

Biology of the Schistosome Genus *Trichobilharzia*

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ABSTRACT

Trichobilharzia is the largest genus within the family Schistosomatidae, covering over 40 species of avian parasites. To clarify the existing confusion in the systematics of the genus, we recommend combining knowledge of life cycles and developmental stages, snail/bird hosts, cytogenetical and molecular data together with morphological criteria for the characterization of particular species. The high specificity of *Trichobilharzia* for the intermediate host is a likely reflection of the ability to avoid the internal defence of specific snails. The spectrum of final hosts (birds) seems to be much wider. The infection of birds – trichobilharziasis – may lead to considerable tissue injuries, caused by eggs of the parasite or migration of immature/mature worms through the body. Most *Trichobilharzia* (visceral species) migrate through the viscera of the host, but nasal species display a neurotropic mode of migration. Due to a low specificity of penetrating cercariae, mammals (including humans) can be attacked. This leads to cercarial dermatitis, predominantly in sensitized hosts. Experimental infections indicate that *Trichobilharzia* never mature in an incompatible (mammalian) host. However, not all cercariae and schistosomula are necessarily trapped and eliminated in the skin, and parasites may migrate throughout the viscera and the nervous system of mammals. These findings suggest that the pathogenicity of *Trichobilharzia* may have been underestimated in the past and health risks associated with trichobilharziasis need to be studied further.

1. INTRODUCTION

Schistosomes are evolutionarily successful parasitic flatworms that are unusual among flukes because they are dioecious and inhabit the circulatory system of vertebrates. The parasites are best known as human pathogens in tropical and subtropical countries (over 200 million people suffering from schistosomiasis;

WHO estimation). A recent discovery revealed that they parasitize not only warm-blooded vertebrates (mammals and birds), but also reptiles, i.e. crocodiles (*Griphobilharzia*; Platt *et al.*, 1991).

Compared to mammalian schistosomes, represented by the well-known genus *Schistosoma*, the genera that infect birds may seem to be of less importance. However, as parasites of birds, the members of *Trichobilharzia* (the largest genus of the family Schistosomatidae) cause trichobilharziasis of ducks and geese (Wojcinski *et al.*, 1987; Graczyk *et al.*, 1993), a disease comparable with schistosomiasis in humans. From a medical viewpoint, these parasites are the causative agent of cercarial dermatitis in humans, an inflammatory reaction of the skin which is now considered as an emerging disease (de Gentile *et al.*, 1996). Moreover, schistosomes of *Trichobilharzia* spp. have been repeatedly reported from the lungs and other organs of mammals, including nervous tissue in the case of *T. regenti*. The obligatory migration of the latter parasite through the central nervous system of birds and mammals is occasionally associated with neuromotor disorders. Clearly, such properties justify continued attention towards these rather underestimated infections.

The last review of *Trichobilharzia* was published nearly 20 years ago (Blair and Islam, 1983). This paper provides updated information on the genus, including descriptions of new species, their molecular characterization, discoveries in parasite behaviour (host-finding), penetration of the host skin, immune evasion by the parasite, parasite migration and associated pathology in vertebrate hosts, and new data on outbreaks of cercarial dermatitis.

2. LIFE CYCLES AND HOSTS

2.1. General Life Cycle

The members of *Trichobilharzia* have a two-host life cycle similar to that of the related genus *Schistosoma*, with water snails and birds as the intermediate and final hosts, respectively (Figure 1). Whereas the cercarial larvae of *Trichobilharzia* spp. released from water snails are encountered relatively frequently in connection with outbreaks of cercarial dermatitis, reports describing adult worms are rare. This is partly due to the fact that *Trichobilharzia* adults possess a thin, threadlike body (20–100 µm diameter) and live hidden inside the host. The adult worms can be recovered only by means of a complicated examination of birds. Consequently, reports on these flukes from birds are limited and data on the life cycles are known mainly from experimental infections of both the intermediate and the final hosts.

The cycle starts with the eggs. The mature and fertilized female (depending on the species, located either in the visceral organs or in the nasal mucosa of

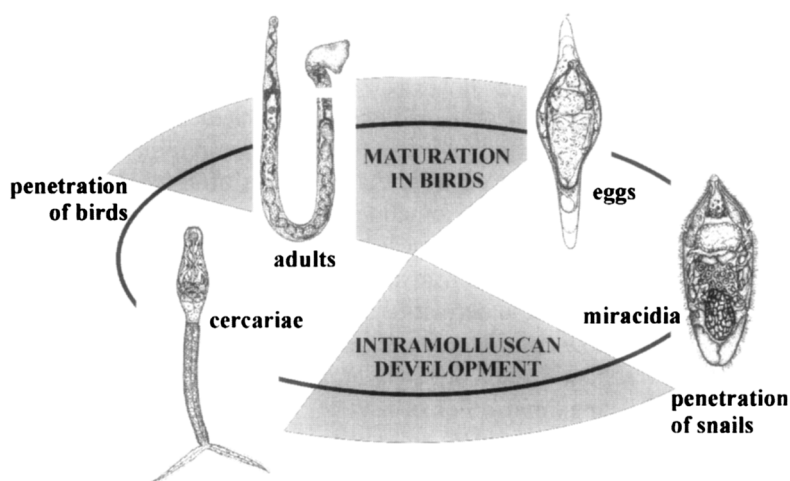


Figure 1 The life cycle of *Trichobilharzia* sp. The miracidium leaves the egg (either in water or in the nasal host tissues) and infects an appropriate intermediate snail host. Inside, the parasite multiplies asexually. The resulting life stage, cercariae, emerges from the snail and searches for the final host – a bird. Once the bird is found, the cercariae penetrate the skin and develop into schistosomula. The parasite then migrates to a preferred location within the host, where sexual maturation, mating and egg laying take place.

the host) contains a single egg. A similar situation with only one uterine egg is well known from other schistosomes, e.g. *S. mansoni* (see Loker, 1983). Usually, the egg of visceral *Trichobilharzia* is deposited in the circulation (capillaries) of the final host and needs to pass through different tissues in order to reach the lumen of the intestine and, subsequently, leave the host with faeces. In the nasal species of *Trichobilharzia*, eggs are deposited in the nasal mucosa. The eggs mature within the host and the released eggs contain fully developed miracidia that are able to hatch. As an exception, miracidia of nasal schistosomes, e.g. *T. regenti*, hatch from the eggs within the host tissues and await water contact (Horák *et al.*, 1998a). Similar behaviour can be expected from other nasal schistosomes and indeed, miracidia of *T. arcuata* hatch in contact with either water or physiological saline (Islam, 1986b). Not all larvae reach the outside environment. Some eggs are trapped in the tissues, where they trigger host reactions (granulomas) and are destroyed.

Once in the water, the miracidium leaves the egg and searches for the intermediate host, i.e. a water snail. The life span of miracidia of *T. szidati* is about 20 h at 20 °C (Neuhaus, 1952a). The species of *Trichobilharzia* usually have a narrow specificity towards the intermediate host, using only one (or closely related) snail species for larval development. Upon finding a proper molluscan host, the miracidium penetrates and develops into a mother sporocyst in the

head-foot region of the snail. The latter stage produces daughter sporocysts which leave the head-foot region and migrate to the hepatopancreas where further development with subsequent release of cercariae continues. The prepatent period within the snails is variable (about 3–10 weeks) and depends on several factors, including temperature. Details on snail-finding and intramolluscan development are provided in section 4.

It is hypothesized that a set of specialized glands serves for the passage of cercariae through the snail tissues (for *T. szidati* see Neuhaus, 1952a) in order to leave the snail. Once in the water, the cercariae need to find and infect a proper final host quickly because of their short life span. If a vertebrate host is found the cercariae penetrate the skin, transform to schistosomula and start to migrate to the preferred location for egg laying. Depending on the parasite species, two different routes are employed to reach these places: migration via the blood circulation or passage through the nervous tissues (peripheral nerves and the central nervous system). The first route is typical for visceral species (e.g. *T. franki*, *T. elvae*); the second one is used by the nasal schistosome *T. regenti*. Detailed information regarding host-finding, penetration, migration and associated pathologies is presented in sections 5 and 6.

2.2. Experimental Life Cycles and *In Vitro* Cultivations

As mentioned above, cercariae of *Trichobilharzia* spp. are frequently encountered in the environment. However, this stage lacks distinguishing characters and the morphological characterization of other developmental stages (primarily adults) is necessary for species diagnosis. Fortunately, parasite life cycles can be realized experimentally if a proper final host (bird) can be selected. Frequently, birds of the family Anatidae represent a suitable host. Birds can be infected percutaneously (by exposing the feet to cercariae) or, in some cases, by applying cercariae perorally (Macy *et al.*, 1955). With compatible intermediate (snail) and final (bird) hosts available in the laboratory, the whole life cycle can be maintained experimentally for many years (Meuleman *et al.*, 1984b). The feasibility of maintaining parasite life cycles can be restricted by (1) laws intended to protect animals, which down-regulate or prohibit the breeding of experimental ducks and other birds and (2) failure of long-term maintenance of snails in the laboratory due to unknown reasons. As far as *Trichobilharzia* spp. from Europe is concerned, host snails such as *Lymnaea stagnalis* and *Stagnicola* spp., and recently also *Radix peregra*/*R. ovata*, are easily kept in the laboratory. By comparison, the maintenance of *R. auricularia* and other snails (physid and small planorbid species) is more difficult.

There are several possible ways to simplify the experimental cycle and to replace certain phases of the snail-parasite co-existence. Largely, these approaches aim to avoid undesirable properties of the molluscan host that

may hamper the parasite cycle and the study of the parasite stages. To avoid an unsuccessful penetration of snails by parasites, the miracidia can be injected directly into the snails (*T. ocellata* into *L. stagnalis*); no harmful effects on the host and parasite have been recorded (Meuleman *et al.*, 1984a). The subsequent stages – mother and daughter sporocysts – can be recovered from snails (Amen and Meuleman, 1992). Moreover, miracidia can transform to mother sporocysts and develop further under *in vitro* conditions either in complex culture media (Mellink and van den Bovenkamp, 1985) supporting a long-term cultivation, or in simplified media enabling a short-term incubation and allowing analysis of parasite surface/excretory/secretory molecules (Schallig *et al.*, 1990). Recent experiments showed that tissue cultures of Bge cells (*Biomphalaria glabrata* embryonic cells) or even a lowered oxygen pressure can support complete larval development of *Schistosoma mansoni* *in vitro* (for a review see Coustau and Yoshino, 2000; Bixler *et al.*, 2001). These approaches may also facilitate cultivation of avian schistosomes.

Two approaches, based on former experiments with the human parasite *S. mansoni* (Clegg, 1965; Samuelson and Stein, 1989; Smyth, 1990), were applied in the *in vitro* development of bird schistosomes from cercariae to schistosomula and adults. Cercariae with mechanically detached tails were incubated in phosphate buffer (300 mOsm, 39 °C) for 5 h to characterize ultra-structural and biochemical changes in the tegument during penetration and cercaria–schistosomulum transformation (Horák *et al.*, 1998b). A nutrient-rich medium based on Earle's saline was used for long-term cultivation of *T. ocellata* to the organogeny stage. The medium contained lactalbumen hydrolysate and duck/chicken serum together with homologous erythrocytes. Although the worms grew and digested erythrocytes, they did not differentiate to the gametogeny stage (Howell and Bourns, 1974).

3. SYSTEMATICS OF THE GENUS *TRICHOBILHARZIA*

3.1. Introductory Notes

A precise determination of species within the genus *Trichobilharzia* is valuable not only from the biological viewpoint (e.g. to document differences in forms and developmental strategies of parasites within vertebrates) but also as a tool to predict health risks. Cercarial dermatitis, caused by cercariae of *Trichobilharzia*, is considered to be an emerging disease of humans (de Gentile *et al.*, 1996). Clearly, the medical and veterinary importance of *Trichobilharzia* spp. increases considerably with the new insights regarding parasite distribution (outbreaks of cercarial dermatitis), life cycles (penetration of both specific and non-specific vertebrate hosts) and pathogenicity (clinical symptoms and

pathologies caused by penetrating and migrating worms). Cercarial dermatitis is sometimes associated with subsequent generalized symptoms. It cannot be excluded that such symptoms are species-related, depending on parasite (species-specific) antigens, survival, and migration within vertebrates. Although limited data are available on migration of particular species of visceral *Trichobilharzia* and clinical consequences, it is evident that nasal schistosomes (*T. regenti*) invade the nervous tissues of birds and mammals and may cause neuromotor disorders (including paralysis).

However, the identification of *Trichobilharzia* species is very difficult work, complicated by the existence of synonyms, incomplete descriptions or incorrect parasite identifications (e.g. liberal and incorrect use of the name *T. ocellata* – see below). In their thorough review of the taxonomy, Blair and Islam (1983) noted that several synonyms and incomplete descriptions occur among the 40 species of *Trichobilharzia*. Historically, this may in part be due to the poor description of the type species, *T. ocellata*. Although the cercariae were identified by La Valette in 1855, and the corresponding adults by Brumpt in 1931, there is still no consensus on what *T. ocellata* (and *Cercaria ocellata*) actually is (Odening, 1996; see below). At present, there is no doubt that the species composition within the genus needs major revision.

3.2. Historical Overview

Trichobilharzia was described for the first time at the larval (cercarial) stage by La Valette (1855) who characterized *Cercaria ocellata* from *Lymnaea stagnalis* collected near Berlin in Germany. Subsequently, the cercariae were reported by several other authors (e.g. Ssnitzin, 1910), even from other intermediate hosts, e.g. from *Radix ovata* (see Mathias, 1930; Wesenberg-Lund, 1934), or from multiple lymnaeid, physid, planorbid and hydrobid/bithynid snails (Kiliass and Frick, 1964; van den Broek, 1965). The adult worms were found by Skrjabin and Zakharov (1920) in Russia in the visceral blood vessels of a duck. The schistosome was named as *T. kossarewi* and a new genus, *Trichobilharzia*, was established. In 1931, Brumpt performed experimental infections with *C. ocellata* and obtained adult worms which were considered to be identical to those of *T. kossarewi* Skrjabin and Zakharov, 1920. Due to the law of priority, the type species was renamed *T. ocellata*. Since then, however, doubts concerning the description of *T. ocellata* from different intermediate hosts (lymnaeids, or even other snail families) have been raised. The cercariae that emerge from different lymnaeids may vary in size and behaviour. Thus, *C. ocellata* probably represents a collective term for several species (see Szidat, 1942 for review).

It should be noted that the description of the type species, *T. ocellata*, is confusing for several reasons. (1) *C. ocellata* La Valette, 1855, as originally described shows features common to all *Trichobilharzia* cercariae. The only

specific marker is that the larvae were isolated from *L. stagnalis* in Germany. (2) The description of *T. kossarewi* Skrjabin and Zakharov, 1920 (later renamed *T. ocellata* by Brumpt, 1931) was based on one male worm, but the presence of a gynaecophoric canal was not observed (or mentioned) by the authors. McMullen and Beaver (1945) in their description of *T. ocellata* suggest that Skrjabin and Zakharov (1920) had been wrong to omit this anatomical feature. (3) Finally, Brumpt's (1931) description of *T. ocellata* (uniting *C. ocellata* sensu La Valette (1855) and *T. kossarewi* sensu Skrjabin and Zakharov (1920) under the name *T. ocellata* (La Valette, 1855) Brumpt, 1931) lacks the most important characters of the species. There is no information in the text on acetabulum, canalis gynaecophorus, seminal vesicles, male genital papilla, intestinal bifurcation and reunion etc. The drawing of the miracidium does not contain several important diagnostic features. Also, the text mentions that compared with *L. stagnalis*, the miracidia of *T. ocellata* exhibited higher affinity towards *Planorbarius corneus*, *Planorbis rotundatus* and *Stagnicola palustris*. The attraction to planorbid snails over *L. stagnalis* is in strong contrast to recent findings (e.g. Kalbe *et al.*, 1997). Since then, several authors have tried to clarify the situation, e.g. by redescribing locally occurring *T. ocellata* (McMullen and Beaver, 1945; Chikami, 1961). These (re)descriptions of *T. ocellata* may deal with the original *T. ocellata*, but they may also characterize other species. For example, McMullen and Beaver (1945) synonymized *T. elvae* with *T. ocellata*; however, their main reason for doing this was the shape of eggs, an insufficient marker (see section 3.4.1).

It remains up to current authors to decide which is the most relevant description of *T. ocellata*, if any. In his excellent review, Odening (1996) proposed two choices. (1) The best description of *T. ocellata* can be selected (e.g. *T. ocellata* sensu McMullen and Beaver (1945); *T. ocellata* sensu Chikami (1961); *T. szidati* sensu Neuhaus (1952a) as the most junior 'synonym') and considered to describe the true *T. ocellata* found originally by La Valette (1855) and Brumpt (1931). (2) The view should be adopted that *T. ocellata* represents a complex of species. In the latter case, some new species will continuously be separated from this complex, as may be deduced from the recent descriptions of European species (*T. franki* Müller and Kimmig, 1994; *T. regenti* Horák *et al.*, 1998a; *T. salmanticensis* Simon-Martin and Simon-Vicente, 1999).

Aside from confusion regarding the type species, additional inconsistencies exist within the genus of *Trichobilharzia*. Two new genera were named in the past: *Pseudobilharziella* Ejsmont, 1929 is a junior synonym of *Trichobilharzia* (Bykhovskaya-Pavlovskaya and Ryzhikov, 1958; Blair and Islam, 1983). *Jilinoibilharzia* has been separated from *Trichobilharzia* (see reviews of Blair and Islam, 1983; Khalil, 2002) and includes the former *T. yokogawai* (Oiso, 1927) McMullen and Beaver, 1945 and *T. brantae* Farr and Blankemeyer, 1956. Finally, *T. tatianae* Spasskaja, 1954 apparently does not belong to the genus *Trichobilharzia* (see Blair and Islam, 1983).

3.3. Diagnosis of the Genus *Trichobilharzia* (based on Farley, 1971; Blair and Islam, 1983; Khalil, 2002)

Among the members of the family Schistosomatidae (also comprising *Schistosomatium*, *Heterobilharzia*, *Schistosoma*, *Bivitellobilharzia*, *Austrobilharzia*, *Orientobilharzia*, *Ornithobilharzia*, *Macrobilharzia*, *Griphobilharzia*, *Bilharziella*, *Jilinobilharzia*, *Gigantobilharzia* and *Dendritobilharzia*), the genus *Trichobilharzia* is the largest in terms of number of species. In the adult stage, males and females are similar and threadlike. Oral and ventral suckers are present in both sexes and the posterior end of the body is spatulate. The gynaecophoric canal is short and does not extend to the posterior end of the body. Testes are numerous, occupying space posterior to the gynaecophoric canal on either side of the common caecum. The elongate seminal vesicle is localized in the anterior part of the body, between the acetabulum and gynaecophoric canal. The males possess small cirrus and their genital pore is at the anterior end of the gynaecophoric canal. An elongate and coiled ovary occurs in the anterior part of the female body. The seminal receptacle is well formed and lies immediately posterior to the ovary. The ootype is anterior to the ovary with the uterus opening immediately posterior to the acetabulum. Adults are parasites of birds.

The position and validity of the genus among other schistosomes is well supported by molecular data. Phylogenetic analyses based on partial sequences of 28S rDNA place the genus of *Trichobilharzia* in the clade of bird schistosomes (Snyder and Loker, 2000).

3.4. Main Diagnostic Criteria; Traditional and New Approaches in Species Diagnosis

Traditionally, the morphology of particular developmental stages is used as a diagnostic criterion. Arguably, the adult morphology is the most valuable characteristic for species identification (Blair and Islam, 1983). Additionally, eggs, miracidia and cercariae can also provide diagnostic characteristics. However, different species may exhibit very similar morphological features, and other life cycle data (intermediate host-specificity, larval behaviour, site of final location within birds) may also be required. Therefore, any new species description should contain not only the characters of adults, but also of other developmental stages (eggs, miracidia, cercariae), data on specificity towards intermediate and final hosts, organ specificity within birds and, facultatively, behaviour of miracidia and cercariae. Additional techniques may highlight further differences between species, e.g. chaetotaxy (characterization of surface papillae of miracidia and cercariae by staining with silver nitrate) and karyology (characterization of chromosomes in the mitotic metaphase plate).

