Scanning electron microscopy of the collar region of *Deropristis inflata* and *Echinostoma revolutum*

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Abstract

Scanning electron microscopy (SEM) was used to study the collar region of *Deropristis inflata* (Molin, 1858), an intestinal digenean of fish, and *Echinostoma revolutum* (Froelich, 1802), an intestinal digenean of birds. The results showed basic differences in the following morphological features. The collar of *D. inflata* was open ventrally and dorsally whereas that of *E. revolutum* was closed dorsally and ventrally, forming a kidney-like ring. The collar of *D. inflata* was located posterior to the oral sucker, some distance behind the anterior end of the body. That of *E. revolutum* was located terminally around the oral sucker. The collar of *D. inflata* had numerous collar spines which varied in size, shape and position. That of *E. revolutum* had 37 collar-spines, mainly homogenous in their general morphology and with a typical arrangement pattern for 37-collar-spined echinostomes. The collar of *D. inflata* had few tegumentary papillae whereas that of *E. revolutum* had abundant papillae. A ventral depressed area occurred in *D. inflata*, between the acetabulum and oral sucker, dividing the collar into two left and right independent parts. The ventral depression in *E. revolutum* extended from the ventral sucker to the posterior end of the collar, and was not divided. In *D. inflata*, tegumentary spines were located on the oral sucker, and the collar was posterior to the sucker. In *E. revolutum*, the oral sucker and the collar lacked tegumentary spines. The region immediately posterior to the collar also lacked spines in *E. revolutum*. The collar pattern of *D. inflata* is undoubtedly more primitive than that of *E. revolutum*.

Introduction

Deropristis inflata (Molin, 1858) is an intestinal digenean of fish (Skrjabin, 1958; Yamaguti, 1971; Dezfuli *et al.*, 1992). *Echinostoma revolutum* (Froelich, 1802), a name often used to refer to numerous digenean species (see Kanev, 1985, 1994 for redescription of *E. revolutum*), is an intestinal digenean of wild and domestic birds. Both species possess a collar with spines, characteristic features of members of the family Echinostomatidae. However, in spite of these characteristics Skrjabin (1958) placed *D. inflata* in the family Deropristidae and Yamaguti (1971) placed *D. inflata* in the family Acanthocolpidae instead of the family Echinostomatidae.

Comparative scanning electron microscopy (SEM) studies of the collar region of *D. inflata* and *E. revolutum* have not been done. The purpose of this study was to use SEM to compare the surface topography of the collar region of *D. inflata* and *E. revolutum*.

