On a New S*pecies of Hysterothylacium* (Nematoda: Anisakidae) from *Cauque mauleanum* (Pisces: Atherinidae) by Brightfield and Scanning Electron Microscopy

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Hysterothylacium geschei *n. sp. (Nematoda, Anisakidae) is described from the intestine of* Cauque mauleanum *(Steindachner) (Pisces: Atherinidae) from Lake Panguipulli (39º43'S; 72º13'W), Chile. Eleven (78.6%) out of 14 fish were infected, with a mean intensity (range) of 14.4 (1-55) worms. The new species can be differentiated from the two previously described species of freshwater fishes from South America by the presence of lateral alae, the number of caudal papillae, and the length of the spicules, oesophagus, intestinal caecum, distance vulva-anterior extremity and the length ratio intestinal caecum: ventricular appendix. From the fishes examined in Lake Panguipulli, including the introduced salmonid species* Oncorhynchus mykiss *(Walbaum) and the authochthonous species* Basilichthys australis *Eigenmann (Atherinidae) and* Percichthys trucha *(Valenciennes) (Percichthyidae), only one specimen of* P. trucha *was found parasitized by a third-stage larva of this species.*

Key words: *Hysterothylacium geschei* n. sp. - Anisakidae - Nematode - fresh water fish - *Cauque maulenaum* - Atherinidae - Chile

Three species of anisakid nematodes of the genus *Hysterothylacium* Ward & Magath, 1917 have been described in South American fishes (Moravec et al. 1997). Among them *Hysterothylacium fortalezae* (Klein, 1973) was described in marine fishes, including *Scomberomorus cavalla* (Cuvier), *Scomberomorus maculatus* Mitchill (Scombridae) and *Harengula clupleola* (Cuvier) (Clupeidae) in the coastal waters of Brazil (Vicente et al. 1985). *Hysterothylacium rhamdiae* Brizzola & Tanzola, 1995 was recovered from the pimelodid catfish *Rhamdia sapo* (Valenciennes) in streams from Buenos Aires province, Argentina (Brizzola & Tanzola 1995), and *Hystherothylacium patagonense* Moravec, Urawa & Coria, 1997 was reported in *Percichthys trucha* (Valenciennes) a Percichthyidae freshwater fish in Lake Alumine Patagonia, Argentina (Moravec et al. 1997).

In Chile *Hysterothylacium* sp. has been registered in autochthonous freshwater fishes such as the atheriniform *Cauque mauleanum* (Steindachner) and *Basilichthys australis* Eigenmann (Torres et al. 1988), *P. trucha* (Torres et al. 1992), and also in introduced salmonids including, *Salmo trutta* (L.) and *Oncorhynchus mykiss* (Walbaum) (Torres et al. 1992, Torres 1995).

In this paper a new species of *Hysterothylacium* is described in South America and is the first to be reported from Chile in the atherinid authochthonous freshwater fish *C. mauleanum*.

MATERIALS AND METHODS

During May 1995, the following species and numbers of adult fishes were caught with 10 and 20 mm mesh gillnets in Lake Panguipulli (39º43'S; 72º13'W): 13 *C. mauleanum*, 14 *B. australis*, 23 *P. trucha* and 30 *O. mykiss*. Lake Panguipulli is located in the pre-Andes mountains and its surface lies at an altitude of 140 m with a maximum depth of 268 m and is of glacial origin (Campos et al. 1981). Nematodes were removed from the intestine of the fish and rinsed in saline, fixed in 10% formalin, cleared in lactophenol for examination and preserved in 70% ethanol. Paraffin-embedded 8 µm transversal sections were stained with hematoxilin and eosin. Drawings were made with a camera lucida connected to a Nikon brightfield microscope.

Some specimens were treated as indicated by Torres et al. (1994) for examination with Scanner Nanolab 2000 (Bausch & Lomb International).

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Measurements are in micrometers unless otherwise stated and include the mean followed by the range in parentheses.