Recently, molecular taxonomy has proved to be a powerful tool for diagnosis; the rDNA of four species has been sequenced (see section 3.4.8.).

The following overview provides descriptions of the main morphological structures of particular developmental stages and those that are important for species identification are discussed. In addition, current knowledge regarding intermediate hosts, location within birds and karyological and molecular characterization is summarized.

3.4.1. Eggs

The eggs (Figure 2) can be spindle-shaped (*T. parocellata*, *T. franki*), crescentic (*T. ocellata*, *T. arcuata*) or even ovoid (*T. corvi*, *T. filiformis*). Usually, they have a projection/small hook at one end. At the time of release from the bird, the eggs already contain a miracidium. In certain cases, the miracidium hatches from the egg within the bird host (*T. regenti*; see Horák *et al.*, 1998a). Some species produce similar eggs (e.g. *T. salmanticensis* and *T. parocellata*). Thus, although the size and shape of eggs are important criteria, they cannot be the reason for synonymization (used for *T. elvae* and *T. ocellata* by McMullen and Beaver, 1945) or species determination (used for *T. ocellata* by Sluimers, 1983).

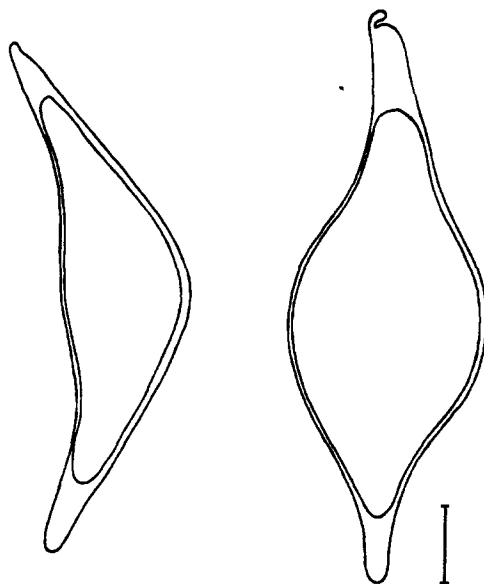


Figure 2 Shape of the eggs of *Trichobilharzia ocellata* (left) and *T. franki* (right). Scale bar = 30 μ m. Reproduced with permission from Kock (2000).

Nevertheless, eggs represent a valuable tool for quick identification of species with different egg morphology that occur sympatrically within the same bird host (e.g. visceral duck schistosomes of *T. ocellata* and *T. franki*).

3.4.2. *Miracidia*

These pyriform larvae (Figure 3) are covered by a mosaic epithelium, composed of intercellular ridges and ciliated plates. The latter are usually arranged in four tiers of 6:9:4:3 = 22 cells. This pattern may differ in some species (e.g. 6:7:5:4 = 22 in *T. corvi*; see Y. Ito, 1960, cited by Blair and Islam, 1983). Two lateral horns are located between the first and second tier. The anterior papilla (terebratorium) contains terminal or lateral openings of the penetration glands. Generally, two gland structures are visible: the apical gland (one or two cells) and the lateral gland (two cells). The posterior part of the body is filled

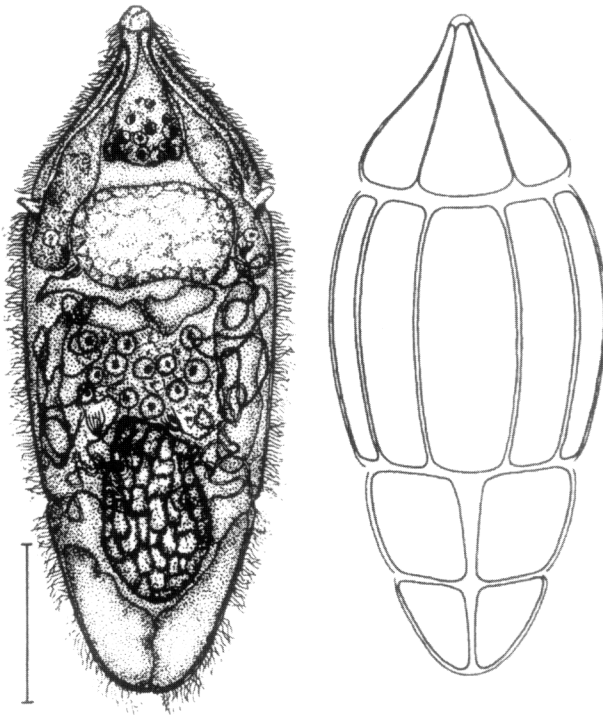


Figure 3 Miracidium of *Trichobilharzia regenti*. General morphology (left) and the arrangement of ciliated plates (right). Scale bar = 25 μ m. Reproduced with permission from Horák *et al.* (1998a).

with clusters of germ cells (these multiply and differentiate to produce daughter sporocysts). Particular species possess different numbers of these germ cell clusters, e.g. one in *T. regenti*, two in *T. ocellata* from Japan, three in *T. corvi*.

Some authors doubt the usefulness of miracidial morphology for species identification, mainly because only a few structures are present to help determination. Usually, the arrangement of ciliated plates, morphology of penetration glands and number of clusters of germ cells were used for distinguishing species. Contrary to the report on heterogeneity in the pattern of ciliated plates in *T. indica* (see Baugh, 1978), we feel that the first two characters mentioned above may represent valid criteria. However, these cannot be used as a stand-alone marker in the absence of additional information concerning other stages or the life cycle. Although considered valid in the past, the value of the number of groups of germ cells has been diminished by the observation that a single mass of germinal cells divided into three groups due to contraction of miracidia of *T. salmanticensis* (see Simon-Martin and Simon-Vicente, 1999).

3.4.3. Sporocysts

The sporocyst stage is usually not mentioned in descriptions of species of *Trichobilharzia* (an exception is, for example, *T. australis*; see Islam, 1986a). This is based on the fact that no distinguishing features are present and morphology does not help in diagnosis. For some species (*T. szidati*; see Neuhaus, 1952a) spination of the anterior end of daughter sporocysts is mentioned. Because of insufficient data and the absence of special structures, we regard sporocysts to be of low value for species identification.

3.4.4. Cercariae

The apharyngate ocellate furcocercariae (Figure 4) were the first stage of *Trichobilharzia* that was described (*C. ocellata* La Valette, 1855). They are composed of the body and tail with furcae. The surface tegument contains spines. Typically, two pigmented eye spots can be found in the first half of the body; their ultrastructure has been studied by van de Roemer and Haas (1984). The anterior end is occupied by a head organ, which contains ducts and openings of the penetration glands. The cercariae have five pairs of penetration glands: two pairs are located dorsally over the acetabulum and three pairs further towards the distal part of the body. The glands differ in their content. Additional to the main penetration glands there are also some other, smaller glands present in the cercarial body (Neuhaus, 1952a). The intestine is

inconspicuous. The excretory system is typically composed of $2[3+3+(1)] = 14$ flame cells; () indicates the flame cell on one side of the tail, [] represents the pattern of cells on one side of the cercaria = body and tail (the excretory system is symmetrical along the longitudinal axis, see Figure 4). Different patterns occur in some species: $(2[3+4+(1)])=16$ in *T. arcuata* (see Islam, 1986b) and *T. australis* (see Blair and Islam, 1983), $(2[3+2+(1)])=12$ in *T. corvi* (see J. Ito, 1960, cited by Blair and Islam, 1983). Moreover, several descriptions list two ciliary clusters within the excretory ducts (*T. szidati*, *T. regenti*, *T. salmanticensis*; see Kolářová and Horák, 1996; Horák *et al.*, 1998a; Simon-Martin and Simon-Vicente, 1999).

Unfortunately, the cercariae of different species closely resemble each other and they cannot usually be used for species identification. Their size cannot be used for diagnosis. Some morphological structures may indicate, but do not prove, the taxonomic position of certain species, e.g. the number and

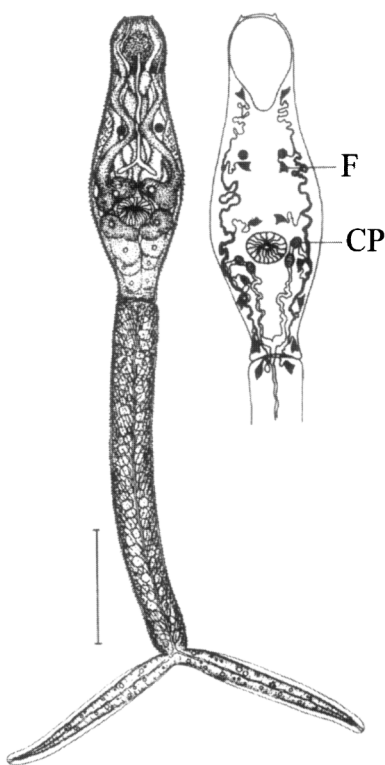


Figure 4 Cercaria of *Trichobilharzia regenti*. General morphology (left) and excretory system with flame cells (right). F, flame cell; CP, ciliary patches within excretory ducts. Scale bar = 100 μ m. Reproduced with permission from Horák *et al.* (1998a).

organization of flame cells in the body. Because several species of *Trichobilharzia* have similar patterns of flame cell organization, other markers have been sought. The distribution of surface sensory papillae (chaetotaxy) has been considered a valuable tool in distinguishing species of cercariae (e.g. Richard, 1971; Bayssade-Dufour and Ow-Yang, 1975; Kolářová and Horák, 1996). These papillae can be observed by staining cercariae with silver nitrate or examining the worms under the scanning electron microscope (Kock and Böckeler, 1998). Chaetotaxy can be used to distinguish certain species with typical papillae patterns, but the value of this method is limited because (1) the distribution of papillae is known for a small number of species only (2) minor differences in papillae positions occur frequently, thus challenging the specificity of this marker and (3) the method requires experienced examiners and many cercariae need to be assessed (usually not all papillae typical of the species are visible in one specimen). Two recent papers clearly demonstrated that chaetotaxy in *Trichobilharzia* studies can be problematic: Kock and Böckeler (1998) did not find any difference when comparing sensory papillae of *T. ocellata* and *T. franki*; and, surprisingly, Gay *et al.* (1999) were able to identify *T. franki* from *R. auricularia* and *T. ocellata* from *L. stagnalis*, and the pattern of *T. franki* was not identical with that reported by Kock and Böckeler (1998).

Evidently, either description of new species or determination of known species based exclusively on cercarial morphology should be avoided. Also, older data should be evaluated carefully, e.g. the findings of *T. ocellata* and *T. szidati* from *Radix peregra* or *R. auricularia* are probably not valid (see sections 3.2 and 3.4.6).

3.4.5. Adults

The isolation of adults from different host organs is a complicated matter and requires attention due to the worm size (usually, the specimens measure between 5 and 10 mm in length and 20–100 µm in diameter) and intimate contact with the host tissues. It is rare to obtain a whole worm intact; frequently only fragments of adults can be recovered. The measurements of the body length/width and of inner organs are generally of limited value for species determination. The worms are able to contract or relax their body such that the diameter (and corresponding length) of body parts may be substantially modified and the shape deformed.

The adults (Figure 5) represent the stage that is most important in species diagnosis (Blair and Islam, 1983). Both sexes have a filiform body of almost uniform width. The body can be covered by tubercles or spines. Usually, the posterior end is spatulate. The oral opening is situated subterminally and surrounded by an oral sucker. The head part of the body has several long sensory

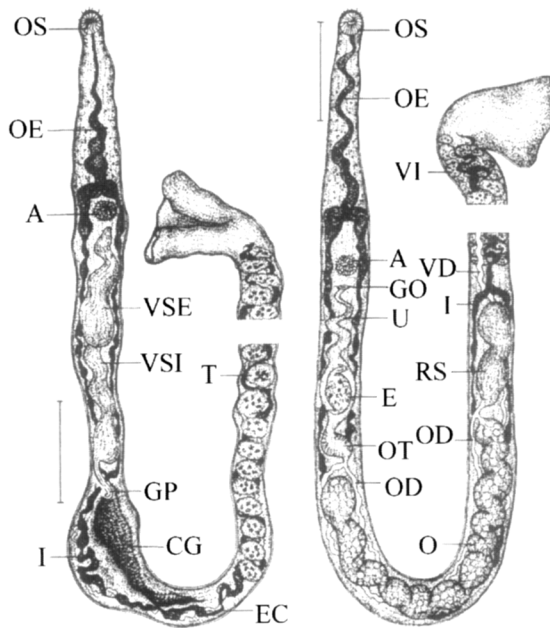


Figure 5 Anterior and posterior end of a male (left) and a female (right) of *Trichobilharzia regenti*. A, acetabulum; CG, canalis gynaecophorus; E, egg; EC, excretory duct; GO, genital opening; GP, genital papilla; I, intestine; O, ovary; OD, oviduct; OE, oesophagus; OS, oral sucker; OT, ootype; RS, receptaculum seminis; T, testes; U, uterus; VD, vitelloduct; VI, vitellaria; VSE, vesicula seminalis externa; VSI, vesicula seminalis interna. Scale bar = 100 μ m. Reproduced with permission from Horák *et al.* (1998a).

cilia. The acetabulum lies very close to the anterior end and it is covered by spines. In males, there is a short canalis gynaecophorus, which is usually decorated with spines. As an exception to the rule, the canalis may be used for diagnosis, as it is unusually long in *T. anatina* (see Fain, 1955, 1956b). The digestive tract lacks a pharynx, and a long oesophagus terminates just before the acetabulum where intestinal bifurcation occurs. The place of reunion of two intestinal branches provides an important taxonomic marker; in some species the reunion is located near the anterior end of the seminal vesicle of males (*T. szidati*, *T. elvae*, *T. corvi*), whereas it is situated posterior to the seminal vesicle in *T. brevis* and *T. australis*. The united caecum runs in curves to the end of the body. The reproduction apparatus of males is composed of numerous testes that lie just behind the canalis gynaecophorus and fill up the body to the posterior end. The number of testes varies in mature males; however, a minimum–maximum range can aid in species identification. The ducts leading forward from the testes terminate in the seminal vesicle, located between the

acetabulum and canalis gynaecophorus; the genital papilla is located at the beginning of the canalis gynaecophorus. In females, the posterior part of the body is filled with vitellaria; towards the anterior seminal receptacle, the ovary, ootype and uterus occur. The genital opening is situated just behind the acetabulum. Only one egg is found within the uterus.

The following features seem most appropriate for species identification: type of surface tegumental structures (spination, tubercles), spination and length of canalis gynaecophorus in relation to the body, place of caecal reunion, position of male genital papilla, number of testes (minimum–maximum range) and proportions (size and shape) of internal organs. The measurements of individual bodies and organs (length and width) may be considered informative but cannot be used as independent criteria. These features are easily influenced by at least two factors: frequently occurring contraction/relaxation of the body parts (including shrinkage caused by fixatives) and intraspecific variability in the size of worms (either within the same host organism or in different final hosts).

3.4.6. Spectrum of intermediate hosts

Larval stages of *Trichobilharzia* spp. develop in freshwater pulmonate snails of two families: Lymnaeidae (all over the world) and Physidae (mostly in North America). At present, the use of a pleurocerid snail, *Semisulcospira libertina*, by *T. corvi* (see J. Ito, 1960, cited by Blair and Islam, 1983) is the only exception known. It is believed that particular species of *Trichobilharzia* have rather narrow specificities towards their intermediate hosts, parasitizing either only one snail species or several, closely related species (Blair and Islam, 1983). For instance, *T. franki* develops exclusively in *R. auricularia* (see Müller and Kimmig, 1994), *T. regenti* in *R. peregra* and *R. ovata* (see Horák *et al.*, 1998a) and so on. Two important aspects of intermediate host specificity should be noted: (1) some species of *Trichobilharzia* may share one snail species (e.g. *L. stagnalis*) as intermediate host (Szidat, 1942; Neuhaus, 1952b; Farley 1971; Blair and Islam, 1983); and (2) laboratory experiments clearly showed that miracidia of some specific, well-defined species (e.g. *T. franki*) prefer *R. auricularia* as intermediate host. However, such behavioural studies showed that, to some degree, *T. franki* is also attracted to other, closely related snail species such as *R. ovata*. Moreover, the behavioural preference for particular host species correlates with the success of parasite development in the snails; after exposure to the parasites, *T. franki* developed in 100% of *R. auricularia* and 10% of *R. ovata* (see Kock, 2001).

The larval stages of '*Trichobilharzia* spp.' or '*Trichobilharzia ocellata*' have also been reported from snails of the family Planorbidae (e.g. *Anisus* sp. with *T. ocellata* reported by Beer and German, 1994), Physidae and

Hydrobiidae/Bithyniidae (Kilius and Frick, 1964; van den Broek, 1965). However, caution is warranted; either *Trichobilharzia* spp. with unknown life cycles (and probably not *T. ocellata*) may develop within these snails or the cercariae belong to another genus (e.g. *Gigantobilharzia* spp.; see Dönges, 1965).

The systematic relationships of the intermediate hosts (snails) are also full of confusions and remain to be resolved. Currently, the designations at the genus and species levels are in flux. Unfortunately, this further complicates the study of host specificity of species of *Trichobilharzia*. Concerning the family of lymnaeid snails, one approach is that all species belong to the genus of *Lymnaea*, whereas another approach recognizes several valid genera (e.g. *Radix*, *Stagnicola*, *Galba*). This second view, supported by molecular data (Bargues *et al.*, 2001), is adopted for this review. Morphologically, the species are distinguished by the shape and size of the shell. However, this can hardly separate closely related forms such as *R. ovata* and *R. peregra*. Additional morphological features such as the colour of the mantle (*Radix* species), surface sculptures of the shell (*Radix* and *Stagnicola* species; Jackiewicz and Koralewska-Batura, 1995), and the examination of the penis and internal reproductive organs (Jackiewicz, 1988) can be used to help diagnosis at the species level. Recently, molecular techniques have been applied to investigate the phylogeny of lymnaeid snails. Comparative analysis of 18S rDNA sequences (Bargues and Mas-Coma, 1997; Bargues *et al.*, 1997) yielded three clades, composed of *Lymnaea-Stagnicola*, *Radix* and *Galba* within the lymnaeids. More recently, a review of European lymnaeids (Bargues *et al.*, 2001), which also considered an extensive analysis of ITS-2 sequences, confirmed the grouping into these three clades, while showing heterogeneity within the genus of *Radix*. Based on support from the molecular data, several other names were introduced in addition to *R. auricularia*, *R. ovata* and *R. peregra*. Interestingly, the molecular analysis suggested that snails previously determined to be *R. peregra* and *R. ovata* (using morphological features) and that serve as the intermediate hosts of *T. regenti* (see Horák *et al.*, 1998a), belong to two other separate species: *R. labiata* and *R. lagotis*. Unfortunately, it seems difficult to distinguish these newly announced species from the morphological viewpoint, and their routine diagnosis in malacology and parasitology is questionable. Taken together, a heterogeneity in susceptibility within certain snail populations for infection by *Trichobilharzia* spp. (and other trematodes) may be due to the occurrence of cryptic (sub)species of snails, classified by traditional methods as belonging to one species. However, ITS-2 data do not distinguish between resistant and susceptible individuals of the same snail species (Bargues *et al.*, 2001), and probably other genetically linked factors play a role in host-parasite compatibility.

