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Goezia spinulosa (Nematoda: Raphidascarididae), a pathogenic parasite of the arapaima *Arapaima gigas* (Osteichthyes)

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Abstract: The nematode *Goezia spinulosa* (Diesing, 1839) (Raphidascarididae) is redescribed based on specimens found in the stomach and intestine of the naturally infected arapaima *Arapaima gigas* (Schinz) from the Mexiana Island, Amazon River Delta, Brazil. Light and electron microscopy examinations revealed some previously unreported or inaccurately described morphological features in the species, such as the position of the excretory pore, phasmids in the male or the number (4) of postanal papillae. The morphology of *G. spinulosa* is compared with that of other four congeneric species parasitizing freshwater fishes in South America. This nematode seems to be one of the most pathogenic parasites of *A. gigas* in the Mexiana Island, which are responsible for a high mortality of cultured arapaima fingerlings. Apparently, the source of *G. spinulosa* infection for arapaima fingerlings cultured in tanks was the infected plankton collected in the localities inhabited by wild arapaimas. Therefore, control measures should include the sterilisation of the plankton before its use as food for fish. A rare infection of *Eustrongylides* sp. larvae (Dioctophymatidae) in arapaima fingerlings was also found (new host record); the larvae were inside swellings on the body surface.

Key words: parasitic nematode, *Goezia spinulosa*, freshwater fish, *Arapaima gigas*, aquaculture, Amazonia, Brazil

The arapaima, *Arapaima gigas* (Schinz, 1822), is a fish of high economic value, endemic of the Amazon region. Due to the extensive fisheries, the fish stocks decreased and *A. gigas* is now considered to be an endangered species by the Convention on International Trade of Endangered Species (CITES) of wild fauna and flora. The arapaima or "pirarucu" is considered an excellent fish for food, reaching the weight of up to 200 kg and 450 cm in length (Froese and Pauly 2008) and, therefore, efforts to cultivate this fish have been made (Venturieri and Bernardino 1999).

Within the Pirarucu Management Project in the Mexiana Island, Amazon River Delta, attempts to culture arapaimas in a fish farm are currently being carried out. However, a high mortality of *A. gigas* fingerlings was observed in culture tanks. Our surveys of their parasites revealed a high infection with *Goezia spinulosa* (Diesing, 1839) (Nematoda: Raphidascarididae). The parasites were found free or penetrating the tissues mainly of the stomach but also the intestine of the fish, sometimes perforating the wall of these organs. Other nematodes of *A. gigas* in the area as *Nilonema senticosum* (Baylis, 1927), *Rumai rumai* Travassos, 1960, *Camallanus tridentatus* (Drasche, 1884) and *Capillostrongyloides arapaimae* Santos, Moravec et Venturieri, 2008 have been dealt with in our previous papers (Santos and Gibson 2007, Santos et al. 2008, Santos and Moravec 2009a, b). *Goezia spinulosa* is redescribed herein based on the examination of freshly collected material from wild and cultured arapaimas. New data on the morphology of adults, inferred from the scanning electron microscopy (SEM) study, comparative observations on the occurrence of the parasite in fingerlings and adult fish, and ways to control the parasite are discussed.

Since its original description by Diesing (1839) from *A. gigas* from an unknown locality in Brazil, *G. spinulosa* from its type host was redescribed several times in Brazil (Drasche 1884, Baylis 1927, Rasheed 1965, Santos et al. 1979) and reported from Peru (see Moravec 1998). However, its morphology remained insufficiently known until Costa et al. (1995) provided data on specimens from *A. gigas* studied with the use of SEM. Nevertheless, the present study revealed some previously unreported or inaccurately described morphological features. *Goezia spinulosa* was also redescribed by Freitas and Lent (1946) and Travassos and Kohn (1965) from specimens collected in perciform fishes *Astronotus ocellatus* (Agassiz) (Cichlidae) and *Micropterus salmoides* (Lacépède) (Centrarchidae), respectively, in Brazil, and by Hamann (1984)

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from the catfish *Pseudoplatystoma corruscans* (Spix et Agassiz) (Pimelodidae) in Argentina. However, the identifications of *G. spinulosa* from perciform and siluriform fishes might not be reliable, because the descriptions vary (see Rasheed 1965), the hosts belong to different fish orders, and *A. gigas*, the type host of *G. spinulosa*, does not naturally occur in the regions from where this parasite has been reported from perciform and siluriform fishes (Moravec et al. 1994).

The life cycle and pathogenicity of *G. spinulosa* have not yet been studied. The only paper reporting *G. spinulosa* larvae from the digestive tract of sick arapaima fingerlings (body length 45–55 mm) unsuccessfully cultured in Lima Campos (Ceará State), Brazil is that of Freitas and Lent (1946). The authors also recorded infective larvae of *G. spinulosa* in the plankton (*Diaptomus* sp.) from the tanks of arapaima. They observed a high mortality associated with *G. spinulosa* infection (adults and larvae found in the body cavity and the digestive tract) in cultured *A. ocellatus* fingerlings fed with plankton from arapaima tanks.

