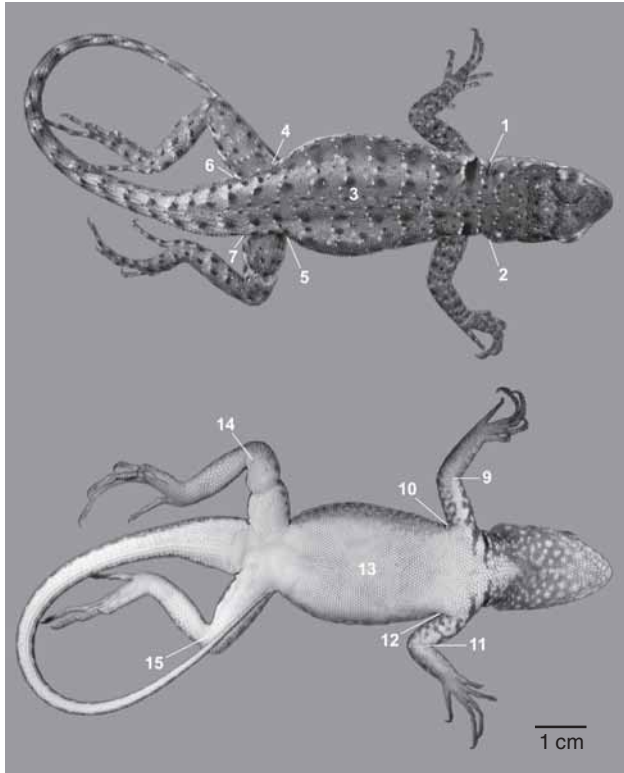


Figure 1. *Tropidurus torquatus* dorsal and ventral views indicating sites of mite infestation, where: (1-2) left and right neck mite pockets, (3) dorsum, (4-5) left and right inguinal mite pockets, (6-7) left and right post femoral region, (8) tail, (9-10) forelimbs, (11-12) right and left armpits, (13) venter, (14-15) hindlimbs.

and in the pattern of distribution of mite larva in different regions of lizard's body, the data were log-transformed, submitted to ordination using a principal component analysis (PCA, correlation matrix). The hypothesis of differences between species, populations and between sexes were tested with a discriminant analysis. The two samples of *T. torquatus* were analyzed independently.

We performed a linear regression analysis to investigate the relationship between host size (SVL) and intensity of infestation. Each analysis was done with all specimens of each species and repeated separately by sex. All statistical tests were executed in the software SYSTAT 7.0, except the Z-test that was executed in the software MICROSTAT.

RESULTS

Table I presents the prevalence and parasitic intensity for the lizards. The highest prevalence value was observed in *Tropidurus itambere*, followed by *T. oreadicus*, and *T. torquatus*. In the last species, the highest prevalence was obtained for Unai population.

The chi-square test indicated that the prevalence of parasitism differed among the four populations of *Tropidurus* ($\chi^2 = 119.977$, $gl = 3$, $p < 0.001$). The Z-test showed that only *T. itambere* and *T. oreadicus* did not differ in prevalence of parasites ($Z = 0.124$, $p = 0.451$). Only *T. torquatus* from Brasília was different from the others with respect to sexual differences in prevalence of mite larvae, but marginally ($Z = 1.677$, $p = 0.047$).

The eigenvalues generated by the PCA and the explained variance are presented in table II (see also Fig. 2). The most important site of infestation was the neck, followed by the inguinal region, which presented the highest values in the first principal component. We observed a tendency of an inverse correlation between principal component values representing the number of mites in the neck and inguinal region, when these were contrasted with those for the axillary region. However, the linear regression test did not confirm the significance of this tendency.

