Rhinella pombali (Baldissera, Caramaschi & Haddad, 2004) is an endemic toad from the Brazilian rainforest, specifically found in transition areas of tropical savannah habitat in the states of Minas Gerais (Baldissera et al. 2004) and Rio de Janeiro (Silveira et al. 2009). Rainforests are critical areas for conservation because their biodiversity is threatened by human impact (Myers et al. 2000). Rhinella pombali is a widespread abundant species and larvae develop in temporary pools adjacent to streams (Baldissera 2008).

Amphibian populations are declining or disappearing in many parts of the world; one of the causes is parasite infestation (Stuart et al. 2004). Parasitism reduces the energy available for tadpole metamorphosis, resulting in organisms with little chance of survival (Miers et al. 2000). Rhinella pombali is a widespread abundant species and larvae develop in temporary pools adjacent to streams (Baldissera 2008).

Ciliate protozoa of the genus Trichodina Ehrenberg, 1838 (Ciliophora: Trichodinidae) are widely known fish ectoparasites which cause severe damage to their hosts, with consequent economic losses in many countries (Lom 1995). Besides infesting fish, trichodinids may parasitize the external surfaces or the urinary bladders of amphibians. Heavy parasite loads may be associated with clinical diseases such as skin discoloration, ulcers, and reddened gills (Poynton & Whitaker 2001). There are reports of endo and ectoparasitism by trichodinids in species of amphibians such as Bufo Laurentii, 1768 (Bufonidae), Rana Linnaeus, 1758 (Ranidae) and Xenopus Wagler, 1827 (Pipidae) (Diller 1928, Lom 1958, Chen 1963, Kattar 1975, Tomava & Golemansky 2001). In Brazil there are reports of T. steini Claparède & Lachmann, 1858 on Bufo ictericus Spix, 1824 tadpoles (Kattar 1975) and T. heterodentata Duncan, 1977 on R. pombali tadpoles (Dias et al. 2009).

Despite the influence of parasitism on amphibian population structure (Stuart et al. 2004), there are few studies on the ecology of trichodinids as frog’s ectoparasites (Tomava & Golemansky 2001, Nieto et al. 2007). In South America there have been no investigations on the subject. An increase in parasite intensity can alter parasite characteristics, for instance causing a decrease in the average size of individuals (Dezfulli et al. 2002, Churcher et al. 2006) due to food restriction and increased competition. These effects are known as density-dependent.
However, density-dependent studies on ciliate ectoparasite populations are scarce.

This is the first quantitative analysis studying the prevalence, abundance, intensity of infestation, and distribution pattern of infrapopulations (all individuals of a parasite species that are on an individual host at a particular time – BUSI et al. 1997) of trichodinids infesting endemic tadpoles in a Brazilian rainforest. We also investigated how those parasitological parameters are related to host weight, and whether infestation intensity influenced the mean diameter of parasites, as a density-dependence effect.

**MATERIAL AND METHODS**

One hundred and twenty tadpoles were collected (IBAMA license number: 447/06-NUFAS-MG) with a sieve (20 cm diameter) in a 100 m transect of a stream in the Sagrado Coração de Jesus Farm (21°49′00.04″S, 43°21′21.67″W). The farm is located in the agricultural area of the city of Juiz de Fora, southeastern Brazil. Eighty of the tadpoles were immediately placed in individual plastic containers with 5 mL 7% formalin solution for fixation. The remaining 40 tadpoles were kept alive in containers with stream water for in vivo observation of ciliates. After analysis, the tadpoles were identified by specialists from the Department of Zoology and Botany, UNESP, São José do Rio Preto, state of São Paulo and deposited in the UNESP collection (DZSJRP – 1317.1). The key used for species identification can be found in BALDISSERA et al. (2004). All tadpoles collected were in stage 26 (GOSNER 1960).

Each fixed tadpole, along with the formalin in the vial, was placed in a Petri dish where the body surface of the host was scraped with a scalpel. The content of the Petri dish was then homogenized and 1 mL was removed for ciliate quantification, which was conducted in a Sedgewick-Rafter chamber by observing all fields at 100x magnification. The number of ciliates found in 1 mL from each host was then multiplied by five to estimate the total number of ciliates present. This methodology includes quantification of ectoparasites which may have come loose from the tadpole body surface in the fixation process.

Ciliate identification was based on the uniform system of specific characteristics proposed by LOM (1958), using impregnated specimens (KLEIN 1958). For morphological details see DEAS et al. (2009). Analysis of slides prepared for trichodinid quantification was carried out under an Olympus BX51 bright field microscope.

