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Short Communication

Intraocular nematode and other parasites associated with *Physalaemus albonotatus* (Anura: Leptodactylidae) from Corrientes, Argentina

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The Menwig Frog, *Physalaemus albonotatus* (Steindachner, 1864), is found in the states of Mato Grosso and Mato Grosso do Sul in Brazil, in Paraguay, and in the Chacoan regions of Bolivia and Argentina (Frost 2018). In Argentina, this species inhabits the provinces of Buenos Aires, Chaco, Corrientes, Entre Rios, Formosa, Misiones and Santa Fe (Vaira *et al.* 2012). Its microhabitat includes vegetation along the shores of temporary or permanent bodies of water; however, these anurans also are found in dry terrestrial environments. Numerically, isopterans are most common in the frog's diet, but volumetrically, ants predominate. *Physalaemus albonotatus* actively forages for prey (Duré 2004).

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The known parasite fauna reported for *Physalaemus albonotatus* from Corrientes (Corrientes Province, Argentina) comprises the nematodes *Strongyloides* sp., *Oswaldocruzia* sp.; *Cosmocerca podicipinus* Baker and Vaucher, 1984 and *C. parva* Travassos, 1925 (adults); *Brevimulticaecum* sp.; *Spiroxys* sp.; *Physaloptera* sp. (larvae) (González and Hamann 2010, 2013, 2015); and the digenean *Travtrema* aff. *stenocotyle* (Cohn, 1902) (metacercariae) (Hamann and González 2009). Mulieri *et al.* (2018) reported the presence of an ectoparasite—the larvae of the sarcophagid fly, *Lepidodexia* (*Notochaeta*) *adelina* Mulieri, Schaefer, Duré and González, 2018—in this species.

We report three novel parasites in a *Physalaemus albonotatus* collected in Corrientes (Argentina)—viz., a nematode larva of the order Rhabditida in the eye, and cystacanths and cosmocercids in the mesentery and the large

intestine, respectively. On the morning of 06 December 2017, one of authors (EFS) captured a female *P. albonotatus* (Figure 1 A, B) in the 8-ha compound of the Centro de Ecología Aplicada del Litoral (CECOAL); this field station in the suburbs of the city of Corrientes (27°29'33.89" S, 58°45'33.63" W) is operated by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina. The frog was caught by hand and taken to the laboratory, where it was weighed and the snout–vent length (SVL) measured (450 g and 26.5 mm, respectively). When captured, an active parasitic nematode was seen moving in the left eye of the frog. (Video was uploaded to YouTube: https://youtu.be/gf588u3jaW8.). This frog was anesthetized topically with a lidocaine 2% cream and necropsied. In addition to its eye, the digestive tract, lungs, liver, kidneys, urinary bladder, coelomic cavity, and musculature were examined. Helminths were observed *in vivo,* counted, fixed in a hot 10% formalin solution and preserved in 70% alcohol. The nematodes were cleared in Amman's lactophenol, mounted on temporary slides, and examined under a light microscope, whereas the acanthocephalans were stained with hydrochloric carmine, cleared in creosote, and mounted permanently in Canada balsam. For morphological examination and measurements, a Leica DM2500 microscope equipped with a drawing tube was used. All measurements are shown in micrometers, unless otherwise stated. Parasite specimens were deposited in the Helminthological Collection of the Museo de La Plata (La Plata, Buenos Aires, Argentina), accession number MLP-He 7490 (Rhabditida, 1 larva), and the Helminthological Collection of the Centro de Ecología Aplicada del Litoral, accession number CECOAL 17120601 (*Centrorhynchus* sp.; 3 cystacanths); CECOAL 17120602 (*Cosmocerca podicipinus*; 1 male); CECOAL 17120603 (*C. parva*; 1 male, 4 females). The specimen of *P. albonotatus* was deposited in the Colección Herpetológica of the Universidad Nacional del Nordeste; accession number UNNEC 13179.

