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ANIMAL SCIENCE

Spinitectus asperus and *Klossinemella iheringi*, intestinal nematodes of *Prochilodus lineatus* (Pisces, Prochilodontidae) from the alluvial plain of the Middle Paraná River, Argentina

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Abstract: *Prochilodus lineatus* has been considered an ecosystem engineer in the Neotropics given its influence on important ecosystem processes, and it is therefore relevant to understand their inter-specific relationships. The association of *P. lineatus* with parasitic helminths was studied in two isolated shallow lakes of the alluvial plain of the Middle Paraná River. Only two species of gastrointestinal nematodes were found: *Spinitectus asperus* (Cystidicolidae) and *Klossinemella iheringi* (Atractidae), with *S. asperus* having higher prevalence and mean intensity values. This is the first record of *K. iheringi* in the Middle Paraná River, Argentina, as well as the southernmost citation of *S. asperus*.

Key words: Endoparasite, fish parasite, Nematoda, middle Paraná River, *sábalo*.

INTRODUCTION

Essentially, the endoparasite community in a host indicates the status of the host organism in the trophic pathways of the ecosystem (Marcogliese 2003). Parasites are affected by biotic and abiotic changes to the environment and are effective indicators of many aspects of host biology; thus, they are extremely useful as management and conservation tools (Marcogliese 2004).

Among the fish species of the Paraná River basin, *Prochilodus lineatus* (Valenciennes 1836) is the most noticeable, due to its abundance and biomass and also because of its role in the trophic webs of the system (Rossi et al. 2007, Scarabotti et al. 2017). This species moves significantly between different habitats in the basin, which show its adaptive response to seasonal variability, synchronizing its reproduction to the flood pulse and associating the main features of its life history with changes in the hydrological cycle (Arthington et al. 2008). Its trophic habits are detritivores, having been recognized that it feeds mainly on epiphyton and the organic matter of the benthos (Emiliani & Brandi 1972, Bowen et al. 1984, Bayo & Cordiviola de Yuan 1996). In studies carried out in the floodplain of the Middle Paraná River, the remarkable significance of the detritus energy pathway has been evidenced (Marchese et al. 2014, Saigo et al. 2016).

In this basin, as with other large South American rivers, important fractions of the production of periphyton and macrophytes enter the aquatic trophic webs in the form of detritus, and the most important trophic pathways link organic matter and detritus with detritivore fish and their piscivorous predators. Consequently, most of the matter and energy of the aquatic food web flow through short

food chains, which favors the efficiency of energy transfer from the resource base to the fish (Lewis Jr. et al. 2001). With its important migratory movements throughout the basin, *P. lineatus* favors the exchanges of matter and energy between different compartments of the system, which is why it has been considered an "ecosystem engineer" in the Neotropics, given its influence on important ecosystem processes such as the nutrient cycle and primary and secondary production (Vitule et al. 2017).

Previous studies on the parasitic nematodes of *P. lineatus* have, for the most part, been carried out in Brazil, where Anisakidae gen. sp., *Contracaecum* sp. type II larva, *Klossinemella* sp., *Procamallanus* (*Spirocamallanus*) *inopinatus, P.* (*S.*) sp., *Procamallanus* sp., *Raphidascaris* sp. and *Spinitectus asperus* were recorded (Moravec et al. 1993, Santos et al. 2003, Lizama et al. 2006, Kohn et al. 2011, Luque et al. 2011, Reis et al. 2017). In Argentina, there is limited knowledge of the parasite fauna associated with native fish species of the Paraná-La Plata River basin (Chemes & Takemoto 2011, Ostrowski de Núñez et al. 2017). However, some studies have been carried out, particularly, on parasite-host interaction in *P. lineatus* (Hamann & Lombardero 1981-1982, Hamann 1982a, Ramallo 1999, Ramallo et al. 2000, 2020, Chemes & Gervasoni 2013).

The present study has enabled us to report on the two species of nematodes found in *P. lineatus*, and has broadened our knowledge of the relationship between the parasite, the host and the different environments of the Middle Paraná River.

