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On the morphology and surface ultrastructure of some parasitic nematodes (Nematoda) of birds (Aves)

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Abstract. The morphology of four species of avian nematodes was studied using scanning electron microscopy (SEM): *Porrocaecum depressum* (Zeder, 1800) from *Buteo buteo*; third- and fourth-stage larvae of *Porrocaecum semiteres* (Zeder, 1800) from *Larus ridibundus* and *Turdus philomelos*; *Acuaria anthuris* (Rudolphi, 1819) from *Corvus frugilegus*; *Cosmocephalus obvelatus* (Creplin, 1825) from *Larus ridibundus*. The examination of the head end of adult *Porrocaecum depressum* and fourth-stage larvae of *P. semiteres* revealed a pattern of labial papillae that is typical of ascaridoid genera. The structure of the head end of third- and fourth-stage larvae of *P. semiteres* seems to be identical with that of the related *P. ensicaudatum*, which occurs in the same species of intermediate and definite host. The fourth-stage larvae of *P. semiteres* was redescribed. A detailed examination of the oral region of *Acuaria anthuris* revealed teeth at the anterior end of the cordons, which may serve to host tissue damage during feeding. The deirids of *A. anthuris* are very small, with a bicuspid tip.

Morphology, surface ultrastructure, nematode, parasite, Aves

INTRODUCTION

The taxonomy of parasitic nematodes of birds (Aves) is often based on incomplete descriptions and drawings. During a recent study of some materials from birds (Frantová 2002) provided by the Helminthological Collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic (ASCR), in České Budějovice, morphology of four common species of nematodes was studied in detail, using light microscopy and scanning electron microscopy (SEM): *Porrocaecum depressum*, *P. semiteres*, *Acuaria anthuris* and *Cosmocephalus obvelatus*.

Porrocaecum depressum is a common parasite of birds of prey (Falconiformes, Strigiformes) and its morphology had been described several times using light microscopy (see Mozgovoy 1953, Hartwich 1975). *P. semiteres* is typical of birds of the order Charadriiformes. It frequently occurs as a third- or fourth-stage larva in the digestive tract of small passerines (especially Turdidae), but is unable to mature in these atypical hosts (Iygis 1967). Descriptions and drawings of third-stage larvae are included in the works of Mozgovoy & Bishaeva (1959), Iygis (1967) and Moravec (1971). There are few accounts of the morphology of the fourth-stage larvae (Iygis 1967). A related species, *P. ensicaudatum* (Zeder, 1800), also uses small passerines (especially Turdidae) as definitive hosts, but is also found as larvae in Charadriiformes (Iygis 1967, 1970). Consequently, larvae of *P. ensicaudatum* and *P. semiteres* can occur in the same species of avian host. They can be distinguished by the ratio of the stomach to the intestinal caecum length (Supryaga & Supryaga 1971, Baruš et al. 1978b). In the genus *Porrocaecum* Railliet et Henry, 1912, only *P. ensicaudatum* was examined using SEM (Wharton 1978, Baruš et al. 1983, McNeill & Anderson 1990a, b).

Tab. 1. Nematodes studied with the aid of SEM

nematode species	host	site	locality
Porrocaecum depressum	*Accipiter gentilis* *Buteo buteo*	duodenum	České Budějovice (1980)
Porrocaecum semiteres, 3rd- and 4th-stage larvae	*Larus ridibundus* *Turdus philomelos*	gizzard	Praha (1978) Klec (1982, 1983) České Budějovice (1999)
Acuaria anthuris	*Corvus frugilegus* L.	gizzard	České Budějovice (1981, 1982)
Cosmocephalus obvelatus	*Larus ridibundus*	oesophagus	Klec

The ultrastructure of the head end larval *P. ensicaudatum* was studied by McNeill & Anderson (1990a, b). The present study focuses on the head end of larval *P. semiteres* with the aim of finding differences.

Acuaria anthuris is a frequent parasite in gizzards of corvids (Corvidae). There are several light microscopy studies that deal with the morphology of this species (see Chabaud & Petter 1961, Skryabin et al. 1965, Baruš et al. 1972). The cordon ultrastructure was studied by Baruš & Majumdar (1975).

Cosmocephalus obvelatus is a parasite of the oesophagus of various piscivorous birds. Its morphology has been studied using light microscopy (Skryabin et al. 1965, Baruš et al. 1978a, Anderson & Wong 1981). SEM micrographs of second- and third-stage larvae of *C. obvelatus* were published by Wong & Anderson (1982).

MATERIALS AND METHODS

All of the nematodes studied were provided by the Helminthological Collection of the Institute of Parasitology, ASCR, České Budějovice. The parasites were collected from birds shot at localities in South Bohemia, Czech Republic, from1977–1983 (Tab. 1). They were fixed and stored in 4% formaldehyde and, after being cleared in glycerine, were identified under an optical microscope. Drawings were made with the aid of a Zeiss microscope drawing attachment. Prior to SEM examination, the specimens selected were washed in 4% formaldehyde, postfixed for 2 hours in 2% aqueous osmium tetraoxide, washed in double-distilled water, dehydrated through a series of increasing concentrations (10–100%) of ethyl alcohol, critical point dried using CO_2 and coated with gold palladium in the POLARON PS 100 sputter coater. The JEOL SEM-6300 scanning electron microscope was used to examine and photograph the specimens.

RESULTS

Family Ascarididae Baird, 1853

***Porrocaecum depressum* (Zeder, 1800) Baylis, 1920**
(Fig. 1)

DESCRIPTION (based on SEM examination of the head ends of 2 males and 2 females). Oral opening triradiate, surrounded by three massive labia (one dorsal and two subventral) and three small

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Fig. 1. *Porrocaecum depressum* (Zeder) from *Buteo buteo*. SEM micrographs. A – head end, apical view; B – inner margin of labium with detail of teeth; C – subventral labium, ventrolateral view. Note double papilla (dp), single papilla (sp) and amphid (a); D – dorsal labium, dorsal view. Note double papillae (dp).

