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Evidence of phoresy by leeches (Hirudinoidea) on *Rhinella abei* (Anura: Bufonidae) in the Atlantic Rainforest in the state of Santa Catarina, southern Brazil

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Abstract. In the Atlantic Rainforest of the Parque Estadual da Serra do Tabuleiro, state of Santa Catarina, southern Brazil, we found *Rhinella abei* (Bufonidae) infested by leeches (Hirudinoidea). We captured 27 toads on the margin of a lagoon both inside and outside water in a survey carried out during one night, and 13 of which had a total of 30 leeches (mean intensity of infestation = 2.3 ± 1.3). We did not observe wounds, scars and/or hemorrhages caused by the leeches on the toads examined, no hemorrhaging after the removal of the leeches, and the leeches removed from the toads were empty of blood. This evidence led us to theorize that the leeches were not parasitizing the toads but had a phoretic relationship. The leeches were found on both dorsal and ventral surfaces (13 on each) of the toads and were predominantly on the toads' axils followed by back and thighs. The average snout-vent length of the toads was 69.2 ± 5.3 mm and their average body mass was 27.1 ± 6.9 g. The number of leeches found on a toad was not related to its snout-vent length or body mass. We are unaware of any previous records of leeches using anurans as dispersal agents, as suggested in the present study.

Keywords. Leeches, phoresy, *Rhinella*, Brazil.

In recent decades records of phoretic associations among aquatic organisms have become more common (Calisto and Goulart, 2000). Phoresy occurs when an organism uses another as a temporary "host" in order to disperse (see Davies et al., 1982; Khan and Frick, 1997; Calisto and Goulart, 2000; Bajerlein and Bloszyk, 2004; Eng et al., 2005; Santos et al., 2005; Zawal, 2006; Beaulieu et al., 2008). The phenomenon has been frequently reported for invertebrates such as Diptera (Calisto and Goulart, 2000), Acari (Bajerlein

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and Bloszyk, 2004; Zawal, 2006; Beaulieu et al., 2008), Nematoda (Eng et al., 2005), and Pseudoscorpionida (Santos et al., 2005) using animals for transport. It may also be widespread in hematophagous species of leeches (Hirudinoidea) to disperse to other water bodies (Davies et al., 1982; Khan and Frick, 1997). For example Khan and Frick (1997) found the leech *Erpobdella punctata* attached to spotted salamanders (*Ambystoma maculatum*) in a pond in South Carolina, USA and concluded that the leech was using the salamander as a phoretic agent. To our knowledge, however, there are no reports of leeches using anurans for transport.

Most species of leeches are considered ectoparasites that feed through sucking the blood and other body fluids from their hosts, and most of the species included in this group are parasites of small invertebrates, such as gastropods, crustaceans, and annelids, although they can also parasite on vertebrates such as reptiles (Readel et al., 2008) and amphibians (Briggler et al., 2001; Toledo, 2005; Loebmann et al., 2008; Romano and Cerbo, 2007; Stead and Pope, 2010; Tiberti and Gentilli, 2010). Generally, leeches obtain food through structures of the foregut as a protractile pharyngeal proboscis or a cutting structure similar to slicing jaws or blades that cut the host tegument and anchor by pressing the mouth against it, and they may eat several times their own weight (Brusca and Brusca, 2003). Also, some leeches produce anticoagulants which allows the maintenance of the hemorrhage even after the removal of the leeches (Brusca and Brusca, 2003; Tiberti and Gentilli, 2010). At least two species of leeches have been reported to be associated with amphibians in southern Brazil, feeding on tadpoles, on juveniles and on adults of anurans of different families (Loebmann et al., 2008).

Rhinella abei (Baldissera Jr., Caramaschi and Haddad, 2004) (Bufonidae), inhabits remnants of the Brazilian Atlantic Rainforest throughout the eastern of the states of Paraná and Santa Catarina and north to the state of Rio Grande do Sul, with its geographic distribution limited by the Serra do Mar to the north and by the Serra Geral to the west (Baldissera Jr. et al., 2004). This species may be encountered in both forested habitats and clearings in natural or anthropogenically modified areas. Studies of *R. abei* are scarce, and are mainly on its distribution and habitat use (Garey, 2007; Lucas, 2008; Armstrong and Conte, 2010; Cunha et al., 2010; Wachlevski and Rocha, 2010), and one study concerning its phylogeography (Thomé et al., 2010). None of these studies mentioned an association of leeches with *R. abei*. In the Atlantic Rainforest of the Parque Estadual da Serra do Tabuleiro, state of Santa Catarina, Brazil, we found individuals of *R. abei* externally infested by leeches. This led us to question whether the leeches were feeding on the toads or were in a phoretic relationship with them.

