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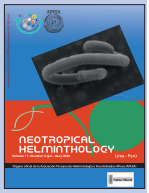

RESEARCH NOTE / NOTA CIENTÍFICA

SUBCUTANEOUS *STRIGEA* SP. (DIPLOSTOMOIDEA: STRIGEIDAE) METACERCARIAE IN RALLIDAE (GRUIFORMES) FROM THE NEOTROPICAL REGION

METACERCARIAS DE *STRIGEA* SP. (DIPLOSTOMOIDEA: STRIGEIDAE) SUBCUTÁNEAS EN RALLIDAE (GRUIFORMES) DE LA REGIÓN NEOTROPICAL

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ABSTRACT

The life cycle of species belonging to *Strigea* remains complex and poorly understood, especially in the Neotropical region, where information about their intermediate hosts is scarce. Rallidae birds are known for their adaptability to aquatic and semi-aquatic environments since they exhibit the ability to disperse and adjust to a wide range of ecological conditions. In this study, we examined seventeen specimens of *Pardirallus maculatus* (Boddaert, 1783) and one of *Pardirallus sanguinolentus* (Swainson, 1838) collected in southern Brazil in search for *Strigea* sp. metacercariae. Metacercariae occurred in 11.1% (2/18) of birds with infection intensity of 12 (*Strigea* sp. 2) and 28 (*Strigea* sp. 1) helminths found in *P. sanguinolentus* and *P. maculatus*, respectively. Metacercariae were found exclusively in the pectoralis muscle region, beneath subcutaneous tissue. These findings suggest the potential that Rallidae birds have to act as intermediate hosts of *Strigea* spp. in that region. They are reinforced by previous observations of small aquatic birds which were identified as prey of Falconiformes (definitive hosts). Thus, our study provides novel information that may assist further research that aims at expanding our understanding of the life cycle of *Strigea* spp. and at enhancing our knowledge of biodiversity by revealing new geographic distribution and intermediate hosts.

Keywords: Digenea – *Pardirallus maculatus* – *Pardirallus sanguinolentus* – plumbeous rail – spotted rail

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RESUMEN

El ciclo de vida de las especies pertenecientes al género *Strigea* sigue siendo complejo y poco comprendido, especialmente en la región neotropical, donde la información sobre sus hospederos intermediarios es escasa. Las aves Rallidae son conocidas por su adaptabilidad en entornos acuáticos y semiacuáticos, demostrando habilidades de dispersión y capacidad de ajuste a una amplia gama de condiciones ecológicas. En este estudio, examinamos diecisiete especímenes de *Pardirallus maculatus* (Boddaert, 1783) y uno de *Pardirallus sanguinolentus* (Swainson, 1838) recolectados en el sur de Brasil en busca de metacercarias de *Strigea* sp. Se encontraron metacercarias en el 11,1% (2/18) de las aves, con una intensidad de infección de 12 (*Strigea* sp. 2) y 28 (*Strigea* sp. 1) helmintos en *P. sanguinolentus* y *P. maculatus*, respectivamente. Las metacercarias se encontraron exclusivamente en la región del músculo pectoral, debajo del tejido subcutáneo. Estos hallazgos sugieren el potencial de las aves Rallidae como hospederos intermediarios de *Strigea* spp. en la región, respaldado por observaciones previas de aves acuáticas pequeñas identificadas como presas de Falconiformes (hospederos definitivos). Por lo tanto, nuestro estudio aporta nueva información que puede ayudar en investigaciones futuras destinadas a ampliar nuestra comprensión del ciclo de vida de *Strigea* spp. y mejora nuestro conocimiento de la biodiversidad al revelar nuevas distribuciones geográficas y hospederos intermediarios.

Palabras clave: Digenea – *Pardirallus maculatus* – *Pardirallus sanguinolentus* – riel moteado – riel plomizo

INTRODUCTION

Strigeidae Railliet 1919 (Digenea) is composed of species with a cup-shaped forebody and holdfast organ in the form of two lobes, which commonly parasitize birds (definitive hosts) (Niewiadomska, 2002). Metacercariae are transmitted trophically through ingestion of intermediate hosts (Niewiadomska & Pojmańska, 2011). *Strigea* Abildgaard, 1790 comprises 45 known species which develop into their adult forms in birds of prey (Lunaschi & Drago, 2013). Unlike other strigeids, species in this genus have four hosts in their life cycle, i. e., mollusks (first-order intermediate hosts), anurans and snakes (second-order intermediate hosts), in which mesocercariae develop, and amphibians, fish, snakes, birds and mammals (third-order intermediate hosts), in which 'tetracotyle' metacercariae develop (Lutz, 1933; Niewiadomska, 2002).

