

New data on the morphology of *Nilonema senticosum* (Nematoda, Philometridae), a parasite of *Arapaima gigas* (Pisces), with notes on another philometrid, *Alinema amazonicum*, in Peru

František Moravec^{1*}, Tomáš Scholz^{1,2}, Roman Kuchta^{1,2}, Iva Dyková^{1,2} and Petr Posel³

¹Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic; ²Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic; ³Fuengirola Zoo/Rain Forest, C/Camilo Jose Cela, 8-10, 29640 Fuengirola (Malaga), Spain

Abstract

Female specimens of a little-known philometrid nematode, *Nilonema senticosum* (Baylis, 1927), were collected from the swimbladder of the arapaima, *Arapaima gigas*, from the Amazon River basin (Iquitos, Loreto District) in Peru. Scanning electron microscopical (SEM) examination, used for the first time in this species, made it possible to reveal some taxonomically important, previously unreported features of *N. senticosum*, such as the presence of minute cephalic papillae (10 papillae in 2 circles) and amphids surrounding the small oral aperture, and to confirm the absence of an anal opening in this species. Males and females of another philometrid, *Alinema amazonicum* (Travassos, 1960), were recovered from the body cavity of the pimelodid catfishes *Calophysus macropterus* and *Brachyplatystoma juruense* (a new host record) from the Amazon River basin (fish market in Iquitos, Loreto District) in Peru. SEM examination, not previously used in the male of *A. amazonicum*, enabled to study in detail the male cephalic and caudal structures.

Key words

Parasitic nematode, *Nilonema*, *Alinema*, Philometridae, freshwater fish, *Arapaima*, *Calophysus*, *Brachyplatystoma*, Amazon River basin, Peru

Introduction

During examination of some fishes from the Amazon River basin in Peru, carried out in April 2005 and April 2006, nematode specimens of the family Philometridae were collected from the swimbladder of the arapaima *Arapaima gigas* (Schinz) (family Arapaimidae), and the mesentery and the body cavity of two species of catfishes, the zamurito *Calophysus macropterus* (Lichtenstein) and the zebra catfish *Brachyplatystoma juruense* (Boulenger) (both family Pimelodidae). The arapaima is distributed in the Amazon River basin only, whereas both species of catfishes in the Amazon and the Orinoco River basins (Froese and Pauly 2006). The philometrid nematodes recovered proved to belong to two insufficiently known species, *Nilonema senticosum* (Baylis, 1927), a specific parasite of arapaima, and *Alinema amazonicum* (Travassos, 1960) parasitizing some pimelodid catfishes of the Amazon River drainage system. An examination by scanning

electron microscopy of the male of *A. amazonicum* and of females of *N. senticosum*, used for the first time in these species, made it possible to recognize the true structure of the male cephalic and caudal ends of the former and to reveal some taxonomically important characters of the latter. The results of this study are presented herein.

Materials and methods

Among some other fish species, 16 specimens of *Calophysus macropterus* (body length 24–40 cm) and 5 specimens of *Brachyplatystoma juruense* (body length 55–60 cm) (both Pimelodidae, Siluriformes) were bought at the fish market (Belén) in Iquitos, Loreto District, Peru, in April 2005 and April 2006, and they were subsequently examined in the laboratory for the presence of helminth parasites. Viscera of 2 cultured *Arapaima gigas* (body length 80 and 95 cm) were obtained for exam-

