Investigations of intermediate host specificity help to elucidate the taxonomic status of *Trichobilharzia ocellata* (Digenea: Schistosomatidae)

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SUMMARY

The avian schistosomatid *Trichobilharzia ocellata* plays an important role as causative agent of cercarial dermatitis of humans in Europe. In order to improve the taxonomic knowledge on this parasite, studies of miracidial chemo-orientation as well as experimental infections of different snail species were conducted using strains of *T. ocellata* and *T. franki*. Both schistosomes exhibited a high intermediate host specificity. The miracidia clearly preferred the SCW (snail-conditioned water) of the respective natural intermediate host to SCW of other sympatric snail species. *T. ocellata* proved to be capable of infecting *Lymnaea stagnalis* and *Stagnicola palustris*, but could not develop in *Radix ovata* or *R. auricularia*. *T. franki* established an infection in specimens of *R. auricularia* and *R. ovata*, but not in *L. stagnalis* or *S. palustris*. The results imply that the intermediate host spectrum of *T. ocellata* is limited to *L. stagnalis* and *S. palustris*. Findings of *T. ocellata* (or *Cercaria ocellata*) that originated from snails of the genus *Radix* are likely to have actually belonged to species such as *T. franki* or *T. regenti*. The assumption that *T. szidati* is synonymous to *T. ocellata* is also discussed.

Key words: intermediate host specificity, miracidium, host-finding, *Trichobilharzia*, Lymnaeidae, Schistosomatidae.

INTRODUCTION

Trichobilharzia ocellata (La Valette St George, 1855) Brumpt, 1931 plays an important role as causative agent of cercarial dermatitis (swimmer's itch) in humans. In spite of extensive morphological examinations, the taxonomic status of this parasite is still uncertain. There is strong evidence that *T.* '*ocellata*' is a collective term for several species grouped under this name (Blair & Islam, 1983; Kolarova & Horak, 1996; Odening, 1996). In the opinion of Odening (1996), investigations of the intermediate host specificity of *T. ocellata* could elucidate this systematic problem. In Europe, *T. ocellata* was not only reported from its original snail host (*Lymnaea stagnalis*), but also from species of the genus *Radix* (e.g. Mathias, 1930; Wesenberg-Lund, 1934) and even from physid, planorbid or hydrobid snails (van den Broek, 1965; Kilias & Frick, 1964). Blair & Islam (1983), however, doubt that *T. ocellata* is able to utilize snail species of different families. Odening (1996) even goes so far as to assume that *T. ocellata* exclusively uses snails of the original host species, *L. stagnalis*, as intermediate hosts. Regarding miracidial chemo-orientation, Brumpt (1931) stated that the attraction of *T. ocellata* miracidia to snails of 8 different species was totally unspecific. Kalbe (Kalbe, Haberl & Haas, 1997; Kalbe, 1998), on the contrary, demonstrated that miracidia of *T. ocellata* were significantly more attracted by snail-conditioned water (SCW) of their original snail host than by SCW of *Galba truncatula*, *Radix peregra*, *Planorbarius corneus* and *Planorbis planorbis*. In view of these controversies, comparative studies of miracidial chemo-orientation as well as experimental infections of different snail species were conducted for strains of *T. ocellata* and *T. franki* in order to assess their molluscan host spectrum and to shed light on the taxonomic status of *T. ocellata*.

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MATERIALS AND METHODS

Parasites

T. ocellata was obtained from experimentally infected specimens of *L. stagnalis* (Linnaeus, 1758) which were supplied by Professor Dr W. Haas (Department of Zoology, University of Erlangen-Nürnberg, Germany). This parasite had originally been isolated from fish ponds near Höchstadt/Aisch (Bavaria, Germany) and has been kept in the laboratory for 8 years using *Anas platyrhynchos* as final host and laboratory-reared *L. stagnalis* as intermediate host. Its identity was verified on the basis of origin, natural host, location of the adult worms, form and size of the eggs, cercarial chaetotaxy and molecular data as discussed in detail by Kock (2000).

