

## Morphological and molecular data for *Leucochloridium* (*Papilloleucochloridium*) *pulchrum* (Trematoda: Leucochloridiidae) recorded for the first time in Argentina

## Datos morfológicos y moleculares de *Leucochloridium* (*Papilloleucochloridium*) *pulchrum* (Trematoda: Leucochloridiidae), registrado por primera vez en la Argentina

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**ABSTRACT:** The members of the genus *Leucochloridium* (Trematoda, Leucochloridiidae) are cosmopolitan parasites from both the cloaca and bursa of Fabricius of birds, mainly Passeriformes. In South America three species of this genus have been reported: *Leucochloridium* (*Neoleucochloridium*) *flavum* Travassos, 1922 from Brazil and Venezuela, *L. (Leucochloridium) parcum* Travassos, 1922 and *L. (Papilloleucochloridium) pulchrum* Fernandes, 1970 from Brazil. No molecular data from leucochloridiids in the Southern hemisphere have been published so far. The aim of this work is to increase the knowledge of the helminth diversity of *Jacana jacana* in Argentina with a combined morphological and molecular approach. Two specimens of *J. jacana* were captured in Formosa province, Argentina, and nine digeneans were recovered from the cloaca of one of them. DNA was extracted and the barcode region of cytochrome c oxidase 1 (CO1) was sequenced for two specimens. Digeneans were identified as *L. (P.) pulchrum* through morphological study. These specimens differed from the original description of *L. (P.) pulchrum* by the smaller size of testes and the presence of minute spines in the anterior region of the tegument. The most similar CO1 sequences on GenBank are those of *Urogonimus macrostomus* (Leucochloridiidae) from *Passer domesticus* in western Canada which differed by a 21% from *L. (P.) pulchrum*. Our study expands the geographic range of Neotropical *Leucochloridium* species with both morphological and molecular data that will be useful for future works on regional diversity and life cycles. This finding constitutes the second report of this species since its original description, and the first record of the genus *Leucochloridium* for Argentina.

**Keywords:** Birds, Cytochrome c oxidase 1, Digenea, Helminths, Jacanidae.

**RESUMEN:** Los miembros del género *Leucochloridium* (Trematoda, Leucochloridiidae) son parásitos cosmopolitas de la cloaca y bolsa de Fabricio de aves, principalmente Paseriformes. En Sudamérica se han reportado tres especies de este género: *Leucochloridium* (*Neoleucochloridium*) *flavum* Travassos, 1922 en Brasil y Venezuela, *L. (Leucochloridium) parcum* Travassos, 1922 y *L. (Papilloleucochloridium) pulchrum* Fernandes, 1970 en Brasil. Hasta el momento, no se han realizado trabajos con enfoques moleculares sobre los leucochlorididos en el hemisferio sur. El objetivo de este trabajo es ampliar el conocimiento de la diversidad de los helmintos de *Jacana jacana*, con nuevos datos morfológicos, geográficos y moleculares de *L. (P.) pulchrum*. Dos especímenes de *J. jacana* fueron capturados en la provincia de Formosa, Argentina, disecados en el campo y nueve digeneos fueron recuperados de la cloaca de uno de ellos. En dos digeneos se extrajo ADN y se generó la secuencia de código de barras de citocromo c oxidasa 1 (CO1). Los digeneos se identificaron como *L. (P.) pulchrum* por sus caracteres morfológicos. A diferencia de la descripción original, estos especímenes tienen testículos más pequeños y tegumento con pequeñas espinas en la región anterior. Las secuencias más similares de CO1 en GenBank son las de *Urogonimus macrostomus* (Leucochloridiidae) de *Passer domesticus* en el oeste de Canadá, que difieren en un 21% de *L. (P.) pulchrum*. Estos datos amplían el rango geográfico de especies neotropicales de *Leucochloridium* y serán útiles para futuros trabajos sobre diversidad regional y ciclos de vida. Este hallazgo constituye el segundo registro de la especie desde su descripción original y el primer registro del género *Leucochloridium* para Argentina.

**Palabras clave:** Aves, Citocromo c oxidasa 1, Digenea, Helmintos, Jacanidae

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## INTRODUCTION

Members of the cosmopolitan genus *Leucochloridium* (Trematoda, Leucochloridiidae) are parasites from both the cloaca and bursa of Fabricius of birds, mainly Passeriformes (Pojmańska, 2002). Members of this genus have long attracted wide attention because of the strange presentation and action of their larval broodsacs in land snails of the family Succineidae (known as amber snails, having a thin and fragile shell) (Moore, 2013). The pulsating and colorful broodsac in the snail's eyestalk seems to be a caterpillar mimic for attracting insectivorous birds, and the infection is associated also with changes in snail behaviour that may enhance predation by birds. Transmission occurs when birds prey on the broodsacs containing many metacercariae (Nakao *et al.*, 2019).