DESCRIPTION

Hysterothylacium geschei n. sp.
(Figs 1-20)

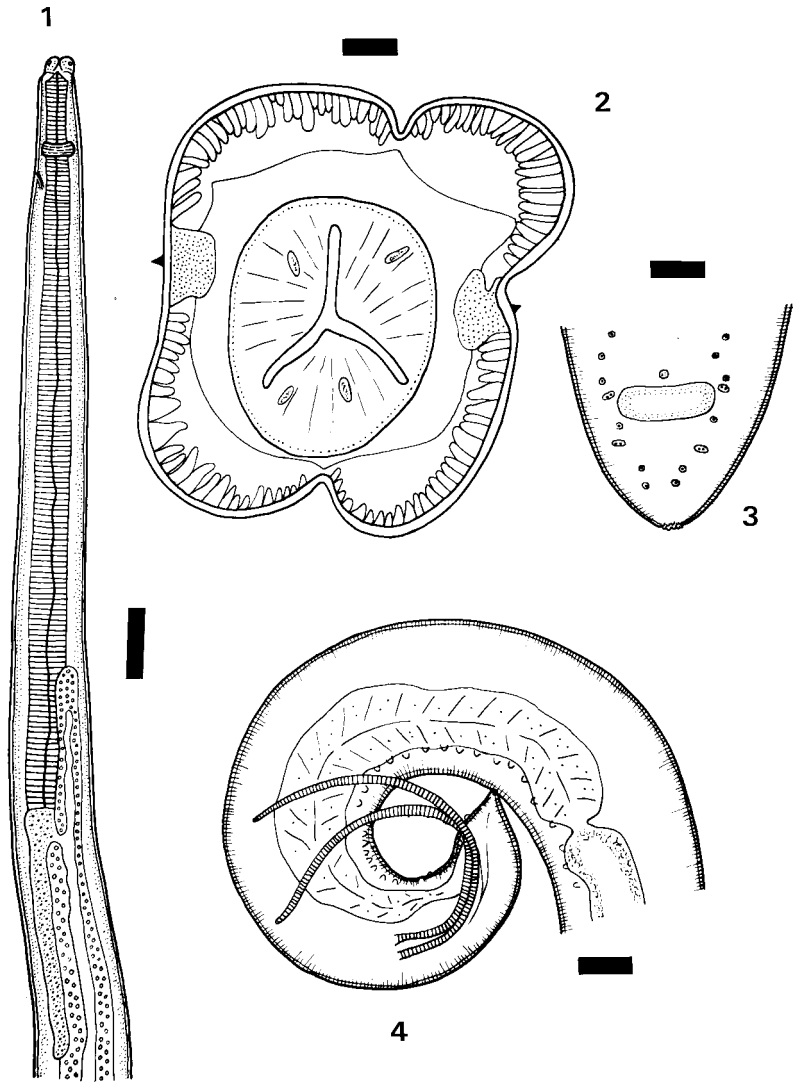
Body thickest in middle region, anteriorly and posteriorly tapering. Dorsal lip slightly wider than subventral lips. Dorsal and subventral lips slightly longer than wider. Interlabia basally wider than long measuring about 1/3 of lip length. Lateral alae very narrow, not evident anteriorly, alae grooves run from base of subventral lips and extending along body up to tail. Alae are 12-18 wide in posterior extremity and 6-8 wide at level of nerve ring. Lips similar in shape, about as long as wide. Flanges widest at posterior half of lip. Dorsal lip with two lateral doubled papillae. Subventral lips with mediolateral doubled papilla; a single lateral papilla and amphid. Excretory pore immediately posterior to nerve ring. Oesophagus 10-15% body length. Nerve ring lying between anterior 10-20% of oesophagus. Ventriculus slightly wider than narrow. Ventricular appendage 25-43% length of oesophagus. Intestinal caecum 22-45% length of oesophagus. Tail ventrally flexed with conical nodulose apex.