*Corresponding author: moravec@paru.cas.cz

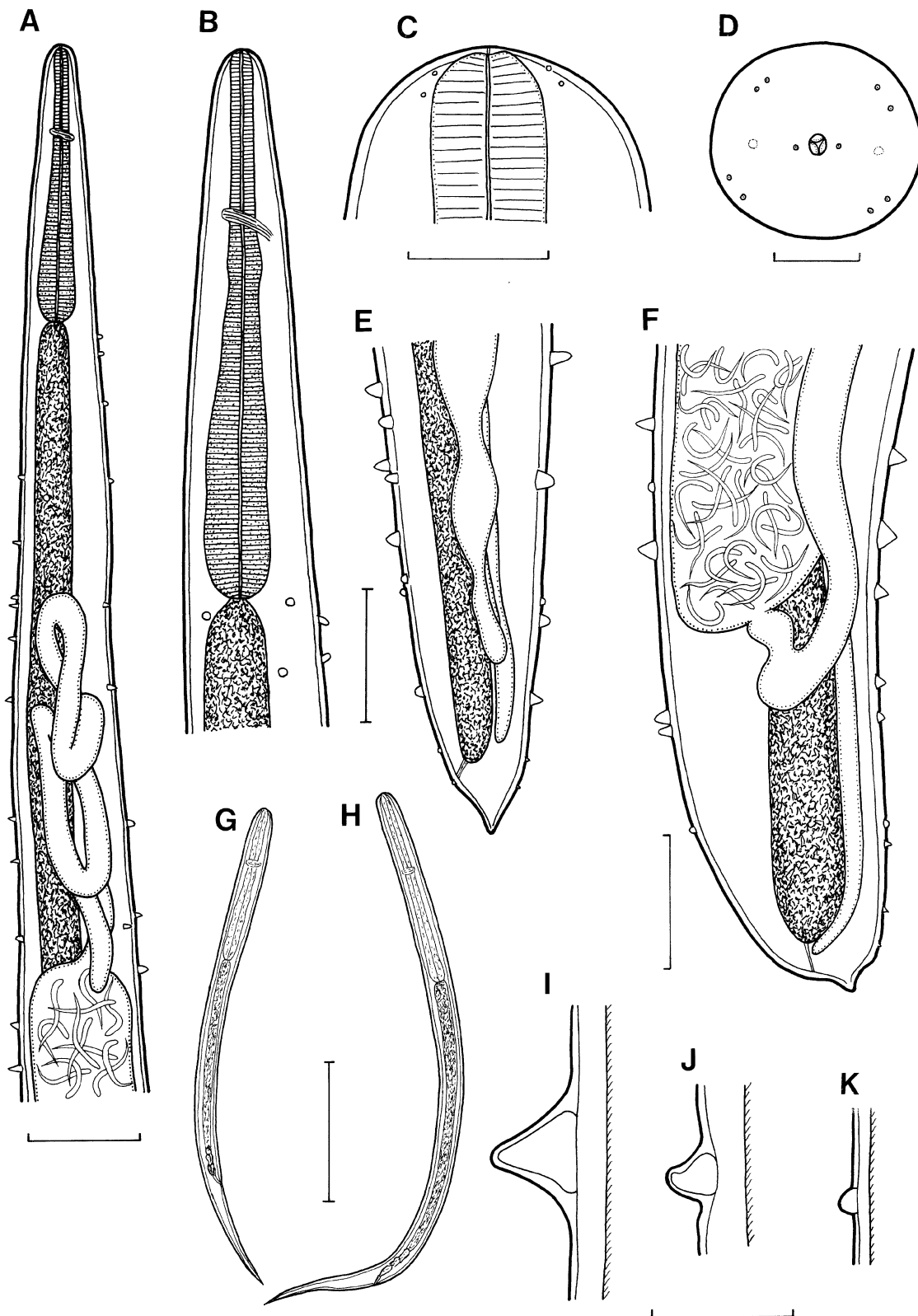


Fig. 1. *Nilonema senticosum* (Baylis, 1927), female: **A** – anterior part of body, lateral view; **B** – oesophageal part of body, lateral view; **C** and **D** – cephalic end, lateral and apical views; **E** – posterior end of subgravid specimen, lateral view; **F** – posterior end of gravid specimen, lateral view; **G** and **H** – larvae from uterus; **I–K** – different shapes of cuticular ornamentations, lateral views. Scale bars: **A** – 0.5 mm; **B**, **E**, **F** – 0.3 mm; **C** – 0.05 mm; **D** – 0.02 mm; **G–K** – 0.1 mm

ination from the fish farm Granja 4 near Iquitos in April 2005. It was impossible to find exact information about localities of the catfishes examined, but it is certain that they came from the nearby water bodies of the Amazon River basin.

The nematodes recovered were fixed in hot 4% formaldehyde solution in physiological saline. For light microscopical examination, the nematodes were cleared with glycerine. Drawings were made with the aid of a Zeiss drawing attachment. Specimens used for scanning electron microscopy were transferred to 4% formaldehyde solution and then postfixed in 1% osmium tetroxide, dehydrated through a graded ethanol series, critical point dried and sputter-coated with gold; they were examined using a JEOL JSM-6300 scanning electron microscope at an accelerating voltage of 15 kV. All measurements are in micrometres unless otherwise stated. The scientific names of fishes follow FishBase (Froese and Pauly 2006). For comparative purposes, the holotype of *Rumai rumai* Travassos, 1960, another philometrid from the arapaima in Brazil, deposited in the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro (Cat. No. 26636), was reexamined during a short stay of T. Scholz in Brazil.

