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PENSOFT

Diplotriaena delirae Pinto & Noronha, 1970 (Nematoda, Diplotriaenidae) in *Pitangus sulphuratus* (Linnaeus, 1766) (Passeriformes, Tyrannidae) from southern Brazil

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Abstract
Diplotriaena delirae Pinto & Noronha, 1970 is known to parasitize *Pitangus sulphuratus* (Linnaeus, 1766) in Peru and in the Midwestern and Southeastern regions of Brazil. Here, specimens of *P. sulphuratus* were collected in the southern state of Rio Grande do Sul, Brazil, and necropsied. Nematodes ($n = 6$) found in these specimens were identified as *D. delirae* based on their morphological traits. This is the first report of *D. delirae* from southern Brazil, expanding the knowledge of the helminth fauna of *P. sulphuratus* in the Neotropical region.

Key words
Nematode; great kiskadee; endoparasite; taxonomy; Neotropical region.

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Introduction

The family Tyrannidae Vigors, 1825 has 4 subfamilies, Elaeninae Cabanis & Heine, 1860, Fluvicolinae Swainson, 1832, Hirundineinae Tello, Moyle, Marchese & Cracraft, 2009, and Tyranninae Vigors, 1825. A total of 66 tyrannid species are known to occur in the Brazilian state of Rio Grande do Sul (Bencke 2010). The tyrannine genus *Pitangus* Swainson, 1827 is monotypic, and includes *Pitangus sulphuratus* (Linnaeus, 1766), known as the Great Kiskadee (Piacentini et al. 2015).

Pitangus sulphuratus is endemic to the New World, where it is distributed from southern Texas, USA, southward as far as Argentina (Sick 1997). This tyrannid has an omnivorous diet, feeding on fruit, invertebrate prey such as arachnids, coleopterans, dipterans, lepidopterans, hymenopterans, and small crustaceans (Argel-de-Oliveira et al. 1998), as well as vertebrates, including amphibians and fish (Andrade 1997), and processed human foods.

A number of helminth taxa are known to parasitize *P. sulphuratus* in Brazil, including the nematodes *Diplotriaena delirae* Pinto & Noronha, 1970, *Deliria gomesae* Vicente, Pinto & Noronha, 1980, and *Skrjabinoclava tupacincai* Freitas, Vicente & Ibañes (1970) (Vicente et al. 1983a, 1995). Species of the genus *Diplotriaena* Railliet & Henry, 1909 have specific parasitic relationships

