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Morphology and chaetotaxy of *Trichobilharzia szidati* Neuhaus, 1952 cercariae (Trematoda: Schistosomatidae: Bilharziellinae)

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Summary

Morphology and chaetotaxy of *Trichobilharzia szidati* Neuhaus, 1952 cercariae from naturally and experimentally infected snails of the family Lymnaeidae from Czech Republic and Northern Austria were studied. Chaetotaxy of cercariae expressed as the number and arrangement of dorsal, ventral and lateral surface papillae of the body and tail is presented and compared with literature describing cercariae of *T. ocellata* group.

Key words: *Trichobilharzia*; Trematoda; cercariae; morphology; chaetotaxy

Introduction

Cercariae of the genus *Trichobilharzia* play an important role as an aetiological agent of cercarial dermatitis of humans in Central Europe: Germany (Dönges, 1965; Kilias and Frick, 1964; Krampitz *et al.*, 1974; Hohorst, 1981; Feiler and Haas, 1988; Effelsberg, 1989; Müller *et al.*, 1993; *etc.*), Austria (Graefe *et al.*, 1973), Slovakia (Farkaš, 1980), Switzerland (Meyer and Dubois, 1954; Eklu-Natey *et al.*, 1985; etc.) and Czech Republic (Ďďárská, 1963; Kolářová *et al.*, 1989; Kolářová *et al.*, 1992). Contrary to the description of *T. szidati* by Neuhaus (1952a), many incomplete data of ocellate furcocercariae without knowledge of the entire trematode life cycle were published and these findings are generally considered to be larval stages of *T. ocellata* (La Valette, 1855) Brumpt, 1931. The taxonomic status of such cercariae is in some critical reviews regarded as *species inquirendae* (Blair and Islam, 1983).

Despite difficulties in discrimination between *T. ocellata* and *T. szidati* (Haas and Pietsch, 1991), our unpublished studies based on miracidial and adult morphology and location of adult worms (Horák and Kolářová, unpublished results) resulted in the confirmation that *T. szidati* represents a valid species.

No data are available on the chaetotaxy of *T. szidati* until now. Richard (1971) mentioned that the evaluation of cercarial chaetotaxy may be an important step towards identification of trematode species. Because the staining of papillae is a simple and quick method, the aim of our study was to present chaetotaxy as a tool for rapid species identification of infective larval stages which are very similar in their morphology among related species.

Here, we present complete morphology of cercariae found in naturally and experimentally infected lymnaeid snails. Emphasis was paid on the excretory system and surface papillae patterns. Our findings were compared with the Neuhaus' (1952a) description of *T. szidati*.

Material and Methods

Cercariae were obtained from naturally or experimentally infected snails of *Lymnaea stagnalis*, *Radix auricularia*, *L. peregra* and *Stagnicola palustris*. Naturally infected snails were collected in Bohemia, Southern Moravia and Northern Austria. Most of Czech localities were noted in our previous paper (Kolářová *et al.*, 1992), new localities with *Trichobilharzia* sp. occurrence were as follows: Hamr (Veselí n. L., Southern Bohemia) where a total of 359 snails of the species *L. stagnalis*, *R. auricularia* and *L. peregra*, and Odening (Northern Austria) where a total of 841 *R. auricularia* were obtained.

Cercariae from naturally infected snails were used for morphological study as well as for supporting infection experiments leading to life cycle observations (not considered in this study). Cercariae released from experimentally infected snails of *L. stagnalis* were compared with those emerged from naturally infected intermediate hosts.

Larval stages of flukes were identified in fresh and permanent mounts. Fresh mounts were stained with Nile blue, permanent mounts were made either of cercariae emerged from infected snails or of squashed tissue of infected snails, both fixed with 4% formaldehyde. Cercariae which were heat-killed in a drop of water on a slide were measured in mounts (modification of Brumpt's method from 1930). Size of cercariae and their organs was assessed in 100 specimens originated from naturally infected hosts: *L. stagnalis* (locality St. Říše, Strmilov and Hamr), *R. auricularia* (locality Příbram, Rozkoš and Odening) and *L. peregra* (locality Hamr). Moreover, size of larval stages was assessed in 100 specimens originated from experimentally infected *L. stagnalis*. Sensory papillae were examined after staining with 1% silver nitrate in distilled water. Permanent mounts were made using Swan embedding medium modified by Holman.