MATERIALS AND METHODS

The fish were obtained from the Fazenda Santo Ambrosio area, Mexiana Island (Amazon River Delta), Pará State (Pirarucu Management Project – IBAMA 005-2007). They were collected either in local natural canals or they came from culture tanks in the arapaima farm, where fingerlings were fed with plankton collected in the canals. A total of 30 specimens of arapaimas, large fish with body length 70–175 cm (n = 12) and fingerlings with body length 6.5–15.0 cm (n = 18), were examined for the presence of helminth parasites. The nematodes recovered were washed in physiological saline and then fixed in hot 4% formaldehyde solution. For light microscopy, the nematodes were cleared with glycerine. Drawings were made with the aid of a Zeiss drawing attachment. After examination, the specimens were stored in vials with 70% ethanol. Measurements are given in micrometres unless otherwise stated.

Photographs were taken with a Nikon camera adapted to a Leica stereomicroscope. For SEM, fixed specimens were post-fixed in 1% osmium tetroxide in phosphate buffer, dehydrated through a graded acetone series, critical-point dried and sputter-coated with gold. The samples were examined using a JEOL ISM-7401F scanning electron microscope at an accelerating voltage of 4 or 8 kV.

RESULTS

Goezia spinulosa (Diesing, 1839) Figs. 1–4

Syn.: *Lecanocephalus spinulosus* Diesing, 1839.

Description: Medium-sized nematodes of whitish to brownish colour. Body elongate, with thick cuticle bearing transverse rows of posteriorly directed spines (Fig. 2 C–E). Rows of spines begin at short distance behind lips; tips of spines closer together in anterior region (Figs. 1 A, 2 A–D, F); rows gradually become more separated and spines longer towards end of oesophagus (Figs. 2 C, F). Cuticular spines longest at region of ventricular appendix, their maximum length being 21–30; distance between rings at this region 41–82; spines in same row not equally long (Fig. 2 E), rarely bifurcated. Lips approximately equal in size, very shallow with deep postlabial groove, margins alate; inner part of each lip with two distinct lobes oriented to oral aperture. Dorsal lip with two double papillae, ventrolateral lips each with one lateral amphid, one single papilla and one double papilla (Fig. 2 B). Dentigerous ridges and interlabia absent. Length (height) of lips in lateral view 41–54. Oral aperture triangular. Oesophagus clavate, forming 4–6% of body length (Fig. 1 A, C). Nerve ring encircling oesophagus approximately at border of its first and second fourths. Excretory pore slightly posterior to level of nerve ring, between 12th and 13th rows of cuticular spines (Figs. 1 C, 2 C). Deirids small, situated at level of 20th row of spines, at about middle of oesophagus length (Figs. 1 C, 2 F, G). Ventriculus narrower than widest level of oesophagus. Ventricular appendix narrow, approximately twice to three-times as long as oesophagus (Fig. 1 A); appendix frequently displaced, forming anterior coil. Intestinal caecum wide, somewhat exceeding anteriorly base of oesophagus end (Fig. 1 A, C). Tail conical, with digitiform process.

Male (5 specimens): Length of body 11.64–20.70 mm, maximum width 476–625. Length (height) of lips 41–54, width of body at level of lips 190–245. Spination of cuticle on posterior end of body gradually disappearing on dorsal side (spines absent dorsally from about posterior fourth of withdrawn spicules to posterior extremity) (Figs. 1 F, G, 3 A). Ventral precloacal region covered with rows of mostly anteriorly oriented triangular spines (Fig. 3 B), with narrow smooth median field (Fig. 3 C); spines at anterior part of this region large, becoming very small posteriorly from about level of 10th pair of preanal papillae (counting from cloacal opening) (Fig. 3 C, E); spines somewhat larger around cloacal opening, forming five distinct rows on posterior cloacal lip (Fig. 3 H). Oesophagus 476–898 long (4–6% of body length). Nerve ring, excretory pore and deirids 163–217, 231–272 and 449–490, respectively, from anterior extremity. Ventriculus 54–82 long and 82–109 wide, length of ventricular appendix 1.40–2.82 mm. Intestinal caecum 68–109 long. Length ratio of caecum and ventricular appendix 1:12–36, that of caecum and oesophageal length 1:2–3. Spicules alate, equal, 429–636 long (2.7–3.7% of body length), with cup-like proximal and narrow distal ends (Fig. 1 B, G). Genital papillae sessile, about 21 pairs. Preanal pairs 16, becoming closer together and more median when approaching cloacal opening; last 10–11 pairs of preanals distinctly smaller than papillae of more anterior pairs; additional, unpaired median preanal papilla present anterior to cloacal opening. One pair of small adanal papillae present (Fig. 2 H). Postanal pairs 4, in two subventral