Prevalence, mean intensity, and abundance values were determined according to BUSI et al. (1997). Tadpoles were separated into four weight classes using the statistics program SPSS 11.5 for Windows to investigate any relationships between these characteristics and mean trichodinid infestation intensity. The non-parametric Kruskal-Wallis test was used to test significant differences in infestation intensity between these classes. The software used for statistical analysis was BioEstat 5.0 (AVRES et al. 2007). The Discrepancy index “D” (POULIN 1993) was calculated to determine the pattern of ectoparasite distribution in the host population. This index measures the difference between the observed parasite distribution and a hypothetical distribution in which all hosts possess the same number of parasites. This D value can range from zero to one; the closer to one, the greater the degree of aggregation, the closer to zero, the greater the uniformity of distribution (POULIN 1993). The discrepancy index was calculated using Quantitative Parasitology 2.0 (ROZSA et al. 2000). Arithmetic means are followed by standard deviation. Values were considered significant when p < 0.05.

The diameters of 30 trichodinids from the same host were determined to check whether infestation intensity influenced average ciliate diameter as a density-dependent effect. In other words, we aimed to ascertain if parasite density affects the size of each individual parasite. Trichodinids from 70 tadpoles were measured, totalling 2100 ciliates. Mean ectoparasite intensity and diameter for each host were submitted to simple linear regression analysis and Pearson’s correlation test.

**RESULTS**

Examination of the body surface of 80 *R. pombali* tadpoles revealed monospecific infestation by *T. heterodentata* (Figs 1-4), and 100% parasitic prevalence. As all hosts examined were infested, the mean intensity value was equal to the mean abundance, which was 695.14 ± 335.12 ciliates/tadpole (ranging from 75-1425 ciliates/tadpole). *Trichodina heterodentata* infrapopulations showed a uniform distribution pattern (D = 0.286) in their host population.

Infestation intensity was positively correlated with host weight: a significant increase in infestation intensity was observed with increased tadpole weight (Tab. I). In other words, heavier tadpoles had a higher parasite load (Fig. 5). The mean *T. heterodentata* infestation intensity in the four host weight classes is shown in figure 6.

Infestation intensity had no significant influence on mean trichodinid diameter, as demonstrated by the simple linear regression analysis (Fig. 7). No significant correlation (p = 0.66, Pearson’s coefficient = 0.053) was observed between infestation intensity and trichodinid diameter, indicating an absence of density-dependent effects on this trait in the ectoparasite infrapopulations studied.

**DISCUSSION**

We observed a significant increase in *T. heterodentata* infestation intensity in heavier tadpoles (Figs 5 and 6). This suggests that the degree of trichodinid infestation may be directly correlated with tadpole weight. Data on the relationship between trichodinid infestation intensity and host weight are scarce in the literature. Also, host size is recognised as a factor that influences parasite intensity and abundance. Larger hosts
Quantitative study of *Trichodina heterodentata* infrapopulations infesting tadpoles

**Figs 1-4**. *Trichodina heterodentata* infesting tadpoles of a Brazilian toad *Rhinella pombali*. (1) Oral region of *R. pombali* infested by *T. heterodentata*, arrows indicate attached parasites. (2) Trichodinids infesting the tadpole tail. Highlighted white spots are the ectoparasites. (3) *Trichodina heterodentata* from life. (4) Details of the *T. heterodentata* adhesive disk evidenced by silver-impregnation method (Klein, 1958). Scale bars: 1-2 = 1 cm; 3-4 = 20 µm.

Can usually harbour a larger number of parasites (Özer 2003). In general, there is an increase in the degree of infection for both ecto- and endoparasites in relation to host growth, possibly as a result of a longer accumulation period or a larger space for the parasite to feed and develop (Rhode 1993). Some authors (Özer & Erdem 1998, Özer 2003) observed a positive correlation between mean trichodinid intensity and fish length. Özer (2007), however, did not find significant differences between mean trichodinid intensity in different fish length classes.

 Defense mechanisms against ectoparasites in amphibians are innate, adaptive immune response, which are less effective in the larval stage, and can directly influence infestation intensity (Green 2001). This fact was also observed by Kattar (1975). He recorded *T. steini* on the larvae of *Xenopus laevis*.
Those studies indicate that parasite intensity may be related to the developmental stage of the tadpole. However, there is no published study on the possible alterations Trichodina spp. infestations cause on the development of amphibians.

One of the most common characteristics of parasitic populations is the tendency to present an aggregated distribution pattern within the host population (Anderson & Gordon 1982). Contrasting with that trend, the populations of T. heterodentata analysed in this study presented a uniform distribution (D = 0.286). According to Poulin (1993), the discrepancy index D indicates that parasite aggregation and mean intensity decrease with increasing prevalence. This hypothesis is supported by our study, in which ectoparasite prevalence and aggregation were shown to be inversely correlated.

