TRICHOBILHARZIA REGENTI n. sp. (SCHISTOSOMATIDAE, BILHARZIELLINAE), A NEW NASAL SCHISTOSOME FROM EUROPE

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Summary :

Members of the genus *Trichobilharzia* are parasitic in visceral or nasal body parts of their avian hosts. The examination of water snails in South Bohemia revealed a schistosome infection in *Radix peregra* snails. The experimental infection of ducklings (*Anas platyrhynchos, Cairina moschata*) confirmed that a new *Trichobilharzia* species – *T. regenti* n. sp. – was found. The adults, eggs and larvae (miracidia and cercariae) of the species were morphologically characterized. The adults occupy the nasal blood vessels where they lay the eggs; the miracidia hatch from the eggs directly in the tissue and leave the host during drinking/feeding of the infected birds.

KEY WORDS : *Trichobilharzia*, Schistosomatidae, nasal schistosome, life cycle, *Radix*.

Résumé : *TRICHOBILHARZIA REGENTI* N. SP. (SCHISTOSOMATIDAE, BILHARZIELLINAE), NOUVEAU SCHISTOSOME NASAL D'EUROPE

Les membres du genre Trichobilharzia *parasitent les régions viscérale et nasale de leurs hôtes aviaires. L'examen de mollusques d'eau douce en Bohème du Sud a montré une infestation par schistosome de* Radix peregra. *L'infestation expérimentale de canetons (*Anas platyrhynchos, Cairina moschata*) confirme la découverte d'une nouvelle espèce de* Trichobilharzia, T. regenti *n. sp. Les formes adultes, les œufs et les larves (miracidies et cercaires) de l'espèce sont caractérisés sur le plan morphologique. Les adultes occupent les vaisseaux sanguins nasaux où ils déposent leurs œufs; les miracidies éclosent directement dans les tissus et quittent l'hôte quand les oiseaux infectés boivent ou s'alimentent.*

MOTS CLÉS : Trichobilharzia, *Schistosomatidae, schistosome nasal, cycle parasitaire,* Radix.

INTRODUCTION

The genus *Trichobilharzia* comprising of about 40 species is the largest genus of the family Schistosomatidae (Blair & Islam, 1983). In the two-host life cycle, the adults parasitize birds whereas the larvae (miracidia) invade different pulmonate snails giving rise to sporocysts. The furcocercariae released from intermediate hosts are known as the causative agent of human cercarial dermatitis.

Adults are parasitic either in visceral or nasal areas of the infected host. The latter, i.e. the parasites of nasal cavity, represent a minor group within the genus. They were reported for the first time by Fain (1955a,b,c, 1956a,b, 1959) in Rwanda, Africa. Later, the parasites were found in India (Lalitha & Alvar, 1960), Australia (Bearup, 1957; Blair & Ottesen, 1979; Blair & Islam, 1983; Islam, 1986a) and Switzerland (Palmer & Ossent, 1984). Until now, seven species of nasal *Trichobilharzia* were described (Table I).

In our contribution, the description of a new nasal *Trichobilharzia* from Central Europe is presented. The species uses *Radix peregra* as intermediate host; anatid birds represent a susceptible final host under laboratory conditions.

Species	Host	Country	Report
T. nasicola	*Anas undulata undulata*	Rwanda	Fain 1955a, 1956a
T. rodhaini	*Hagedashia hagedash*	Rwanda	Fain 1955a, 1956a
T. spinulata	*Alopochen aegyptiacus* *Plectropterus gambensis*	Rwanda	Fain 1955a, 1956a
T. aureliani	*Podiceps cristatus infuscatus* *Poliocephalus ruficollis capensis*	Rwanda	Fain 1956a
T. duboisi	*Nettapus auritus*	Rwanda	Fain 1959
T. australis	*Anas superciliosa*	Australia	Blair & Islam 1983
T. arcuata	*Dendrocygna arcuata*	Australia	Islam 1986a

Table I. – Descriptions of nasal *Trichobilharzia* species.

MATERIALS AND METHODS

During the period June-September 1997, snails of the species *Radix peregra peregra* and *R. p. ovata* were collected in two South Bohemian ponds: Žoldánka and Podkadovský, both near

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Blatná, district Strakonice. In the laboratory, the snails were exposed to illumination and in case of schistosome infection, the released cercariae were processed further.

A part of the cercariae was used for experimental infection of ducklings and chickens. The infection dose varied with respect to the number and size of infected snails and ranged approx. from 50 to 500 cercariae/one bird. Ducklings of the species *Anas platyrhynchos f. domestica* (one specimen) and *Cairina moschata f. domestica* (nine specimens) and chickens *Gallus gallus f. domestica* (two specimens) were infected by the cercariae as described by Meuleman *et al.* (1984). Starting on day 10 p.i., the bird faeces were tested for the presence of schistosome eggs. The birds were decapitated between 18-30 d.p.i. and their organs (intestine, liver, kidney, lungs, heart, nasal area) and adjacent blood vessels were examined for the presence of schistosome eggs and/or adults by tearing of the organs in 0.85 % saline.

