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PARASITISM OF *HYLODES PHYLLODES* (ANURA: CYCLORAMPHIDAE) BY *HANNEMANIA* SP. (ACARI: TROMBICULIDAE) IN AN AREA OF ATLANTIC FOREST, ILHA GRANDE, SOUTHEASTERN BRAZIL

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Parasitism of *Hylodes phyllodes* (Anura: Cycloramphidae) by *Hannemania* sp. (Acari: Trombiculidae) in an area of Atlantic Forest, Ilha Grande, southeastern Brazil

HATANO F.H.*, GETTINGER D.**, VAN SLUYS M.* & ROCHA C.F.D.*

Summary:
We studied some parameters of the parasitism by the mite *Hannemania* sp. on the endemic frog *Hylodes phyllodes* in the Atlantic Forest of Ilha Grande (Rio de Janeiro State, Brazil). Prevalence, mean abundance, mean intensity and total intensity of infestation, body regions infected, and host sexual differences in parasitism rate of the larvae of *Hannemania* sp. on individuals of *H. phyllodes* were assessed. Prevalence was 86.5 % (N = 37; total of 1,745 larvae of *Hannemania* sp) for male hosts and 91.7 % (N =12; total of 330 larvae) for female hosts, with no significant difference between the sexes. Overall prevalence of *Hannemania* sp. on *H. phyllodes* was 87.7 %. Mean intensity of infestation in males (54.5 ± 42.5; range 1-173 larvae) was higher than in females (29.9 ± 47.6; range 1-166). We conclude that the rates of intensity, abundance, and prevalence of *Hannemania* larvae parasitizing *Hylodes phyllodes* at Ilha Grande were considerably high, suggesting that this species of anuran constitutes a relevant host for this mite species to complete its life-cycle in the area. Differences between males and females in infestation rates probably reflect their differential use of space in the forest.

KEY WORDS : *Hylodes phyllodes*, *Hannemania*, parasitism, mite infestation, Atlantic Forest, Ilha Grande, Brazil.

Résumé : Parasitisme d'*Hylodes phyllodes* (Anura : Cycloramphidae) par *Hannemania* sp. (Acari : Trombiculidae) dans une zone de la forêt atlantique d'Ilha Grande, au sud-est du Brésil

Nous avons étudié quelques paramètres du parasitisme par les larves de l'acarien Hannemania *sp. sur la grenouille* Hylodes phyllodes *dans la forêt atlantique d'Ilha Grande (État de Rio de Janeiro, Brésil). Nous avons évalué la fréquence, l'abondance moyenne, les intensités d'infestation moyenne et totale, les régions du corps infestées, et les différences entre mâles et femelles de* H. phyllodes *vis-à-vis des taux de parasitisme des larves d'Hannemania. La fréquence était de 86,5 % (N = 37; total de 1745 larves d'*Hannemania *analysées) sur les hôtes mâles et de 91,7 % (N = 12; total de 330 larves) sur les hôtes femelles, et cette différence n'était pas significative. La fréquence globale des larves de* Hannemania *sp. sur* H. phyllodes *était de 87,7 %. L'intensité d'infestation moyenne sur les mâles (54,5 ± 42,5; 1-173) était plus élevée que sur les femelles (29,9 ± 47,6; 1-166). Nous concluons que les taux de parasitisme des larves de Hannemania sur* H. phyllodes *à Ilha Grande étaient considérablement élevés. Ces résultats suggèrent que cette grenouille est un hôte important pour que l'accarien finisse son cycle biologique à Ilha Grande. Les différences entre les mâles et les femelles en ce qui concerne les taux d'infestation sont probablement le résultat des differences dans leur utilisation de l'espace dans la forêt.*

MOTS CLÉS : Hylodes phyllodes, Hannemania, *parasitisme, infestation de la mite, forêt atlantique, Ilha Grande, Brésil.*

