

Some new data on the morphology of *Cystidicola farionis* (Nematoda, Cystidicolidae), a swimbladder parasite of salmonids, as revealed by SEM

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■ Abstract

Scanning electron microscopy studies of nematode specimens of *Cystidicola farionis* Fischer, 1798, a swimbladder parasite mainly of salmonids, made it possible to describe in detail the cephalic structure as well as some additional morphological features of this widely distributed nematode and, accordingly, to correct the generic diagnosis of *Cystidicola* Fischer, 1798. Similarly to species of the most related genera *Comephoronema* Layman, 1933 and *Salvelinema* Trofimenko, 1962, the males of *C. farionis* (a type species of the genus) possess well-developed ventral preloacal ridges (area rugosa). As shown by this study, the genus *Cystidicola* differs from *Comephoronema* and *Salvelinema* mainly in the structure of the mouth, particularly in the presence of four well-demarcated labia, large circumoral teeth, and in the character of pseudolabia.

■ Key words

Cystidicola farionis, nematodes, swimbladder parasites, morphology, *Osmerus*, fish, Poland

■ Introduction

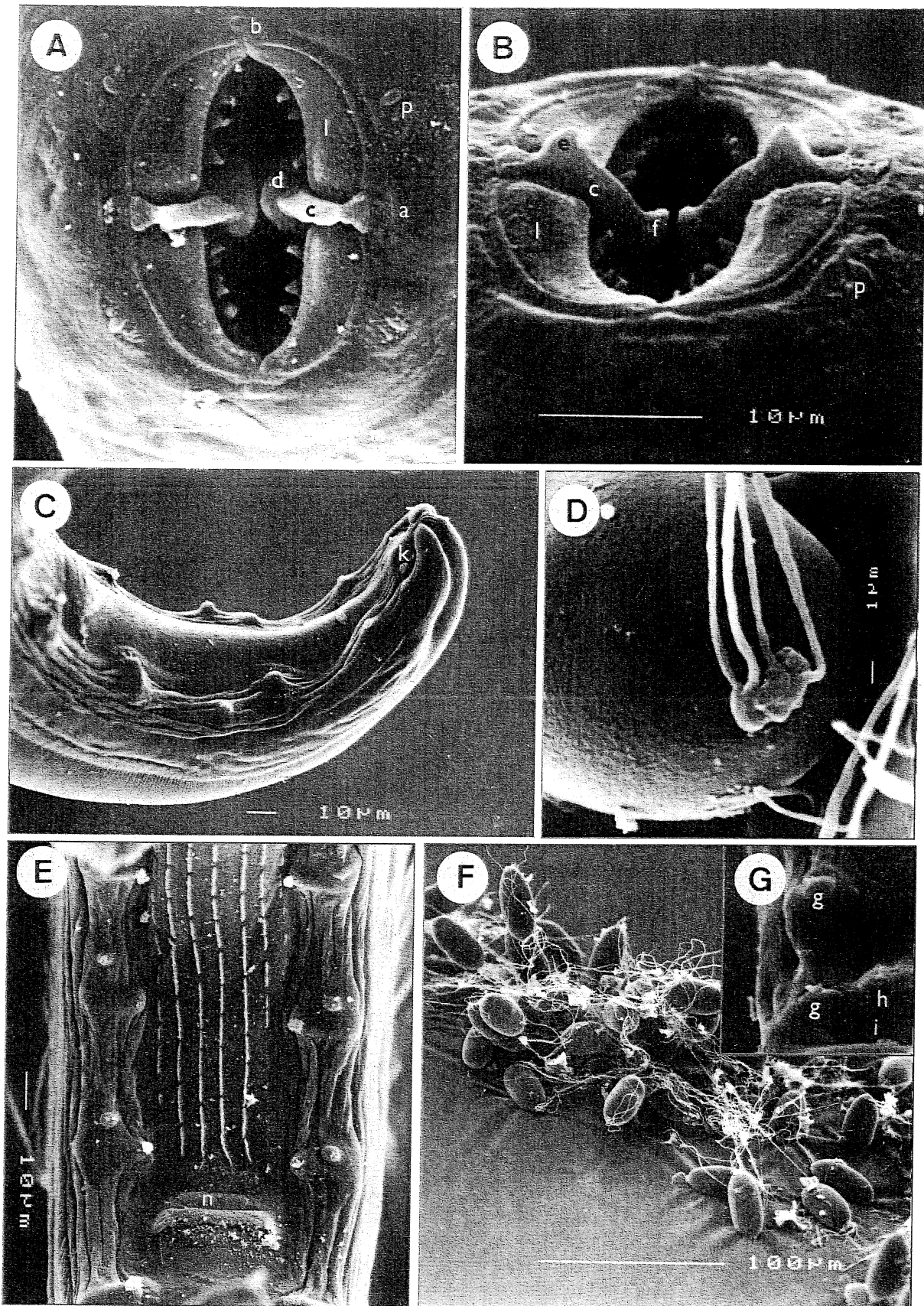
Cystidicola farionis Fischer, 1798 is a common, widely spread swimbladder parasite of salmonid and osmerid fishes in Europe, palaeartic Asia and North America (Moravec 1994). Although this highly pathogenic nematode was known to helminthologists as early as at the end of the eighteenth century, the knowledge of its morphology remained poor for a long time. It concerns particularly the structure of the mouth, which is very important for the identification and systematics of cystidicolid nematodes (e.g., Margolis 1977, Apy and Anderson 1982, Ko 1986, Fagerholm and Berland 1988, Moravec 1996, Moravec and Nagasawa 1999).

