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VERSITA

Cercarial dimensions and surface structures as a tool for species determination of *Trichobilharzia* spp.

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Abstract

Cercariae of bird schistosomes are traditionally considered to be very similar in their morphological characteristics. In order to solve the problem, we tested some methods which might be suitable for cercarial differentiation. Fourteen isolates of three *Trichobilharzia* species (*T. szidati*, *T. franki*, *T. regenti*) occurring sympatrically in Central Europe were used. Dimensions of individual cercariae do not represent a useful criterion for identification, because the intraspecific variability exceeds the interspecific one. On the other hand, chaetotaxy appears a promising way for discrimination, although some sensory papillae do not stain sufficiently with silver nitrate. The papillary pattern (i.e. number and relative position of papillae) is specific for all *Trichobilharzia* species studied by us. Therefore, we compiled an identification key for the three *Trichobilharzia* species. In addition, we tried to find species-specific surface saccharide epitopes; none of the labeled lectin probes can be used as a species-specific marker.

Keywords

Trematoda, schistosomes, *Trichobilharzia*, cercaria, chaetotaxy, surface carbohydrates

Introduction

Cercariae of bird schistosomes emerge from intermediate snail hosts and actively penetrate the skin of birds and mammals. In birds the infection culminates by worm maturation and egg production. Particular species migrate through various tissues to the place of final location. This migration, depending on the parasite and the affected tissue, may result in severe symptoms, e.g., leg paralysis and orientation and balance disorders as indicated by infections of neurotropic *Trichobilharzia regenti*. In mammalian hosts including man, the repeated skin infections are accompanied by inflammatory reactions called cercarial dermatitis (swimmers' itch, clam-diggers disease), and precise mechanisms and time of parasite killing by sensitized hosts remain to be clarified (Horák *et al*. 2002). Concerning the taxonomy of schistosomes, nine genera of the family Schistosomatidae parasitize birds, and *Trichobilharzia* spp. are the best known and worldwide occurring member. Cercariae of this genus develop in various snail species and have frequently been assigned as *T. ocellata* due to their mutual similarity. In the second half of the twentieth century several new species occurring sympatrically in Europe were described: *T. szidati* Neuhaus, 1952, *T. franki* Müller et Kimmig, 1994, *T. regenti* Horák, Kolářová et Dvořák, 1998 and *T. salmanticensis* Simon Martin et Simon Vincente, 1999. Some recent findings indicate that the species spectrum of *Trichobilharzia* and other bird schistosomes in Europe is even broader (Rudolfová and Podhorský, unpublished results). In addition, as a result of morphological and molecular analyses, the frequently reported fluke *T. ocellata* should be considered as *species inquirenda* or a complex of species (e.g. Rudolfová *et al*. 2005).

Because of differences in their life cycles, host specificity and pathogenicity, precise determination of bird schistosomes seems to be essential, particularly in the assessment of risk factors in areas of distribution. The adults may possess valuable morphological characteristics used in the traditional determination of bird schistosomes (Blair and Islam 1983). On the other hand, cercariae are known by their apparent uniformity (Horák *et al*. 2002), as they are released from infected snail hosts in high quantities. In the past, the number and arrangement of cercarial gland cells and sensory papillae (chaetotaxy), dimensions and behavior, and intermediate host specificity were used for species determination, but taxonomic value of these characteristics was subjected to doubt (Blair and Islam 1983, Horák *et al*. 2002).

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Table I. List of *Trichobilharzia* isolates used in this study

Isolate number	Species determination	Locality	Snails kept at	Intermediate host	Size of intermediate host (mm)
1	*T. regenti*	laboratory strain – Prague	22°C	*R. peregra s. l.*	15–18 × 7–10
2	*T. regenti*	laboratory strain – Prague	15°C	*R. peregra s. l.*	9–13 × 5–7
3,4*	*T. regenti*	laboratory strain – Prague	15°C	*R. peregra s. l.*	15–18 × 7–10
5	*T. szidati*	Dubovec pond (Třeboň district)	18°C	*L. stagnalis*	38–45 × 18–20
6,7*	*T. franki*	Olbramovice pond (Benešov district)	22°C	*R. auricularia*	23 × 12
8	*T. szidati*	Vlkov pond (Třeboň district)	18°C	*L. stagnalis*	38–45 × 18–20
9	*T. szidati*	Švarcenberk pond (Třeboň district)	15°C	*L. stagnalis*	46 × 23
10	*T. szidati*	Smyslov pond (Blatná district)	22°C	*L. stagnalis*	38–45 × 18–20
11	*T. szidati*	Smyslov pond (Blatná district)	15°C	*L. stagnalis*	38–45 × 18–20
12	*T. ocellata***	laboratory strain – Erlangen	15°C	*L. stagnalis*	47 × 22
13	*T. ocellata***	laboratory strain – Amsterdam	22°C	*L. stagnalis*	17 × 10
14	*T. szidati*	Švarcenberk pond (Třeboň district)	22°C	*L. stagnalis*	46 × 23