The morphological characterization (the position and shape of organs) of adult schistosomes was made on fresh unfixed material using Nomarski interference contrast, while all measurements were made on fixed and mounted worms. The worms were fixed in hot 4% formaldehyde overnight. Then, they were washed and stained either by hematoxylin-eosin or by Gomori (a trichrome). The stained worms were dehydrated in ethanol and toluene and mounted in Histoclad (Clay Adams, USA).

The size and shape of eggs was determined on fresh, unfixed material. Morphology of miracidia and cercariae was observed on native larvae (interference contrast) as well as on formaldehyde-fixed worms. The fixed larvae were measured and their inner morphology was evaluated by staining of whole larvae as well as their histological sections in JB-4 resin (Polysciences). Borax-carmine, hematoxylin-eosin, Giemsa and Gomori were used as stains. In case of miracidial plate counting, surface labelling by fluorescein-conjugated lectins (*Arachis hypogaea* agglutinin and *Ricinus communis* agglutinin; 100 µg/ml in Tris buffer, pH 7.8; see Horák, 1995 for details) served as a marker. Sensory papillae of 100 cercariae were examined after staining in 2% silver nitrate in distilled water; permanent mounts were made using Swan embedding medium modified by Holman. Chaetotaxical nomenclature of Richard (1971) has been used for evaluation.

Freshly emerged miracidia were also used for infection experiments. A total of 21 *R. peregra* snails (shell height about 7-10 mm) collected in natural ponds and free of trematode infection as well as 20 specimens (shell height about 10 mm) of laboratory reared *Lymnaea stagnalis* snails were exposed individually to five miracidia for two hours.

The type material is deposited in the Natural History Museum, Vienna, Austria: NHMWien ZOOEV 3681: holotype (anterior end of a male); 3682: allotype (anterior end of a female); 3683: paratype (anterior and posterior ends of a male).

RESULTS

Prevalence of the natural infection

In the locality Žoldánka, 422 specimens of *R. peregra* were collected and two snails (0.5%) were positive for schistosome cercariae. In the pond Podkadovský, 613 *R. peregra* snails were collected and 16 specimens (2.6%) were infected by schistosomes. The members of both subspecies – *R. p. peregra* and *R. p. ovata* – released the schistosome larvae. Although other species of snails were also examined (*Lymnaea stagnalis*: 105 specimens; *L. palustris*: 41 specimens; *Physa acuta*: three specimens), no infection by schistosomes was revealed. However, in 19 out of the total 945 *R. auricularia* snails (2%), a schistosome infection was revealed. The systematic position (species identification) of these schistosome larvae needs to be further studied and, therefore, they are not the subject of the below mentioned descriptions.

Laboratory infection of birds

Ten ducklings and two chickens were infected by schistosome cercariae from *R. peregra*. Whereas the infection developed in all ducklings, the chickens remained infection-free. Both duck species *A. platyrhynchos* and *C. moschata* represent susceptible final host. Worms were found in the nasal area; other organs were infection-free. Although adult worms were recovered by day 15 p.i. already, the prepatent period (detection of eggs in nasal secretions) was about 20 days p.i.; no eggs were found in faeces. Dissection of the nasal area showed that adult worms are localized partly within blood vessels and partly in soft nasal tissue outside vessels. Course of infection is probably of short duration: although two ducklings were heavily infected and released high number of miracidia (see below), they possessed only one-two worms in nasal area by day 25 p.i.

Adult schistosomes

No intact adult worm was recovered from nasal tissue. Therefore, total length was not measured. With respect to body parts being found, total length of males is estimated to be about 11 mm. All other measurements were made on fragments from thirteen males and six females (Table II and III). The longest fragment from a male was 5.22 mm, from a female 7.11 mm.

	No. Measured	Mean ± SD (mm)
Maximum width[1]	20	0.049 ± 0.007
Width before spatulate end	6	0.058 ± 0.016
Width at posterior end	7	0.132 ± 0.018
Length of spatulate end	7	0.121 ± 0.038
Oral sucker	10	0.039 ± 0.004 × 0.032 ± 0.005
Acetabulum to anterior end	5	0.333 ± 0.073
Acetabulum	9	0.034 ± 0.006 × 0.029 ± 0.006
Length of gynaecophoric canal[2]	12	0.280 ± 0.029
Width of gynaecophoric canal[3]	11	0.079 ± 0.009
Caecal reunion to acetabulum	5	0.247 ± 0.041
Oesophagus[4]	7	0.265 ± 0.045
Seminal vesicle	7	0.247 ± 0.020 × 0.026 ± 0.002
Vesicula seminalis externa[5*]	3	0.140 ± 0.034 × 0.028 ± 0.001
Vesicula seminalis interna[6*]	2	0.104 ± 0.023
Genital papilla	3	0.015 ± 0.001
Size of testes	24	0.039 ± 0.008 × 0.028 ± 0.008
Number of testes	1	> 120
1st testis to gynaecophoric canal[7]	5	0.058 ± 0.011
Last testis to posterior end	5	0.028 ± 0.002
Ejaculatory duct	3	0.030 ± 0.004

Values obtained from extremely contracted/relaxed worms (not included in the above presented means and SD):
[1] min. 0.028, max. 0.101; [2] min. 0.230, max. 0.387; [3] min. 0.064, max. 0.113; [4] min. 0.150, max. 0.368; [5] max. 0.223 × 0.035; [6] max. 0.219 × 0.023; [7] max. 0.230.
* Vesiculae seminales externa and interna were not distinguishable in all specimens; therefore, seminal vesicle as a whole is also reported.