Despite the fact that *C. farionis*, originally described by Fischer (1798) from brown trout, *Salmo trutta* m. *fario* L., in Europe, is a type species of the genus *Cystidicola* Fischer, 1798, the presence of buccal teeth in this species was first reported only by Spasskiy and Rakova (1958) in specimens from salmonids in Kamchatka, the Russian Far East. Later descriptions of the cephalic structures of *C. farionis* from Europe by Rasheed (1966) and Ko and Anderson (1969), based on light microscopy studies, showed considerable differences in their observations. An inadequate knowledge of the morphology of *C. farionis* and other related forms fre-

quently resulted in confusions as to the generic appurtenance of the latter. *C. farionis* has so far been examined by scanning electron microscopy (SEM) only by Fagerholm (1982); he added some morphological details, but did not mention some others. Recent SEM study of *C. farionis* obtained from smelt, *Osmerus eperlanus* (L.), from Poland revealed a true detailed cephalic structure of this nematode and confirmed some other morphological features mostly not previously reported in this species. The results are presented herein.

■ Materials and methods

Adult specimens of *C. farionis* from the collection of the Department of Invertebrate Zoology, University of Gdańsk, were used for SEM study. They were from the material collected from the swimbladder of *Osmerus eperlanus* caught in the Gulf of Gdańsk, Poland on 8th March 2001. Originally these nematodes were fixed in Berland's fluid and cleared in glycerine for light microscopical examination; later they were stored in 70% ethanol. For SEM examination, the nematodes were transferred to 4% formaldehyde, postfixed in 1% osmium tetroxide, dehydrated through graded ethanol and acetone and then subjected to critical point drying. The specimens



were sputter-coated with gold and examined with a JSM-6300 scanning electron microscope at an accelerating voltage of 15 kV. The remaining specimens of *C. farionis* not used for SEM were deposited in the helminthological collection of the Institute of Parasitology, ASCR, in České Budějovice (Cat. No. N-65).

Results

The SEM examination of the cephalic end of *C. farionis* shows that the oral aperture is oval, dorsoventrally elongated, surrounded by four very low, well demarcated submedian labia, two subdorsal and two subventral, with broad base (Fig. 1A, B), forming dorsolateral and dorsoventral smooth margins of oral aperture; no row of numerous small teeth in the form of fine serrations was observed on the labial inner margins. In an apical view, each labium is broad near the respective pseudolabium and gradually narrows towards its dorsal or ventral end. The separation of both subdorsal and both subventral labia is very short, but distinct. The cuticle near the site where both subdorsal and both subventral labia come into contact forms a small papilla-like structure. Sublabia (according to the terminology by Margolis 1977) absent.

The lateral pseudolabia (Fig. 1A, B) are very narrow, rod-like in an apical view, with slightly broader external ends; the inner end of each pseudolabium forms a distinct small, anteriorly oriented protuberance (Fig. 1B) and is continuous with a broad, rounded base, which is an anterior extension of the lateral wall of the buccal cavity (Fig. 1A). Each pseudolabium bears a large conical, anteriorly directed protuberance near the edge of the oral aperture, from which its upper part is gradually depressed towards the inner pseudolabial protuberance (Fig. 1B). The mouth is armed with 16 large, conical circumoral (dorsal, ventral and lateral) teeth, four in each quadrant; exceptionally some of these teeth may be subdivided into two so that the total number of teeth in one quadrant may be up to seven (Fig. 1A).