Tetracotyles (metacercariae) are mainly found encapsulated and encysted in subcutaneous tissue, intercostal muscles, inner thighs and the neck region, but also in bird pectoralis (intermediate hosts), with no records in the body cavity (Pearson, 1959; Awad & Al-Tameemi 2014). A metacercaria is composed of a fibrous capsule with a viscous fluid separating the capsule from the cyst (Wittrock *et al.*, 1991).

Rallidae birds have representatives in all habitable continents. They are both aquatic and semi-aquatic birds with remarkable ability to disperse and adapt to a wide range of ecological conditions (García *et al.*, 2014). *Pardirallus maculatus* (Boddaert, 1783) is typically associated with areas of aquatic vegetation in wetlands, since it depends on this ecological structure for foraging and nesting (Navas, 1991). The species is recorded from North America to the southern continent (De La Peña & Rumboll, 1998). Its diet consists mainly of insects but may also include mollusks and small vertebrates, such as tadpoles and fish, as food resources (Navas, 1991). *Pardirallus sanguinolentus* (Swainson, 1838) is a small-sized species found in the Americas; it inhabits all types of wetlands with vegetation that provides shelter, nesting resources and foraging opportunities (Clements *et al.*, 2023). The species is insectivorous but also consumes mollusks, crustaceans and plants (Goodal *et al.*, 1951; Taylor & Bonan, 1996).

Even though there are records of birds which are as hosts of metacercariae of the species *Strigea*, there is scarce information on their life cycle. Metacercariae (tetracotyles) have been described in Brazil parasitizing *Sterna* sp. (Laridae) (Lutz, 1933). In Germany and Iraq, they have been associated with *Buteo buteo* (Linnaeus, 1758) (Accipitridae) (Krone & Streich, 2000) and *Ardeola ralloides* (Scopoli, 1769) (Ardeidae) (Awad & Al-Tameemi, 2014), respectively. In the Neotropical region, there are no records of other birds that participate in the cycle as intermediate hosts, in addition to the previously described definitive hosts (Lunaschi & Drago, 2013). Therefore, this study aims at recording *P. maculatus* and *P. sanguinolentus* as intermediate hosts of *Strigea* spp. and at providing morphometric information on cysts and metacercariae.

MATERIAL AND METHODS

Seventeen specimens of *P. maculatus* and one specimen of *P. sanguinolentus* collected in Pelotas (31°44'45.6"S - 52°21'43.3"W), Rio Grande do Sul (RS), Brazil, were examined. The birds were donated by the Núcleo de Reabilitação da Fauna Silvestre and Centro de Triagem de Animais Silvestres which belongs to the Universidade Federal de Pelotas (NURFS/CETAS/UFPel) where the animals died during the rehabilitation process. The hosts were individually frozen and subsequently necropsied.

In the necropsy process, skin was detached from muscles. All organs were removed and individually separated (esophagus, trachea, proventriculus, gizzard, heart, lungs, stomach, small and large intestines, ceca, kidneys, liver and cloaca). Then, they were washed through a 150µm mesh sieve. Metacercariae were counted and isolated from host tissues and removed from cysts by preparation needles. Metacercariae were fixed in cold AFA (70ºGL ethanol, 37% formalin and glacial acetic acid) for 48 hours and preserved in 70ºGL ethanol. Specimens were stained with Langeron's carmine and mounted in Canada balsam. Helminth identification followed Niewiadomska (2002) and Hamann *et al.* (2023). Voucher specimens were deposited in the Helminthological Collection at the Instituto Oswaldo Cruz (CHIOC) Rio de Janeiro, Brazil (CHIOC 39648, 39649, 40269a-j, 40285a-f).

Measurements are expressed as micrometers (µm) and minimum and maximum values are shown between parentheses. Parasitological indices of prevalence and mean abundance of infection were estimated in agreement with Bush *et al.* (1997). Illustrations were created in ink and assembled by the GIMP 2.10 software program.

Ethic aspects: For this study, formal consent is not required.

RESULTS

Two (11.1% - one of each species) out of eighteen birds under investigation were parasitized by *Strigea* spp. metacercariae. Infection intensities were 28 metacercariae – with mean abundance of 1.64 and prevalence of 5.8% – in *P. maculatus* and 12 metacercariae in *P. sanguinolentus*. Metacercariae were found only in chest muscle region beneath subcutaneous tissue (Fig. 1). Two morphotypes were identified, i. e., one in each host species. *Strigea* spp. 1 occurred in *P. maculatus* while *Strigea* sp. 2 was found in *P. sanguinolentus*.