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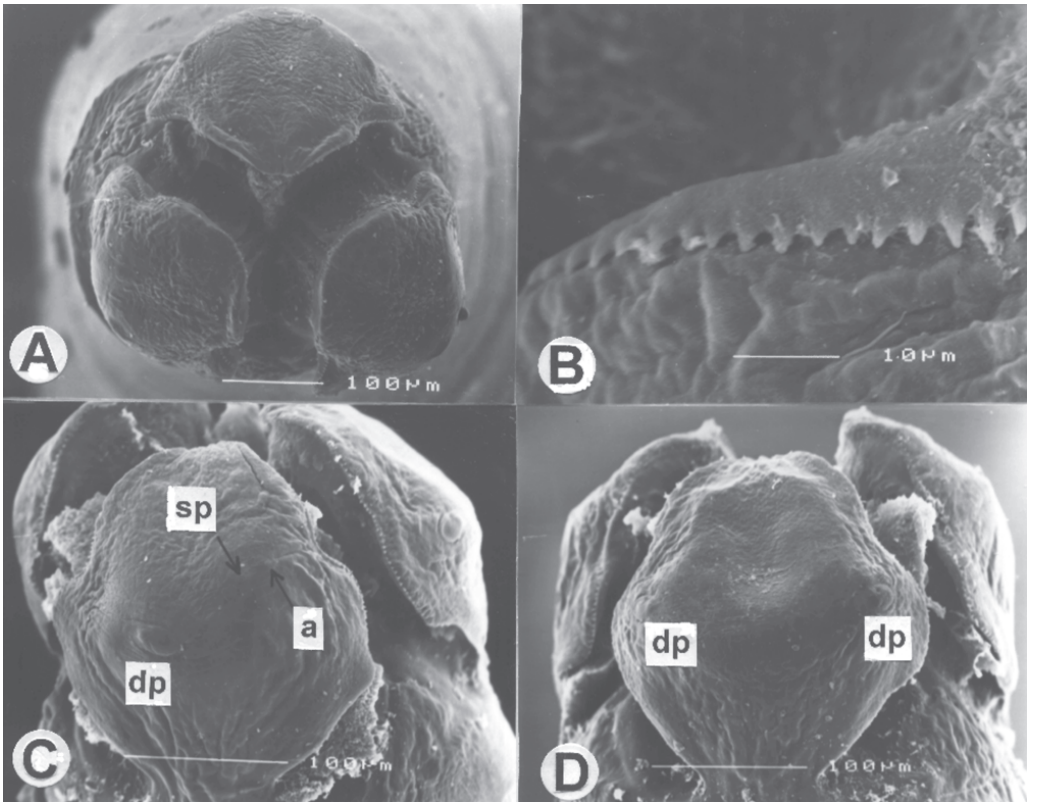
Fig. 2. *Porrocaecum semiteres* (Zeder) from *Larus ridibundus* (A) and *P. ensicaudatum* (Zeder) from *Turdus philomelos* (B). Third-stage larvae, lateral view. Scale bar in mm.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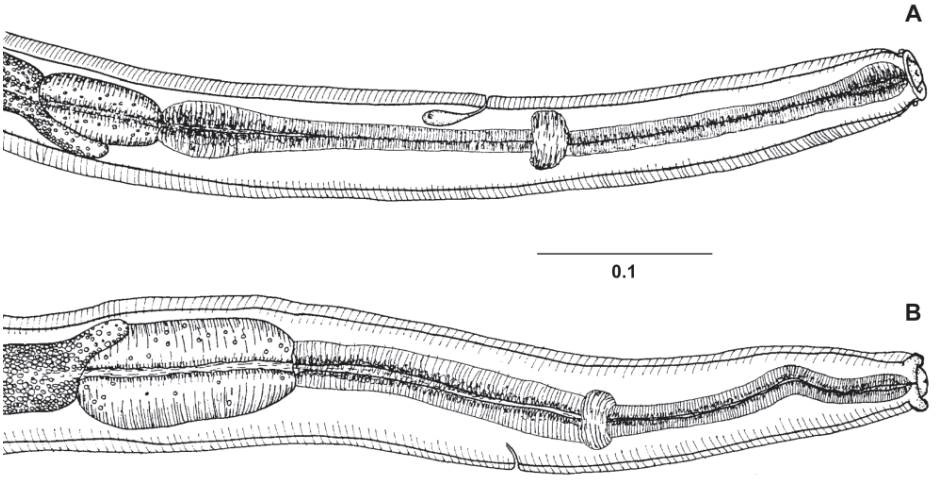
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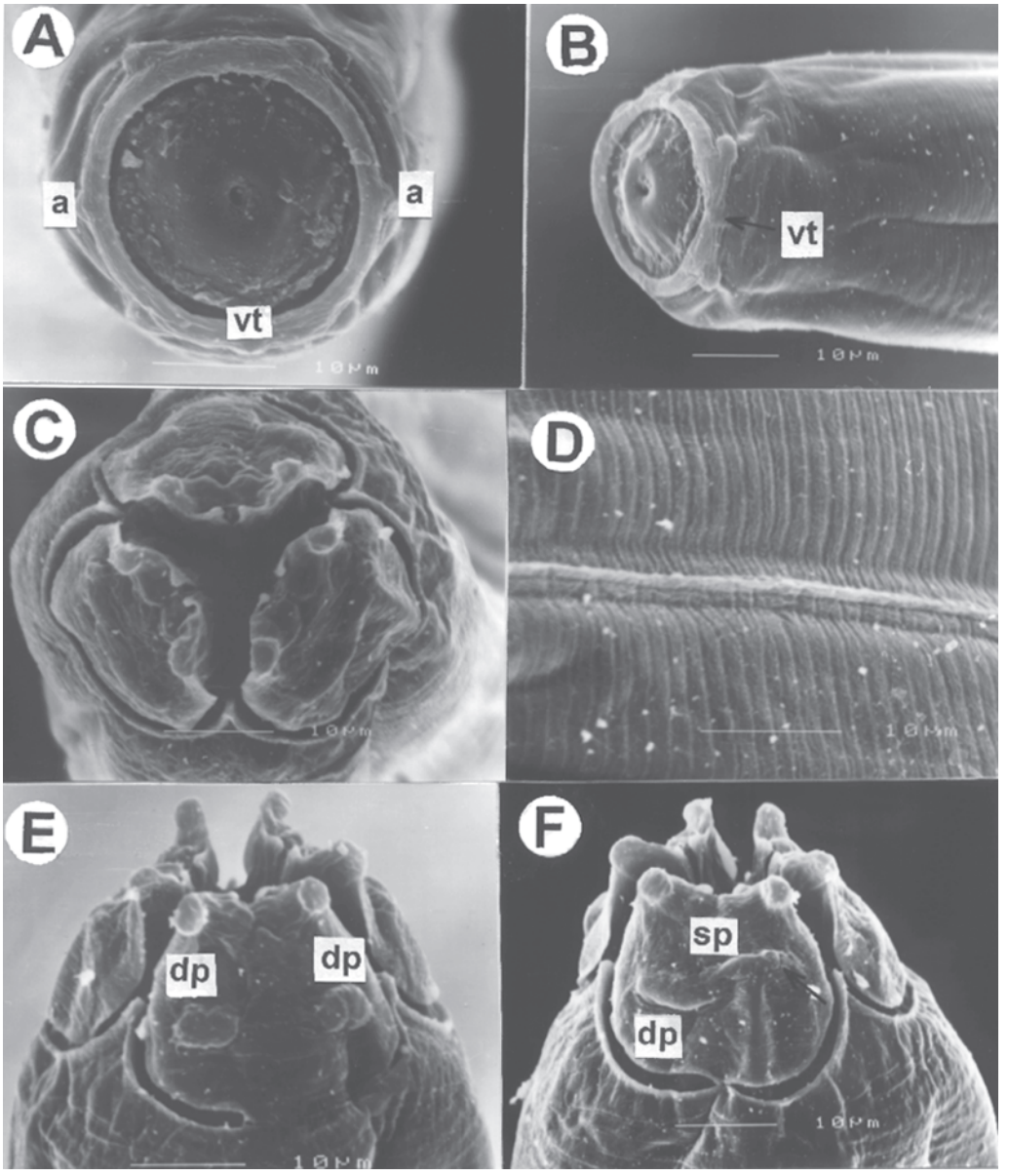