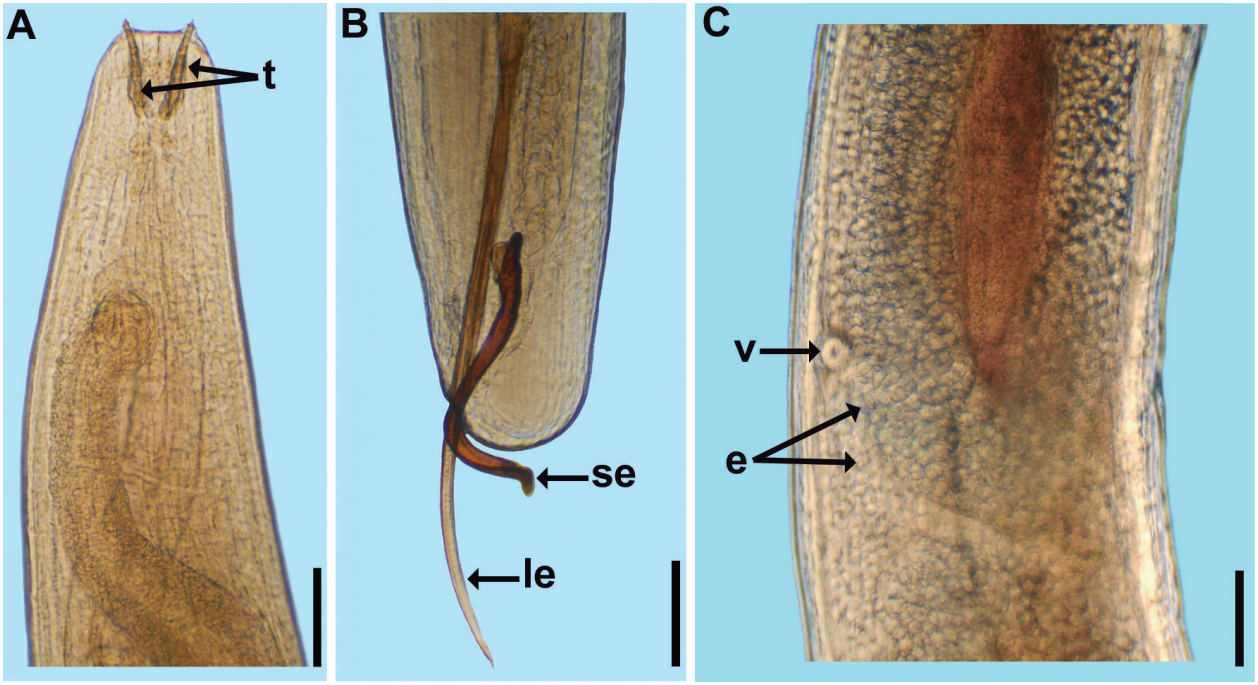

Figure 1. Photomicrographs of *Diplotriaena delirae* Pinto & Noronha, 1970. **A.** Lateral view of the anterior extremity showing the tridents (t). Scale bar = 580µm. **B.** Posterior extremity of a male, showing the large (le) and small (se) spicules. Scale bar = 230µm. **C.** Vulvar region of the female showing the vulva (v) and eggs (e). Scale bar = 200 µm.

with bird hosts, occurring in families as diverse as the Accipitridae Vigors, 1824, Corvidae Leach, 1820, Picidae Leach, 1820, Icteridae Vigors, 1825, Dendrocolaptidae Gray, 1840, Formicariidae Gray, 1840, Thraupidae Cabanis,1847, Hirundinidae Rafinesque, 1815, Turdidae Rafinesque, 1815, Furnariidae Gray, 1840, and Tyrannidae Vigors, 1825 (Vicente et al. 1983b). Our study provides the first record of *D. delirae* in *P. sulphuratus* from Rio Grande do Sul, extending the distribution of *D. delirae* to the southernmost state of Brazil.

Methods

In 2015 and 2016, a research team from the Wild Animal Rescue Center (CETAS) of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) retrieved 4 specimens of *P. sulphuratus* from the margins of the RS-040 state highway between the towns of Viamão (30°08′08.0″ S, 050°51′27.5″ W) and Capivari do Sul (30°09′11.5″ S, 050°26′45.9″ W), in the state of Rio Grande do Sul, Brazil. The bird specimens were taken to the laboratory of invertebrate zoology at the Brazilian Lutheran University (ULBRA) in Canoas for necropsy. The nematodes found in the specimens were fixed in A.F.A. (70° GL ethanol; formalin 37%; glacial acetic acid) at 65 °C for 48 h and then stored in 70° GL ethanol (Amato and Amato 2010). The internal structures were visualized in specimens mounted temporarily and cleared using Amann's lactophenol (Humason 1979).

The species was identified based on the morphological traits and morphometry of the specimens (Pinto and Noronha 1970, Vicente et al. 1983b, 1995). Nematode systematics followed De Ley and Blaxter (2002). The measurements were obtained under a light microscope with an ocular micrometer and are given in micrometers (µm), unless otherwise indicated. In the text, these measurements are presented as the range followed (between parentheses) by the mean, and standard deviation. The ecological terminology (prevalence, mean intensity, and mean abundance of infections) follows Bush et al. (1997). Photomicrographs were taken using a Nikon Coolpix S3300 camera attached to the microscope. Voucher specimens of the nematode were deposited in the Helminthological Collection (CHMU) in the ULBRA Natural Sciences Museum in Canoas, Rio Grande do Sul, Brazil.

Results

Phylum Nematoda Rudolphi, 1808
Class Chromadorea Inglis, 1953
Subclass Chromadoria Pearse, 1942
Order Rhabditida Chitwood, 1933
Suborder Spirurina Railliet & Henry, 1915
Infraorder Spiruromorpha De Ley & Blaxter, 2002
Superfamily Diplotriaenoidea Skrjabin, 1916
Family Diplotriaenidae Anderson, 1958
Subfamily Diplotriaenidae Skrjabin, 1916
Genus *Diplotriaena* Railliet & Henry, 1909

***Diplotriaena delirae* Pinto & Noronha, 1970**
Figure 1

New records. Brazil: Rio Grande do Sul state: Municipality of Capivari do Sul (30°09′11.5″ S, 050°26′45.9″ W) (Fig. 2). Host: *Pitangus sulphuratus* (Linnaeus, 1766). Site of infection: coelomic cavity. Collection of parasites:

