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DIOECOCESTUS PARONAI FUHRMANN, 1900 (CESTODA, CYCLOPHYLLIDEA) PARASITE OF THE WHITE-TUFTED GREBE *ROLLANDIA ROLLAND* (QUOY AND GAIMARD, 1824)(PODICIPEDIFORMES) AND A REVIEW OF THE HOST SPECIFICITY IN DIOECOCESTIDAE

DIOECOCESTUS PARONAI FUHRMANN, 1900 (CESTODA, CYCLOPHYLLIDEA) PARÁSITO DEL MACÁ COMÚN *ROLLANDIA ROLLAND* (QUOY AND GAIMARD, 1824)(PODICIPEDIFORMES) Y REVISIÓN DE LA ESPECIFICIDAD HOSPEDATORIA EN DIOECOCESTIDAE

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ABSTRACT

The family Dioecocestidae Southwell, 1930 largely confined to grebes, has a confounding and poorly known systematic composition. Most of its members are known only from their original descriptions and were not studied since then. Based in two tapeworms with completely separate sexes, *Dioecocestus paronai* Fuhrmann, 1900 was primarily consigned to *Plegadis guarauna* (Linnaeus 1766) as the natural host and Buenos Aires, Argentina, as the type locality. In the present study specimens of *D. paronai* are described, data on its biology and host distribution are provided. There were no significant differences in the prevalences of males and females infesting grebes. Nor in the abundances between host sexes. The variance/mean ratio was 0.426 and the parameter of contagion K= ─0.36, showing an underdispersed pattern of distribution. Following its original description, not only was *P. guarauna* never cited again as host of this tapeworm but the remaining six species of the genus *Dioecocestus* Fuhrmann, 1900 are grebe specialists in the five continents and *D. paronai* was nevermore found from ibises. The white-tufted grebe *Rollandia rolland* (Quoy & Gaimard, 1824) is rectified as the natural host and it is confirmed the marked specificity of the family Dioecocestidae in grebes.

Keywords: Cestoda – *Dioecocestus* – Podicipediformes – *Rollandia rolland*

RESUMEN

La composición sistemática de la familia Dioecocestidae Southwell, 1930 ampliamente confinada a los macáes o zampullines, es confusa y muy pobremente conocida. La mayoría de sus integrantes se conocen solo a partir de sus descripciones originales y varias no han sido vueltas a estudiar. *Dioecocestus paronai* Fuhrmann, 1900 descripta sobre la base de dos individuos con sexos completamente separados, fue primariamente asignada a *Plegadis guarauna* (Linnaeus, 1766) como hospedador natural y tuvo a Buenos Aires, Argentina como su localidad típica. En el presente estudio se describen, se aportan datos acerca de su biología y distribución en el hospedador de especímenes adultos de *D. paronai*. No se hallaron diferencias estadísticamente significativas en cuanto a la prevalencia entre sexos de los hospedadores como tampoco entre las abundancias parasitarias. El coeficiente de dispersión varianza/media fue de 0.426 y el parámetro de contagio K= ─0.36, ambos revelaron un patrón de distribución subdisperso o regular en la población de hospedadores. Desde su descripción original, *P. guarauna* nunca más fue citado como hospedador de esta especie, además las restantes seis especies del género *Dioecocestus* son todas especialistas de macáes en los cinco continentes y *D. paronai* nunca más fue hallado en ibises. En el presente trabajo se rectifica al macá común *Rollandia rolland* (Quoy & Gaimard, 1824) como el hospedador natural de la especie y se confirma la marcada especificidad de grupo de la familia Dioecocestidae en macáes.

Palabras clave: Cestoda – *Dioecocestus* – Podicipediformes – *Rollandia rolland*

