



## Neotropical Helminthology



RESEARCH NOTE / NOTA CIENTÍFICA


### NEW FRESHWATER FISH HOSTS OF GORDIID (NEMATOMORPHA) LARVAE IN THE SOUTH OF CHILE

### NUEVOS PECES DULCEACUICOLAS HOSPEDEROS DE LARVAS DE GORDIACEOS (NEMATOMORPHA) EN EL SUR DE CHILE

Patricio Torres-Hevia<sup>1\*</sup>

Instituto de Parasitología, Facultad de Medicina, Edificio de Ciencias Biomédicas, Isla Teja, Universidad Austral de Chile, Valdivia, Chile

\*Corresponding author: ptorres@uach.cl

Patricio Torres-Hevia:  <https://orcid.org/0000-0002-9882-0183>

## ABSTRACT

Nematomorpha Vejdovsky, 1886 includes most of its species in the taxon Gordiida, presenting a freshwater free-living adult and a parasitic larval stage that live in some aquatic invertebrates, amphibians and fish. Present results documented morphological characteristics of unencysted and encysted larvae of gordiids in the intestines of two new freshwater fish hosts, *Aplochiton zebra* Jenyns, 1842 (Osmeriformes, Galaxiidae) and *Trichomycterus areolatus* Valenciennes, 1846 (Siluriformes: Trichomycteridae), in the south of Chile.

**Keywords:** *Aplochiton* – Chile – fish – Nematomorpha – parasites – *Trichomycterus*

## RESUMEN

Nematomorpha Vejdovsky, 1886 incluye la mayoría de sus especies en el taxón Gordiida, incluyendo un estado adulto de vida libre dulceacuícola y un estado larvario parasítico que vive en algunos invertebrados acuáticos, anfibios y peces. Los resultados del presente estudio documentan las características morfológicas de larvas enquistadas y no enquistadas de gordiáceos en dos nuevos peces hospederos dulceacuícolas, *Aplochiton zebra* Jenyns, 1842 (Osmeriformes, Galaxiidae) y *Trichomycterus areolatus* Valenciennes, 1846 (Siluriformes, Trichomycteridae), en el sur de Chile.

**Palabras clave:** *Aplochiton* – Chile – Nematomorpha – parásitos – peces – *Trichomycterus*

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## INTRODUCTION

The Phylum Nematomorpha Vejdovsky, 1886 includes around 350 species distributed in the Gordiida, with freshwater species, and Nectonematida taxa with five marine species (Hanelt *et al.*, 2005; Szmygiel *et al.*, 2014). Gordiida species, commonly called horsehair worms, in their adult stage reproduce in freshwater environments where they lay eggs that develop into larvae (Hanelt *et al.*, 2005). The larvae hatch and establish as parasites after being ingested by paratenic hosts, including aquatic insects (Coleoptera, Diptera, Trichoptera and Ephemeroptera), snails, annelids, crustaceans (amphipods and copepods), amphibians and fish. In these hosts, larvae secrete a cystic wall when located in their tissues or body cavity (Poinar & Doelman, 1974; Hanelt & Janovy, 2003; Hanelt *et al.*, 2012). Aquatic insects predated by terrestrial arthropods (orthopterans, coleopterans and mantids) are infected by the larval stage. As they develop into their adult stage, host behavior is manipulated by the parasite so that they look for and jumps into water; here, the parasite abandon their host to mate (Bolek & Coggins, 2002; Hanelt & Janovy, 2003; Hanelt *et al.*, 2012; Barquin *et al.*, 2015).

The records of encysted or unencysted gordiids in fish are scarce and are related to natural infections by *G. aquaticus* in *Misgurnus fossilis* (Linnaeus, 1758), *Lampetra fluviatilis* (Linnaeus, 1758), *Lampetra planeri* (Bloch, 1784), *Thymallus thymallus* (Linnaeus, 1758), *Phoxinus phoxinus* (Linnaeus, 1758), and *Salmo* sp. (Villot, 1881; Von Linstow, 1898; Malmqvist & Moravec, 1978), as well as infections by *Gordius* sp. in *Barbatula barbatula* (Linnaeus, 1758) (syn. *Barbatulus barbatulus*), *Rutilus rutilus* (Linnaeus, 1758), *Leuciscus idus* (Linnaeus, 1758), and *Carassius carassius* (Linnaeus, 1758) (Zhokhov & Molodozhnikova, 2008), in Europe. Additionally, the experimental infection by *Gordius robustus* Leidy, 1851, *P. varius* and *Chordodes morgani* Montgomery, 1898 has been verified in *Notropis stramineus* (Cope, 1865) (syn. *Notropis ludibundus*) in the USA (Hanelt & Janovy, 2003). Natural infections by unidentified gordiid cysts was identified in *Galaxias vulgaris* Stokell, 1949 and *Gobiomorphus breviceps* (Stokell, 1939) in