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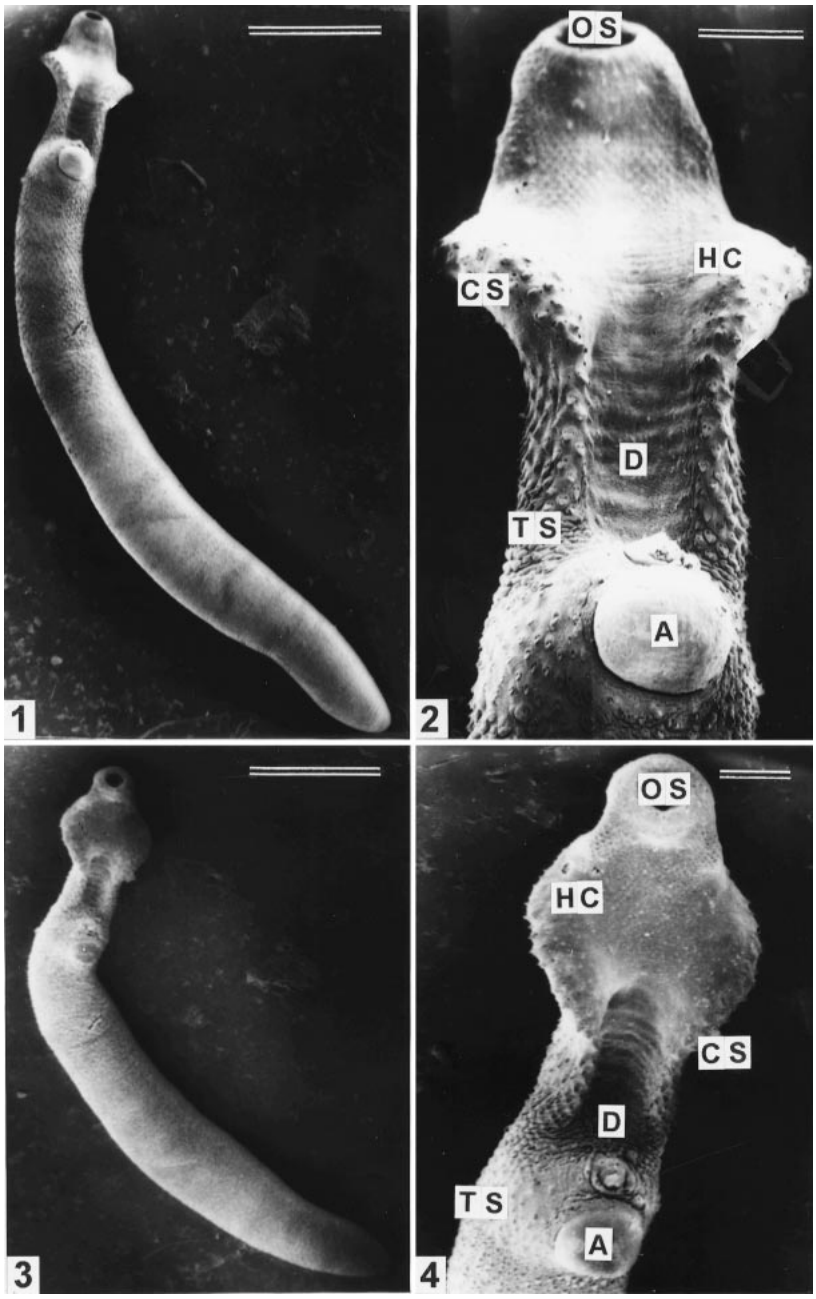

Figs 1–4. SEM of *Deropristis inflata*. Fig. 1. Ventral view of adult. Bar = 500 μm. Fig. 2. Anterior region of adult: oral sucker (OS), head collar (HC), depressed area (D), acetabulum (A), tegumentary spines (TS) and collar spines (CS). Bar = 100 μm. Fig. 3. High angle view of adult. Bar = 500 μm. Fig. 4. Anterior end of adult: oral sucker (OS), head collar (HC), depressed area (D), acetabulum (A), tegumentary spines (TS) and collar spines (CS). Bar = 100 μm.

Materials and methods

Twenty-three adults of *Deropristis inflata*, collected from the intestines of naturally infected European eels (*Anguilla anguilla* (L.) from the Comacchio lagoons, in the northern Adriatic Sea, Italy were used. Forty-one adults of *Echinostoma revolutum* obtained from the intestines of experimentally infected, domestic chickens (*Gallus gallus*) in Bulgaria, at 21 days post infection, were examined. The fish and chickens were necropsied, worms were removed

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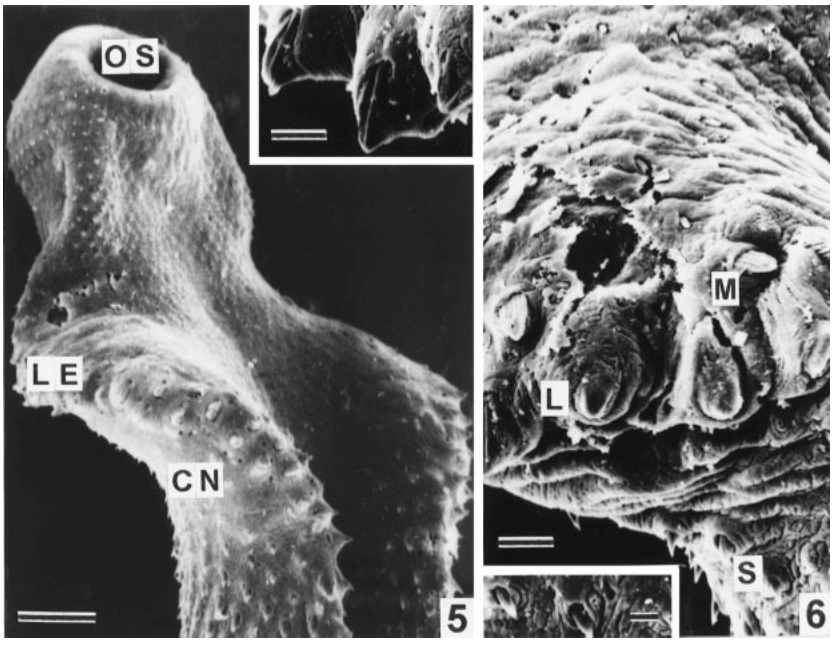