INTRODUCTION

Compared to other vertebrates, amphibians are rarely parasitized by ectoparasitic arthropods (Wohltmann *et al.*, 2006; Arthur, 1962). Only a few species of mites have evolved special ecological relationships with amphibian hosts (Ewing, 1926; Hyland, 1950; Duszynski & Jones, 1973; Wohltmann *et al.*, 2006). The parasitic mites associated with amphibians belong to the families Trombiculidae and Leeuwenhoekiidae, in which the larvae (= chigger) are parasitic (Hyland, 1950). The other active stages (deutonymphs and adults) of Trombiculid and Leeuwenhoekid mites are free-living predators in the soil (Hyland, 1950; Flechtmann, 1975; Freitas *et al.*, 1984). The larvae of *Endotrombicula* Ewing 1931 (Trombiculidae), *Vercammenia* Audy & Nadchatram 1957 (Trombiculidae) and *Hannemania* Oudemans 1911 (Leeuwenhoekiidae) parasitize the integument of Amphibians by invading the epidermal tissues of the host and forming intradermal nodules (Hyland, 1961; Duszinski & Jones, 1973; Welbourn & Loomis, 1975). The genus *Endotrombicula* is known only from African anurans and *Vercammenia* is known only from Malaysian and Australian anurans. The Leeuwenhoekid genus, *Hannemania* is associated with anurans and salamanders in the New World (Hyland, 1961; Duszynski & Jones, 1973; Welbourn & Loomis, 1975; McAllister, 1991; McAllister *et al.*, 1995; Jung *et al.*, 2001; Wohltmann *et al.*, 2006).

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The prevalence of *Hannemania* varies among host species in different localities (Duncan & Highton, 1979; Welbourn & Loomis, 1975; McAllister, 1991). Some studies show differential use of the host body by the larval *Hannemania* (McAllister, 1991; McAllister *et al.*, 1995; Jung *et al.*, 2001). Jung *et al.* (2001) found the infestation rate by larval *Hannemania* differed between juveniles and adults and between adult males and females of *Rana berlandieri*.

The remaining information available about *Hannemania* refers to host records and host/parasite relationships (Welbourn & Loomis, 1975; McAllister, 1991; McAllister *et al.*, 1995; Jung *et al.*, 2001; Goldberg *et al.*, 2002), and to histochemical studies (Hyland, 1961; Duszynski & Jones, 1973; Duszynski & Jones, 1975; Wohltmann *et al.*, 2006).

The torrent frog *Hylodes phyllodes* (Cycloramphidae, Hylodinae) was described from the Atlantic Forest region of Boracéia (23º 38' S, 45º 50' W), in São Paulo state, southeastern Brazil (Heyer & Cocroft, 1986) and is restricted to the Atlantic Forest of São Paulo and Rio de Janeiro states, in southeast Brazil. These frogs inhabit streams within primary and secondary forests (Heyer & Cocroft, 1986; Rocha *et al.*, 1997; Hatano *et al.*, 2002).

Previous observations of *H. phyllodes* from Ilha Grande (Rio de Janeiro State) show that males and females are commonly parasitized by larval *Hannemania*. Although there is no information on how the mites find their host in the wild, it is likely these mites are present in *H. phyllodes* microhabitat where contact between parasite and host occurs. The frequency the frog uses a particular microhabitats may potentially favor increased infestation rate. Therefore, considering that males and females of *H. phyllodes* differ in the microhabitats used (males predominantly use rocks and woody snags in rivers and streams, while females predominantly use the litter of the forest floor) (Hatano, 2004), we hypothesize that differences will occur in the rate of infestation between sexes of *H. phyllodes*. We also hypothesize there will be intersexual differences in infestation among body regions.

In this study we analyzed some ecological and parasitological parameters of *Hannemania* sp. on *H. phyllodes* at Ilha Grande. We looked at the following questions: 1) What are the prevalence, mean abundance, mean intensity and parasitic intensity of infestation of the larval *Hannemania* sp. on individuals *Hylodes phyllodes*? 2) How does the infestation vary between the body regions of individual *H. phyllodes*? 3) Do these parameters differ between male and female *H. phyllodes*? 4) Does the intensity of infestation affect the host body condition? 5) Is the intensity of infestation affected by host body size?

MATERIALS AND METHODS

Study area

The study was conducted in an area of Atlantic Forest of Ilha Grande (23º 11' S, 44º 12' W), and island in the south of Rio de Janeiro State, southeastern Brazil. The annual rainfall is approximately 2,200 mm (Central nuclear de Angra – NUCLEN), and the mean annual temperature is approximately 22.5º C, with the mean maximum in February (25.7º C) and the mean minimum in July (19.6º C).

Ilha Grande has an area of approximately 193 km^2, covered by Atlantic Forest at different levels of regeneration due to human disturbances (Araújo & Oliveira, 1988). Some remnants of relatively undisturbed forest can still be found in inaccessible central parts of the island (Rocha *et al.*, 2000).