Female (11 mature specimens). Body 46.9 (32.3-73.2) mm long by 1.0 (0.7-1.5) mm maximum width. Dorsal lips 176 (156-220) long by 165 (145-195) maximum width; subventral lips 160 (151-184) long by 146 (130-155) wide. Interlabia 82.6 (65-93). Nerve ring 717 (601-844) from anterior end. Excretory pore 882.6 (701-1138) from anterior end. Oesophagus 5.2 (3.3-7.6) mm long by 391.3 (286-558) maximum width at posterior one third. Oesophagus 11.4 (10-15)% length of body. Ventriculus 304.0 (243-358) long by 304 (257-458) maximum wide. Ventricular appendix 1.6 (1.2-2.2) mm long by 244.5 (157-372) maximum wide. Intestinal caecum 1.7 (1.0-2.4) long by 268.0 (215-358) maximum wide. Ratio and percentage of ventricular appendix to oesophagus length 1:0.3 (1:0.2-0.4) and 31.8 (25-43)%, respectively. Length caecum: ventricular appendix 1:0.9 (1:0.6-1:1.3). Intestinal caecum 34.1 (23-45)% length of oesophagus. Vulva 13.3 (11.0-18.1) mm from anterior end; 31.2 (22-40)% length of body. Vagina muscular directed posteriorly. Vulvar area not swollen. Uteri opposed. Tail 295.0 (229-327) long with conical nodulose apex. Eggs spherical, thin walled, smooth. Eggs in proximal uterus 74 (71-85) in diameter.

Male (9 mature specimens). Body 33.8 (24.7-47.2) mm long by 0.7 (0.5-1.0) mm maximum width. Dorsal lip 169.7 (140-202) long by 177 (158-186) maximum wide; subventral lips 162.4 (145-192) long by 143.7 (134-165) wide. Interlabia 80.6 (73-91) long. Nerve ring 632 (480-744) from anterior end. Excretory pore 825 (670-972) from anterior end. Oesophagus 4.3 (3.3-5.7) mm long by 294 (229-358) maximum width at posterior one third. Oesophagus 12.8 (11-15)% length of body. Ventricule 231.8 (186-300) long by 233.7 (186-315) wide. Ventricular appendix 1.5 (1-2.1) mm long by 240 (157-343) maximum wide. Intestinal caecum 1.2 (0.9-1.7) long by 209 (143-257) maximum wide. Ratio and percentage of ventricular appendix with respect to oesophagus length 1:0.3 (1:0.3-0.4) and 34.8 (26-43)% respectively. Length ratio intestinal caecum: ventricular appendix 1:1.2 (1:0.9-1.7). Intestinal caecum 29.4 (22-39)% length of oesophagus. Ejaculatory duct 1978 (1673-2174) long by 246.7 (143-358) wide, 6 (4-8)% length of body. Spicules 1362 (758-2259) long by 35.2 (29-58) maximum wide, 67.8 (40-96)% of ejaculatory duct and 4.1 (2-5)% of body length. Tail 138.6 (114-200), conical nodulose apex. Twenty-eight to 34 pairs of pre-anal subventral papillae, 4 post-anal and one pair ad-anal. Medio ventral papilla on anterior cloacal lip.

Fourth-stage larva (30 specimens). Body 19.5 (11.7-34.3) long by 0.5 (0.3-0.7) mm maximum width. Dorsal lip 101.2 (54-163) long by 109.3 (61-152) wide; subventral lips 115.6 (61-147) long by 113 (72-140) wide. Interlabia 61.6 (46-72) long. Nerve ring 406.3 (300-586) from anterior end. Excretory pore 608.7 (407-715) from anterior end. Oesophagus 2.4 (1.6-4.8 mm) long by 203.2 (143-315) width at posterior one third. Oesophagus 12.2 (10-15)% length of body. Ventricule 168.3 (114-257) long by 172.5 (114-254) wide. Ventricular appendix 919.5 (572-1459) long by 148.3 (72-215) maximum wide. Intestinal caecum 923.8 (586-1430) long by 133.9 (72-215) maximum wide. Ratio and percentage of ventricular appendix respect to oesophagus length 1:0.4 (1:0.2-0.5) and 39.3 (24-53)% respectively. Length ratio intestinal caecum: ventricular appendix 1:1 (1:0.7-1.4). Intestinal caecum 39.2 (29-53)% length of oesophagus. Tail 150.4 (106-286) long with conical nodulose apex.