INTRODUCTION

Storer (2000), in his monograph on the fauna of metazoan parasites of grebes emphasizes, among other concepts, that the cestodes dominate the fauna of intestinal helminths. Also, the family Dioecocestidae Southwell, 1930 largely confined to grebes, has a poorly known systematic composition. In fact, in the Storer's systematic list of cestodes parasitizing grebes, *Dioecocestus paronai* described by Fuhrmann (1900) is not considered. Parona, who worked at Genoa, had received from the National Museum of Buenos Aires, two specimens of an unknown tapeworm, apparently parasiting a white-faced ibis, *Plegadis guarauna* (Linnaeus, 1766) (Ciconiiformes, Threskiornithidae) and he forwarded them to Otto Fuhrmann, in Neuchatel, for identification. Based on the novel finding of the complete separation of sexes in a tapeworm, Fuhrman proposed the genus *Dioecocestus* with *paronai* as type species, consigning *P. guarauna* as the natural host and Buenos Aires, Argentina, as the type locality. From its original description, not only the species was never cited again but the remaining six species of the genus *Dioecocestus* are grebe specialists in the five continents. This fact has a significant influence in assessing both the host specificity of the dioecocestids as well as aspects of the co-evolution between cestodes and grebes. The aim of the present study is to describe the tapeworms recorded from *Rollandia rolland* (Quoy & Gaimard, 1824) to provide data on its biology and host distribution and to evaluate its host specificity.

MATERIAL AND METHODS

The helminths compose a collection made in wetlands from Buenos Aires province between 1983-2018: Chascomús lagoon (LCH) 35°35'29" S 58°01'28" W), Mar Chiquita lagoon (AMCH) (37°46' S and 57°27' W), Villa Arias (38°52'00" S 62°05'00" W) and Los Talas ponds (LT) (34°55'44" S and 57°43'04" W). The sample of hosts consisted of 40 *R. rolland* (19 females, 19 males and 2 indeterminate). The tapeworms were preserved in 10% formalin and stained with Langeron´s hydrochloric carmine. The identification was made by morphology and morphometry. The measurements are given in µm unless otherwise indicated. For microanatomy studies, the worms were fixed in buffered formalin, dehydrated in ethilic alcohols, embedded in paraffin, sectioned at 5-7 µm thick and colored with hematoxylin and

eosin. Microphotographs were taken with a Motic BA 200 optical microscope. Voucher material was deposited in the Helminthological Collection of the Museum of La Plata (Argentina). The variance/mean ratio (coefficient of dispersion) and the contagion parameter, K, were determined to assess the distribution patterns at the level of infrapopulations. The parasitological indexes follow Bush *et al.* (1997). The mean abundances and prevalences were compared according to the sex of the hosts using the Mann-Whitney *U* test, (IBM SPSS version 23), and the Z statistic (Morales & Pino, 1987), respectively. The level of significance used was $\alpha = 0.05$.

Ethic Aspects

Corresponding scientific hunting permits were obtained from the Faune Direction of the Agricultural Ministry, Buenos Aires province, Argentina.

RESULTS

Description (based on 1 male and 1 female, fixed and stained). The species presents the sexes completely separated. The male has a total length of 110 mm and a maximum width of 2.60 mm, while the female reaches 140 mm long and 3.86 mm wide. The scolex is rudimentary, in the form of a low anterior prominence, 450 wide, 160 long and 100 thick, probably not functional as a fixation organ. Four primordia of circular suckers 45 in diameter with a slender orbicular musculature and narrow frontal medial sulcus (Fig. 1). There are neither rostellum nor hooks. The strobila consists of a long chain of craspedote proglottids, with a region of maturity occupying approximately the anterior third of the strobila, whose proglottid are wider than long (80 x 1280 at 1 mm from the scolex, 384 x 2440 at 8 mm from the scolex).

Towards the posterior end the morphology of the segments changes becoming longer and of approximately quadrangular (2200 x 2600). The male strobila presents a set of duplicated gonads in an anterior transverse field of the medullary parenchyma (Fig. 2). Numerous ovoid testes of 48 x 32 cluster between the longitudinal excretory vessels. They do not form lateral fields, as in other *Dioecocestus* spp., but form a single stratum that runs posteriorly and dorsoventrally. It was not possible to quantify the number of testes. Two vas deferens run as rectilinear conduits, from the gonadal mass towards both margins of the segment, passing between the excretory vessels (Fig. 3). After enter the robust cirrus sac, they expand to give rise an internal seminal vesicle. The cirrus sac with strong muscular walls measures 180 in length in the middle zone of the strobila and reaches 520 in the last proglottids. This observation is highlighted since the testes quickly deplete their gametes, then become rudimentary and disappear completely from the parenchyma in the posterior strobila. However, both the segment as a whole and the cirrus sac, continue growing with uncertain functional explanation (Fig. 4). The cirrus is robust and is completely covered by spines "L" shaped with a sharp and retrograde tooth (Figs. 5ab).