In South America three species of this genus have been reported: *Leucochloridium* (*Neoleucochloridium*) *flavum* Travassos, 1922, parasitizing the cloaca of jacanid, rallid, and ardeid birds from Brazil and Venezuela, *L. (Leucochloridium) parcum* Travassos, 1922 in the cloaca from passeriform birds, and *L. (Papilloleucochloridium) pulchrum* Fernandes, 1970 in the cloaca of jacanid birds from Brazil (Fernandes *et al.*, 2015). Only two leucochloridiids are known from Argentina: *Pojmanskia riosae* Zamparo, Brooks and Causey, 2003, found in the cloaca of the great antshrike *Taraba major* (Vieillot) (Passeriformes: Thamnophilidae) in Pirané, Formosa province (Lunaschi *et al.*, 2014), and *Urotocus fusiformis* McIntosh, 1935, parasitizing the rectum of the huet huet *Pteroptochos tarnii* (King) (Passeriformes: Rhinocryptidae) from the Nahuel Huapí National Park, Río Negro province (Flores *et al.*, 2003). Leucochloridiids in the Northern hemisphere have been repeatedly studied with integrated molecular and morphological approaches (Casey *et al.*, 2003; Iwaki *et al.*, 2009; Locke *et al.*, 2012; Rząd *et al.*, 2014; Heneberg *et al.*, 2016; Kim *et al.*, 2019; Nakao *et al.*, 2019), but such work has not yet begun in the Southern hemisphere.

The aim of this work is to increase the knowledge of the helminth diversity of the wattled jacana, *Jacana jacana* (Linnaeus) (Charadriiformes: Jacanidae), with new morphological, geographical, and molecular data from *L. (P.) pulchrum*.

## MATERIALS AND METHODS

Two specimens of *Jacana jacana* were collected at La Marcela farm (26° 17'35''S, 59° 08'38''W), Pirané, Formosa province, Argentina, with an authorization from the Ministerio de la Producción y Ambiente of Formosa province. Birds were killed with a shotgun, dissected in the field, and their viscera were analysed immediately after capture. Digeneans were removed and preserved in 70% ethanol. In the laboratory the specimens were stained with hydrochloric carmine,

dehydrated, cleared in xylene, and mounted in Canada balsam. Measurements are given in micrometres (µm), as range followed by mean in parentheses. Vouchers of parasites were deposited in the Helminthological Collection from the Museo de La Plata (MLP-He), La Plata, Argentina. The DNA was extracted from two digenean specimens recovered from a single host, such as in Ivanova *et al.* (2006). The barcode sequence of CO1 was generated using the PCR protocols and MplatCOX1d primers of Moszczyńska *et al.* (2009) from the Canadian Centre for DNA Barcoding (CCDB, Guelph, Ontario, Canada). Electropherograms were aligned in the Geneious Prime 2019 2.3 (www.geneious.com), and contigs were compared with the published data using BLAST (Altschul *et al.* 1990) through separate alignments in MEGA X (Kumar *et al.*, 2018) with sequences from leucochloridiids found in literature searches.

## RESULTS

The examination of two *Jacana jacana* individuals revealed the presence of nine digeneans in the cloaca of one of them.

*Leucochloridium (Papilloleucochloridium) pulchrum* (Fig. 1, Table 1)

Description (based on four specimens): Body oval with rounded anterior end, tapered posterior end. Ratio of body length to body width 1: 1.75 - 2.02 (1.89). Tegument covered with minute spines, extending from anterior end to acetabular region. Oral sucker well developed, large, subterminal, and rounded. Ventral sucker equatorial, smaller than oral sucker. Sucker width ratio 1: 0.8 - 0.94 (0.86). Prepharynx absent. Pharynx well developed, contiguous or overlapping oral sucker. Oesophagus absent. Intestinal bifurcation in pharyngeal zone, caeca long, extending to 203-266 (232) from posterior end. Genital pore median, dorso-subterminal. Cirrus pouch posttesticular. Gonads rounded, intercaecal, arranged in triangle, situated in the posterior third of body. Anterior testis dextral; posterior testis submedian; ovary intertesticular. Vitellarium in two lateral fields, extending from pharyngeal level to posterior end; small follicles mainly extracaecal. Uterus forming numerous coils, ascending to level of caecal bifurcation, traversing body laterally between pharynx and ventral sucker, and descending to genital pore, occupying entire intercaecal area, sometimes extending beyond caeca. Eggs small, numerous. Excretory vesicle and excretory pore not observed.