Results

Nilonema senticosum (Baylis, 1927) (Figs 1 and 2)

Syn.: *Philometra senticosa* Baylis, 1927.

Description of female (7 gravid specimens; measurements of 1 subgravid specimen in parentheses): Body of fixed specimens whitish, with distinct brown-coloured intestine displaced by uterus to body wall, cylindrical, somewhat tapering at both ends. Length of body 110.00–136.30 (105.09) mm, maximum width 789–911 (707). Maximum width/length ratio of body 1:121–162 (1:149). Almost whole body covered with numerous, irregularly scattered cuticular ornamentations in form from small bosses to large cone-shaped excrescences up to 54–82 (68) high (Figs 1I–K and 2E–G); cuticular ornamentations absent from oesophageal region of body. Anterior end blunt, simple, cephalic papillae difficult to find. Oral aperture circular to somewhat oval, surrounded by eight minute external cephalic papillae arranged in four submedian pairs and one pair of more prominent internal lateral papillae (Figs 1D and 2A–C); pair of small lateral, poorly developed amphids present at some distance outside internal papillae (Fig. 1D). Bottom of mouth formed by surfaces of three lobular oesophageal sectors. Oesophagus initiating immediately below mouth opening; its length 1.20–1.46 (1.33) mm, representing 1 (1%) of body length; it consists of anterior cylindrical portion 68–82 (54) wide and somewhat darker posterior broader portion with maximum width 150–177 (150); oesophageal glands indistinct. Ventriculus absent. Nerve ring encircling oesophagus 326–422 (394) from anterior extremity. Intestine straight, of approximately equal width throughout, ending blindly at short distance anterior to posterior extremity, being attached by short ligament to ventral body wall. Posterior end of body con-

ical; length of caudal projection from site of ligament attachment to posterior extremity 24–54 (68). Ovaries reflected, situated near body ends; anterior ovary and uterus by far not reaching posterior end of oesophagus (Fig. 1A). Uterus occupying most space of body. In gravid specimens it contains large number of larvae 354–366 long and 12–15 wide, with rounded anterior and sharply pointed posterior ends; length of oesophagus 111–117 (30–33% of body length), of tail 51–63 (14–17% of body length). Uterus of subgravid specimen containing numerous eggs.

Male: Unknown.

Host: Arapaima, *Arapaima gigas* (Arapaimidae, Osteoglossiformes).

Site of infection: Swimbladder.

Locality: Fish farm Granja 4, Iquitos, Amazon River basin, Loreto District, Peru (collected 10 April 2005).

Prevalence and intensity: In both fish specimens examined 1 and 7 nematodes recovered.

Deposition of voucher specimens: Helminthological Collection of the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, in České Budějovice (Cat. No. N-852) and Parasite Collection of the Museo de Historia Natural, UNMSM, in Lima, Peru (Cat. No. 2171).

Comments: This species was originally described by Baylis (1927) from many large females collected from the swimbladder of *Arapaima gigas*, sent to the then British Museum (Natural History) (= the present Natural History Museum, London) from Brazil. In spite of certain differences in the structure of the oesophagus and the mouth, and the presence of the conspicuous thorny cuticular ornamentations, he assigned it only provisionally to *Philometra* Costa, 1845. Later Travassos (1960) reported females of the same species, under the name *Philometroides senticosa* (Baylis, 1927), from the body cavity of *A. gigas* from the Pará State, Brazil, providing their short description and drawings. In her revisional work, Rasheed (1963) transferred this species to *Nilonema* Khalil, 1960. Based on female specimens deposited in the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro, *N. senticosum* was later reported from *A. gigas* of the Amazon River basin in Brazil by Santos *et al.* (1979), Vicente and Jardim (1980) and Vicente *et al.* (1985), who had taken over the original drawings by Travassos (1960) in their papers. Outside Brazil, *N. senticosum* was listed from *A. gigas* of the Amazon River basin (Pacaya River) in Peru (Cordóva 1974, Tantaleán *et al.* 1985, Sarmiento *et al.* 1999).