Fig 3. *Porrocaecum semiteres* (Zeder) from *Turdus philomelos* (A, B) and *Larus ridibundus* (C–F). SEM micrographs. A – cephalic extremity of third-stage larva, apical view. Note subdorsal and subventral papillae (arrows), amphids (a) and ventral tooth (vt); B – cephalic extremity of 3rd stage larva, ventral view. Note ventral tooth (vt); C – head end of 4th stage larva, apical view; D – lateral alae of 4th stage larva; E – dorsal labium of 4th stage larva. Note two double papillae (dp); F – subventral labium of 4th stage larva. Note double papilla (dp), single papilla (sp) and amphid (arrow).

Tab. 2. The dimensions (mm) of third- and fourth-stage larvae of *Porrocaecum semiteres* (Zeder, 1800) from *Larus ridibundus* and *Turdus philomelos*

	third-stage larvae	fourth-stage larvae male	female
body length	3.448 (3.376–3.520)	8.08 (6.82–9.34)	7.952 (3.712–12.192)
maximum width	0.088 (0.080–0.096)	0.216 (0.144–0.288)	0.200 (0.112–0.288)
nerve ring – anterior	0.217 (0.213–0.221)	0.300 (0.271–0.328)	0.289 (0.210–0.368)
excretory pore – anterior	0.219 (0.218–0.220)	0.364 (0.328–0.400)	0.351 (0.253–0.448)
length of muscular oesophagus	0.419 (0.410–0.428)	1.016 (0.784–1.248)	0.862 (0.435–1.288)
length of ventriculus	0.054 (0.045–0.063)	0.128 (0.105–0.151)	0.113 (0.076– 0.150)
intestinal caecum	0.038 (0.030–0.045)	0.059 (0.050–0.068)	0.108 (0.068–0.148)
ratio of length of ventriculus to that of caecum	1:0.70 (1:0.67–0.71)	1:0.67 (1:0.48–0.85)	1:0.9 (1:0.63–1:1.23)
genital primordium – anterior	1.746 (1.723–1.768)		
vulva – anterior			4.188 (2.096–6.280)
length of spicule primordia		0.134 (0.068–0.200)	
tail	0.145 (0.126–0.164)	0.164 (0.126–0.202)	0.199 (0.125–0.272)

interlabia (Fig. 1A). Cuticle of head end smooth. Labia hexagonal in shape, very narrow at base, widest in middle. Anterior half of labia divided by longitudinal shallow median depression into two lobes. Each lobe supported by a finger-like projection, bluntly terminating close to anterior margin of labium. Anterior margins of labia rounded, rimmed with single row of teeth (Fig. 1B). Teeth conical, with rounded tips, usually longer than wide, expanding laterally and caudally to last third of labia. Dorsal labium with two double papillae situated mediolaterally (Fig. 1D). Each subventral labium with one double papilla situated medioventrally, and a single papilla and amphid situated anterolaterally (Fig. 1C). Interlabia indistinct, triangular in shape, reaching first third of labia.
Comment. Based on light microscopy, the specimens studied showed the dimensions and morphology consistent with the description given by Mozgovoy (1953)

Porrocaecum semiteres (Zeder, 1800) Baylis, 1920
(Figs 2, 3, 4; Tab. 2)