On the other hand, the female strobila has a bilobed ovary central and dorsal to a compact vitelline gland (Fig. 6). The vagina is a blind straight tube that runs towards the margin. As a taxonomical feature in dioecocestids, the vagina ends blind near the margin and alternating irregularly along the strobila. The gravid multilobed uterus (Fig. 7) fills the medulla and compresses both bands of longitudinal musculature towards the margins, exceeding the osmoregulatory vessels. Embryonated eggs measure 56 x 48, have a thin vitelline membrane and a thin embryophore that protects a subsferic oncosphere of 32 x 24, with three pairs of embryonic hooks of 16 in length (Fig. 8).

Taxonomic summary

Dioecocestus paronai Furhmann, 1900 (Cyclophyllidea, Dioecocestidae)

Host: *Rollandia rolland* (Quoy & Gaimard, 1824) (Podicipediformes)

Location: Small intestine

Geographic localization: Several ponds and lagoons from Buenos Aires province (Argentina)

Voucher material: One male, mounted and stained, accession number = MLP-He 7567 (MLP helminthological collection)

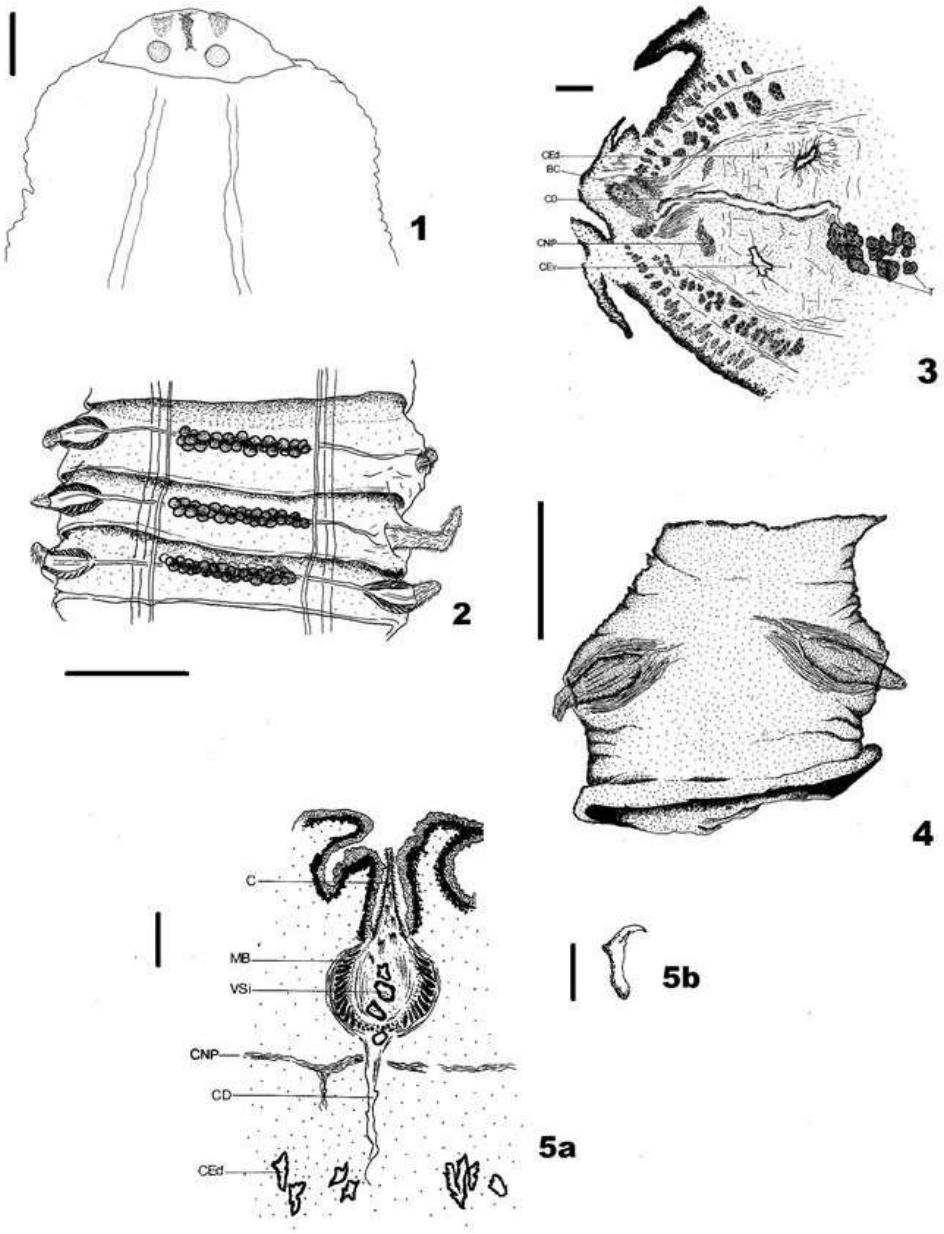
Prevalence= 82.5% (33/40) (males= 89%; females= 79%)

