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MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

Digenean trematodes in freshwater snails in the
surroundings of Vienna with a focus on species involved
in human infections

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of
Master of Science (MSc)

Wien, 2016 / Vienna 2016

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on
the student record sheet:

A 066 834

Studienrichtung lt. Studienblatt /
degree programme as it appears on
the student record sheet:

Masterstudium Molekulare Biologie

Betreut von / Supervisor:

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1. Introduction

1.1. Digenean Trematodes

1.1.1. Classification

Phylogenies are reconstructed by morphological as well as molecular means. In order to verify molecular phylogenies, complementary data sets from morphological studies are needed; to comprehend morphological homology, phylogenetic evidence from molecular data is necessary. Morphological and molecular data thus go hand in hand in the construction of evidenced phylogeny models and cannot be viewed as substitutes for each other. However, morphology-based character matrices for flatworms are rare in the systematic literature; vast amounts of morphological data are indeed available (especially through the use of electron microscopy), but these data are poorly structured. A further shortcoming of flatworm-related morphological data is that the informative values of characteristics (e.g. size, ratios, and the relative position of an organ) are problematic; presence/absence characteristics are instead powerful (LITTLEWOOD et al. 1998). According to LITTLEWOOD et al. (1998), these problems with morphological data sets lead to the conclusion that molecular techniques represent the only means by which phylogenies can be estimated cladistically. However, molecular analysis may also be problematic, as molecular and morphological classification are rarely completely concordant. For this reason, the generic level is most reliably identified with the help of the three-volume *Keys to the Trematoda* of GIBSON et al. (2002, cited in GIBSON et al. 2014). For closely related species, morphological means fail and molecular analysis must be used.

The taxonomic hierarchy of digenean trematodes is illustrated in table 1. Flatworms (Platyhelminthes) are characterized by bilateral symmetry, a hermaphroditic way of life, and usually a dorsoventrally flattened body; they also possess three germinal sheets. Instead of a coelom, they exhibit a parenchyma filling the space between ectoderm and endoderm (LUCIUS & LOOS-FRANK 2008, WESTHEIDE & RIEGER 1996). Depending on the species, the parenchyma may serve as a circulatory system for materials, provide skeletal support, store nutrients and oxygen, support motility, and interact with other tissues (CONN 1993).

Table 1. Taxonomic hierarchy of digenean trematodes (ITIS REPORT 2013).

Kingdom	Animalia
Subkingdom	Bilateria
Phylum	Platyhelminthes
Subphylum	Neodermata
Class	Trematoda
Subclass	Digenea

Parasitic flatworms constitute the group Neodermata (GIBSON et al. 2014). The term 'Neodermata' derives from the fact that the larvae shed their ciliated epidermis once they reach their host. A new envelope, the neodermis, then emerges from mesodermal cells. This modified syncytial epidermis is important for the uptake and secretion of material, e.g. the release of immunosuppressive substances (LUCIUS & LOOS-FRANK 2008).

In discussing further characteristics of Neodermata, LITTLEWOOD (2006) refer to the two-cell weir of the protonephridia, the electron-dense collars of the epithelial sensory receptors, the complete incorporation of the axonemes into the sperm body, and the existence of two long and one short insertions in nuclear small subunit ribosomal DNA.

Parasitism of Neodermata presumably developed very early in evolution, which suggests that the early parasitic flatworms were parasites of invertebrates. Parasitism of vertebrates emerged much later (WEBB 2004).

The Neodermata group comprises the Monogenea, the Cestoda (tapeworms), and the Trematoda (flukes) (figure 1) (LUCIUS & LOOS-FRANK 2008). The Trematoda are divided into two subclasses: the Digenea and the Aspidogastrea (FRIED 1997). The most important difference between these two groups is that in contrast to aspidogastreans, digeneans have an alternation of generations (GIBSON

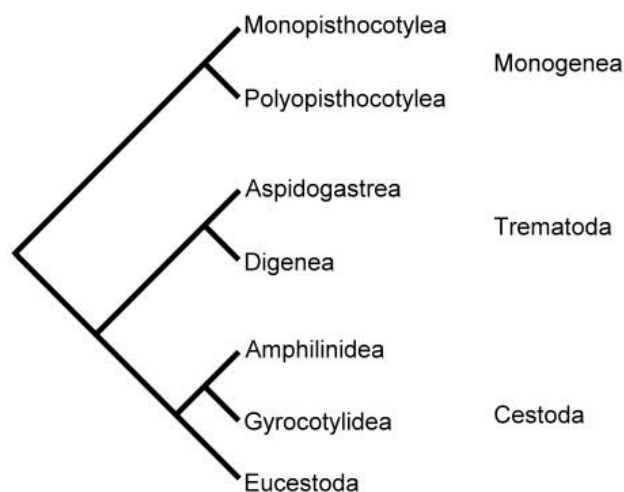


Figure 1. Phylogeny of Neodermata. Modified from LUCIUS and LOOS-FRANK 2008.

1996). This feature also sets digeneans apart from other helminths (such as nematodes) that do not reproduce asexually (HASEEB & FRIED 1997).

The Digenea comprise approximately 8,000 species (ASPÖCK & WALOCHNIK 2007) and more than 2,500 genera, which are grouped into four orders: the Strigeatida, the Echinostomida, the Plagiorchiida, and the Opisthorchiida (GIBSON 2002). The morphological species identification of digeneans is problematic, as digeneans may show age or host-induced phenotypic plasticity (NOLAN & CRIBB 2005, cited in HAYWARD 2010); as such, identification using traditional methods is practically impossible. The actual dimensions of digenean diversity are therefore still uncertain (HAYWARD 2010).

1.1.2. Geographical Distribution

Digenea are very common parasites of almost all vertebrates (FELIU et al. 2006), and their distribution depends on several factors. As digeneans are dependent on intermediate hosts, their occurrence is limited to each host's geographical distribution (HASEEB & FRIED 1997). Some trematodes are confined to only one species as intermediate host (HASEEB & FRIED 1997), whereas others are able to exploit different species (with some host species being parasitized more frequently or at higher intensities than others) (SMITH et al. 2007). Host range and diversity thus significantly determine parasite occurrence and diversity (HASEEB & FRIED 1997).

Habitat structure also plays an important role, as it affects the chance of contact between the parasite and its potential host (SOUSA & GROSHOLZ 1991, cited in SMITH et al. 2007). According to LAFFERTY et al. (2005), habitats with vegetation exhibit a higher prevalence of trematodes in snail intermediate hosts than mudflats. A possible explanation is that habitats with vegetation are richer in host species diversity (SMITH et al. 2007).