Third-stage larva (based on 16 specimens). Body 17.7 (11.3-22.2) long by 0.45 (0.3-0.5) mm maximum width. Head rounded with ventral cephalic tooth. Nerve ring 390 (295-472) from anterior end. Excretory pore 567.4 (404-624) from anterior end. Oesophagus 2.1 (1.6-2.6 mm) long by 148 (129-172) maximum width at posterior one third. Oesophagus 12.0 (10-13)% length of body. Ventricule 143.8 (86-186) long by 135.8 (90-186) wide. Ventricular appendix 977.9 (401-1230) long by 149.1 (86-186) maximum width. Intestinal caecum 855.3



Adults of *Hysterothylacium geschei* n. sp. Camera lucida drawings. Fig. 1: anterior end of male. Bar = 386 µm. Fig. 2: histological section posterior to nerve ring in a female. Bar = 30 µm. Fig. 3: tail of male, ventral view. Bar = 73 µm. Fig. 4: posteriorextremity, in lateral view. Bar = 190 µm.

(615-1144) long by 108 (86-157) maximum width. Ratio and percentage of ventricular appendix respect to oesophagus length 1:0.5 (1: 0.4-0.6) and 46.3 (38-56)% respectively. Length ratio intestinal caecum: ventricular appendix 1:1.2 (1: 0.9-1.7). Intestinal caecum 40.5 (32-49)% length of oesophagus. Tail conical with single spine 112.4 (86-143).

Host: *Cauque mauleanum* (Steindachner, 1876) (Atherinidae).

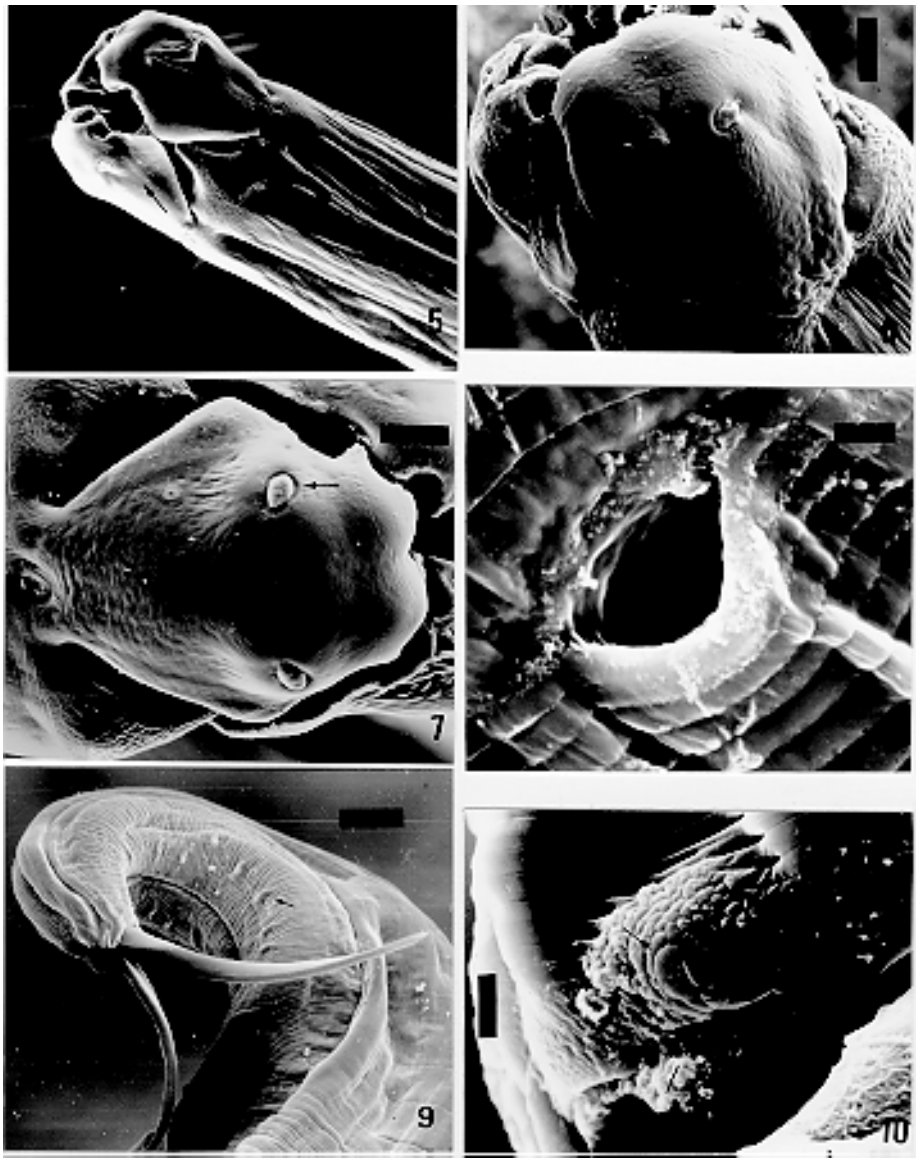
Site: intestine.