The morphology of nematodes of the present material is, more or less, in agreement with the original description of *Philometra senticosa* given by Baylis (1927). However, neither Baylis (op. cit.) nor subsequent authors observed any cephalic papillae in this species. Cephalic papillae were neither found in females of the only other representative of *Nilonema*, *N. gymnarchi* Khalil, 1960 (the type species of the genus), a parasite of the lung-like swimbladder of *Gymnarchus niloticus* Cuvier (Gymnarchidae, Osteoglossiformes) in Africa (Khalil 1960, Khalil and Polling 1997). However, as revealed by SEM, used in a *Nilonema* species for the first time, the

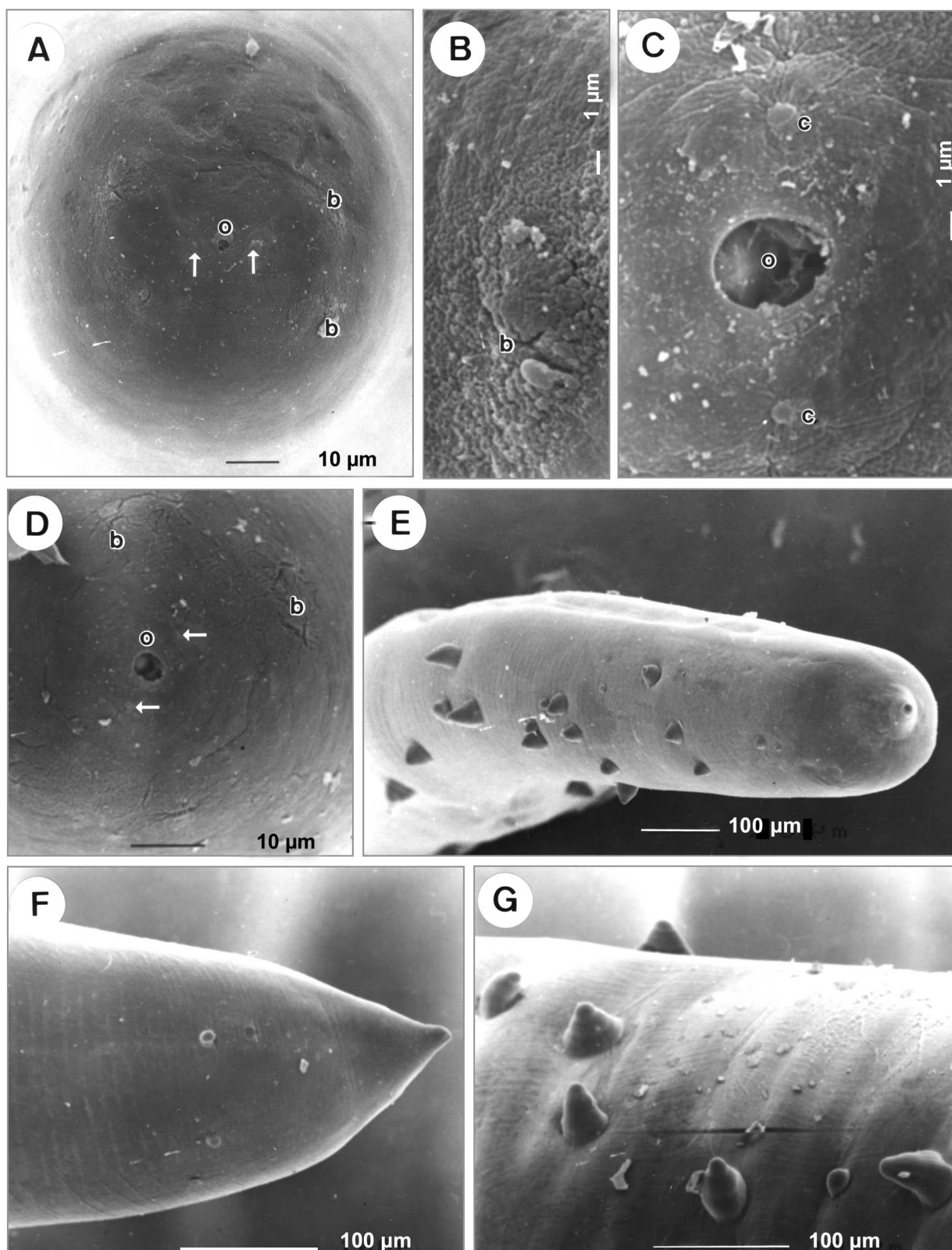


Fig. 2. *Nilonema senticosum* (Baylis, 1927), scanning electron micrographs of gravid female: **A** – cephalic end, apical view (arrows show lateral cephalic papillae), **B** – pair of submedian cephalic papillae of external circle, **C** – oral aperture with nearby pair of lateral cephalic papillae of internal circle, apical view, **D** – cephalic end of another specimen, apical view (arrows show lateral cephalic papillae), **E** – caudal end of body with large conical cuticular ornamentations, ventral view, **F** – posterior extremity, ventral view, **G** – cuticular ornamentations. **Abbreviations:** b – pair of minute submedian cephalic papillae of external circle, c – lateral papilla of internal circle, o – oral aperture

females of *N. senticosum* possess cephalic papillae that are of a similar arrangement as in other philometrids (see Moravec 2004, 2006), but they are very small and not easily visible; the oral aperture is markedly small for a philometrid.

Travassos (1960) and subsequent Brazilian authors reported the presence of an anal opening in females of *N. senticosum*, but this was not confirmed in the present study; the SEM examination showed (Fig. 2E, F) the absence of the anus, as originally observed by Baylis (1927). In this it resembles a great majority of philometrids in which the intestine ends blindly, being attached by a ligament to the body wall of the caudal end (Moravec 2004).