New Zealand (Blair, 1983), and in *Galaxias maculatus* (Jenyns, 1842) in Chile (Torres *et al.*, 2017).

The objective of this research note is to report and describe the morphology of gordiid larvae in two new fish hosts in the Lingue River and Riñihue Lake, in the south of Chile.

## MATERIAL AND METHODS

During January 2014 and 2015, 12 fish were collected distributed in four *Aplochiton zebra* Jenyns, 1842 (Osmeriformes, Galaxiidae) [three in the Lingue River (39°26'60''S; 73°13'60''W): one female and two with undetermined sex (US), with 9.3-10.6 cm of body length (BL) and 1 female with 10.8 cm BL in the Riñihue Lake (39°50'36''S; 72°17'39''W)], and eight *Trichomycterus areolatus* Valenciennes, 1846 (Siluriformes: Trichomycteridae) [three females in the Lingue River with 6.0-6.9 cm BL and two females and 3 males in the Riñihue Lake with 4.5-6.7 cm BL]. All the fish were caught, with authorization from the Subsecretaria de Pesca y Acuicultura (Resolution No. 2430/2013), by electric fishing and taken alive to the Institute of Parasitology, kept in aquariums at 4°C until dissection and examination within 48 hr after its capture.

Gordiids were searched in the stomach, intestines, liver, spleen and gonads. The stomach and intestines were opened longitudinally and cut into 2-3 cm long sections. With the contents of the stomach and intestines of each section, preparations were made between slides and coverslips and observing in a light microscope with 40X and 100X. Subsequently, each section was placed between slides and coverslips adding saline solution with slight compression, examining with a light microscope. The other organs were minced and then made preparations between slides and coverslips with slight compression, observing under the microscope. Number of encysted and unencysted larvae of gordiids was determined. The morphological characteristics registered correspond to those proposed by Hanelt & Janovy (2002). Voucher specimens were deposited in the Collection of the Museo de Zoología, Universidad

de Concepción, Chile.

**Ethic aspects:** The research adhered to ethical aimed at reducing the minimum possible damage to the animals under study as well as the application of biosafety standards in field and laboratory work.

## RESULTS AND DISCUSSION

The number of infected / examined fish (range of parasite number / infected fish) was as follows for each host and locality: *A. zebra*: Lingue River 1/1 (280 unencysted larvae), Riñihue Lake 2/3 (7-11 encysted larvae); *T. areolatus*: Lingue River 2/3 (7-12 encysted larvae), Riñihue Lake 5/5 (1-122 encysted and unencysted larvae). All gordiids were alive in the intestines of the hosts. Table 1 and Fig. 1 show the morphological characteristics of encysted and unencysted larvae.