Locality: Lake Panguipulli (39º 43' S; 72º 13' W), Chile.

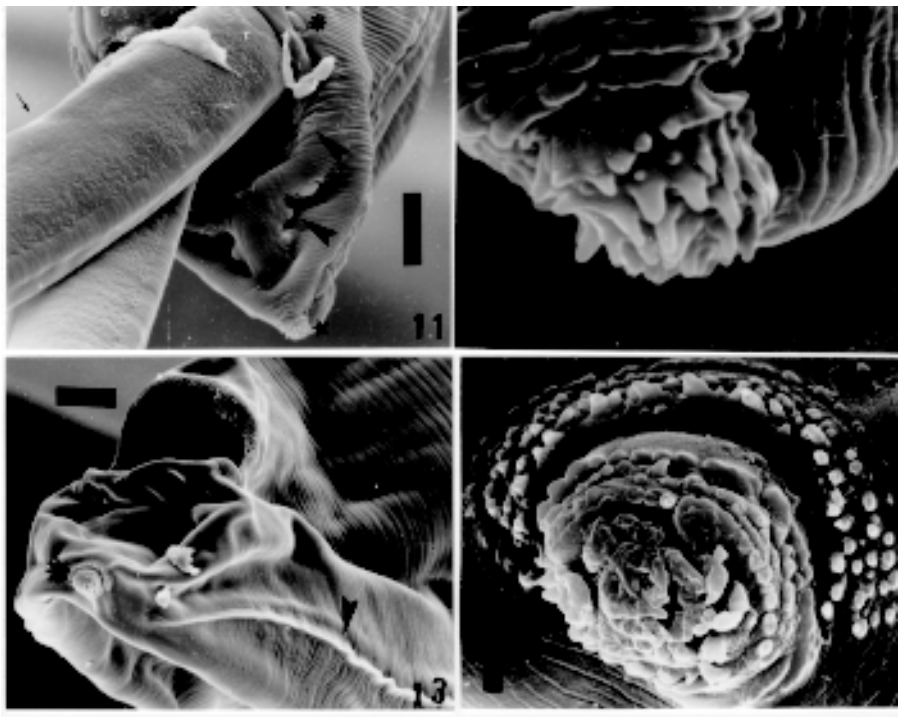
Specimens: Instituto de Parasitología Collection, Universidad Austral de Chile (IPUAT) No. 0254 (Holotype); No. 0255 (Allotype); No. 0256-0258 (Paratypes).