Description. Third-stage larvae: 10 specimens studied using light microscopy (Fig. 2, Tab. 2), 2 of which were used for SEM (Figs 3A, B). Anterior end bluntly rounded, with smooth rounded circular rim of cuticle (Fig. 3A). Labia absent. Two single cephalic papillae subdorsally and two subventrally at base of cuticular rim. Two amphids situated laterally on either side. One ventral tooth present (Fig. 3A, B). Oral opening circular. Cuticle with distinct annulation and lateral alae. Excretory porus slightly posterior to nerve ring. Oesophagus with anterior muscular portion and posterior ventriculus. Intestine dark, with rudimentary, anteriorly directed dorsal caecum. Caecum longer than half the length of ventriculus. Ventral genital primordium positioned slightly posterior to mid body. Sexes not distinguishable. Anus subventral. Tail tapered and pointed.
Comment. Based on light microscopy, the specimens showed the dimensions and morphology consistent with the description given by Iygis (1967) and Okulewicz (1979). Larvae of *P. semiteres* were distinguished from those of the related *P. ensicaudatum* on the basis of the ratio of ventriculus and intestinal caecum lengths by Supryaga & Supryaga (1971) and Baruš et al. (1978b) (Fig. 2, Tab. 2).

Fourth-stage larvae (Figs 3C–F, 4): Oral opening triradiate. Three labia (one dorsal and two subventral) and three interlabia present (Fig. 3C). Labia widest at base, with anterior margins projecting in two horn-like processes. Dorsal labium with two prominent oval double papilae situated mediolaterally (Fig. 3E). Subventral labia with one medioventral double papilla and single papilla, and amphid situated mediolaterally (Fig. 3F). Three pointed interlabia, half the length of labia. Cuticle with distinct annulations. Lateral alae present from cervical region to last quarter of body (Fig. 3D). Excretory porus slightly posterior to nerve ring. Oesophagus with anterior muscular portion and posterior ventriculus. Intestine dark to light brown, with rudimentary, anteriorly directed dorsal caecum. Caecum longer than half length of ventriculus. Caudal extremity tapered, with rounded tip. One dorsal and two subventral large rectal cells present. Anus subventral.

Male: 10 specimens studied using light microscopy (Fig. 4E, Tab. 2), 2 of which were used for SEM. Two spicule primordia of similar shape and length.

Female: 10 specimens studied using light microscopy (Figs 4B, C, D, Tab. 2), 2 of them used for SEM. Vulva situated slightly anterior to midbody, uteri without eggs.

Comment. The ratio between the ventriculus and caecum lengths in the specimens studied corresponded to that given by Baruš et al. (1978b).

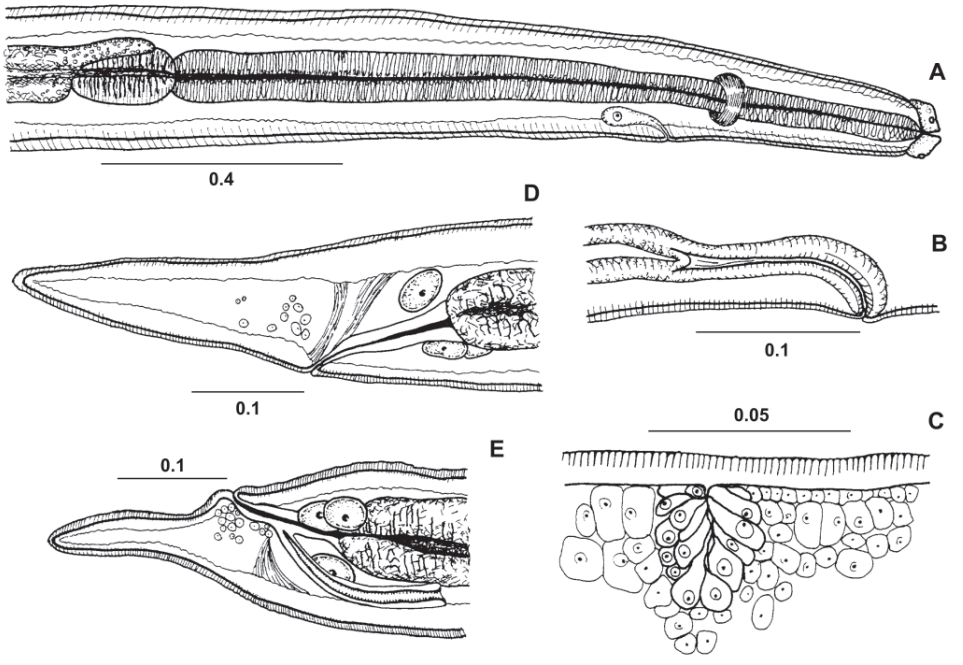

Fig. 4. *Porrocaecum semiteres* (Zeder) from *Larus ridibundus* and *Turdus philomelos*, fourth-stage larvae. A – cephalic extremity, lateral view; B – vulva region (in females 10.2–12.2 long); C – vulva region (in females 3.7–4.4 long); D – caudal extremity of female; E – caudal extremity of male. Scale bars in mm.