Table II. – Measurements of male *Trichobilharzia* regenti n.sp.

	No. Measured	Mean ± SD (mm)
Width of ovarian region	21	0.062 ± 0.010
Width before spatulate end	2	0.043 ± 0.018
Width at posterior end	2	0.122 ± 0.058
Length of spatulate end	2	0.179 ± 0.073
Oral sucker	5	0.037 ± 0.002 × 0.030 ± 0.002
Acetabulum	6	0.034 ± 0.002 × 0.029 ± 0.002
Acetabulum to anterior end	3	0.297 ± 0.011
Oesophagus	5	0.242 ± 0.012
Caecal reunion to acetabulum	3	0.805 ± 0.162
Ovary	4	0.317 ± 0.029 × 0.022 ± 0.003
Ovary to acetabulum	3	0.223 ± 0.029
Seminal receptacle	5	0.093 ± 0.008 × 0.038 ± 0.010
Vitellaria to seminal receptacle	2	0.123 ± 0.005
Vitellaria	10	0.040 ± 0.005 × 0.014 ± 0.004
Vitellaria to posterior end	2	0.042 ± 0.006
Mature eggs in nasal mucus	1	0.274 ± 0.094
Immature eggs in nasal mucus	15	0.199 ± 0.017 × 0.044 ± 0.005

Table III. – Measurements of female *Trichobilharzia regenti* n.sp.

• Male (Fig. 1A, Table II) has filiform body of almost uniform width which is covered in anterior part by sporadic tubercles; their number was not determined. Also, several pigmented spots occur in this area. Surface of worms is longitudinally striated. Tegument of oral sucker bears anteriorly several cilia of putative sensory function. Sucker encompasses the oral opening which is situated subterminally. There is a relatively short and wide gynaecophoric canal (ventral groove), walls of which are covered by 0.003-0.005 mm long spines. The posterior end (tail) is broadened and in fresh worms coil-shaped. Reproductive system consists of numerous testes (more than 120), collecting duct and vesicula seminalis. Slightly oval testes start close to posterior end of gynaecophoric canal and continue nearly to body end. Seminal vesicle is usually divided into external and internal parts. Ejaculatory duct opens on genital papilla lying at the beginning of gynaecophoric canal. Concerning digestive tract, long oesophagus passes from oral opening to caecal bifurcation lying just before acetabulum, surface of which is covered by spines. Reunion of caeca was observed at posterior end of or behind seminal vesicle. This position may slightly vary in contracted/dilated worms. Reunited intestine continues dorsal to gynaecophoric canal and its sinuous course in testes area ends just before coiled tail. Intestine, mainly in bifurcation part, was filled by dark-brown pigment, probably hematin created during haemoglobin digestion. As far as excretory system is concerned, it was impossible to count number of flame cells; collecting duct visible throughout entire body contained numerous ciliary patches.

• Female (Fig. 1B, Table III) is also filiform and its surface characteristics correspond with those of males. Posterior body end (tail) is usually club-shaped but not so extremely as in males; coiled end was never observed. Morphology of suckers and digestive tract is generally the same as in males. Reunion of caeca is localized behind seminal receptacle; this feature seems to be stable. Intestine is visibly more filled by brown pigment; therefore, sinuous intestine in posterior body parts represents, by its brown colour, the most dominant organ. Concerning reproductive system, long tubular ovary followed by seminal receptacle are the most visible organs in anterior body part. Numerous vitelline follicles are localized between seminal receptacle and body end. Granular vitelloduct passes ovary area forward and opens in ootype area before ovary. Also, oviduct starting at posterior ovary end and joining seminal receptacle duct, turns back and passes ovary area to ootype lying before ovary. Short and sometimes curved uterus continues from ootype and ends by genital opening just behind acetabulum. Exclusively one egg was found in ootype/uterus. Although usually described in other *Trichobilharzia* species, Laurer's canal was not observed. Excretory system is basically the same as in males.

Eggs

Thirty one eggs were measured in total. The size of elongate eggs (Fig. 1C) is 0.289 ± 0.029 mm ×