*Cercariae were isolated twice during two consecutive days. **Molecular analysis showed that these strains of "*T. ocellata*" belong to the species *T. szidati* (see Rudolfová *et al*. 2005).

Differences in cercarial dimensions were reported in numerous papers in the past. Szidat (1942) separated three new species (primarily labeled as *T. ocellata*) based on their size. Neuhaus (1952) observed some level of variability between cercariae from the snails *Lymnaea stagnalis* and *Radix ovata*. In order to clarify such data, some authors attempted to identify factors influencing cercarial size: Dönges (1964) found that dimensions depend on temperature (strigeid cercariae developing in snails at 15°C were significantly larger than those developing at 24°C). Such variability might also be caused by the age difference of intermediate hosts from which cercariae were produced (Neuhaus 1952). Furthermore, cercariae of different species resemble each other in size (Horák *et al*. 2002), and use of morphometric characteristics in the determination of different species may not always be accurate.

Chaetotaxy has been studied on cercariae of various trematode species and several authors have shown important differences between certain species (e.g. Bayssade-Dufour *et al*. 1989, Niewiadomska 1996). In bird schistosomes, chaetotaxy of cercariae was described for one species of the genus *Austrobilharzia* (Abdul-Salam and Screelatha 2004) and several species of the genus *Trichobilharzia*: e.g. *T. ocellata sensu lato* (Richard 1971, Kock and Böckeler 1998, Gay *et al*. 1999), *T. australis* (Blair and Islam 1983), *T. szidati* (Kolářová and Horák 1996), *T. franki* (Kock and Böckeler 1998, Gay *et al*. 1999) and *T. regenti* (Horák *et al*. 1998). Generally, *Trichobilharzia* chaetotaxy at the interspecific level is believed to be of limited value (see Horák *et al*. 2002 for review) which is supported by dissimilar conclusions in two papers: *T. franki* and *T. ocellata sensu lato* exhibited the same papillae pattern in the study by Kock and Böckeler (1998), whereas Gay *et al*. (1999) reported difference between the two species in the position of one papilla.

The surface of free-living cercariae is covered by a relatively thick protective glycocalyx. Its carbohydrate composition (glycosylation) has been studied using fluorescein- or otherwise labeled lectins as markers of surface changes during schistosome development. Surface glycosylation was described for cercariae of *Schistosoma mansoni* (Linder 1984), *T. szidati* (Horák and Mikeš 1995, Horák *et al*. 1997) and *T. regenti* (Blažová and Horák 2005). These studies indicated differences in binding of ConA, LCA, RCA-I, SBA, WGA and PWM lectins. In the case of other helminths, the application of labeled lectins was also used to characterize species-specific differences, e.g., for infective larvae of *Onchocerca* spp. (Hagen *et al*. 1994). Therefore, species-specific distribution of surface saccharides might represent a useful criterion in the identification of larvae of *Trichobilharzia* spp. This hypothesis was also tested in this study.

Materials and methods

Parasites: In all experiments cercariae of three European species of the genus *Trichobilharzia* (*T. szidati*, *T. regenti* and *T. franki*) were compared. Cercariae of *T. franki* and some samples of *T. szidati* were obtained from naturally infected snails *Radix auricularia* and *Lymnaea stagnalis*, respectively. Freshwater snails were collected in several ponds in Central and Southern Bohemia during the period 2003–2005. Cercariae of *T. regenti* and the remaining samples of *T. szidati* were obtained from experimentally infected snails *R. peregra sensu lato** and *L. stagnalis*, respectively; the life cycle of these isolates has been maintained in the laboratory (Charles University in Prague) for many years. The origin of isolates is shown in Table I.; their species identity was checked by molecular tools (Rudolfová *et al*. 2005). In the laboratory the infected snails were kept individually in glass beakers and exposed to artificial light for 1 hour. The released cercariae were used for subsequent experiments.

*Species within the snail genus *Radix* are known for their morphological plasticity, and molecular tools need to be employed to confirm the validity of morphologically-defined species. Therefore, the system (and species spectrum) of European *Radix* snails is under reconstruction.