Results

Morphology and chaetotaxy of cercariae from naturally and experimentally infected snails from all localities were identical.

a) *Morphology of cercariae*

Size of cercarial body (Fig.1) is 290—390 µm x 70—80 µm and of tail stem 330—456 x 370—463 µm. Two furcae measure 192—246 x 24—28 µm each bearing finfolds clearly visible in distal half of dorsal and ventral margins.

Tail is fastened to body by diagonal muscle fibers arising from lateral wall of posterior end of the body and attached to the corresponding part of proximal end of the tail. Tail stem consists of longitudinal and circular muscle fibers.

Tegument of body and tail contains spines. Length of spines of the body is about 1 µm, of stem and furcae about 3 µm, on the latter two parts less dense than on the body.

Anterior part of the body possesses a large muscular organ - a head organ (83—97 µm x 46—59 µm) with ducts and openings of penetration glands. Digestive tract starts on ventral side of the head organ, proceeds to muscular oesophagus (19—22 x 19 µm) which branches into two short caeca, these reach the area facing the first pair of penetration glands.

Ventral sucker - acetabulum (26—42 x 24—47 µm) is surrounded by 2 pairs of coarsely granular penetration glands. Other 3 pairs of unicellular glands with finely granular content are located in posterior part of cercarial body, 5 pairs of ducts pass from glands forwards to the head organ. Genital primordium (18—27 µm x 9—18 µm) consisting of small cells lies behind acetabulum between 2 pairs of penetration glands. Lateral parts of the