Trematode distribution and transmission are further influenced by ecological constraints. According to PAULL and JOHNSON (2011), the eggs of the trematode *Ribeiroia* develop four times faster at 26 °C than at 17 °C and do not hatch at 12 °C. POULIN (2006) suggests that global warming may not only change the geographical distribution of parasitic diseases, but could also result in a higher proliferation of their infectious stages and support the transmission of parasites, which would lead to an increase in their local abundance.

In addition, humans also have a strong impact. Anthropogenic changes such as nutrient inflow and the introduction of non-indigenous species may cause alterations to the parasite fauna (BARBER & POULIN 2002), while the worldwide transportation of animals increases the risk of introducing pathogenic organisms to previously unaffected areas (TATEM et al. 2006). High salinity or eutrophized water and other water characteristics (such as changing water levels) can exclude particular snail species from a locality and inhibit parasite transmission and snail reproduction (MAS-COMA et al. 2009).

1.1.3. General Life Cycle

Digeneans have complex life cycles with diverse developmental stages, and the variations make it difficult to make broad generalizations. However, all digenean trematodes need a final host in order to mature to sexually reproducing adults and at least one intermediate host for completing their life cycle (HASEEB & FRIED 1997, OPENSTAX COLLEGE 2013).

A generalized life cycle of digenean trematodes is illustrated in figure 2. The egg is released from the final host (LUCIUS & LOOS-FRANK 2008), which is commonly a vertebrate (although some trematodes exploit invertebrates as final host, e.g. *Hirudicolotrema richardsoni* parasitizes freshwater leeches) (HASEEB & FRIED 1997). As the eggs of most species are not embryonated when released, the larvae (or the miracidium) require water to develop (LUCIUS & LOOS-FRANK 2008). Factors such as light, temperature, oxygen, and salinity activate the miracidium and cause it to hatch and penetrate the first intermediate host (LUCIUS & LOOS-FRANK 2008), which is commonly a mollusc and most frequently a snail (HASEEB & FRIED 1997). The miracidium thereby sheds its ciliated epithelium and the neodermis develops. At this stage the miracidium settles in the snail's tissue and transforms into a mother sporocyst. Depending on the species, the sporocyst generates either daughter sporocysts (e.g. *Schistosoma*, *Dicrocoelium*) or rediae (e.g. *Fasciola*, *Heterophyes*), both of which migrate into the midgut gland. A large number of cercariae develop and when triggered by certain stimuli (such as light) leave through a birth pore and prospect the final host. There they mature to adult organisms and undergo sexual reproduction, which results in the release of new eggs (LUCIUS & LOOS-FRANK 2008).

However, some trematodes require further intermediate hosts, which could be molluscs, other invertebrates or even vertebrates (HASEEB & FRIED 1997). Within the second intermediate host, the cercariae migrate into different tissues, i.e. the musculature, midgut gland and other organs (but never the gut), and convert into metacercariae. As they have to be ingested by the final host, the last intermediate host is an animal that belongs to the final host's food chain. In the case of an herbivorous final host, the encystation occurs on plants (e.g. *Fasciola*) or in an animal that is accidentally picked up with herbal food (e.g. *Dicrocoelium* in ants taken up by sheep). However, a few species do not develop any metacercariae at all, e.g. the superfamily Schistosomatoidea (LUCIUS & LOOS-FRANK 2008). As the metacercariae pass through the stomach and the duodenum, the metacercarial envelope dissolves, the pre-adult digeneans are released, and adult worms develop (LUCIUS & LOOS-FRANK 1997).

As most helminths do not increase in quantity in the final host, the worm burden is completely dependent on the dose of infection (HASEEB & FRIED 1997).

The reproductive potential of trematode larvae is very high and allows successful transmission even in vast and physiologically challenging environments. Hundreds to thousands of cercariae emerge every day from snails infected with miracidia, e.g. a sole *Littorina littorea* parasitized by *Cryptocotyle lingua* releases 3,300 cercariae per day. In contrast, little is known about the fecundity of adult digeneans (HASEEB & FRIED 1997).

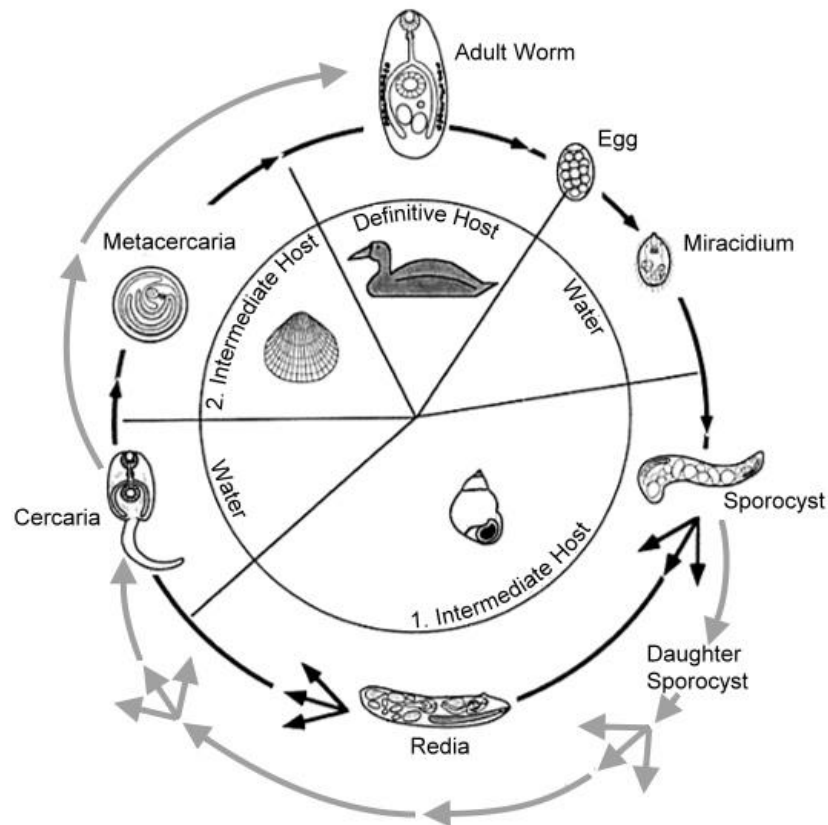


Figure 2. General life cycle of digenean trematodes. Modified from LOOS-FRANK and GOTTSTEIN 2006.

