

ARGENTOPHILIC STRUCTURES OF THE MIRACIDIUM OF *ECHINOCHASMUS PERFOLIATUS* (TREMATODA: ECHINOSMATIDAE)

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Summary :

Argentophilic structures of the miracidium of *Echinochasmus perfoliatus* were described from material collected in the vicinity of Vladivostok, Far East of Russia. Impregnated with 0.5% solution of AgNO₃ miracidium showed 21 epidermal plates arranged in four rows: 6 + 9 + 4 + 2. Up to 23 papilla-like structures on the terebratorium were arranged along three axes and in four groups. A single papilla was located at the base of each of ventral and dorsal epidermal plates of the first row. Two papillae were located at the base of each of lateral epidermal plates of the first row. The eyespots were located posterior to the first row of plates. Two excretory pores were located anterior to the last row of plates. The results obtained were compared with the argentophilic structures of closely related species of the genus *Echinochasmus*.

KEY WORDS : *Echinochasmus perfoliatus*, miracidia, argentophilic structures.

Résumé :

STRUCTURES ARGYROPHILES DU MIRACIDIUM DE *ECHINOCHASMUS PERFOLIATUS* (TREMATODA: ECHINOSMATIDAE)
Les auteurs décrivent les structures argyrophiles du miracidium d'*Echinochasmus perfoliatus*. Les poissons contenant les métacercaires à l'origine de la souche ont été récoltés dans les environs de Vladivostok (Russie extrême orientale). Les miracidiums imprégnés dans une solution de AgNO₃ à 0,5 %, portent 21 cellules ciliées disposées sur quatre rangées: 6 + 9 + 4 + 2. Sur le terebratorium on observe jusqu'à 23 structures identiques à des papilles, alignées selon trois axes et formant quatre groupes. Une papille est située à la base de chaque cellule dorsale et ventrale de la première rangée et deux papilles à la base de chaque cellule latérale de cette même rangée. Les ocelles sont situés au-dessous de la première rangée de cellules ciliées. Les deux pores excréteurs sont placés au-dessus des cellules ciliées de la dernière rangée. Les résultats obtenus sont comparés à ceux connus chez les autres espèces du genre.

MOTS CLÉS : *Echinochasmus perfoliatus*, miracidium, structures argyrophiles.

Echinochasmus perfoliatus (Ratz, 1908) is an intestinal parasite of mammals and birds, occurring in Europe and Asia. Various freshwater snails act as first intermediate host. The cercariae of this 24-collar-spined echinostome are known to exhibit broad specificity towards the second intermediate fish host. The life history and morphology of all developing stages have been studied (Tanabe, 1915; Ciurea, 1920; Muto, 1921; Otagaki & Kanemitsu, 1953). Detailed studies on the structure of the miracidia of this species are not available, although Sahai & Shrivastava (1970) identified the epidermal plate pattern as 6:6:4:2. In the present study, silver impregnation was used to examine the argentophilic structures of *E. perfoliatus* miracidia.

MATERIAL AND METHODS

Carp (*Cyprinus carpio*), caught in the irrigation canals near Vladivostok in the Far East of Russia, were found infected with 24-collar-