T. franki was isolated from specimens of the

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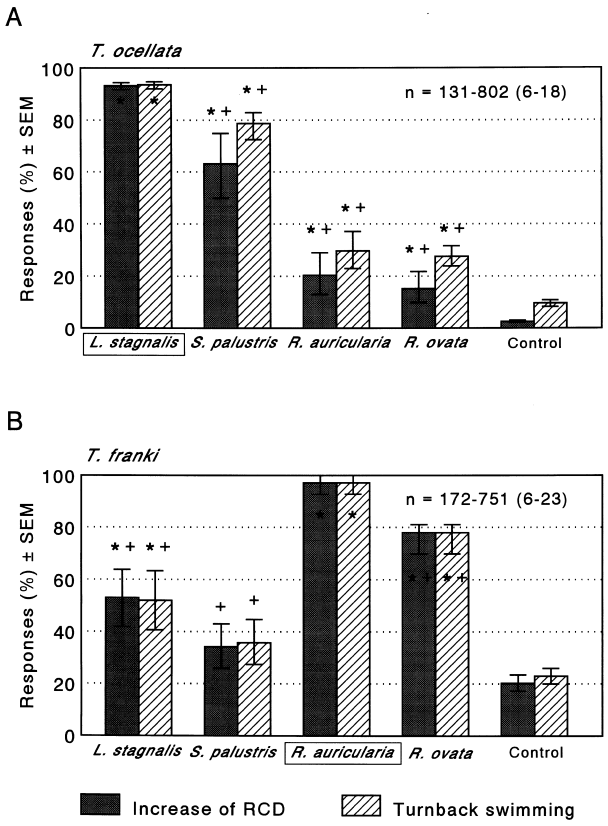

Fig. 1. Miracidial responses to snail-conditioned water (SCW) of different lymnaeid species. (A) *Trichobilharzia ocellata*; (B) *T. franki*. * $P < 0{\cdot}05$ *vs* control; $^{+}P < 0{\cdot}05$ *vs* SCW from the respective original host snail (framed).

original intermediate host, *Radix auricularia* (Linnaeus, 1758), that had been collected at the Tunisee near Freiburg, Germany (locus typicus).

Miracidia of both parasites were obtained from bird faeces using sidearm flasks (Dönges, 1966) and were used in behavioural studies within 3 h and in infection experiments within 1 h after hatching.

The intermediate hosts

Snails of the species *L. stagnalis* (Linnaeus, 1758), *Stagnicola palustris* (O. F. Müller, 1774), *Radix ovata* (Draparnaud, 1805) and *R. auricularia* (Linnaeus, 1758) were collected at different lakes in Schleswig-Holstein, Germany. They were kept for breeding in constantly aerated tanks (10–20 l) with dechlorinated tap water at 20 °C and a 12 h light–12 h dark photoperiod and fed *ad libitum* with lettuce and Tetra Min. Only uninfected, laboratory-reared snails were used for the production of snail-conditioned water and for infection experiments.

Miracidial chemo-orientation

Snail-conditioned water (SCW) was produced by incubating living snails for 2 h in dechlorinated tap water at a concentration of 0·5 g snails per ml of water (0·5 g snails × 2 h/ml) as described by Kalbe (1998). It was then pre-filtered employing fibreglass filters (Millipore A 25) and stored at −20 °C. Before use, SCW was diluted 1:10 with dechlorinated tap water.

Chemo-orientation of the miracidia was examined using a one-arm chamber according to the method described by Haberl *et al.* (1995). The following behavioural patterns were quantified: increase of rate of change of direction (RCD) when entering and turnback swimming when leaving the test substrate section. Test substrates, the water containing the miracidia and the water used to fill up the chamber were all adjusted to pH 7·0 with phosphate buffer (final concentration 5 mM). Experiments were carried out at 20 ± 1 °C. A blind protocol was used for every experiment to avoid any subjective evaluation of the behavioural pattern shown by the miracidia. The data obtained were arcsine transformed and analysed using SPSS statistical analysis software. Standard errors of means were retransformed after computation. Means were compared by applying the multiple *t*-test procedure according to Tukey with a significance level of 0·05.

Snail exposures

Juvenile snails were exposed to 5 miracidia each for 12 h in 2 ml vol. Eppendorf tubes filled with dechlorinated tap water. From day 28 post-exposure (p.e.) onwards, they were tested weekly for the emission of cercariae. At week 10 p.e., specimens that had not shed any cercariae were examined for the presence of sporocysts.

RESULTS

Miracidial chemo-orientation

When substrates of different sympatric snails were offered, miracidia of *T. ocellata* and *T. franki* both responded with the highest intensity to SCW of their original host snails (*L. stagnalis* respectively *R. auricularia*) (Fig. 1A, B). *T. ocellata* miracidia also showed a strong response to SCW of *S. palustris* and *T. franki* miracidia responded to SCW of *R. ovata*.

Snail exposures

T. ocellata infected *L. stagnalis* and *S. palustris*, but did not develop in *R. ovata* or in *R. auricularia* (Table 1). *T. franki* established an infection in specimens of *R. auricularia* and *R. ovata*, but not in *L. stagnalis* or in *S. palustris*.