MATERIALS AND METHODS

The fish were collected between November 2013 and April 2014, in two isolated lentic environments, Del Medio Lake (n = 11), connected to the Colastiné River (31°45´9"S; 60°45´49"W) and La Chicana Lake (n = 8), associated with the Coronda River (31°38´20"S; 60°28´48"W). It should be noted that both lakes are close to secondary channels of the Middle Paraná system and that the collections were made in periods of low water (Fig. 1). The main environmental parameters are detailed in Table I.

Fish collection was authorized by the Ministry of Environment of the Santa Fe province (resolution 036/18) and the care and use of animals complied with guidelines and policies of the National Research Council of Argentina (CONICET 2005). Fishes were identified, measured and weighed in the field, and then were euthanized (by percussive stunning and subsequent medullar denervation). The specimens obtained in Del Medio Lake had a mean standard length (SL) = 33.8 cm (30.2 - 60.6) and weight = 832.7 g (734 - 924). Those from La Chicana Lake measured SL = 26.9 cm (14.3 - 32.3) and weighed = 622.5 g (103 - 1034). The digestive tracts were separated and preserved in 10% formalin. In the Laboratory of Natural Sciences of the Research Group in Ichtyoparasitology and Ichthyology of the Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Santa Fe, Argentina, the dissections, the exhaustive review under a stereoscopic microscope and the separation of endoparasites were performed and prepared according to Eiras et al. (2006). Parasite morphometric characteristics were carried out using a Nikon E200 binocular microscope with a graduated scale. Taxonomic keys and original articles were consulted for identification. Nematodes were stored in the Zoological Collection of the Provincial Museum Florentino Ameghino (MFA-ZI-Nd), in Santa Fe, Argentina. Measurements are expressed in microns, indicating the average value followed by the range, and the number of measurements made is shown in parentheses.