Table II. Dimensions of cercariae of *T. szidati*, *T. franki* and *T. regenti*

Morphometric characters		*T. szidati* (n = 251)		*T. franki* (n = 64)		*T. regenti* (n = 124)		Correlation coefficient
		mean	SD	mean	SD	mean	SD	
1	Body length (μm)	293	36	316	14	333	48	0.24
2	Tail stem length (μm)	399	40	480	30	421	31	0.76
3	Furca length (μm)	273	30	318	18	285	23	0.64
4	Total length (μm)	965	76	1114	51	1039	81	0.71
5	Body width (μm)	63	12	67	7	78	14	0.11
6	Tail stem width (μm)	47	6	55	5	59	9	0.41
7	Head organ length (μm)	65	10	75	6	76	14	0.39
8	Head organ width (μm)	50	6	53	4	58	6	0.21
9	Anterior end – acetabulum (μm)	191	28	223	18	219	38	0.43
10	Anterior end – eye (μm)	131	16	150	10	145	20	0.47
11	Body length/total length	0.30	0.03	0.28	0.01	0.32	0.03	0.32
12	Tail stem length/total length	0.41	0.03	0.43	0.01	0.41	0.02	0.29
13	Furca length/total length	0.28	0.02	0.29	0.01	0.28	0.02	0.06
14	Acetabulum – anterior end/body length ratio	0.65	0.06	0.71	0.05	0.66	0.05	0.44
15	Eye – anterior end/body length ratio	0.45	0.03	0.48	0.02	0.44	0.03	0.36
16	Head organ width and length ratio	0.79	0.16	0.71	0.12	0.79	0.15	0.23
17	Body width and length ratio	0.22	0.05	0.21	0.02	0.24	0.07	0.07
18	Tail stem width and length ratio	0.12	0.02	0.11	0.01	0.14	0.02	0.07
19	Head organ width and length ratio	0.22	0.03	0.24	0.02	0.23	0.03	0.24

Measurement of cercariae: Snails infected by schistosomes were kept in different temperatures for 30 days and then sorted by size (Table I). Cercariae were fixed in 2% buffered formaldehyde (pH 7.4) and measured under the light microscope (30 individuals of each isolate) using Quick Photo Micro software (Olympus C&S). In total, 19 morphometric characters were observed (Table II). To demonstrate potential effect of temperature and/or snail size on dimensions of cercariae, four different sets of data were evaluated: (1) Individuals of *T. regenti* (samples 1, 3 and 4) originated from snails of the same size kept at different temperatures. (2) Individuals of *T. szidati* (samples 9, 10, 11 and 14) originated from snails of the same size kept at different temperatures. (3) Individuals of *T. regenti* (samples 2, 3 and 4) originated from snails of different sizes kept at the same temperature. (4) Individuals of *T. szidati* (samples 10, 13 and 14) originated from snails of different sizes kept at the same temperature. *Trichobilharzia franki* developing in *R. auricularia* was not analyzed with respect to the influence of temperature and/or snail size. In order to demonstrate whether cercarial dimensions are species-specific, all samples (Nos. 1–14) were analyzed using (i) characters of all individuals from each sample as well as (ii) the average of each character within each sample.

Staining and distribution of sensory papillae: Chaetotaxy was evaluated after staining of the samples (Nos. 1, 6 and 14) with silver nitrate. Suspension of freshly emerged cercariae was mixed with the same volume of 1% silver nitrate and stored in the dark for 10 min. The cercariae were then washed thoroughly for ten times in distilled water to remove traces of soluble silver nitrate, and exposed to light for 10 min. Under the light microscope, chaetotaxy patterns were recorded from a minimum of 80 specimens in each sample. For this purpose, the nomenclature of Richard (1971) was used: distribution of papillae reflects the central nervous system of a hypothetical cercaria, comprising of three cephalic (CI-CIII), three preacetabular (AI-AIII), one median (MI) and three postacetabular (PI-PIII) commissures; with papillae also situated at the acetabulum (SI-SII), tail (U) and furcae (F). Differences found in the number/distribution of papillae were quantitatively assessed; the differences found in angles formed by lines connecting papillae were measured using the graphic program analySIS (Soft Imaging System GmbH Munster).

Statistical analysis: For all sets of data, Principal Component Analysis (PCA) with all individuals as well as population averages was carried out. PCA was calculated in the program Canoco (Ter Braak and Šmilauer 1998). Morphometric characters without normal distribution were square root transformed. Basic descriptive characteristics were calculated for each individual morphometric character by the program SAS (SAS Institute Inc. Cary).