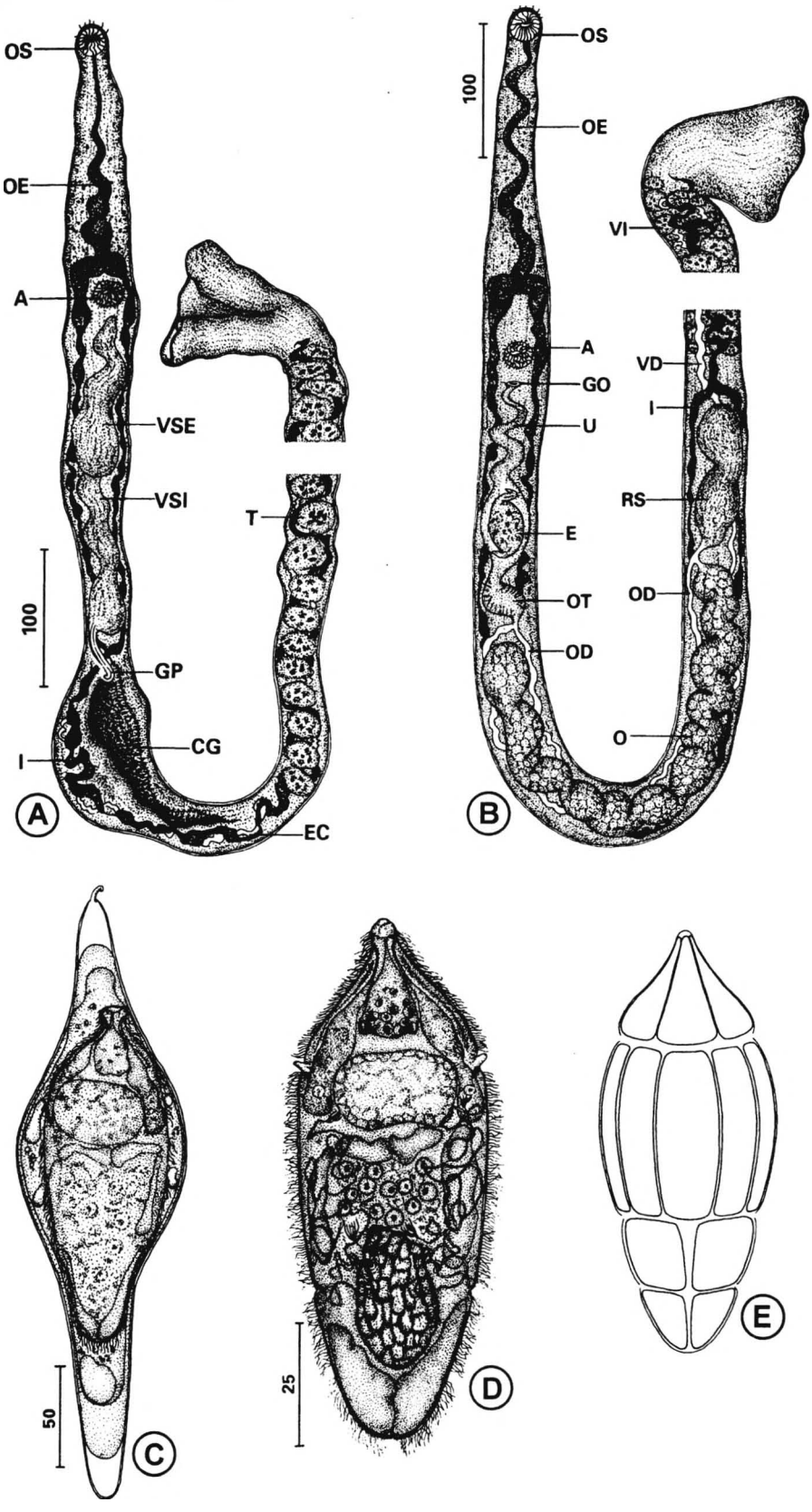

Fig. 1. – *Trichobilharzia regenti* n. sp. A: anterior and posterior end of a male ; B: anterior and posterior end of a female ; C: egg ; D: miracidium ; E: miracidial plates. A, acetabulum ; CG, canalis gynaecophorus ; E, egg ; EC, excretory duct ; GO, genital opening ; GP, genital papilla ; I, intestine ; O, ovary ; OD, oviduct ; OE, oesophagus ; OS, oral sucker ; OT, ootype ; RS, receptaculum seminis ; T, testes ; U, uterus ; VD, vitelloduct ; VI, vitellaria ; VSE, vesicula seminalis externa ; VSI, vesicula seminalis interna. Scale in µm.

0.089 ± 0.013 mm (mean ± SD); variability in shape was characterized as length:width ratio and this parameter ranged between 2.8-4.6. Eggs are nearly symmetrical about to long axis and have two extremities (poles) – one is longer with rounded end and one is shorter with slender and curved process at top. Within few days formation of miracidia is completed. Mature eggs are very fragile and tend to disrupt once under slight pressure (e.g. coverslip).

Miracidia

Fifty specimens were measured in total. Size of pyriform miracidia (Fig. 1D) was 0.106 ± 0.015mm × 0.041 ± 0.005 mm (mean ± SD). On surface, ciliated plates are arranged in four tiers and pattern is 6:9:4:3; second tier is the longest one (Fig. 1E). On border between first and second tier, two lateral horns are localized. Under surface layer numerous vacuoles of undetermined shape and function are located. Within miracidia there are two kinds of glands in anterior region – one apical gland (head gland) which is coarsely granular and light-blue-stained using Giemsa and two long lateral glands reaching posterior end of neural mass which are finely granular and pink-stained using Giemsa. Both types of glands open at miracidial anterior end (terebratorium) – apical gland terminally and two lateral glands subterminally. Apical gland is followed by nerve mass surrounded by circle of cells. Distal half of body is filled by one group of germinal cells. Group of rounded cells of unknown function occupies region between germinal cells and neural mass. Miracidia possess two pairs of flame cells; first and second pairs lie at anterior and posterior level of second ciliated tier, respectively. Excretory system opens at border between second and third tier of ciliated plates.