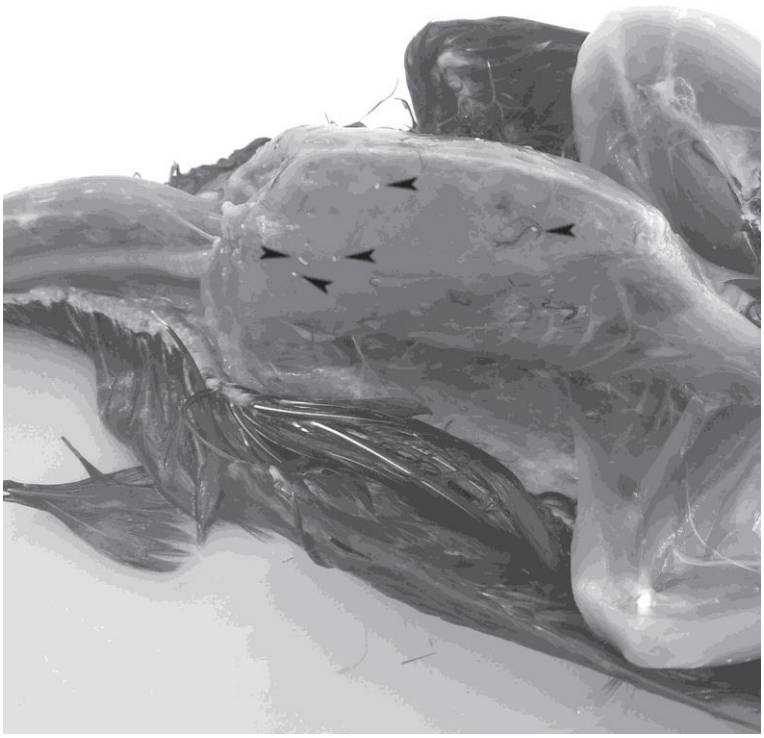

Figure 1 - Cysts in the pectoral muscle region in *Pardirallus maculatus* (Boddaert, 1783) (Rallidae) from southern Brazil.

***Strigea* sp. 1** (Fig. 2 A - B)

Total length of the metacercariae (tetracotyle) (Fig. 2A) is 410±25 (390 – 450) and its width is 278±16 (260 – 300). Oral sucker is 50±4 (45 – 55) long and 60±4 (55 – 65) wide. Ventral sucker is 72±8 (65 – 85) long and 110±8 (100 – 120) wide. Left pseudosucker is 100±16 (80 – 120) long and 80±8 (70 – 90) wide. Right pseudosucker is 80±8 (70 – 90) long and 100±16 (80 – 120) wide.

Round cysts (Fig. 2B) consist of three layers; both outer layers are thicker than the inner one. The first outer layer is fibrous and originates from the host's immune response while the third layer, near the metacercariae, is hyaline and fragile. Total length of the cyst is 574±52 µm (560 – 640) and its width is 453±52 (400 – 515). The first outer layer is 60±9 (50 – 75) thick while the second layer is 70±7 (60 – 80) thick and the hyaline layer closer to the metacercariae is 69±5 (60 – 75) thick.

***Strigea* sp. 2** (Fig. 2 C - D)

Total length of the metacercariae (tetracotyle) (Fig. 2C) is 458±28 (430 – 500) and its width is 208±13 (200 – 230). Oral sucker is 80±2 (78 – 84) long and 70±4 (65 – 75) wide. Ventral sucker is 80±4 (75 – 85) long and 97±4 (90 – 100) wide. Left pseudosucker is 90±6 (80 – 95) long and 77±4 (70 – 80) wide. Right pseudosucker is 77±4 (70 – 80) long and 90±6 (80 – 95) wide.

Oval cysts (Fig. 2 D) are composed of two layers; the outer layer is thicker than the inner one, which is near the metacercariae and is hyaline and fragile. The metacercariae occupies most of the cyst. Total length is 510±25 (490 – 550) and width is 315±17 (290 – 330). The outer layer is 37±2 (35 – 40) thick while the inner layer is 37±2 (35 – 40) at the most distant ends of metacercariae.