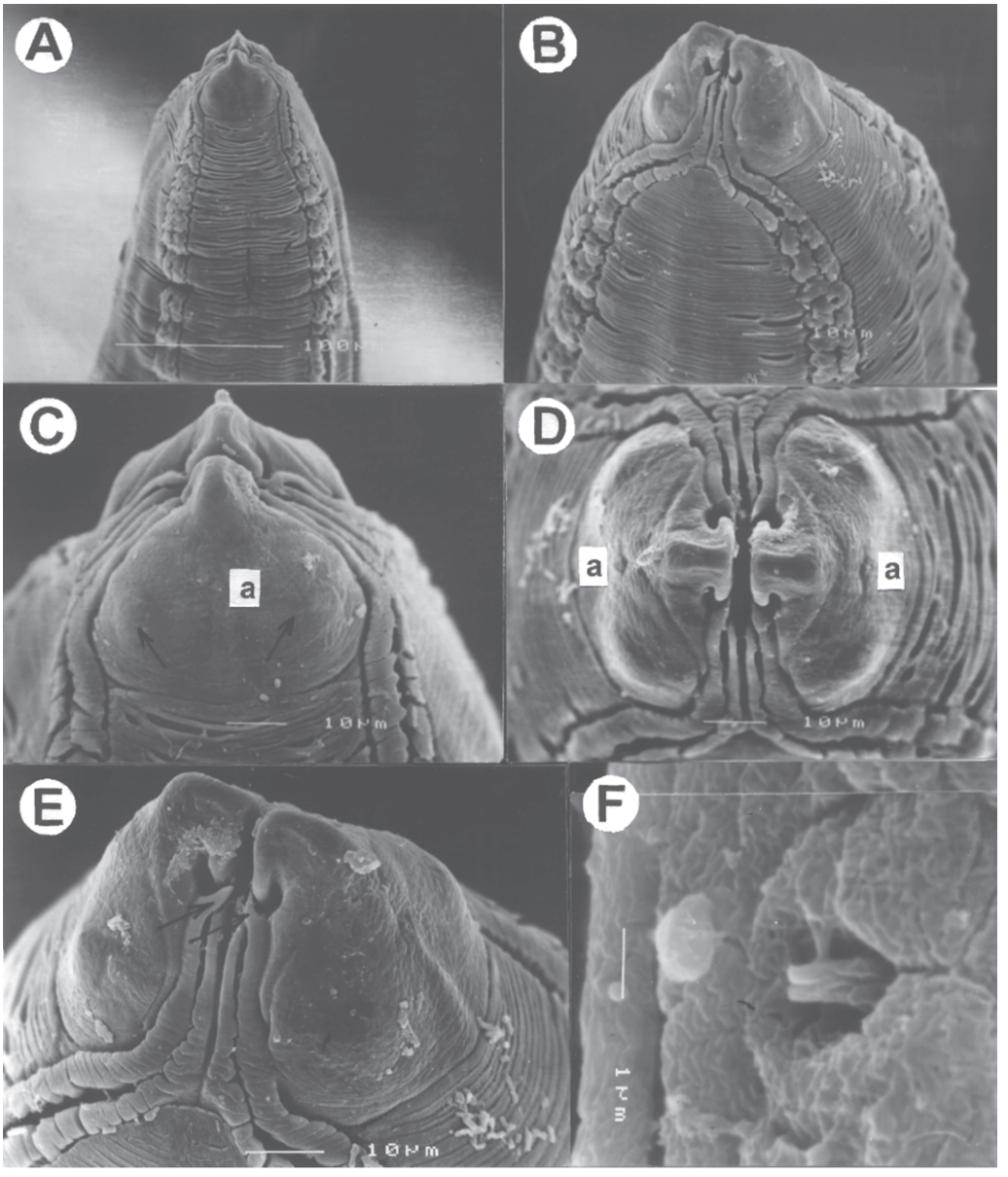

Fig. 5. *Acuaria anthuris* (Rudolphi) from *Corvus frugilegus*. SEM micrographs. A – cephalic extremity, lateral view. Note deirid (arrow); B – cephalic extremity, ventrolateral view; C – head end, lateral view. Note amphid (a) and labial papillae (arrows); D – head end, apical view. Note amphids (a); E – head end, ventrolateral view. Note cordons with anteriorly projecting teeth (arrows); F – deirid.