All females of the present material from the host's swimbladder were large and, except for one large subgravid specimen, all were larvigerous. Also Baylis (1927) had large worms (90–125 mm long) from the swimbladder of the arapaima, comparable with our specimens. Tantaleán *et al.* (1985) also reported *N. senticosum* from the swimbladder. On the other hand, Travassos (1960) and other Brazilian authors reported this parasite from the host's body cavity. However, their materials included small females (ca. 10–60 mm long) in addition to large ones. It can be deduced that the principle site of infection by gravid females of *N. senticosum* is the host's swimbladder (acting as a "lung" in the arapaima), whereas younger forms and, exceptionally, some gravid worms occur in the body cavity. Gravid females of the related African species, *Nilonema gymnarchi*, have the same localization (swimbladder) in the host (Khalil 1960); the hosts of both *N. senticosum* and *N. gymnarchi* belong to the same fish order Osteoglossiformes. Males of both *Nilonema* spp. are unknown.

Travassos (1960) described another philometrid genus and species, *Rumai rumai* Travassos, 1960, based on a gravid female collected from the body cavity of *Arapaima gigas* from the Amazon River in Brazil; the male of this species is unknown. A recent reexamination of the holotype of *R. rumai* confirmed that its morphology considerably differs from that of *Nilonema senticosum* (see Moravec 2006).

***Alinema amazonicum* (Travassos, 1960) (Fig. 3)**

Syns: *Philometra amazonica* Travassos, 1960, *Philometra* (*Alinema*) *alii* Rasheed, 1963.

Hosts: Zamurito *Calophrysus macropterus* and zebra catfish *Brachyplatystoma juruense* (both Pimelodidae, Siluriformes).

Site of infection: Mesentery, body cavity.

Locality: Amazon River basin (fish market in Iquitos, Loreto District), Peru (collected 14, 20 and 22 April 2005 and 5 April 2006).

Prevalence and intensity: *C. macropterus*: 25% (4 fish infected/16 fish examined); intensity 1–13 specimens. *B. juruense*: 1/5, 5.

Deposition of voucher specimens: Helminthological Collection of the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, in České Budějovice (Cat. No. N-831).

Comments: Based on specimens from *Calophrysus macropterus* from Peru, this species has recently been redescribed by Moravec *et al.* (2006); the authors synonymized *Philometra alii* Rasheed, 1963 with *Philometra amazonica* Travassos, 1960 and validated the genus *Alinema* Rasheed, 1963 to accommodate this species. They studied gravid females also by SEM, but this method could not be applied for the only available male specimen.