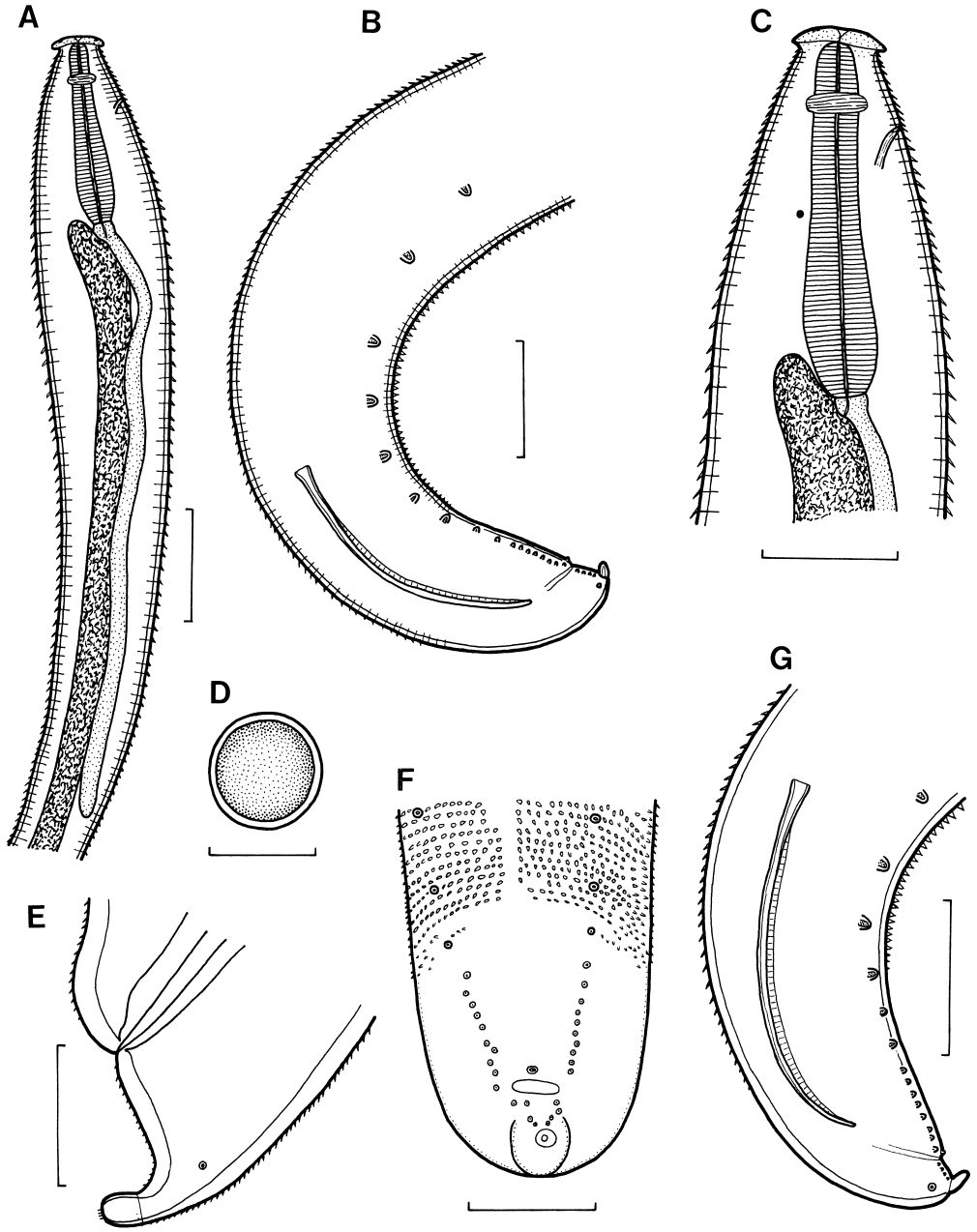

Fig. 1. *Goezia spinulosa* (Diesing, 1839). **A** – anterior part of male body, lateral view; **B** – posterior end of male, lateral view; **C** – anterior end of male, lateral view; **D** – egg; **E** – tail of gravid female, lateral view; **F**, **G** – caudal end of male, ventral and lateral views. Scale bars: A = 500 μm; B, G = 200 μm; C = 300 μm; D = 30 μm; E, F = 100 μm.

rows; first pair more median, situated immediately posterior to cloacal opening, papillae of second pair appearing to be doubled (Figs. 1 F, G, 3 D–H). Tail conical, 78–114 long including digitiform process 12–18 long, without any terminal spinous structures (Fig. 3 F, G). Phasmids situated laterally at base of caudal process (Fig. 3 F).

