SEM VERIFICATION OF ARMATURE AND CHAETOTAXY OF *DIPLOSTOMUM PARACAUDUM* (ILES, 1959) CERCARIA (DIGenea, DIPLOSTOMIDAE)

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SUMMARY

Light microscopy observations on armature and chaetotaxy of *Diplostomum paracaudum* (Iles, 1959) were verified in scanning electron microscopy. The new findings concern some details of

armature and outer structure of sensory cells. They show a variety in cilia length and structure of a collar. The specific distribution of particular sensilla forms is observed.

RÉSUMÉ : Vérification au microscope électronique à balayage de l'armature et de la chétotaxie de la cercaire de *Diplostomum paracaudum* (Iles, 1959) (Digenea, Diplostomidae).

Nous avons vérifié au microscope électronique à balayage (MES) les observations précédentes sur l'armature et sur la chétotaxie de la cercaire de *Diplostomum paracaudum* (Iles, 1959). Les nouvelles observations concernant certains détails sur les épines et sur

la structure externe des cellules sensorielles. Mise en évidence de différences dans la longueur des cils et dans la structure du collier. La distribution spécifique des différents types de papilles est étudiée.

INTRODUCTION

Light microscopy observations on armature and chaetotaxy of *Diplostomum paracaudum* (Iles, 1959) Shigin, 1977 cercariae were published by Shigin (1977) and Niewiadomska (1989). The structures were recently re-examined using scanning electron microscopy. SEM observations showed some new details concerning, among other things, a variety of ciliary sensilla and their different distribution. SEM observations on surface structures of *Diplostomum* cercariae have not been published till now.

MATERIALS AND METHODS

Cercariae of *D. paracaudum* originated from naturally infected snail, *Galba palustris* (O. F. Müll.), from Mazurian Lakeland, Poland, and *Radix peregra ovata* (Drap.) from North Bohemia, Czechoslovakia. The cercariae from Poland were killed by dropping Karnovski fluid into the water, and fixed and stored in pure Karnovski fluid (up to one year). The cercariae from Czechoslovakia were killed with hot (70° C) 4 % formaldehyde. Both material were postfixed in 2 % OsO₄ solution in 0.1 M cacodylate buffer, washed, dehydrated in a series of alcohol, and critical point dried. The specimens were coated with gold

and then examined with a Tesla BS-300 scanning electron microscope at 15 kV.

RESULTS

Body armature (fig. 1)

Preoral spines: the central group (16-18 spines arranged in 3-4 rows) forms a triangle; the lateral groups (1-2 small spines) lie near the outlets of penetration glands (fig. 1 H, J). The post-oral spines (6-7 alternating rows diminishing in size) surround the spineless body tip and the outlets of the penetration glands (fig. 1 D, H). Beyond the posterior row there is a large area with small sparse spines in the central part. This area is distally limited by the first of ten transverse rows of spines surrounding the anterior body part. All ten rows on the dorsal side and eight on the ventral side are full of spines, with additional spines on the lateral and ventral sides in five anterior rows (fig. 1 A, B, C, D, K). From the posterior margin of the ventral sucker and behind the last transverse row, small sparse spines cover the ventral and lateral body sides, spines are less numerous on the dorsal surface leaving a wide spineless area (fig. 1 A, B, C). On the ventral sucker spines arranged in three rows can be observed (fig. 2 B). On the ventral and dorsal sides of the tail stem and the ventral and dorsal furca margins there are spine-like structures rather than spines (fig. 1 E, G).

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Chaetotaxy (figs. 1, 2)

The SEM picture of sensilla showed a variety in cilia length and structure of a collar. The following form can be distinguished: (a) with a long free cilium and a wide protruding collar (fig. 2 D); (b) with a short cilium and a tight collar (fig. 2 A); (c) with a short cilium and no visible collar (fig. 1 H); and (d) with a cilium of intermediate length and no information about the collar (fig. 2 E). Sensilla with a long cilium are situated on the dorsal side and laterally near the end of the body, and on the tail stem; sensilla with short cilium and a collar—on the ventral, lateral and dorsal sides of the body; with short cilium and no visible collar—around the oral opening and on the ventral sucker; with the cilium of intermediate length—on the furcae.

The distribution of different sensilla in the chaetotaxy pattern was as follows.

Cephalic cycle I (CI) comprises 20 sensilla (on each body side), i. e. nine sensilla more than were visible in light microscope. Two ventral sensilla enter the inner margin of the oral sucker, and two dorsal ones lie on either side of the central group of preoral spines. On the outer margin of the oral sucker and along the depression containing the outlets of penetration glands, 1 + 9 sensilla are in line as far as the lateral group of preoral spines. Dorsally there is a group of six sensilla (on the apical side of the depression) and one on the opposite side. Only two sensilla lying opposite each other at the midlength of the depression have a short cilium with a collar, the remaining ones have no visible collar (fig. 1 D, H, I).

The CII cycle comprises sensilla lying among the last rows of post-oral spines. Of two lateral groups of 5-6 sensilla revealed by AgNO₃ impregnation only 2-3 (with short cilium and a collar in the more ventral group and with a short cilium and no visible collar in more dorsal one) were observed in the SEM picture. The missing sensilla may be unciliated or multiciliated sensory pits in the structure (fig. 1 D, J).

Sensilla of CIII cycle are distributed between post-oral spines and the first transverse row. Apart from the dorsal pair with long cilia, they have a short cilium and a visible collar (figs. 1 D, J, K, 2 A, D).

The AI cycle—sensilla are distributed between the 1st and 3rd transverse rows of spines, but two dorsal sensilla

(with the short cilium and a collar) lie before the first row. Each of the second dorsal pair has a long cilium and a collar. The lateral group of sensilla are enclosed by the 2nd and 3rd transverse rows of spines but, of 5 sensilla revealed by AgNO₃ impregnation, only 3 were visible in the SEM (fig. 2 A, D).