The mouth is surrounded by four simple submedian cephalic papillae and two small lateral amphids, both situated outside the labia and pseudolabia (Fig. 1A). The body cuticle is slightly transversely striated.

The male posterior end is provided with narrow caudal alae and well-developed, longitudinal ventral precloacal cuticular ridges (area rugosa) (Fig. 1E). The present study confirms that there are 14–18 pairs of subventral pedunculated preanal papillae arranged in couples, the papillae of one pair in each couple (usually of a more posterior one), being situat-

ed more ventrally. There are five pairs of subventral pedunculated postanal papillae and an additional pair of small sessile papillae situated ventrally at the level of the last subventral pair (Fig. 1G). These are followed by a pair of weakly developed phasmids. A median elevated area is present between the fifth and the sixth pairs of postanal papillae (Fig. 1C). The anterior cloacal lip bears a median, transverse elongated formation of an elevated cuticle (Fig. 1E).

Fully developed (larvated) eggs were found to bear a tuft of several (mostly 2–8) thread-like filaments at each pole (Fig. 1F); no lateral filaments were found. The filaments grow out from a common flat basal formation on the egg pole (Fig. 1D).

Discussion

The general morphology of nematodes of the present material corresponds to *Cystidicola farionis*, as it has been described in the papers by Dujardin (1845), Leiper (1908), Baylis (1934), Spasskiy and Rakova (1958), Ko and Anderson (1969) or Fagerholm (1982), differing only in some morphological details, and there is no doubt that they belong to this species. Although the presence of buccal teeth was reported in North American species of *Cystidicola* by Skinner (1930, 1931) and White and Cable (1942) and was assumed in European *C. farionis* by Campana-Rouget (1955), Spasskiy and Rakova (1958) were the first to describe them in *C. farionis* from the Far East; the last-named authors observed 16 (four in each quadrant) circumoral teeth and two conical outgrowths of pseudolabia situated at sides the oral aperture. Rasheed (1966) gave drawings of the cephalic end of *C. farionis* based on European specimens and she illustrated 4–7 circumoral teeth in each quadrant of the oral aperture and did not observe any median dorsal and ventral teeth, which corresponds to our findings; on the contrary, she illustrated eight cephalic papillae, but the present study shows that only four papillae are present. Ko and Anderson (1969) working with specimens of *C. farionis* from *Salvelinus alpinus* (L.) from England described and illustrated two rows of teeth, an external row made up of one large ventral, one large dorsal and two prominent lateral, and an internal row of 16 large teeth, four in each quadrant formed by subdorsal, subventral and lateral teeth; in contrast to North American *Cystidicola* spp., the authors did not observe any small teeth in the form of fine serrations in the external row, but they believed it was because only smaller nematode specimens were examined. Since *C. farionis* is the type species of *Cystidicola*, and because the identical arrangement of buccal teeth was allegedly found by them in

Fig. 1. *Cystidicola farionis* Fischer, 1798, SEM micrographs: **A** and **B** – cephalic end, apical and dorsoventral views; **C** – tail of male, lateral view; **D** – polar filaments on fully developed egg; **E** – precloacal region with cuticular ridges, ventral view; **F** – eggs with polar filaments; **G** – caudal papillae of last three pairs, ventral view; **a** – amphid, **b** – papilla-like formation near junction of labia, **c** – pseudolabium, **d** – anterior extension of lateral wall of buccal cavity, **e** – outer pseudolabial protuberance, **f** – inner pseudolabial protuberance, **g** – subventral papillae of fourth and fifth postanal pairs, **h** – ventral papilla of sixth postanal pair, **i** – phasmid, **k** – caudal median elevated area, **l** – labium, **n** – median precloacal transverse elongated formation, **p** – cephalic papilla

North American *Cystidicola* spp., Ko and Anderson (1969) re-diagnosed *Cystidicola* so that this type of arrangement of buccal teeth was included into the generic diagnosis. They failed to describe the submedian labia and the actual structure of the pseudolabia.