Female (5 gravid specimens): Body 21.91–23.33 mm long and 625–694 wide. Spination of cuticle throughout body length including digitiform process of tail; rings of spines interrupted by narrow lateral smooth fields at anal region (Fig. 2 H). Maximum length of spines 27–30. Length (height) of lips 54, width of body at level of lips 231–258. Oesophagus 0.99–1.07 mm long (4–5% of body length). Nerve ring, excretory pore and deirids 177–204, 258–272 and 448–454, respectively, from anterior extremity. Ventriculus measuring 68 × 109–122, length of ventricular appendix 1.73–3.17. Intestinal caecum 95–98 long. Length ratio of caecum and ventricular appendix 1:32–33, that of caecum and oesophageal length 1:1.7–3.0. Rectum hyaline tube. Vulva opening 8.36–9.79 mm

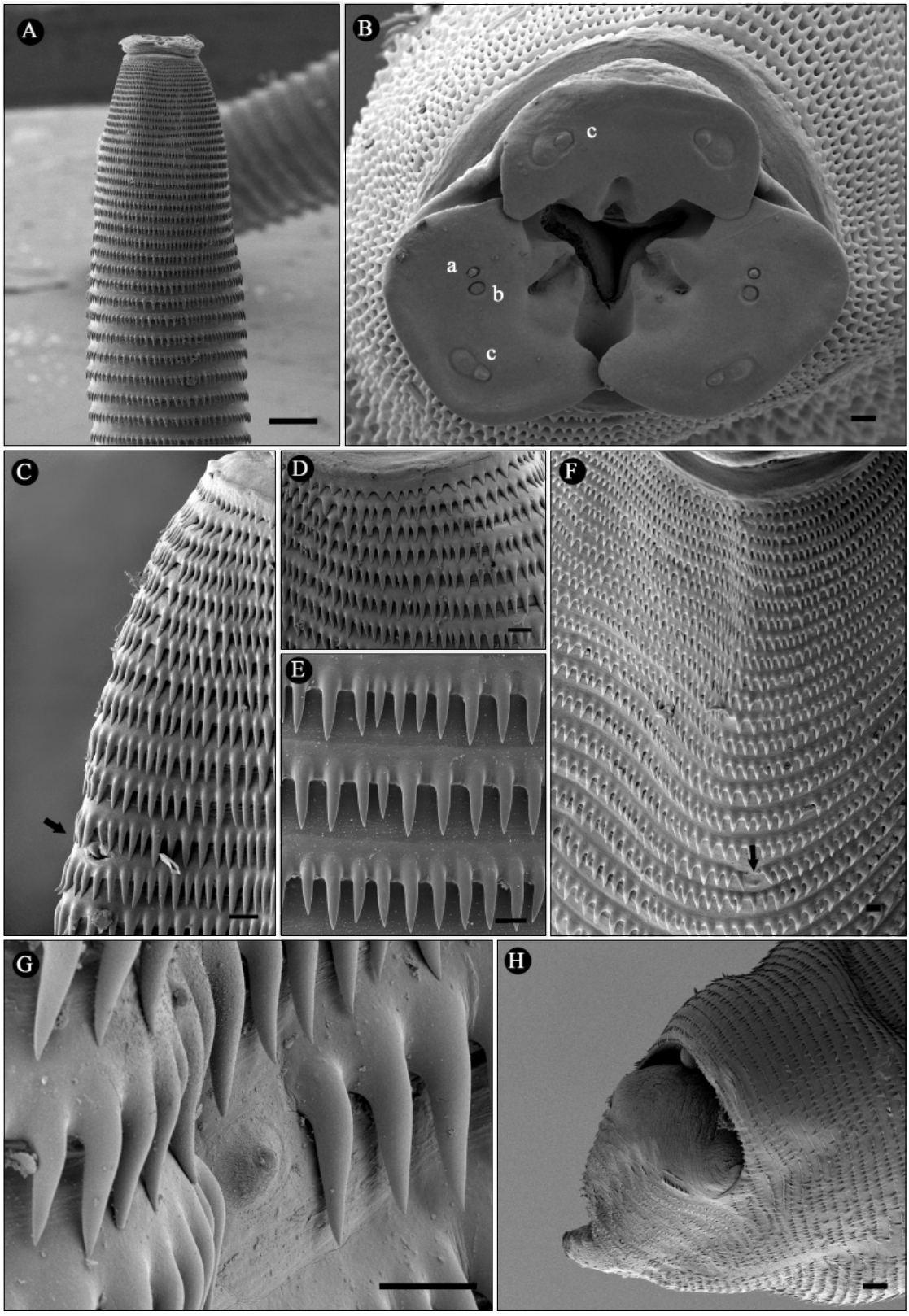

Fig. 2. *Goezia spinulosa* (Diesing, 1839), scanning electron micrographs. **A** – anterior end of body, lateral view; **B** – cephalic end, apical view; **C** – location of excretory pore (arrow) between 12th and 13th rows of spines; **D**, **E** – cuticular spines at anteriormost rows and those in more posterior region, respectively; **F** – location of deirid (arrow) at level of 20th row of spines; **G** – detail of deirid; **H** – female tail, sublateral view. *Abbreviations*: a – amphid; b – single labial papilla; c – double labial papilla. Scale bars: A = 100 μm; B–H = 10 μm.