3.4.7. Spectrum of bird hosts and final location

It seems that the spectrum of final hosts (suitable birds) is broader than that of intermediate hosts. This can partly be due to the fact that cercariae of *Trichobilharzia* possess an ability to penetrate practically any warm-blooded animal (Haas and van de Roemer, 1998). Yet unknown factors (the immune response might be one of them) allow the subsequent development and maturation of *Trichobilharzia* parasites in birds, but not in mammals. Because larval parasite stages, i.e. cercariae, released from snails have been encountered frequently, these were used to experimentally infect birds. From the successful development of, for example, *T. physellae* in canaries, mallards and pigeons (McMullen and Beaver, 1945) and *T. corvi* in chickens (J. Ito, 1960, cited by Blair and Islam, 1983), it is clear that species of *Trichobilharzia* do not specifically depend on water birds, as might be deduced from their aquatic intermediate snail hosts. This fact has been confirmed by examining naturally infected birds. Concerning the spectrum of bird orders, besides Anseriformes, some species of *Trichobilharzia* have been found in Podicipediformes (*T. aureliani*; see Fain, 1956b), Ciconiiformes (*T. rodhaini*; see Fain, 1955), Coraciiformes (*T. cerylei*; see Fain, 1956b) and Passeriformes (e.g. *T. corvi*; see Yamaguti, 1941).

Once inside the final host, the parasites mature and lay eggs in a specific site. Generally, the species of *Trichobilharzia* can be divided into two main groups: visceral schistosomes (e.g. *T. brevis*, *T. stagnicolae*, *T. szidati*, *T. franki*) and nasal schistosomes (e.g. *T. arcuata*, *T. australis*, *T. regenti*). Whereas the nasal schistosomes exclusively inhabit the nasal soft tissues, the visceral parasites may inhabit different sites within the body. This preference is not particularly strict and parasites can usually be found in blood vessels of the intestine (*T. franki*; see Müller and Kimmig, 1994), cloaca (*T. kegonsensis*; see Brackett, 1942), liver (*T. franki*; see Müller and Kimmig, 1994), and in portal (*T. querquedulae*; see McLeod, 1937) and mesenterial (*T. maeграithi*; see Kruatrachue *et al.*, 1968) vessels. Some species (*T. szidati*, *T. stagnicolae*, *T. elvae* and *T. physellae*) have been discovered within the tissues of the intestinal wall (including muscles) and associated vessels (McMullen and Beaver, 1945; Neuhaus, 1952a).

3.4.8. Cytogenetical and molecular analyses

To date, karyological studies and rDNA sequencing have been applied to *Trichobilharzia* for species characterization. So far, karyotypes from nine species (isolates) have been characterized, i.e. *T. physellae* (Short and Menzel, 1960), *T. stagnicolae* A (Short and Menzel, 1960), *T. stagnicolae* B (Short and Menzel, 1960), *Trichobilharzia* sp. 1 (Barshene *et al.*, 1989),

Trichobilharzia sp. 2 (Barshene *et al.*, 1989), *T. szidati* (Barshene and Stanivichiute, 1993), *T. szidati* (Špakulová *et al.*, 1996), *T. franki* (Špakulová *et al.*, 1997) and *T. regenti* (Špakulová *et al.*, 2001). Špakulová *et al.* (1997) have reviewed the variability in chromosome patterns for the genus *Trichobilharzia*. Generally, the species show similar patterns of chromosomes in metaphase plates, having $2n = 16$ with 14 autosomes and 2 gonosomes. The sex-determining mechanism is ZZ in males and ZW in females. Only *T. stagnicola* B (Short and Menzel, 1960) was found to possess $2n = 18$. The fifth autosome pair is usually satellited (Špakulová *et al.*, 1997). The main differences between species can be observed in the sex chromosomes. Characterization of *T. regenti*, the only nasal schistosome among these otherwise visceral schistosomes, disclosed the presence of one or two supernumerary B chromosomes in more than 60% of the analyzed cells from male and female sporocysts (Špakulová *et al.*, 2001), the mode of formation of which is unclear. At present, karyological mapping should be considered as a supplementary diagnostic tool, as it cannot be used as a stand-alone method for species identification. This is based on the limited number of species/isolates of *Trichobilharzia* that were karyologically characterized. Moreover, the older descriptions do not contain information on sex chromosomes (Short and Menzel, 1960; Barshene *et al.*, 1989; Barshene and Stanivichiute, 1993) or satellites (Barshene *et al.*, 1989; Barshene and Stanivichiute, 1993). However, re-examination of the older descriptions and characterization of additional species of *Trichobilharzia* may allow future use of karyotypes for determination of species.

Recently, the validity of species and phylogenetic relationships have been tested by sequencing rDNA. This approach was initiated by the study of evolutionary relationships among the Schistosomatidae (Snyder and Loker, 2000) in which partial sequences of the 28S rRNA gene were obtained and used in phylogenetic analyses. A clearly defined position of the genus *Trichobilharzia* (*T. ocellata* as the representative of the genus; strain isolated in Germany from *L. stagnalis*) within the clade of avian schistosomes was confirmed. Consecutive studies, involving sequence data derived from the internal transcribed spacer regions 1 and 2 (ITS-1, ITS-2), reported on interspecific differences and the positions of particular species within the genus. To date, these studies have been limited to a small number of European isolates of *Trichobilharzia* (*T. ocellata*, *T. szidati*, *T. franki*, *T. regenti*, *Trichobilharzia* sp.). Nevertheless, two important conclusions can be drawn from these preliminary data. (1) The European strains of *T. ocellata* and *T. szidati* isolated from *L. stagnalis* (and also from *Stagnicola palustris*; see Kock, 2000) show sequence identity of >99% and belong to one clade (perhaps species) (Kock, 2000; J. Rudolfová, J. Dvořák and P. Horák, unpublished data). With this approach it should be possible to resolve the important dilemma of the composition of the genus *Trichobilharzia* (see also Odening, 1996; Kock, 2000). Should *T. szidati*

Table 1 Overview of species with known developmental stages and life cycles

<i>Trichobilharzia</i> species	Distribution	Natural and/or experimental bird hosts	Footnote
Nasal species using lymnaeid snails:			
<i>T. arcuata</i> Islam, 1986	Australia	Anseriformes, Columbiformes	
<i>T. australis</i> Blair and Islam, 1983	Australia	Anseriformes	
<i>T. regenti</i> Horák <i>et al.</i> , 1998	Europe	Anseriformes	1b,c
Visceral species using lymnaeid snails:			
<i>T. alaskensis</i> Harkema <i>et al.</i> , 1957	N. America	Anseriformes	2,3
<i>T. brevis</i> Basch, 1966	Asia	Anseriformes	
<i>T. elvae</i> (Miller, 1923) McMullen and Beaver, 1945	N. America	Anseriformes, Passeriformes	
<i>T. franki</i> Müller and Kimmig, 1994	Europe	Anseriformes	1b,c
<i>T. jequitibaensis</i> Leite <i>et al.</i> , 1978	S. America	Anseriformes	
<i>T. jianensis</i> Liu <i>et al.</i> , 1977	Asia	Anseriformes	2,4
<i>T. limnaeae</i> Yamaguti, 1971	Asia	Anseriformes	2
<i>T. maeграithi</i> Kruatrachue <i>et al.</i> , 1968	Asia	Anseriformes	
<i>T. ocellata</i> (LaValette, 1855) Brumpt, 1931	Europe, N. America, Asia	Anseriformes	1a,b
<i>T. paoi</i> (Kung <i>et al.</i> , 1960) Tang and Tang, 1962	Asia	Anseriformes	2,4
<i>T. parocellata</i> (Johnston and Simpson, 1939) Islam and Copeman, 1980	Australia	Anseriformes	
<i>T. salmanticensis</i> Simon-Martin and Simon-Vicente, 1999	Europe	Anseriformes	
<i>T. stagnicolae</i> (Talbot, 1936) McMullen and Beaver, 1945	N. America	Passeriformes	
<i>T. szidati</i> Neuhaus, 1952	Europe	Anseriformes	1b,c
Visceral species using physid snails:			
<i>T. adamsi</i> Edwards and Jansch, 1955	N. America	Anseriformes	
<i>T. cameroni</i> Wu, 1953	N. America	Anseriformes, Passeriformes	
<i>T. jequitibaensis</i> Leite <i>et al.</i> , 1978	S. America	Anseriformes	
<i>T. oregonensis</i> (Macfarlane and Macy, 1946) Macy and Moore, 1953	N. America	Anseriformes	2,3
<i>T. physellae</i> (Talbot, 1936) McMullen and Beaver, 1945	N. America	Anseriformes, Columbiformes, Passeriformes	

Table 1 continued

<i>Trichobilharzia</i> species	Distribution	Natural and/or experimental bird hosts	Footnote
<i>T. querquedulae</i> (McLeod, 1937) McMullen and Beaver, 1945	N. America	Anseriformes	
Visceral species using pleurocerid snails:			
<i>T. corvi</i> (Yamaguti, 1941) McMullen and Beaver, 1945	Asia	Passeriformes, Galliformes	

Synonymy of the following species has been questioned:
T. querquedulae – *T. physellae* – *T. limnaeae*
(*T. kossarewi*) – *T. ocellata* – *T. szidati* – *T. elvae* (see section 3.2.). Because of the confusion about what is meant by *T. ocellata*, the species was described also from other snail families, e.g. Physidae, Planorbidae and Hydrobiidae/Bithyniidae (genus Bithynia) (Kiliyas and Frick, 1964; van den Broek, 1965). In fact, these findings most probably represent other species of *Trichobilharzia* or even other genera of bird schistosomes.
¹ The species is characterized by rDNA sequences already (Snyder and Loker, 2000^a; Kock, 2000^b; Dvořák *et al.*, 2002^c).
² The original description was not available to the authors; the information is based on Blair and Islam (1983).
³ The original description was not available to the authors; the information is based on Farley (1971).
⁴ The original description was not available to the authors, the information is based on Simon-Martin and Simon-Vicente (1999).

be synonymized with *T. ocellata* (*T. szidati* as a junior synonym), or should *T. ocellata* remain as a complex of species – *species inquirendae* (due to historical uncertainty as to what *T. ocellata* is)? To find a relevant answer is important because so-called *T. ocellata* is held in several laboratories across the world. (2) *Trichobilharzia* sp., *T. regenti* and *T. franki* isolated from *Radix* species (*R. ovata*, *R. peregra*, *R. auricularia*) form one clade, based on either ITS-1 or ITS-2 sequence data (Kock, 2000; Dvořák *et al.*, 2002). It can be hypothesized that the close relationship between these species is reflected in the sharing of one genus of snails (*Radix*) as the intermediate host. The different location within the final host (nasal tissue for *T. regenti* and visceral vessels for *T. franki*) is probably of subsidiary importance within the framework of rDNA analysis.

3.5. Final Notes on Systematics

It is vital that all new descriptions of *Trichobilharzia* species should be as complete as possible (from the viewpoint of morphology, life cycles, host specificity and molecular analysis). New descriptions should also be based on up-to-date information regarding the latest taxonomic situation within the genus of *Trichobilharzia*. Two recent examples demonstrate some of the

pitfalls that ultimately may further contribute to the general taxonomic confusion. (1) *T. salmanticensis* (Simon-Martin and Simon-Vicente, 1999), although described from Spain, was compared and diagnosed with respect to the Asian/South American species of the genus (*T. paoi*, *T. maeграithi*, *T. jianensis*, *T. jequitibaensis*). Based on the key available from Blair and Islam (1983), these were the most closely related forms. However, since the publication of the review by Blair and Islam (1983), additional European members of the genus (*T. franki* and *T. regenti*) have been described. Unfortunately, these new and probably highly relevant species of *Trichobilharzia* have not been included in their analysis. (2) In a molecular study, Picard and Jousson (2001) isolated cercariae of *Trichobilharzia* spp. from *R. ovata* and *R. auricularia* and characterized their ITS-1 sequences. They also obtained sequences from one adult *Trichobilharzia* worm recovered from the nasal cavity of a duck. All sequences were entered into the GenBank database (accession codes AJ312041–AJ312049; February 2002) with *T. ocellata* as the source organism. However, recent experiments (Müller and Kimmig, 1994; Kalbe *et al.*, 1997; Horák *et al.*, 1998a; Simon-Martin and Simon-Vicente, 1999; Kock, 2000, 2001) demonstrated a narrow intermediate host specificity of particular species of *Trichobilharzia*. This implies that the parasites isolated from European *R. ovata* and *R. auricularia* are likely to be *T. regenti*/*T. salmanticensis* and *T. franki*, respectively. Moreover, the nasal *Trichobilharzia* does not definitely belong to *T. ocellata*. In conclusion, assigning these sequences to *T. ocellata* may be less than accurate, and it can confuse future use of the GenBank data for phylogenetic studies.

3.6. Overview of Species

Ideally, in full agreement with the suggestions of Blair and Islam (1983) and Müller and Kimmig (1994), future descriptions of any *Trichobilharzia* must combine data on morphology of the adults and of other developmental stages, life cycles including intermediate and final host specificity and preferred location within the final host. Also, new approaches to characterize the species should be included; isolates with similar morphology may be distinguished by molecular characterization of the species (sequencing of rDNA or other genes).

Currently available descriptions lack much of this vital information. Thus, it is impossible to construct a clear key for species determination. An attempt to organize the species of *Trichobilharzia* into a key has been realized in Blair and Islam's (1983) valuable review; the difficulties encountered in constructing the key are clearly presented by the authors. Rather than trying to construct another key of *Trichobilharzia* species, we have provided a list of species described from all over the world (Tables 1 and 2).

Table 2 Overview of species with partly known developmental stages and life cycles (intermediate hosts unknown)

<i>Trichobilharzia</i> species	Distribution	Natural and/or experimental bird hosts	Footnote
Nasal species:			
<i>T. aureliani</i> Fain, 1956	Africa	Podicipediformes	
<i>T. duboisi</i> Fain, 1959	Africa	Anseriformes	
<i>T. nasicola</i> Fain, 1955	Africa	Anseriformes	
<i>T. rodhaini</i> Fain, 1955	Africa	Ciconiiformes, Anseriformes	
<i>T. spinulata</i> Fain, 1955	Africa	Anseriformes	
Visceral species:			
<i>T. anatina</i> Fain, 1955	Africa	Anseriformes	
<i>T. berghei</i> Fain, 1955	Africa	Anseriformes	
<i>T. burnetti</i> (Brackett, 1942) McMullen and Beaver, 1945	N. America	Anseriformes	
<i>T. cerylei</i> Fain, 1956	Africa	Coraciiformes	
<i>T. filiformis</i> (Szidat, 1938) McMullen and Beaver, 1945	Europe	Anseriformes	
<i>T. guandongensis</i> Tsai <i>et al.</i> , 1979	Asia	Not mentioned	2
<i>T. horiconensis</i> (Brackett, 1942) McMullen and Beaver, 1945	N. America	Anseriformes	
<i>T. indica</i> Baugh, 1963	Asia	Anseriformes	
<i>T. kegonsensis</i> (Brackett, 1942) McMullen and Beaver, 1945	N. America	Anseriformes	
<i>T. kossarewi</i> Skrjabin and Zakharov, 1920	Europe	Anseriformes	1,2,3
<i>T. kowalewskii</i> (Ejsmont, 1929) McMullen and Beaver, 1945	Europe, Asia	Anseriformes	
<i>T. littlebi</i> (Byrd, 1956) Farley, 1971	N. America	Passeriformes	2,3
<i>T. lonchurae</i> (Fischthal and Kuntz, 1973) Tsai <i>et al.</i> , 1979	Asia	Passeriformes	2
<i>T. schoutedeni</i> Fain, 1955	Africa	Anseriformes	
<i>T. waubesensis</i> (Brackett, 1942) 5 McMullen and Beaver, 194	N. America	Anseriformes	
<i>T. zongshani</i> Tsai <i>et al.</i> , 1979	Asia	not mentioned	2

Synonymy of the following species has been questioned:

T. kegonsensis – *T. horiconensis* – *T. burnetti* – *T. waubesensis*.

¹ Because it is impossible to be certain that *T. kossarewi* is a synonym of *T. ocellata* (Blair and Islam, 1983 and section 3.2.), the former species is treated in this table separately as an insufficiently described *Trichobilharzia*.

² The original description was not available to the authors; the information is based on Blair and Islam (1983).

³ The original description was not available to the authors; the information is based on Farley (1971).

4. DEVELOPMENT IN INTERMEDIATE HOSTS

4.1. Host-Finding and Penetration by Miracidia

Adult avian schistosomes produce eggs that, after maturation in the host, contain fully developed miracidia. The eggs of visceral schistosomes leave the avian host with the faeces. Within a short time (5–10 min, *T. szidati*, Neuhaus, 1952a; 2–3 h in *T. cameroni*, Wu, 1953) of being deposited in surface water, physiological cues such as increased light and change in osmotic pressure cause miracidia to hatch from the eggs (Meuleman *et al.*, 1984b; Xu and Dresden, 1990; Kalbe *et al.*, 1997). A nasal schistosome, such as *T. regenti*, deposits eggs in the nasal cavity of birds and miracidia hatch in the nasal mucosa, which may then be released into the water when the bird feeds or drinks (Horák *et al.*, 1998a). The body surface of a miracidium is covered with plates that bear cilia. Miracidia are fast swimmers, propelled by the beating action of these cilia. Miracidia lack a digestive system and cannot feed. The energy for swimming activity is derived from the breakdown of endogenously stored glycogen, mainly via the Krebs cycle (Tielens *et al.*, 1991). Consequently, miracidia can only swim for up to 20 h at 20 °C (*T. szidati*; see Neuhaus, 1952a) before they have depleted their energy reserves and die. In order to survive, miracidia must infect a snail within this time interval. Snails are obligatory intermediate hosts; the internal physiology of a suitable snail provides nutrients and other support for the differentiation and asexual reproduction of schistosome larvae.

Miracidia display specialized behaviours that enable them to find a host snail (reviewed by Haas and Haberl, 1997; Haas, 2000). After hatching from eggs, miracidia disperse by swimming in a relatively fast and linear fashion. This increases the chance for individual miracidia to find a host and it minimizes high infection rates in single snails. A preference for certain environmental conditions (light, temperature and gravity) causes miracidia to locate to microenvironments that are preferred by snail hosts. Snails release various secreted/excreted products (SEP), which results in an 'active space' of (gradients of) snail-generated chemicals around potential hosts. Miracidia are attracted by the macromolecular components of snail-SEP, which consist of glycoproteins larger than 30 kDa, termed 'miracidia-attracting glycoproteins' (MAGs) (Kalbe *et al.*, 2000). Upon entry into an active space of a snail, miracidia of *T. ocellata* change their linear swimming behaviour by increasing the rate of change of direction (RCD). Additionally, *T. ocellata* miracidia display a turn-back response (consisting of a sharp 180° turn) if the chemical cues fall below a threshold value (Kalbe *et al.*, 1997; Kock, 2001). In an aquatic environment, where currents may prevent the formation of linear chemical gradients, these markedly non-directional behaviours may be optimal for miracidia to locate and ultimately contact a moving snail.

If the appropriate cues are absent at the initial contact with a snail, miracidia resume their swimming behaviour and move away (contact without return response). The presence of pertinent macromolecular glycoconjugates in the mucus on the snail's surface causes the miracidia to remain close to the skin of the snail and to display 'repeated investigation' and 'attachment' behaviours (MacInnis 1965; Haas *et al.*, 1991; Haberl and Haas, 1992). Once attachment has occurred, clues of undetermined nature trigger penetration, effected by pulsing movements and release of the contents of apical penetration glands. These secretions remain to be characterized but they probably contain proteolytic enzymes that help miracidia to penetrate through the skin of the snail.