Maximum recovery of miracidia was by days 24 and 25 p.i. Miracidia show photo-positive and geo-negative orientation. They hatch from eggs in water as well as in saline. Examination of nasal area showed that miracidia are able to leave eggs even within nasal tissue; nasal area of ducklings being without contact with water for 10-12 h contained several freely moving miracidia. After rinsing out nasal cavity with tap water, exclusively free miracidia and no eggs were found in the water.

Cercariae

Measurements of body parts and organs of twenty two cercariae released from *R. peregra* are presented in Table IV. Gross morphology of ocellate furcocercariae (Fig. 2A) is basically the same as for *T. ocellata* type. Surface is covered by fine spines which appear to be of the same length on body and tail stem. Localization of silver-stained sensory papillae is shown on Figure 3 and Table V. Dominant head organ fills anterior body end. Acetabulum lies behind body centre. One pair of eye spots is localized at half distance bet-

	No. Measured	Mean ± SD (mm)
Total length	16	0.759 ± 0.049
Length of body	21	0.225 ± 0.032
Maximum width of body	21	0.055 ± 0.005
Length of tail stem	22	0.331 ± 0.020
Maximum width of tail stem	22	0.049 ± 0.009
Length of furca	16	0.206 ± 0.021
Length of anterior organ	21	0.077 ± 0.004
Maximum width of anterior organ	21	0.044 ± 0.003
Diameter of acetabulum	21	0.027 ± 0.005
Acetabulum to anterior end	21	0.124 ± 0.013
Diameter of eye spot	21	0.010 ± 0.001
Eye spot to anterior end	21	0.081 ± 0.004

Table IV. – Measurements of cercariae *Trichobilharzia regenti* n.sp.

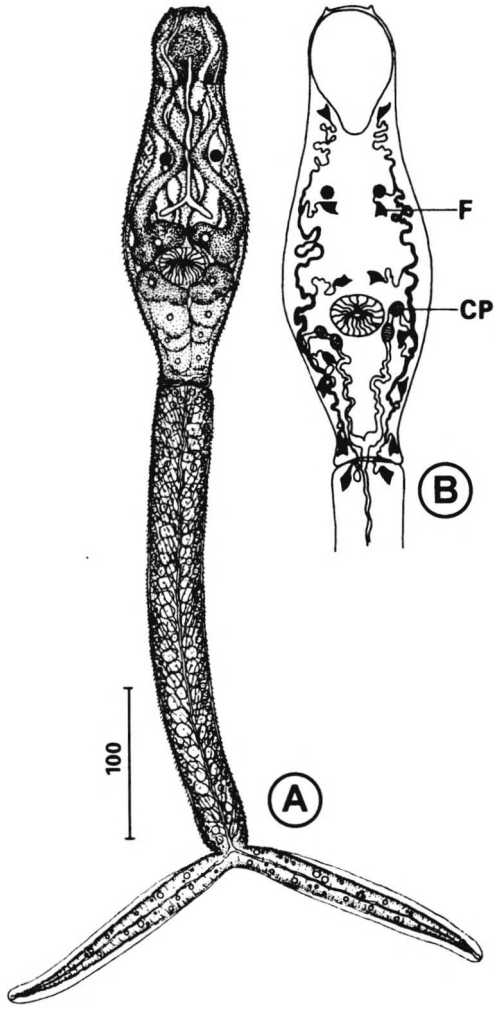

Fig. 2. – *Trichobilharzia regenti* n. sp. A: cercaria; B: excretory system of cercarial body. F, flame cell; CP, ciliary patches. Scale in µm.