Mean abundance= 1.15

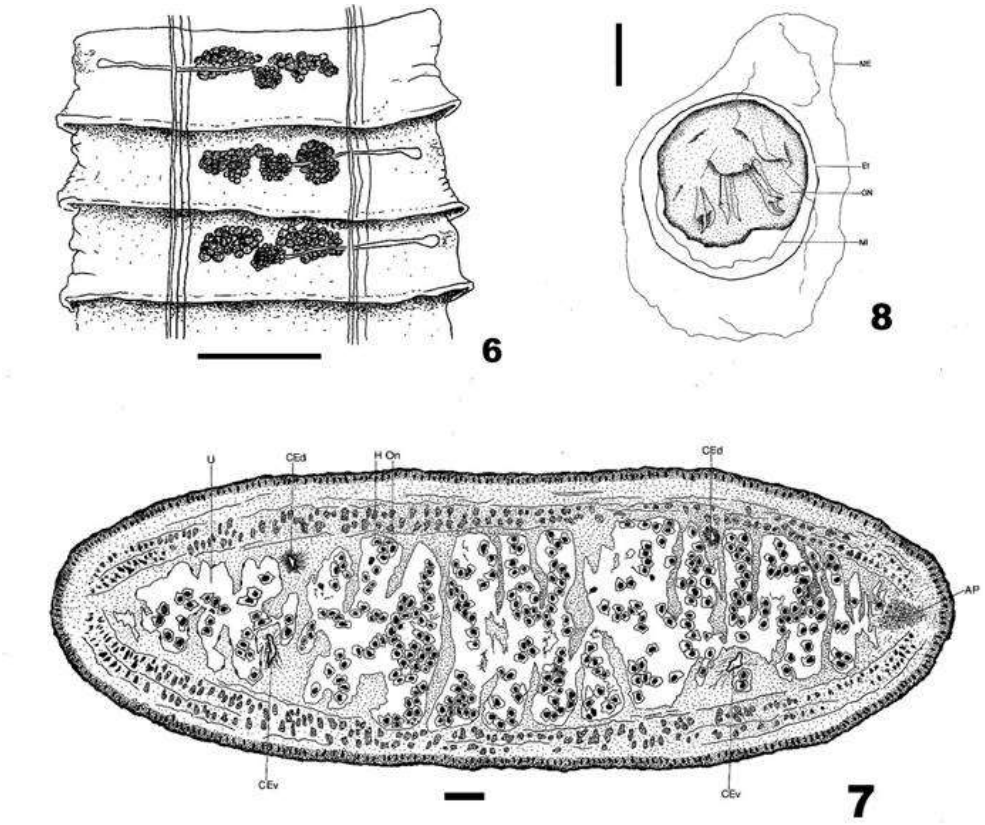
Mean intensity= 1.39

Distribution on hosts: Thirteen of the birds (32,5%) hosted a pair of adults, a male and a female in each

intestine, but the remaining, from 20 birds, were in *postmortem* degradation. The worms occupied 75% of the lumen of the small intestine. The variance/mean ratio was 0.426 and the parameter of contagion K= ─0.36. No juvenile form or larval stages assignable to *Dioecocestus* Fuhrmann, 1900 were found. In respect to the parasitological indexes in our sample, there were not significative differences in the prevalences of males and females of grebes (Z= 0.588 p> 0.05). Nor in the abundances between sexes (U Mann-Whitney= 144.00 p>0.05).