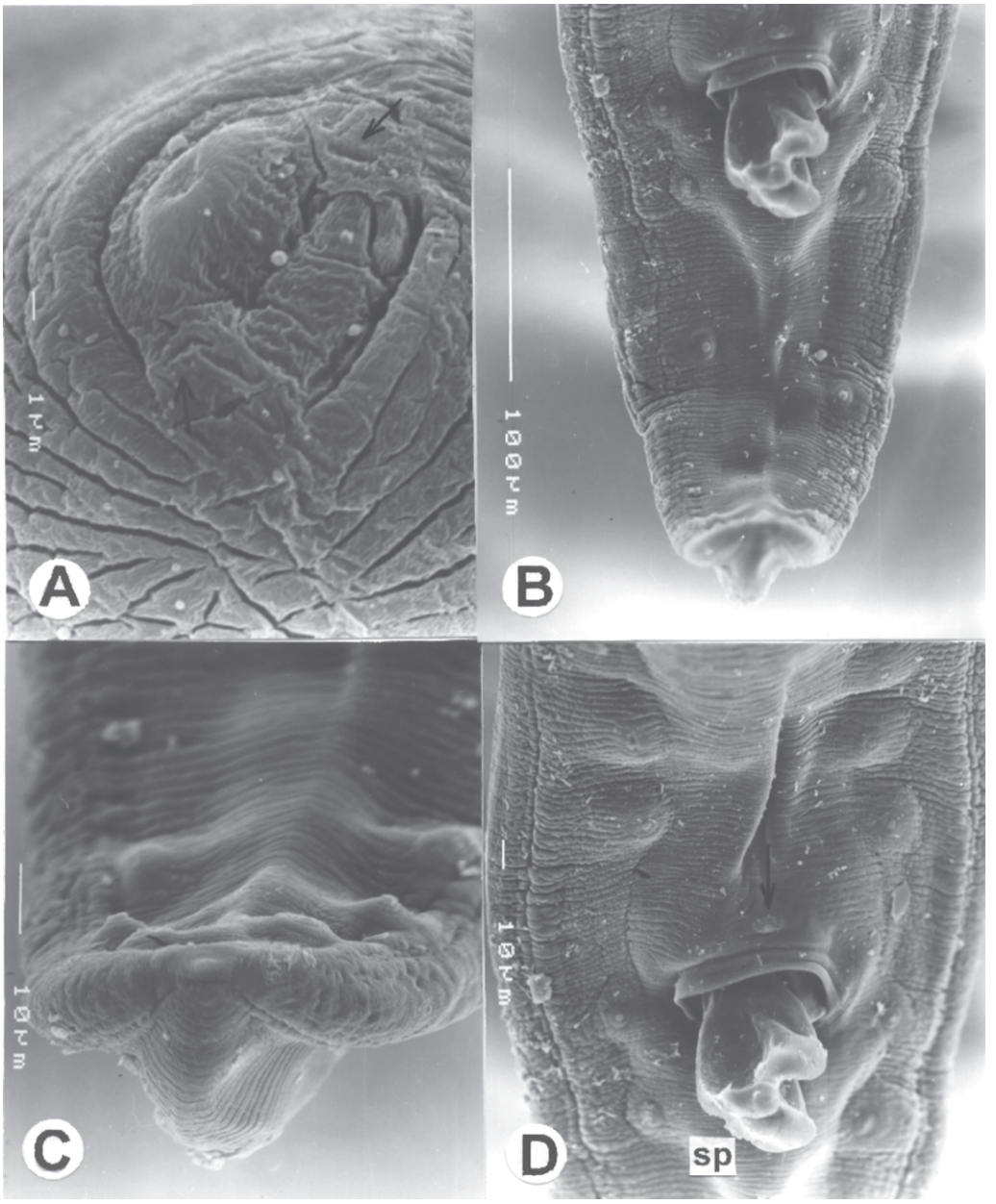

Fig. 6. *Acuaria anthuris* (Rudolphi) from *Corvus frugilegus*. SEM micrographs. A – tail of female, ventral view. Note phasmids (arrows); B – caudal extremity of male, ventral view. Single median precloacal papilla and three pairs of postcloacal papillae; C – tail of male, ventral view. Two pairs of postcloacal papillae and single pair of terminal phasmids; D – cloacal region of male. Note distal end of spicule (sp) and single median precloacal papilla (arrow).

Family Acuariidae Railliet, Henry et Sisoff, 1912

Acuaria anthuris (Rudolphi, 1819) Railliet, Henry et Sisoff, 1912
(Figs 5, 6)

Description (based on SEM examination of 2 male and 2 female adult specimens). Medium sized nematodes. Males smaller than females, with ventrally coiled tail. Cuticle with prominent annulation. Head end with two lateral pseudolabia, separated from rest of body by a transverse groove (Figs 5B, D, E). Pseudolabia massive, with smooth cuticle, triangular in shape, widest at base, tapering anteriorly to rounded tip. Each pseudolabium medially divided by a longitudinal ridge, extending approximately to second third of length of pseudolabia, to level of shallow transverse groove separating tips. Single prominent papillae laterally on both sides of pseudolabium (Figs 5C, E). Amphid situated medially in middle of pseudolabium (Fig. 5C). Tips free, apparently mobile. Distinct curve formed dorsally and ventrally at inner edge of each tip, giving it a nose-like appearance (Fig. 5E). Cordons in form of two laterodorsal and two lateroventral longitudinal cords, slightly exceeding body surface and separated from neighbouring cuticle by a deep grooves. Each cordon consisting of two parallel unconnected cords, transverselly divided into prominent ridges corresponding with annulation of neighbouring cuticle; first ridge at anterior end of both cords in form of tooth (Fig. 5E). Cordons beginning dorsally (ventrally) at level of curved inner edge of pseudolabial tip, then running between psedolabia and diverging laterodorsally (lateroventrally) at their bases; extending to second third of body. Deirids small, poorly visible, with bicuspid tip (Fig. 5F). Phasmids terminal (Figs 6A, C). Caudal extremity curved ventrally (Fig. 6B). Caudal lateral alae present, bearing four pairs of preanal and six pairs of postanal papillae. Two pairs of postanal papillae just posterior to cloaca, a solitary pair in middle of tail and three pairs near tip of tail (Figs 6B, C). Single papilla anteriomedian to cloaca (Fig. 6D).
Comment. The specimens were larger than with the dimensions given by Baruš et al. (1972) for nematodes from the same host.

Cosmocephalus obvelatus (Creplin, 1825) Seurat, 1919
(Figs 7, 8)

Description (based on SEM examination of 2 males and 1 female specimen). Medium sized nematodes. Cuticle with fine annulation. Lateral alae present, beginning at level of deirids and extending to posterior quarter of body (Fig. 7A). Two rather small lateral pseudolabia, rhomboid in shape, widest in middle, narrowing towards tips and bases (Figs 7B, C, G). Tips rounded and protruding (Figs 7D, E); two median ridges separating dorsal and ventral pore on each tip (Fig. 7F). Median part of pseudolabium convex, bearing prominent amphid (Fig. 7G). Median margins of pseudolabia projecting dorsally and ventrally into lobes (Fig. 7C), each with indistinct papilla. Two conspicuous cordons (dorsal and ventral) restricted to head end, with dense transverse striation; beginning dorsally (ventrally) to mouth, then copying shape of pseudolabia, running posteriorly, recurrent and anastomosing (Fig. 7B). Deirids large, with bicuspid tip (Fig. 8C). Excretory porus situated ventrally, slightly posterior to level of deirids (Fig. 8A). Prominent phasmids, situated subventrally near tip of tail (Fig. 8B). Caudal extremity of male with caudal alae bearing four pairs of preanal and five pairs of postanal pedunculate papillae (Figs 8D, E). Two pairs of small sessile papillae present at tip of tail, near last pair of pedunculate papillae. Two spicules, dissimilar in size and shape: right spicule short and massive, left long and slender. Caudal extremity of female tapered and round (Fig. 8B). Knob-like projection at tip of tail, clearly visible in light microscopy, indistinct (probably shrank or damaged during preparation).

COMMENT. These specimens were studied previously (Frantová 2002); they are smaller compared than the dimensions given by Anderson & Wong (1981).