Figs 5–6. SEM of *Deropristis inflata*. Fig. 5. Lateral view of the anterior end of *Deropristis inflata*: lateral edge (LE), oral sucker (OS) and enlarged cobra-like neck (CN). Bar = 50 μm. Inset is an enlarged view of two collar spines at the edge. Bar = 10 μm. Fig. 6. Three types of collar spines – small (S), middle (M) and large (L). Bar = 10 μm. Inset is an enlarged view of the small spines. Bar = 5 μm.

quickly from the intestines, and washed in saline solution. They were then fixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, washed in buffer and placed in 20% glycerol for 25 h (Dobbs, 1972) to remove debris from the body of the worms. Worms were then postfixed for 2 h in 1% osmium tetroxide at 4°C and washed again in cacodylate buffer for 3 h. Specimens of *D. inflata* were dehydrated in a graded ethanol series and critical point dried. After mounting, specimens were coated with gold in an Edwards S 150 sputter coater and examined with a Cambridge Stereoscan S 360 (for *D. inflata*) and a Tesla BS 300 scanning electron microscope (for *E. revolutum*).

Results

The results of the SEM study on *Deropristis inflata* and *Echinostoma revolutum* are shown in figs 1–6 and 7–15, respectively. Both species possess an oral collar. In *D. inflata* the oral collar opens ventrally (figs 1–5) and is an enlarged tegumentary flap with a cobra-like shape, situated posterior to the oral sucker (fig. 5). In *E. revolutum*, the head collar is a kidney-shaped ring (figs 7, 10).

In *D. inflata*, the collar is located posterior to the oral sucker (figs 1–4). The ventral view of adult worms (figs 2, 4) shows that the posterior end of the collar is located midway between the oral and ventral sucker. In *E. revolutum*, the collar is located at the anterior end of the body (fig. 7) and the oral sucker located mainly in the middle of the collar (figs 7, 10).

In *D. inflata*, the collar is covered with numerous collar spines which differ in size, shape and position. These spines are about 15 μm long (figs 5, 6) and are located on the lateral edge of the collar; they are triangular in shape with a sharp edge dorsally (fig. 5, inset). A few, average-sized collar spines, about 7.5 μm long, are found on the ventral surface of the collar, near the larger spines (fig. 6); they are long, dorsoventrally flattened with a clear lateral edge and bilateral symmetrically sharpened end. Hundreds of small spines, about 6 μm long are found on the collar on both the ventral and dorsal surfaces (figs. 5, 6). They are soft cylindrical hooks with sharp ends. An enlarged view of those spines (fig. 6 inset) show that they are located in crater-like holes in the tegument. In *E. revolutum*, the collar contains 37-collar-spines (figs 7–15). These spines are larger with a cylindrical body covered by the tegument and have free conical ends (figs 7–15). Some spines are found surrounded by an outer layer of tegument-like material (figs 11, 12). All 37 collar-spines are located at the edge of the collar, forming a typical formula as follows. Fifteen spines are located dorsally in a double row (fig. 14). Eight of these spines are oral and relatively small in size. Seven are aboral and relatively large in size. These 15 spines represent three groups: one group of three spines (two oral and one aboral) are located in the middle (mid-central) of the double row (fig. 15). Their combined number gives the total number of the collar-spine formula. Two groups of six spines each (three oral and three aboral) are located on each side left and right, of the mid-central three spines. Six lateral spines are arranged in a single row on each left and right lateral side of the collar (figs 7,