1.1.4. Form and Function

1.1.4.1. Egg

An exemplary digenean egg is illustrated in figure 3. Digenean eggs typically have an oval form and are averagely 100 µm in length, with a width that is approximately one-third of its length (ESTEBAN et al. 2014, FRIED 1997). In most cases the egg has an operculum at one end (LUCIUS & LOOS-FRANK 2008), while the other end may have a knob. The egg capsule is very robust. The egg is secreted through the female gonopore and leaves the host, typically with its faeces (although the eggs of *Schistosoma haematobium* are passed in urine, those of *Paragonimus westermani* through sputum, and those of *Trichobilharzia regenti* through the nasal mucosa) (DVOŘÁK et al. 2002, FRIED 1997).

In the absence of oxygen and a watery environment, an egg faces desiccation and death. Furthermore, its development often depends on temperature and light. The eggs of most of the trematode species (e.g. *Echinostoma* and *Fasciola*) are not yet fully developed

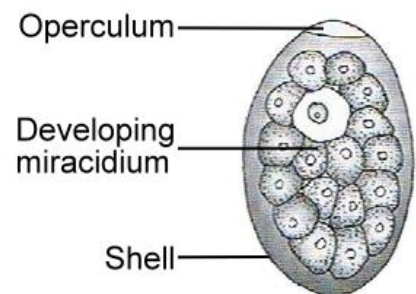


Figure 3. Egg of *Fasciola hepatica*. Modified from LUCIUS and LOOS-FRANK 2008.

when released and embryonation requires oxygen, light and water (KEARN 1998). In some cases, egg development occurs only after ingestion; for instance, the eggs of *Haematoloechus medioplexus* need gut factors of planorbid snails for their hatching process. In contrast, the eggs of schistosomes are completely developed when released from the host (FRIED 1997).

1.1.4.2. Miracidium

The miracidium is an elongated larva that is approximately 100 µm in length and contains ciliated epidermal plates. Underlying circular and longitudinal muscles allow locomotion and the cilia are shed once the miracidium penetrates the first intermediate host. Figure 4 depicts the typical miracidial structure. Miracidia usually have eye-spots and an apical papilla at their anterior end that facilitate sensory perception. In addition to glandular and excretory systems, they also possess a large basal ganglion with peripherally situated cell bodies. The germ cells, which produce the future sporocysts, are imbedded in the parenchyma in the miracidium's posterior end (FRIED 1997, LUCIUS & LOOS-FRANK 2008).

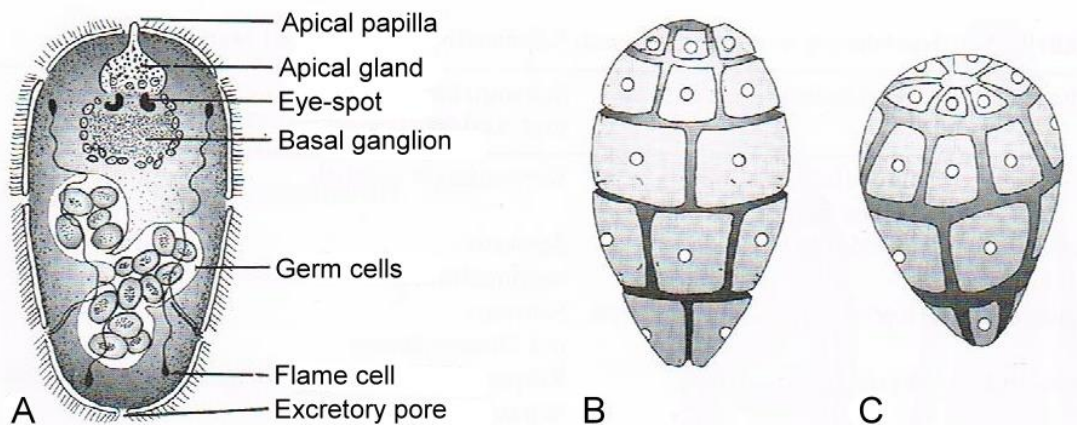


Figure 4. Typical shape of a miracidium. **A.** Inner structures of a miracidium. **B.** The epidermal plates of the family Fasciolidae are typically arranged in five rows of six, six, three, four, and two cells. **C.** The epidermal plates of the family Schistosomatidae are typically arranged in four rows of six, eight, four, and three cells. The cilia are not shown. Modified from LUCIUS and LOOS-FRANK 2008.

1.1.4.3. Sporocyst

Sporocysts have a comparatively bare structure and lack a locomotor system, mouth and digestive tract (figure 5). They have a long, sac-like shape and are covered with a syncytial tegument with microvilli that interdigitate with host tissue. These microvilli make it difficult to remove intact sporocysts from host tissue. The so-called mother sporocyst contains germinal cells, which produce either daughter sporocysts or rediae. Daughter

sporocysts give rise to further generations of sporocysts or to cercariae. Some sporocysts possess a birth pore, but in most cases they disrupt in order to set larvae free (FRIED 1997).

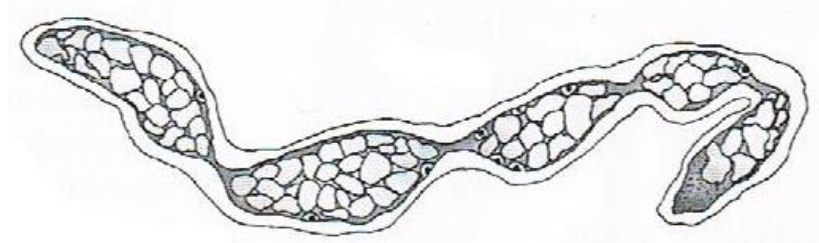


Figure 5. Typical sac-like shape of a sporocyst (LUCIUS & LOOS-FRANK 2008).

1.1.4.4. Redia

Rediae also have an elongated sac-like structure; however, they are more complex than sporocysts and do possess a mouth, pharynx and gut (figure 6). They give rise to further generations of rediae before cercariae emerge. In contrast to sporocysts, rediae migrate within the snail tissue and can often be found in the midgut gland and gonads. While rediae of most species feed only on the snail host, some are predatory on larvae of even their own species (FRIED 1997, LUCIUS & LOOS-FRANK 2008).

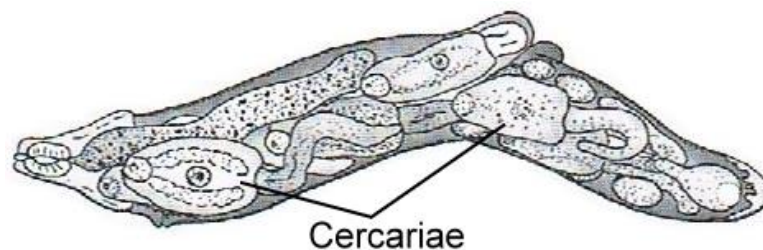


Figure 6. Typical sac-like shape of a redia. Cercariae can be seen in the inside and a mouth exists at the anterior end. Modified from LUCIUS and LOOS-FRANK 2008.