Figures 1-5. **1**. *Dioecocestus paronai* Fuhrmann, 1900 (*in toto*). Anterior end showing the rudimentary scolex and suckers. Scale-bar= 0.1 mm. 2. Male strobila (*in toto*). Scale-bar= 1 mm. 3. Male genitalia terminal (detail from histological section). Abbreviations: BC= cirrus pouch, CD= deferent duct, CEd= dorsal excretory vessel, CEv= central excretory vessel, CNP= principal nervus chord. Scale-bar= 0.1 mm. 4. Sterile proglottid from the posterior end (*in toto*). Scale-bar= 1 mm. 5a. Cirrus pouch (detail from histological section). Abbreviations: C= cirrus, MB= muscular wall of the pouch, VSi= internal seminal vesicule. Scale-bar= 0.1 mm. 5b. tegumentary spine of the cirrus. Scale-bar= 0.01 mm.



Figures 6-8. 6. Female strobila (in toto). Scale-bar= 1 mm. 7. Gravid segment (detail from histological section). Abbreviations: H= egg, On= oncosphere, U= uterus. Scale-bar= 0.1 mm. 8. Mature egg (in toto). Abbreviations: Ef= embryophore, ME= outer envelope, MI= inner envelope, ON= oncosphere. Scale-bar= 0.01 mm.

DISCUSSION

Based in two tapeworms with completely separate sexes, Fuhrmann (1900) described *D. paronai*. These worms apparently had been collected in a white-faced ibis, *P. guarauna* (sic) in Buenos Aires, Argentina. It was considered a type of a genus, so far unknown, whose most significant feature was the dioecy. The same year, Fuhrmann, studied a sample of eleven tapeworms from *Podiceps collaris* (sic) and *Podiceps grisegena* (Boddaert, 1783) from collections of Mehlis, Krabbe, Muller and Rudolphi, which were designated as *Taenia aspera* Mehlis, 1831 and *T. lanceolata* Rudolphi, 1805. Like *D. paronai*, these cestodes had the peculiarity of having separate sexes in different strobilae, although unlike the former, they have scolex with a rostellum armed with 30 hooks, elliptical and more voluminous cirrus sac, a discoidal vitelline gland and larger eggs. Based in these differences, Fuhrmann proposed to meet them in a new taxon nominated *Dioecocestus asper* (Mehlis, 1831). Later, they were successively described *D. acotylus* Fuhrmann, 1904 in the least grebe, *Tachybaptus dominicus* (Linnaeus, 1766) from South Texas, Jamaica, Cuba, Haiti and North of Brazil, *D. novaeguineae* Fuhrmann, 1914, in the Australian little grebe, *T. novaehollandiae* and T. *ruficollis* in New Guinea, *D. fuhrmanni* Linton, 1925 in the red-necked grebe *P. grisegena* and *P. auritus* (Linnaeus, 1758) in Alaska and Yakutia, *D. fevita* Meggitt, 1933 in *T. ruficollis* in India and *D. cablei*

(Siddiqi, 1960) in the same host and site. Also, Furhmann (1909) renamed *D. novaehollandiae* (Krefft, 1871) in the little grebe *T. ruficollis* (Pallas, 1764) from Australia. But Ryzhikov & Tolkacheva (1981) synonymized *D. novaehollandiae* with *D. asper*. So, the six species cited above have the particularity that all of them are specialists in grebes, and four of them were described in the same host, *T. ruficollis*. This situation carries to a poor known and confounding systematic status in the family Dioecocestidae. Storer (2000) stated that the Australian material needs checking. Fuhrmann (1907) ranked all dioecious species in a new family, Acoleinidae, whose type genus, *Acoleus* Fuhrmann, 1899, shared with *Dioecocestus* some features of interest, especially the absence of external vaginal opening, arrangement of the testicular mass, two layers of parenchymatic muscular bands and the shape of the uterus. Ransom (1909) amended the name of the family in question as *Acoleidae* which brought together the genera *Acoleus* Fuhrmann, 1899, *Gyrocoelia* Fuhrmann, 1899, *Dioecocestus* Fuhrmann, 1900, *Diplophallus* Fuhrmann, 1900 and *Shipleya* Fuhrmann, 1908. Later, Southwell (1930) relocated *Dioecocestus*, *Shipleya* and *Gyrocoelia* into a new family, Dioecocestidae. Yamaguti (1959) recognized the subfamily Gyrocoeliinae for the genera *Gyrocoelia*, *Shipleya* and *Infula* Burt, 1939 and Tolkacheva (1979) raised the group to the family level, Gyrocoeliidae.