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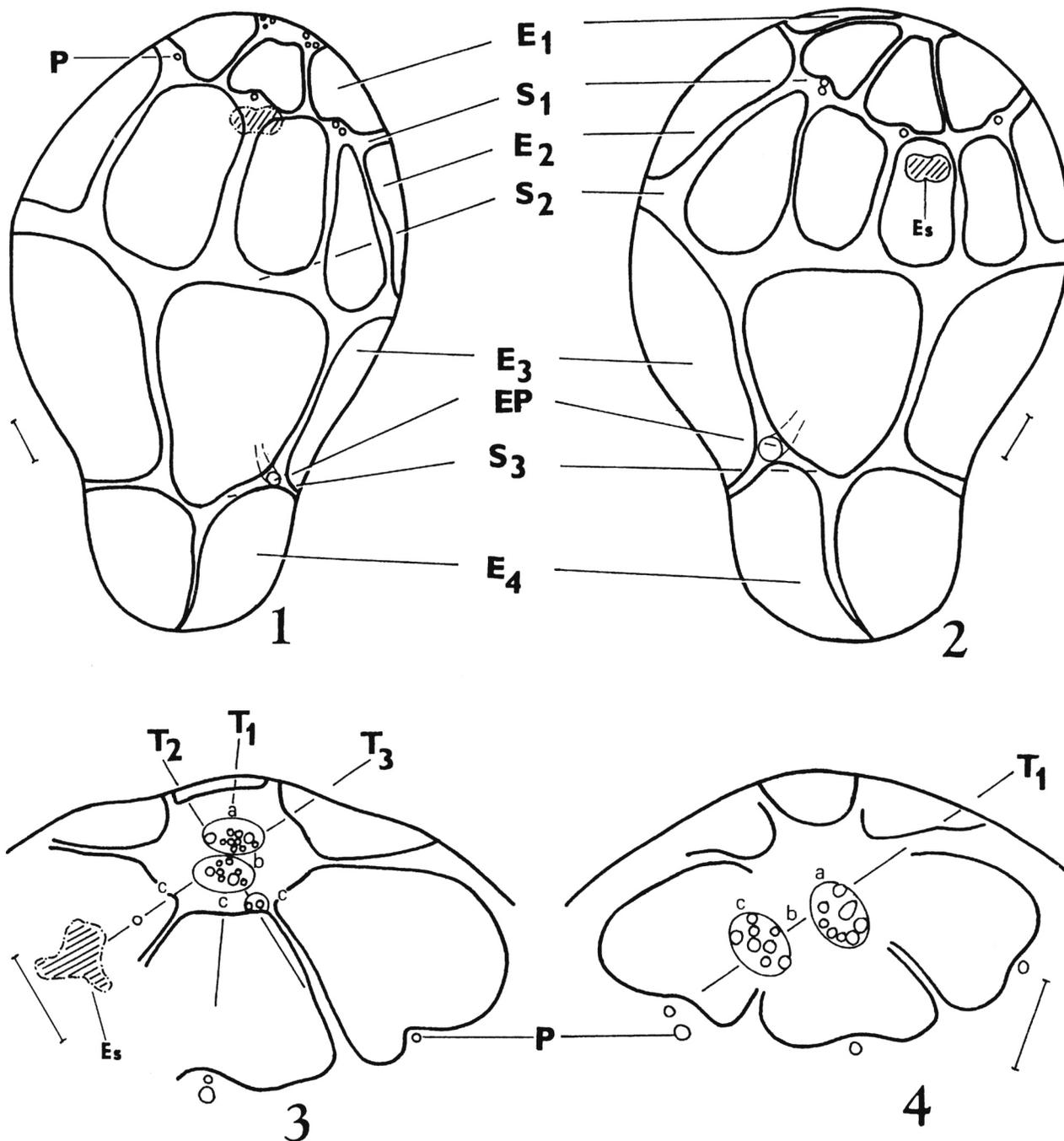
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spined echinostome metacercariae. Laboratory-raised rats (*Rattus rattus*) fed with metacercariae became infected. Egg cultures prepared by teasing the uterus of adults were airshipped to Sofia for incubation and further experimental studies. Techniques used for collecting, incubation, hatching and impregnation of the miracidia were as described in earlier studies (Dimitrov *et al.*, 1991; Dimitrov & Kanev, 1992). The exact locality of all structures are described according to the nomenclature of Dimitrov *et al.*, (1995). Photomicrographs and drawings were made using a light microscope "Opton" equipped with videomat and camera lucida. All measurements are in micrometers.

RESULTS

Miracidia (n = 25) prepared in silver nitrate had a body length 101.5-125.2 and body width of 73.5-82.36. A total of about 52 argentophilic structures per miracidium were described. The eyespots were also seen in impregnated miracidia.

Twenty-one epidermal plates (E) (Figs. 1, 2) were arranged in four rows. The first (anterior) row (E₁) had six triangular plates, two ventral (E₁V), two dorsal (E₁D) and two lateral (E₁L) (one on each side); they were 14-



Figs. 1-4. – Argentophilic structures of *Echinobasmus perfoliatus* miracidia. Fig. 1. Whole body of a miracidia in ventral position showing epidermal plates (E) arranged in four rows (E1-E4), the interepidermal space (S) in three levels (S1-S3), the papillae on the body (P), the eyespots (Es) and the excretory pores (EP). Fig. 2. Whole body of a miracidia in dorsolateral position. Fig. 3. Apical view of the terebratorium (T) surrounded by six epidermal plates (PI) of the first (anterior) row (E1), showing 21 papilla-like structures (PI). Fig. 4. Apical view of the terebratorium (T) of miracidia with 16 visible papillae in T1 a,b,c. Scale bars are 10 micrometers.

