

New records of endoparasites for the Casque-headed Treefrog *Trachycephalus nigromaculatus* (Anura: Hylidae)

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The tribe Lophophylini (Anura, Hylidae, Hylinae) contains most of the so-called “casque-headed” treefrogs, as well as other species without cranial casquing (Blotto et al., 2021). It is a speciose and geographically wide-ranging Neotropical group, with representatives distributed throughout most of tropical and subtropical South America, as well as tropical Mexico, Central America and the Caribbean Islands, with one species having been introduced into the southern continental United States and the Hawaiian Islands (Frost, 2023). The genus *Trachycephalus* Tschudi, 1838 (currently comprising 18 species) is particularly widespread, ranging from Mexico to southern Brazil and northern Argentina (Frost, 2023). In spite of that, there is still relatively little information available on the endoparasite fauna associated with *Trachycephalus* spp. (Bursey and Brooks, 2004; Campião et al., 2014, 2015, 2016; Draghi et al., 2015; Velarde-Aguilar et al., 2015; da Graça et al., 2017; Pereira et al., 2017). Most of that information pertains to individuals identified as *T. typhoni* (Linnaeus, 1758) [= *T. venulosus* (Laurenti, 1768) or *Phrynohyas venulosa* (Laurenti, 1768)], which is currently known to comprise a species complex (see Ron et al., 2016; Zaracho et al., 2018). Data for most species outside the *T. typhoni* complex is, at present, very limited or non-existent.

One of the species for which data on endoparasites is notably scarce is *Trachycephalus nigromaculatus*

Tschudi, 1838, despite its wide geographic distribution (which spans much of northeastern, southeastern and central Brazil; Frost, 2023) and large body size (reaching ca. 100 mm SVL; Izecksohn and Carvalho-Silva, 2001). So far, the only endoparasites recorded in *T. nigromaculatus* are monogeneans of the genus *Polystoma*, first reported by Kohn et al. (1978) from the urinary bladder of some specimens. Four decades later, Du Preez and Domingues (2019) re-examined that material and, based on it, plus some additional specimens from the same host species, described a new species, *Polystoma knoffi*. In this note, we present some data that contribute to the knowledge of the endoparasite fauna associated with *Trachycephalus nigromaculatus*.

On 15 January 2016, during one study on the local pond-breeding anuran assemblages, two adult male *T. nigromaculatus* (96.0 mm and 99.5 mm in SVL; Fig. 1) were collected around a temporary pond (22.4607°S, 42.7765°W; SIRGAS2000; 24 m elevation) in an anthropically disturbed area in the surroundings of the Reserva Ecológica de Guapiaçu, Municipality of Cachoeiras de Macacu, Rio de Janeiro State, Brazil. The specimens were later deposited at the amphibian



Figure 1. Adult male *Trachycephalus nigromaculatus* (UNIRIO 6751; SVL = 96 mm) from Cachoeiras de Macacu, State of Rio de Janeiro, Brazil. Photograph by D. Vrcibradic.

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collection of the Universidade Federal do Estado do Rio de Janeiro (voucher numbers: UNIRIO 6751-52). At the laboratory, their digestive tracts, liver, lungs, and urinary bladders were removed and examined under a stereomicroscope for the presence of endoparasites. All parasites found were temporarily mounted on slides for identification. Monogeneans were rinsed several times in tap water for one hour, stained overnight in a weak solution of acetocarmine, dehydrated, cleared in xylene and mounted using Dammar gum. The other endoparasites were cleared in lactophenol for the mounts.

We recorded three parasite species in the frogs: the platyhelminth *Polystoma knoffi* Du Preez and Domingues, 2019 (Monogeneoidea, Polystomatidae), the nematode *Parapharyngodon* aff. *alvarengai* Freitas, 1957 (Oxyurida, Pharyngodonidae) and the annelid *Dero (Allodero) lutzi* Michaelsen, 1926 (Oligochaeta, Naididae) (Table 1). The parasites were deposited at the parasitological collections of the Instituto Oswaldo Cruz, in Rio de Janeiro, Brazil (*P. knoffi*: CHIOC 39911a-c), and of the Universidade Federal do Ceará, in Fortaleza, Brazil (*D. lutzi*: CPUFC 479; *Pa.* aff. *alvarengai* CPUFC 480-83).

Specimens of monogeneoidean platyhelminths recovered from the urinary bladder were identified as *Polystoma knoffi* by the morphology and morphometry of the anchors, as well as by the number of genital spines presented in the male copulatory organ (nine in *P. knoffi*). This species was originally described from *T. nigromaculatus* and has been previously reported from the Municipalities of Rio de Janeiro and Nova Iguaçu, in Rio de Janeiro State (Du Preez and Domingues, 2019). Considering the data from the present survey, this trematode remains being known only from the state of Rio de Janeiro and from a single host species. This agrees with the notion that members of the genus *Polystoma* tend to be strongly host-specific (Du Preez and Kok, 1997; Fan et al., 2020).

The identification of nematodes was proceeded by observing the morphological characteristics with taxonomic importance according to dichotomous keys and morphometric tables from various studies (Vicente et al., 1993; Pereira et al., 2017; Ferreira et al., 2021). Our specimens partially matched the diagnosis of *Parapharyngodon alvarengai*, but also had some differences from the latter such as the smaller body size of females, eggs with greater dimensions and thicker shells, and the shorter spicule and different arrangement of the caudal papillae in males. We thus refer to our specimens as *Parapharyngodon* aff. *alvarengai*, but it is possible that it represents an undescribed species. However, assessing this will require more detailed examination of the material and is beyond the scope of the present note. In any case, *Trachycephalus nigromaculatus* represents a new host for nematodes in the genus *Parapharyngodon*.

The parasitic annelid *Dero (Allodero) lutzi* has previously been reported from the urinary tracts of several Brazilian frog species (mostly hylids), including two other species of *Trachycephalus* (*T. mesophaeus* and *T. typhoni*; Oda et al., 2015; da Graça et al., 2017). *Trachycephalus nigromaculatus* thus represents the third species of the genus reported as host for *D. lutzi*.

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Table 1. Endoparasites found in two specimens of the treefrog *Trachycephalus nigromaculatus* from Cachoeiras de Macacu, State of Rio de Janeiro, Brazil. Total intensity (i.e., number of individuals) for each parasite species in each infection site is given in parentheses.

Infection site	UNIRIO 6751 (SVL = 96 mm)	UNIRIO 6752 (SVL = 99.5 mm)
Small intestine	<i>Parapharyngodon</i> aff. <i>alvarengai</i> (3)	<i>Parapharyngodon</i> aff. <i>alvarengai</i> (35)
Large intestine	<i>Parapharyngodon</i> aff. <i>alvarengai</i> (30)	<i>Parapharyngodon</i> aff. <i>alvarengai</i> (8)
Urinary bladder	<i>Dero (Allodero) lutzi</i> (1)	<i>Polystoma knoffi</i> (6)

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