From its original description by Fuhrmann (1900), *D. paronai* was nevermore found from ibises. The present authors looked for helminths in two species of ibises from wetlands of Buenos Aires province (MCH, LT and CH), the white-faced ibis *Plegadis chihi* (Vieillot 1817)(n= 5) and the bared-faced ibis *Phimosus infuscatus* (Lichtenstein, 1823) (n= 2) but did not found any species of tapeworms. Digiani (2000) examined the digenean and cestode fauna of *P. chihi* (n= 64) from several wetlands at Buenos Aires province and recorded by the first time *Hymenolepis megalops* (Nitzch in Creplin, 1829) (Cestoda, Hymenolepididae)(10.7% prevalence). Georgiev and Vaucher (2000) described *Chimaerula bonai* (Cestoda, Dilepididae) based on four specimens of *Ph. infuscatus* from Paraguay (100% prevalence). Martínez-Haro *et al.* (2012) recorded one individual of *Cyclustera ralli* (Underwood et Dronen, 1986) Bona 1994 (Cestoda, Dilepididae) in one *P. chihi* from the state of Mexico. Scheer *et al.* (2019) examined the helminth assemblages of *Ph. infuscatus* from Southern Brazil, from twenty-eight birds and cited the presence of *Megalacanthus* sp. (Cestoda, Dilepididae) (89.28% prevalence). None of the mentioned authors recorded *D. paronai* in ibises. This fact, along to the present finding in 32 of 38 *R. rolland* (82.5% prevalence) suggest that the white-tufted grebe is the natural host in Argentina. Storer (2000, p. 20) emphasized that *D. paronai* represents an exception in the host specificity of the members of Dioecocestidae as specialists of grebes. The present finding confirm that all dioecocestids are grebe specialists. Moreover, based on criteria about the levels of host specificity proposed by Palm & Caira (2008), *D. paronai* would be a oioxenous species, showing a high degree of specificity.

Fuhrmann supossed that the scolex unfortunately was degraded in both of the worms he examined (p.50: "*Der Scolex war bei beiden Exemplaren leider abgerissen*"). Ryzhikov & Tolkacheva (1978) on the basis of the scolex morphology divided the genus *Dioecocestus* into four groups and considered *D. paronai* belonging to the third one: "scolex without rostellum or suckers". Our observations shown that really *D. paronai* has four rudimentary suckers and so, it should be grouped into the fourth morphological group: "scolex with suckers but no rostellum", along *D. cablei* from *T. ruficollis* in India.

Few measurements were recorded in Furhmann´s original description. The tapeworms of *R. rolland* were larger than the Fuhrmann's specimens (110 mm vs 70mm –males- and 140mm vs 60 mm –females-) . However the worms described here agree in general terms with the original description. The presence of a rudimentary scolex without rostellum and hooks, arrangement of the testes into a central mass, not divided in two fields, absence of vagina opening, inner muscle bundles well developed in two longitudinal layers, the structure of the cirrus sac and the cirrus are similiar in our material, all these features allow us to confirm the specific status.

In respect to the distribution of *D. paronai* within the host population, both the coefficient of dispersion and the contagion parameter showed a regular or underdispersed pattern (Morales & Pino,

1987). Underdispersed frecuency distributions are uncommon among helminth parasites (Esch & Fernandez, 2013). Have been proposed four possible causes to explain this fenomenon. Among them it could be that parasite recruitment rates are equal to death rates. As we pointed above, several tapeworms were retained in *postmortem* condition suggesting that the worms died and degraded into the gut. Here, both the immune response of the grebes and strong intraspecific competition between worms may occur to prevent further infections.

The present study reports the parasitism of *D. paronai* in *R. rolland* by the first time and confirm the host specificity of all members in Dioecocestidae by grebes.

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