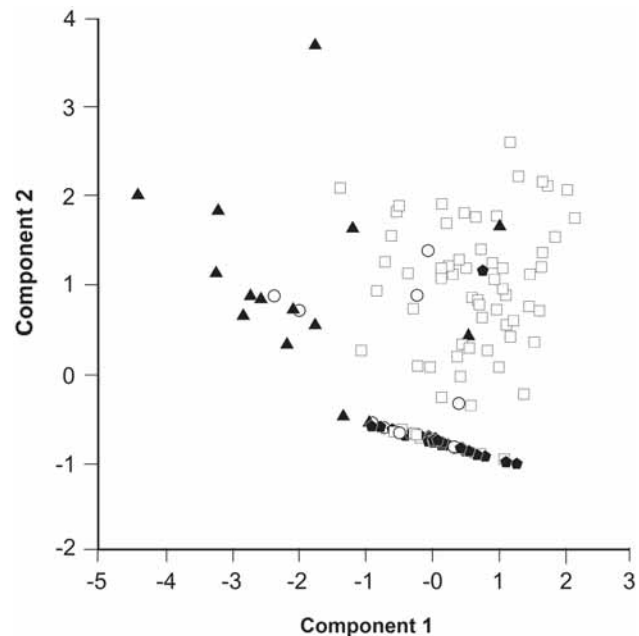


Figure 2. Scatterplot of the first and second principal components from principal component analysis for distribution of mites on the most important sites of infestation for the three species of *Tropidurus* analysed. Square: *Tropidurus itambere*, Pentagon: *T. oreadicus*, Circle: *T. torquatus* from Brasília, Distrito Federal, Triangle: *T. torquatus* from Unai, Minas Gerais.

The discriminant analysis for PCA scores (using neck, axillary and inguinal sites) indicated differences in the intensity of infection for the three lizard species (Wilks' $\lambda = 0.259$; $F = 54.933$; $df = 9.666$; $p < 0.001$). *Tropidurus itambere* was the most heavily parasitized species, followed by *T. oreadicus*, and

Table I. Mean size (snout-vent length in mm) + standard deviation, number of specimens, prevalence, and mean intensity of parasitism by sex and total for *Tropidurus* populations. (DF) Distrito Federal, (MG) Minas Gerais.

Species	Size			Specimens			Prevalence (%)			Mean Intensity of Infestation		
		Male	Female	Total	Male	Female	Total	Male	Female	Total		
<i>Tropidurus itambere</i>	57.63 ± 9.73	50	35	85	92.00	82.86	88.23	46.36 ± 47.40	22.83 ± 24.43	36.67 ± 41.09		
<i>Tropidurus oreadicus</i>	67.82 ± 14.47	60	37	97	86.67	89.19	87.63	17.82 ± 24.96	11.43 ± 11.75	15.38 ± 21.08		
<i>Tropidurus torquatus</i> , DF	88.62 ± 15.74	27	48	75	11.11	20.83	17.33	2.15 ± 6.64	1.87 ± 4.49	1.97 ± 5.43		
<i>Tropidurus torquatus</i> , MG	90.97 ± 13.04	14	9	23	78.57	44.44	65.22	11.64 ± 18.19	12.89 ± 26.24	12.13 ± 21.09		

Table II. Eigenvalues and explained variance by the components (%) obtained using principal components analysis (PCA), based on infestation values.

Site of Infestation	Components		
	1	2	3
Armpit	-0.063	0.994	0.083
Lateral neck	0.822	-0.034	0.568
Inguinal region	0.818	0.112	-0.565
Explained variance	44.966	33.425	21.608

T. torquatus from Unaí. The lowest intensity of infestation was found in *T. torquatus* from Brasília (Tab. I). The results of the discriminant analysis did not indicate differences in intensity of infestation between sexes, for any species.

The intensity of infestation for each body region of the lizards is presented in table III. In *Tropidurus itambere* and *T. oreadicus* the highest intensity of infestation occurred in mite pockets on the neck and inguinal region and the lowest occurred in the axillary region (note that *T. oreadicus* had no parasites in the axillary region, and both species lack mite pockets in this region). *Tropidurus torquatus* presented the most similar distribution of ectoparasites in the three major areas of the body (neck, axillary, and inguinal region). Of the three species, *T. torquatus* was the only where parasites were present in other regions of body besides neck, axillary, and inguinal regions, although at low densities. According to the average index of dispersion (AID > 1) and adjust to the Poisson (random) distribution (Tab. IV), the distribution of parasites over the body was aggregated for all species of *Tropidurus*.