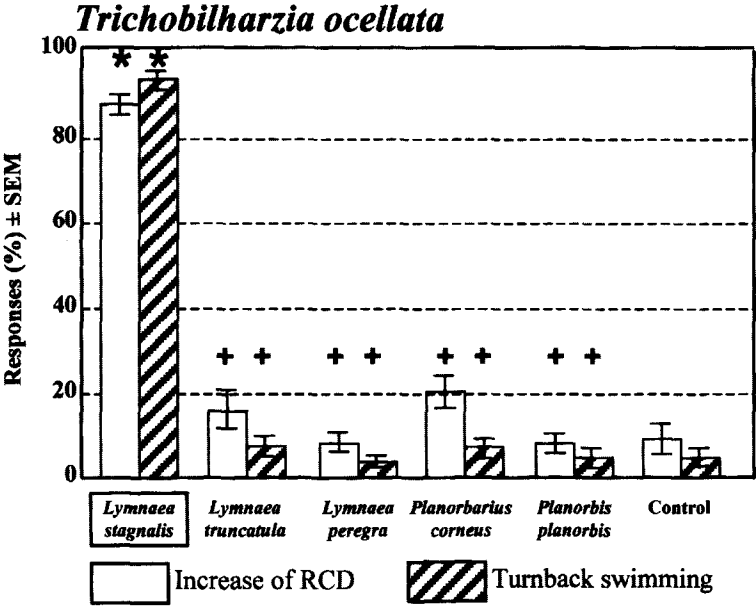


Figure 6 Preference in host-finding by miracidia of *Trichobilharzia ocellata*. The miracidia employ snail-released chemicals for short-range orientation towards a host. Upon detection of appropriate cues, the miracidia change their linear swimming behaviour by increasing the rate of change of direction (RCD). The miracidia also display a turnback swimming response (a sharp 180° turn) if the chemical cues fall below a threshold concentration. *Trichobilharzia ocellata* initiated host-finding behaviour in response to snail-conditioned water (SCW) containing chemicals released by the host snail, *Lymnaea stagnalis* (boxed). SCW derived from non-host snails such as *Lymnaea truncatula*, *Lymnaea peregra* (both Lymnaeidae), *Planorbarius corneus*, and *Planorbis planorbis* (both Planorbidae) failed to evoke significant host-finding behaviour. This greatly increases the likelihood that *Trichobilharzia ocellata* penetrates a suitable host in which it can develop successfully. Control = water, $n = 189-685$, * $P < 0.05$ versus control, + $P < 0.05$ versus response to SCW derived from *Lymnaea stagnalis*. Reproduced with permission from Kalbe *et al.* (1997).

Interestingly, not all schistosome species penetrate a snail through this route. Some schistosome species preferentially enter through natural openings such as the mouth or rectum of snails (Loker, 1978; Xia and Jourdane, 1991).

The reliance on snail-released chemicals to locate a host provides miracidia with a degree of specificity regarding the snail that they infect (Kalbe *et al.*, 1996). The MAGs produced by different snail species may have similar peptide backbones, but differ in saccharide composition. Miracidia are thought to differentiate between these glycosylation patterns in order to preferentially locate and infect particular snail species (Figure 6; Kalbe *et al.*, 2000; Kock, 2001). Such selective abilities become relevant as a particular schistosome species may successfully infect some snails, but fails to establish in other snail species that prove to be unsuitable as a host. For instance, *T. franki* establishes infections

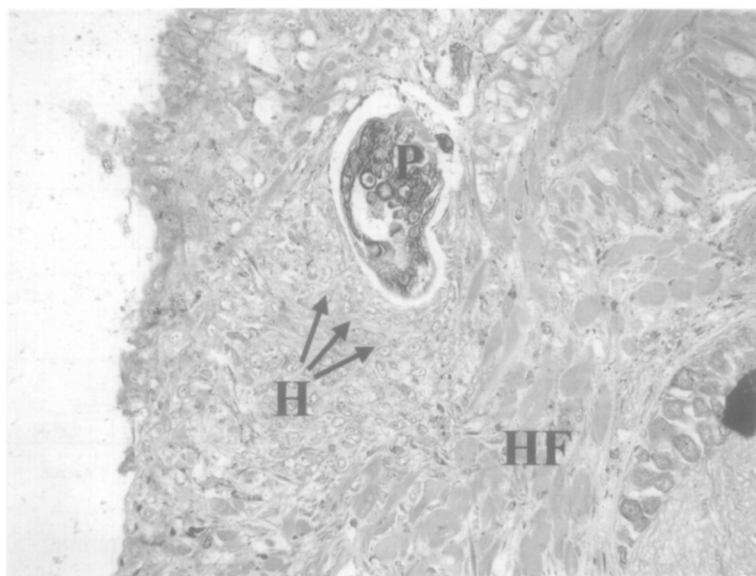


Figure 7 Cross-section showing a critical phase in the interaction between an avian schistosome *Trichobilharzia regenti* and a snail *Lymnaea stagnalis*. Only hours after penetration, the parasite (P) resides in the margin of the head-foot of the snail (HF), and is developing from a miracidium into a mother sporocyst. The combination of the biological properties of both the parasite and the host decides whether the parasite will succeed, or whether it will be defeated by the host. To survive and establish an infection, the parasite requires a suitable intramolluscan environment. Also, the parasite must prevent a defence response from the snail. These conditions are met within host snails such as *Radix peregra* or *Radix ovata*. In this case, however, *Trichobilharzia regenti* penetrated into *Lymnaea stagnalis*, which is unsuitable as host. The parasite will die either from inadequate physiological conditions (such as lack of appropriate nutrients) or due to a defence response from the snail. The aggregation of haemocytes (H; phagocytic snail defence blood cells) indicates that this parasite will soon be encapsulated and eliminated. Giemsa staining. Author: L. Trefil, Parasitology, Charles University, Prague, Czech Republic.

in the snails *Radix auricularia* and *R. ovata*, but not in other lymnaeid snails such as *Lymnaea stagnalis* or *Stagnicola palustris*, whereas the reverse pattern applies to *T. ocellata* (Kock, 2001). The range of suitable intermediate hosts can be restricted to species- or even strain-level of the snail species present in a given environment. The augmented host-finding behaviour in response to *L. stagnalis*-released chemicals increases the likelihood that *T. ocellata* encounters a suitable host. However, a preference for some snails does not always prevent miracidia from penetrating non-host snails (e.g. Grassi *et al.*, 2001; Kock, 2001; Figure 7). Additionally, miracidia do not seem to avoid unsuitable strains of a particular host species. Physiological and immunobiological factors that decide the fate of schistosome parasites within snails are discussed below.

4.2. Intramolluscan Development: Stages and Infection Course

The entry into a snail host marks a transition from free-living to a parasitic phase in the life cycle of an avian schistosome. Studies of the avian schistosome *T. ocellata* (Sluiters, 1981; Amen *et al.*, 1991; Amen and Meuleman, 1992), supplemented with some observations from other schistosomes, provide an overview of the parasite stages, the asexual multiplication and the infection course within the snail.

After penetrating into a snail host, a miracidium undergoes dramatic changes in morphology and metabolism as it transforms into a mother sporocyst, the next larval life stage. During and after penetration, the parasite sheds the cilia-bearing epithelial plates. A new body covering forms from cytoplasmic extensions of cells that lie below a superficial muscle layer. The extended, anuclear cell projections (termed 'intercellular ridges') fuse to form a syncytial tegument that has a continuous cytoplasm without intervening cell membranes, and that remains connected with the lower-lying nucleated cell bodies (Meuleman *et al.*, 1978). The syncytial tegument represents a living interface that facilitates intimate metabolic interactions between the parasite and the internal environment of the snail host. The aerobic metabolism present in miracidia changes to a more fermentative metabolism. This allows sporocysts to be facultative anaerobes that can adjust to the variable conditions (from aerobe to anaerobe) that may occur inside the snail host (Tielens *et al.*, 1991, 1992).

At 4 days post-infection (p.i.), mother sporocysts of *T. ocellata* were present in subepidermal blood vessels and sinusoids of *L. stagnalis*, close to the site of penetration (Sluiters, 1981). Once fully transformed, mother sporocysts migrated further into the head-foot tissues of the snail. Additionally, germinal cells inside the mother sporocysts started to develop into daughter sporocysts, the next larval stage. From day 7 to day 11, individual mother sporocysts contained many maturing daughter sporocysts. Most mother sporocysts remained in the head-foot, but some migrated to the ovotestis or digestive

gland of the host. From day 12 onwards, mature daughter sporocysts emerged from the mother sporocysts and resided in the head-foot and the regions of the ovotestis or digestive gland. Individual mother sporocysts degenerated after releasing many daughters, however, intact mother sporocysts containing developing daughter sporocysts were still present at 33 days p.i.

On day 19, cercarial development from germinal cells inside individual daughter sporocysts became obvious. Daughter sporocysts now also occurred in the kidney, but mechanical damage to the organs of the host was not observed. After about a week, mature cercaria emerged from daughter sporocysts, migrated through blood vessels to the pulmonary region and left the snail by penetration of the body wall. It is hypothesized that a set of specialized glands serves for the passing of cercariae through the snail tissues (for *T. szidati*, see Neuhaus, 1952a).

The intramolluscan development of *T. ocellata* is temperature dependent. If snails were maintained at 20 °C, the infections reached patency (shedding of cercaria) at about 7 weeks p.i. (Amen *et al.*, 1991; Amen and Meuleman, 1992). At 25 °C, the prepatent time interval decreased to 4–5 weeks (Sluiter *et al.*, 1980; Sluiter, 1981). Parasite development (daughter sporocysts and cercaria) and cercarial shedding was observed to continue up to 83 days p.i. (Sluiter, 1981); the shedding of cercaria from *L. stagnalis* continued for longer than 123 days p.i. with *T. ocellata*, after which the experiment was terminated (Sluiter *et al.*, 1980). A single miracidium infection yielded thousands of cercaria, due to the asexual multiplication associated with each transition between the intramolluscan life stages. Infection with multiple miracidia increased the output of cercariae even further (Sluiter *et al.*, 1980).

4.3. Effects on the Snail Host

Infection with an avian schistosome has severe consequences for a snail. Remarkably, *T. ocellata* causes little or no apparent physical injury to host snails; infected snails usually live equally as long as non-infected control snails (Sluiter *et al.*, 1980; Sluiter, 1981). However, avian schistosomes actively and dramatically modify the internal physiology of a snail to maximize the resources (such as nutrients and space) that they derive from the host (for reviews see de Jong-Brink, 1995; Thompson, 1997).

To optimally obtain nutrients, schistosomes induce changes in the metabolism and biochemistry of the host. Infected snails have heightened metabolism (increased heart rate and respiration) and display changes in internal levels of proteins, carbohydrates, nitrogen and lipids (e.g. Meyer *et al.*, 1986; Thompson, 1997).

Trichobilharzia ocellata-infected *L. stagnalis* have increased body and shell size (McClelland and Bourns 1969; Sluiter *et al.*, 1980). The giant growth or

'gigantism' results from an increase in the haemolymph volume; the dry weight of infected snails increases only slightly relative to control snails (Joosse and van Elke, 1986). The increase in growth coincides with the occurrence of mature daughter sporocysts. *Trichobilharzia ocellata* causes gigantism probably to ensure adequate space for larval development within the snail. This phenomenon is considered to result from parasite-mediated modulation of the neuroendocrinology of the snail but the underlying mechanisms remain unclear (de Jong-Brink, 1995). It should be noted that gigantism does not occur in all schistosoma–snail associations (Thompson, 1997).

Generally, schistosoma infection causes 'parasitic castration', the cessation of reproduction in a snail host. Thus, resources (nutrients and space) that otherwise are used for reproduction become available to the parasite. If *T. ocellata* infects juvenile snails, the reproductive organs of *L. stagnalis* do not mature and these snails never produce eggs (Sluiters, 1981). *Trichobilharzia ocellata* retards but does not prevent the development of the reproductive organs in older *L. stagnalis*. Initially, these snails even have an increased production of eggs, but egg-laying ceases when the infection becomes patent (Schallig *et al.*, 1991). Parasitic castration is effected in an indirect fashion. Developing cercariae of *T. ocellata* contain a factor that causes *L. stagnalis* to release 'schistosomin'. Schistosomin inhibits ovoposition through antagonizing several female gonadotropic hormones (Hordijk *et al.*, 1991; Schallig *et al.*, 1992; de Jong-Brink *et al.*, 1992, 1995).

Hoek *et al.* (1997) demonstrated that infection with *T. ocellata* caused changes in the mRNA expression profile of the host, *L. stagnalis*. Among the affected transcripts were several neuroendocrine factors. By affecting the transcription of these factors, *T. ocellata* may indirectly change the physiology of the host. The changes that occur in the host are generally considered to be beneficial to the parasites. The extent of changes in host metabolism is variable, correlating with consecutive transitions between the different stages of intramolluscan larvae. Apparently, schistosoma-mediated manipulations of the host physiology and metabolism ensure optimal usage of host resources for larval development while allowing the host to recover from periods of metabolic exhaustion (Thompson, 1997). It has been proposed to view an infected snail as an extended phenotype of the parasite (Dawkins, 1990). Obviously, the ability to shift the resources from host reproduction towards parasite development, thus reducing the fitness of the host to zero, makes an avian schistosoma a severe pathogen for snails.

4.4. Internal Defence Reactions of Snail Intermediate Hosts

4.4.1. General comments on snail (lymnaeid) immunobiology

Not all snail species are suitable hosts for schistosomes. For instance, digenean infection has never been observed from *Crepidula fornicata*, a marine

gastropod (Pechenik *et al.*, 2001). Field studies show that the incidence of schistosoma infection in snail populations is usually low (Loy and Haas, 2001). Potentially, local adaptation reduces the suitability of snails as hosts for sympatric strains of schistosomes (Morand *et al.*, 1996). However, snails can also be naturally resistant to schistosomes (e.g. Joubert *et al.*, 1990; Grassi *et al.*, 2001). The resistance has a genetic basis. Selective breeding can yield snail strains that do not support parasite development (e.g. Richards *et al.*, 1992; Langand and Morand, 1998).

Snails possess potent internal defences that are capable of eliminating invading pathogens, including schistosome larvae (for reviews see: van der Knaap and Loker, 1990; Adema and Loker, 1997; Lardans and Dissous, 1998). In combination, the genetic backgrounds of both the parasite and the host decide the fate of an invading parasite. A snail in which a parasite cannot develop due to lack of nutrients or inappropriate pH is an unsuitable host. Other snails may provide the correct internal environment for a particular schistosome, but mount an internal defence and eliminate the invader. This renders the snail immunobiologically incompatible or resistant. In a suitable snail host, the internal defences fail to eliminate an invading schistosome, resulting in an immunobiologically compatible parasite–host association.

Snails (and invertebrates in general) lack lymphocytic defences (no antibodies and T-cell receptors, no immunological memory) and employ internal defences of an innate type. Lectins mediate self-non-self differentiation by pattern recognition of repetitive carbohydrate moieties that characterize the surface of pathogens (Janeway, 1989). Snail lectins occur in soluble form, and associated with the cell membrane of defence cells. Recent molecular studies suggest that snails produce complex and highly diverse lectins (Kurachi *et al.*, 1998; Zhang *et al.*, 2001). The binding of a snail lectin to pathogen-associated carbohydrates is thought to activate a snail defence response, comprising humoral and cellular components (van der Knaap *et al.*, 1982, 1983; Richards and Renwrautz, 1991; Horák and van der Knaap, 1997; Horák and Deme, 1998; Horák *et al.*, 1998c).

The humoral defences of snails include several agglutinins and toxic activities (for review see Adema and Loker, 1997). Study of gene expression in *L. stagnalis* after infection with *T. ocellata* (Hoek *et al.*, 1996, 1997) identified a cDNA transcript that was named molluscan defence molecule (MDM), based on sequence similarities with the haemolin, a soluble immune protein with opsonic properties, present in insects (Schmidt *et al.*, 1993). Planorbid snails produced increased amounts of several parasite-reactive plasma polypeptides in response to echinostome parasites. Among these are lectins of a large and diverse gene family of fibrinogen-related proteins (FREPs). FREPs have been found in several planorbid species; no sequence data are available to suggest that lymnaeid snails also employ FREPs (Adema *et al.*, 1997; Zhang, 2001). The functional aspects of both MDM and FREPs remain to be clarified further.

Cell-free plasma of several lymnaeid and planorbid snails contained an activity that killed incompatible intramolluscan trematode larvae *in vitro* (Sapp and Loker, 2000).

Haemocytes, phagocytic cells that circulate in the blood, constitute the major cellular defences of snails. Activated haemocytes migrate towards and then phagocytose or encapsulate pathogens. The internalized pathogens are eliminated by cell-mediated cytotoxicity. *In vitro* killing of incompatible schistosoma larvae by haemocytes of *L. stagnalis* and other snails involves lysosomal enzymes and toxic chemicals such as reactive oxygen intermediates (Dikkeboom *et al.*, 1988; Adema *et al.*, 1993, 1994; Hahn *et al.*, 2001a) and nitric oxide (Hahn *et al.*, 2001b).

Several factors such as age of snails, pollution and the presence of other parasites reduce the functioning of the internal defences of snails (Dikkeboom *et al.*, 1985; Russo and Lagadic, 2000). Generally, however, snails have potent defences that successfully eliminate invading pathogens (Richards *et al.*, 1992; Grassi *et al.*, 2001). It is remarkable then that in some combinations, snails fail to eliminate an invader and become infected by schistosome parasites.

4.4.2. Parasite-induced changes in cellular and humoral immunity

Schistosomes employ both passive and active strategies to survive the internal defences of snails in order to establish a successful infection. However, circumventing the host defences is likely to be a highly complex process that is successful only in a limited range of snail strains or species. Upon entry into a snail, a schistosome will deploy its particular survival strategies to try and prevent a host defence response. Only the defence system of a suitable snail is rendered ineffective; the internal defences of resistant snails remain unaffected and eliminate the schistosome parasite. It is likely that this leads to the restricted immunobiological compatibility between schistosomes and snails.

A schistosome can passively display surface moieties that are not recognized as foreign to evade recognition as non-self. If an invading pathogen is not detected, the internal defence system of the snail remains inactive. Indeed, the composition of carbohydrate moieties on the surface of *T. ocellata* and *T. szidati* changes considerably when a miracidium transforms into a sporocyst (Gerhardus *et al.*, 1991; Horák, 1995), but the functional significance remains to be determined. Schistosomes may also mask their surface with host-like epitopes. This can be achieved by absorbing host-derived factors onto the surface (molecular masking). *Trichobilharzia ocellata* may employ this strategy: immediately after emerging from daughter sporocysts, cercaria were observed to acquire host-like factors onto their surface that bind antisera raised against plasma of the host, *L. stagnalis* (Roder *et al.*, 1977; van der Knaap *et al.*, 1985).

Schistosomes also display molecular mimicry, the expression of host-like determinants from genes of the parasite itself. The question has been raised whether similar gene products protect the parasite or whether sequence similarities between parasite and host have resulted from convergent evolution (Yoshino and Boswell, 1986; Damian, 1987; Dissous and Capron, 1995).

Schistosomes also actively inhibit host defences. After the initial observation that echinostome parasites rendered host snails susceptible to schistosomes for which they were normally resistant (Lie *et al.*, 1976), it was determined that schistosome parasites also reduce the defensive capabilities of snails (Lie and Heyneman, 1977). The interference hypothesis (Lie, 1982) proposes that schistosomes and other trematode parasites interfere with the function of snail haemocytes in order to survive within the host. *Trichobilharzia ocellata* mediates interference indirectly by releasing secreted and/or excreted products (SEP) that interact with the central nervous system (CNS) of the *L. stagnalis* to affect the internal defence system of the snail (Amen and de Jong-Brink, 1992). Concordant with these findings is the demonstration of altered gene expression in the CNS of *L. stagnalis* after infection with *T. ocellata* (Hoek *et al.*, 1997). Among the suppressed messages was that of the putative defence factor MDM (Hoek *et al.*, 1996). It is assumed that this reduces the efficiency of host defences, benefiting the parasite. Changes in the expression levels of neuroendocrine factors (Hoek *et al.*, 1997) are also thought to affect the host defences (de Jong-Brink, 1995).