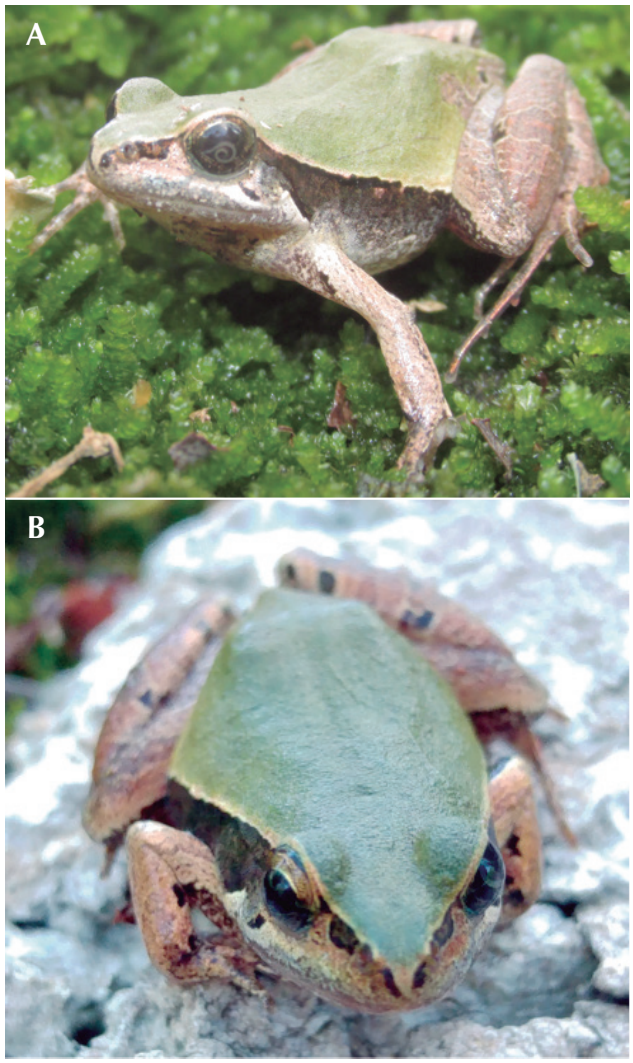

Figure 1. Larva of Rhabditida in the left eye of *Physalaemus albonotatus* from Corrientes, Argentina (**A**). Frontal view of the same specimen showing both eyes (**B**).

The nematode larva was found in the left eye, specifically, in the aqueous humor of *Physalaemus albonotatus;* this was confirmed by dissecting this organ. Macroscopically, no lesions were noted on the eye (Figure 1A) and no parasites were present in the right eye (Figure 1B). Several nematodes also were found in this frog: *Cosmocerca podicipinus* (1 male) and *C. parva* (1 male and 4 females) in the large intestine, and cystacanths of the genus *Centrorhynchus* sp. (3 larvae) in the mesentery.

The larva found in the eye has following characteristics (Figure 2): body gradually tapered anteriorly and posteriorly; total length 4.35 mm; width at level of vulva 170. Anterior end truncated, posterior end tapering. Body cuticle thin. Oral opening rounded, surrounded by six small lips arranged in two lateral groups of three each. Buccal capsule 17 wide and 13 deep. Short vestibulum leading to cavity in anterior part of esophagus funnel-shaped in lateral view. Esophagus with prominent corpus, isthmus and pear-like posterior bulb 492 length corresponding to 11.3% of body length; esophageal bulb 79 wide. Nerve ring and excretory pore situated at 215 and 260, respectively, from anterior end. Excretory glands slightly subequal in size and shape, with rounded posterior parts, situated subventrally, reaching beginning of intestine. Larger gland 470 long, smaller one 460 long. Reproductive system didelphic amphidelphic. Vulva post equatorial, with prominent lips, located at 1.99 mm from posterior end. Rectum short, thin and tubular; posterior part of tail gradually tapering, tail length 185.

Based on these morphological characters, we place this larva in the Order Rhabditida Chitwood, 1933. Although more specific identification is not possible, the possession of non-inflated cuticle, six lips arranged in two lateral groups of three, two well-developed excretory glands, and the typical rhabdiasid reproductive system, suggest that it may be a female of the gonochoristic generation of the genus *Serpentirhabdias* Tkach, Kuzmin and Snyder, 2014. Typically, larval rhabdiasid nematodes are lung-dwelling nematodes that parasitize snakes by penetrating the lungs through the mucosa. Thus, it is possible that the immature *Serpentirhabdias* that we found in *Physalaemus albonotatus* may represent an accidental infection.