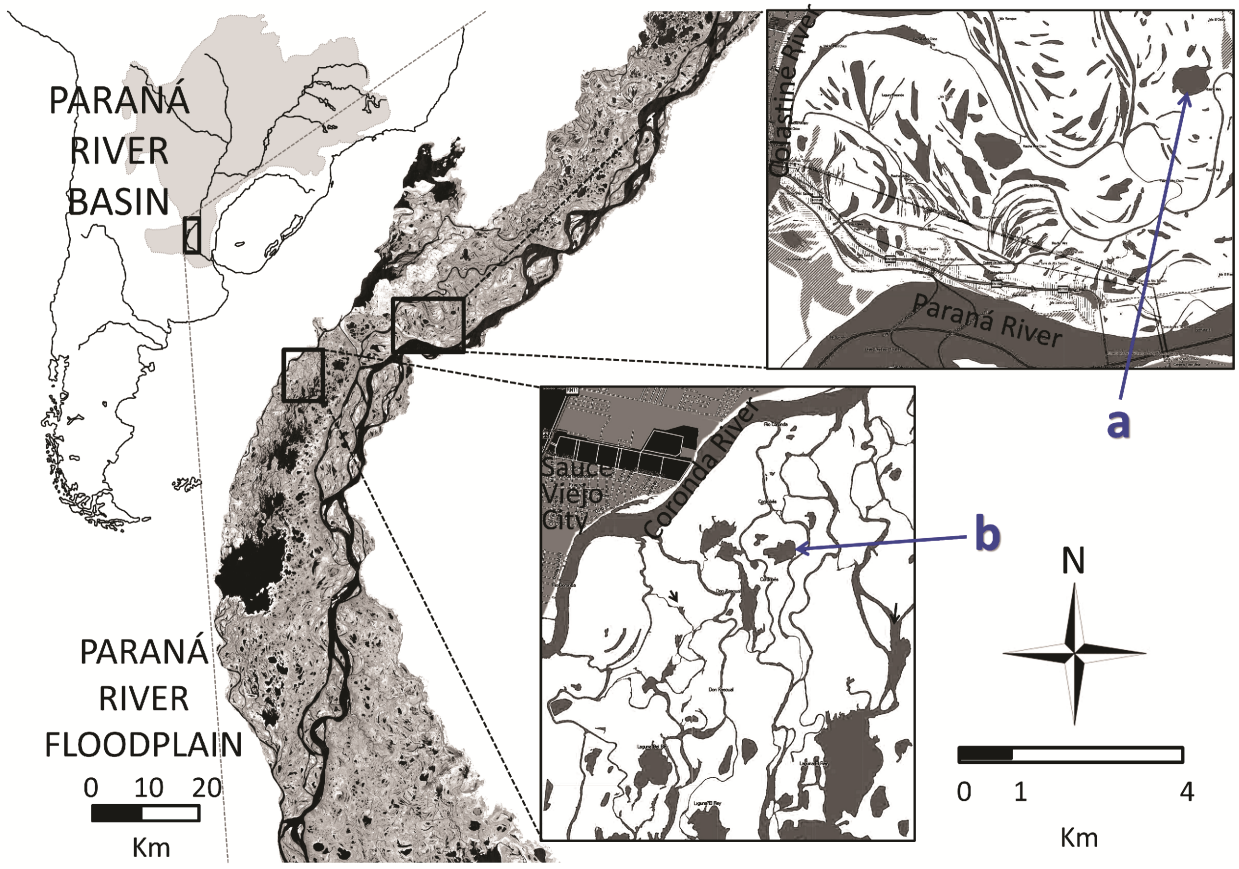

Figure 1. **Lentic environments of the Middle Paraná System. a. Del Medio Lake, b. La Chicana Lake (adapted from Devercelli et al. 2016).**

Table I. Environmental characteristics of the aquatic systems considered (taken from Devercelli et al. 2016), obtained in two lakes of the Middle Paraná System, Argentina.

Lake	Area (m^2)	pH ± SD	Conductivity (mS. cm-1) ± SD	Dissolved oxygen (% saturation) ± SD	Secchi transparence (cm) ± SD	Vegetation cober (%) range
Del Medio	64.90	7.1 ± 0.6	70 ± 20	111.2 ± 11.1	54 ± 36	21-40
La Chicana	4.88	7.4 ± 0.5	290 ± 41	49.7 ± 4.5	66 ± 30	61-100

RESULTS

In both lentic environments, the presence of *S. asperus* was found in the intestinal tract of *P. lineatus*, while *K. iheringi* was only observed in *P. lineatus* from La Chicana Lake. The parasitic prevalence and mean abundance of infestation obtained for *S. asperus* were higher than those obtained for *K. iheringi*. The diagnosis of both species is presented below.