The results of the Mann-Whitney test for differences in intensity of parasitism between body sides indicated that the intensity of parasitism of the inguinal region of *T. itambere* (U = 4349.0, $\chi^2 = 5.91$, df = 169, p = 0.015) and neck region of *T. oreadicus* (U = 5959.0, $\chi^2 = 10.54$, df = 193, p = 0.001) are significantly different. Intensity of parasitism was significantly related to body size in *Tropidurus itambere* (male: $r^2 = 0.309$, p = 0.000; female: $r^2 = 0.176$, p = 0.026; total: $r^2 = 0.261$, p = 0.000) and marginally in *T. oreadicus*, only for the pooled sample ($r^2 = 0.046$, p = 0.058). However, the r^2 values for these species were low.

DISCUSSION

At first glance, one could assume that the similarity in prevalence of mites between *T. oreadicus* and *T. itambere* could be the result of the great similarity in morphology (mostly body shape) and ecology (especially in the use of microhabitats in rocky outcrops and prey items) between these syntopic species (FARIA & ARAUJO 2004). In addition, parasites may not select hosts. When compared to the other two lizard species, *Tropidurus torquatus* had a lower prevalence of mites. Differences in prevalence and intensity of infestation may be related to oscillations in mite populations from different habitats of the lizards. Another possibility is that differences are associated to mite pockets and skin fold morphology. For instance, in *T. torquatus* deep mite pockets are absent. Therefore, this lizard may be more prone to lose their ectoparasites during the collecting and fixation handling processes, resulting in a false lower intensity, and perhaps prevalence. This may be evidenced by studies conducted using freshly collected and killed specimens of *T. torquatus*, in which higher mite intensities and prevalence than in the present study were found (CUNHA-BARROS & ROCHA 2000, CUNHA-BARROS *et al.* 2003).

When the samples of *T. torquatus* from different Cerrado localities are compared, their prevalence of mites differed significantly. The lowest prevalence was recorded in lizards from urban and periurban areas of Brasília; this may be related to decrease in parasite densities in degraded areas. Indeed, as described by CLOPTON & GOLD (1993), populations of *Eutrombicula alfreddugesi* are susceptible to environmental variations, presenting density changes through a gradient of varying degrees of degradation.

Assuming our samples are representative of the populations from which they were collected, the equal prevalence between sexes for all the species (except for *T. torquatus* from Brasília) may be the result of low selectivity by the parasites. Only in *T. torquatus* from Brasília did the sexes differ marginally in parasite prevalence, which may possibly be due to the rarity of parasitism (low prevalence) in the urban and periurban populations. In this case, even small variation in the sample could have caused the perceived differences between sexes, generating a false result.

The use of the mite pockets and skin folds by the larvae

Table III. Average intensity of infestation for *Tropidurus* populations per site of infestation. (DF) Distrito Federal, (MG) Minas Gerais.

Site of Infestation	<i>T. itambere</i>	<i>T. oreadicus</i>	<i>T. torquatus</i> , DF	<i>T. torquatus</i> , MG
Armpit	0.01 ± 0.11	–	0.17 ± 0.84	3.22 ± 5.26
Right armpit	0.01 ± 0.11	–	0.04 ± 0.25	1.30 ± 2.53
Left armpit	–	–	0.13 ± 0.81	1.91 ± 4.64
Lateral neck	21.46 ± 27.86	15.04 ± 20.98	1.53 ± 4.50	3.83 ± 6.46
Right lateral neck	13.45 ± 20.77	9.76 ± 17.41	0.64 ± 2.35	2.09 ± 3.92
Left lateral neck	8.01 ± 13.24	5.28 ± 8.83	0.87 ± 2.81	1.74 ± 2.88
Inguinal region	15.20 ± 24.75	0.17 ± 1.72	0.27 ± 1.47	3.70 ± 10.22
Right inguinal region	9.85 ± 17.29	0.09 ± 0.91	0.13 ± 0.93	2.26 ± 6.22
Left inguinal region	5.35 ± 12.84	0.08 ± 0.81	0.14 ± 1.04	1.43 ± 4.23
Dorsum	–	–	–	0.09 ± 0.42
Post-femoral region	–	–	–	0.83 ± 3.75
Right post-femoral region	–	–	–	0.22 ± 0.85
Left post-femoral region	–	–	–	0.70 ± 2.92

* *Tropidurus torquatus* presented parasites out of the granular neck region (0.478 ± 1.880).