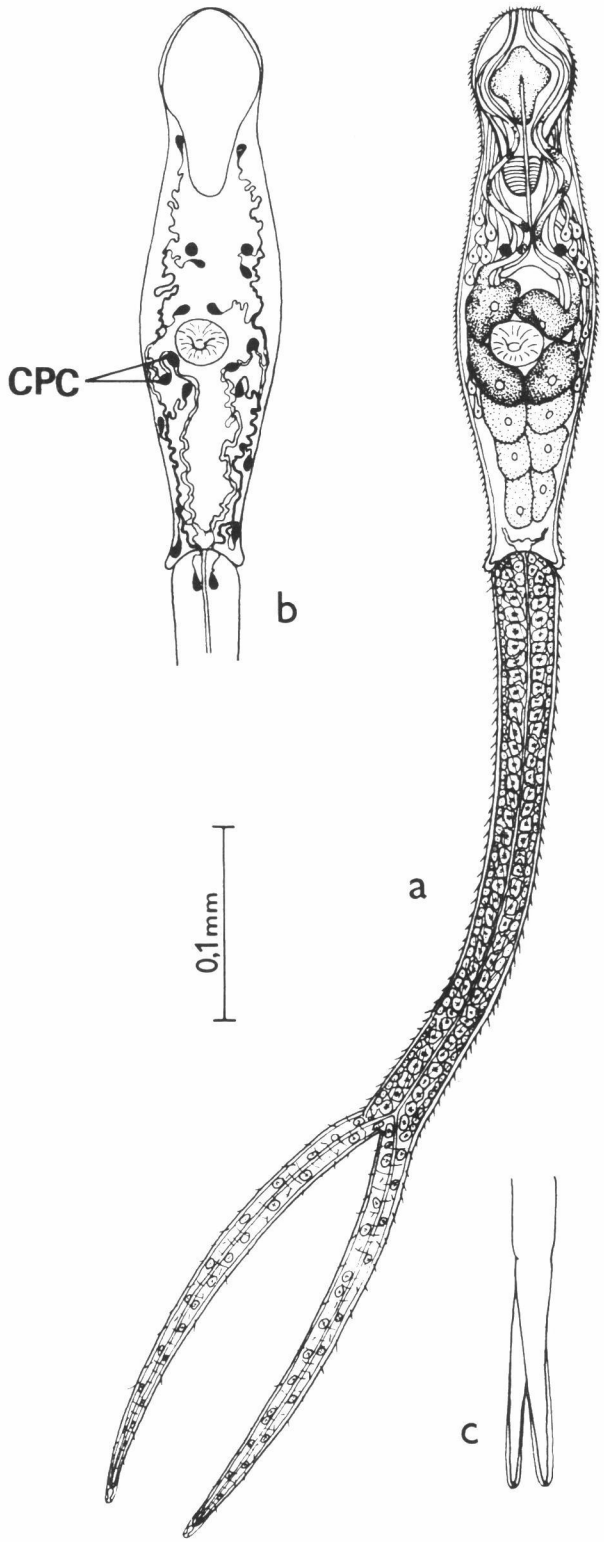

Fig. 1. *Trichobilharzia szidati* Neuhaus, 1952: a—cercaria, b—scheme of excretory system of cercaria with 7 pairs of flame cells and 2 pairs of clusters of propellant cilia (CPC); c—furcae with finfolds

body contain about 13 pairs of escape gland cells with ducts passing to the anterior organ.

One pair of pigmented eyspots (8 μm in diameter) is situated on dorsal part of the body.

Excretory system consists of 7 pairs of flame cells, 2 pairs of propellant cilia and ducts. Six pairs of flame cells are located in the body, 1 pair at the base of the tail stem. Their distribution is illustrated in Fig.1.

On each side of the body, 3 flame cells are followed by the anterior collecting duct in front of acetabulum, 3 flame cells are followed by the posterior collecting duct. Common collecting duct inside with 2 clusters of propellant cilia starts in junction of anterior and posterior collecting ducts on each side of the body. These ducts pass into posterior extremity where they unit and form a bladder before entering top of the tail stem. The main excretory duct passes along center of the tail stem and branches into furcae. Excretory-secretory products leave cercarial body through terminal pores at tops of furcae. Each pore penetrates furcal finfold. Flame cell formula can be expressed either without clusters of cilia as 2[3+3+(1)]=14 or with clusters of cilia as 2[(3+3+1)+ +2]=18.

b) *Chaetotaxy of cercariae*

Sensory papillae are arranged in distinct pattern on body, tail stem and furcae. Their distribution is shown in Fig.2, and Table 1.

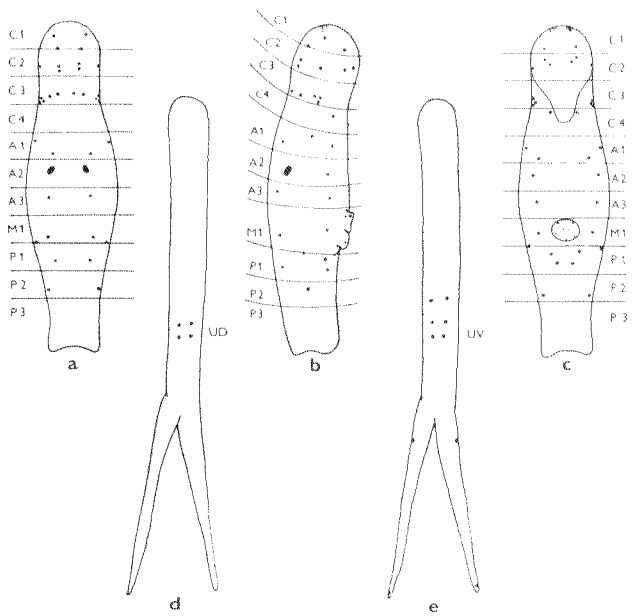

Fig. 2. Distribution of papillae on the body and tail of cercariae of *Trichobilharzia szidati* Neuhaus, 1952
a — c: body (a—dorsal, b—lateral and c—ventral view), d—e: (d — dorsal and e — ventral view)