The newly collected material from Peru consisted of three males and fourteen fragmented nongravid and subgravid females from *Calophrysus macropterus*, and five nongravid and subgravid females (body length 16–98 mm) from *Brachyplatystoma juruense*. The males were 5.06–6.54 mm long and their maximum width was 95 µm; the right spicule, the left spicule and the gubernaculum measured 720–830 µm, 405–510 µm and 329–432 µm, respectively. A pair of larger preanal papillae situated some distance anterior to the cloacal aperture, as reported by Moravec *et al.* (2006), was not observed in the three specimens of the present material.

A SEM examination of one specimen, used for the first time in the male of *A. amazonicum*, revealed the presence of small, inner tooth-like supports of the circumoral rim of the mouth and a narrow ring of somewhat elevated cuticle surrounding the oral aperture; in contrast to females, the external ring of cephalic papillae was formed by only four, comparatively large submedian papillae (vs. eight in the female), and the inner ring consisted of two lateral papillae only (vs. six in the female) (Fig. 3A, B). Three pairs of caudal papillae were found (one preanal, one adanal and one postanal) (Fig. 3C); the last pair of postanal papillae illustrated by Moravec *et al.* (2006) were probably phasmids, which were not visible in the specimen studied by SEM, because its dorsal side was not clean; the preanal papillae were situated ventrally; a poorly developed U-shaped caudal mound (Fig. 3C), which is present in males of most philometrid species (Moravec 2004, 2006), was observed in *A. amazonicum* for the first time.

A remarkable feature of the male of *A. amazonicum*, revealed by SEM, was the presence of ventral cuticular ornamentations in the precloacal region (Fig. 3D, F); they were formed by many transverse bands of irregular longitudinal, somewhat granular formations, alternating with transverse bands of almost smooth cuticle with equally spaced longitudinal slit-like depressions (Fig. 3F). Ventral cuticular ornamentations (area rugosa) frequently occur in some spirurine nematodes, but they have not yet been reported from members of the family Philometridae and they rarely occur in other representatives of the Dracunculoidea (Moravec 2004); Moravec and Nagasawa (1999) observed such ornamentations (but of a different type) in the nematode *Philonema oncorhynchi* Kuitunen-Ekbaum, 1933, a parasite of the abdominal cavity of salmonids; this species was originally listed in the Philometridae, but recently Moravec (2006) transferred it to the family Micropleuridae.

Alinema amazonicum has so far been reported only from pimelodid catfishes of the Amazon River basin in Brazil