The study was carried out in a remnant of dense ombrophilous forest in the Parque Estadual da Serra do Tabuleiro (PEST) (27°44'S, 48°48'W), Santo Amaro da Imperatriz municipality, state of Santa Catarina, southern Brazil. The PEST is one of the most relevant Conservation Units of Integral Protection in Santa Catarina because it shelters five of the vegetal physiognomies occurring in the state. Moreover, it represents an important fitogeographic divisor, presenting high local biodiversity (Klein, 1981), including several anuran species (Wachlevski and Rocha, 2010). The climate in the area has a mean annual rainfall of 1200 mm and a mean annual temperature of approximately 20.5 °C (CECCA, 1997).

The individuals of *Rhinella abei* were collected in July 2009 during one night of survey using visual encounter survey methods (hereafter VES) (Crump and Scott, 1994). We

searched for anurans along the shoreline of a permanent lagoon that the toads used as reproductive site. The gender of the toads was identified by their vocalizations. In this species, males vocalize a release call when they are laterally compressed on both sides of the body in the region between abdomen and thorax, while females do not (personal observation). We carefully examined all body surfaces of each toad captured for leeches or evidence of parasitism (hemorrhages, wounds and/or scars) caused by the leeches. We recorded the region of the body (arm, axils, back, eye, foot, forearm, groin, and thigh) where leeches or scars were found and how many leeches were found attached to each region. The frequency (f, in %) of use of the body regions was represented by the percentage of leeches found attached to each region. We also measured the snout-vent length (SVL) of the toads using a caliper (precision of 0.1 mm) and the body mass using a dynamometer Pesola© with 100 g of capacity (precision of 0.1 g).

The prevalence of infestation on *R. abei* by the leeches was represented by the percentage of anurans found infested among all toads sampled and examined, and the intensity of infestation was estimated by the mean number of leeches encountered attached on the anurans infested. To evaluate whether there were relationships between the number of leeches and toads' SVL and between the number of leeches and toads' mass we performed Simple Regression Analysis (Zar, 1999), using only infested individuals.

We captured 27 individuals of *Rhinella abei* during the VES, of which 26 were males and one was a female. The identified female did not vocalize and was the largest (both SVL and body mass) toad sampled in this study, matching the expected sexual dimorphism in this species with females larger than males (see Baldissera Jr. et al., 2004). We found a total of 30 leeches on 13 *R. abei* (range: 0 – 4, n = 27); the female had one leech attached on the ventral surface of the right thigh. As we sampled only one female of *R. abei* during this study we cannot say if a particular sex may be more or less related to leech phoresys as found by Khan and Frick (1997).

In this study, all infested toads did not present evidence of being parasitized by leeches; we never observed the presence of wounds, hemorrhages or scars in their teguments caused by action of leeches as would be expected (Linnaeus, 1758; Tiberti and Gentilli, 2010). Also, none of the leeches were turgid, indicating that they were not currently consuming the blood of the toads. Furthermore, the leeches were removed by hand from the body surface of the toads without any evidence of causing injuries on the toads' tegument. These observations suggest that the relationship between *R. abei* and the leeches was not parasitism but phoresy.

The average intensity of infestation was 2.3 ± 1.3 leeches per individual toad. The only other study we could find that reported an intensity of infestation by phoretic leeches on amphibians found 1.04 leeches per salamander (Khan and Frick, 1997). The leeches mainly occupied the toads' axils (n = 7, f = 23.3%), followed by the back and the thighs (both n = 6, f = 20.0%) (Fig. 1). In a phoretic association between salamanders *Ambystoma maculatum* (Shaw, 1802) (Ambystomatidae) and leeches *Erpobdella punctata* (Leidy, 1870) (Erpobdellidae) the phoronts attached mainly to the regions near the forelimbs of the salamanders (Khan and Frick, 1997). Briggler et al. (2001) reported the parasitism of four species of anurans and two of salamanders by the leech *Desserobdella picta* (Verrill, 1872) (Glossiphoniidae) and observed a preference of *Bufo americanus* by the leeches, which occupied predominantly the axillaries regions of the front limbs. In another study,