However, the present SEM study shows clearly that there are neither dorsal and ventral teeth present in the oral aperture nor any serrations on the inner edge of submedian labia in *C. farionis*. Although Fagerholm (1982) did not describe the cephalic structures of this species in detail, the same is visible on his SEM micrographs. Probably Ko and Anderson (1969) considered small dorsal and ventral papilla-like structures on the cuticle near the sites where labia come into contact to be dorsal and ventral buccal teeth of the external row; these structures, located outside the oral aperture, seemed not to be present in all specimens.

The lateral teeth of the external row mentioned by Ko and Anderson (1969) are apparently identical with the conspicuous lateral protuberances on the most anterior surface of pseudolabia, which frequently occur also in species of some other cystidicolid genera. Since there is no resemblance of these structures to the buccal teeth of other nematodes, Ko (1986) recommended to term them pseudolabial protuberances. The presence of inner pseudolabial protuberances in *C. farionis* is first described in the present paper and is not known in other cystidicolid nematodes.

The present study shows that the number of subdorsal, subventral and lateral buccal teeth is mostly 16 in *C. farionis*, usually four in each quadrant, but exceptionally some teeth in one or more quadrants may be subdivided into two so that up to seven teeth per quadrant may be present. A similar number and distribution of buccal teeth was found by Margolis (1967) in *Cystidicola* sp. from *Oncorhynchus nerka* (Walbaum) from Alaska, USA.

Considering the above discussion, the diagnosis of *Cystidicola* should be modified as follows:

Habronematoidea, Cystidicolidae. Pseudolabia very small, with marked pseudolabial protuberances. Oral aperture dorsoventrally elongated, surrounded by four submedian labia. Mouth armed with at least 16 large subdorsal, subventral and lateral buccal teeth, usually four in each quadrant. Vestibule (stoma) long and slender. Caudal alae in male well developed, supported by many pedunculate papillae; preanal papillae numerous, arranged in double pairs. Spicules unequal and dissimilar. Vulva slightly posterior to middle of body. Uterus amphidelphic. Mature eggs embryonated, bearing polar filaments or lateral floats. Parasites of swimbladder of fishes.

Type species: *C. farionis* Fischer, 1798.

The numbers and the arrangement of male caudal papillae of specimens of the present material are in accordance with observations by Ko and Anderson (1969), Moravec (1971) and Fagerholm (1982). The presence of a median elevated area between the fifth and the sixth pairs of postanal papillae and a median transverse formation on the anterior cloacal lip were observed in *C. farionis* also by Fagerholm (1982).

Leiper (1908) reported the presence of 2–3 polar filaments on the fully developed eggs of *C. farionis* from *Salvelinus alpinus* from England, but Berghe (1935) found numerous filaments at the poles and the sides of eggs in this species. While only polar filaments were found in *C. farionis* by Spasskiy and Rakova (1958), Moravec (1971) and Fagerholm (1982), Ko and Anderson (1969) reported only polar filaments in specimens from *S. alpinus* from England, but both polar and lateral filaments in those from *Salmo trutta* L. from England. In the present material, only polar filaments occurred. Moravec and Ergens (1970) mention that the differences in the number of observed egg filaments may be due to the fact that the authors described eggs at various developmental stages. In our opinion, the presence of lateral filaments in *C. farionis* is questionable and these data are probably due to inaccurate observations; when too many filaments are present on the egg, sometimes it is difficult to determine under the light microscope from what part of the egg surface they actually grow out.

Species of the related genus *Salvelinema* Trofimenko, 1962 are also swimbladder parasites of salmonids and, in fact, originally they were described as members of *Cystidicola*. Another closely related genus is *Comephoronema* Layman, 1933 including intestinal parasites of cottocomephorids and burbot [*Lota lota* (L.)]. Species of both *Salvelinema* and *Comephoronema* are morphologically very similar to those of *Cystidicola* and, for example, these are the only genera within the Habronematoidea the species of which possess relatively numerous doubled preanal papillae (pairs of papillae arranged in couples) in the male. Recent SEM studies of the cephalic ends of species of *Salvelinema* and *Comephoronema* (Moravec and Nagasawa 1999, Moravec, Hanzelová and Scholz – unpublished) confirmed the absence of buccal teeth (as well as some other differences in the structure of the mouth) in them. Consequently, the presence of distinct buccal teeth in *Cystidicola* spp. can be considered the most important morphological feature distinguishing *Cystidicola* from *Comephoronema* and *Salvelinema*.

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