New data on the acanthocephalan *Neoechinorhynchus villoldoi* Vizcaíno, 1992 (Neoechinorhynchidae: Acanthocephala), based on specimens found in *Austrolebias bellottii* (Steindachner, 1881) (Rivulidae: Cyprinodontiformes) from Punta Indio, Argentina

Martin Miguel Montes,\(^1\) Jorge Barneche,\(^1\) Ignacio García,\(^2\) Sebastian Preisz\(^3\), Sergio Roberto Martorelli\(^1\)

\(^1\)Centro de Estudios Parasitológicos y Vectores (CEPAVE), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata (CCT, CONICET-UNLP), Calle 120 s/n e/ 61 y 62, La Plata, Argentina. \(^2\)Instituto de Limnología “Dr. Raúl A. Ringuelet”, CONICET, Boulevard 120 y 62, La Plata, Argentina. \(^3\)Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, Calle 120 entre 60 y 61, La Plata, Argentina.

**Corresponding author:** Martin Miguel Montes, martinmiguelmontes@gmail.com

**Abstract**

In a survey of parasites on the killfish, *Austrolebias bellottii* from Punta Indio and Magdalena, Argentina, the acanthocephalan *Neoechinorhynchus villoldoi* was found. This parasite had not been recorded since 1992 when it was described for the first time parasitizing *Corydoras paleatus* (Jenyns, 1842). The type material of *N. villoldoi* was examined, and with addition of the new specimens, the measurements range was extended. This is the first record of an acanthocephalan in the annual fish, *A. bellottii*.

**Key words**

Metazoan parasite; new record; annual fishes; pampa; temporary ponds.

**Introduction**

Data on metazoan parasites of South American annual killfish is scarce. Delgado and Garcia (2015) reported 2 larvae of *Contracaecum* spp. nematode in *Austrolebias* spp. from Uruguay, and Luque et al. (2011) mentioned the presence of the nematode *Hedruris iheringi* Pereira & Vaz, 1933 in *Austrolebias bellottii* (Steindachner, 1881) from Brazil. Due to a lack of information in Argentina, we began to search for parasites on *A. bellottii*.

Among genera of the Neotropical fish fauna, the South American killfish genus *Austrolebias* Costa, 1998 are the most numerous annual fishes distributed in southern Bolivia, southern Brazil, Paraguay, northeastern Argentina and Uruguay with 44 valid species (Costa 2010, Loueiro et al. 2011, Nielsen and Pillet 2015). The genus *Austrolebias* inhabits temporary wetlands formed during rainy periods and die when the ponds dry out (Costa 2003, Costa 2006). Most species bury their eggs in the substrate, the embryos survive through dry periods in a diapause inside the resistant eggs and hatch at the beginning of the
The fishes grow fast, mostly in the first months of their life, reach the sexual maturity early and begin to spawn (Walford and Liu 1965, Gonzalves et al. 2011).

The short life cycle, the ecological importance, the aquarist interest of this fish, and the scant information on metazoan parasites in killifishes moved us to study the acanthocephalan *Neoechinorhynchus villoldoi* Vizcaíno, 1992. Based on reexamination of the type material from

Corydoras paleatus (Jenyns, 1842) and the new specimens found parasitizing *A. bellottii*, we extend the range of measurements of this parasite. This is the first record of *N. villoldoi* on *A. bellottii*.

**Methods**

Fishes (Figs 1, 2) were sampled between October and November 2015 from 2 temporary puddles separated from each other by 38 km (Fig. 3). The puddles (Figs 4, 5) were selected randomly and named: site 1 (Punta Indio, 35°18.932’ S, 057°13.176’ W) and site 2 (Magdalena, 35° 04.865’ S, 057°31.627’ W). The collections were made with a frame of 1 × 0.50 m and a net-mesh of 0.5 × 0.5 cm. The specimens were transported to the lab in plastic bags with oxygenated water from the collection sites. The fishes were sacrificed by medullar section. The viscera were examined for parasites under a binocular microscope. The acanthocephalans found were cleared as temporary mounts with Lacto-phenol or stained with dehydrated chlorhidric carmin mounted in Canada balsam, and studied under an Olympus Bx51 microscope (Tokyo, Japan). The drawings were made with a drawing tube. The structures were photographed with an AmScope MU 1000 10 MP digital camera (USA) attached to the microscope and measured using ImageJ software (Schneider et al. 2012). The mean followed by minimum and maximum values in parenthesis are given in micrometers (µm), unless stated otherwise. Figures 1 and 2 were photographed with a Canon 7D camera with a Canon 100 mm 2.8 macro lens and Figures 4 and 5 with a
Montes et al. | Neoechinorhynchus villoldoi parasitizing Austrolebias bellottii

Canon 50 mm 1.8 Macro lens. The vouchers were deposited in the Helminthological Collection of the Museo de La Plata, Argentina, under the number MLP-He 7150.

Examined material included all the type series of Neoechinorhynchus villoldoi, 1537 C, 1539 C, 1541-44 C and 1547-50 C, in the Helminthological Collection of Museo de la Plata.

Results

The prevalence, median intensity and abundance of N. villoldoi from A. bellottii are provided in Table 1.