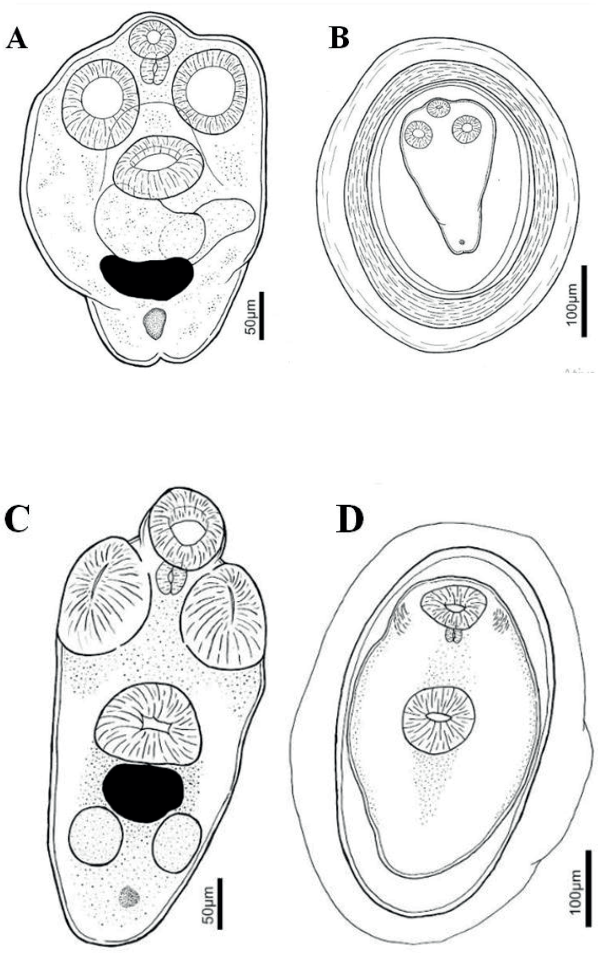

Figure 2. Metacercaria (A, C) and cyst (B, D) of *Strigea* spp. associated with Rallidae birds from southern Brazil. **A – B.** *Strigea* sp. 1 found in *Pardirallus maculatus* (Boddaert, 1783). **C – D.** *Strigea* sp. 2 found in *Pardirallus sanguinolentus* (Swainson, 1838).

DISCUSSION

The life cycle of *Strigea* species is poorly understood and complex, especially in the Neotropical region, where there are few reports about their hosts (Lunaschi *et al.*, 2007). Parasitic relations between intermediate hosts and *Strigea* spp. are influenced by the diversity and structure of the environment in which they are found (Lutz 1933; Pearson, 1959). Furthermore, complexity of the life cycle depends on three intermediate hosts for metacercariae to develop and complete their cycle in definitive hosts (Lutz, 1933).

Findings of encysted metacercariae in subcutaneous tissue of Rallidae birds from southern Brazil corroborate information reported by Krone & Streich (2000) and Awad & Al-Tameemi (2014). A study carried out by Krone & Streich (2000) examined 77 birds (*B. Buteo*) from three locations in Germany and found prevalence ranging from 10% to 58% and infection intensity from 2 to 76 metacercariae found in the neck connective tissue. Awad & Al-Tameemi (2014) do not mention the number of metacercariae found in each host. Infection indices in Rallidae birds were higher than the ones found in *A. ralloides* (8.6%)(Awad & Al-Tameemi, 2014) and lower than the ones found in *B. buteo* (41%) (Krone & Streich, 2000). Although encysted metacercariae have been found in *B. buteo* (Krone & Streich, 2000) which is normally considered a definitive host (Lunaschi 2013), Krone & Streich (2000) pointed out that, in this record, the falconiform would be acting as a third-order intermediate host.

The authors suggested that high prevalence of metacercariae in *B. buteo* may be linked to abundance of flooded areas, which results in high availability of second-order intermediate hosts (Krone & Streich, 2000). In contrast, findings of this study suggest that Rallidae birds may potentially play the role of intermediate hosts of *Strigea* spp. in the region. It is based on the previous observation that small aquatic birds have been identified as part of the diet of falconiforms in the Neotropical region (Silva, 1997; Bó *et al.*, 2007). This pattern may be explained by the active hunting behavior of birds of prey in open areas, as reported by Bó *et al.* (2007).

This study is the first record of *Strigea* metacercariae in Rallidae birds and increases our understanding of the diversity of helminths associated with them and the life cycle of strigeids in the Neotropical region. Need for further studies to comprehend the role of these birds in the transmission dynamics of digeneans is evident. Furthermore, it is essential to consider the significance of parasitic diversity in ecosystems, especially because human activities have altered trophic dynamics and, consequently, have affected parasitic biodiversity (Gómez & Nichols, 2013; Strona, 2015). Thus, documentation of new trophic interactions plays a crucial role in supporting conservation strategies.

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