Order: Habronematoidea

Family: Cystidicolidae Skrjabin 1946

***Spinitectus asperus* Travassos, Artigas et Pereira 1928 (Fig. 2)**

Synonymous: *S. jamudensis* Thatcher & Padilha 1977

Sites of infestation: stomach and intestine

Locality: Del Medio Lake

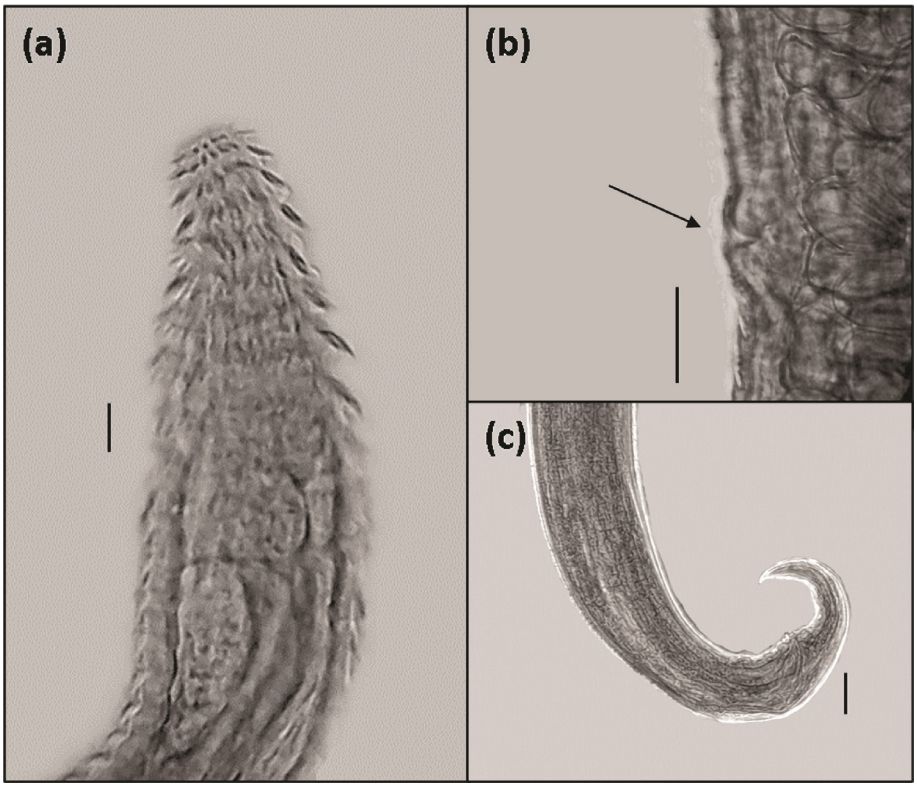

Figure 2. ***Spinitectus asperus*** **(Cystidicolidae). a. Anterior end. Scale bars: 20 µm. b. Vulva in the middle third of the female; c. Caudal portion of male. Scale bars: 30 µm.**

Prevalence and Mean intensity: 72.7 % (8 infected fish / 11 examined fish) and 4.63 (range 1-21) nematodes per infected fish

Locality: La Chicana Lake

Prevalence and Mean intensity: 62.5 % (5 infected fish / 8 examined fish) and 6.4 (range 2-12) nematodes per infected fish

Specimens deposited: MFA-ZI-Nd 113 (female), MFA-ZI-Nd 114 (male)

Description: Small nematodes. Cuticle with transverse rings with retrospines. Initially, the rings are relatively distant from each other, reducing the distance near the forelimb and then after spreading towards the hind limb, they disappear. Spines are fine and pointed, the longest of which were at the level of the muscular esophagus. The mouth has indistinct lips; funnel-shaped or cylindrical oral cavity; two-part esophagus. Parasites of the digestive tracts of fish.

Females: (11 specimens). Total body length: 8981.4 (5830-12699, n = 9) and maximum width 233.9 (158-291, n = 11). Distance from the anterior end to the first ring of spines: 10 (n = 1). Distance between the first ring of spines and the second: 9.2 (6-10, n = 8); between the second and third rings: 10 (8-15, n = 8) and between the third and fourth: 13.4 (7-18, n = 8). Distance from the last ring of spines to posterior end: 31 (29-33, n = 2). Ring spines length: 12.6 (9-17, n = 14). Length of prostomium: 147 (121-173, n = 2). Long muscular esophagus: 62 and long glandular esophagus: 192. Vulva in the middle third. Oviparous. Small, ellipsoidal eggs, 29.6 long (22-42, n = 12) and 16.5 wide (10-24, n = 12), sometimes with polar filaments. Tail length: 204.4 (130-281, n = 5).

Males: (8 specimens). Total body length: 4818 (3805-5743, n = 8) and maximum width: 163 (110-228, n = 8). Distance between the first ring of spines and the second: 9.5 (8-11, n = 2); between the second and third ring: 8.5 (8-9, n = 2) and between the third and fourth: 11.5 (9-14, n = 2). Length of the spines of the rings: 11.8 (8-18, n = 8). Length of prostomium: 144. Muscular esophagus

length: 68 and glandular esophagus length: 452. Unequal spicules, the largest (left): 219 (116-293, n = 3) and the smallest (right): 76.8 (54-90, n = 5). Hind limb rolled up; tail wing narrow; pre-anal and post-anal papillae present (numerous 10 to 15 pairs). Tail length: 120.5 (105-136, n = 2).