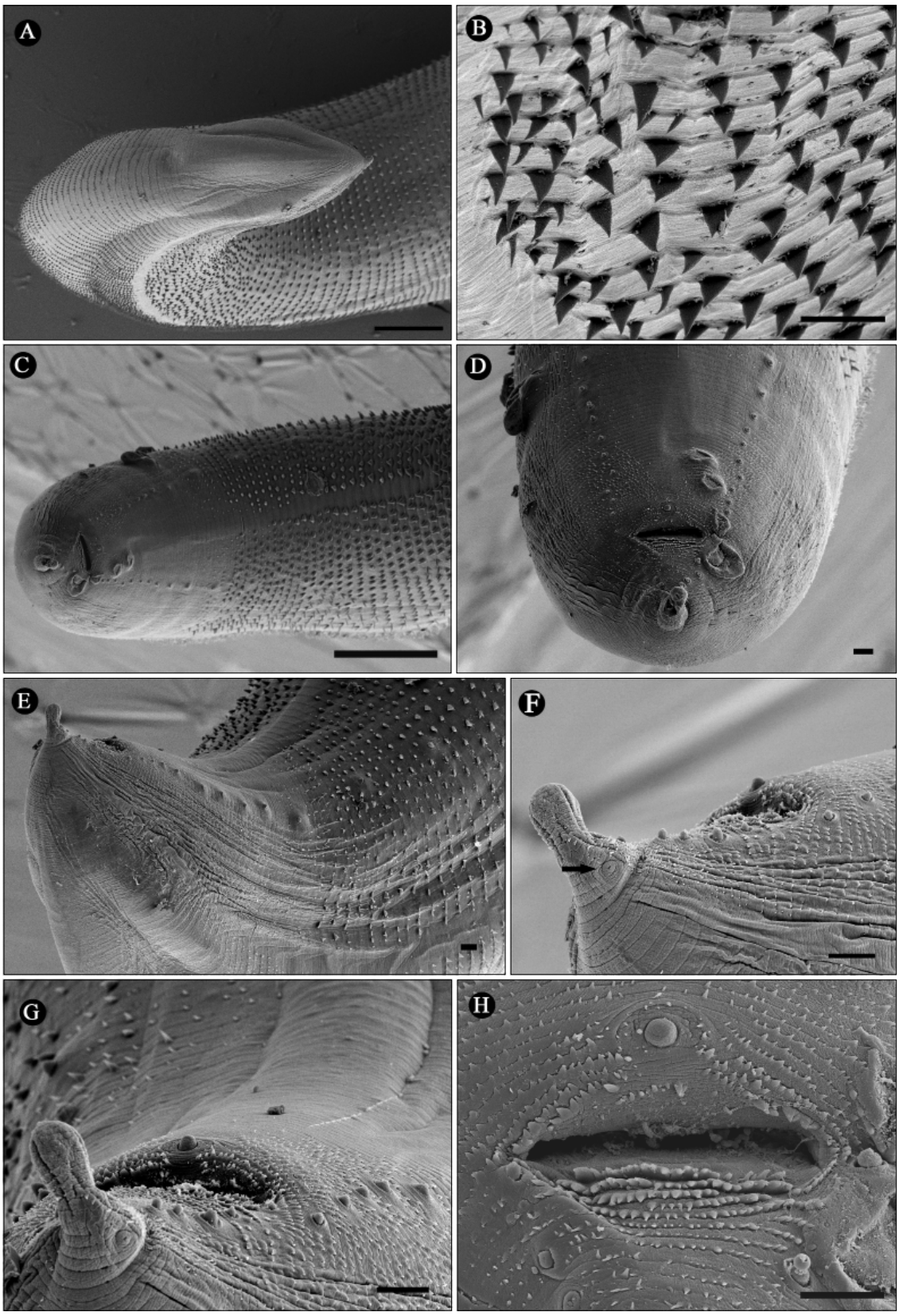

Fig. 3. *Goezia spinulosa* (Diesing, 1839), scanning electron micrographs. **A** – caudal end of male, dorsal view; **B** – spines on ventral precloacal region; **C** – posterior end of male, ventral view; **D**, **E** – male caudal end, ventral and lateral views; **F**, **G** – region of cloaca, lateral and ventrolateral views (arrow indicates phasmid); **H** – cloacal opening, ventral view. Scale bars: A, C – 100 μm; B, D–H = 10 μm.

or 36–44% of body length from anterior extremity; vulval lips not elevated. Vagina directed posteriorly from vulva. Eggs with smooth thin shell, almost spherical, 36–39 in diameter, with uncleaved content (Fig. 1 D). Tail conical, 147–150 including digitiform process 33–41 long; latter with about 4–5 minute terminal spinous structures (Figs. 1 E, 2 H). Phasmids situated somewhat anteriorly to caudal process (Figs. 1 E, 2 H).

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

Sites of infection: Adults mainly in stomach, less often in intestine; larvae in digestive tract and mostly encapsulated in body cavity (mesentery, surface of stomach and pyloric caeca).

Locality: Fazenda Santo Ambrosio (00°05'30"S, 49°34'50"W), Mexiana Island (Amazon River Delta), State of Pará, Brazil.

Prevalence and intensity: 90% (27 fish infected/30 fish examined); 1–228 nematodes per fish.

Deposition of specimens: Instituto Oswaldo Cruz, Rio de Janeiro (CHIOC 35612); Helminthological Collection of the Institute of Parasitology, Biology Centre of ASCR, České Budějovice (Cat. No. N-911).