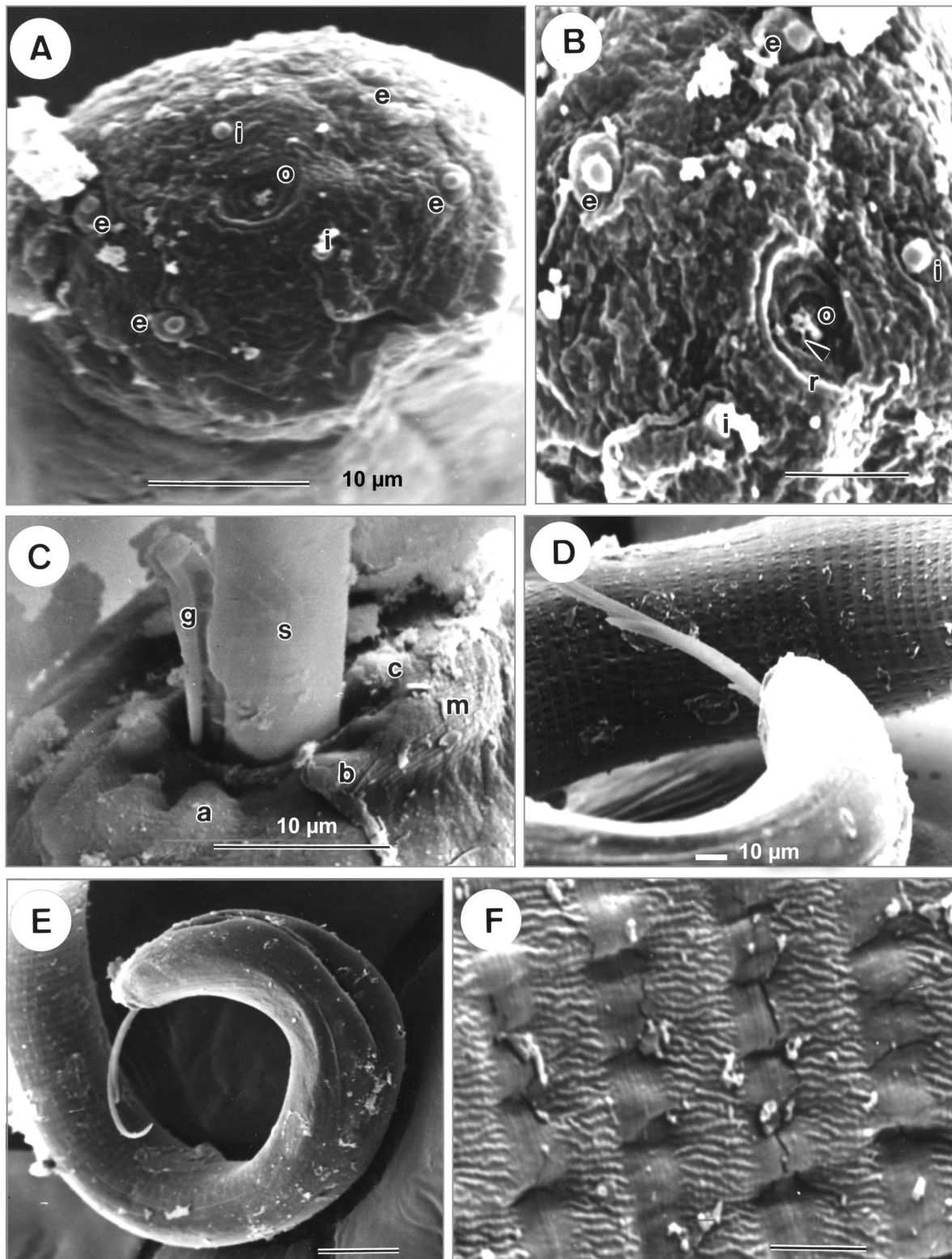


Fig. 3. *Alinema amazonicum* (Travassos, 1960), scanning electron micrographs of male: **A** – cephalic end, subapical view; **B** – region of mouth, subapical view; arrow shows inner tooth-like supports of circumoral rim; **C** – region of cloaca, subventral view; **D** – caudal end with distinct ventral precloacal cuticular ornamentations; **E** – caudal end, lateral view; **F** – detail of ventral precloacal cuticular ornamentations. Scale bars: A and C – 10 µm; B and F – 5 µm; E – 50 µm. **Abbreviations:** a – preanal papilla, b – adanal papilla, c – postanal papilla, e – external submedian cephalic papilla, g – gubernaculum, i – internal lateral cephalic papilla, m – caudal mound, o – oral aperture, r – ring around oral aperture, s – spicule

(Travassos 1960, Rasheed 1963, Inglis and Ogden 1964, Santos *et al.* 1979, Vicente and Jardim 1980, Vicente *et al.* 1985) and Peru (Moravec *et al.* 2006). Its principle host seems to be the zamurito *Calophysus macropterus*, which is the type host, less frequently it has been reported from the pati *Luciopimelodus pati* (Valenciennes), the flatwhiskered catfish *Pinirampus pirinampus* (Spix et Agassiz) and the leopard catfish *Perrunichthys perruno* Schultz (all Pimelodidae). The present finding of *A. amazonicum* in the zebra catfish *Brachyplatystoma juruense* represents a new host record.

Acknowledgements. The authors are deeply indebted to all Peruvian colleagues, who helped them collect fish or their intestines for parasitological examination, especially to Ivan Vásquez Valera, owner of the fish farm Granja 4 and Martin Mortenthaler, Acuario Río Momon, Iquitos, Fernando G. Poma Castillo, José Rivera, Luis Vásquez Uribe, Pamela Porturas, and Alfonso Shapiama (Director), all Vice-ministerio de Pesquería, Oficina Regional, Iquitos, to Emer Gloria Pizango Paima (Head), Marina del Aguila, Rossana Cubas Guerra, Department of Hydrobiology, Faculty of Biological Sciences, National University of Peruvian Amazonia (UNAP), Iquitos, Loreto Province, and to Edgar R. Panduro Noronha, OAFA, Iquitos. Logistic help and support of the Government of the Loreto Region (GOREL), Iquitos, including its office (Delegación) in Lima (Germani Merino Gastelu), and Embassies of Peru in Prague and of the Czech Republic in Lima, are greatly appreciated. Thanks are due to the staff of the Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre of the ASCR, in České Budějovice for technical assistance, and Irena Husáková, Department of Helminthology at the same Institute, for help with illustrations. Dr. Delly Noronha, Curator of the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro, made it possible to reexamine the holotype of *Rumai rumai*. This study was supported by the grant No. 524/06/0170 from the Grant Agency of the Czech Republic. The stays in Peru were financially supported by the Grant Agency of the Czech Republic (projects Nos. 524/04/0342 and 524/03/H133) and the Institute of Parasitology, ASCR, České Budějovice (research projects Nos Z60220518 and LC522).