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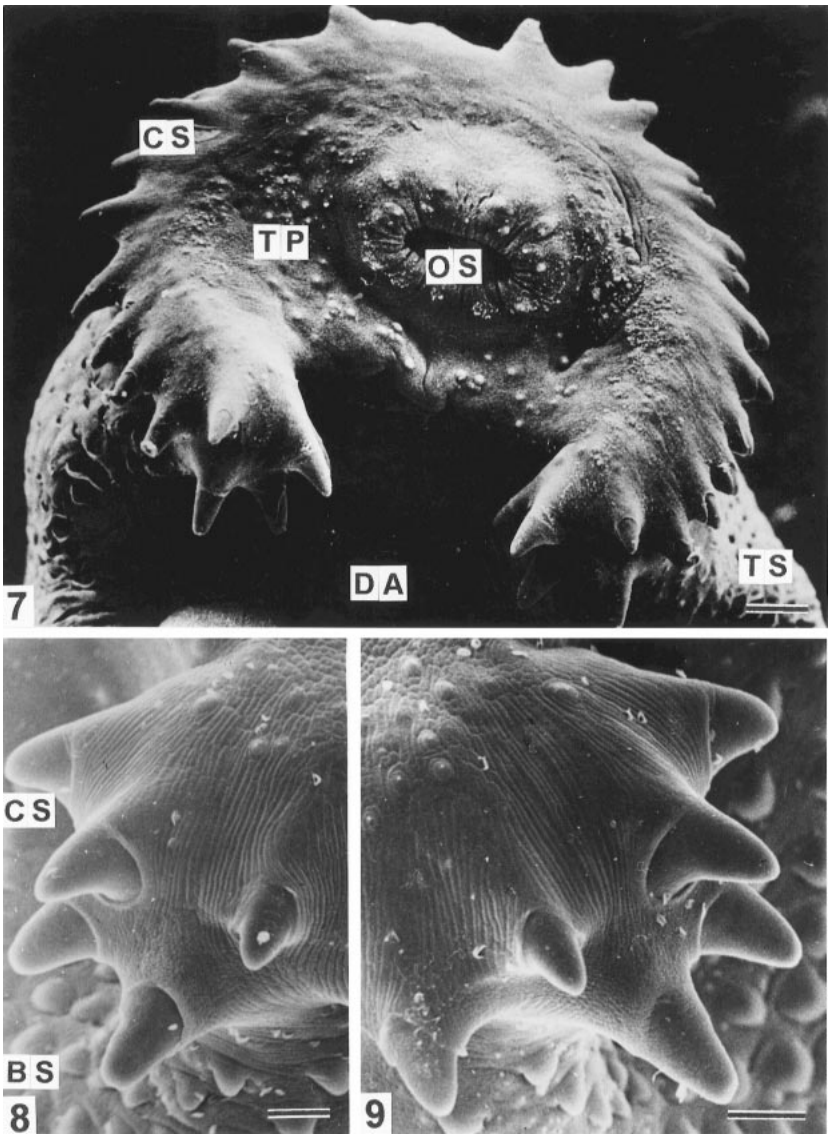

Figs 7–9. SEM of the collar and collar spines of *Echinostoma revolutum*. Fig. 7. Ventral view of collar: oral sucker (OS), tegumentary papillae (TP), collar spines (CS), depressed area (DA) and tegumentary spines (TS). Bar = 50 μm. Fig. 8. Enlarged view of five corner spines (CS), body spines (BS) and tegumentary surface free of tegumentary spines. Bar = 25 μm. Fig. 9. Enlarged view of collar spines, tegumentary papillae and tegumentary spines. Bar = 25 μm.

10). These spines are more or less equal in size and equidistant from spine to spine. Four large spines are located in each left and right ventral corner of the collar. (figs 8, 9, 13). Two of them are oral: one medio (ventro)-oral which is usually the smallest, and one latero (dorso)-oral which possesses a typical asymmetrically formed conical end. Two spines are aboral: one medio (ventro)-aboral and one latero (dorso)-aboral, which is the largest of all the corner spines. One spine is located on each left and right side where the lateral portion of the collar joins the ventral (corner) portion (figs 7–10, 13). These two spines are the smallest of all the collar spines. There is an intermediate chain connecting the four corner spines with the six lateral spines.