Tab. 1. Chatotaxy of *Trichobilharzia szidati* cercariae from the locality Strmilov

Body		
Dorsal papillae	**Lateral papillae**	**Ventral papillae**
1 C I	6—9 C I	1 C I
3 C II	1 C II	2 C II
2 C III	3 C III	C III
C IV	C IV	1 C IV
1 A I	1 A I	1 A I
A II	A II	1 A II
1 A III	A III	1 A III
1 M I	M I	1 M I
1 P I	1 P I	2 P I
P II	1 P II	P II
Acetabulum		
5 S I	2 S II	
Tail		
4 U D	6 U V	2 F V

Discussion

Morphology of cercariae in our study was basically identical to N e u h a u s' description of *T. szidati* (1952a,b). The morphology, location and appearance of other developmental stages confirmed this determination (H o r á k and K o l á ř o v á - unpublished results). Intraspecific variability of cercariae was in agreement with N e u h a u s' observation (1952a,b). Only one difference in the length of finfolds was documented.

As shown in Fig.1c, finfolds are clearly developed up to the first half of furcae. This result might arise due to differences in fixation procedure.

Having regard to two clusters of propellant ciliae present inside collecting ducts, N e u h a u s (1952a) suggested to include these cilia into the flame cell formula 2[(3+3+1)+2]. Despite the clear evidence that the clusters are a part of the excretory system, many authors did not include these clusters into the flame cell formula of other schistosome species (M a c F a r l a n e, 1979; P e n n e r, 1950; E d w a r d and J a n s c h, 1955; B l a i r and I s l a m, 1983, *etc.*), probably due to the fact that they do not represent a terminal flame cell.

In contrast to *T. szidati* (Neuhaus 1952a), the taxonomic status of *T. ocellata* (La Valette, 1855) is uncertain (C o r t, 1950; F a r l e y, 1971; B l a i r and I s l a m, 1983). D u b o i s (1929), M a t h i a s (1930), T a y l o r and B a y l i s (1930), V o g e l (1930), B r u m p t

Table 2. Differences in chaetotaxy of three species of *Trichobilharzia*

Cycle	*T. szidati*	*T.* cf. *ocellata* Eklu-Natey *et al.* (1985)	*T. ocellata* Richard (1971)
C III L	3	3	2
M I L	0	1	0
P I L	1	0	1
S I	5	5	3
F V	2	2	1

(1931), Wesenberg-Lund (1934) and others assume that *Cercaria ocellata* is the sole representative of its group in Europe. On the other hand, Szidat (1942), Neuhaus (1952a) and Meyer and Dubois (1954) demonstrated that such cercariae may belong to several species grouped under the name *C. ocellata*. Feiler and Haas (1988) suggested that *T. szidati* (Neuhaus 1952a) is in synonymy with *T. ocellata*.

The taxonomic position of *T. ocellata* could also be approached by chaetotaxical study of cercariae. Present information on the chaetotaxy of *Trichobilharzia* species is, however, scanty. Richard (1971) described chaetotaxy of *T. ocellata*. Eklu-Natey *et al.* (1985) referred to the distribution of papillae of *T.* cf. *ocellata*, Bayssade-Dufour and Ow-Yang (1975) informed about the distribution of papillae in *T. brevis*, chaetotaxy of *T. australis* was described in the study of Blair and Islam (1983).

Table 2 summarizes foreign and our data concerning differences in chaetotaxy of *Trichobilharzia* furcocercariae found in Europe: A clear difference was observed between *T. ocellata* (Richard, 1971) and two other "species", i. e., *T. szidati* (Czech origin) and *T.* cf. *ocellata* (Eklu-Natey *et al.*, 1985). Richard (1971) noted two papillae on CIIIL, three papillae on SI and one FV papilla. She did not refer any papilla on the MIL position. Our observations as well as those of Eklu-Natey *et al.* (1985) brought the evidence of three papillae on CIIIL, five on SI and two FV papillae. *T. szidati* in our collection differs from *T.* cf. *ocellata* (Eklu-Natey *et al.*, 1985) in position of one papilla in MIL area. We observed variation in the position of this papilla which changed from MIL to PIL areas, but was mostly on PIL. We feel, however, that an examination of the same cercaria by two independent observers could lead to different results as the limits of MIL and PIL are not strictly defined. From this point of view, we conclude that cercariae from our collection and those studied by Eklu-Natey *et al.* (1985) both belong to the same species.