**Figure 1.** *Leucochloridium (Papilloleucochloridium) pulchrum*, entire worm, ventral view. Scale bar = 500  $\mu$ m.

**Table 1. Morphometric data for *Leucochloridium (Papilloleucochloridium) pulchrum* from Neotropical birds.**

Host	<i>Jacana jacana</i>	<i>Jacana jacana jacana</i>
Country	Argentina	Brazil
Source	Present study	Fernandes (1970)
Body length	2371 - 2714 (2600)	2460 - 3240
Body width	1286 - 1514 (1378)	1100 - 1520
Forebody	971 - 1171 (1100)	---
Oral sucker	687 - 943 x 812 - 909 (797 x 858)	740 - 1000 x 760 - 940
Ventral sucker	609 - 783 x 687 - 803 (706 x 735)	580 - 840 x 600 - 860
Pharynx	179 - 213 x 242 - 266 (198 x 256)	160 - 220 x 200 - 280*
Ovary	130 - 135 x 174 - 193 (133 x 183)	80 - 140 x 160 - 200
Anterior Testis	222 x 208 - 266 (237)	540 - 640 x 330 - 450
Posterior testis	131 - 217 x 232 - 242 (174 x 237)	460 - 680 x 360 - 430
Eggs	21 - 26 x 14 - 17 (24 x 15)	20 - 25 x 15 - 20

\*width given as 2280 by Fernandes (1970)

Measurements correspond to length x width. Range followed by mean in parentheses.

#### Taxonomic summary

Host: *Jacana jacana* (Linnaeus) (Charadriiformes: Jacanidae).

Site of infection: cloaca.

Locality: La Marcela farm (26° 17' 35" S, 59° 08' 38" W), Pirané, Formosa province, Argentina.

Deposited material: MLP-He 7521 (four specimens).

Distribution and hosts: This species was also found parasitizing *Jacana jacana jacana* (L.) [cited as *Jacana spinosa jacana* (L.)] from Soóretama, Espírito Santo state (type locality) and Salobra, Mato Grosso state, Brazil (Fernandes, 1970).

There were no differences in the 621-bp CO1 sequences obtained from both specimens (GenBank accessions MT456826-7). The most similar sequences on GenBank are those of *Urogonimus macrostomus* (Leucochloridiidae) from the house sparrow (*Passer domesticus*) in western Canada (Locke et al., 2012), which differed by a 21% (uncorrected p) from *L. (P.) pulchrum*.

#### DISCUSSION

The specimens here studied are morphologically similar to those described by Fernandes (1970) from Brazil differing only in having smaller testes and small spines on the anterior tegument. We noted that Pojmańska (2002) mentioned tegumental spines as features from both the family Leucochloridiidae Poche,

1907, and the subgenus *Papilloleucochloridium* Bakke, 1980.

DNA sequences of leucochloridiids generated in other studies (Casey et al., 2003; Iwaki et al., 2009; Rząd et al., 2014; Heneberg et al., 2016; Kim et al., 2019; Nakao et al., 2019) are from nuclear or mitochondrial markers such as CO1, but other than the barcode region studied herein. Thus, the molecular comparison with leucochloridiids is currently limited to the homologous CO1 data from *U. macrostomus* sampled in Canada (Locke et al., 2012).

The wattled jacana is a shorebird with a wide distribution in the Neotropical region from Panama to central Argentina (BirdLife International, 2020). Its diet is mainly composed of insects, but also crustaceans, molluscs, small fishes, and seeds (De la Peña, 2019). Examined bird stomachs in the present study contained insects, planorbid and ampullarid snails, but not succinids.

Despite its broad distribution in Argentina, the helminth fauna of the wattled jacana is scarcely known. To date, only two species of helminths are known from Argentine populations of this host, namely *Athesmia heterolecithodes* (Braun, 1899) (Digenea, Dicrocoeliidae), and *Echinostoma jacanae* Lunaschi, Drago and Núñez, 2018 (Digenea, Echinostomatidae) from the Buenos Aires province (Digiani, 2000; Lunaschi et al., 2018).

This finding constitutes the second report of *L. (P.) pulchrum* since its original description, and the first record of the genus *Leucochloridium* for Argentina. The data provided here will be a useful resource for future studies of the life cycle, distribution, and diversity of leucochloridiids in Argentina and the Neotropical region.

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