In *D. inflata*, a deep depressed area is closed between the left and right lateral edge of the body posteriorly from the acetabulum and genital atrium, and at the anterior it is open (figs 1–5). Because of this depression, the left and right ventral portions of the head collar are divided into two separate left and right parts. In *E. revolutum*, a depressed area is organized like that of *D. inflata* except in the anterior end where it ends at the level of the posterior end of the head collar. This junction appeared as a tegumentary edge, connecting both the left and right

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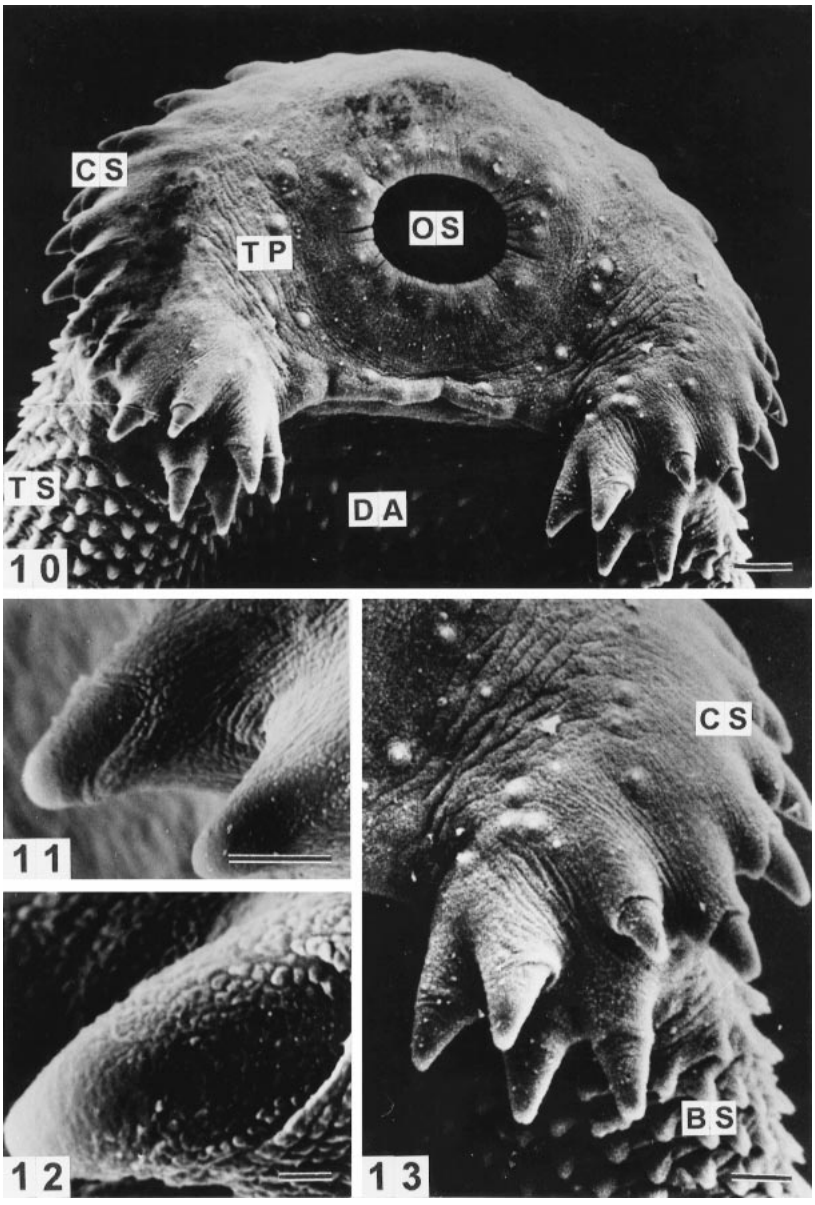

Figs 10–13. SEM of the collar and collar spines of *Echinostoma revolutum*. Fig. 10 Ventral view of collar: oral sucker (OS), tegumentary papillae (TP), collar spines (CS), depressed area (DA) and tegumentary spines (TS). Bar = 50 μm. Figs 11, 12. Enlarged view of collar spines. Bars = 25 μm and 5 μm respectively. Fig. 13. Enlarged view of collar spines (CS), body spines (BS) and tegumentary surface free of tegumentary spines. Bar = 25 μm.

sides of the collar (figs 7, 10). This ridge is solid, prominent and devoid of any spines.