Collecting methods and analysis

We collected individuals of *Hylodes phyllodes* between January 1997 and April, 2003. The individuals were killed in 50 % alcohol, fixed (10 % formaline) and later preserved in 70 % alcohol. We chose to treat the species at the genus level: *Hannemania* sp., because the taxonomy of the species are not well known. Voucher specimens together with the frog host were deposited in the Museu Nacional, Rio de Janeiro, numbers MNRJ No 35191-35222 and MNRJ No 35244-35262. Microscopic analysis of the external morphology of all mite larvae from *H. phyllodes* on Ilha Grande can be assumed to belong to a single species of *Hannemania*.

In the laboratory, all individuals were carefully examined for ectoparasites (*Hannemania* larvae) under a stereomicroscope, snout-vent measured (SVL, in mm), and weighed to the nearest 0.1 g using an electronic balance (Mettler). Every single nodule on each host was carefully opened using a probe and the larvae were quantified.

The number of larvae on each of 14 host body regions was recorded. The regions of the host body were characterized as follows: head, gular region, dorsum, venter, lateral body, dorsal hand, ventral hand, dorsal foot, ventral foot, dorsal arm, ventral arm, dorsal leg, ventral leg, and axillary region. The proportion of mites found in each body region in relation to the total number of mites found under the skin of the frog indicated the proportion of parasitism in each body region. We followed the terminology (prevalence, total intensity of infestation, mean intensity of infestation, mean abundance) of Bush *et al.* (1997).

Differences in mean intensity of infestation between male and female hosts were tested with Mann-Whitney test (Zar, 1999). The effect of host body size (SVL) on the intensity of infestation by *Hannemania* sp. was

analyzed by simple regression analysis (for both sexes separately). Intersexual differences in parasite prevalence were tested with Z-test for proportions (Zar, 1999).

To estimate the niche breadth of *Hannemania* sp. on different parts of the host body for individuals of each sex of *H. phyllodes*, we used the Simpson Index of diversity (Zar, 1999):

$$B_{ij} = \frac{1}{\sum_{i=1}^{n} pi^2}$$

Where *pi* is the proportion of individuals of *Hannemania* sp. associated to each body region on *H. phyllodes*.

To evaluate the degree of similarity in microhabitats used by mites on the body of males and females *H. phyllodes*, we used the MacArthur and Levins (Pianka, 1986) index of similarity:

$$O_{jk} = \frac{\sum_{i=1}^{n} pij \times pik}{\sqrt{\sum_{i=1}^{n} pij^2 \sum_{i=1}^{n} pik^2}}$$

Where *j* and *k* are the number of mites on each body region of male and of female *Hylodes phyllodes*. The values of overlap can range from zero (absence of overlap) to 1 (complete overlap).

The effect of the intensity of infestation on the body condition of individuals frogs was estimated using a simple regression analysis between intensity of infestation and the respective residuals of the relationship of frog body mass and frog body length (SVL). For this analysis we included only males, since the sample size for females was too small.

RESULTS

A total of 49 adults of *Hylodes phyllodes* were collected, 37 males and 12 females. Of the males, 32 were parasitized (prevalence of 86.5 %) and of the 12 females collected, 11 were parasitized (prevalence of 91.7 %) by larval *Hannemania* sp. (Table I). In some cases there was more than one mite per nodule. There was no significant difference in mite prevalences between sexes (Z = -0.478; P = 0.316). The overall prevalence of *Hannemania* sp. on *H. phyllodes* was 87.7 %.

The mean intensity of infestation in *H. phyllodes* males was 54.5 (± 42.5 SD) larvae per host, with a range of 1-173 larvae, whereas the females had 29.9 (± 47.6 SD) larvae per host, with a range of 1-166 larvae (Table I). Of the 49 hosts examined, six (five males and one female) were infested by more than 100 larvae. The intensity of infestation by *Hannemania* larvae differed between males and females (Mann-Whitney, U = 85.0; p = 0.01). The mean mite abundance was 47.1 (± 43.7) larvae per host individual for males of *H. phyllodes*, whereas for females it was 27.4 (± 46.2) larvae (Table I).

The most parasitized body regions were: the dorsal legs (31.2 %), the lateral body (27.2 %) and the dorsum (20.3 %) (Table II). The most notable difference in the distribution of the mites was that, in males, the dorsal region of the legs (32 %) was the most infested, followed by the lateral body (26.9 %), while in females, the region most infested was the lateral body (28.6 %), followed by the dorsal region of the legs (27.3 %). The body regions least utilized by *Hannemania* on males was the ventral hand, the ventral foot and the head (Table II). In the female the least utilized body region was the ventral hand, gular region and the dorsal hand (Table II). The overall body size did not significantly affect the intensity of mite parasitism in males ($F_{1.30}$ = 3.384, P = 0.07) or females ($F_{1.9}$ = 0.180, P = 0.68).