The SEP released by schistosomes mediate direct interference effects towards snail haemocytes. Co-incubation of snail haemocytes with SEP collected from larval schistosomes or with sporocysts inhibits several functional properties of these defence cells such as intracellular protein synthesis (Lodes *et al.*, 1991), haemocyte motility (Lodes and Yoshino, 1990) and production of oxygen radicals (Connors *et al.*, 1991). *Trichobilharzia ocellata* inhibits phagocytosis (Amen *et al.*, 1992), efficiency of encapsulation (Adema *et al.*, 1994) and bacterial killing (Nuñez *et al.*, 1994) by haemocytes from *L. stagnalis*. In the latter case, the killing activity of *L. stagnalis* haemocytes was inhibited by a 40-kDa protein component of SEP from *T. ocellata*. Intriguingly, the 40-kDa factor did not affect the bactericidal activity from haemocytes of a non-host snail *Planorbarius corneus* (Nuñez and de Jong-Brink, 1997; Nuñez *et al.*, 1997). This finding seems to underline the fact that the capabilities for immunomodulation of schistosomes are restricted to affect only host snails. At this time, sequence information is not available for any candidate interference factors.

Clearly, much remains to be learned about the close and specific association between schistosomes and snails to understand fully how the larval development in the intermediate snail host culminates in the release of cercariae.

5. DEVELOPMENT IN VERTEBRATE HOSTS

5.1. Host-finding

The mature cercariae are released from snails in high quantities, especially on sunny days preceded by an overcast period. Cercariae are thought to use secretions of specialized glands to pass through the snail tissues (*T. szidati*, see Neuhaus, 1952a). Free-living cercariae need to infect a final host quickly as the parasites have only a short life span after emerging from the snail into the water (1–1.5 day at 24 °C; Neuhaus, 1952a). Characterization of the host-finding behaviour of *T. ocellata* (Haas 1992, 2001 for reviews) revealed that the cercariae exhibit a positive phototactic and a geonegative orientation and can swim both backwards and forwards (Figure 8). A forward swimming response towards shadows, water turbulence and touch affords a high probability of contact with a final host (Feiler and Haas, 1988a).

Prior to an initial contact, cercariae are selectively attracted to, and attach to, substrates that display physical and chemical properties of warm-blooded vertebrate skin. Not all warm surfaces are equally attractive; cercariae of *T. ocellata* can differentiate their preferred temperature with a sensitivity of only 1 °C (Feiler and Haas, 1988a). Cercariae also react to chemical signals. *Trichobilharzia* sp. cercariae released from *Physa acuta* were attracted by linoleic acid (Graczyk and Shiff, 2000), a fatty acid that also attracts *S. mansoni* (Shiff *et al.*, 1993; Shiff and Graczyk, 1994). Skin surface lipids such as ceramides and cholesterol are important stimuli for attachment of *T. ocellata* (Feiler and Haas, 1988b). Feathers covered by the uropygial gland secretions lack these particular lipids allowing cercariae to preferentially attach to the skin of birds. Due to a similar lipid composition of the skin of birds and mammals, the cercariae are not able to avoid penetration into mammals and cause cercarial dermatitis (see section 6.2.1.).

5.2. Penetration of the Vertebrate Skin

After contact with the host the cercariae of *T. ocellata* show several behavioural patterns: attachment to the host, enduring contact, leech-like creeping to a suitable entry site (wrinkles in the skin or openings of hair follicles) and penetration (Figure 8). On average, the creeping behaviour lasts 8 s, ranging from 0 to 80 s (Haas and Haberl, 1997; Haas and van de Roemer, 1998). Not all cercariae initiate penetration efforts following attachment. Appleton and Brock (1986) estimated that 85.2% of *Trichobilharzia* sp. cercariae (released from *Lymnaea natalensis*) attempted to penetrate mouse skin. After 1 h, 59% of the cercariae had proceeded to enter the skin, whereas the remaining 41% were

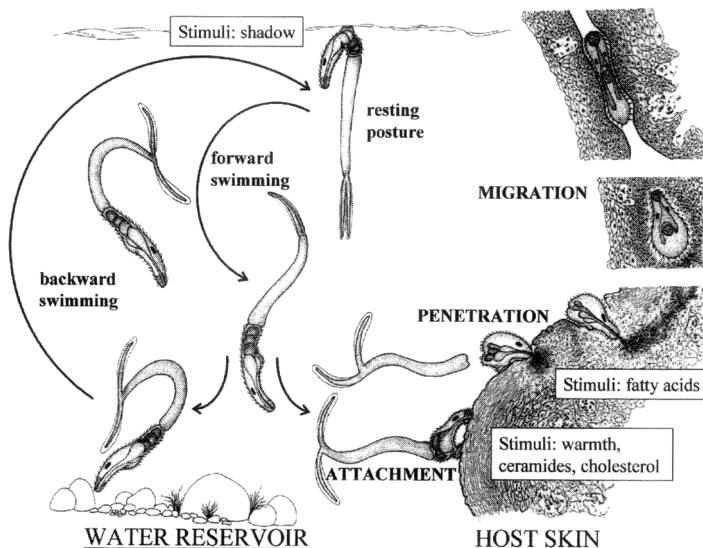


Figure 8 Behaviour of *Trichobilharzia* before and after the penetration into a vertebrate host (behaviour of free-living cercariae modified after Haas, 1992).

The free-living cercarial stage of *T. ocellata* shows phototactic and geonegative orientation. Additionally, it displays four types of behaviour: (1) Resting posture is a motionless position in which parasites cling to the water surface with the acetabulum, the tail hangs downward and the furcae are relaxed; cercariae do not respond to water currents or touch. (2) Forward swimming (body first). This behaviour is stimulated mainly by passing shadows. The direction of swimming is directed away from the light source; the furcae of the tail are folded together; only in this phase do cercariae respond to thermal and chemical vertebrate cues that induce attachment. (3) Backward swimming (tail first) towards light (upward) is stimulated by a contact with inappropriate substrates. (4) During the passive phase cercariae sink down using the furcae as a drag anchor. The active phase (2 or 3) alternates with the passive phase (1 or 4).

After contact with the host, *T. ocellata* shows four patterns of behaviour: (1) attachment to the host (stimulated by warmth and surface lipids – ceramides and cholesterol); (2) enduring contact; (3) creeping to a suitable entry site; and (4) penetration (stimulated by fatty acids). At the start and during penetration, the contents of the penetration glands are released. The penetration process is accompanied by penetration movements (contractions and elongations of the body, mechanically supported by the spines that cover the body), shedding of the tail and transformation of the tegument. The gland secretions surround the schistosomulum and line the entry tunnel. After the initial phase of skin infection, visceral *Trichobilharzia* species usually enter the lymphatic or venous system whereas nasal *Trichobilharzia* migrate via peripheral nerves.

still attached by the suckers to the epidermis. Comparable observations were made from *T. regenti* (Hrádková and Horák, 2002). Perhaps cercariae do not always encounter appropriate signals to initiate penetration of the host. However, cercariae can also establish (experimental) infection perorally, penetrating mucosa of the oral cavity (Macy *et al.*, 1955). This suggests that cercariae are not highly restricted in recognizing and penetrating the different epithelia of a vertebrate.

Whereas warmth alone has no effect, penetration is stimulated by free fatty acids (saturated acids with 9–14 C atoms and unsaturated acids; the penetration-stimulating effect of the latter acids increases with the number of double bonds; Feiler and Haas, 1988a; Haas and van de Roemer, 1998). The high levels of such fatty acids in human skin stimulate higher rates of cercarial penetration in humans than in the natural duck host. The nature of stimulatory host signals may be species- or genus-specific; another bird schistosome, *Austrobilharzia terrigalensis*, initiates penetration in response to free sterols, whereas fatty acids have only a weak effect (Clegg, 1969).

The cercariae start the penetration process by attaching the head organ to the skin surface, in a similar fashion as described for human schistosomes (*S. mansoni*). Like *S. mansoni* (Howells *et al.*, 1975), *T. ocellata* sheds the tail at this time by the contraction of a sphincter muscle at the cercarial hindbody (Haas and van de Roemer, 1998). Sometimes the tail is shed earlier during the creeping phase. The contents of the penetration glands are released onto the skin. These contain proteolytic enzymes, including elastase, a 30-kDa serine proteinase (*T. ocellata*; see Bahgat *et al.*, 2001; Bahgat and Ruppel, 2002). This can be observed histologically by a distinct lysis of the stratum corneum (Bourns *et al.*, 1973; Haas and van de Roemer, 1998). Moreover, detection with fluorescein-labelled lectins showed that gland deposits surround the penetrating parasite (*T. szidati*) and line the resulting entry tunnel in the host tissue (Horák *et al.*, 1998b). The gland secretions contain additional types of bioactive molecules. *Trichobilharzia szidati* releases a lectin specific for β -1,3-glucan and glycosaminoglycans that may be involved in recognition of host connective tissue and activation of parasite effector molecules (e.g. proteases). Other than carbohydrate-binding properties, no additional activities have been detected for this lectin (Horák *et al.*, 1997). The lytic attack on the skin and underlying tissues, combined with intermittent contractions of the body which is covered with tegumental spines, allows cercariae to disrupt and penetrate the skin of the host. Usually, cercariae of *T. ocellata* penetrate in a nearly surface-parallel direction to the stratum corneum before entering deeper tissue layers. On average, the parasites pass through the stratum corneum within 4–5 min and the stratum germinativum is penetrated at 3 h p.i. (Bourns *et al.*, 1973; Haas and van de Roemer, 1998).

5.3. Cercaria–Schistosomulum Transformation and Immune Evasion

With the transition from free-living to parasitic life inside the final host, the parasite undergoes a second transformation, from cercaria to schistosomulum. This transformation is accompanied by a substantial reconstruction of the surface. The thick cercarial glycocalyx is shed, which considerably changes the antigenic properties of the parasite. This is demonstrated by low recognition of the parasite's surface by lectin probes and anti-cercarial antibodies. This state remains for several hours or even days and may represent a way to escape recognition by the host immune system (Horák *et al.*, 1998b). The outer tegumental membrane is replaced by a double membrane (Horák *et al.*, 1998b) that is typical for all blood-dwelling adult trematodes (spirorchids, sanguinicolids, schistosomes; McLaren and Hockley, 1977). The outer membrane of human schistosomes is thought to also have immune-evasive properties.

Additional factors may protect the parasite from immune attack. The cercariae of *T. ocellata* possess enzymes that convert C₂₀ polyunsaturated fatty acids (most notably arachidonic acid) into eicosanoids (lipid mediators) such as prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids. Interestingly, eicosanoids are produced at levels similar to those of human schistosomes (Nevhutalu *et al.*, 1993). These bioactive compounds may cause vasodilatation in host tissues, and suppress immune functions such as superoxide production by human neutrophils (Nevhutalu *et al.*, 1993) and the migration of epidermal Langerhans cells, which play a key role in the immune defence system (Angeli *et al.*, 2001).

5.4. Migration

Shortly after completion of the transformation, schistosomula of the visceral species of *Trichobilharzia* leave the skin and enter the blood vessels of the host to mature and spend their adult life in the circulatory system (see section 5.4.5 for information on nasal species). Adult parasites usually reside in portal or intestinal veins of naturally infected hosts, sites that are typical also for species of the genus *Schistosoma* (for review see Basch, 1991). Based on this similarity, it is expected that *Trichobilharzia* follows the same migration route as *Schistosoma* (for review see Bayssade-Dufour *et al.*, 1994), entering lymphatic or venous vessels and continuing via the right heart to the lungs, the left heart and the systemic circulation to the final location. The receptors and mechanisms that guide the path of migration through the host are not understood (Basch, 1991). Interestingly, the parasites migrate along similar routes in both (immunologically and physiologically) compatible final (bird) hosts and incompatible hosts. However, parasites do not start reproduction in incompatible hosts and die at various intervals p.i.

5.4.1. Visceral schistosomes in the skin phase

(a) *Compatible hosts.* A comparison of the number of cercariae that penetrated the skin and the number of worms recovered from internal organs indicates that many parasites fail to cross the skin. Ellis *et al.* (1975) suggested that schistosomula that are unable to break the cornified layers are permanently trapped in the skin. The passage of schistosomes through the skin seems to depend mainly on the immunity status of the host (see section 6.1.4.). Relative to primary infections, the number of *T. ocellata* schistosomula trapped in the skin of ducks increased with repeated challenge infections (Bourns *et al.*, 1973; Ellis *et al.*, 1975; Rau *et al.*, 1975). Successful schistosomula of *T. ocellata* leave the duck skin within 3 days p.i. (Bourns *et al.*, 1973). *Trichobilharzia* migrates rapidly after entering the circulation, especially in comparison with *S. mansoni* (Haas and Pietsch, 1991). By days 3 and 4 p.i., 36% of the parasites originally present in the skin were detected in various internal organs (Bourns *et al.*, 1973; Haas and Pietsch, 1991).

(b) *Incompatible hosts.* Avian schistosomes, including *Trichobilharzia* spp., may or may not be able to cross the skin of an inappropriate vertebrate host. For instance, *Cercaria parocellata* did not penetrate beyond the epidermis of humans (Macfarlane, 1952). As observed from the bird schistosome *A. terri-galensis*, failure to penetrate results in increased mortality of parasites in the skin (Rai and Clegg, 1968). In contrast, some schistosomula of *T. ocellata* leave the mouse skin soon after penetration, although others can still be extracted from the skin by day 4 p.i. (Haas and Pietsch, 1991). As in the bird hosts, survival of the parasites depends heavily on the immune status of the host (see section 6.2.1.). In previously unsensitized rabbits *T. ocellata* and *T. stagnicola* survived up to 48 h p.i. However, many of the worms had died in the skin of sensitized rabbits by 6 h p.i. (Olivier and Weinstein, 1953).

5.4.2. Visceral schistosomes in the lung phase

(a) *Compatible host.* On the way towards the systemic circulation, the lungs represent the first target organ that is invaded by migrating schistosomula, as reported for several species: *T. cameroni*, *T. oregonensis*, *T. parocellata* and *T. ocellata* (McMullen and Beaver, 1945; Wu, 1953; Macy *et al.*, 1955; Islam and Copeman, 1986). Within 1–2 days after penetrating the skin *T. ocellata* invades the lungs and remains there between 4 and 16 days p.i. During this time the parasites grow and feed on the host blood (Bourns *et al.*, 1973; Ellis *et al.*, 1975; Haas and Pietsch, 1991; Horák and Kolářová, 2000). Once in the lungs the parasites leave the venous system and probably remain extravascularly in the air spaces for a certain amount of time. They migrate from air capillaries and parabronchi to secondary bronchi, then invade the bronchial epithelium

and gain entrance into vessels (Bourns *et al.*, 1973). The lungs represent a difficult barrier and only some schistosomula are able to pass through. Even during primoinfections of compatible hosts, schistosomula are frequently trapped during the lung passage. In contrast to the lungs, a lower recovery rate for *T. ocellata* worms was reported for duck livers and intestine (e.g. Haas and Pietsch, 1991).

(b) *Incompatible host.* In several cases, avian schistosomes invade the lungs of incompatible hosts. This was first described for schistosomula of *T. stagnicolae* in Swiss mice, *T. ocellata* in Swiss mice, hamsters and monkeys, and *T. physellae* in mice at 4–7 day intervals p.i. (Olivier, 1953). A pulmonary phase was also recorded from mice infected with *T. brevis* (Basch, 1966), *Trichobilharzia* sp. (Appleton and Brock, 1986) and *T. szidati* (Horák and Kolářová, 2000). Similar observations were reported for other schistosome genera, i.e. *Austrobilharzia*, *Bilharziella* and *Ornithobilharzia* (Bacha *et al.*, 1982; Horák and Kolářová, 2000; Bayssade-Dufour *et al.*, 2001). This suggests that lung migrations are common to all visceral species of schistosomes.

The migrating schistosomula can reach the lungs within 10 h p.i. (*T. ocellata* in mouse; Haas and Pietsch, 1991). Usually, the survival time in an incompatible host is short. However, *T. szidati* worms may persist in the mouse lungs for up to 10 days p.i. (Horák and Kolářová, 2000). Haas and Pietsch (1991) reported that, despite a significant decrease in parasite number within the first 5 days, schistosomula of *T. ocellata* survived in the lungs of mice up to 6 days p.i.

Subsequently, worms disappear from the incompatible hosts. The factors influencing survival of lung schistosomula are poorly understood. Schistosomula of *T. szidati* seem metabolically active in the lungs of incompatible hosts; their gut contains a dark pigment evidently derived from digestion of erythrocytes of the host (Horák and Kolářová, 2000). Perhaps the parasites are eliminated by a host immune response. However, electron microscopy of *T. szidati* in the lungs of mice showed no damage to the tegument and an absence of host immune cells (Horák and Kolářová, 2000). The authors hypothesized that failure of the parasite during a primary infection of an incompatible host may result from some immunologically unrelated factors (absence of essential nutrients, etc.). A comparison of the pulmonary phase of infection showed that *T. szidati* schistosomula grow and feed rapidly in a duck, whereas in a mouse they seem to be inhibited in development (Horák and Kolářová, 2000). As suggested by Kolářová (2001), survival of parasites in the lungs can also be influenced by their size; schistosomula of certain species can be too large to enter lung capillaries and to migrate in a non-specific host.

5.4.3. Visceral schistosomes in the liver phase, final location and egg production

(a) *Compatible host.* After re-entering the systemic circulation, the parasites migrate to a final location. In most cases, adult schistosomes concentrate in the portal or intestinal veins of the venous system. This suggests that the schistosomula of visceral avian schistosomes develop into adults in the portal veins, similar to *Schistosoma* spp. The few available data indicate that the prepatent period for *Trichobilharzia* infections is about 12–14 days (Neuhaus, 1952a; Basch, 1966; Bourns *et al.*, 1973; Meuleman *et al.*, 1984b). The stimuli that guide the migration of adult worms and the exact location for egg-laying remain to be fully characterized. However, adult worms of different species appear to favour particular sites for oviposition. Depending on the species of *Trichobilharzia*, parasite eggs have been detected in the intestinal wall of various parts of the small and large intestines, including the cloaca of birds (McMullen and Beaver, 1945; Wu, 1953; Yamaguti, 1971). The migration of adults to sites where egg-laying occurs may be similar to that proposed for *A. variglandis* (Wood and Bacha, 1983). The female worms travel from vessels of the intestinal serosa through the muscularis externa and crawl by means of body contractions into small veins of the mucosa. After the release of eggs, the worms return to the serosa. This allows the small mucosal veins to contract around the newly deposited eggs, keeping them in that place.

The eggs pass from the mucosal vessels through the connective tissue of the lamina propria into the intestinal lumen. As suggested for human schistosomes, the progression of eggs may be due to a combination of host inflammatory reactions and egg-derived proteases, resulting in lysis of the tissues. The finding of frequent accumulations of leucocytes around eggs located within blood vessels supports this view (Wood and Bacha, 1983). Muscular contractions of the host may further help the eggs to pass through the connective tissue into the intestinal lumen (Bloch, 1980). Finally, the eggs are released from the intestines with the faeces when the bird defecates.

It is not clear whether adult avian schistosomes spend their entire life in the portal and/or intestinal veins. On at least two occasions, adult worms have been encountered in the intestinal tissues. Adult *T. ocellata* migrate to veins of the small intestine and subsequently penetrate the mucosa, sometimes even approaching the tops of villi (Bourns *et al.*, 1973). Similarly, Neuhaus (1952a) recorded mature adults of *T. szidati* in muscularis submucosa and the mucosa of the intestinal wall. Both *T. ocellata* and *T. szidati* disappeared from the host intestines about day 21 p.i. This time also coincides with the end of the patency of infection; the bird host ceases to release parasite eggs in the faeces (Bourns *et al.*, 1973; P. Horák, unpublished data). Because all schistosomes normally occur in the blood system, their location in the intestinal tissues may represent a terminal phase of the infection. Hypothetically, the flukes escape from the

blood vessels during this phase and are destroyed in the tissues or enter the intestinal lumen, die and leave the host. The death of the adult worms could explain the observed brevity of *Trichobilharzia* infections.