In *D. inflata*, tegumentary spines are spread out in the collar area (figs 2, 4–6): on the collar itself; on the oral sucker (situated anterior to the collar); and on the tegument surface posterior to the collar. The spines of the oral sucker are the smallest, while those of the body posterior to the collar are the largest. There are small spines on the collar, which are similar to those on the oral sucker and also large spines similar to those behind the collar. In *E. revolutum*, the collar and the oral sucker are devoid of tegumentary spines. A short (about 200 μm width) neck-like space surrounding the posterior end of the collar like a ring is also devoid of spines. The first row of tegumentary spines are found beyond this spineless ring (fig. 14). The first (anterior) spines are bigger, conspicuous and are scale-like structures arranged in longitudinal rows. Toward the posterior end they are smaller in size, more widely spread out, and irregular in distribution.

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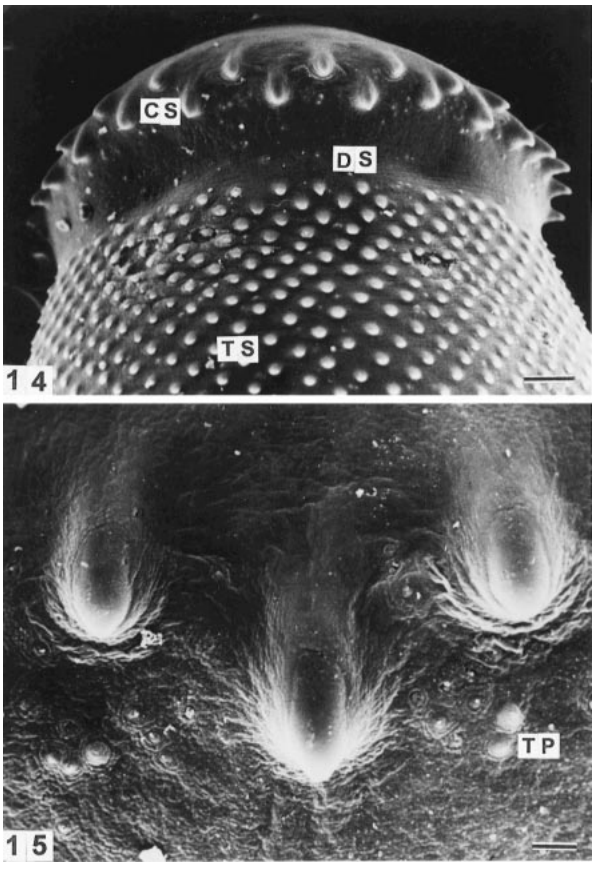

Figs 14–15. SEM of the collar and collar spines of *Echinostoma revolutum*. Fig. 14. Dorsal view showing double rows of collar spines (CS) and tegumentary spines (TS) separated by a space devoid of spines (DS). Bar = 50 μm. Fig. 15. Enlarged view of the three central spines shown in fig. 14. Note the abundance of tegumentary papillae (TP). Bar = 10 μm.

In *D. inflata*, the head collar possesses few tegumentary papillae, which are widely dispersed (fig. 6). In *E. revolutum*, a large number of tegumentary papillae occur on the ventral and dorsal collar surfaces (figs 7, 10).

Discussion

The collar region of *D. inflata* and *E. revolutum* appeared in different forms, showing the basic morphological differences of these worms.

In *D. inflata*, the collar area including its shape, and position and size, and the shape and arrangement of the collar spines is similar to the collar arrangement in intestinal parasites of crocodiles which Odhner (1902) referred to as *Stephanoprora ornata*. *Deropristis inflata* and *S. ornata* and their respective fish and crocodile hosts are considered older and more primitive in an evolutionary sense. This suggests that their pattern of oral collar and collar spines may be relatively primitive.

In *E. revolutum*, the collar topography was found to be similar to that of *E. trivolvis* and *E. caproni* (referred to before Kanev, 1985 as *E. revolutum* and *E. liei*, respectively) described by Smales & Blankespoor (1984), Fried & Fujino (1984), Donovick & Fried (1988), Fried *et al.* (1990), Kruse *et al.* (1992), Ursone & Fried (1995) and Fujino *et al.* (1995). In the aforementioned papers SEM information and photomicrographs of details of the collar region were incomplete. For this reason, detailed comparisons between this study and the aforementioned studies were not possible. However, it is well known that *E. revolutum* and the 37-collar-spined allies of the *E. revolutum* group and their avian and mammalian hosts, including humans, are considered relatively new in an evolutionary sense. We therefore consider their head collar pattern as more advanced than that of *D. inflata*.