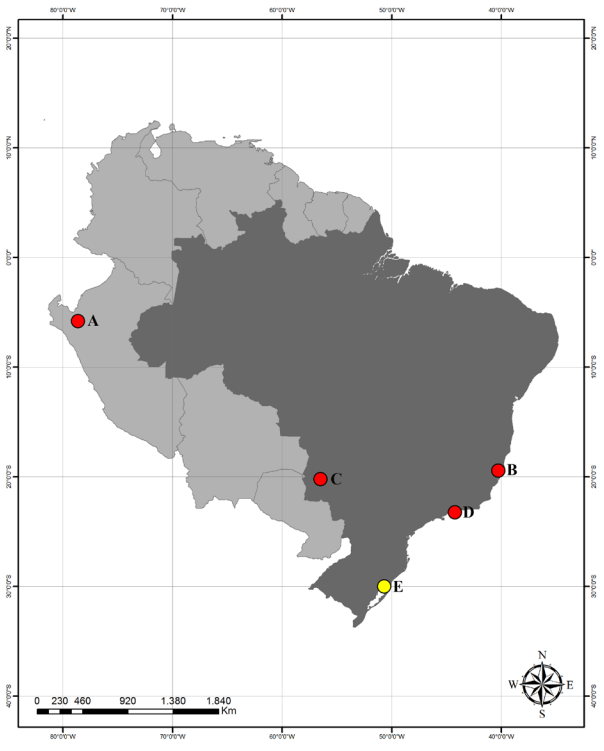

Figure 2. Map showing known geographic distribution (A–D) and the new record (E) of *Diplotriaena delirae* Pinto & Noronha, 1970. A. Province of Jaén, Peru. B. Linhares, state of Espírito Santo, Brazil. C. Salobra, state of Mato Grosso do Sul, Brazil. D. Angra dos Reis, state of Rio de Janeiro, Brazil. E. Capivari do Sul, state of Rio Grande do Sul, Brazil.

10 May 2017; identification of parasites: 5 March 2018. Voucher specimens of helminths deposited: CHMU-265-1-2-6-male; CHMU-265-1-1-6-female.

Identification. Nematodes with slender body and transversely striated cuticle. Rudimentary buccal capsule. Anterior extremity with two chitinous structures in trident shape with little apparent roughness, tapering finely toward the anterior extremity. Esophagus divided into 2 portions, glandular portion longer than the muscular portion. No excretory pore observed.

Males (n = 5). Body 25.8–28.3 mm (26.7 ± 1 mm) long, 0.55–0.77 mm (0.65 ± 0.9 mm) wide. Trident 196–265 (224 ± 19) long. Nerve ring 216–274 (235 ± 24) from anterior extremity. Muscular esophagus 0.37 mm long and glandular esophagus 2.45 mm long. Spicules unequal, smallest curved, 0.57–0.64 mm (0.60 ± 0.03 mm) long; largest 1.03–1.07 mm (1.05± 0.02 mm) long. Seven pairs of caudal papillae, of which, 3 pairs are precloacal and 4 pairs are post-cloacal.

Female (n = 1). Body 55.4 mm long, 0.75 mm wide. Tridents 216 and 245 long. Nerve ring 245 from anterior extremity. Female amphidelphic and oviparous with posequatorial vulva. Vulva 42.9 mm from anterior extremity. Uterus directly opposite one another. Eggs with thin shell, 49 long, 29 wide. Subterminal anus.

The identification of the species of the genus *Diplotriaena* Railliet & Henry, 1909 is based primarily on the shape and size of the tridents and spicules (Vicente et al. 1983b, 1995). *Diplotriaena delirae* is closely related to *Diplotriaena attenuata-verrucosa* (Molina, 1858) Henry & Ozoux, 1909, *Diplotriaena henryi* Blanc, 1919, and *Diplotriaena zederi* Pinto, Vicente & Noronha, 1981 due to its rough trident. However, *D. delirae* is closest to *D. henryi* due to its less apparent roughness, but can be distinguished from this species by the presence of tridents of more than 0.21 mm in length.