Etymology: the species is named in honour of Dr Walter Gesche from Instituto de Salud Publica, Universidad Austral de Chile, who collaborated in our investigations related to zoonotic fish infections up to his death in 1995.

Prevalence and intensity: 11 (78.6%) out of 14 fishes were infected with 159 specimens, with mean intensity (range) of 14.4 (1-55) worms per infected fish.



Adults of *Hysterothylacium geschei* n.sp. Scanning electron micrographs. Fig. 5: anterior region of a male showing lips (arrow), interlabium (arrow head) and alae groove (asterisk). Bar = 69.2 µm. Fig. 6: subventral lip showing mediolateral doubled papilla (arrow), single lateral papilla (arrow head) and amphid (asterisk). Bar = 29 µm. Fig. 7: dorsal lip with two lateral doubled papillae (arrow). Bar = 36.5 µm. Fig. 8: excretor pore. Bar = 3.36 µm. Fig. 9: posterior extremity showing pre-anal papillae (arrow) and spicules (arrow head). Bar = 113.9 µm. Fig. 10: medio ventral papilla (arrow) on anterior cloacal lip. Bar = 5.5 µm.



Adults of *Hysterothylacium geschei* n.sp. Scanning electron micrographs. Fig. 11: tail of male showing spicules (arrow), post-anal (arrow head), adanal papillae (asterisk), and conical nodulose apex (star). Bar = 21.9 µm. Fig. 12: idem, detail of the conical nodulose apex, lateral view. Bar = 35 µm. Fig. 13: tail of female, anus (arrow), alae (arrow head), conical nodulose apex (asterisk). Bar = 55 µm. Fig. 14: idem, detail of the conical nodulose apex. Bar = 7.2 µm.

REMARKS

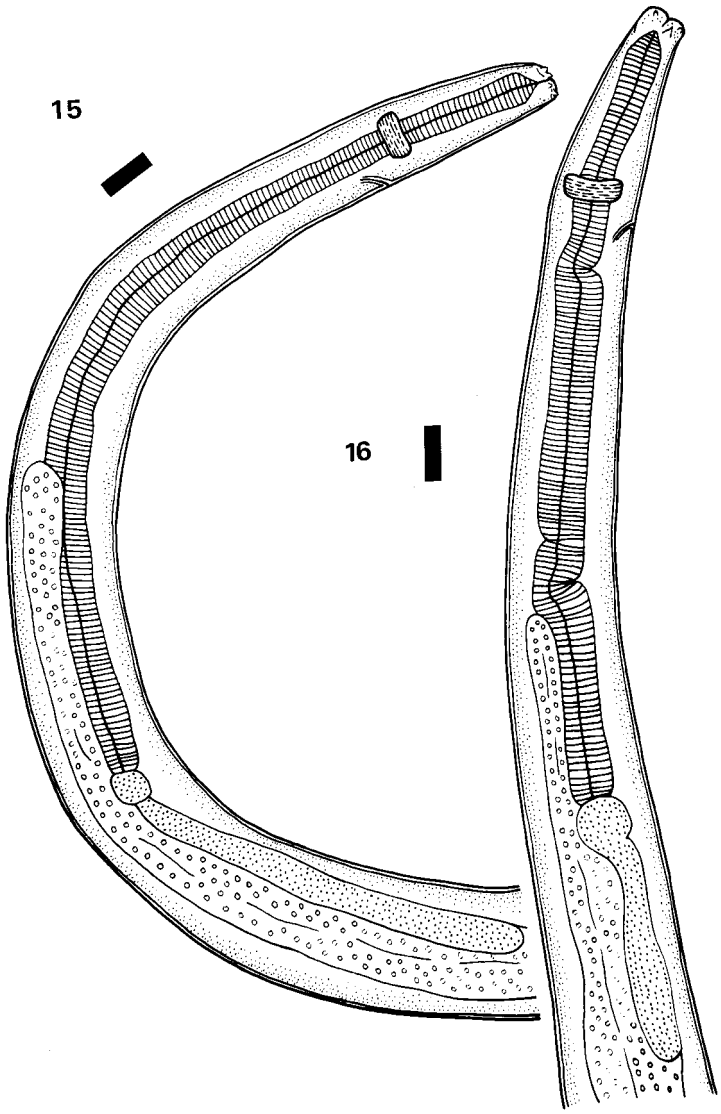
Up to the present, about 55 species of the genus *Hysterothylacium* have been described from marine, estuarine and freshwater fishes (Bruce et al. 1994, Moravec et al. 1997). Of the three species of *Hysterothylacium* described in South America, only *H. rhamdiae* and *H. patagonense* parasitize freshwater fishes (Brizzola & Tanzola 1995, Moravec et al. 1997).