	Prevalence (N)	Mean intensity (± DP)	Mean abundance (± DP)	Range
Males	86.5 (37)	54.5 (± 42.5)	47.1 (± 43.7)	1-173
Females	91.7 (12)	29.9 (± 47.6)	27.4 (± 46.2)	1-166
Total	87.7 (49)	48.2 (± 44.6)	42.3 (± 44.7)	1-173

Table I. – Prevalence (%), mean intensity, mean abundance and range of infestation by *Hannemania* sp. larvae in individuals of the frog *Hylodes phyllodes* (N = 49) at Ilha Grande, Angra dos Reis, Rio de Janeiro, Brazil. Number of individuals are in parenthesis.

Body region	Males (N = 1,745 larvae)	Females (N = 330 larvae)	Total (2,075 larvae)
HE	0.8	1.8	1.0
GU	1.2	0.3	1.1
DO	20.5	19.1	20.3
VR	1.4	5.8	2.1
LB	26.9	28.6	27.2
DRH	1.7	0.6	1.5
VRH	–	–	–
DRF	1.1	0.9	1.1
VRF	0.5	1.5	0.6
DRA	4.0	4.1	4.0
VRA	3.1	7.6	3.8
DRL	32.0	27.3	31.2
VPL	4.2	1.5	3.8
AR	2.6	0.9	2.3

Table II. – Proportion of the infestation intensity by *Hannemania* sp. larvae in different regions of the body (microhabitats) of 32 males and 11 females *Hylodes phyllodes* at Ilha Grande, in Rio de Janeiro state, Brazil. (head = HE; gular region = GU; dorsum = DO; ventral region = VR; lateral body = LB; dorsal hand = DRH; ventral hand = VRH; dorsal foot = DRF; ventral foot = VRF; dorsal arm = DRA; ventral arm = VRA; dorsal leg = DRL; ventral leg = VRL; armpit region = AR).

The spatial niche breadth of *Hannemania* larvae on the host body was similar in males (B_{ij} = 4.49) and females (B_{ij} = 4.88) and there was a significant overlap in the microhabitat use by mites between males and females (O_{jk} = 0.98).

The intensity of infestation by *Hannemania* sp. larvae did not affect the body condition of adult males ($F_{1,30}$ = 0.506, P = 0.48).

During the sampling period, we collected four adult individuals of another stream-dwelling anuran, *Crossodactylus gaudichaudii* (Hylodinae), that were also parasitized by larvae of *Hannemania* sp. This species occurs sympatrically and syntopically with *H. phyllodes* in forest streams in Ilha Grande.

DISCUSSION

More than 80 % of the male and female *Hylodes phyllodes* examined were infested by larval *Hannemania* sp. The intensity of infestation did not differ between sexes and was not affected by the host body size.

The large proportion (87.7 %) of individuals of both sexes and of different body sizes of *H. phyllodes* which were parasitized by *Hannemania* sp. suggests that this frog is an important host for the mite to complete its life cycle. We also found one *H. phyllodes* tadpole, from the same area, with a *Hannemania* sp. parasitizing its dermis (personal observation).

In addition, we also observed four adults of *Crossodactylus gaudichaudii*, a sympatric species who lives in the same streams, and were also parasitized by this mite genus. This is suggestive that the cycle of parasitism of *Hannemania* sp. in Ilha Grande may be associated with the stream environment, small rivers with rocks inside and on the edges. Other anurans occurring in Ilha Grande (e.g. *Adenomera marmorata, Eleutherodactylus binotatus, E. guentheri, E. parvus*, *Zachaenus parvulus*, *Myersiella microps*, *Chiasmocleis* sp. and *Bufo ornatus*), but which live on the forest floor at the study area, did not present evidence of parasitism by *Hannemania* sp. (Rocha *et al.*, 2000; Rocha *et al.*, 2001; Van Sluys *et al.*, 2001).

A high parasitism by *Hannemania* has also been observed in amphibians in North America. Goldberg *et al.* (2002) studied the ectoparasitism of two anuran species (*Bufo mazatlanensis* and *Rana tarahumarae*), in Sonora, Mexico, and found 95 % of the *R. tarahumarae* (n = 42) with *H. monticola*; of 20 % of the *B. mazatlanensis* (n = 20) with *H. bufonis* and of 70 % of the same individuals of *B. mazatlanensis* with *H. hylae*. Similarly, Jung *et al.* (2001) in Texas, United States, found two species of *Hannemania* (*H. hylae* and *Hannemania* sp.) infesting *Rana berlandieri* and one species (*H. hylae*) infesting *Hyla arenicolor* and *Eleutherodactylus guttilatus*, with high prevalence (> 65 %) on most host species. McAllister *et al.* (1995) found that 68 % of the salamander *Desmognathus brimleyorum* analyzed were parasitized by *Hannemania* larvae. The prevalence values found in these studies of *H. phyllodes* from Ilha Grande indicate that *Hannemania* larvae can be high in some amphibian populations. Taxonomic studies are necessary to identify the *Hannemania* larvae of *H. phyllodes* and confirm this prevalence.