In endoparasitic helminth populations, there is generally an inverse relationship between body size and infestation intensity. In ectoparasite populations, by contrast, this relationship is often positive (Poulin 1999). We did not observe the latter trend in our trichodinid population, and found no significant relationship between mean ectoparasite diameter and infestation intensity, possibly because the regulation of parasite size as a density-dependent effect is common in larger species and when resources are limited (Poulin 1999). Density-dependent effects occur in parasite populations with aggregate distribution, when there is greater competition for food and

![Graph](image)

Figure 6. Infestation intensity by *Trichodina heterodentata* on *Rhinella pombali* tadpoles weight classes. The boxes represent the distribution of infestation intensity within the classes. Host weight classes = 1: 0.01-0.049 g; 2: 0.05-0.056 g; 3: 0.057-0.086 g; 4: 0.087-0.447 g.

![Graph](image)

Figure 7. Linear regression analysis between the mean diameter of the *Trichodina heterodentata* and infestation intensity over 70 *Rhinella pombali* tadpoles.

<table>
<thead>
<tr>
<th>Host weight classes (g)</th>
<th>Mean intensity ± S.D.</th>
<th>Comparison between classes</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classe 1: 0.010-0.049 (n = 20)</td>
<td>562.0 ± 341.2</td>
<td>1x2</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x3</td>
<td>0.03</td>
</tr>
<tr>
<td>Classe 2: 0.050-0.056 (n = 20)</td>
<td>662.2 ± 300.5</td>
<td>1x4</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x3</td>
<td>0.12</td>
</tr>
<tr>
<td>Classe 3: 0.057-0.086 (n = 21)</td>
<td>785.4 ± 325.0</td>
<td>3x4</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4x2</td>
<td>0.03</td>
</tr>
<tr>
<td>Classe 4: 0.087-0.447 (n = 19)</td>
<td>816.3 ± 402.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
space (Poulin 1999). Density-dependent mechanisms can only act if there is competition for space on the host, or when an immune response against the parasite is in place (Boré 1991, Poulin 2007). As trichodinids are not obligatory parasites, i.e., they can be found feeding and reproducing freely in the water, unattached to a host, these microorganisms may face less competition for space and food.

The high infestation intensities (26-1410 ciliates/tadpole) and prevalence observed in this study may be an evidence of environmental degradation. Palm & Döderlein (1999) recommended the use of Trichodina sp. prevalence and parasite density data as a biological indicator for comparing between organically polluted and non-polluted areas. They suggested that such parameters are related to bacterial biomass in the environment. Ogut & Palm (2005) showed that the prevalence of trichodinids is higher in organically polluted sites, and advocated that this parameter can be used to indicate levels of organic pollution. Trichodinids also have four characteristics of good bioindicators, as proposed by Mackenzie (1983): they are easy to remove from their host, easy to identify at the generic level, able to respond quickly to changing environmental conditions, and are non-pathogenic at low intensities. These aspects strongly suggest that the prevalence and intensity of trichodinid infestations might be useful as an individual measure for assessing the biological integrity of aquatic ecosystems.

Most studies on Trichodina discuss the morphology and taxonomy of the included species, and focus on their economic impact on fish farming. Most reports on ectoparasitism by Trichodina heterodentata concern fish from different families (Dove & O’Donoghue 2005, Martins et al. 2010). Trichodina heterodentata was recently documented parasitizing the channel catfish Ictalurus punctatus in Brazil (Rafinesque, 1818) (Martins et al. 2010). According to Van As & Bason (1992), it is one of the trichodinid species with the widest distribution, and has low specificity, being able to infest a wide range of vertebrate and invertebrate hosts in both marine and freshwater environments (Dove & O’Donoghue 2005, Martins et al. 2010). Therefore, quantitative studies of trichodinid parasitism in other host groups, especially endemic species, are also important for conservation.

In summary, this study presented a quantitative analysis of parasitism by T. heterodentata, an introduced ectoparasite infesting a toad species endemic to the Brazilian rainforest. Our results show that the ectoparasite has a high prevalence, high infestation intensity, and uniform distribution pattern on the host population. We also found a positive correlation between parasite abundance and host weight. Additionally, there is no significant density-dependent effect on the mean diameter of T. heterodentata.

These results highlight the importance of understanding the quantitative aspects of parasite species commonly found parasitizing fish and other introduced hosts in biodiversity conservation studies.

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LITERATURE CITED


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(Ciliophora: Trichodinidae) infesting tadpoles of Rhinella pombali (Anura: Bufonidae) in the Neotropical area. 