Binding of FITC-labeled lectins: The same isolates as for the above mentioned chaetotaxy (Nos. 1, 6 and 14) were used. Cercariae were transferred into 10 mM Hepes buffer (Sigma), pH 7.8, supplemented with 2 mM $CaCl_2$ and 2 mM $MnCl_2$. Twenty one FITC-labeled lectins were used at 20 μg/ml final concentration; lectins, source organisms and saccharide inhibitors are listed in Table III. Lectins were obtained from Vector Labs or Molecular Probes (HPA). Inhibitors were purchased from Sigma-Aldrich. Cercariae were treated with lectin solution for 20 min, with or without addition of 0.5 M inhibiting saccharide (except for PWM, WGA and WGA_{suc}, for which the inhibitor concentrations were 300 mg/ml). They were then transferred onto microscopic slides and observed using a fluorescence Olympus BX51 microscope. The presence of 0.05% Procain (Léčiva a.s., Czech Rep.) immobilized cercariae for taking photos; cercariae shed their tails and relaxed within 10 min. The experiment was repeated three

Table III. Binding of FITC-labeled lectins to the surface of cercariae of the genus *Trichobilharzia.* Comparison of results obtained in previous studies and those of the present study

Lectin	Isolated from	Inhibitor	T.s.[1,2]		T.r.[3]	T.f.		T.r.		T.s.	
			body	tail	body	body	tail	body	tail	body	tail
PWM	*Phytolacca americana*	oligo-N-acetyl-D-glucosamine	+	+	–	0	0	0	0	0	0
WGA	*Triticum vulgaris*	oligo-N-acetyl-D-glucosamine	++	–	–	+	+–	+–	–	+	–
WGA_{suc}	succinylated *Triticum vulgaris* lectin	oligo-N-acetyl-D-glucosamine	0	0	0	+–	+–	+–	+–	+	–
BS-II	*Bandeiraea simplicifolia*	N-acetyl-D-glucosamine	–	–	–	0	0	0	0	0	0
LTA	*Lotus tetragonolobus*	L-fucose	++	++	+	++	++	++	++	++	++
UEA-I	*Ulex europaeus*	L-fucose	++	++	++	++	++	++	++	+	+
HPA	*Helix pomatia*	N-acetyl-D-galactosamine, N-acetyl-D-glucosamine	–	–	–	–	–	–	–	–	–
DBA	*Dolichos biflorus*	N-acetyl-D-galactosamine	0	0	–	–	–	–	–	–	–
PNA	*Arachis hypogaea*	D-galactose	–	–	–	–	–	–	–	+	+
GSL	*Griffonia simplicifolia*	D-galactose, N-acetyl-D-galactosamine	0	0	0	–	–	–	–	–	–
GSL-II	*Griffonia simplicifolia*	D-galactose, N-acetyl-D-galactosamine	0	0	0	–	–	–	–	–	–
SJA	*Sophora japonica*	N-acetyl-D-galactosamine	0	0	0	–	–	–	–	–	–
JAC	*Artocarpus integrifolia*	N-acetyl-D-galactosamine	0	0	0	++	++	++	++	++	++
SBA	*Glycine max*	N-acetyl-D-galactosamine	+	–	–	+	–	–	–	–	–
RCA-I	*Ricinus communis*	lactose	++	++	–	+	+	++	++	++!	++!
ECL	*Erythrina cristagalli*	lactose	0	0	0	+–	+–	–	–	–	–
PSA	*Pisum sativum*	D-methylmannose, D-methylglucose	++	++	+	++	++	++	++	++	++
LCA	*Lens culinaris*	D-methylmannose, D-methylglucose	+	+	–	+	+	++	++	++	++
ConA	*Canavalia ensiformis*	D-methylmannose, D-methylglucose	–	–	+	+	+	+	+	++	++
PHA-E	*Phaseolus vulgaris* erythroagglutinin	complex specificity – no inhibitor used	0	0	0	–	–	–	–	–	–
PHA-L	*Phaseolus vulgaris* leucoagglutinin	complex specificity – no inhibitor used	0	0	0	–	–	–	–	–	–

Species: T.s. – *Trichobilharzia szidati*; T.r. – *Trichobilharzia regenti*; T.f. – *Trichobilharzia franki*. Rating: ++ strong fluorescence; + moderate fluorescence; +– weak fluorescence; – no fluorescence; 0 not tested; ! non-specific reaction. Published results: [1]Horák *at al*. (1997); [2]Horák and Mikeš (1995); [3]Blažová and Horák (2005).