Occurrence of *Goezia spinulosa* in wild and cultured arapaimas

Goezia spinulosa seems to be one of the most pathogenic parasites of tank-reared arapaimas in Mexiana Island. Marked differences were observed in the degree of infection between large fish (body length 70–175 cm) and conspecific fingerlings (6.5–15 cm). Large fish harboured adult and larval *G. spinulosa* located mainly inside the stomach, with the intensity of 4–228 (mean 54) nematodes per fish; only two adult fish had 1 and 3 nematodes in the intestine. In contrast to large fish, fingerlings were parasitized exclusively by *G. spinulosa* larvae and juveniles, which were mostly found encapsulated on the surface of the stomach and pyloric caeca and in the host's mesentery (smaller larvae), less frequently in the lumen of the stomach (larger larvae and subadult individuals). The intensity in fingerlings ranged within 1–50 (mean 18) parasites, with a total prevalence of 94%.

In small arapaimas the stomach tissue is thin and highly liable to the attachment of *G. spinulosa* which frequently causes perforations, penetrating the mesentery of the fish (Fig. 4 E). The stomach wall of larger fish is thick (Fig. 4 A) and adults of *G. spinulosa* were observed to penetrate deeply into the stomach mucosa to cause ulcers sometimes with more than 50 nematodes involved (Fig. 4 B–D), but without perforations into the mesentery.

Other nematode parasite recorded from *Arapaima gigas*

In addition to *Goezia spinulosa* and the above mentioned *Camallanus tridentatus*, *Capillostrongyloides arapaimae*, *Nilonema senticosum* and *Rumai rumai*, all common parasites of *A. gigas* in this locality, the following larval nematode was rarely also recorded from this fish host:

Eustrongylides sp. larvae Fig. 4 F

Larvae of the genus *Eustrongylides* Jägerskiöld, 1909 (Dioctophymatidae) were found in swellings on the body surface of a cultured arapaima fingerling (body length 7.9 cm) (prevalence in fingerlings 6%: 1 infected/18 examined; intensity 2).

DISCUSSION

Even though *Goezia spinulosa* was several times described based on specimens from the type host *Arapaima gigas* (see above), some taxonomically important characters remained undescribed or were described inaccurately; for example, it is interesting that such an important feature as the position of the excretory pore has not been described in this species to date. Costa et al. (1995) gave a good description of *G. spinulosa* based on SEM examination, but they also failed to observe the excretory pore; otherwise, the present study can confirm most features found by them (sometimes with slight differences). Thus, for the first time, the excretory pore, the phasmids of the male, and the characteristic spination of the cloacal opening are described. Although Costa et al. (1995) reported six pairs of postanal papillae (as also did Freitas and Lent 1946) in this species, Fig. 3 D–H of this paper indicates that only four pairs are present; in fact, only four pairs of postanals are visible in fig. 8 in the paper by Costa et al. (1995); the formation at the level of the largest (double?) papilla designated as a papilla on their fig. 9 may well be an artifact, because no such formations are visible on Fig. 3 F–H of this paper. Only four postanal pairs are also reported for other South American *Goezia* spp.

To date, five species of *Goezia* Zeder, 1800 are known from freshwater fishes in South America: *G. spinulosa*, *G. intermedia* Rasheed, 1965, *G. brasiliensis* Moravec, Kohn et Fernandes, 1994, *G. brevicaeca* Moravec, Kohn et Fernandes, 1994, and *G. leporini* Martins et Yoshitoshi, 2003 (see Moravec 1998, Martins and Yoshitoshi 2003, Thatcher 2006). Whereas the first species has been mainly reported from *Arapaima gigas* (Arapaimidae, Osteoglossiformes) from Brazil and Peru, *G. intermedia* is known from *Cichla ocellaris* Schneider (Cichlidae, Perciformes) in Guyana, both *G. brasiliensis* and *G. brevicaeca* mainly from *Brycon hilarii* (Valenciennes) (Characidae, Characiformes) in the Paraná River basin in Brazil, and *G. leporini* from *Leporinus macrocephalus* Garavello et Britski (Anostomidae, Characiformes) also in the Paraná River basin in Brazil. *Goezia brasiliensis* differs from *G. spinulosa* mainly in much longer (802 μm) spicules and less numerous (10) pairs of preanal papillae, whereas both *G. brevicaeca* and *G. intermedia* differ in distinctly shorter spicules not exceeding 410 μm; moreover, *G. brevicae-*