The sensilla of AII cycle, mostly with a short cilium and collar, are present between the 3rd and 6th transverse rows. Only two pairs (one with long cilia and a collar) lie between the 3rd and 5th or 3rd and 4th transverse row (fig. 2 D).

The AIII cycle is distributed between 6th and 9th rows. Cycle M is not present.

Sensilla of PI cycle are situated behind the 10th transverse row of spines. Apart from one dorsal pair with long cilia the sensilla have a short cilium and a collar (fig. 2 F).

The PII cycle comprises only one pair of sensilla with long cilia and a collar on lateral sides (fig. 2 C, G).

The PIH cycle is comprised of one or two pairs of sensilla with a short cilium and collar.

Ventral sucker—three sensilla are situated on the inner margin and six on the outer one. Sensilla have a short cilium and no visible collar (fig. 2 B).

On the tail stem the sensilla with a long cilium and a collar were visible on the whole length (figs. 1 E, 2 C, E). Only one pair with intermediate cilium length was situated dorsally and ventrally near the bifurcation. The same type of sensilla were situated on the margin of the furcae (fig. 2 E).

Taking into account the SEM results the following chaetotaxy pattern can be presented:

2c CIV + 9c + (5c + 2b) CIL + 0 CID
 3b CIIV + (2b + 3?) + (3c + 2?) CIIL + 2b CIID
 1b + 2b CIIV + 1b + 1b CIIL + 1a CIID
 1b + 1b AIV + 2b + 2? AIL + 1b + 1a AID
 1b + 1b AIIV + 1b AIIL + 1b + 1a AIID
 1b + 1b AIIIV + 1b AIIIL + 0 AIIID
 OM
 1b + 1b PIV + 1b (2b) PIL + 1a PID
 0 PIIV + 1a PIIL + 0 PIID
 2b (1b) PIHIV + 0 PIHIL + 0 PIHID
 3c SI + 6c SII
 tail stem 46-55a + 2d; furcae 15-25d

FIG. 1. — Armature and sensilla in *D. paracaudum* cercaria. Micrographs. A) Ventral body side. $\times 1,260$. B) Dorsal body side. $\times 1,020$. C) Latero-ventral view. $\times 1,080$. D) Anterior tip with post-oral spines (PS) and sparse spines (SS). Transverse rows (R) with additional spines. $\times 2,220$. E) Tail stem in the region of bifurcation with spine-like structures on the surface. $\times 1,800$. F) Spines in higher magnification. $\times 7,200$. G) Spine-like structures in higher magnification. $\times 3,600$. H) Mouth of cercaria surrounded with preoral spines (PRS) and ciliary sensilla (C). Arrowheads indicate ventral and dorsal sensilla. Post-oral spines (PS) surrounding the mouth and spineless body tip (SBT). $\times 6,000$. I) Outlets of penetration glands (o) with two sensilla with short cilium and a tight collar (type b) (arrowheads). C—ciliary sensilla with a short cilium and no visible collar (type c). $\times 6,000$. J) Sensilla of CII cycle (CII) forming two lateral groups (L). One lateral and two dorsal sensilla of CIII are visible before the first transverse row of spines. $\times 3,400$. K) Lateral sensilla of CII, CIII, AI, AII and AIII lying between the transverse rows. Note the additional spines in the first five rows, $\times 2,280$.

FIG. 2. — Sensilla in *D. paracaudum* cercaria. Micrographs. A) The ventral b type sensory cilia in CIII and AI cycles. $\times 4,800$. B) The c type sensory endings in SII cycle on ventral sucker. $\times 4,200$. C) The a type (with long cilium and a wide protruding collar) sensilla in PII cycle and on the tail stem (arrowheads). $\times 3,600$. D) Dorsal body sensilla of a (arrowhead) and b (arrow) types arranged in CIII, AI and AII cycles. $\times 2,700$. E) Furca with the d type (intermediate cilium and no visible collar) of sensory cilia (arrowheads). $\times 1,560$. F) The a type PID sensillum (arrowhead). $\times 2,220$. G) Lateral sensillum of a type in PII cycle. $\times 4,200$.

DISCUSSION

The SEM picture extends Niewiadomska's observations obtained in the light microscope (1987). Regarding the body armature, it is seen that all ten transverse rows are com-

plete on the dorsal and 8 on the ventral side instead of 6 and 8, respectively, observed in LM. On the dorsal side a spineless area between the last transverse row and spines covering the posterior body part is demonstrated. Although on LM observations the tail stem and the furca were reco-

gnized as spineless, SEM showed spine-like structures on these body parts. Generally, the shape and distribution of spines in the SEM picture were clearly visible.

The SEM provided more details in relation to chaetotaxy. It was possible to demonstrate a different outer structure of sensilla connected with length of cilium, presence or absence of collar, and its structure. These differences undoubtedly correspond with the differences in inner structure of sensilla as was found in TEM investigations of sensory endings of *D. pseudospathaceum* cercaria (Czubaj and Niewiadomska, unpublished data). It was also found that the distribution of different sensilla types is stable. New data were obtained about the number, structure and arrangement of CI cycle of cephalic sensilla which was impossible to investigate in LM. The position of sensilla in relation to transverse rows of spines may change, e. g. dorsal sensilla of the AII cycle may be arranged between two or three transverse rows.

A disadvantage of SEM investigations is the limited number of observed specimens which hinder observation of variability in body armature and chaetotaxy. However we consider the inclusion of the variety of sensilla in chaetotaxy to have considerable importance in establishing a characteristic pattern for species or indicating the relationships.

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