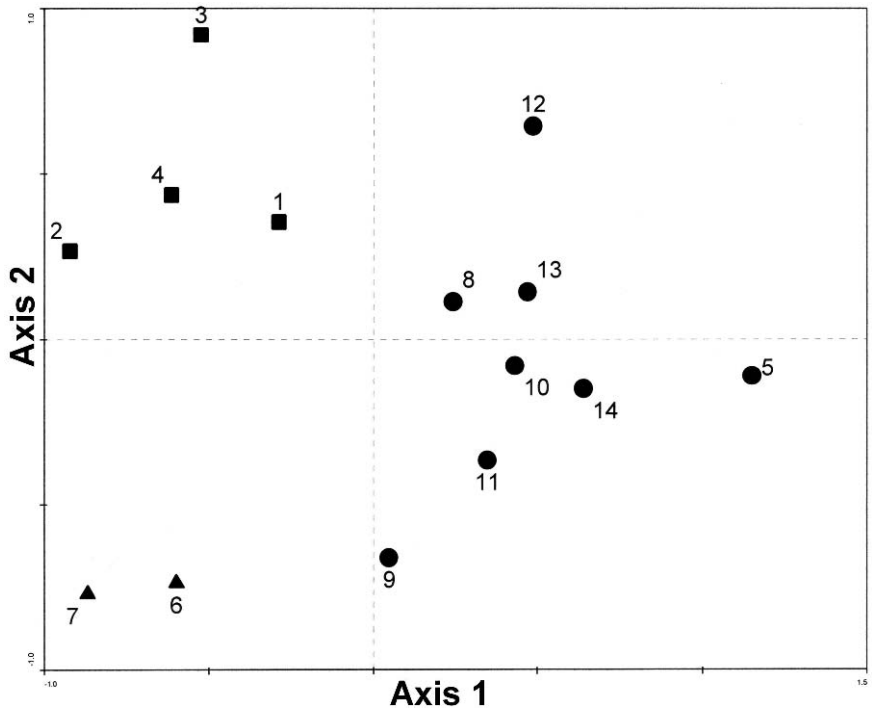

Fig. 1. Principle component analysis (PCA) of morphometric data. *Trichobilharzia szidati* is represented by black circles, *T. franki* by triangles and *T. regenti* by squares. The numbers next to these symbols correspond with the isolates in Table I. The first axis goes through the maximum variation in the data and explains 37.5% of variability, the second one 24.4%. All individual species form separated groups. According to the Axis 1, *T. szidati* is distinguished from *T. franki* + *T. regenti* (*T. franki* and *T. regenti* are not distinguishable). Additionally Axis 2 distinguishes *T. franki* and *T. regenti*

times and the results compared with previous studies accomplished with *T. szidati* and *T. regenti*.

Results

Morphometric characteristics: Morphometric characters did not significantly influence each other and, therefore, all measurements were included in the analysis. For mean and standard deviation values and correlation coefficients of each cercarial character see Table II. PCA (not shown) demonstrated that water temperature, snail size as well as parasite species classifications do not substantially influence the dimensions of cercariae. Therefore, the data were also processed using population averages (Fig. 1). The ordination diagram showed that samples of individual species form clearly separated groups and, therefore, morphometric characteristics processed in this way can be used for the identification of *Trichobilhar-*

Table IV. Overview of sensory papillae on the surface of all three species (*T. szidati*, *T. franki* and *T. regenti*)

Segment of cercariae	**Number of sensory papillae** ventral view	lateral view	dorsal view
CI	1	hardly remarkable	1
CII	2	1	3
CIII	0	3	2
CIV	1	0	0
AI		1	1
AII	3–6	0	0
AIII	(depending on species)	0	1
MI		0	1
PI	2	1	1
PII	0	1	0
SI	hardly remarkable	–	–
SII	2	–	–
U	0	6	4
F	2	0	2

Table V. Chaetotaxy characters of cercariae of *T. szidati*, *T. franki* and *T. regenti*

Chaetotaxy characters	*T. szidati* (n = 81)		*T. franki* (n = 84)		*T. regenti* (n = 82)	
	mean	SD	mean	SD	mean	SD
Angle A	97°	10	105°	10	88°	10
Angle B	176°	7.8	152°	6.6	157°	7.3
No. of ventral papillae in segments AI-MI	3.6	0.51	4.8	0.49	5.5	0.56

A and B represent angles demonstrated in Figure 2; SD – standard deviation.

zia species in our study. Non-significant variability (not exceeding the level of interspecific variability) caused by sample preparation was shown for isolates 3+4 and 6+7 (pairs of samples originated from identical conditions).