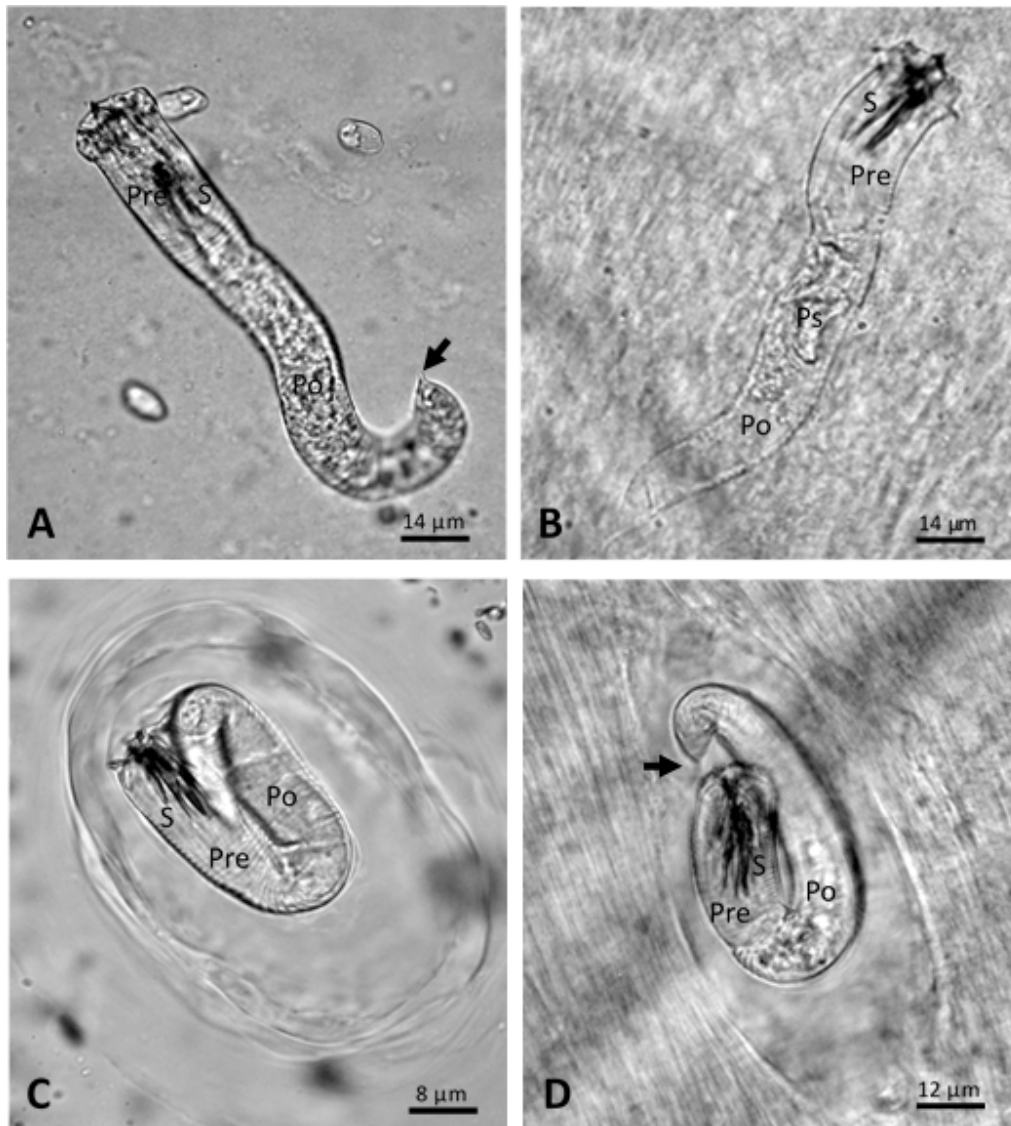
In other studies, in *G. vulgaris* and *G. cotidianus*, encysted larvae have been recorded in the intestines, esophagus, stomach, and liver (Blair,

1983), in *T. thymallus* and an unidentified species of *Salmo* (Villot, 1881) and *N. ludibundus* (Hanelt & Janovy, 2003) in intestines; and in *G. maculatus* in intestines and liver (Torres *et al.*, 2017). For *Gordius robustus*, *P. varius* and *C. morgani* in experimental infections of *N. stramineus*, the encysted larvae were found in the intestines, observing a similar response from the host, including the formation of granulomas around the cysts, which in some cases could destroy them (Hanelt & Janovy, 2003). In *G. maculatus*, the presence of inflammatory infiltrate associated with gordiid encysted larvae was not observed, however, although most of them were viable, a smaller proportion showed dead larvae (Torres *et al.*, 2017).

The characteristics of unencysted larvae (measurements of the preseptum and postseptum, the absence of longer outer hooks on the outer ring of the preseptum, and the presence of only one terminal spine in the postseptum) and encysted ones (larvae folded twice without spines on the preseptum and encysted larvae length) in both hosts (Table 1) suggest they correspond to the *Gordius* genus according to the descriptions of the

**Table 1.** Morphological characteristics (mean and range in  $\mu\text{m}$ ) of larvae and cysts of gordiids in *Aplocheilichthys zebra* Jenyns, 1842 and *Trichomycterus areolatus* Valenciennes, 1846 from Lingue River<sup>1</sup> and Riñihue Lake<sup>2</sup> in the south of Chile.