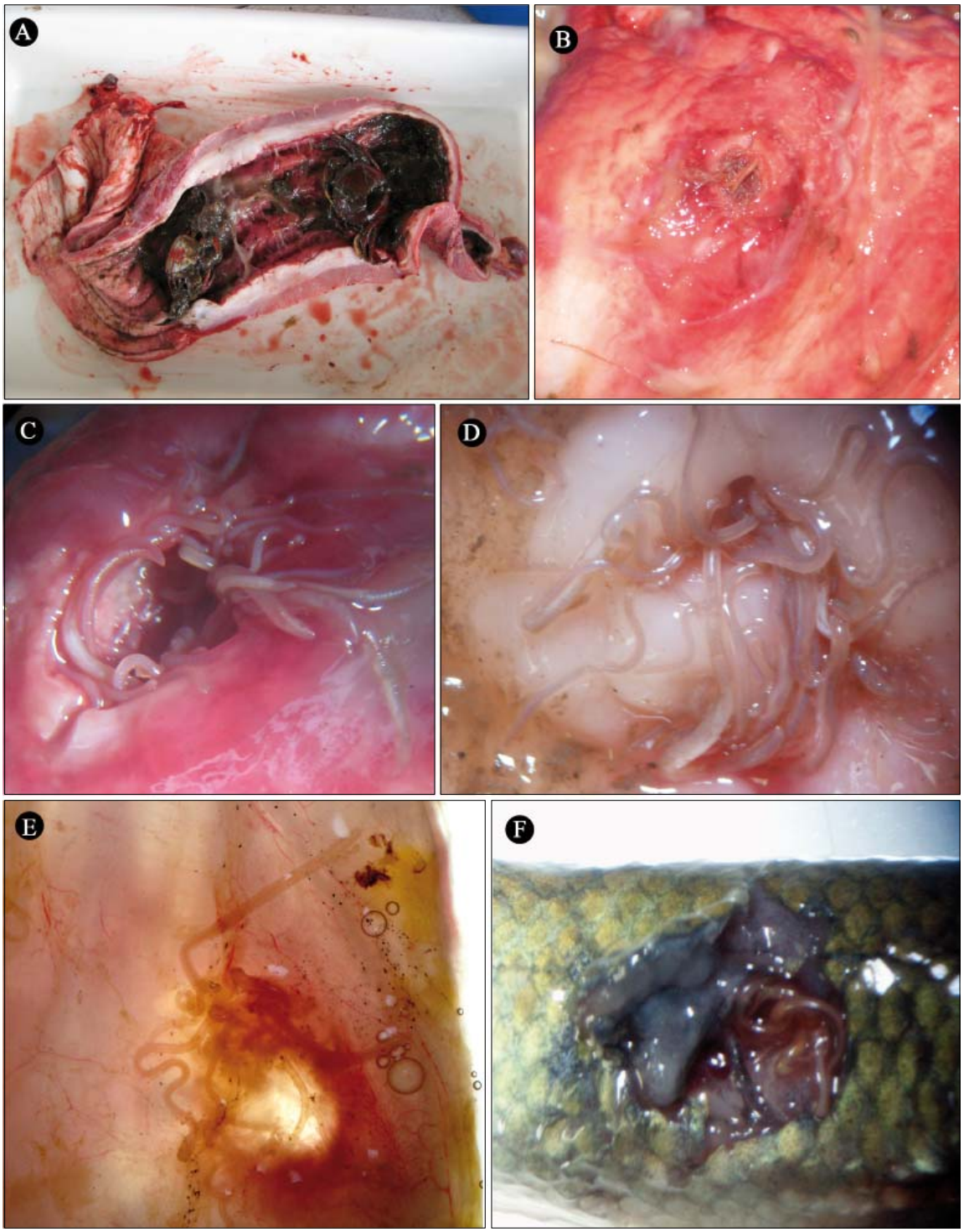

Fig. 4. A–E – *Goezia spinulosa* in stomach of arapaima. **A** – cut-open stomach in large arapaima to show its thick walls (×0.25); **B** – low ulcer in large arapaima with *G. spinulosa* specimens (×2); **C**, **D** – deep ulcers in large arapaimas with concentrations of many *Goezia* specimens (both ×6); **E** – perforation of stomach wall by *Goezia* specimens in small arapaima (elver) (×4). **F** – *Eustrongylides* sp. larva inside of open tumour on skin of arapaima elver (×5).

ca possesses an intestinal caecum not reaching the end of the oesophagus, and *G. intermedia* has a markedly plump body and a thick, rounded female tail. *Goezia leporini* differs from *G. spinulosa* mainly in having more pairs (18–23) of preanal papillae and probably also in possessing well-developed cuticular spines surrounding the unpaired preanal papilla.

As noted, the life cycle of *Goezia spinulosa* is unknown. Of many described *Goezia* spp., it was only studied by Mozgovoy et al. (1971) in *G. ascaroides* (Goeze, 1782), a parasite of the European catfish *Silurus glanis* Linnaeus, and it can be expected that the life-cycle pattern of *G. spinulosa* is similar. According to Mozgovoy et al. (1971), the gravid *G. ascaroides* females are localized inside the swellings in the stomach wall of catfishes where they also lay their eggs; these pass through the aperture in the swelling into the stomach from where they progress through the intestine and are released, along with the host's faeces, into the water. The eggs in water develop further and after 3–4 days (at 25–28 °C) the second-stage larvae hatch from egg shells. The freely swimming larvae are eaten by the copepod intermediate host, *Diaptomus castor* (Jurine), in the body cavity of which they undergo their second moult and change into the third-stage larvae; these are already infective for the fish. After 15 days in the copepod, the larvae are 1.65–1.71 mm long.