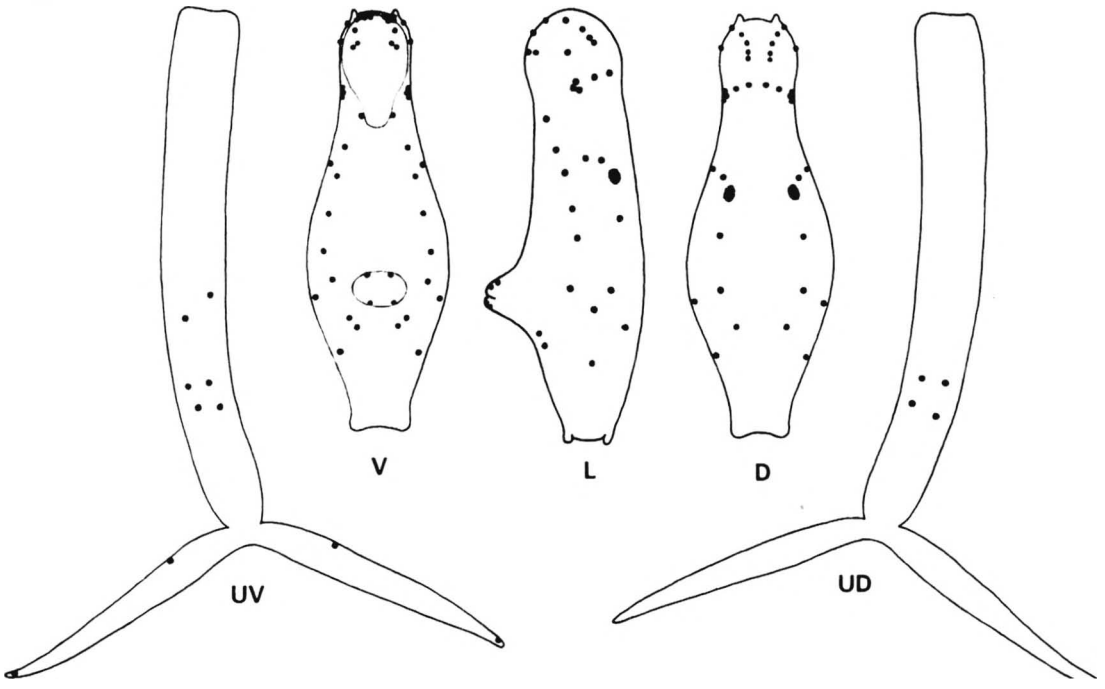

Fig. 3. – Surface sensory papillae of *Trichobilharzia regenti* n. sp. cercariae. V, ventral body view ; L, lateral body view ; D, dorsal body view ; UV, ventral tail view ; UD, dorsal tail view.

Body			
Position	Ventral papillae	Lateral papillae	Dorsal papillae
CI	1	6-7	1
CII	2	1	3
CIII	0	3	2
CIV	1	0	0
AI	2	1	1
AII	1	0	0
AIII	1	0	1
MI	1	1	1
PI	2	1	1
PII	0	0	0
Tail			
	2 FV	6 UV	4 UD

Table V. – Chaetotaxy of *Trichobilharzia regenti* n.sp. cercariae.

ween head organ and acetabulum. Intestine with bifurcation and two short caeca is localized centrally along with body axis; it starts subterminally in head organ and ends before circumacetabular glands. Two pairs of circumacetabular penetration glands surround acetabulum; posterior body part is filled by three pairs of postacetabular penetration glands. Content of circumacetabular glands remains unstained whereas that of postacetabular glands is pink-stained using Giemsa. Massive gland ducts pass cercarial body forward thru head organ and open at anterior end. Excretory system is composed of seven pairs of flame cells and two pairs of cilia patches within collecting ducts. Flame cell formula is 2 [3 + 3 + (1)] = 14 (Fig. 2B). Excretory system leads into excretory junction at body base and continues throughout tail stem and furcae. Tail stem is obliquely striped and filled by star-shaped cells; furcae are cross-striped and finfolds are well developed nearly along entire furcae. Furcae end in 0.015 mm long thorns.

Cercariae exhibit photo-positive reaction. However, they do not strongly attach to the container wall. They often float in water column and finally, they also rest at the bottom. They become active by shadow or mechanical stimuli when they quickly swim to water surface.

Laboratory infection of snails

Although ten *R. peregra* snails died during experiment, remaining eleven specimens became infected; no infection established in *L. stagnalis* (20 specimens). Prepatent period under laboratory conditions was only estimated – after three weeks p.i. no cercariae were shed, whereas by five weeks p.i. snails releasing schistosome larvae were detected.

Systematic summary

Species: *Trichobilharzia regenti* n.sp.

Type host (experimental infection): *Anas platyrhynchos f. dom.* L., *Cairina moschata f. dom.* (L.)

Intermediate host: *Radix peregra peregra, R. p. ovata* (natural and experimental hosts)

Site of infection: blood vessels and mucous tissue of the nasal cavity

Type locality: Blatná, South Bohemia, Czech Republic

Prevalence in the intermediate host: 0.5-2.6%

Deposition of types: Natural History Museum, Vienna, Austria: NHMWien ZOOEV 3681: holotype (anterior end of a male); 3682: allotype (anterior end of a female); 3683: paratype (anterior and posterior ends of a male).

Etymology: regent is a governor of a kingdom/principality; the title was frequently used in South Bohemia (i.e. the area where the schistosome comes from) in the Middle Ages. At present the name represents a famous South Bohemian brewery (founded in 1379).

DISCUSSION

So far we know, only *Trichobilharzia* species causing visceral infections of birds were systematically described from Europe. The site of infection (nasal area) as well as the morphological characterization proved that *T. regenti* n.sp. is a new European schistosome species; it cannot be confused with the sympatrically occurring species *T. ocellata* (LaValette, 1855) Brumpt, 1931, *T. szidati* Neuhaus, 1952 and *T. franki* Müller & Kimmig, 1994.

One report on *Trichobilharzia* sp. in nasal cavity of mute swans from Switzerland has been published (Palmer & Ossent, 1984). However, the report is centred mainly on histopathological evaluation; it lacks a deeper morphological characterization and species description as well as detail data on the parasite life cycle. Due to the death of the experimentally infected *R. peregra* snails in the Palmer's and Ossent's experiments, it is impossible to say whether the intermediate host specificity of that parasite was the same as in our case. Nevertheless, *L. stagnalis* appeared to be resistant to the infection as has also been proved in our experiments.