The fate of the majority of adult worms in the 'postpatent' period remains to be determined, although it is generally assumed that adult worms die soon after depositing their eggs. However, a limited number of worms may leave the intestinal area to return to the liver. Mature *T. parocellata* were found in portal veins of the liver in a duck at 86 days p.i. (Islam and Copeman, 1986), and *T. ocellata* was observed in the latter organ even at 248 and 370 days p.i. (Bourns *et al.*, 1973). Interestingly, dead and degenerating females of *T. physellae* were found within the interlobular bile ducts (Pence and Rhodes, 1982). Remarkably, adult parasites survive for some time in dead hosts; Szidat (1938) recovered living worms from decomposing birds that had died several days earlier.

The factors that determine the life span of these parasites remain unclear. Nevertheless, the patency of *Trichobilharzia* infections and potentially also the life span of adult avian schistosomes are considerably shorter than those of human schistosomes (for review see Basch, 1991).

(b) *Incompatible host.* Immature worms are rarely found in the liver of non-specific hosts. However, radiolabelled *T. ocellata* were sporadically detected in the mouse liver and intestine on days 2–5 p.i. (Haas and Pietsch, 1991). The longevity of these parasites in the liver remains unknown, but appears to be shorter than that in the lungs.

5.4.4. Egg dissemination and atypical migration of visceral schistosomes

As occurs in human schistosomiasis (for review see Bacha *et al.*, 1982), part of the eggs of *Trichobilharzia* species fails to leave the bird host and is disseminated via the circulatory system to the liver, lungs and other organs (McMullen and Beaver, 1945; Wu, 1953). The number of eggs in various organs, and associated pathology (see section 6), increase with higher worm burdens. In heavy infections, adult flukes may reside in atypical locations within the host. The infection by 300–400 specimens of *T. physellae* resulted in equal distribution of worms over the lungs, liver and intestine of a canary (McMullen and Beaver, 1945). It is likely that additional observations of adult parasites in atypical sites, e.g. *T. ocellata* in the lungs (Bourns *et al.*, 1973) and *Trichobilharzia* sp. in gonadal, gastric, oesophageal, jugular, femoral and thyroidal veins (Wojcinski *et al.*, 1987) and in interlobular bile ducts (Pence and Rhodes, 1982), were due to high infection doses also.

5.4.5. *Nasal schistosomes*

Some schistosome species mature in the nasal area of a final host. Of only nine species that were described to date, *S. nasale* belongs to the genus *Schistosoma*. The others are representatives of the genus *Trichobilharzia* (Horák *et al.*, 1998a). Except for *T. regenti*, there are no details available regarding the migration routes to the nasal tissues.

(a) *Compatible host*. The migration route of a nasal schistosome such as *T. regenti* differs dramatically from those of visceral schistosomes (Horák *et al.*, 1999; Hrádková and Horák, 2002). After penetrating the skin of a duck, *T. regenti* invades peripheral nerves. The schistosomula then migrate further through the central nervous system (CNS), progressing through the spinal cord and the brain. Ultimately, the schistosomula locate to blood vessels in the nasal area where they complete their maturation to adult worms, mate and produce eggs. The progression through the host CNS is fast: schistosomula can be detected in peripheral nerves by day 1.5 p.i., in the spinal cord by day 2 p.i. and in the brain by day 12 p.i. (Hrádková and Horák, 2002). The nasal area is reached by day 13 p.i. (Horák *et al.*, 1999). Eggs are produced by females as early as 14 days p.i. (Horák *et al.*, 1999). Contrary to visceral schistosomes, miracidia hatch from the eggs directly in the nasal soft tissues (Horák *et al.*, 1998a). The life span of *T. regenti* in a duck is estimated to be 23–25 days. The end of the patent period appears to be characterized by migration of adults from vessels into the surrounding extravasal tissues of the nasal area (Horák *et al.*, 1999; Kolářová *et al.*, 2001).

(b) *Incompatible host*. Infection of *T. regenti* in mice is also accompanied by invasion by schistosomula of the CNS. The parasites enter the peripheral nerves, migrate to the spinal cord (by day 2 p.i.), and reach the brain by day 3 p.i. (Hrádková and Horák, 2002). Contrary to the establishment and reproduction of worms in ducks, the infection of mice fails and results in the death of parasites in various parts of the CNS (Horák and Kolářová, 2001). The parasites triggered a host immune response (see section 6.2.2.). This can partly explain the incomplete development of the worms (Kolářová *et al.*, 2001).

A comparative study of the migration of *T. regenti* in suitable versus unsuitable (including immunodeficient and immunocompetent) vertebrate hosts disclosed differences in the rate of migration during the first 3 days p.i. (Hrádková and Horák, 2002). During this time, worms were recovered from the synsacral and thoracic spinal cord of ducks. In several strains of mice tested, the *T. regenti* had progressed further, having reached the cervical spinal cord and medulla oblongata, and in SCID mice, even the cerebellum. Differences in body size of the host may also affect the rate of migration, as suggested for visceral schistosomes (McMullen and Beaver, 1945). The migration rate of worms was faster in the CNS of SCID mice than in that of immunocompetent (BALB/c and hr/hr) mice (Hrádková and Horák, 2002).

Interestingly, the highest numbers of worms were found in the synsacral spinal cord of ducks and the thoracic spinal cord of mice. Many parasites remained in these locations (also during the later phases of infection) and only a small percentage of worms migrated further.

On rare occasions, juveniles of *T. regenti* and *T. arcuata* were observed in the lungs of both duck and murine hosts (Islam, 1986b; Horák *et al.*, 1999; Hrádková and Horák, 2002). If these parasites retain the ability to reach their final location, it can be hypothesized that these parasites use the arterial system of the host to migrate from the lungs to the nasal area of a duck.

6. VERTEBRATE IMMUNE RESPONSE AND PATHOLOGY

Schistosomes are considered to be highly pathogenic for migratory waterfowl (Graczyk *et al.*, 1993). The general paucity of reports dealing with clinical aspects, pathology and immunity of trichobilharziasis can be explained in part by the ease with which the parasites can be overlooked during gross necropsy due to their small size and occurrence within blood vessels (Wojcinski *et al.*, 1987). The available data focus mostly on symptoms of patent infections in birds. However, recent investigations have elucidated that immature flukes also may cause severe tissue injuries. Therefore, the spectrum of syndromes is probably wider than previously considered.

Cercarial dermatitis in mammals is but one familiar aspect of the pathology that bird schistosomes cause in incompatible hosts. Despite the failure to develop fully, the parasites penetrate and migrate in mammals during early phases of infection in a similar fashion to that observed in avian hosts. These novel findings warrant that the role of these parasites in other pathological changes of tissues and organs should be reassessed (Horák and Kolářová, 2001).

6.1. Compatible Hosts

6.1.1. Factors influencing severity of infections

Particular schistosome infections in waterfowl differ in severity. In general, pathogenicity of each species is determined by parasite biology (e.g. metabolism, feeding, mobility) and morphology (e.g. size), preferred site of the adult worms, the worm load and the duration of infection. Severe damage to tissue and organs results from accumulation of numerous parasites in vessels and dissemination of many eggs of the parasite to various organs of the host (Wojcinski *et al.*, 1987). Whereas mild infections with visceral schistosomes often are subclinical or even asymptomatic (Macy *et al.*, 1955; Basch, 1966),

even a small number of nasal schistosomes in close proximity to the brain may cause severe syndromes. The severity of injuries may progress with time as the parasites persist in the host.

Also immature schistosomes may severely impair the host. In some instances, invasion of the lungs by high numbers of visceral schistosomes induces sufficient lung damage to cause death (McMullen and Beaver, 1945; Wu, 1953). During the prepatent period of infection, migrating schistosomula of *T. regenti* can mechanically damage and destroy nervous tissue. Potentially, the parasite also releases metabolites and other secretory or excretory products that may be neurotoxic. The number of parasites additively increases the pathology (Kolářová *et al.*, 2001).

The resulting clinical picture is influenced by innate susceptibility and suitability as well as by acquired immunity of the hosts. In the field, the prevalence of *Trichobilharzia* infections differs among various avian species (e.g. Appleton, 1986; Loken *et al.*, 1995) and usually the prevalence in adults is lower than in young birds (Guth *et al.*, 1979). The study of sera from birds that were repeatedly infected showed that acquired immunity increases with age and previous exposure to *Trichobilharzia* parasites. *Trichobilharzia*-released antigens evoke immune reactions around the parasites. Dead worms induce the strongest responses, probably due to the release of large amounts of antigens (Kolářová *et al.*, 2001). Specifically, *T. regenti*-infected birds produce antibodies against gut-associated antigens (GAA) of adult worms (Kouřilová and Kolářová, 2002). It is of interest that repeated infections by cercariae result in a reduction of severity of *T. ocellata* infection in birds (Ellis *et al.*, 1975; Rau *et al.*, 1975). Nevertheless, transfer of lymphoid cells and/or immune serum did not convey significant levels of passive immunity to *T. ocellata* in ducks (Bourns and Ellis, 1975).

6.1.2. Clinical features

The majority of syndromes that are usually associated with trichobilharziasis occur during the patent phase of infection, due to injuries to tissues and organs that develop in close proximity to the sites where egg-laying worms of *Trichobilharzia* reside. However, cercarial invasion into the skin and the migration of immature parasites also cause various disorders. For instance, pulmonary symptoms may appear shortly after the penetration by visceral schistosomes. Infections of ducks by *T. regenti* showed that the prepatent phase can be manifested by neuromotor symptoms (balance or orientation disorders and pareses) due to the migration of this nasal schistosome through the CNS of the host (Horák *et al.*, 1999).

Whereas birds appear to tolerate mild infections with visceral schistosomes relatively well (Macy *et al.*, 1955; Basch, 1966), heavy infections may cause

swelling of feet and legs, emaciation, weight loss and stunted growth (Macy *et al.*, 1955; Wojcinski *et al.*, 1987). Moreover, death was ascribed to *Trichobilharzia* infections: in birds infected experimentally with *T. ocellata*, *T. oregonensis* and *T. elvae* (Brumpt, 1931; Macy *et al.*, 1955), and in wildfowl harbouring *T. physellae* (Pence and Rhodes, 1982), *Trichobilharzia* sp. (Graczyk *et al.*, 1993) and *Trichobilharzia* sp. + *Dendritobilharzia* sp. (Wojcinski *et al.*, 1987). The patent period of *T. regenti* infections can be associated with bleeding of the nasal mucosa (Horák *et al.*, 1999).

The dissemination of eggs to various organs and tissues may cause non-typical symptoms during the chronic phase. These include proliferative conjunctivitis in Hawaiian goose due to *Trichobilharzia* sp. eggs (Schmäschke *et al.*, 1992) and neurological problems displayed by birds that have eggs of *Trichobilharzia* sp. in the brain (Graczyk *et al.*, 1993).

6.1.3. Gross pathology

Trichobilharzia infections are initially characterized by skin symptoms. Penetration of *T. szidati* and *T. regenti* cercariae in skin of duck frequently leads to rapidly developing macular eruptions (Horák *et al.*, unpublished data), followed by crust formation and scaling of the affected tissues at 2–3 days p.i. (Ellis *et al.*, 1975). Subsequently, migrating schistosomula may cause haemorrhage, inflammation and subsequent consolidation of the tissue in the lungs (McMullen and Beaver, 1945) and, in cases of heavy infections, enlargement and abscessation of the liver (McLeod and Little, 1942).

Patent infections with low numbers of parasites may result in two different types of lesions (Pence and Rhodes, 1982). (1) Minor lesions associated with the eggs occur mostly in the liver and intestines. The surface of livers of ducks infected with *T. brevis* (Basch, 1966) and *T. oregonensis* (Macy *et al.*, 1955) displayed white cysts 150 and 175 µm in diameter, respectively. Potentially, severe infections cause livers to enlarge and have a pale aspect, and cross-sections disclose a friability of the liver tissue (Pence and Rhodes, 1982). The intestinal mucosa contains granulomas (evident as reddish and soot-like spots), although weak infections are limited to some sloughing of the internal intestinal wall (McLeod and Little, 1942). The egg dissemination by venous circulation may also result in lesions in other organs. In Hawaiian geese, whitish proliferative alterations occurred in the sclera, nictitating membrane and conjunctiva, accompanied occasionally by microabscesses (Schmäschke *et al.*, 1992). (2) The accumulation of numerous adults in mesenteric veins leads to major lesions. These encompass thrombosis, thickening and congestion of the intestine with a granular fibrino-haemorrhagic exudate adherent to the mucosal surface (due to host immune reactions), pulmonary congestion and hepatomegaly (Wojcinski *et al.*, 1987).

On occasion, the presence of egg-laying nasal schistosomes causes haemorrhages and petechiae in the nasal cavity of birds (Fain, 1956a; Horák *et al.*, 1999); an undetermined *Trichobilharzia* species caused rhinitis in a swan (Palmer and Ossent, 1984).

6.1.4. Histopathology and immunology

The main pathological alterations caused by bird schistosomes in a host are probably due to eggs rather than to adult parasites (Szidat and Wigand, 1934; Szidat, 1938). Nevertheless, in some cases, tissue lesions can be attributed also to immature and mature schistosomes (Pence and Rhodes, 1982).

(a) *Skin lesions.* Soon after invasion (5 min p.i.), the parasites cause a distinct lysis of the *stratum corneum* in the duck skin (Bourns *et al.*, 1973; Haas and van de Roemer, 1998). Later on, the parasites are surrounded by an inflammatory reaction, the intensity of which depends on the timing of infection and immune status of the afflicted host. In chickens and pigeons initially infected by *Ornithobilharzia canaliculata*, the inflammation around the parasites was observed as early as 12 h p.i. Later, cell infiltration (histiocytes and heterophils) became stronger, accompanied by perivascular infiltration in the dermis (Morales *et al.*, 1971). However, the reaction in the skin of initially infected animals is probably not strong enough to prevent the subsequent migration of schistosomula. The data of Ellis *et al.* (1975) on worm load reduction in ducks challenged by *T. ocellata* suggest that repeated infections result in stimulation of host immunity to the extent that it increases the trapping of parasites in the host skin. A similar effect was described for *Trichobilharzia* infections of an incompatible host (Figure 9a; Kouřilová and Kolářová, 2001) or for *S. mansoni* in a compatible host (von Lichtenberg and Ritchie, 1961).

(b) *Lung lesions.* Lung lesions are caused by migrating schistosomula (usually visceral schistosomes); the parasites occupy air spaces (Bourns *et al.*, 1973; Ellis *et al.*, 1975) and feed on red blood cells. During early initial infections, the parasites evoke only weak inflammations. Challenge infections of birds led to stronger cellular infiltration (predominantly lymphoid cells) around *T. ocellata*. The observation of dead and dying worms in the centre of these inflammations in the lungs confirmed the effectiveness of the immune response in immune hosts (Ellis *et al.*, 1975). During the chronic phase, the eggs of *Trichobilharzia* incited minor lesions, varying from none to marked granulomatous reactions (Wojcinski *et al.*, 1987). In heavy infections, adults of *O. canaliculata* invaded the lungs of pigeons. The vascular system displayed medial hyperplasia, lymphotic endarteritis and segmental proliferation of vascular endothelium. The tissue of the lungs contained granulomatous reactions (consisting mostly of macrophages, histiocytes, heterophils and lymphocytes) that surrounded remnants of dead parasites (Morales *et al.*, 1971).

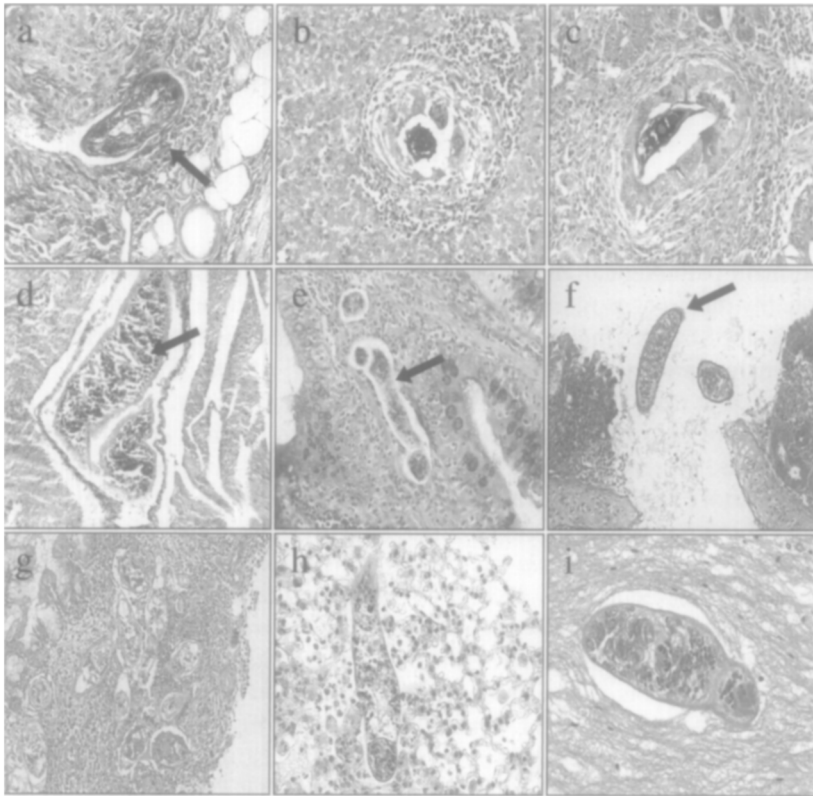


Figure 9 Histopathological findings in compatible and incompatible hosts infected either by nasal (*Trichobilharzia regenti*) or visceral (*T. szidati*) bird schistosomes. (a) *T. regenti* schistosomula in the skin of a mouse, reinfected for the fifth time; 4 h p.i. (PAS). The schistosomula approaches the epidermis (stratum reticulare). An inflammatory reaction, consisting of eosinophils, neutrophils and macrophages (arrow) surrounds the parasite. (b) An egg of *T. szidati* in the liver of a duck, 21 days after primary infection (PAS). Usually, eggs are surrounded by inflammatory cells, an inner layer of giant cells and histiocytes, lymphocytes, plasma cells and macrophages towards the periphery. (c) An egg of *T. szidati* in the muscularis mucosae of the intestine of a duck (PAS). The cellular reaction is similar to that in (b). (d) *Trichobilharzia* sp. mature flukes (arrow), located intravascularly in the intestinal vessels of a naturally infected whooper swan (HE). (e) *T. szidati* in the duck intestinal wall – primary infection of lamina propria; 21 days p.i. (PAS). In certain cases the worms (arrow) reside in an extravascular location. (f) Adults of *T. regenti* in the duck nasal mucosa, 16 days after primary infection (HE). Worms are located intra- or extravascularly (arrow). (g) The eggs of *T. regenti* in the duck nasal mucosa, 16 days after primary infection (HE). Several miracidia occur free in the mucosa. Some miracidia are surrounded by a dense accumulation of eosinophils, heterophils, histiocytes and mononuclear cells, including voluminous giant cells. (h) *T. szidati* schistosomula in the lungs during primary infection of a mouse; 3 days p.i. (PAS). The immune reaction consisted of lymphocytes, neutrophils and macrophages. (i) *T. regenti* immature worms in the white matter of the thoracic spinal cord during primary infection of mice; 3 days p.i. (HE). Note the slight cellular infiltration around the parasites. Author of Figure 9a,i: P. Kouřilová, Department of Tropical Medicine, Charles University, Prague, Czech Republic. Staining: periodic acid-Schiff reaction (PAS); hematoxylin-eosin (HE).