1.1.4.5. Cercaria

Cercariae are released either by daughter sporocysts or rediae. The most conspicuous attribute of these short-lived juvenile digeneans is usually a tail, which is used for swimming (FRIED 1997, LUCIUS & LOOS-FRANK 2008).

The appearance of cercariae can differ and may depend on the type of host being infected (figure 7). Cercariae may have eye-spots like those of *Paramphistomum*, although those which infect arthropods possess an apical stylet. Echinostome cercariae feature a head collar of spines around their oral sucker (LUCIUS & LOOS-FRANK 2008).

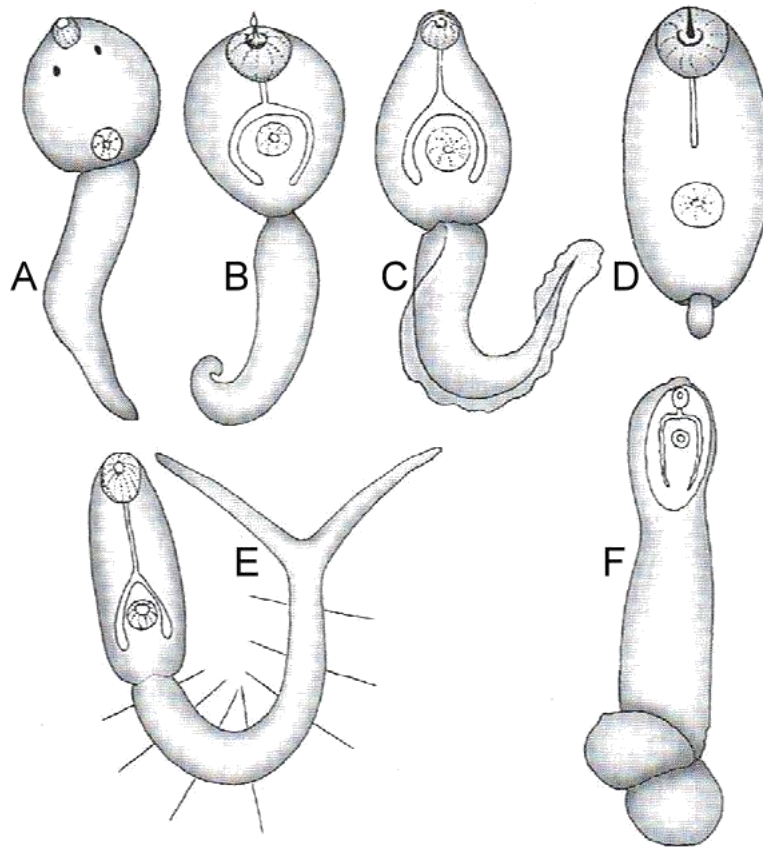


Figure 7. Different types of cercariae. **A.** gymnocephal amphistome. **B.** gymnocephal xiphidocerc. **C.** gymnocephal lophocerc. **D.** microcerc xiphidocerc. **E.** furco-trichocerc. **F.** cystocerc furcocerc. Modified from LUCIUS and LOOS-FRANK 2008.

1.1.4.6. Metacercaria

Numerous species develop metacercariae as a quiescent stage. The cercariae therefore detach their tail and release cystogenous material produced by their glands in order to form a hyaline and elastic envelope, or metacercarial cyst (figure 8) (FRIED 1997, LUCIUS & LOOS-FRANK 1997). Depending on the species, the envelope consists of up to five layers, which makes the cyst particularly resistant. The metacercaria can be directly infectious for the definitive host or may need up to a day to become infective (FRIED 1997).



Figure 8. Metacercaria of *Metagonimus yokogawai*. The cercaria inside is ventrally bent (HONG & SEO 1969).

1.1.4.7. Adult

The typical shape of adult digeneans is illustrated in figure 9. As digeneans belong to the Platyhelminthes, most are elongated and dorsoventrally flattened. Body size usually ranges between 1 and 5 mm in length, although some larger species (such as *Fasciola gigantica*) exceed 3 cm. While some species are pigmented, most are semi-transparent or even transparent. The majority are distome and therefore have two suckers: an oral sucker that embraces the mouth and a ventral sucker (or acetabulum) that serves only for adhesion. However, some species possess only an oral sucker (e.g. monostomes) (FRIED 1997, LUCIUS & LOOS-FRANK 1997). Their final location in the definitive host is in most cases the digestive tract (particularly the small intestine), but some species reside in other body parts; for instance, *Paragonimus* resides in the respiratory tract and schistosomes reside in blood vessels (LUCIUS & LOOS-FRANK 1997). The latter is also special because it is the sole family that is dioecious; all other digeneans are hermaphrodites.