Biology: The genus *Spinitectus* includes a group of parasitic nematodes whose life cycle includes an arthropod intermediate host and a vertebrate definitive host (F.A. Christian, unpublished data). In general, nematodes with cuticular spines invade the intestinal mucosa of the host, thus *Spinitectus* species are among those which cause the most severe inflammation of the intestinal wall (Thatcher 2006).

Comments: Cited for the first time in Argentina by Hamann (1982a), found in the stomach of *P. lineatus* obtained from the confluence zone between the Paraguay and Paraná rivers, in the province of Corrientes (Fig. 4).

Order: Cosmocercoidea Railliet 1916

Family: Atractidae Railliet 1917

***Klossinemella iheringi* Travassos, Artigas et Pereira 1928 (Fig. 3)**

Synonyms: *Monhysterides iheringi* Travassos, Artigas & Pereira, 1928 (Gállego-Berenguer 1947). *Klossiella iheringi* Costa 1960.

Site of infestation: Intestine

Locality: La Chicana Lake

Prevalence and Intensity of infestation: 12.5 % (1 infected fish / 8 fish examined) and 4 nematodes per infected fish.

Specimens deposited: MFA-ZI-Nd 115 (female and male)

Description: Fusiform body, thin cuticle and slightly striated transversely. Bilabiate mouth, bilobed with the appearance of a tetralabiate mouth, presenting interlabies, totaling six small lips; both chitinized formations. Forelimbs are armed with sclerotized pieces; these are

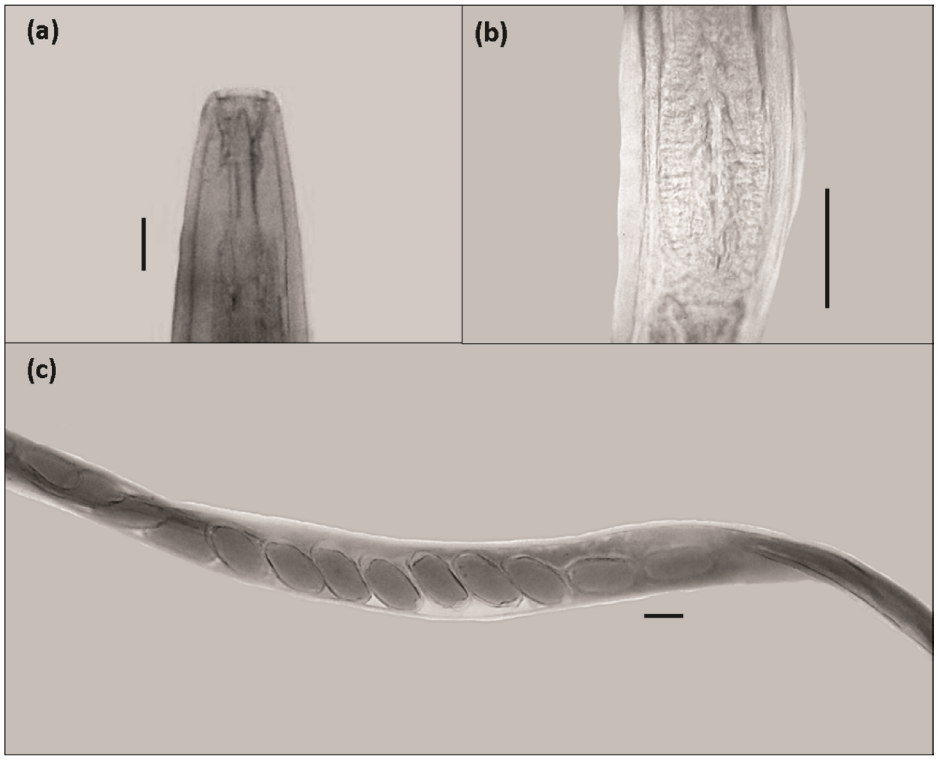

Figure 3. *Klossinemella iheringi* (Atractidae). a. Anterior end. Scale bars: 20 µm. b. Esophageal bulb. Scale bars: 30 µm. c. Gravid female eggs. Scale bars: 40 µm.