DISCUSSION

T. ocellata and *T. franki* revealed a highly specific miracidial host-finding behaviour and a rather narrow intermediate host spectrum. The miracidia of both parasites could clearly differentiate between SCW of the original host snails and SCW of other sympatric snails. Infections established only in snail

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Table 1. Infection rates in snails of different lymnaeid species after exposure to miracidia of *Trichobilharzia ocellata* and *T. franki*

	T. ocellata		*T. franki*	
	Percentage infected	No. of snails*	Percentage infected	No. of snails*
Lymnaea stagnalis	80	157	0	34
Stagnicola palustris	50	26	0	30
Radix ovata	0	37	9·8	41
Radix auricularia	0	30	100	26

* No. of exposed snails which survived the infection experiments.

species which have proved to be closely related (Bargues & Mas-Coma, 1997; Bargues *et al*. 1997) such as *L. stagnalis* and *S. palustris* for *T. ocellata*, respectively, and *R. auricularia* and *R. ovata* for *T. franki*. Therefore, it seems very doubtful that *T. ocellata* is able to utilize specimens of the genus *Radix* or even planorbid, physid and hydrobid snails. Apparently, several species were seen by van den Broek) and Kilias & Frick (1964) as was also suggested by Blair & Islam (1983). Findings of *T. ocellata* that originated from snails of the genus *Radix* are likely to have actually belonged to species such as *T. franki* Müller & Kimmig, 1994 or *T. regenti* Horak, Kolarova & Dvorak, 1998.

Thus assuming that, in Europe, *T. ocellata* exclusively uses snails of the species *L. stagnalis* and *S. palustris* as intermediate hosts, the question arises as to how important this knowledge is for the systematic classification of this parasite. *L. stagnalis* is also utilized by *T. szidati* as intermediate host. However, morphological data (Sluiters, 1983; van de Roemer, 1984; Haas-Lupold, 1986; Feiler & Haas, 1988*a*, *b*; Odening, 1996; Kock, 2000) as well as preliminary molecular data (Kock, 2000) strongly imply that *T. ocellata* and *T. szidati* are synonymous species. The fact that *T. ocellata* was incapable of infecting specimens of *R. ovata* appears to stand in contradiction to this assumption, since Neuhaus (1952) also described *R. ovata*, in addition to *L. stagnalis*, as original intermediate host species of *T. szidati*. On the other hand it is not quite clear if he actually employed cercariae from *R. ovata* when establishing the experimental life-cycle of this parasite. He mentioned that he exclusively used specimens of *L. stagnalis* when re-transmitting *T. szidati* from the definitive host to the snail host, but did not say specifically from which snail host species the cercariae had originated that he used to infect the definitive host in the first place. Thus, there is no real proof that specimens of *R. ovata* can actually function as intermediate hosts of *T. szidati*. Neuhaus (1952) may have actually been dealing with 2 different species. This fact has also been observed by Odening (1996) who therefore proceeded from the assumption that Neuhaus (1952) only used cercariae from *L. stagnalis* when studying the life-cycle of *T. szidati*. Consequently the name *T. szidati* would refer exclusively to the schistosomatid that Neuhaus (1952) isolated from specimens of *L. stagnalis*. In this case, *T. szidati* may not be a valid species and the schistosomatid examined by Neuhaus (1952) may have been nothing else than *T. ocellata*, as was also suggested by Sluiters (1983) and Odening (1996). The *Trichobilharzia* species that Neuhaus (1952) isolated from specimens of *R. ovata* may have been *T. franki* or *T. regenti* or a species for which the cercarial stage is still unknown (e.g. *T. filiformis* (Szidat, 1938) McMullen & Beaver, 1945 or *T. kowalewskii* (Ejsmont, 1929) McMullen & Beaver, 1945) respectively a species yet to be described for Europe.

S. palustris was also reported as intermediate host of *C. neocellata* Szidat, 1942 and *C. pseudocellata* Szidat, 1934 in Europe. However, Szidat established these 2 species exclusively on the basis of common cercarial characters which were found to be inadequate for the systematic classification of *Trichobilharzia* species (Blair & Islam, 1983; Kock, 2000). Therefore, it appears that the establishment of these 2 species was not justified and that the schistosomatids examined by Szidat (Szidat & Wigand, 1934; Szidat, 1942) actually belonged to the species *T. ocellata*.

To complete the picture, it should be pointed out that *T. ocellata* has also been described from the USA (e.g. Olivier, 1953), Canada (e.g. Bourns, Ellis & Rau, 1973), Japan (Chikami, 1961), Russia (Eastern Siberia) (Bykhovskaya-Pavlovskaya & Ryzhikov, 1958) and Uzbekistan (Azimov, 1977), but the identity of the respective parasite which was studied in each case is questionable (Odening, 1996). Extensive experimental work will be necessary to confirm their taxonomic status and demonstrate their molluscan host spectrum.

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