(c) *Liver lesions.* Schistosome eggs (Figure 9b) are carried to the liver via the portal veins during the chronic phase of infection (e.g. Wojcinski *et al.*, 1987). The eggs usually cause granulomatous reactions in the liver (Graczyk *et al.*, 1993). Adult *Trichobilharzia* also cause liver lesions. Obstructive fibrosis of the portal triads may develop in case of heavy infections, during which adults may invade the bile ducts also (Pence and Rhodes, 1982). Dead worms, if transported to the liver (by the circulation), elicit mixed granulocytic inflammation. These large lesions are accompanied by tissue necrosis around the worms (Pence and Rhodes, 1982).

(d) *Intestinal lesions.* Intestinal lesions are mostly due to eggs. The eggs are usually located in close proximity to adult worms. Whereas slight immune reactions occur around viable eggs, granulomas are often associated with eggs that undergo degeneration or mineralization (Figure 9c; McLeod and Little, 1942; Wojcinski *et al.*, 1987). Additional pathology may be caused by loosely aggregated groups of adult *Trichobilharzia* worms in serosal and mesenteric blood vessels (Figure 9d; McLeod and Little, 1942), and by adult worms that are located extravascularly in the intestinal tissue (Figure 9e). Accumulation of numerous worms may be accompanied by thrombi in the veins and subsequent extensive haemorrhage and perivascular mononuclear cell infiltration around vessels (Wojcinski *et al.*, 1987). Heavy and chronic infections may result in thickening of the intestinal wall (Wojcinski *et al.*, 1987) similar to that caused by *Austroilharzia variglandis* (Wood and Bacha, 1983).

(e) *CNS lesions.* The nervous tissue incurs damage from eggs of visceral and nasal schistosomes that are distributed by the circulatory system. In a similar way to that described for *Dendritobilharzia* sp. (Levine *et al.*, 1956; Wilson *et al.*, 1982), eggs of *Trichobilharzia* caused granulomatous encephalitis in waterfowl. Disseminated granulomas containing centrally located eggs surrounded by giant cells, macrophages, lymphocytes, heterophils and fibroblasts were noted in close proximity to the blood vessels of the *cerebrum* and *cerebellum* (Graczyk *et al.*, 1993).

The affinity of immature worms of nasal schistosomes (*T. regenti*) for nervous tissue can cause severe injury due to migration and establishment of parasites in the CNS (Kolářová *et al.*, 2001). The presence of the worms induces inflammatory reactions and pathological changes in the nervous tissue (spongiosis, eosinophilic perivascularitis and dystrophic and necrotic changes of neurons). Usually, more pronounced inflammatory reactions develop at sites with numerous parasites or around older, dead and disintegrating worms.

(f) *Nasal lesions.* The lesions are produced by eggs and adults of nasal schistosomes (Fain 1956a; Kolářová *et al.*, 2001). Whereas the eggs were detected only in the nasal mucosa, the adults (Figure 9f) were located intra- and extravascularly (Kolářová *et al.*, 2001). Pathological changes of the tissues surrounding adult worms have not been observed to date, but it is likely that these will be similar to those described for visceral schistosomes in other parts

of the body. Ageing of the eggs resulted in various host reactions (Figure 9g), ranging from focal accumulation of cells to the formation of granulomas (Kolářová *et al.*, 2001), similar to those described for visceral schistosomes (e.g. Wood and Bacha, 1983). In some cases, the observation of free miracidia surrounded by an inflammatory reaction (Kolářová *et al.*, 2001) supported the notion that these miracidia hatch directly in the host tissues (Horák *et al.*, 1998a).

(g) *Other findings.* Severe infections with visceral species of *Trichobilharzia* may result in occurrence of eggs, and both immature and adult parasites in unusual sites (Wojcinski *et al.*, 1987). In addition to the portal and mesenteric veins (normal locations), parasites were also discovered in caudal renal, pulmonary, gonadal, gastric, oesophageal, jugular, femoral and thyroidal veins of birds. Eggs were detected in liver, colon, small intestine, lungs, kidney, spleen as well as in tissues of adrenal/gonads, brain, gizzard, proventriculus, oesophagus, skeletal muscles and foot web. Schmäscke *et al.* (1992) reported lesions containing *Trichobilharzia* sp. eggs with viable miracidia in the sclera, nictitating membrane and conjunctiva of geese. Parasites and eggs can induce immune reactions and pathology in all these unusual locations.

6.1.5. Diagnosis and therapy

The diagnosis of *Trichobilharzia* infections is focused primarily on microscopical detection of eggs in bird excrements. Sedimentation techniques (Kassai, 1999) facilitate the recovery of eggs with developed miracidia from faecal samples of birds infected with visceral schistosomes. Egg concentrations are usually low, and this necessitates examination of whole droppings (Appleton, 1983). This is achieved by filtering and concentration (by means of a formol–ether technique) of the samples prior to microscopical examination (Appleton, 1986). In the case of nasal schistosomes, fresh exudates from nasal cavities are examined for eggs or free miracidia. Samples can be obtained also by rinsing the nasal cavity with saline or water (Horák, unpublished data). In some cases, tissue biopsies can be examined histologically (Schmäscke *et al.*, 1992).

Necropsy can reveal eggs in intestinal and nasal scrapings; adults and eggs can be detected in squashed tissue samples or in stained cross sections. Immunodiagnostic tests are not used routinely in veterinary practice, but they can be helpful in some cases. Antibodies against gut-associated antigens (GAA) of adult flukes can be detected from ducks harbouring patent *T. regenti* infections by means of indirect immunofluorescence and cross-sections of adult schistosomes (IFAT) (Kouřilová and Kolářová, 2002). Although sensitive, this assay cross-reacted with GAA of different schistosome species (including *Schistosoma*).

Praziquantel seems to be a highly effective antihelminthic for the treatment of bird schistosome infections (Blankespoor and Reimink, 1991; Schmäscke *et al.*, 1992; Müller *et al.*, 1993; Reimink *et al.*, 1995). A threefold application of 200 mg to ducks with patent *Trichobilharzia* infections at 24-h intervals led to a permanent reduction in the release of eggs with viable miracidia (Müller *et al.*, 1993). A single dose of 200 mg/kg significantly reduced parasite load in common mergansers (Blankespoor *et al.*, 2001). Doses of 22 mg/duck/day, applied for 1 week during prepatency, prevented the development of schistosomes completely (Müller *et al.*, 1993).

6.2. Incompatible Hosts

6.2.1. Cercarial dermatitis

Cercarial dermatitis is an allergic response to the penetration of schistosome larvae into mammalian skin (Cort, 1928). It develops after repeated contacts (host sensitization) with the cercariae (Olivier, 1949). Cercariae of the genus *Trichobilharzia* are the most common causative agent (Kolářová *et al.*, 1997).

(a) *Clinical features and gross pathology.* The skin syndromes develop in various mammals, including, for example, rabbits and dogs (Herber, 1938; Augustine and Weller, 1949; Olivier, 1953) but have mostly been described from afflicted humans (e.g. Cort, 1928; Vogel, 1930a; Brackett, 1940; Olivier, 1949; Haemmerli, 1953; Gianotti and Luvoni, 1958; Bearup and Langsford, 1966; Margono, 1968; Hoeffler, 1977; Blair and Copeman, 1977; Appleton and Lethbridge, 1979; Eklun-Natey *et al.*, 1985; Baird and Wear, 1987; Wiley *et al.*, 1992; Chamot *et al.*, 1998). The associated symptoms show only minor differences between humans and animals.

Grossly, cercarial dermatitis represents a maculopapular skin eruption associated with intense itching. Cercarial penetration into the skin results in a prickling sensation (primary itching) within 4–20 min, persisting for up to 1 h. Transitory maculae (up to 10 mm in diameter) appear at the sites of penetration of each cercaria. Local urticarial reactions have also been reported as discrete indurated papulae that reach 1–5 mm in diameter. Although the maculae usually disappear in a few hours, they may persist to be replaced by distinct and indurated papulae of 3–5 mm in diameter (10–15 h p.i.) The development of papulae is accompanied by intense itching (secondary itching). An erythematous zone may develop around the papulae with a central punctum. When the papulae are confluent, the whole exposed area may be elevated and oedematous. On the second or third day p.i., vesicles (1–2 mm in diameter) may form on the papulae and these often rupture when rubbed or scratched. Papulae regress progressively after about 4 days and usually disappear by day 10 p.i. to leave a pigmented spot (1–4 mm in diameter) that persists for a month or

longer. Sporadic and intermittent pruritus may accompany the skin eruption; it tends to disappear within several days. The time course of skin symptoms depends on the sensitivity of the afflicted person (Olivier, 1949).

Only mild reactions occur during initial infections, and the disease manifests itself only by development of maculae. If papulae develop, they are usually small (1–2 mm), innocuous and inconspicuous. Usually, no secondary itching, oedema or diffuse erythema are detected. Sometimes a delayed formation (as late as 8 days p.i.) of small papulae was noted. In sensibilized hosts, papulae may occur within one hour of penetration by cercariae. In this case, the papulae have vesicles and increase to 3–8 mm in diameter. They are associated with erythema (10–100 mm in diameter), oedema and intensive pruritus. The symptoms may persist for a relatively long period, at least 20 days in one case (Bechtold *et al.*, 1997). The erythema starts to regress by day 3 p.i.; however, the size depends among other things on the extent of rubbing or scratching induced by the pruritus. Excoriation may occur with severe itching. Pustules do not form unless the lesions become secondarily infected. Generalized skin reactions are rare but may start by day 2 p.i. and persist for 2 days. The rash consists of small (about 2 mm), hard and pink papulae, which are very mildly and intermittently itchy (Olivier, 1949).

Japanese rice planters frequently suffer considerably from repeated infections by cercariae. In severe cases, the patients develop a chronic type of dermatitis called 'koganbyo' in which papulo-vesicular eruptions appear mainly on the dorsal side of hands and fingers; sloughing and injury of the skin is extensive (Oda, 1973).

Massive infections in humans may also cause fever, limb swelling, nausea and diarrhea. Skin lesions occur only on parts of the body that were in contact with cercariae, including parts under swimming suits. The itching can range from negligible to unbearable, potentially creating a noted insomnia (Chamot *et al.*, 1998).

Sensibilization may persist for years in humans (Olivier, 1949). Although few data are available on the development of resistance against cercarial dermatitis, Macfarlane (1949) reported immune individuals among adults, but not in children. Repeated contacts with various species of cercariae might lead to desensibilization (Berg and Reiter, 1960).

All representatives of the genus *Trichobilharzia*, and other non-human schistosomes, seem to produce similar symptoms when infecting humans (Brackett, 1940; Cort, 1950; Batten, 1956; Malek and Armstrong, 1967; Appleton and Lethbridge, 1979; Wiley *et al.*, 1992). There are no significant differences between dermatitis occurring in fresh and salt water bodies (Kolářová *et al.*, 1989). By contrast, human schistosomes usually induce milder skin reactions (Basch, 1991). This difference may stem from an inability of *T. ocellata* to produce a molecule analogous to the 16.8-kDa anti-inflammatory factor from *S. mansoni* (Ramaswamy *et al.*, 1996). In the

case of the latter parasite, the activation of the receptor for parasite-derived prostaglandin D2 dramatically reduced the skin hypersensitivity response after challenge (Angeli *et al.*, 2001).

Generally, it is difficult to distinguish whether skin reactions were caused by bird or human schistosomes. In some areas, bird and human schistosomes may co-occur, sometimes even in the same snail species (Nassi, 1987). The similarity in symptoms underlines the necessity to consider all travellers to be positive for schistosomiasis if they acquired 'cercarial dermatitis' in areas that are endemic for human schistosomes.

(b) *Histopathology and immunology.* Pathological effects associated with penetration of *Trichobilharzia* cercariae into mammals primarily depend on the sensitivity of the afflicted hosts. No marked differences were noted for cellular responses either of various mammalian species against one *Trichobilharzia* species or one mammal against different species (and genera) of bird schistosomes. Initial infections of rabbit and mouse skin by *Trichobilharzia* (Figure 9a) exhibited a mixed inflammatory exudate with few eosinophils and neutrophils, starting by 6 h p.i. (Augustine and Weller, 1949; Olivier, 1953; Kouřilová and Kolářová, 2001). Repeated infections of mice simultaneously resulted in subacute oedematous dermatitis and increased numbers of schistosomula trapped in the skin (Kouřilová, 2001), similar to that recorded from mice exposed to *Gigantobilharzia* (Batten, 1956) and *Ornithobilharzia* (Bayssade-Dufour *et al.*, 2001). The infiltration by melanophages and sinusal hyperplasia, with accumulation of melanin-laden macrophages in subcutaneous lymph nodes, was detected only 30 min after the last challenge of mice infected with *Ornithobilharzia* sp.; the mobilization of melanotic pigment is probably secondary due to pruritus and scratching of the tissue (Bayssade-Dufour *et al.*, 2001).

Strong inflammation around the worms in humans probably arises in hosts that have been infected repeatedly. The observations on this topic were made from sections of biopsies taken from sites with papulae (e.g. Vogel, 1930b; Brackett, 1940; Macfarlane, 1949; Haemmerli, 1953; Gianotti and Luvoni, 1958; Margono, 1968; Appleton, 1984). The occurrence of papulae suggests that the skin biopsies were obtained from sensitized subjects. The most comprehensive study of Haemmerli (1953) described a three-phase cellular response (leucocytic, lymphocytic and histiocytic), which led to the elimination of *Trichobilharzia* schistosomula. During the leucocytic phase (3–9 h p.i.), the parasites were intact. The tegument of the parasite started to degrade at 24 h p.i. (lymphocytic phase) and schistosomula were completely destroyed during the histiocytic phase (36–52 h p.i.). The process was accompanied by perivascularitis and spongiosis of the area surrounding the parasites. Tissue repair (72 h p.i.) was accompanied by parakeratosis with reduced perivascular infiltration in the surrounding tissue; afterwards, cell melanotic pigmentation occurred (100 h p.i.).

The progression of the skin reactions described above may be determined by the degree of host sensibilization (Gay *et al.*, 1999). Whereas a mild human reaction to *T. ocellata* and *T. szidati* comprised only leucocytic vascularitis with perivascular dermal infiltrates of lymphomonocytic and polynuclear type, a strong reaction in another patient was characterized by lymphocytic vascularitis with lymphocytic dermatitis that occurred as early as 10 h p.i.

The use of IFAT- and ELISA-based methods disclosed that *T. szidati* infection in mice led to production of antibodies specific for cercariae of *T. szidati* and *S. mansoni* (Kolářová *et al.*, 1994). However, the mouse antisera did not react with (skin and lung phase) schistosomula (Horák *et al.*, 1998b). It was observed that the parasite loses the main antigenic structure when the cercariae lose the carbohydrate-rich glycocalyx during penetration of the host skin.

6.2.2. Additional syndromes

Trichobilharzia infections may cause other types of symptoms in various species of mammals. Under certain circumstances (e.g. first contact with cercariae), the transformed schistosomula can escape and migrate in the mammalian body (for review see Horák and Kolářová, 2001). Schistosomula of various bird schistosomes (*Trichobilharzia* sp., *T. ocellata*, *T. szidati*, *T. stagnicola*, *T. physellae*, *T. brevis* as well as *Bilharziella polonica* and *Ornithobilharzia* sp.) were detected in the lungs of mice, hamsters, rabbits, rhesus monkeys and gerbils (Olivier, 1953; Appleton and Brock, 1986; Haas and Pietsch, 1991; Horák and Kolářová, 2000; Bayssade-Dufour *et al.*, 2001), and additionally in organs such as liver, kidney, heart and intestines (Haas and Pietsch, 1991). In some of these animals, initial infection resulted in minute petechiae in the lungs. During the early phases of such infections in mice the majority of these lesions were bright red, distinct, discrete and apparently distributed at random over the pleural surface of the lungs. In some cases, deeper, larger and dark red haemorrhages with diffuse and indistinct margins developed with progression of the infection (Olivier, 1953). The lungs of *Ornithobilharzia*-infected gerbils contained numerous whitish nodules alongside pulmonary and collateral arteries, and the peribronchial trees. Some nodules were scattered in the parenchyma (Bayssade-Dufour *et al.*, 2001).

In the lungs of *T. szidati*-initially infected mice, many schistosomula (Figure 9h) resided in the air spaces on day 4 p.i. (L. Kolářová, unpublished data). Immune reactions developed around many intact parasites; the inflammations were most intense around older schistosomula (Moravcová and Kolářová, 2001).

Previously exposed mammals generally develop stronger immune responses against the parasites. Examination of the lungs of gerbils that were re-infected with *Ornithobilharzia* disclosed that most parasites suffered damage to the tegument. Moreover, lymphocytic vasculitis occurred around live worms and worms that had incurred some tegumental damage. The resulting pathology was accompanied by macrophage alveolitis in the peripheral lung parenchyma (Bayssade-Dufour *et al.*, 2001).

Immature parasites of *T. regenti*, a nasal species of *Trichobilharzia*, are neurotropic and also migrate through the CNS of mammals. Histological examination disclosed both intact (Figure 9i) and destroyed worms in the nervous tissue of mice (Horák *et al.*, 1999). Compared with birds, mice displayed more vigorous cellular responses to the parasites. The strong inflammatory reactions around disintegrating parasites were associated with dystrophic and necrotic changes of neurons in the surrounding tissues (Kolářová *et al.*, 2001). The intense inflammatory response may in part be the reason why *T. regenti* fails to develop completely in a non-specific host. The establishment of *T. regenti* in the CNS, and the associated pathology, may lead to changes in neurobehavioural reactions of affected mammals, similar to those described from human neuroinfections by the genus *Schistosoma* (Scrimgeour and Gajdusek 1985; Lambertucci, 1993). However, in the latter case, the pathology develops due to the dissemination of parasite eggs to the CNS.

6.2.3. *Diagnosis and therapy*

Differential diagnosis of cercarial dermatitis is difficult. The resulting lesions resemble those that result from dermatitis of bacterial etiology, contact dermatitis or insect bites from chiggers, fleas and mosquitoes (Hoeffler, 1977). However, clinical and epidemiological data, together with parasitological and immunological diagnostic methods, may combine to identify cercariae as the correct etiological agent. Three criteria are the most important among other clinical and epidemiological data: (1) recent history of a contact with natural water bodies (i.e. within the previous 96 h); (2) development of a papular dermatitis with severe itching between 12 and 24 h after exposure; and (3) distribution of these lesions only on parts of the body that were immersed in the water (Appleton, 1984).

Infrequently, schistosomula can be detected in skin biopsies taken from sites where papulae have developed. However, this method can only be successful shortly after exposure. The parasites are usually destroyed in (or migrate out of) the human skin within 24–72 h p.i. (e.g. Haemmerli, 1953).

Additionally, laboratory tests that monitor the immune reactions in patients can aid in the diagnosis of cercarial dermatitis. Cercarial dermatitis may be

associated with increased eosinophil counts, as observed in human volunteers exposed to *Heterobilharzia americana* (Malek and Armstrong, 1967). Skin tests have been developed, based on the evaluation of erythema that develops around injected antigens, prepared by homogenization of either cercariae of bird schistosomes (Macfarlane, 1949) or adults of *S. mansoni* (Cort, 1936; Augustine and Weller, 1949; Moore *et al.*, 1968; Blackburn and Ma, 1971; Wiedermann *et al.*, 1973; Krampitz *et al.*, 1974) or *S. japonicum* (Hsü and Ameel, 1956). Unfortunately, these tests lack specificity and are frequently insensitive. Therefore, they cannot be used for definitive diagnosis of cercarial dermatitis (Wiederman *et al.*, 1973).

Other methods rely on detection of antibody responses for diagnosis. The 'Cercarienhüllenreaktion', a complement fixation test, IFAT and ELISA have been used to assess the titres of specific antibodies against cercariae (of either *Trichobilharzia* or *Schistosoma*) or against homogenates of *S. mansoni* adults (Vogel and Minning, 1949a,b; Hendricks and Cort, 1956; Moore *et al.*, 1968; Knight and Worms, 1972; Krampitz *et al.*, 1974; Kimmig and Meier, 1985; Kolářová *et al.*, 1994; Pilz *et al.*, 1995). Although these techniques are more sensitive than skin tests, they are not specific. Thus, they cannot be performed for a differential diagnosis between cercarial dermatitis and human schistosomiasis. Recently, an IFAT approach to test the antibody reactivity with schistosomular and adult GAA of both *Trichobilharzia* and *Schistosoma* species allowed the correct distinction of antisera. Thus, this particular method seems promising for differential diagnosis of cercarial dermatitis caused by bird schistosomes (Kouřilová and Kolářová, 2002).