As far as the seven nasal *Trichobilharzia* species are concerned, they differ from *T. regenti* n.sp. in the following major morphological features (size differences are not considered): (1) The caeca of males reunite at the middle part of the seminal vesicle (*T. nasicola* Fain, 1955; *T. rodhaini* Fain, 1955; *T. spinulata* Fain, 1955; *T. duboisi* Fain, 1959; *T. arcuata* Islam, 1986a); in *T. regenti* n.sp., although varying to a certain degree, the reunion was found at the posterior level of or even behind the seminal vesicle. (2) The posterior end of males is only slightly broadened (*T. nasicola, T. spinulata*); in *T. regenti* n. sp. the posterior end is strikingly broadened and coil-shaped. In none of the formerly described avian *Trichobilharzia* species the coil-shaped posterior end of males was noted. (3) The surface papillae (tubercles) appear also at the posterior end of males (*T. rodhaini*), or the surface is covered by 0.006-0.010 mm spines (*T. spinulata*). These morphological features were not found in *T. regenti* n.sp. males. (4) The female caeca reunite at the posterior ovarial end (*T. spinulata*) or at the level of receptaculum seminis (*T. arcuata*), while in *T. regenti* n.sp. the reunion lies behind the seminal receptacle. (5) Contrary to *T. regenti* n.sp. females, no tubercles were noted at the anterior end of *T. arcuata* females. (6) The eggs of all seven nasal *Trichobilharzia* (*T. nasicola, T. rodhaini, T. spinulata, T. duboisi, T. aureliani* Fain, 1956; *T. arcuata, T. australis* Blair & Islam, 1983) seem to have different size, shape, or both. In case of some *Trichobilharzia* species from Rwanda, the above mentioned morphological differences may be of limited value due to the low number of adult flukes observed in the original descriptions, i.e., the intraspecific variability of particular characters is poorly known.

As the life cycle of only two nasal *Trichobilharzia* species was completed (Blair & Islam, 1983; Islam, 1986a,b), the morphological and/or behavioural comparison on the larval level was made exclusively between *T. regenti* n. sp. and *T. australis/T. arcuata.* (1) The arrangement of miracidial anterior gland cells of *T. arcuata/T. australis* is different from that observed in *T. regenti* n. sp. where the two lateral, finely-granular, and elongate glands reach the posterior part of the neural mass. (2) *T. arcuata* miracidia possess two groups of germinal cells whereas those of *T. regenti* n.sp. only one germinal mass. (3) The number of cercarial flame cells is 16 in *T. arcuata* and *T. australis*; cercariae of *T. regenti* n. sp. have the usual *Trichobilharzia* number (14 flame cells). (4) The finfolds of *T. arcuata* cercariae are present only on two thirds of furcae whereas in *T. regenti* n. sp. the finfolds cover nearly the entire furcae. (5) The distribution pattern of the silver-stained cercarial papillae of *T. regenti* n. sp. differs from that in *T. arcuata/T. australis.* (6) The resting behaviour of *T. regenti* n. sp. cercariae seems to be different from that of *T. australis* and *T. arcuata.*

Although clear differences in cercarial chetotaxy exist between *T. regenti* n. sp. and *T. australis/T. arcuata* (e.g., ventral papillae C IV and A I and dorsal papillae C II), a complete comparison is rather impossible. This is based on the fact that lateral body (*T. arcuata*) and ventral/dorsal tail (*T. arcuata, T. australis*) views are not drawn in the original descriptions (Blair & Islam, 1983; Islam, 1986a) and papillae positions are not defined. Therefore, the comparison of *T. regenti* n.sp. with *T. arcuata/T. australis* arose from our personal evaluation of Blair's and Islam's drawings.

It appears that the European *Trichobilharzia* species exhibit a strong intermediate host specificity. The ori-

ginal descriptions of *T. ocellata* as well as *T. szidati* mention *L. stagnalis* as the intermediate host (LaValette, 1855; Brumpt, 1931; Neuhaus, 1952); *T. franki* develops exclusively in *R. auricularia* (Müller & Kimmig, 1994) and *T. regenti* n. sp. in *R. peregra*. The confusion arisen from numerous descriptions of *Cercaria ocellata* from different snail hosts, i.e., without knowledge of the adults and the parasite life cycles, is not considered in this discussion. However, it can be hypothesized that the European findings of *C. ocellata* from *R. peregra* (e.g., Našincová, 1992) might belong to *T. regenti* n. sp. The specificity of *Trichobilharzia* parasites to the intermediate hosts is supported by Kalbe's *et al.* (1997) observation that *T. ocellata* (the strain is identical to the *T. szidati* stock) miracidia are attracted exclusively to *L. stagnalis* and not to *Galba truncatula*, *R. peregra* or even planorbid snails. The susceptibility of a snail host to a trematode infection is probably genetically linked; the 18S rDNA sequence analysis of lymnaeid snails shows that *L. stagnalis* (the original host of *T. ocellata*/*T. szidati*) belongs to a different group than *R. auricularia*/*R. peregra* (snail hosts of the recently described species *T. franki* and *T. regenti* n. sp., respectively). This snail grouping seems to be reflected by transmission pattern of other trematodes, e.g., fasciolid flukes (Bargues & Mas-Coma, 1997).

The nasal avian schistosomes seem to be well adapted to the transmission. As already mentioned by Islam (1986a,b), the miracidia hatch from eggs in pond water as well as in 0.85 % saline. We observed, moreover, that free miracidia are present in the nasal tissue. They might hatch directly in the nasal area either without a stimulus or after a short contact with water incoming during the duck drinking/feeding. The examination of water after rinsing out the nasal cavity showed that only freely-moving miracidia and no eggs were present. Taken together, the miracidia leave the eggs and their duck host via openings of the bill/nasal cavity during the duck drinking/feeding. The incomplete border between the bill (mouth) and the nasal cavity might contribute to this mode of transmission.