This study raises doubts about the present systematic position of digeneans such as *D. inflata* which possess an oral collar and crown of collar spines, but are not included in the family Echinostomatidae. It is well known that the family Echinostomatidae was founded by Dietz (1909) for classifying in a common family all digeneans which possess an oral collar and collar spines. Skrjabin (1958) placed *D. inflata* in the family Deropristidae and Yamaguti (1971) placed *D. inflata* in the family Acanthocolpidae instead of the Echinostomatidae. More observations on similarities and differences between *D. inflata* and representatives of the family Echinostomatidae are necessary to clarify the present taxonomic situation.

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References

Dezfuli, B.S., Fioravanti, M.L., Onestini, S. & Rossi, R. (1992) *Deropristis inflata* (Plathyhelminthes: Digenea) parasite of *Anguilla anguilla* (L): morphology of parasite and intestinal pathology of host. *Parasitologia* **34,** 205–206.

Dietz, E. (1909) Die Echinostomiden der Vogel. Inaugural-Dissertation, Konigsberg.

Dobbs, G.H. (1972) A method for preservation of soft tissues of marine teleosts for scanning electron microscopy. *Tissue and Cell* **4,** 687–690.

Donovick, R.A. & Fried, B. (1988) Scanning electron microscopy of *Echinostoma revolutum* and *E. liei* from

https://doi.org/10.1017/S0022149X00700186 Published online by Cambridge University Press

domestic chicks. *Journal of the Pennsylvania Academy of Science* **62,** 78–82.

Fried, B. & Fujino, T. (1984) Scanning electron microscopy of *Echinostoma revolutum* (Trematoda) during development in the chick embryo and the domestic chick. *International Journal for Parasitology* **14,** 75–85.

Fried, B., Irwin, S.W.B. & Lowry, S.F. (1990) Scanning electron microscopy of *Echinostoma trivolvis* and *E. caproni* (Trematoda) adults from experimental infections in the golden hamster. *Journal of Natural History* **24,** 433–440.

Fujino, T., Fried, B. & Hosier, D.W. (1995) The expulsion of *Echinostoma trivolvis* (Trematoda) from ICR mice: extension/retraction mechanisms and ultrastructure of the collar spines. *Parasitology Research* **80,** 581–587.

Kanev, I. (1985) On the morphology, biology, ecology and taxonomy of *Echinostoma revolutum* group (Trematoda: Echinostomatidae: *Echinostoma*). DSc thesis, Bulgarian Academy of Sciences, Sofia, 467 pp. (in Bulgarian with English summary).

Kanev, I. (1994) Life cycle, delimitation and redescription of *Echinostoma revolutum* (Froelich, 1802) (Trematoda: Echinostomatidae). *Systematic Parasitology* **28,** 125–144.

Kruse, D.M., Hosier, D.W. & Fried, B. (1992) The expulsion of *Echinostoma trivolvis* (Trematoda) from ICR mice: scanning electron microscopy of the worms. *Parasitology Research* **78,** 74–77.

Odhner, TH. (1902) Trematoden aus Reptilien nebst allgemeinen systematischen Bemerkungen. *Vetenskaps-Akademiens Forhandlingar. Stockholm* **1,** 19–45.

Skrjabin, K.I. (1958) *Trematodes of animals and man. Essentials of trematodology.* Vol. 15, Izdatelstvo Akademiia Nauk SSSR, pp. 33–37.

Smales, L.R. & Blankespoor, H.D. (1984) *Echinostoma revolutum* (Froelich, 1802) Loos, 1899 and (Schrank, 1788) Luhe, 1909 (Echinostomatidae, Digenea): scanning electron microscopy of the tegumental surfaces. *Journal of Helminthology* **58,** 187–195.

Ursone, R.L. & Fried, B. (1995) Light and scanning electron microscopy of *Echinostoma caproni* (Trematoda) during maturation in ICR mice. *Parasitology Research* **81,** 45–51.

Yamaguti, S. (1971) *Synopsis of digenetic trematodes of vertebrates.* Vol. 1. p. 122. Tokyo, Japan, Keigaku Publishing Co.

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