Number and distribution of sensory papillae: We analyzed papillae distribution of all three species (Table IV). Concerning particular segments, the results indicated differences in the number of AI-MI papillae in the ventral area, and in the distribution of papillae in the AI and CIV segments in relation to the AI-AIII ventral papillae (Table V, Fig. 2). Ordination diagram (PCA) based on papillary patterns of all individuals showed nearly discrete groups formed by particular *Trichobilharzia* species (Fig. 3); basic statistics (Table V) allowed to construct an identification key (Table VI).

Cercarial surface saccharides. The results of lectin binding to cercariae of particular species are summarized in Table III. Cercariae of *T. regenti* (isolate 1) were recognized by lectins with specificity for L-fucose (LTA, UEA-I), D-mannose/D-glucose (PSA, LCA, moderate reaction with ConA) and N-acetyl-D-galactosamine/D-galactose (RCA-I, JAC). Cercariae of *T. szidati* (isolate 14) were labeled by lectins with specificity for L-fucose (LTA, moderate reaction with UEA-I), D-mannose/D-glucose (PSA, LCA, ConA) and N-acetyl-D-galactosamine/D-galactose (RCA-I, JAC, moderate reaction with PNA). Except for PNA, cercarial lectin binding pattern in both species seemed to be identical (PNA bound to *T. szidati* cercariae in two out of three experiments). *Trichobilharzia franki* (isolate 6) showed similar glycosylation of the tegument as in other species (isolates 1 and 14); the cercariae were recognized by LTA, UEA-I, PSA and JAC, moderate reaction was obtained with LCA, ConA and RCA-I. A different reaction was obtained with SBA which recognized *T. franki*, but gave no reaction with the other two species. No discriminative lectin markers distinguishing *T. regenti*, *T. szidati* or *T. franki* cercariae were found.

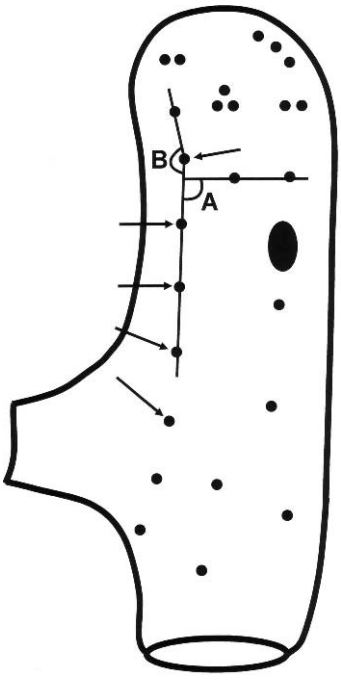

Fig. 2. Schematic drawing of chaetotaxy (lateral view) with demonstration of papillae discriminating cercariae of individual *Trichobilharzia* species. Ventral papillae AI-MI are marked by arrows; the angle between the lines connecting AI-AIII ventral papillae and AI papillae (one lateral and one dorsal) is marked A; the angle between the lines connecting AI-AIII ventral papillae and AI-CIV papillae is marked B

Discussion

Dimensions are traditionally considered to be one of the most important criteria in the determination of living organisms, as indicated in species descriptions. In the case of schistosomes (i.e., adults as well as cercariae) the data on dimensions may cause confusion due to the contractility of worms, and/or temperature (Dönges 1964), snail size (Neuhaus 1952) and fixative (Meyer and Dubois 1954) dependent variabilities. However, the size of some *Trichobilharzia* cercariae was used to establish new species (Szidat 1942). Recent views regarded morphometric criteria as an unsuitable tool for *Trichobilharzia* determination (Blair and Islam 1983, Horák *et al*. 2002).

PCA diagrams constructed from morphometric characteristics of all cercariae (*T. regenti*, *T. szidati* and *T. franki*), i.e., of datasets including either all individuals or particular subsets of data (isolates differing only in snail size or temperature during development of snails/parasites), showed no correlation between a character and a species. Statistical analyses demonstrated high intraspecific and low interspecific variabilities. It can be concluded that the pursued environmental/physiological conditions had no influence on cercarial size.