In conclusion, a new species of nasal *Trichobilharzia*, *T. regenti* n. sp., has morphologically been characterized. The description concerns the adults, eggs, miracidia and cercariae. A strict intermediate host specificity and a new mode of *Trichobilharzia* transmission has been pointed out.

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REFERENCES

Bargues M.D. & Mas-Coma S. Phylogenetic analysis of lymnaeid snails based on 18S rDNA sequences. *Molecular Biology and Evolution*, 1997, *14*, 569-577.

Bearup A.J. Schistosomes in the nasal passages of aquatic birds. *Australian Journal of Science*, 1957, *19*, 163.

Blair D. & Islam K.S. 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*. *Systematic Parasitology*, 1983, *5*, 89-117.

Blair D. & Ottesen P. Nasal schistosomiasis in Australian anatids. *Journal of Parasitology*, 1979, *65*, 982-984.

Brumpt E. *Cercaria ocellata* déterminant la dermatite des nageurs, provient d'une bilharzie des canards. *Comptes Rendus de l'Académie des Sciences Paris*, 1931, *193*, 612-614.

Fain A. Recherches sur les schistosomes d'oiseaux au Ruanda-Urundi (Congo Belge). Découverte d'une nouvelle bilharziose aviaire: La trichobilharziose nasale, et description de schistosomes nouveaux. Note préliminaire. *Revue de Zoologie et de Botanique Africaines*, 1955a, *51*, 373-387.

Fain A. Étude sur les schistosomes d'oiseaux au Ruanda-Urundi (Congo, Belge). Découverte d'une nouvelle bilharziose aviaire: la trichobilharziose nasale, et description de schistosomes nouveaux. Note préliminaire. *Revue de Zoologie et de Botanique Africaines*, 1955b, *51*, 373-387.

Fain A. Une nouvelle bilharziose des oiseaux: La trichobilharziose nasale. Remarque sur l'importance des schistosomes d'oiseaux en pathologie humaine. Note préliminaire. *Annales de la Société belge de Médecine tropicale*, 1955c, *35*, 323-327.

Fain A. Les schistosomes d'oiseaux du genre *Trichobilharzia* Skrjabin et Zakharow, 1920 au Ruanda-Urundi. *Ibidem*, 1956a, *54*, 147-178.

Fain A. Nasal trichobilharziasis: a new avian schistosomiasis. *Nature*, 1956b, *177*, 389.

Fain A. Un nouveau schistosome du genre *Trichobilharzia* dans les fosses nasales du canard nain. *Revue de Zoologie et de Botanique Africaines*, 1959, *60*, 227-232.

Horák P. Developmentally regulated expression of surface carbohydrate residues on larval stages of the avian schistosome *Trichobilharzia szidati*. *Folia Parasitologica*, 1995, *42*, 255-265.

Islam K.S. The morphology and life-cycle of *Trichobilharzia arcuata* n. sp. (Schistosomatidae: Bilharziellinae) a nasal schistosome of water whistle ducks (*Dendrocygna arcuata*) in Australia. *Systematic Parasitology*, 1986a, *8*, 117-128.

Islam K.S. Development of *Trichobilharzia australis* Blair & Islam, 1983 in the snail, *Lymnaea lessoni* Deshayes and in an experimental definitive host, the Muscovy duck. *Journal of Helminthology*, 1986b, *60*, 301-306.

Kalbe M., Haberl B. & Haas W. Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails. *Parasitology Research*, 1997, *83*, 806-812.

Lalitha C.M. & Alwar V.S. Parasites of domestic ducks (*Anas boschas domesticus*) in Madras. *Indian Veterinary Journal*, 1960, *37*, 179-181.

La Valette St. George A.J.H. Symbolae ad trematodum evolutionis historiam. Dissertation, Berolius 1855, 1-42.

Meuleman E.A., Huyer A.R. & Mooij J.H. Maintenance of the life cycle of *Trichobilharzia ocellata* via the duck *Anas platyrhynchos* and the pond snail *Lymnaea stagnalis*. *Netherlands Journal of Zoology*, 1984, *34*, 414-417.

Müller V. & Kimmig P. *Trichobilharzia franki* n.sp. - die Ursache für Badedermatitiden in südwestdeutschen Baggerseen. *Applied Parasitology*, 1994, *35*, 12-31.

Našincová V. Trematode developmental stages in Czech water snails and life cycles of selected species of the family Omphalometridae and Echinostomatidae. PhD Thesis. Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice, 1992, 1-268.

Neuhaus W. Biologie und Entwicklung von *Trichobilharzia szidati* n.sp. (Trematoda, Schistosomatidae), einem Erreger von Dermatitis beim Menschen. *Zeitschrift für Parasitenkunde*, 1952, *15*, 203-266.

Palmer D. & Ossent P. Nasal schistosomiasis in Mute Swans in Switzerland. *Revue suisse de Zoologie*, 1984, *91*, 709-715.

Richard J. La chétotaxie des cercaires. Valeur systématique et phylétique. *Mémoires du Museum National d'Histoire Naturelle*, 1971, Série A, *57*, 1-179.

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