Discussion

The specimens examined here presented morphological measurements generally similar to those reported for *D. delirae* in previous studies (Pinto and Noronha 1970, Vicente et al. 1983b, Hon et al. 2013). While the measurements of our specimens were closest to those recorded by Hon et al. (2013), they are generally smaller than those reported by Pinto and Noronha (1970) and Vicente et al. (1983b) (Table 1).

To date, 12 *Diplotriaena* species have been reported from Brazil: *Diplotriaena agelaius* (Walton, 1927) Anderson, 1959, *Diplotriaena americana* Walton, 1927, *D. attenuata-verrucosa*, *Diplotriaena bargusinica* Skrjabin, 1917, *D. delirae*, *Diplotriaena falconis* (Connal, 1912) Blanc, 1919, *D. henry*, *Diplotriaena microspiculum* Pinto & Noronha, 1971, *Diplotriaena ozouxi* Railliet & Henry, 1909, *Diplotriaena sylvinae* Pinto & Noronha, 1970, *Diplotriaena ursulae* Pinto & Noronha, 1971, and *D. zederi* (Vicente et al. 1995).

Previous reports of *D. delirae* include the following hosts and localities (Fig. 2): *Myiarchus tyrannulus* (Statius Müller, 1776) from Jaén province, Peru (Hon et al. 2013), and *P. sulphuratus* from the Brazilian towns of Angra dos Reis, in Rio de Janeiro state (Pinto and Noronha 1970), Linhares in Espírito Santo, and Salobra in Mato Grosso do Sul (Vicente et al. 1983a). This is the first report of *D. delirae* parasitizing *P. sulphuratus* in Rio Grande do Sul, extending the distribution of *D. delirae* to the southernmost state of Brazil.

During its life cycle, the female *D. delirae* produces eggs that enter the air sacs of the bird host, and subsequently migrate to the throat and then the feces, where they reach the environment (Chabaud 1955, Anderson 2000). The eggs in the feces are ingested by insects, possibly the intermediate hosts, which are the prey of bird hosts in which the parasite completes its development (Anderson 2000). Given these features of the parasite's life cycle, the presence of *D. delirae* in *P. sulphuratus* reflects its omnivorous diet under natural conditions, which includes insects (Argel-de-Oliveira et al. 1998).

The microhabitat of the adult *D. delirae* is the air sacs of its birds hosts, although most studies have identified erroneously the coelomic cavity as the site of infection (Anderson 2000). The presence of *D. delirae* in the coelomic cavity of the specimens of *P. sulphuratus* examined by us is likely due to the impact of the vehicles that collided with the birds on the highway. The prevalence

Table 1. Comparison of the morphological traits (mm) of *Diplotriaena delirae* Pinto & Noronha, 1970 from various studies.

Reference	Present study		Pinto and Noronha (1970)		Vicente et al. (1983b)		Hon (2013)	
Sex of nematode	Male	Female	Male	Female	Male	Female	Male	Female
Trident	0.2–0.26	0.22–0.24	0.29	0.31	0.21–0.28	0.22–0.28	0.18–0.25	0.18–0.3
Nerve ring (from anterior end)	0.22–0.27	0.25	0.37	0.4	0.3–0.33	0.32–0.4	0.25–0.31	0.22–0.34
Small spicule	0.57–0.64	—	0.61	—	0.59–0.65	—	0.43–0.77	—
Large spicule	1.03–1.05	—	1.19	—	1.00–1.09	—	0.52–1.04	—

of *D. delirae* was 25%, with a mean abundance of 1.5 helminths per host. The study of the helminths collected from animals killed by traffic can provide important insights into the composition of the parasitic fauna of a given region, as well as aspects of the relationship with hosts and the life cycle of the parasite in specific environments. Despite this potential, the analysis of the helminth fauna of roadkill specimens is limited by the typically small numbers of specimens collected from this source.

Animals killed by traffic may provide a good source of specimens for the analysis of the helminth fauna of wild vertebrates, including endangered species (Gallas et al. 2014), and the systematic retrieval of carcasses should be stimulated as an alternative strategy for the collection of specimens without the need to sacrifice the hosts. Parasitological research provides important insights into the quality of the environment, which contributes to the development of effective conservation measures for wild species and the environments they inhabit (Lafferty 1997).

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Authors' Contributions

JCM necropsied the hosts, processed and identified the helminths, prepared the figures and wrote the text; DMFS processed the helminths and prepared the figures; MG identified the helminths, prepared the figures and wrote the text; EFS identified the helminths, wrote the text; EP wrote the text.

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