Description of N. villoldoi found in A. bellottii. Small, cylindrical, with gigantic hypodermic nuclei 1 or 2 dorsal and 1–3 ventral (not drawn), posterior end rounded. Genital pore subterminal in both sexes. Small proboscis, cylindrical, 18 hooks in 3 circles of 6 hooks each. First 2 circles with similar hooks lengths; third circle of hooks significantly smaller (Figs 6–9). Nerve ganglion posterior to the receptacle of the proboscis. Lemnisci short, sub-equal, barely longer than the receptacle of the proboscis.

Male (Figs 10, 11) shorter than female, reproductive system consisting of 2 contiguous testicles, cement gland syncytial, rounded reservoir of cement, elongated Säfftigen’s pouch. Copulatory pouch spade-shaped (Figs 12, 13).

Table 1. Number of specimens of Austrolebias bellottii examined (N). Prevalence of infection, mean intensity and mean abundance of Neoechinorhynchus villoldoi in sample sites.

<table>
<thead>
<tr>
<th></th>
<th>Prevalence of infection</th>
<th>Mean intensity</th>
<th>Mean abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punta Indio (sample site 1)</td>
<td>10</td>
<td>80%</td>
<td>3.13</td>
</tr>
<tr>
<td>Madariaga (sample site 2)</td>
<td>22</td>
<td>0%</td>
<td>0</td>
</tr>
</tbody>
</table>

Figures 6–9. Proboscis of Neoechinorhynchus villoldoi found in Austrolebias bellottii. 6, 7. Male proboscis. 8, 9. Female proboscis; 9, stained with chloridric carmin. Scale bars: Figs 6, 7, 9 = 20 µm, Fig. 8 = 16 µm.
type locality or (b) the similarity in the host feeding habits. Site 1 at Punta Indio is 7 km away from Villoldo stream (the type locality of *N. villoldoi*) but 35 km from site 2. The distance between sites could be one of the reasons for the absence of this parasite in fishes from Magdalena (site 1).

*Corydoras paleatus* (the type host) and *A. bellottii* are fishes with different habitat but perhaps they prey over the same food item. The diets of both species are represented mainly by microcrustaceans (Cladocera, Copepoda, and Ostracoda), and immature aquatic insects (Chironomidae) (Escalante 1983, Grosman 2002, Laufer 2009, Keppeler 2014). Better knowledge of the life cycle of *N. villoldoi* could clarify if this parasite uses 1, 2 or more intermediate hosts, and if both fishes prey over the same or different intermediate host?"

Knowledge of the parasite fauna of *A. bellottii* is important because this fish forms a link between micro- and macroecosystems. Killifishes mainly feed on microcrustaceans and immature aquatic insects, and killifishes serve as food for aquatic birds (Keppeler et al. 2016). They are also important fishes for aquarists due to the high number of species, bright colors, size and the relatively easy breeding in captivity.

There are many species of *Austrolebias*, and the genus has a wide distribution, and therefore we suspect that our record *N. villoldoi* in *A. bellottii* is only the first of a several new records (or new species) of adult metazoan parasites and larval stages waiting for to be discovered in these “killis”.

Acknowledgements

We thank Monica Casciaro and Monica Rodriguez of the Dirección y Subdirección de Flora y Fauna de la Provincia de Buenos Aires for the permits to collect fishes, Samanta Faiad of the Departamento de Dibujo e Ilustración Científica del Museo de La Plata for the line drawings, Marcos Gastón Cavallo for helping in the sample collection, Cristian Rodriguez for the construction of the fishing gear, and Cecilia Gabellone and Carlos Romero for reading the manuscript and helping to translate it to English. We also want to thank to the Consejo Nacional de Ciencia y Tecnología (CONICET) for financial support of this research.

Authors’ Contributions

MM identified the acanthocephalan, measured them, and wrote the text. JB collected the fishes and parasites and stained the acanthocephalan. IG collected the samples, determined the fishes, and wrote the text (the part on the fishes). SP found the sample site, collected the samples, and photographed the male and female of *A. bellottii* and sample sites. SM identified the acanthocephalan, wrote the text.
Figures 14–18. Female reproductive of *Neoechinorhynchus villaldi* found in *Austrolebias bellottii* and eggs. 14, 15 Juvenile female. 16, 17. Reproductive female. 18. Eggs. Abbreviations: CR = cement reservoir, SP = Säfftigen's pouch. Scale bars: Figs 14–15, 17 = 40 µm, Fig. 16 = 50 µm, Fig. 18 = 8 µm
Table 2. Comparison of *Neoechinorhynchus villoldoi* measurements between males and females of *Austrolebias bellottii* (present study), original description (Vizcaino 1992) and type material. Abbreviations: Max = maximum length, W = width, * not measured, ** material in poor stage of conservation.

<table>
<thead>
<tr>
<th>Host</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present study</td>
<td>Vizcaino 1992</td>
</tr>
<tr>
<td>A. bellottii</td>
<td>1322 (1043–2897)</td>
<td>2071 (895–6013)</td>
</tr>
<tr>
<td>Anterior circle of hooks</td>
<td>5 (4–5)</td>
<td>24 (23–26)</td>
</tr>
<tr>
<td>Middle circle of hooks</td>
<td>5 (4–5)</td>
<td>25 (16–27)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>Total length</th>
<th>Max. width</th>
<th>Proboscis</th>
<th>Anterior circle of hooks</th>
<th>Middle circle of hooks</th>
<th>Posterior circle of hooks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>1322</td>
<td>2071</td>
<td>5 (8–5)</td>
<td>6 (5–8)</td>
<td>5 (5–6)</td>
<td>5 (5–8)</td>
</tr>
</tbody>
</table>

References