DISCUSSION

Ascarididae

The present SEM study of the head end ultrastructure of *Porrocaecum depressum* confirmed, with few exceptions, the descriptions given by Mozgovoy (1953) and Hartwich (1975). There is a difference in the description of labial papillae, which are small and indistinct in this species, compared, for example, with *P. ensicaudatum* (Baruš et al. 1983, McNeill & Anderson 1990a, b). The papillae on the dorsal labium and the medioventral papilla on each subventral labium are double. There are a single papilla and an amphid anterolaterally on both subventral labia. The pattern of papillae is consistent with the above mentioned light microscopy studies and similar in all ascaridoid genera.

The head end ultrastructure of third- and fourth-stage larvae of *P. semiteres* did not differ from that of *P. ensicaudatum* (McNeill & Anderson 1990a, b). The cuticular rim with four single papillae, two ampids and a single ventral tooth in the third-stage larvae is replaced by three labia with four double and two single papillae, and two amphids in the fourth-stage larvae. The fourth-stage larvae have a pattern of labial papillae similar to that of the adults. The head end structure of adult *P. semiteres*, as seen in light microscope, seems to be almost identical with that of *P. ensicaudatum* (see Mozgovoy 1953, Hartwich 1975). Adults of both genera can be distinguished as lateral alae are absent in adult *P. ensicaudatum* and the ratio of the length of the ventriculus to that of the intestinal caecum is 1: 0.64–1.2 in *P. semiteres* (Baruš et al. 1978b) and 1 : 0.15–0.36 in *P. ensicaudatum* (Supryaga & Supryaga 1971). The ratio of the length of the ventriculus to that of the intestinal caecum seems to be the only difference between larval *P. ensicaudatum* and *P. semiteres*.

Acuariidae

Acuaria anthuris has been described several times using ligth microscopy (Chabaud & Petter 1961, Skryabin et al. 1965, Baruš et al. 1972). These descriptions differ mainly in the number of postanal papillae in the male. Chabaud & Petter (1961) stated that the number of the latter varied depending on the host: those from *Pica* and *Garrulus* had 6 pairs of postcloacal papillae and one pair of terminal phasmids; those from *Corvus* mostly 7 pairs of postcloacal papillae and one pair of phasmids, with rare specimens that have the same pattern as in *Pica* and *Garrulus*. Baruš et al. (1972) observed 7–8 pairs of postcloacal papillae in the nematodes parasitic in *Corvus frugilegus*. The present SEM study of specimens from *C. frugilegus*, revealed 6 pairs of postcloacal papillae and one pair of terminal phasmids in males; there were no difference in the specimens examined using light microscopy. The ultrastructure of the cordons was studied by Baruš & Majumdar (1975). The present study completes the previous description by adding details of the organization of the cordons at the level of the oral opening. The anterior ends of cordons project into teeth, which may serve to host tissue damage during feeding. Data on deirids is scarce, only their position has previously been mentioned (see Skryabin et al. 1965). The bicuspid tips are typical of *C. obvelatus* and other acuariid species, but there can be intraspecific variations in the shape of deirids and the number of tips (Moravec, unpubl. data). The SEM study of *C. obvelatus* fully confirmed the data presented by Anderson & Wong 1981).