Based on both the complete description by Neuhaus (1952a) which differs from other descriptions (e.g., Richard, 1981) and our additional study of morphology and chaetotaxy, we regard the examined cercariae as belonging to the species *T. szidati*. In our opinion, *T. ocellata* (La Valette, 1855) Brumpt, 1931 represents a complex of species containing without deeper knowledge of all developmental stages such cryptic species as were *T. szidati* (Neuhaus, 1952a) and *T. franki* (Müller *et al.*, 1994) before 1952 and 1994, respectively.

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References

Bayssade-Dufour, Ow-Yang D. B. (1975): The sensory receptors of two cercariae from Malaysia, *Trichobilharzia brevis* (Schistosomatidae) and *Haplorchis pumilio* (Heterophyidae). *Southeast Asian J. Trop. Med. Publ. Health,* 6: 338—342

Blair D., Islam K. S. (1983): The life cycle and morphology of *Trichobilharzia australis* n. sp. (Digenea:Schistosomatidae) from the nasal blood vessels of the black duck (*Anas superciliosa*) in Australia with a review of the genus *Trichobilharzia*. *Syst. Parasitol.*, 5: 89—117

Brumpt E. (1930): Cycle évolutif complet de *Schistosoma bovis*. Infection naturelle en corse et infection expérimentale de *Bulinus contortus*. *Ann. Parasit. Hum. Comp.*, 8: 17—50

Brumpt E. (1931): *Cercaria ocellata* determinant la dermatite des nageurs, provient d'une bilharzie des canards. *Compt. Rend. Acad. Sci. Paris,* 193: 612—614

Cort W. W. (1950): Studies on schistosome dermatitis. IX. Status of knowledge after more than 20 years. *Am. J. Hyg.* 52: 251—307

Dönges J. (1965): Schistosomatiden-Cercarien Süddeutschland (Ein Beitrag zur Kenntnis dermatitiserregender Trematodenlarven). *Z. Tropenmed. Parasit.*, 16: 305—321

Dubois G. (1929): Les cercaires de la région de Neuchatel. *Bull. Soc. Neuchat. Sci.*, 53: 2—177

Edwards D. K., Jansch M. E. (1955): Two new species of dermatitis-producing schistosome cercarie from Cultus Lake, British Columbia. *Can. J. Zool.*, 33: 182—194

Eklu-Natey D. T., Al-Khudri M., Gauthey D., Dubois J. P., Wuest J., Vaucher C., Huiggel H. (1985): Epidemiologie de la dermatite des baigneurs et morphologie de

Trichobilharzia cf. *ocellata* dans le lac Léman. *Revue Suisse Zool.*, 92: 939—953

Farkaš J. (1980): Cerkariendermatitis bei einem Ancler. *Dermatol. Monatsschr.*, 166: 747—750

Farley J. (1971): A review of the family S c h i s t o s o m a t i d a e excluding the genus *Schistosoma* from mammals. *J. Helminthol.*, 45: 289—320

Feiler W., Haas V. (1988): Host finding in *Trichobilharzia ocellata* cercariae: swimming and attachment to the host. *Parasitology*, 96: 493—505

Graefe G., Aspöck H., Picher O. (1973): Auftreten von Badedermatitis in Österreich und Möglichkeiten ihrer Bekämpfung. *Zbl. Bakt. Hyg. I. Abt. Orig.*, A 225: 398—405

Haas W., Pietsch V. (1991): Migration of *Trichobilharzia ocellata* schistosomula in the duck and in the abnormal murine host. *Parasitol. Res.*, 77: 642—644