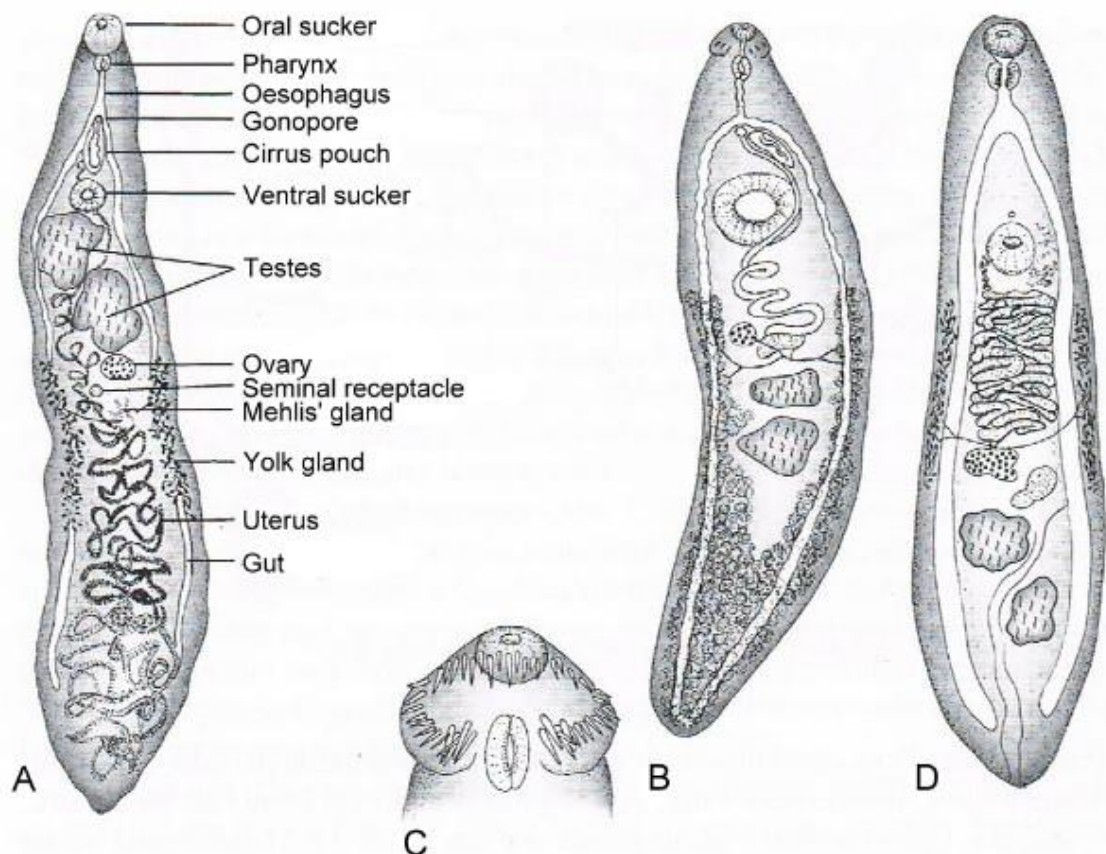


Figure 9. Adult digeneans. **A.** *Dicrocoelium dendriticum*. **B.** *Echinostoma hortense*. **C.** Head collar of spines of an echinostome species. **D.** *Opisthorchis felinus*. Modified from LUCIUS and LOOS-FRANK 2008.

1.1.5. Medical Importance

Trematodes include a group of parasitic flatworms that have medical and veterinary importance. In contrast to aspidogastriids, which parasitize in molluscs, fish and chelonians (GIBSON 2002), digeneans have a more serious impact on people and domestic animals as they are mainly internal parasites and may cause severe diseases in humans (FRIED 1997). Approximately 120 species are known to infect humans (ASPÖCK & WALOCHNIK 2002). The Centre for Agriculture and Bioscience (CAB) International identified the number of times some of the most medically important digeneans were cited in the literature from 1972 to 1995 (table 2). Its findings reveal that the most medically important digeneans by far are the genera *Schistosoma* (the causative agents of the waterborne disease schistosomiasis) and *Fasciola* (the liver flukes that cause fasciolosis) (FRIED 1997). The former affects approximately 200 million people in the tropics and leads to severe symptoms including organ enlargements (OPENSTAX COLLEGE 2013), while the latter is considered to be a neglected trematodiosis that has re-emerged as a human disease (ALBA et al. 2016). Both diseases have medical as well as veterinary relevance, which explains their abundant citation (FRIED 1997).

Table 2. Number of citations listed in CAB International from 1972 to 1995 for some medically important digeneans and their diseases (FRIED 1997).

Trematode Genus	No. of Citations	Disease	No. of Citations
<i>Schistosoma</i>	14,623	Schistosomiasis	7,541
<i>Fasciola</i>	6,377	Fasciolosis	2,667
<i>Paragonimus</i>	1,499	Paragonimiasis	585
<i>Opisthorchis</i>	1,278	Opisthorchiasis	359
<i>Clonorchis</i>	952	Clonorchiasis	359
<i>Echinostoma</i>	710	Echinostomiasis	29

According to ARYA et al. (2016), trematodes are an emerging group of parasites in tropical countries. Southeast Asia is one of the world's hotspots for parasitic infections, in particular zoonotic and vector-borne diseases (LIM & VYTHILINGAM 2013). The reason for this is two-fold. First, the combination of fast urbanization and exponential population growth found in this region has led to increased food production, agriculture, livestock and land use and thereby caused pathogens (including parasites) to adapt to the altered conditions. Second, antiparasitic drugs are being used inappropriately. In general, distribution and prevalence of parasitic infections depend predominantly on economic development and hygiene, as factors such as poor sanitation play an important role in spreading faecal-orally transmitted pathogens. This is why countries with increasing economic prosperity have almost no parasitic infections (LIM & VYTHILINGAM 2013).

Table 3 shows the prevalence and local relevance of some foodborne trematodioses in Southeast Asia. The data make it clear that parasitic diseases caused by trematodes are still a significant burden to public health in this region (MOHAMED-NOR 2013).

Table 3. Prevalence data for foodborne trematode infections in Southeast Asia. Modified from MOHAMED-NOR 2013.

Country	Prevalence of foodborne trematodes	Year reported
(1) <i>Clonorchis sinensis</i>		
Vietnam	51.5%	2007
	32.2%	2011
Thailand	23% (Central)	2009
(2) <i>Opisthorchis viverrini</i>		
Cambodia	4.6%	2012
Lao PDR	50% (Southern provinces)	2000
	23% (Thakhek), 15% (Vientiane)	2003
	85% (Southern region)	2007
Thailand	9.6-19.3%	2003
	2.1-70.8% (Khon Kaen District)	2004
	7-13% (Khukan District)	2004
	64% (Central)	2009
Vietnam	21%	2004
(3) <i>Fasciola</i> spp.		
Lao PDR	2.4% (stool examination), 13.8% (serology)	2008
(4) <i>Paragonimus westermani</i>		
Lao PDR	51% (villagers), 14.5% (school children)	2008
Vietnam	12.7% (Sinho District), 3.3% (Luc Yen District)	2011
Thailand	10% (samples of the 1980s), 4.9% (samples of 2005)	2008
	15.8% (samples of 1988)	1988
	0.51% (samples of 2000)	2001

1.2. Intermediate Hosts

The primary intermediate host of trematodes is commonly a mollusc and typically a snail, mostly with a strict host specificity (HASEEB & FRIED 1997, LUCIUS & LOOS-FRANK 1997). Snails of the family Lymnaeidae (e.g. *Radix* spp. and *Lymnaea stagnalis*) are known to serve as intermediate hosts for different digenean species (APO 2013a,b, FALTÝNKOVÁ et al. 2007, FUCHS 2002, HUŇOVÁ et al. 2012, SOLDÁNOVÁ et al. 2013). They are right-handed, pulmonate freshwater snails with a wide distribution. Their mantle cavity functions as a lung and the eyes are situated at the base of cephalic triangular tentacles. Figure 10 reveals the general body plan of gastropods (FECHTER & FALKNER 1989,

SANDHALL 1973). Although they are hermaphroditic, they prefer to cross-fertilize if possible. They are most common in calcium-rich, stagnant, or slowly flowing shallow waters with stony or muddy banks and submerged vegetation (ADW 2000, WHITE-MCLEAN 2011).