Various species of fishes including small catfishes serve as paratenic hosts of *G. ascaroides*, in which they are localized in the wall of the stomach and intestine, usually in individual capsules or groups of capsules, sometimes in huge numbers. The definitive host (catfish) acquires infection either directly by ingestion of infected intermediate host (copepod) or paratenic host (forage fish). In the definitive host, the nematode larvae undergo two more moults before they become mature. The prepatent period of *G. ascaroides* was estimated to be about two months.

The similarity of the *G. spinulosa* life cycle with that of *G. ascaroides* is, for example, apparent from that both species utilize congeneric intermediate hosts (*Diaptomus* spp.) and their infective larvae from copepods are of approximately the same size; Freitas and Lent (1946) considered such larvae from copepods to be second-stage larvae, but they did not study their development; the body length of these larvae indicates that, in fact, they represented the third larval stage.

The present results indicate that only larger arapaimas serve as the definitive hosts of *G. spinulosa*, being mostly infected by feeding on fish paratenic hosts (small forage fishes), whereas arapaima fingerlings serve largely only as the parasite's paratenic hosts, the only source of infection of which is the infected plankton (copepods harbouring infective larvae). The presence of small *G. spinulosa* larvae, frequently in large numbers, in the abdominal cavity of arapaima fingerlings suggests that, after ingestion of infected plankton, the liberated third-stage nematode larvae penetrate through the gut wall to encapsulate in the body cavity of the fingerlings; this is probably possible due to weak defence reactions of the host's organism and to thin walls of the digestive tube of the fingerlings. As Moravec (1970) observed in another raphidascaridid nematode, *Raphidascaris acus* (Bloch, 1779), the phase when its infective larvae penetrated in large numbers through the intestinal wall of small fish intermediate hosts was followed by a shock reaction of the fish and might bring about the fish death. Mozgovoy et al. (1971) observed high numbers of *G. ascaroides* larvae encapsulated in the wall of the stomach and the intestine of young catfishes, with up to 8–12 capsules on 1 cm^2 of the intestinal wall. It is not known how long these larvae remain viable, but after some time they are probably killed by the host's tissue reaction and are calcified as observed in *R. acus* (see Moravec 1970). No encapsulated *G. spinulosa* larvae were found in large specimens of *A. gigas* in this study.

Adults and larger juvenile individuals of *G. spinulosa*, mostly gathered in stomach swellings or ulcers, were found in larger *A. gigas*. Nematode juveniles were also recorded in the stomach mucosa, sometimes in swellings, in larger arapaima fingerlings (body length >15 cm); sometimes, the stomach wall was perforated in sites where swellings were formed. A perforation of the stomach associated with *G. ascaroides* in catfishes has also been reported by Mozgovoy et al. (1971).

Species of *Goezia* are generally considered to be pathogenic to their fish hosts (Moravec 1994). For example, Freitas and Lent (1946) observed high mortality of cultured *Astronotus ocellatus* due to *G. spinulosa* infection in Brazil, some data concerning the pathogenicity of *G. ascaroides* in the former Soviet Union were provided by Mozgovoy et al. (1971), fish mortalities associated with *Goezia* sp. were reported in central Florida, USA (see Moravec 1998), and the effects of *G. leporini* infection on the haematological characteristics of cultivated *Leporinus macrocephalus* were studied by Martins et al. (2004) in Brazil.

In the Mexiana Island, *G. spinulosa* seems to be one of the most pathogenic parasites of *A. gigas* (see above). It influences negatively the health condition of this fish, especially young fish, and contributes substantially to the high mortality of arapaima fingerlings in the culture. The most serious damage to the fish by this parasite is probably caused, in addition to other effects, by the migration of *G. spinulosa* third-stage larvae through the wall of the digestive tract and by the mechanical destruction of the stomach mucosa, with occasional perforations of the stomach wall. Since the only source of *G. spinulosa* infection for arapaima fry and fingerlings is the infected plankton collected in the localities inhabited by wild arapaimas, it would be possible to prevent this parasitosis in tank-reared arapaima fingerlings either by feeding them on artificial food (pellets), which they do not accept easily, or by sterilising their natural food (plankton and small forage fishes) by cooking or deep-freezing (around

-20 °C), which would kill the parasite's infective larvae. Initial attempts to keep arapaima fingerlings in culture tanks on cooked natural food during a few weeks resulted in a considerable decrease in their mortality (own data).

Arapaima fingerling represents a new host record for the *Eustrongylides* larvae found inside swellings on the body surface; the fish may be considered a second intermediate or paratenic host. Adult *Eustrongylides* spp. are common parasites in the proventriculus of fish-eating birds. Aquatic oligochaetes serve as intermediate hosts, whereas different fishes act as the second intermediate or paratenic hosts (Moravec 1998, Anderson 2000). Fourth-stage larvae of *Eustrongylides* are occasionally found as the parasites of humans after being ingested along with raw fish (Eberhard et al. 1989).

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