Y-shaped. Depending on the contraction of the mouth, the sclerotic parts can be oriented in different ways. Esophagus made up of two portions, the anterior is muscular and cylindrical and the posterior is glandular and has a rudimentary bulb equipped with valves. The nerve ring is located in the anterior part of the second portion of the esophagus. Right and small intestine. Straight like a simple hyaline tube. The tail in both sexes is long and pointed. Parasites of fish intestine.

Females: (3 specimens). Total body length: 6837 (5542-7747, n = 3). Maximum body width: 120.3 (108-138, n = 3). Oral cavity, length: 24 (22-25, n = 2) and maximum width: 18 (15-21, n = 2). Buccal ornamentation or sclerotized pieces, length: 23.5 (22-25, n = 2). Length of the esophagus: 498 (n = 1), length of the anterior part: 509 (280-738, n = 2) and length of the posterior part (including the esophageal bulb): 308 (218-398, n = 2). Maximum width of the esophageal bulb: 44.5 (34-55, n = 2). Female monodelphs, prodelphs, viviparous, with vulva located above the anus, with anterior direction. Sacciform uterus, in gravid females, containing developing eggs and/or up to 1 to 6 well-developed larvae, simple ovary, short ovary, slightly coiled. Subulate tail. Eggs length: 72.1 (54-89, n = 9) and width: 41.3 (36-51, n = 9). Tail length: 428 (n = 1).

Male: (1 specimen). Total length of the body: 6648, maximum width of the body: 89. Oral cavity, length: 16 and maximum width: 20. Length of the lips of the mouth: 2. Sclerotized part: 20. Esophagus, entire length: 1138. Excretory pore located: 238 from the anterior end of the body. Two unequal striated spicules, the larger (left) length: 84, and the smaller (right) length: 50. Gubernacle present. Males with the hind limb rolled in a spiral, presenting 4 pairs of pre-anal papillae and 5 post-anal papillae. Thin tail, with a pointed end.

Biology: Direct evolutionary cycle, adult females can release larvae into the host's intestine, and these develop until they reach sexual maturity. The transmission of parasites to new hosts occurs by the elimination of larvae in feces (Costa et al. 1968).

Comments: Recorded in the digestive tract of freshwater fish from Brazil, such as *Piaractus brachypomis* (type host), *Mileus* sp., *Mylesinus paraschomburgkii*, *Myloplus asterias*, *Leporinus copelandi*, *L. fasciatus*, *Hoplias malabaricus*, *P. lineatus*, *Schizodon naspterutum*, *Tetrana nasopterina* gen sp.; only once found in Argentina, in the intestine of *Pterodoras granulosus* (Costa et al. 1968, Hamann 1982b, Moravec & Thatcher 1997, Reis et al. 2017) (Fig. 4).

DISCUSSION

There are few records of intestinal nematodes associated with *P. lineatus*. In the Upper Paraná River plain (Brazil), Lizama et al. (2005) recorded nematode *Raphidascaris* sp, in a very low prevalence (approximately 2%). In the case of *S. asperus*, in various studies carried out in Neotropical environments, it was found in different parts of the digestive tract of Prochilodontidae, including *P. lineatus*, *P. scrofa*, *P. argenteus* and *P. reticulatus* (Travassos et al. 1928, Fernandes et al. 1982, Petter & Morand 1988, Ramallo 1999, Santos et al. 2003, Monteiro et al. 2009, Reis et al. 2017, Leite et al. 2018, Lehun et al. 2020, Ramallo et al. 2020). In Argentina, the records of *S. asperus* parasitizing *P. lineatus* correspond to the Paraná River in the area of its confluence with the Paraguay River, Corrientes (Hamann 1982a), the Río Hondo reservoir, Santiago del Estero (Ramallo 1999) and the Pilcomayo River, Salta (Ramallo et al. 2020). The present record in environments of the Middle Paraná, in the province of Santa Fe, constitutes the southernmost detection of this species.