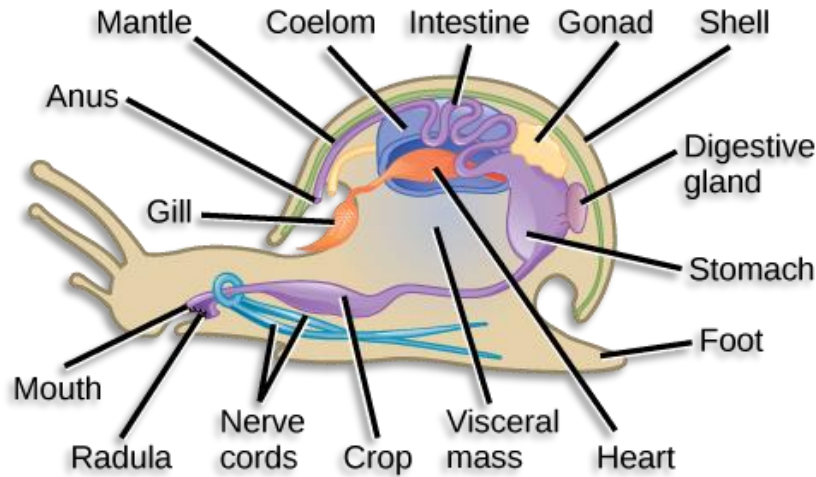


Figure 10. Anatomy of gastropods (OPENSTAX COLLEGE 2013).

1.2.1. *Radix* spp. MONTFORT, 1810

The genus *Radix* is widely distributed over the northern hemisphere (figure 11) (DMG 2016, SCHNIEBS et al. 2011, SEDDON et al. 2014a). However, the type species *R. auricularia* is expected to be extirpated in Africa (GBIF 2016, SEDDON et al. 2014a). Today, both *R. auricularia* and the species *R. labiata* and *R. balthica* appear predominantly in Europe and Asia, although *R. auricularia* has also been introduced to North America (WELTER SCHULTES 2013a,b,c, SEDDON et al. 2014a). In contrast, *R. lagotis* seems to be



Figure 11. Range map of the *Radix* genus. Orange: Extant. Green: Probably extant. Modified from IUCN 2014.

limited to the Danube Basin (WELTER SCHULTES 2015, PROSCHWITZ 2011).

1.2.1.1. *Radix auricularia* (LINNAEUS, 1758)

R. auricularia is named after its ear-like shell, which has a maximum size of 35 mm (figure 12A) (FECHTER & FALKNER 1989). It inhabits a variety of different waterbodies, including lakes, brooks, wet fields, brackish waters, and puddles; thanks to its ability to adapt to anthropogenic habitats, it can even thrive in irrigation canals and pools. Due to this adaptability, it is considered an invasive species. *R. auricularia* is used as food for both humans and animals (for instance, it is used to feed poultry and fish in Asia) (SEDON et al. 2014a).

1.2.1.2. *Radix lagotis* (SCHRANK, 1803)

R. lagotis is depicted in figure 12B (WELTER SCHULTES 2015). *R. lagotis* is morphologically hard to distinguish from *R. balthica*. Little information is available on *R. lagotis*, but it is known that it lives in ponds, rivers, and lakes (PROSCHWITZ 2011, SCHNIEBS et al. 2011). Its shell has a maximum length of 25 mm (WELTER SCHULTES 2015).

1.2.1.3. *Radix labiata* (ROSSMÄSSLER, 1835)

R. labiata is known to resist drought periods remarkably better than the other *Radix* species (FECHTER & FALKNER 1989). This species, which is up to 20 mm long, usually lives



Figure 12. *Radix* spp. **A.** *Radix auricularia* (WHITE-MCLEAN 2011). **B.** *Radix lagotis* (WELTER SCHULTES 2013d). **C.** *Radix labiata* (NATURBIOTOP 2015). **D.** *Radix balthica* (CUMMING n.d.).

in stagnant or slowly flowing waterbodies. Figure 12C illustrates the typical appearance of this species (WELTER SCHULTES 2013b).

1.2.1.4. *Radix balthica* (LINNAEUS, 1758)

R. balthica has a maximum length of 20 mm and looks similar to a small *R. auricularia* (figure 12D). This species can be found in a wide range of habitats. Its preferred habitats are rivers, creeks, and streamlets as well as standing waters. *R. balthica* is highly tolerant to pH, salinity, and temperature, although water pollution and changes in water regime are problematic (SCHNIEBS 2011, WELTER SCHULTES 2013d, SEDDON et al. 2014b).

1.2.2. *Lymnaea stagnalis* (LINNAEUS, 1758)

L. stagnalis was first described by Linnaeus in 1758. With a maximum length of 70 mm, it is the biggest pond snail species; together with *Planorbarius corneus* (Planorbidae), it is also among the most common and conspicuous species (figure 13) (FALTÝNKOVÁ et al. 2007, FECHTER & FALKNER 1989). It prefers slow or stagnant waterbodies with dense vegetation and muddy sand or crushed stone ground. *L. stagnalis* has a wide distribution (figure 14) and is used as food for humans in Russia (BUDHA et al. 2010, FECHTER & FALKNER 1989).



Figure 13. *Lymnaea stagnalis* feeding on an algal biofilm (PETERS n.d.).



Figure 14. Range map of *Lymnaea stagnalis* (IUCN 2010).

1.3. *Trichobilharzia* Species

Trichobilharzia is a genus within the family Schistosomatidae, which is characterized by certain features that are unique within the trematode class: Adult members of this family are dioecious and inhabit the blood vessels of their definitive hosts (LUCIUS & LOOS-FRANK 1997). This family comprises various medically relevant species. Although they occur particularly in the tropics and subtropics (where they cause human bilharziosis), several pathogenic species of this family can also be found in the temperate zone. This includes the so-called bird schistosomes, which belong to the subfamily Bilharziellinae (HORÁK & KOLÁŘOVÁ 1997). Cercariae of the genus *Trichobilharzia*, which is a prominent member of this subfamily, may cause cercarial dermatitis (SCHETS et al. 2008). With more than 40 species, it is the largest genus of the family Schistosomatidae (HORÁK et al. 2002). However, knowledge on *Trichobilharzia* is still insufficient. As morphological criteria are limited, morphological species determination is inadequate; as a result, molecular analyses are indispensable (BRANT & LOKER 2009, DVOŘÁK et al. 2002).