H. rhamdiae, described from the pimelodid catfish *R. sapo* from Argentina, differs from *H. geschei* n. sp. in possessing shorter lips (40-100), intestinal caecum (372-876), spicules (100-200) and distance vulva-anterior extremity (4.6-10 mm). Also, *H. rhamdiae* differs from *H. geschei* because the spicules are 0.4-1.3% of body length and the ventricular appendix is approximately two to six times longer than the intestinal caecum and in having fewer pre-anal papillae (27-31 pairs) and larger number of post-anal papillae (7 pairs) (Brizzola & Tanzola 1995).

H. patagonense described from *P. trucha* from Patagonia, Argentina has a smaller number of pre-anal papillae (20-22 pairs), a larger number of post-anal papillae (7 pairs) and lateral alae are absent (Moravec et al. 1997) when compared to *H. geschei*.

The third species of *Hysterothylacium*, *H. fortalezae*, described in South America was recovered from marine fishes including *S. cavalla*, *S. maculatus* (Scombridae) and *H. clupleola* (Clupeidae) (Vicente et al. 1985) sampled in Brazilian coastal waters. This species has also been registered from *Oligoplites saurus* (Bloch & Schneider) (Carangidae) and *Mycteroperca bonaci* (Poey) (Serranidae) in Horn Island, Mississippi and in the Biscayne Bay, Florida (Deardorff & Overstreet 1981).

H. fortalezae can be distinguished from *H. geschei* by having shorter oesophagus (0.9-2.1 mm), intestinal caecum (144-360) and length of vulva-anterior extremity (5.5-6.5 mm) (Deardorff & Overstreet 1981, Vicente et al. 1985). Also, *H.*