The intensity of parasitism of *Hannemania* sp. on *Hylodes phyllodes* at Ilha Grande was considerably high in both sexes. Goldberg *et al.* (2002) did not encounter intensities as high as were found in the present study [e.g. *Rana tarahumarae* infested with *Hannemania monticola* (5 ± 2, range 4-8); *Bufo mazatlanensis* infested with *H. bufonis* (2 ± 1, range 1-4); *Bufo mazatlanensis* infested with *H. hylae* (5 ± 4, range 1-12)]. The comparatively elevated intensity of infestation of *Hannemania* sp. on *Hylodes phyllodes* at Ilha Grande is consistent with the idea that this frog constitutes a primary host in the area.

Our data indicated that the distribution of larval *Hannemania* on the host was similar between males and females with an overlap in microhabitat by mites between males and females (98 %), which indicates a similar spatial pattern in terms of occupation of host body by the mite.

Mean intensity of infestation was higher in males than females *H. phyllodes*. This may reflect a difference in the behavior of males and females (*i.e.* males are always in or close to streams, perched in vocalization sites, on the rocks, snags and lianas, while females remain predominantly on the litter of the forest floor) (Hatano, 2004). Jung *et al.* (2001) also found a significant difference in infestation rates by *Hannemania* larvae between the sexes of *Rana berlandieri* living along the Rio Grande, in Texas. Host individuals collected in localities distant from the river were not infested, leading the authors to suggest two hypotheses: 1) the higher rates of infestation and densities of parasites are associated in areas with higher densities of hosts, 2) considering the post-larval stages of development that are free-living in the soil, they can survive better near the margins of streams than in dryer environments. These hypotheses reinforce the idea proposed above of the tendency of the males of *Hylodes phyllodes* to present a higher rate of infestation than females due to differences in microhabitats used. Males of *H. phyllodes*, by remaining in the interior or along the rocky borders of creeks and streams, tend to be aggregated near the water, whereas females, in general, remain on the litter floor of the forest. It is possible that the free-living mites encounter more appropriate conditions on the edge of rocky rivers

compared to the forest floor. Future studies must be carried out to detect differences in densities of these free-living mites, within and away from the streams and rivers.

The most infested regions of the hosts' bodies were the lateral body and the hind limbs, particularly the thighs. The parts of the body least utilized by the mites were the ventral and feet. Jung *et al*. (2001) found the same result for *Rana berlandieri*. The parts of the body least utilized by the mites were the ventral hands and feet. A probable explanation could be that the ventral region of the hands and feet are regions of the body that are usually in contact with the substrate, making it difficult for the larvae to become permanently attached, or that constitute microhabitats with less appropriate physicochemical conditions for the mites. Rey (1992) suggested that, in general, ectoparasites occur heterogeneously on the host´s body because they have preference for regions with most appropriate physicochemical conditions for their development. Among the individuals of *Hylodes phyllodes*, body size differences between the sexes did not produce differences in the rates of infestation. Also, body size did not affect the intensity of infestation indicating that individuals of different sizes tend to be similarly parasitized. Also, host body condition was not affected by the intensity of parasitism. This suggests that mites do not affect the condition of *H. phyllodes*, even in high densities. It may also be possible this effect was not detectable with the statistical analysis employed. Rey (1992) suggested that the pathological nature of the parasitism is incidental, with the occurrence of the lesions not being constant with an energetic loss by the host. This apparent absence of impairment for the host can indicate a relationship between the anuran and the parasite that is a result of a long period of coevolution, with reciprocal adjustments and equilibrium (Cheng, 1964; Ávila-Pires, 1989). However, we must also consider that the host may be injured at physiological levels not detectable in the present study (e.g. reproduction).

We conclude that the rates of intensity, abundance, and prevalence of the larval *Hannemania* sp. that parasitized the population of *Hylodes phyllodes* at Ilha Grande was high, suggesting that this anuran is an important host in the area for this. The differences between males and females probably reflect the difference in microhabitats used.

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