1.3.1. Geographical Distribution

The geographical distribution of *Trichobilharzia* spp. is directly connected to that of its intermediate hosts. As species of the family Lymnaeidae and members of Physidae can all play the host role, *Trichobilharzia* spp. are distributed globally (HORÁK & KOLÁŘOVÁ 1997, HORÁK et al. 2015). Seven *Trichobilharzia* species occur in Europe, namely *T. regenti* HORÁK, KOLÁŘOVÁ & DVOŘÁK, 1998, *T. szidati* NEUHAUS, 1952, *T. franki* MÜLLER & KIMMIG, 1994, *T. salmanticensis* SIMON-MARTIN & SIMON-VINCENTE, 1999, *T. kowalewskii* (EJSMONT, 1929), *T. filiformis* (SZIDAT, 1938), and *T. ocellata* (LA VALETTE, 1855), although the systematic position of the last three species mentioned is still uncertain (ASPÖCK et al. 2002, AUER & ASPÖCK 2002, HORÁK et al. 1998).

1.3.2. Life Cycle

Figure 15 illustrates the life cycle of *Trichobilharzia* spp. In contrast to their sister genus *Schistosoma* (which parasitizes in humans), adult *Trichobilharzia* exclusively use birds as definitive host, in which they reach sexual maturity (DVOŘÁK et al. 1999, Soldánová et al. 2013). When the eggs are released, they enter water. The fully developed miracidium then hatches and infects a suitable snail intermediate host, in which asexual reproduction occurs. The miracidium develops into a mother sporocyst, which gives rise to several generations of daughter sporocysts. Free-swimming furcocercariae are released

in the aquatic environment, where they penetrate the skin of an appropriate avian definitive host. Once there they shed their tail and transform into schistosomula, which then migrate to the preferred site in the body and mature into adult worms (CDC 2012, SOLDÁNOVÁ et al. 2013).

Most *Trichobilharzia* species (such as *T. szidati* and *T. franki*) migrate in visceral organs and associated blood vessels (HORÁK et al. 1998, SOLDÁNOVÁ et al. 2013). Migration of these “visceral schistosomes” occurs through the circulatory system and the eggs are released with the host’s faeces (CDC 2012, SOLDÁNOVÁ et al. 2013).

In contrast, eight species (including *T. regenti*) settle in the nasal area (HORÁK et al. 1998). Cercariae of these “nasal schistosomes” migrate through the peripheral nerves and central nervous system of the final host. The eggs are deposited in the nasal mucosa, where the miracidia hatch directly and leave the host once it has contact with water (SOLDÁNOVÁ et al. 2013).

Both migration routes as well as the presence of adults and eggs can damage the host’s organs, and “nasal schistosomes” may neurologically harm the host as they feed on nervous tissue (CHANOVÁ et al. 2012, KOLÁŘOVÁ et al. 2013, SOLDÁNOVÁ et al. 2013).

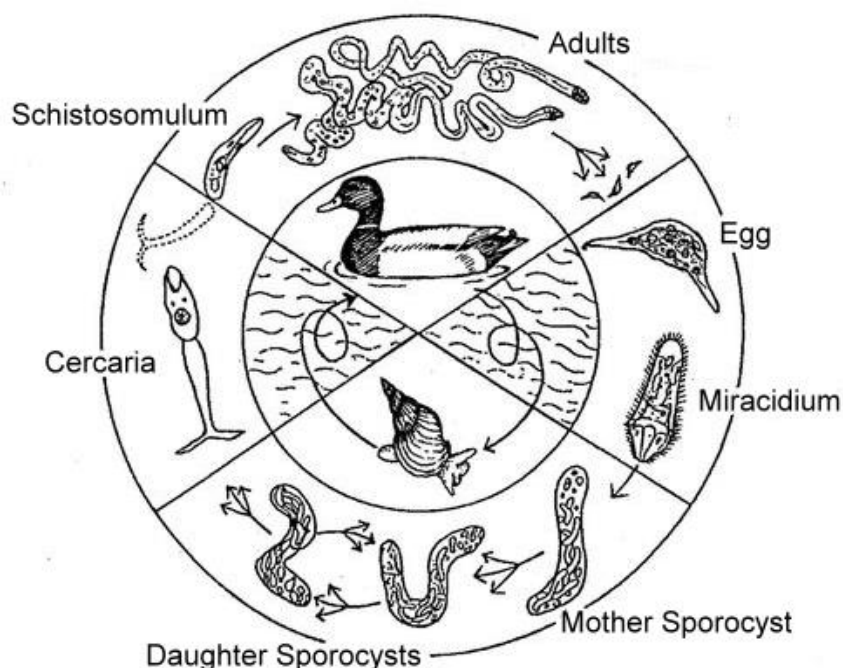


Figure 15. Life cycle of *Trichobilharzia* spp. Modified from HAAS n.d.

Although the most important final hosts of bird schistosomes are waterfowl of the family Anatidae (KORSUNENKO et al. 2010), humans swimming in a burdened waterbody can become infested when furcocercariae accidentally penetrate their skin. As a human is a dead-end host (ASPÖCK et al. 2002), the cercariae are unable to complete their life cycle and they die (HOF & DÖRRIES 2009, LUCIUS & LOOS-FRANK 1997).

1.3.3. Morphology

1.3.3.1. Cercaria

The cercariae are characterized by a bifurcated tail that enables swimming locomotion in water and two eye-spots at the head (figure 16) (DVOŘÁK et al. 1999, KOLÁŘOVÁ et al. 1999). As the connection between the head and the tail is relatively weak, the tail can easily be released upon penetration of the schistosomulum into the skin (CEJKA 1998). According to HORÁK et al. (1998), their body surface is covered by fine spines.

Cercariae of different *Trichobilharzia* species are so similar that they are hardly distinguishable (DVOŘÁK et al. 1999).



Figure 16. Lateral view of an ocellate furcocercaria of *T. regenti*. The acetabulum is protruded (arrow). Scale bar: 200 μ m (Horák et al. 2015).

1.3.3.2. Adult

The adults have a filiform body shape (figure 17). Males and females are morphologically almost identical, apart from their reproductive systems. The total body length of *T. regenti* males is estimated to be about 11 mm (HORÁK et al. 1998). While no information on the body size of females was found, the female in figure 17 seems to have a length of approximately 4.5 mm. That the female is smaller than the male conforms to the measurements of body parts made by HORÁK et al. (1998).