Figure 4. Records of *Spinitectus asperus* and *Klossinemella iheringi* in South America.

The finding of *S. asperus* in fish species that inhabit different hydrographic basins may indicate that the transmission of these parasites to their definitive hosts can occur through nearby intermediate hosts or those that are more abundant in their habitats. To explain the presence of this heteroxene nematode parasitizing a detritivore species, it is interesting to consider that F.A. Christian (unpublished data) confirmed under experimental conditions that the larvae of Isopoda, Ephemeroptera or Amphipoda are involved in the life cycle of *S. asperus* as intermediate hosts. When reviewing the contents of the digestive tract of the fish in the present study, arthropod remains were found, but they were too small and incomplete for us to carry out their taxonomic recognition. For their part, Saigo et al. (2015) identified the presence of nymphs of *Campsurus violaceus* Needham & Murphy 1924, *America baetis* Klunge 1992 and *Caenis* Stephens 1835 (Ephemeroptera) in these environments and recorded amphipods which are also common. Probably by consuming large quantities of benthic organisms, *Prochilodus lineatus* can incorporate arthropods with larvae of this nematode.

In relation to *K. iheringi*, it was recorded in the swim bladder of *P. lineatus*, in Mogi Guaçu, Cachoeira de Emas (São Paulo), while in the Upper Paraná River floodplain, the genera found were associated with the intestine of *Pimelodus maculatus*, a siluriform, abundant in the region (Reis et al. 2017, Lehun et al. 2020). The present study constitutes the first record of *K. iheringi* associated with *P. lineatus* in Argentina and in the Middle Paraná River. Its life cycle is direct, monoxenous (Moravec 1998); therefore, infestation could occur by ingestion of larvae in the feces dissolved in the benthos.

The fact of having recorded *K. iheringi* in La Chicana, but not in Del Medio Lake, could be related to the evidence that the fish obtained in the latter were older. Several studies show that larger hosts may have greater immunity than smaller fish, since an important aspect of the specific immune response of bony fish is immunological memory (Rubio Godoy 2010). On the other hand, there were differences in the limnological conditions of the two water bodies, which can be linked to the health status of the fish. La Chicana Lake, isolated from the nearest lotic course, small in size and during a dry period, presented high conductivity and less oxygenation than Del Medio Lake. As it has been shown, when fish are weakened by stressors, the effects of endoparasites can be worsened by the consequent depression of defense mechanisms, so an unfavorable environmental context favors the invasion of the host by a greater number of parasites (Santos et al. 2003). Thus, the limnological conditions of this shallow environment could have favored the

transmission of *K. iheringi* through contact with the eggs released into the detritus, the food source of *P. lineatus*. This fish, a basal species in trophic networks, participates in different energy transfer pathways that link the aquatic system and nearby terrestrial systems. The juveniles and adults of *P. lineatus* are preyed upon by the large predators of the river, such as surubíes (*Pseudoplatystoma* spp.) and dorados (*Salminus brasiliensis*). But numerous species of aquatic birds also include shad in their diet, such as the white heron (*Egretta alba egretta*), the biguá (*Phalacrocorax olivaceus*) and the "kingfishers" (*Ceryle torquata*, *Chloroceryle amazona* and *Chloroceryle americana*) (Beltzer & Oliveros 1981, 1987, Oliveros & Beltzer 1983). Considering these relationships, it is of considerable value to continue these studies to understand in greater detail the ecological role of ichthyoparasites in these complex trophic networks of the Paraná River.

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Author contributions

SBC and SG carried out the laboratory work on parasites; LR analyzed the food content of the digestive tracts and carried out an exhaustive bibliographic review. MAPL analyzed data and their interpretation. All authors analyzed the results and contributed equally to the writing and reviewing of the manuscript.