At present, the therapy of cercarial dermatitis is only symptomatic. Abirritant powders may be applied to the skin and, in serious cases, the use of systemic antihistaminics (tablets or better in gels) or mild corticosteroids should be considered (Hoeffler, 1977).

7. EPIDEMIOLOGY OF CERCARIAL DERMATITIS

Cercarial dermatitis has been recognized since historical times. In 1847, Fujii (cited by Oda, 1973) reported skin eruptions in Japanese rice planters. Naegeli (1923) described clinical symptoms of this 'water-borne' disease in Germany, hypothesizing that it was caused by 'animal plancton'. Finally, in 1928, Cort in North America identified the causative agent and the disease was named schistosome dermatitis. Many reports have accumulated since and the infection is presently known under different names in various areas of the world, e.g. swimmer's itch (Christensen and Green, 1928), Zerkarien-Dermatitis (Vogel, 1930b), dermatite des nageurs (Brumpt, 1931), bouton de canicule des baigneurs (Desportes, 1944/45), kabure (Fujii, 1847, cited by

Oda, 1973), koganbyo (Tanabe, 1948), sawah itch (Buckley, 1938), etc. In most areas, the parasite species that cause human cercarial dermatitis have not been precisely identified due to a variety of practical problems and technical difficulties (see section 3). This is unfortunate as unequivocal identification of the causative agent (including data on life cycles) is a prerequisite for estimation of health risks and for designing control measures (see sections 3 and 6).

7.1. Distribution

With the exception of Antarctica, cercarial dermatitis occurs globally (Hoeffler, 1982). It has been reported from North and Central America (USA, Canada, Haiti, Salvador, Mexico, Cuba), Australia, New Zealand, Asia (Formosa, Malaysia, Philippines) (for review, see Hoeffler, 1982), Japan (Oda, 1973; Suzuki and Kawanaka, 1980), India (Narain *et al.*, 1994; Agrawal *et al.*, 2000), Thailand (Kullavanijaya and Wongwaisayawan, 1993), Vietnam (Landmann *et al.*, 1961), China (Liu *et al.*, 1977), Iran (Sabha and Malek, 1979), Africa (South Africa; Appleton, 1984) and Europe (Denmark, Finland, Great Britain, the Netherlands, Slovak Republic, Czech Republic) (for review see Kolářová *et al.*, 1989), Austria (Graefe *et al.*, 1973; Dvořák *et al.*, 1999), European countries of the former Soviet Union (e.g. Raisyte, 1974; Tsyркunov, 1987; Beer and German, 1994), France (Léger and Martin-Löehr, 1999), Germany (for review see Allgöwer and Matuschka, 1993), Iceland (Kolářová *et al.*, 1999), Italy (Nobile *et al.*, 1996; Golo *et al.*, 1998), Norway (Thune, 1994), Spain (Simon-Vicente, 1983), Sweden (Thors and Linder, 2001) and Switzerland (Eklu-Natey *et al.*, 1985; Chamot, 1998).

Probably, the disease is most prominently distributed along the major flyways of the migratory birds: for example, in North America, the dermatitis seems to concentrate along the Mississippi flyway (Jarcho and van Burkalow, 1952) and the Pacific distribution may follow the Neoarctic–Hawaiian, Asiatic–Palauan and Japanese–Marianan flyways (Chu, 1958). According to this view, the Palaearctic–African bird migration (Moreau, 1972) may influence the distribution of cercarial dermatitis in Europe and Africa.

The transient presence of waterfowl is essential for establishment of the parasite life cycle in a particular water reservoir. Most of the birds probably become infected in nesting areas; however, in some cases, birds may also transport mature (within the host) or larval (within snails transported on bird legs) schistosomes to and from the wintering locations (Woodruff and Mulvey, 1997; Wesselingh *et al.*, 1999). Thus, bird migrations may be expected to introduce certain parasite species into new geographical areas. However, no data are available to suggest that avian schistosomes have expanded their geographical range in this fashion.

7.2. Epidemiology

Cercarial dermatitis is a common, non-communicable cutaneous disease that is widely neglected. The occurrence of the disease depends upon complex interactions among the parasites, molluscan and definitive hosts, and humans as they share the same environment under conditions that favour parasite transmission (Hoeffler, 1982). Depending on the causative species, cercarial dermatitis can develop in fresh- or salt-water bodies (for review see Horák and Kolářová, 2001). At present, the most frequently reported cercariae belong to the genus *Trichobilharzia* (Horák and Kolářová, 2001), larval development of which takes place only in freshwater bodies.

Cercarial dermatitis occurs seasonally. It is most prevalent during warmer months when both the release of cercariae from snail intermediate hosts and the number of people that have contact with water reach peak levels (Cort *et al.*, 1940; Appleton and Lethbridge, 1979). In addition to factors that relate to the biology of the parasites, the frequency of human cercarial dermatitis also depends on the behaviour of bathers (duration of swimming), previous contact with the parasite (history of cercarial dermatitis) and, possibly, individual susceptibility to the cercariae. A multivariate analysis (Chamot *et al.*, 1998) showed that the time of day, barometric pressure, maximum air temperature and duration of swimming activity strongly predict not only the incidence of cercarial dermatitis but also the number of skin lesions.

A history of cercarial dermatitis should be assumed if strong skin reactions occur early after infection (Chamot *et al.*, 1998). Both sexes and all age groups can be affected equally under similar conditions (Chamot *et al.*, 1998). However, the time interval of exposure may differ for various age groups. Children between 5 and 9 years are at highest risk because they spend more time in the water during recreational activities (Appleton and Lethbridge, 1979). In rice planters or pickers, for instance, adults were the most affected group (Appleton, 1984).

At present, cercarial dermatitis is regarded as an emerging disease (de Gentile *et al.*, 1996). High eutrophication of water reservoirs (Allgöwer and Effelsberg, 1991), colonization of ponds by susceptible snails and by nesting ducks, combined with long periods of sunshine in the summer, are important factors that have led to recent increases in the number of outbreaks of cercarial dermatitis (de Gentile *et al.*, 1996). Also, the disease may be acquired when cleaning an aquarium containing field-collected snails that harbor avian schistosomes (Bastert *et al.*, 1998; Fölster-Holst *et al.*, 2001).

7.3. Identification of Potential Transmission Sites for Cercarial Dermatitis

The occurrence of cercarial dermatitis is delineated by the distribution of intermediate hosts infected by schistosomes. With respect to species-specific physical, chemical and biological requirements, freshwater snails live in various habitats, differing in size and origin (natural or man-made). Usually, the distribution of snails within larger habitats is discontinuous, often due to a preference for aquatic vegetation that offers protection and sites for egg-laying (Sturrock, 1993). Identification of reservoirs that pose a risk for humans relies on detection of bird schistosomes.

The examination of water samples may disclose the presence of cercariae in a particular water body. However, this is usually difficult due to low parasite concentrations, and various methods of cercariometry – filtration, the use of phototactic response equipment and continuous flow centrifugation – have been developed to detect (human) schistosomes (for review see Théron, 1986); perhaps these may also be applied in the search for bird schistosomes.

Moreover, cercariae can be ‘collected’ by a trap containing a matrix with unsaturated fatty acids (linoleic acid). This substrate stimulates the attachment and penetration by cercariae. Any immobilized larvae are subsequently visualized for counting. This method to monitor schistosome-infested waters was primarily developed for *S. mansoni* (Shiff *et al.*, 1993; Shiff and Graczyk, 1994), but could be applied also for *Trichobilharzia* (Graczyk and Shiff, 2000). Molecular methods can further increase the sensitivity of detection, e.g. a PCR assay based on a highly repeated sequence of *S. haematobium* allows detection of single cercariae and as little as 10 fg of schistosomal DNA (Hamburger *et al.*, 2001).

Determination of field-collected cercarial species is a complicated matter, as described in section 3. Surprisingly, the findings of bird schistosome cercariae are frequently confirmed by experimental infections of volunteers. Several novel insights into the potential pathogenic effects of these parasites (see section 5) warrant caution against performing experimental infections in humans.

The source of infection is identified mainly by examination of water snails. Two methods are commonly used: cercarial emergence and dissection of snails. Exclusive use of cercarial emergence underestimates the value of parasite prevalence and overlooks parasites that are present but not shed by snails at the time of examination. This method yielded false results in 59.1% of cases when compared with the dissection of snails (Curtis and Hubbard, 1990). The occurrence of the parasites fluctuates during the year; these differences are due to cyclic reproduction and changes in the age distribution within snail populations. It is recommended to examine snails of various sizes to determine parasite prevalence more accurately (Hurley *et al.*, 1994).

Higher numbers of infected snails increase the infection risk for humans.

However, *Trichobilharzia* infection rates in lymnaeids are usually low, ranging between 0.3 and 5.2% (Meyer, 1964; van den Broek, 1965; Dönges, 1965; Bearup and Langsford, 1966; Graefe *et al.*, 1973; Wills *et al.*, 1976; Kolářová *et al.*, 1992; Müller and Kimmig, 1994; Hurley *et al.*, 1994; Loken *et al.*, 1995; Niewiadomska *et al.*, 1997; Väyrynen *et al.*, 2000; Loy and Haas, 2001). Except for *L. auricularia*, prevalences that exceed 5% are exceptional and may falsely result from examination of only a low number of snails (Loy and Haas, 2001). The high prevalences of parasites (22 and 26%), recorded from *L. auricularia* in Bohemia and Germany, respectively (Kolářová *et al.*, 1989; Müller and Kimmig, 1994), may result from disturbances in the ecological balance of the lakes that were sampled, leading to increased sizes of snail populations (Kolářová *et al.*, 1989).

The proportion of infected snails at any time depends upon complex interactions of biotic and abiotic factors, including susceptibility of particular snail hosts, and number, distribution and behaviour of definitive hosts. Environmental factors such as temperature are also important (Webbe, 1982). It appears that most snails become infected during the first summer of their life (Hoeffler, 1982). Snail infections in the late summer probably secure survival of schistosomes in the water body. It is obvious that snails containing parasites overwinter and cercarial shedding starts during warm days of the next spring. In this way, the parasite infects new birds when they return to their breeding areas (McMullen and Beaver, 1945; Jarcho and van Burkalow, 1952). On the other hand, summer infections of the snails might be responsible for distribution of schistosomes to the bird wintering areas.

Most commonly, infections of birds by avian schistosomes are diagnosed through faecal examination. This approach is appropriate to detect visceral schistosomes, but it will fail to show the presence of nasal schistosomes. The latter parasites are also common in waterfowl (Rudolfová *et al.*, in press). Therefore, different methods to examine birds should be applied to obtain correct values for prevalence of infection (see also section 6.1.5).

The prevalence of trichobilharziasis in birds can be quite high in comparison with snails. Faecal surveys indicated the following prevalences of *Trichobilharzia* spp.: 60.5% in spurwing goose (Appleton, 1986), 69.6% in domestic duck (Liu *et al.*, 1977), 83.9% in common merganser, 2.6% in Canada goose and 4.9% in gulls (Loken *et al.*, 1995). Guth *et al.* (1979) demonstrated prevalences of 12.0 and 46.3% of visceral schistosomiasis in adult and young Anseriformes, respectively. The investigation of wildfowl (genus *Aythya* and *Anas*) during two consecutive years showed that prevalence of nasal schistosomes was constant at 24.0% (Rudolfová *et al.*, in press).

Migratory birds probably do not acquire heavy infections because they leave the infective habitat, whereas non-migratory birds may suffer severely from trichobilharziasis and die (Wilson *et al.*, 1982). However, the properties

of resident snail populations that determine susceptibility or resistance can lead to different infection rates at particular localities.

7.4. Control

Effective control of cercarial dermatitis must be based on analysis of an entire biological system. Only then can the interruption of the schistosome life cycle be achieved. Intervention efforts can be directed at the level of either intermediate or definitive hosts. The control of intermediate hosts is usually aimed at elimination of snails and/or prevention of snail reproduction. At the definitive host level, the focus is to eliminate schistosome infection in birds.

The control of snail populations may employ chemical, mechanical and biological approaches (reviewed by Allgöwer and Matuschka, 1993). Since the first use of copper sulphate (Taylor and Baylis, 1930) to kill snails in an effort to control cercarial dermatitis, various chemical compounds that are toxic for snails (molluscicides) have been used for this purpose. Many of these preparations are derived from various copper compounds (e.g. Brackett, 1939; McMullen and Brackett, 1948). The use of these compounds may yield inconsistent results, depending on abiotic properties of the water to be treated and the experience of personnel involved in the control (for review see Blankespoor and Reimink, 1991). For example, treatment of a water body with copper sulphate can cause copper to precipitate as copper carbonates. Accumulation of copper in the reservoir sediments can cause death of various aquatic organisms and reduce the recreational value of the lake. Surprisingly, after treatment with a copper-based molluscicide, snails continued to reproduce and spread at a rapid rate (Blankespoor and Reimink, 1991). There is even evidence that, over an extended time, snail populations develop (partial) resistance to copper sulphate (Blankespoor *et al.*, 1985).

Among other synthetic compounds, such as B-2 (sodium 2,5-dichloro-4-bromophenol) or NaPCP (sodium pentachloropentate niclosamide), niclosamide (Bayluscide®) is still the molluscicide of choice. Although it is expensive, niclosamide is highly effective against all life stages of the snail and also against schistosome larvae. Whereas niclosamide is non-toxic for humans, domestic animals and crops, it is, unfortunately, toxic for fish (for review see Perrett and Whitfield, 1996). Moreover, the application of niclosamide did not prevent the re-colonization of sites by remaining snails (Lardans and Dissous, 1998). A promising alternative is provided by the ongoing development of plant molluscicides (Singh and Singh, 2000). However, the availability of these preparations and their effectiveness depend on the local situation and native flora (Perrett and Whitfield, 1996). Finally, the application of various bacterial products could be considered for control of snails and parasites. The eluate of *Bacillus thuringiensis israelensis* contains a

water-soluble M-exotoxin which is toxic for *S. mansoni* and *T. szidati* cercariae and corresponding snail hosts (Horák *et al.*, 1996).

The removal of aquatic vegetation and snails from swimming areas can be an effective control measure (e.g. Graefe *et al.*, 1973). Virtually all snails can also be eliminated from large bodies of water by mechanical disturbance of the snail habitat (using boats mounted with rototillers or tractors equipped with a rake), provided that the intervention is performed in shallow (littoral) areas with high concentration of snails at the time of reproduction and early development (Leighton *et al.*, 2000). Alternatively, small lakes can be emptied temporarily to dry out the bottom, remove mud and cover places reserved for bathing with a new layer of river sand (Kolářová *et al.*, 1989).

Generally, biological control of bird schistosomes aims to regulate or reduce snail and/or parasite populations. The fitness of schistosome intermediate hosts can be affected significantly by removal of food sources and introduction of natural predators (e.g. fish) or competing snail species (resistant to schistosomes) (Haas, 1985; Allgöwer and Matuschka, 1993). The introduction of parasites that attack or compete with larval schistosomes within the snail host, or that induce high mortality or castration of snails has been explored (for review see Lim and Heyneman, 1972). For example, intramolluscan larvae of echinostome parasites are predators of schistosome larvae that may co-occur in the same snail. Introduction of (eggs of) *Echinostoma audyi* led to some level of control of intramolluscan larvae of *T. brevis* in the snail *Lymnaea rubiginosa* (Ow-Yang *et al.*, 1970; Lie and Ow-Yang, 1973). Many factors affect the feasibility of biological control, and these approaches should be taken only under semi-controlled conditions, such as in small lakes. Also it may take considerable time before these approaches yield demonstrable effects. Because of this, these methods have little practical value.

At the level of definitive hosts, control efforts have explored prevention of bird contact with infested waters and chemotherapy of birds. Whereas the first approach (e.g. relocation of breeding bird host species to other areas; Blankespoor and Reimink, 1991) is difficult to implement on a large scale in the field, the treatment of birds with antihelminthics is rather promising. Based on a study in which birds were captured and treated with praziquantel to kill adult worms, Blankespoor and Reimink (1991) concluded that treatment of birds is more effective than the use of copper sulphate; also it is less expensive and has no detrimental effects on the environment.

7.5. Prophylaxis

Theoretically, human infections are prevented by prohibiting access to water that harbours cercariae of avian schistosomes. However, various restrictions in water activities are usually not respected by bathers (Allgöwer and Matuschka,

1993; Chamot *et al.*, 1998). Some protection against cercarial dermatitis can be achieved by rubbing the skin to full dryness immediately after water contact, before cercarial penetration occurs (Brackett, 1939; Haas and van de Roemer, 1998). A thorough coating of the body with vaseline provides complete protection against infection (McLeod and Little, 1942). The value of protective clothing (long trousers, hip boots and waders) is unpredictable. Recently, *N,N*-diethyl-*m*-toluamide (DEET) was found to have a repellent effect against various cercariae of the genus *Schistosoma* (Salafsky *et al.*, 1999, 2001); when incorporated in liposomes ('lipodeet'), the application on skin was 100% effective in preventing penetration by cercariae. A similar prophylactic effect can be expected against penetration by schistosome larvae from genera that cause cercarial dermatitis.

8. CONCLUSION

The association between avian schistosomes of the genus *Trichobilharzia* and cercarial dermatitis in mammals has been recognized for many decades. However, difficulties in obtaining adult parasites from birds, combined with extensive morphological similarities, continue to complicate both the unequivocal identification at species level and the characterization of specific biological properties of distinct species within the genus *Trichobilharzia*. The systematics of the genus requires a major revision, including clarification of the position and validity of the type species *T. ocellata*. New, more extensive criteria to identify a species of *Trichobilharzia*, combining morphology, characteristics of the life cycle and molecular sequence data, may begin to resolve this issue.

Species of *Trichobilharzia* display a remarkable specificity towards (species of) snail intermediate hosts. The parasite modifies the physiology and internal defences of a snail to complete intramolluscan development successfully. The transmission of a *Trichobilharzia* species by only certain snails is thought to reflect the highly specialized nature of these complex interactions between the parasite and the intermediate host.

As parasites, *Trichobilharzia* species are serious pathogens for birds. Comparable to pathology caused by parasites of the genus *Schistosoma* in mammals, trichobilharziasis is accompanied by injuries due to parasites that migrate through the viscera or the CNS (in the case of nasal species of *Trichobilharzia*) of birds. Moreover, adult worms and parasite eggs that have become trapped in the tissues evoke the formation of granuloma.

The ability of cercariae to penetrate into mammals is linked to more than just cercarial dermatitis alone. Study of primary infections in rodents revealed that some immature *Trichobilharzia* worms also migrate through the body of

mammals. Strikingly, they are also neurotropic in mammals. The invasion of nasal species of *Trichobilharzia* into the nervous tissues of experimental rodents potentially led to neuromotor disorders. This suggests that cercarial dermatitis, the elimination of penetrating cercariae in the skin, represents a protective reaction of a sensitized organism against the parasite. At present, there are no data on the pathology of primary infections in large mammals and humans. However, the cosmopolitan occurrence of *Trichobilharzia* species and the frequent exposure of animals and humans to these parasites (cercarial dermatitis is an emerging disease) provide ample justification to intensify study of clinical and pathological aspects related to trichobilharziasis in mammals, including humans.

In summary, species of the genus *Trichobilharzia* display striking transitions in morphology, a complex life-cycle and fascinating properties that enable these parasites to survive in intermediate and final hosts. The inconspicuous nature of their endoparasitic life style may have led to a considerable underestimation of the pathogenicity and medical/veterinary importance of *Trichobilharzia*.

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