In the case of population means, there is a chance for species identification, because particular samples formed three isolated clusters (for *T. szidati*, *T. franki* and *T. regenti*) (Fig. 1). Based on the correlation coefficient (Table II) we selected the most suitable characters for particular species (morphometric characters Nos. 2, 3, 4, 6, 10 and 14) and, therefore, we were able to classify all 14 samples used in our study. On the other hand, the organisms studied in the past (*Trichobilharzia* species used by Neuhaus 1952, Müller and Kimmig 1994, Kock 2000) were not correctly classified by our method. The discrepancies might be caused by different methods of fixation and/or high intraspecific variability described in our study for *T. szidati* (4 wild and 2 laboratory

Table VI. Determination key for *Trichobilharzia* cercariae based on chaetotaxy characters. Due to intraspecific variability, prevailing character value needs to be used for identification (we recommend to use at least 20 individuals to make any conclusion)

Determination character	Value of character	Species determination
No. of ventral papillae in segments A and M	3 or 4	continue to 1
	5 or 6	continue to 2
1: Angle B		
The angle between the line made from papillae in segments AI-AIII on ventral	more than 163°	*T. szidati*
surface and the line made from papillae in AI-CIV segments on ventral surface	less than 163°	*T. franki*
2: Angle A		
The angle between the line made from papillae in segments AI-AIII on ventral	more than 97°	*T. franki*
surface and the line made from papillae in AI segment (on lateral and dorsal surface)	less than 97°	*T. regenti*

The drawing of determination characters can be found in Figure 2.

strains), but not for *T. franki* and *T. regenti* (1 strain from each species).

Papillary patterns were reported from various trematode species, e.g., *Diplostomum* sp. (Niewiadomska 1996) and some species of the family Schistosomatidae (Bayssade-Dufour *et al*. 1989). Chaetotaxy of bird schistosome cercariae was studied by several authors. Surface papillae and their distribution reflecting morphology of the nervous system are traditionally considered to be hardly influenced by environmental conditions and, therefore, they are probably subjected to slow change during evolution.

Gay *et al*. (1999) observed one dorsal papilla in PI area, a location of which was used as a marker for discrimination between cercariae of *T. ocellata sensu lato* and *T. franki*. This difference was confirmed neither by us nor by the other authors (Kock and Böckeler 1998). In addition, we analyzed diversity of ventral papillae in area AI-MI (observed e.g. by Horák *et al*. 1998 for *T. regenti*) and concluded that the no-