Table IV. Average index of dispersion (AID), chi-squared value (χ^2), adjust to the Poisson distribution and pattern of distribution of mites for *Tropidurus* populations. (DF) Distrito Federal, (MG) Minas Gerais. For all the species: $p = 0.000$ and distribution = aggregated.

Species	N	DF	AID	χ^2
<i>Tropidurus itambere</i>	74	14	19.698	275.776
<i>Tropidurus oreadicus</i>	85	14	12.135	169.895
<i>Tropidurus torquatus</i> , DF	13	14	6.174	86.444
<i>Tropidurus torquatus</i> , MG	16	14	4.889	68.449

of *E. alfreddugesi* may be related to two main factors that appear to influence the pattern of distribution of parasites over the lizard's body. First, the larvae may actively search for the pockets and folds. Second, skin folds and mite pockets (with small and granular scales) may offer protection against mechanical impacts, insulation, and dehydration (CUNHA-BARROS & ROCHA 2000, CUNHA-BARROS *et al.* 2003, GARCIA-DE-LA-PEÑA *et al.* 2004) assuring that, once the larvae is fixed there, it may not be easily removed. Therefore, the probability of finding larvae at that site is increased. *Tropidurus itambere* has two pockets in the neck and another in the inguinal region, and had the highest intensity of infestation. *Tropidurus oreadicus* has only two deep and oblique pockets in the neck region, and had the second highest intensity of infestation. In *T. torquatus*, which has only shallow skin folds (not forming "true" pockets), the intensity of infestation was the lowest among the three species compared here.

The relationship between skin folds and pockets, and the gradient of intensity of infestation could indicate a drawback in possessing these structures. However, as proposed by ARNOLD (1986), investment in specialized areas for mite installation

could save more important areas of the lizard's body against infestation, actually diminishing the overall damage caused by the parasites.

Alternatively, other factors may explain the increase in intensity of infestation in specific regions of the body (folds and pockets). One possibility is that guarding and protecting a set of mites may play an important role in decreasing the chances of parasite exchanges and transmission of diseases. Our suggestion adds another advantage in offering protection to the mites to ARNOLD'S (1986) hypothesis. Tests of such a hypothesis may be possible as we learn more about the relationships between mites and the transmissions of hemoparasites to lizards.

Intensity of infestation was similar between the sides of the lizard's bodies, except in the inguinal region of *T. itambere* and the neck of *T. oreadicus*. This may suggest that the sites of infestation are not isolated and that mites may migrate from site to site. Another possibility is that incoming mites may only fix themselves in sites not yet overcrowded. With a continuous influx of newly arriving mites, all sites tend to become equally infested.

Although the relationship between body size and intensity of infestation should be expected (older and larger lizards presenting a higher load than younger and smaller ones), assuming all size classes use the same microhabitats, this assumption was not strongly supported by our data. Despite the observed tendency of increasing intensity of infestation with increasing host size (only for *T. itambere* and *T. oreadicus*), the fit (r^2) was relatively weak. No correlation between lizard body size and parasitic infestation was described by some workers (CUNHA-BARROS *et al.* 2003, GARCIA-DE-LA-PEÑA *et al.* 2004). However, this lack of relationship does not hold true for all lizards (CUNHA-BARROS & ROCHA 2000). Even when data from the same

species from different areas are compared, as in the case of *T. torquatus* (CUNHA-BARROS & ROCHA 2000, CUNHA-BARROS *et al.* 2003), this relationship may vary. These variable results point to the need of further work on this subject.

ACKNOWLEDGEMENTS

To Marília de C. Brasil Sato (UFRRJ), for the identification of the mite larvae and revision of the manuscript; Mary S. Linn (The University of Oklahoma) and Joseph R. Mendelson III (The Atlanta Zoo), for the last revision of the text; Roberta R. da Silva (UFRRJ), and Renato Gomes Faria (UnB), for help in data recording; Guarino R. Colli and Mariana Zatz (UnB), for their attention during our visit to the herpetological collection of this institution (CHUNB).

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