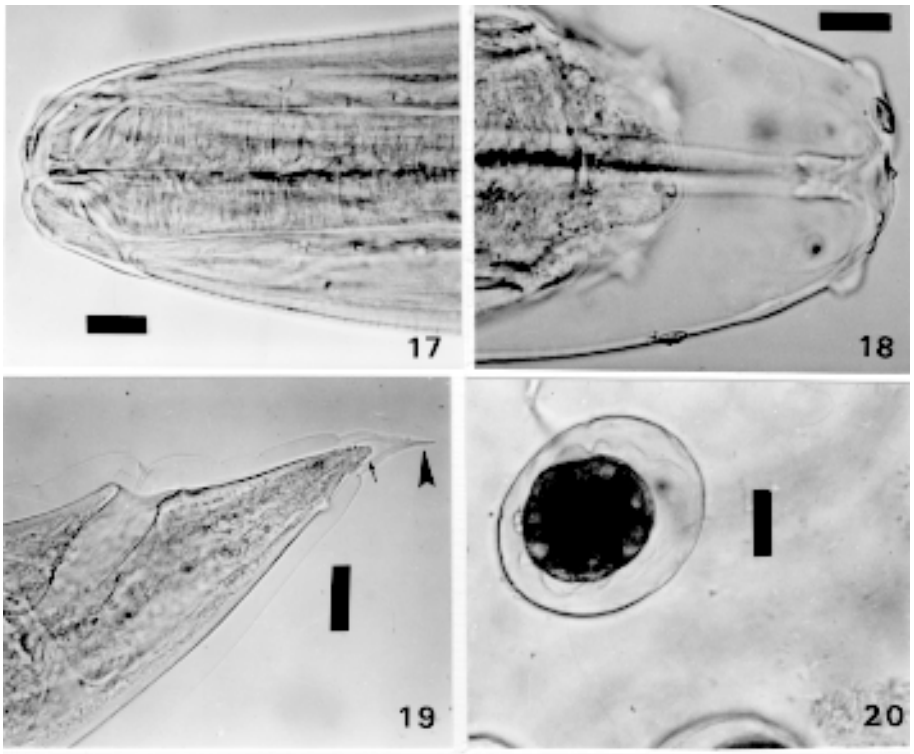


Larvae of *Hysterothylacium geschei* n. sp. Camera lucida drawings. Fig. 15: anterior end of third stage larvae. Bar = 133 µm. Fig. 16: anterior region of fourth stage larvae. Bar = 143 µm.

fortalezae showed ratio for lengths of ventricular appendix to oesophagus (1: 1.6-1.8) and intestinal caecum to ventricular appendix lengths (1: 2.1-3.9) that were up to 1:1, and have a smaller number of pre-anal papillae (13-25 pairs), a larger number of post-anal papillae (8 pairs) and no adanal papillae (Deardorff & Overstreet 1981).

H. patagonense is the species most similar to *H. geschei* and its definitive host, *P. trucha*, is an endemic fish from the south of Chile and Argentina (Campos 1985). Larvae of the third stage of *H. patagonense* also have been registered in *S. trutta*, *O. mykiss* and *Salvelinus fontinalis* (Mitchill) all introduced salmonids in South America in Lake Alumine, Argentina. Moravec et al. (1997) considered that salmonids could act as paratenic or transport hosts.

The host for *H. geschei*, *C. mauleanum*, is an autochthonous fish from Chile, distributed approximately between 33º-42º Lat. S. (Campos 1985). The parasitological analysis of *O. mykiss*, *S. trutta*, *P. trucha* and *B. australis* only revealed the presence of a L3 in one specimen of *P. trucha*, which could represent an accidental infection from the consumption of some common prey which is the definitive host of *H. geschei*. In this context, *P.*



Light micrographs of *Hysterothylacium geschei* n. sp. Fig. 17: cephalic extremity of third stage larva. Bar = 47 µm. Fig. 18: cephalic extremity of fourth stage larva ensheathed in the cuticle of the third stage larva. Bar = 31 µm. Fig. 19: idem, caudal extremity showing conical nodulose apex (arrow) of fourth stage larva and spine (arrow head) in the caudal region of cuticle of the third stage larva. Bar = 46 µm. Fig. 20: egg. Bar = 21 µm.

trucha and *C. mauleanum* consume decapod crustaceans such as *Aegla* spp. (Arenas 1978, Torres et al. 1988) that have been reported as hosts of L3 of *Hysterothylacium* sp. in the Lake Ranco, Chile (Torres & Jara 1987).

Infections by juvenile stages of *Hysterothylacium* sp. have been reported in the River Valdivia Basin in *C. mauleanum* in the River Calle-Calle and in the Lake Calafquen, as well as in *B. australis* in Lake Riñihue, with prevalences of 3.6%, 28.6% and 1.7%, respectively. Mean intensities did not exceed 1.7 worms per host (Torres et al. 1988). Infection by *Hysterothylacium* in different rivers and lakes of the River Valdivia basin has not been observed in the introduced salmonids *O. mykiss* and *S. trutta* (Torres et al. 1991). In 211 adult specimens of *O. mykiss* from eight lakes situated between 39º-41º South lat., only one male specimen of *Hysterothylacium* was recovered in the Lake Ranco (Torres 1995). In other lakes, including Lake Tagua-Tagua (41º39'S) and Lake Yelcho (43º16'S) unidentified species of *Hysterothylacium* have been reported in *O. mykiss* and *S. trutta* respectively (Torres et al. 1992).

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