23.2 long and 15.4-22.04 wide at the base. In the second row (E_2) there were nine plates, one medioventral (E_{2MV}), two ventrolateral (E_{2VL}), two lateral (E_{2L}), two dorsolateral (E_{2DL}), and two dorsal (E_{2D}); two lateral epidermal plates were triangular, 19.6-30.1 long and 9-14 wide at the base; the other plates in the second row were rectangular, 19.6-30.1 long and 7.7-17.5 wide. The third row (E_3) included four square plates, two ventrolateral (E_{3VL}) and two dorsolateral (E_{3DL}) and were 27.3-53.3 long and 20-40.6 wide. The fourth row (E_4) consisted of two subtriangular in ventrodorsolateral positions (E_{4VDL}), 29.4-35.9 long and 38.5-69.6 wide at the anterior end. The exact position of the epidermal plates are:

$$6E_1 + 9E_2 + 4E_3 + 2E_4 = 21$$

$$E_1 = 1 + 1E_{1V}, 1 + 1E_{1L}, 1 + 1E_{1D}$$

$$E_2 = 1E_{2MV}, 1 + 1E_{2VL}, 1 + 1E_{2L}, 1 + 1E_{2DL}, 1 + 1E_{2D}$$

$$E_3 = 1 + 1E_{3VL}, 1 + 1E_{3DL}$$

$$E_4 = 1 + 1E_{4VDL}$$

A maximum of 23 papilla-like structures (Figs. 3, 4) was observed on the terebratorium (T). They were arranged on three axes (T_1 , T_2 and T_3) and were distributed in four groups (T_{1a} , T_{1c} , T_{2c} and T_{3c}). Small and large structures, about 1 and 2.5 in diameter, respectively, occurred in the groups T_{1a} and T_{1c} . The arrangement of papilla-like structures in the terebratorium is not completely symmetrical due to variations in their number and position. The papilla formula is:

$$T = T_1 + T_2 + T_3 = 16 - 23$$

$$T_1 = 8 - 10T_{1a} + 0T_{1b} + 8 - 10T_{1c}$$

$$T_2 = 0T_{2a} + 0 - 2T_{2c}$$

$$T_3 = 0T_{3a} + 0 - 1T_{3c}$$

Eight papillae on the body (P) were found in the space (S) between the first (E_1) and second row (E_2) of plates (Figs. 1, 2, 3, 4). A single papilla was located at the base of each of the ventral and dorsal, and two were located at the base of each lateral epidermal plate. Two of them were ventral (PV), four were lateral (PL), and two were dorsal (PD). Their number and arrangement are:

$$P = 8S_1$$

$$8S_1 = 1 + 1PV, 2 + 2PL, 1 + 1PD$$

Two eyespots (E_s) and two excretory pores (EP) were also seen. The eyespots were formed by three pairs of crystalline lenses. The length of the eyespots was about 8 in diameter, they were located dorsally, close to the interepidermal space (S_1) (Figs. 1, 2, 3). The excretory pores opened laterally between the third (E_3) and fourth (E_4) row of epidermal plates, on the third interepidermal space, i.e., S_3 (Figs. 1, 2).

DISCUSSION

Miracidia considered to belong to the species of the genus *Echinochasmus* were described with four different numbers and arrangement of epidermal plates as follows: a pattern of

17 epidermal plates, arranged in four rows: 6:6:3:2 was described by Yamaguti (1942) for miracidia of *Echinochasmus tobi* (Yamaguti, 1939). A pattern of 18 epidermal plates arranged in four rows: 6:6:4:2 was described by Karmanova (1971, 1973) for miracidia of *E. bursicola* (Creplin, 1837) and for *E. spinosus* Odhner, 1910, and by Sahai & Srivastava (1970) for miracidia of a parasite from India identified as *E. perfoliatus*. A pattern of 20 epidermal plates, arranged in four rows: 6:8:4:2 was described by Karmanova (1974) for miracidia of *E. coaxatus* (Dietz, 1909). A pattern of 21 epidermal plates is described in this paper for miracidia of *E. perfoliatus*.

Significant differences were found in respect of the number and arrangement of the papillae on the terebratorium and on the body. Karmanova (1974) described five papillae on the terebratorium of *E. coaxatus* miracidia while in this paper we found up to 23 papillae for the *E. perfoliatus* miracidia. Also Karmanova (1974) established that there were six papillae between the epidermal plates of first (E_1) and second (E_2) rows. In the present paper we found eight papillae for miracidia of *E. perfoliatus*. Compared structures are easy visible (exception: small papillae on the terebratorium) by different silver impregnated methods. These differences could be explained, because *E. coaxatus* and *E. perfoliatus* are two well established species using different vertebrate and invertebrate animals as final, first and second intermediate hosts. For the same reason, it is possible to explain some of the other differences presented above. However, it is difficult to explain why Sahai & Srivastava (1970) and we in this paper found different numbers and different patterns of arrangement of papillae of miracidia from Asian *E. perfoliatus*. Based on previous studies (Kanev, 1994, Kanev *et al.*, 1993, 1994a,b, 1995a,b) where adults and larvae of different species, even of different genera have been found incorrectly described with a common name as members of a common species, we suggest that perhaps the name *E. perfoliatus* was used for description of miracidia belonging to different species. Working in Europe, we compared adult worms of *E. perfoliatus* from its type locality in mid-Europe with the adults from Vladivostok region, used in this study, and found no significant differences between them.

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