Adult stages of nematodes can be found in different organs of amphibian and reptile hosts; according to Goater and Goater (2001), the small intestine is the most common habitat for

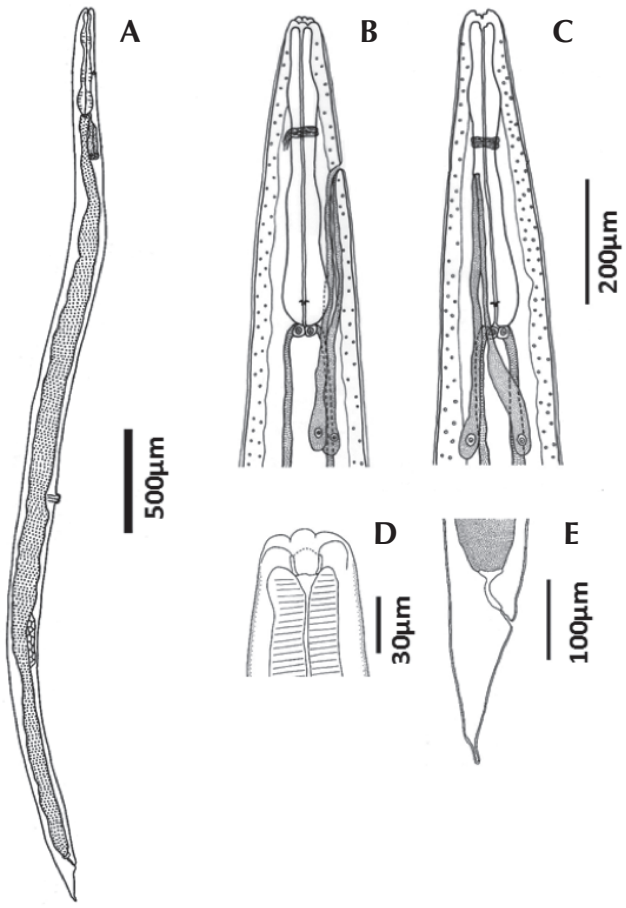

Figure 2. Larva of Rhabditida from the eye of *Physalaemus albonotatus* from Corrientes, Argentina. General, lateral view (**A**). Anterior extremity, lateral view (**B**), and ventral view (**C**). Detail of anterior extremity (**D**). Posterior extremity, lateral view (**E**).

roundworm parasites. Campião *et al.* (2014, 2015) reported that the most common helminths in South American amphibians are gastrointestinal nematodes of the families Cosmocercidae (Raillet, 1916, subfam.) Travassos, 1925; Kathlaniidae (Lane, 1914 subfam.) Travassos, 1918; Molineidae (Skrjabin and Schulz, 1937, subfam.) Durette-Desset and Chabaud, 1977; Physalopteridae (Railliet, 1893 subfam.) Leiper, 1908; and lungworms of the family Rhabdiasidae Railliet, 1915.

Our findings agree with those of Campião *et al.* (2014, 2015) because we primarily found cosmocercid nematodes infecting *P. albonotatus.*

Moreover, like González and Hamann (2013, 2015), we found the intestinal nematode species *Cosmocerca podicipinus* and *C. parva.*

The presence of cystacanths of *Centrorhynchus* sp. in the mesenteries of the host, suggest that *P. albonatus* may represent an additional paratenic host for this acanthocephalan. Santos and Amato (2010) found that the toad, *Rhinella fernandezae* (Gallardo, 1957), also is a paratenic host of *Centrorhynchus* sp. in the south of Brazil (state of Rio Grande do Sul). Hamann *et al.* (2006) report these cystacanths in the mesenteries of *Leptodactylus latinasus* Jiménez de la Espada, 1875, from Corrientes, Argentina (the same locality of the present study) suggesting that other species of anurans might be involved in the life cycle of this parasite in this region.

Nematode infections of anuran eyes seem relatively uncommon. Some are associated lesions on the eye. Imai *et al.* (2009) reported rhabditoid larva of the free-living nematode *Caenorhabditis elegans* Maupas, 1900 in Indonesian *Megophrys montana* (Kuhl and Van Hasselt, 1822) (Megophryidae) that caused ophthalmic and neural disease in this host. Additionally, rhabditoid larvae have been observed in the eyes of various introduced and native species of Australian anurans (Kelehear *et al.* 2010, Pizzatto *et al.* 2010). These have been seen crawling over the surface of the eye (Kelehear *et al.* 2011), as well as lodged in the periocular tissue—e.g., conjunctiva, extraocular muscles, connective tissue between the sclera and the skin, and embedded in the sclera or beneath the retina (Pizzatto *et al.* 2010). In the case of *Physalaemus albonotatus,* the larval nematode moving freely in the aqueous humor, and no macroscopic lesion was observed in the eye.

Finally, we highlight that this study reports for the first time the accidental presence of Rhabditid nematodes naturally infecting the eye of a species of anuran and the infection of cystacanths of the genus *Centrorhynchus* contributing to the knowledge of the diversity of helminths associated with *P. albonotatus,* from Corrientes Argentina.

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