Characteristics	<i>A. zebra</i> <sup>1</sup>	<i>A. zebra</i> <sup>2</sup>	<i>T. areolatus</i> <sup>1</sup>	<i>T. areolatus</i> <sup>2</sup>
Unencysted larvae	n = 5	n = 8		n = 7
Preseptum length	39.5 (30-45)	44.7 (16-59)		35.2 (19-44)
Preseptum width	17.8 (14-20)	17.0 (11-22)		16.9 (11-21)
Postseptum length	100.4 (62-121)	97.2 (19-161)		83.1 (45-110)
Postseptum width	16.2 (14-18)	14.9 (9-20)		16.3 (9-21)
Stylet length	21.9 (17-31)	23.6 (11-35)		17.4 (10-21)
Stylet width	6.9 (5-9)	10.6 (9-14)		4.9 (4-7)
Pseudointestine length	28.5 (18-39)	31.2 (18-46)		30.9 (20-38)
Pseudointestine width	19.8 (12-27)	10.6 (9-14)		9.8 (8-12)
Encyst larvae			n = 6	n = 5
Total length			52.5 (40-70)	66.5 (49-98)
Total width			41.7 (30-55)	26.1 (19-41)
Cyst wall length			13.1 (9-18)	14.3 (8-22)
Cyst wall width			13.8 (9-19)	3.2 (2-6)
Cyst larva length			26.5 (21-35)	37.9 (28-54)
Cyst larva width			14.1 (10-18)	19.8 (15-28)
Cyst larval folding			2	2



**Figure 1.** A. Unencysted larva of gordiid found in the intestine of *Aplochiton zebra* from Lingue River (arrow: terminal spine). B. Unencysted larva of gordiid found in the intestine of *A. zebra* from Riñihue Lake. C. Encysted larva found in the intestine of *Trichomycterus areolatus* from Lingue River. D. Encysted larva found in the intestine of *T. areolatus* from Riñihue Lake (arrow: terminal spine). Abbreviations: Pre = pre-septum. Po = post-septum. Ps = pseudointestine. S = stylet.

unencysted and encysted *Gordius* spp. larvae in the northern hemisphere (Hanel & Janovy, 2002; Szmygiel *et al.*, 2014; Harkins *et al.*, 2016). Previously, in the Valdivia River basin, where the Riñihue Lake is located, a female of *Gordius* genus was registered, which could correspond to *G. robustus* (Schmidt-Rhaesa, 2016).

The new hosts of gordiids consume different prey through which they could acquire the infection, for example, *A. zebra* is a consumer of chironomids,

Ephemeroptera or Trichoptera while *T. areolatus* consumes gastropods, oligochaetes, small crustaceans and insects, such as Ephemeroptera (Ruiz & Marchant, 2004), all groups registered as paratenic hosts (Poinar & Doelman, 1974; Hanelt & Janovy, 2003; Hanelt *et al.*, 2012).

In Chile, nematomorphs have been scarcely investigated, describing only seven species in their adult stage, among them *Beatogordius latastei* (Camerano, 1895), *Neochordodes talensis*

(Camerano, 1897), *Neochordodes meridionalis* (Carvalho & Feio, 1950), *Gordius paranensis* Camerano, 1892, *Gordius austrinus* Villalobos, Zanca & Ibarra-Vidal, 2005, *G. robustus* Leidy, 1851, and *Gordionus enigmaticus* Villalobos, Zanca & Ibarra-Vidal, 2005 (De Villalobos *et al.*, 2005).

Up to day, life cycles of the described species are unknown.