References

- Baylis H.A. 1927. Some parasitic worms from *Arapaima gigas* (teleostean fish) with a description of *Philometra senticosus* n. sp. (Filarioidea). *Parasitology*, 19, 35–47.
- Cordóva R.V. 1974. Incidencia de bacterias y parásitos en “paiche” *Arapaima gigas* (Cuvier). *IV Congreso Nacional de Biología, Trujillo, Perú*. Libro de Resúmenes, 121–122 (ex Tantaleán *et al.* 1985).
- Froese R., Pauly D. (Eds.). 2006. FishBase. World Wide Web electronic publication. www.fishbase.org, August 2006.
- Inglis W.G., Ogden C.G. 1964. Miscellanea nematodologica. IV. The male of *Alinema alii* Rasheed, 1963. *Annals and Magazine of Natural History, Ser.* 13, 7, 523–525.
- Khalil L.F. 1960. On a new nematode, *Nilonema gymnarchi* gen. et sp. nov., (Dracunculidae), from a freshwater fish in the Sudan. *Journal of Helminthology*, 34, 55–58.
- Khalil L.F., Polling L. 1997. Check list of the helminth parasites of African freshwater fishes. University of the North, Pietersburg, Republic of South Africa.
- Moravec F. 2004. Some aspects of the taxonomy and biology of dracunculoid nematodes parasitic in fishes: a review. *Folia Parasitologica*, 51, 1–13.
- Moravec F. 2006. Dracunculoid and anguillicoloid nematodes of vertebrates. Academia, Prague, in press.
- Moravec F., Nagasawa K. 1999. New data on the morphology of *Philonema oncorhynchi* Kuitunen-Ekbaum, 1933 (Nematoda: Dracunculoidea) from the abdominal cavity of Pacific salmon (*Oncorhynchus* spp.). *Systematic Parasitology*, 43, 67–74.
- Moravec F., Scholz T., Dyková I., Kuchta R., Fiala I., Kohn A. 2006. Redescription of *Alinema amazonicum* (Travassos, 1960) n. comb., a philometrid nematode with unusual morphology. *Journal of Parasitology*, 92, 138–144.
- Rasheed S. 1963. A revision of the genus *Philometra* Costa, 1845. *Journal of Helminthology*, 37, 89–130.
- Santos E., Vicente J.J., Jardim C.R. 1979. Helmintos de peixes de rios amazônicos da Coleção Helminológica do Instituto Oswaldo Cruz. II. Nematoda. *Atas da Sociedade de Biologia do Rio de Janeiro*, 20, 11–19.
- Sarmiento L., Tantaleán M., Huiza A. 1999. Nemátodos parásitos del hombre y de los animales en el Perú. *Revista Peruana de Parasitología*, 14, 9–65.
- Tantaleán M., Huiza A., Hurtado E. 1985. Helmintos parásitos de peces de agua dulce del Perú. Universidad Nacional del Altiplano, Facultad de Medicina Veterinaria y Zootecnia, Instituto de Investigaciones para el Desarrollo Social del Altiplano, Puno.
- Travassos L. 1960. Sobre nematódeos cavitários de peixes do Rio Amazonas. *Atas da Sociedade de Biologia do Rio de Janeiro*, 4, 15–20.
- Vicente J.J., Jardim C.R. 1980. Filarídeos da Coleção Helminológica do Instituto Oswaldo Cruz. I. Peixes, anfíbios e répteis. *Atas da Sociedade de Biologia do Rio de Janeiro*, 21, 47–57.
- Vicente J.J., Rodrigues H.O., Gomes D.C. 1985. Nematóides do Brasil. 1ª parte: Nematóides de peixes. *Atas da Sociedade de Biologia do Rio de Janeiro*, 25, 1–79.