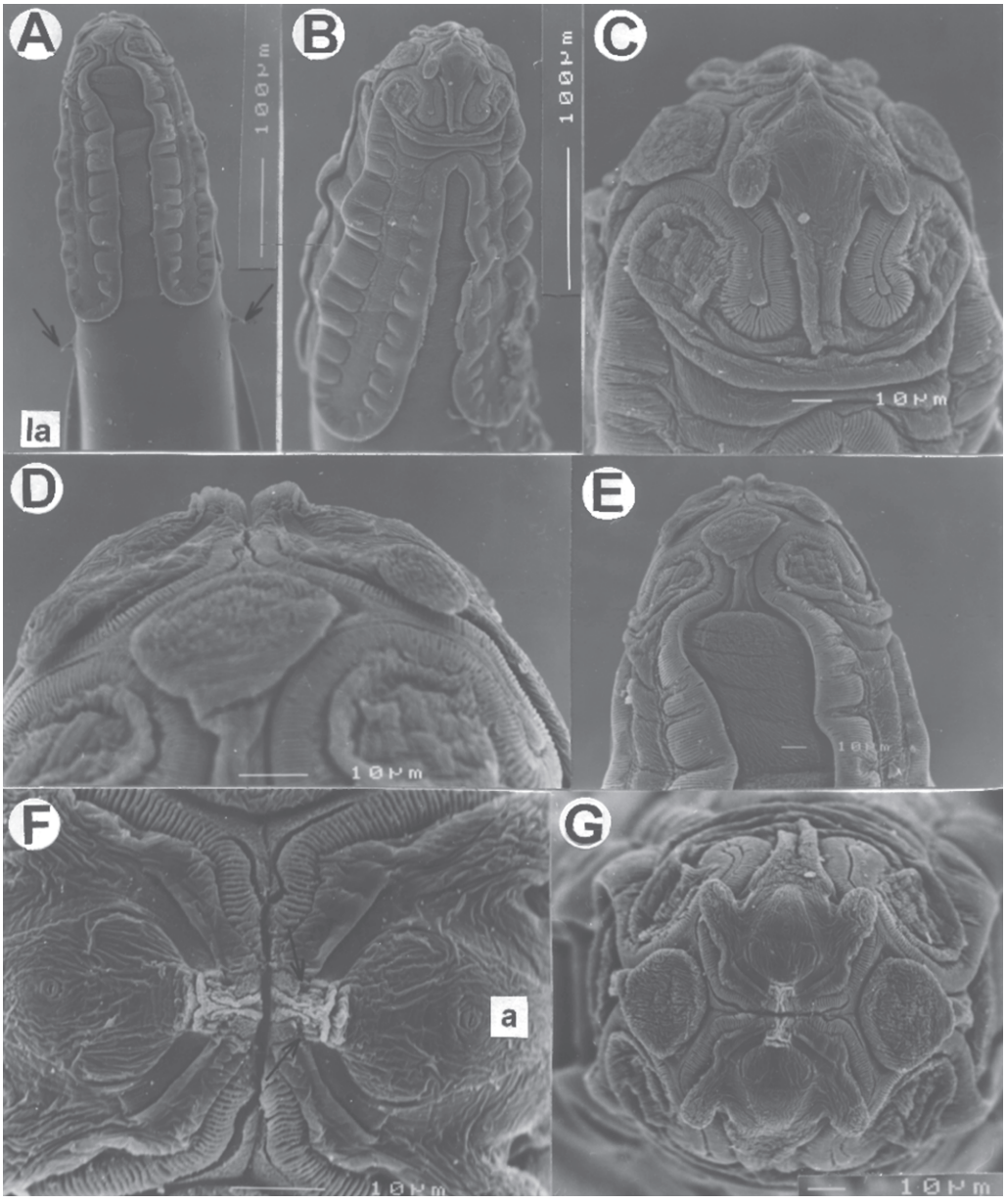

Fig. 7. *Cosmocephalus obvelatus* (Creplin) from *Larus ridibundus*. SEM micrographs. A – cephalic extremity, dorsoventral view. Note deirids (arrows) and lateral ala (la); B – head end, lateral view; C – detail of pseudolabium, lateral view; D – detail of pseudolabia, dorsoventral view; E – head end, dorsoventral view; F – detail of pseudolabia, apical view. Note pores (arrowhead) and amphid (a); G – head end, apical view.

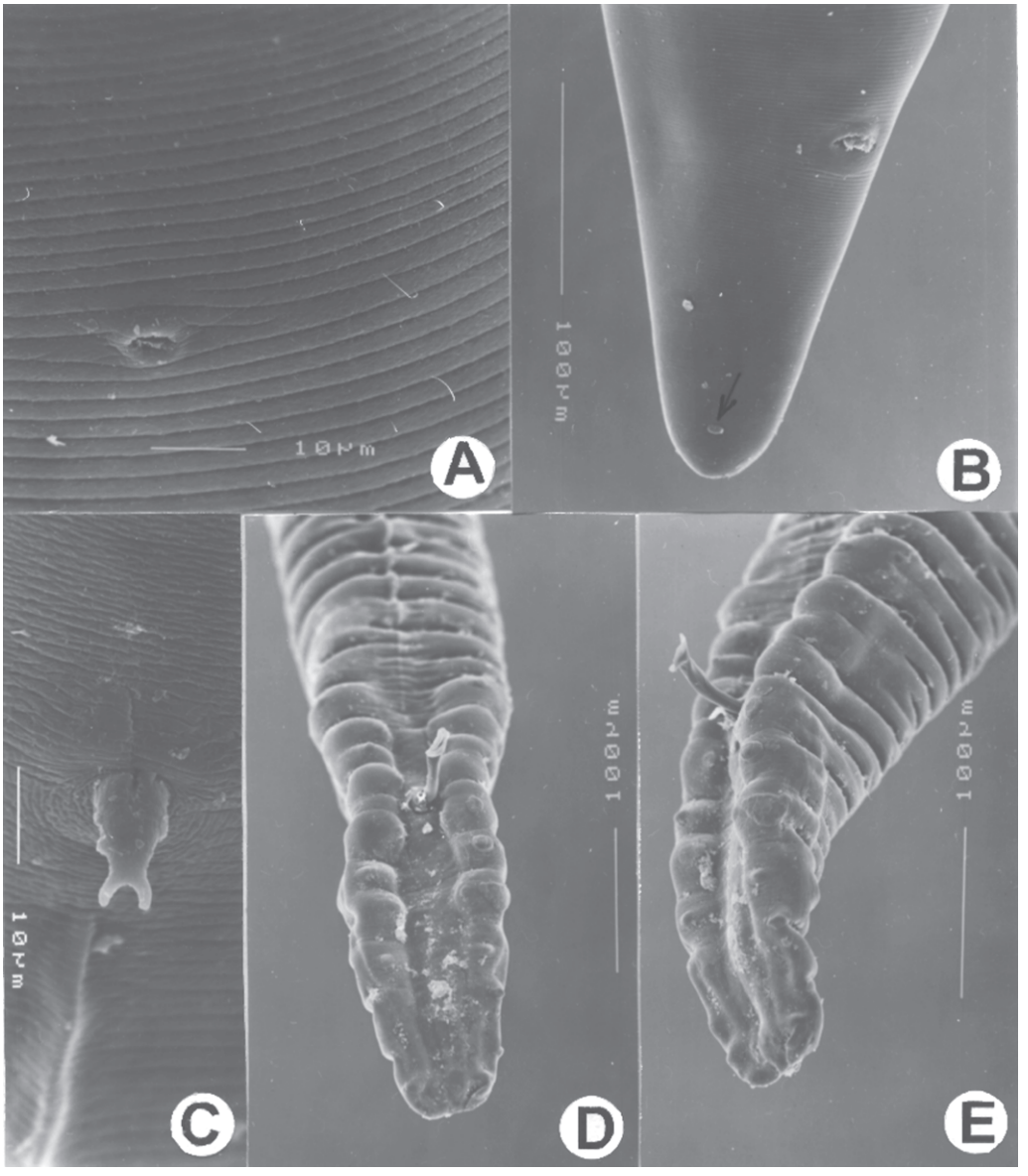

Fig. 8. *Cosmocephalus obvelatus* (Creplin) from *Larus ridibundus*. SEM micrographs. A – excretory pore, ventral view; B – caudal extremity of female, subventral view. Note phasmid (arrow); C – deirid, lateral view; D – caudal extremity of male, ventral view; E – caudal extremity of male, sublateral view. Note distal end of left (slender) spicule.

A c k n o w l e d g e m e n t s

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