Hohorst W. (1981): Schnecken als Brutstätten parasitischer Würmer. *Nat. Mus.*, 111: 60—69

Kilias R., Frick W.(1964): Die Zwischenwirtsschnecken wichtiger einheimischer Haus- und Nutztierhelminthen. *Angew. Parasitol.*, 5: 13—45

Kolářová L., Gottwaldová V., Čechová D., Ševcová M. (1989): The occurrence of cercarial dermatitis in Central Bohemia. *Zbl. Hyg.*, 189: 1—13

Kolářová L., Horák P., Fajfrlík K. (1992): Cercariae of *Trichobilharzia szidati* Neuhaus, 1952 (Trematoda: S c h i s t o s o m a t i d a e): The causative agent of cercarial dermatitis in Bohemia and Moravia. *Folia Parasitol.*, 39: 399—400

Krampitz H. E., Piekarski G., Saathoff M., Weber A. (1974): Zerkarien-Dermatitis. *Münch. med. Wschr.*, 116: 1491—1496

La Valette St. George A. J. H. (1855): *Symbolae ad trematodum evolutionis historiam*. Dissertation, Berolius

Mac Farlane W. V. (1949): Schistosome dermatitis in New Zealand Part. I. The Parasite. *Am. J. Hyg.*, 50: 43—51

Mathias P. (1930): Sur *Cercaria ocellata* La Valette. *Ann. Paras. Hum. Comp.*, 8: 151—160

Meyer P. O., Dubois G. (1954): Dermatite humaine causée par des furcocercaries ocellés dans les bains publies de Zurich. *Bull. Soc. Neuchat. Sc.*, 77: 81—110

Müller V., Kimmig P., Frank W. (1993): Die Wirkung von Praziquantel auf *Trichobilharzia* (Digenea, S c h i s t o s o m a t i d a e), einen Verursacher von Badedermatitiden beim Menschen. *Appl. Parasitol.*, 34: 187—201

Müller V., Kimmig P. (1994): *Trichobilharzia franki* n.sp. - die Ursache für Badedermatitiden in südwestdeutschen Baggerseen. *Appl. Parasitol.*, 35: 12—31

Neuhaus W. (1952a): Biologie und Entwicklung von *Trichobilharzia szidati* n. sp. (Trematoda, S c h i s t o s o m a t i d a e), einem Erreger von Dermatitis beim Menschen. *Z. Parasitenkde*, 15: 203—266

Neuhaus W. (1952b): Der Einfluß des Zwischenwirtes auf die Gestalt der Cercarien von *Trichobilharzia szidati* Neuhaus, 1951 und ihre systematische Kennzeichnung. *Zool. Anz.*, 148: 275—285

Richard J. (1971): La chétotaxie des cercaires. Valeur systématique et phylétique. *Mém. Mus. Nat. Hist.*, Série A Zool. 67: 1—179

Szidat L. (1942): Was ist *Cercaria ocellata* La Valette? Morphologische und entwicklungsgeschichtliche Untersuchungen über den Erreger der europäischen Cercarien-Dermatitis des Menschen. *Dtsch. Tropmed. Zeitsch.*, 46: 481—497, 504—524

Taylor E. L., Baylis H. A. (1930): Observation and experiments on a dermatitis-producing cercaria and other cercariae from *Lymnaea stagnalis* in Great Britain. *Trans. R. Soc. Trop. Med. Hyg.*, 24: 219—244

van den Broek E. (1965): Some recent cases of Asian schistosomiasis and schistosome dermatitis in the Netherlands. *Trop. Geogr. Med.*, 17: 229—235

Vogel H. (1930): Cercarien-dermatitis in Deutschland. *Klin. Wschr.*, 9: 883—886

Wesenberg-Lund C. (1934): Contributions to the development of Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. *Mem. Acad. Roy. Sc. Lettr.*, Danmark: 1—223

Ždárská Z. (1963): Trematode larval stages from fresh water snails in Czechoslovakia. *Čs. parasitol.*, 10: 207—262