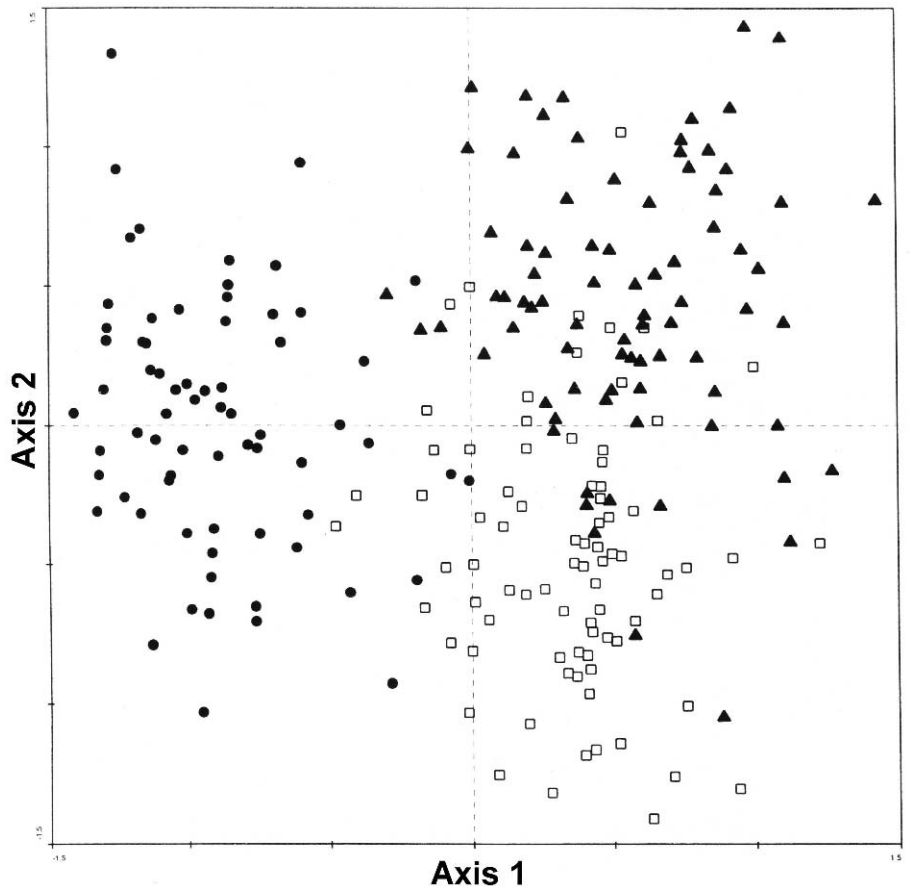

Fig. 3. Principle component analysis (PCA) of chaetotaxy data. *Trichobilharzia szidati* is represented by black circles, *T. regenti* by black triangles and *T. franki* by empty squares. The first axis explains 52.3% of variability of the data, the second one 35.7%. There is a clear trend of group formation for cercariae belonging to the same species. According to the Axis 1, *T. szidati* is distinguished from *T. franki* + *T. regenti* (*T. franki* and *T. regenti* are not distinguishable). Additionally, Axis 2 distinguishes *T. franki* and *T. regenti*

menclature based on segments represented by commissures of the nervous system is problematic. The number of papillae in this area (containing 4 segments) varies from 3 (*T. szidati*) to 6 (*T. regenti*). It means that individual papillae do not correspond to the number of commissures, or the number of commissures is not stable.

Surprisingly, using other sets of papillae and the angles given by lines connecting them (Fig. 2), our isolates of *T. szidati*, *T. franki* and *T. regenti* could be distinguished (Tables V and VI). Our analysis of surface papillae also confirmed that cercariae of *T. szidati* (*sensu* Kolářová and Horák 1996) and *T. ocellata sensu lato* (mentioned by some other authors in the past; see Rudolfová *et al*. 2005) belong to the same species.

We also checked cercarial papillae using SEM (unpublished data) which allows a precise discrimination between sensory papillae and gland openings. Contrary to this advantage there was no other benefit for species determination. For example, comparing our data to those of Kolářová and Horák (1996), Horák *et al*. (1998), Kock and Böckeler (1998) and Richard (1971) shows that, using light microscopy, interpretation of papillae patterns in the apical area of cercariae is subjective, mainly due to the misclassification of gland openings situated in the same area. Similarly, acetabular papillae (segment S1) could hardly be recognized and some unknown gland openings at the top of the acetabulum might erroneously be counted as papillae (unpublished results).

FITC-labeled lectins were successfully used to determine some species, for example toxic dinoflagellates (Rhodes and Haywood 1995), *Bacillus anthracis* (Cole *et al*. 1984) and infective larvae of *Onchocerca* spp. (Hagen *et al*. 1994). Also in trematodes, cercarial saccharides seem to be unique for certain genera (e.g. different glycosylation of *Echinostoma*, *Schistosoma* and *Trichobilharzia*; Hůzová, unpublished results). In monogeneans, moreover, surface saccharide residues exhibit specific distribution patterns at least for some species (Schabuss *et al*. 2003, Frýzková 2004).

Surface glycosylation of *T. regenti* and *T. szidati* cercariae was previously described (Horák and Mikeš 1995, Horák *et al*. 1997, Frýzková 2004, Blažová and Horák 2005) showing differences in binding of particular lectins (see Table III for comparison). In our study, the reactions of most lectins with particular species were identical (11 lectins: LTA, JAC, PSA, HPA, DBA, GSL, GSL-II, SJA, PHA-E, PHA-L, BS-II). Minor differences were found with 7 lectins (WGA, WGA_{suc}, UEA-I, RCA-I, ECL, LCA and ConA), and more pronounced differences with 2 lectins (PNA, SBA). In this case, results dissimilar to the older studies (i.e., the binding of six lectins: PNA, WGA, SBA, RCA-I, LCA and ConA) were recorded. Therefore, such lectin reactions might be influenced by different experimental conditions, lectin quality/origin and/or parasite strain. At present we do not dispose of data showing convincingly species-specific binding of lectin probes to *Trichobilharzia* cercariae.

We can conclude that the isolates of *Trichobilharzia* cercariae analyzed in our study can be distinguished by specific distribution of sensory papillae, but not by cercarial dimensions or surface glycosylation. Although chaetotaxy seems to be a rapid/cheap method providing reproducible results in our experiments, its suitability to discriminate against other *Trichobilharzia* cercariae needs to be tested with more samples (isolates and species). Of course, in laboratories employing molecular techniques the DNA analysis of cercarial samples represents a more rigorous alternative.

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