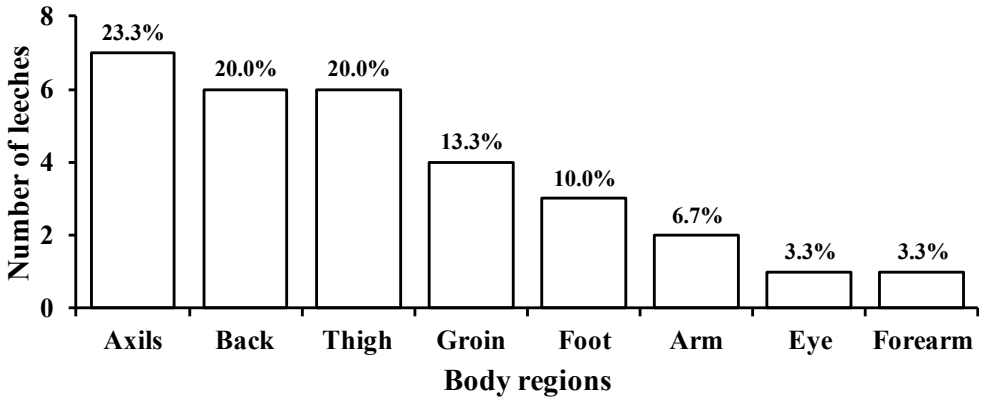

Fig. 1. Number of leeches (n = 30) recorded attached on each of the body regions (n = 8) of *Rhinella abei* (n = 13) in the Atlantic Rainforest of the Parque Estadual da Serra do Tabuleiro, in the state of Santa Catarina, southern Brazil. Values above the bars indicate the frequency (%) of leeches on each body region.

leeches were observed attached to the legs or to the skin at the base of their vocal sac of anurans of different families (Bufonidae, Leptodactylidae, Cycloramphidae and Hylidae) (Loebmann et al., 2008). The study of Loebmann et al. (2008) reported the presence of leeches on the animals' bodies but did not report evidence of wounds, scars or hemorrhages caused by leeches, and therefore may have been incorrectly reported as parasitism instead of phoresy. However, under laboratorial conditions, one toad *Rhinella arenarum* (Hensel, 1867) exposed to 15 leeches of the same species found attached on *Rhinella dorbignyi* (Duméril and Bibron, 1841) died quickly (less than 20 minutes; Loebmann et al., 2008). In general, based on a review of available studies and our own, it seems that the leeches are predominantly found on the limbs and on the axillaries regions of anurans (Khan and Frick, 1997; Briggler et al., 2001; Tiberti and Gentilli, 2010; Loebmann et al., 2008), probably because the leeches attached there are less vulnerable to being removed by contact to the substrate and/or by action of the anurans.

We found leeches on almost half (48.1%) of the *R. abei* sampled in Serra do Tabuleiro. Similarly Khan and Frick (1997) found phoront leeches on 49.1% of the salamanders they sampled. Another study that found a parasitic relationship between frogs (*Rana temporaria*) and leeches found leeches on 7 of 15 (46.7%) frogs, although four of the remaining eight frogs had dark globular clusters on their skin (Tiberti and Gentilli, 2010). In four (9.5%) of the 42 adults anurans of different species were found at least one leech (Loebmann et al., 2008). The average SVL of the toads was 69.2 ± 5.3 mm (range: 60.8 – 86.0 mm) and their average body mass was 27.1 ± 6.9 g (range: 19 – 55). We predicted that larger toads would carry more leeches due to their greater available surface area, but neither SVL nor body mass were related with the number of leeches found on the toads' bodies ($F_{1,11}$ = 0.194, P = 0.668and $F_{1,11}$ = 0.196, P = 0.666, respectively). We also expected that more leeches would be attached to dorsal surfaces of the toad's body compared to ventral surfaces, because on dorsal surfaces the leeches would be less vulnerable to direct contact with the substrate, decreasing the possibility of being mechanically removed from

the tegument. However, we found no difference in the rate of occupation on the dorsal and ventral surfaces with 13 leeches on each. However, in this study the back of the toads was the second most occupied body region following axils.

There are only a small number of studies reporting phoresy with vertebrates as "hosts" (Davies et al., 1982; Khan and Frick, 1997), and no reports of anurans as the dispersal agents. While our study involved a fairly small number of individuals collected at one site in one month, it still provides evidence of a possible phoretic relationship between anurans *R. abei* and at least one unidentified species of leech. Additional research is needed to verify this relationship both spatially and temporally and to determine the species of leeches involved.

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