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## BIBLIOGRAPHIC REFERENCES

- Barquin, A, McGehee, B, Sedam, RT, Gordy WL, Hanelt, B, & Wise de Valdez, MR. 2015. *Calling behavior of male Acheta domesticus crickets infected with Paragordius varius (Nematomorpha: Gordiida)*. Journal of Parasitology, vol. 101, pp. 393-397.
- Blair, D. 1983. *Larval horsehair worms (Nematomorpha) from the tissues of native freshwater fish in New Zealand*. New Zealand Journal of Zoology, vol. 10, pp. 341-343.
- Bolek, MG & Coggins JR. 2002. *Seasonal occurrence, morphology, and observations on the life history of Gordius difficilis (Nematomorpha: Gordioidea) from southeastern Wisconsin, U.S.A.* Journal of Parasitology, vol. 88, pp. 287-294.
- De Villalobos, C, Zanca, F & Ibarra-Vidal H. 2005. *Redescription and new records of freshwater Nematomorpha (Gordiida) from Chile, with the description of two new species*. Revista Chilena de Historia Natural, vol. 78, pp. 673-686.
- Hanelt, B & Janovy, J. 2002. *Morphometric analysis of nonadult characters of common species of American gordiids (Nematomorpha: Gordioidea)*. Journal of Parasitology, vol. 88, pp. 557-562.
- Hanelt, B & Janovy, J. 2003. *Spanning the gap: identification of natural paratenic hosts of horsehair worms (Nematomorpha: Gordioidea) by experimental determination of paratenic host specificity*. Invertebrate Biology, vol. 122, pp. 12-18.
- Hanelt, B, Thomas, F & Schmidt-Rhaesa, A. 2005. *Biology of the Phylum Nematomorpha*. Advances in Parasitology, vol. 59, pp. 243-305.
- Hanelt, B, Bolek MG & Schmidt-Rhaesa, A. 2012. *Going solo: discovery of the first parthenogenetic gordiid (Nematomorpha: Gordiida)*. PLoS ONE 7e34472.
- Harkins, C, Shannon, R, Papes, M, Schmidt-Rhaesa, A, Hanelt, B & Bolek, MG. 2016. *Using gordiid cysts to discover the hidden diversity, potential distribution and new species of gordiids (Phylum Nematomorpha)*. Zootaxa, vol. 4088, pp. 515-530.
- Malmqvist, B & Moravec, F. 1978. *Gordius aquaticus larvae found in the lampreys Lampetra planeri (Bloch) and L. fluviatilis (L.)*. Folia Parasitologica, vol. 25, pp. 40.
- Poinar, GO & Doelman, JJ. 1974. *A reexamination of Neochordodes occidentalis (Montg.) comb. N. (Chordodidae: Gordioidea): larval penetration and defense reaction in Culex pipiens L.* Journal of Parasitology, vol. 60, pp. 327-335.
- Ruiz, VH & Marchant, M. 2004. *Ictiofauna de aguas continentales chilenas*. Universidad de Concepción, Concepción, Chile. 355 pp.
- Schmidt-Rhaesa, A. 2016. *The collection of Nematomorpha in the zoological museum Hamburg, including description of a new species, Chordodes jelkae sp. n.* Zoosystematics Evolution, vol. 92, pp. 211-250.
- Szmygiel, C, Schmidt-Rhaesa, A, Hanelt, B & Bolek MG. 2014. *Comparative descriptions of non-adult stages of four genera of gordiids (Phylum: Nematomorpha)*. Zootaxa, vol. 3768, pp. 101-118.
- Torres, P, Leyán, V & Lamilla, J. 2017. *Cyst stages of gordiids (Nematomorpha) and other eukaryotic parasites from the inanga,*

- Galaxias maculatus* (*Osmeriformes: Galaxiidae*), in the *Lingue River, southern Chile*. *Comparative Parasitology*, vol. 84, pp. 72-79.
- Villot, MA. 1881. *Nouvelles recherches sur l'organisation et le développement des gorgiens*. *Annales des Sciences Naturelles, Zoologie et paléontologie*, vol. 11, pp. 1-44.
- Von Linstow, OFB. 1898. *Helminthologische Beobachtungen. Zur Entwicklungsgeschichte von Gordius aquaticus Gmel.* *Archiv für Mikroskopische Anatomie*, vol. 51, pp. 747-763.
- Zhokhov, AE & Molodozhnikova NM. 2008. *Taxonomic diversity of parasites in agnathans and fishes from the Volga River Basin V. Nematoda and Gordiacea*. *Parazitologiya*, vol. 42, pp. 114-128.

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