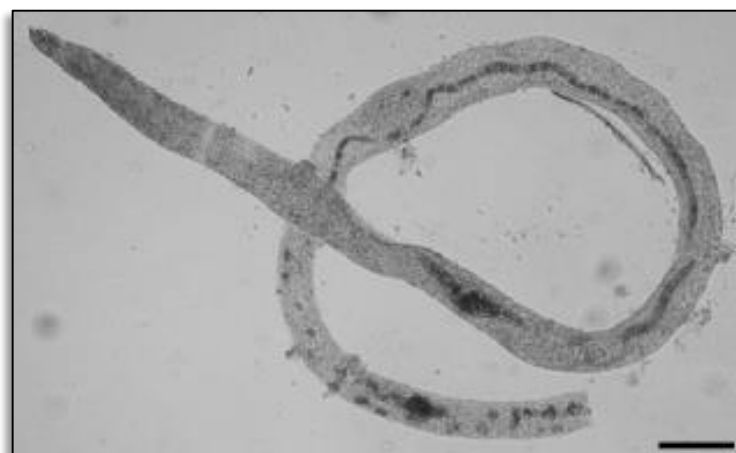


Figure 17. Adult female *Trichobilharzia* sp. Scale bar: 200 μ m (SKIRNISON et al. 2009).

1.3.4. Swimmer's Itch

1.3.4.1. Epidemiology

Swimmer's itch (also: cercarial dermatitis) is a waterborne allergic reaction to the penetration of bird schistosomes through the skin of swimming humans (MORLEY 2016a). Bird schistosomes are abundant and successful pathogens whose transmission to a definitive host is influenced by many abiotic and biotic factors (HORÁK & KOLÁŘOVÁ 2011). Reports of cercarial dermatitis have been made in North America, Australia, New Zealand, Asia, Africa (KOLÁŘOVÁ et al. 1999, KUMAR 1999), and at least 17 European countries (as

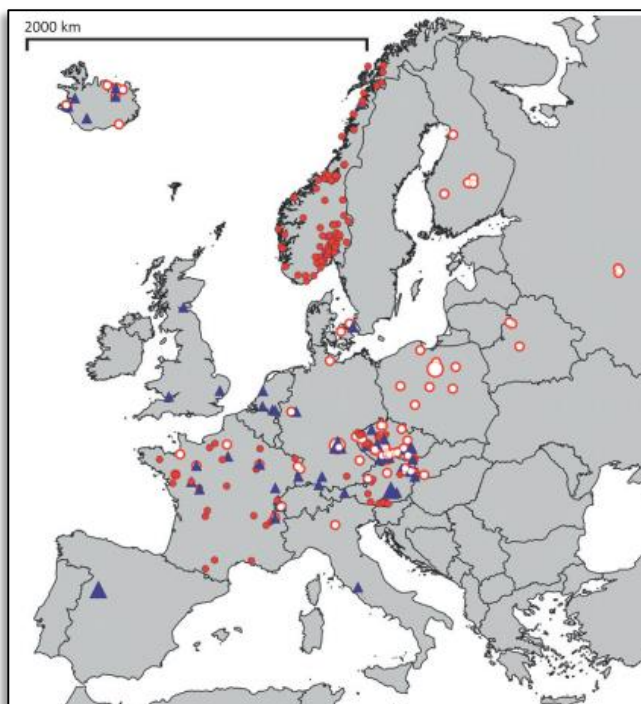


Figure 18. Occurrence of bird schistosomes and reported cases of cercarial dermatitis. Hollow red circles: bird schistosomes. Filled red circles: cases of cercarial dermatitis. Blue triangles: both (SOLDÁNOVÁ et al. 2013).

shown in figure 18). The wide dispersion of this disease illustrates its importance in Europe; however, its prevalence is still underestimated due to its benign pathology and the possibility of confusing it with insect bites or allergic reactions (which leads to many cases remaining unreported) (SOLDÁNOVÁ et al. 2013). In Europe, several foci with high prevalence are known, including regions in both Germany and Austria (AUER et al. 1999, FERTÉ et al. 2005). Most cases have been registered in eutrophic lakes and man-made pond systems used for fish farming, given that species richness is often higher under eutrophic conditions (SOLDÁNOVÁ et al. 2013). Due to continuing anthropogenic changes in the environment and increasing eutrophication, the distribution of bird schistosomes is expanding and cercarial dermatitis has been recognized a re-emerging disease in Europe (HORÁK & KOLÁŘOVÁ 2011, DE GENTILE et al. 1996, cited in HÖRWEG et al. 2006, FERTÉ et al. 2005, SOLDÁNOVÁ et al. 2013). However, the infection rate in European snails is generally low (ranging between 0.05 and 5.0%), whereas the prevalence in birds is up to 74.5%. In eutrophic environments, the infection rates in snails might be much higher, occasionally exceeding 40% (SOLDÁNOVÁ et al. 2013).

As the name “swimmer's itch” suggests, this disease is associated with water activities; as such it is reported predominantly during the summer months, when people pursue

recreational activities in nature (such as swimming in waterbodies) (KOLÁŘOVÁ et al. 1999).

1.3.4.2. Causative Agents

Cercariae of different species of bird schistosomes are known to be the causative agent of human cercarial dermatitis (FERTÉ et al. 2005, HORÁK et al. 2015). In addition to *Trichobilharzia* species, which are the most frequent cause of this disease (KORSUNENKO et al. 2010), infections may also be caused by related genera (including *Gigantobilharzia*, *Bilharziella*, *Dendritobilharzia*, *Austrobilharzia*, and *Allobilharzia*) (AUER & ASPÖCK 2002, KOLÁŘOVÁ et al. 1999, SOLDÁNOVÁ et al. 2013).

1.3.4.3. Clinical Appearance

Swimmer's itch is a transient skin reaction to the entering furcocercariae. Within a few hours after they have penetrated the skin, maculopapular skin eruptions that are characterized by intense itching emerge. Fever, local oedema and enlarged lymph nodes also occur occasionally (KOLÁŘOVÁ et al. 1999, SKÍRNISSON et al. 2009). As the disease is an allergic reaction, the degree of symptoms depends on whether and how often the person has previously been exposed to the furcocercariae. Figure 19 illustrates the development of skin eruptions in a sensitized volunteer.



Figure 19. Development of skin eruptions on the dorsal (1) and ventral (2) parts of the left hand of a sensitized volunteer who has been infected experimentally with *T. szidati*. The photos were taken one (A), two (B), three (C), and four (D) days post infection. **A:** Formation of macules. **B-D:** Formation of papules with vesicles. In B1, a noticeable swelling of the hand can be seen (HORÁK et al. 2015).

The disease typically appears on the legs of an individual who has waded in warm, shallow waters that comprise dense vegetation, as this is primarily where cercariae sojourn (TREMAINE et al. 2009). However, further body parts can be exposed to the surface of the water and thus be infected during swimming.

As a human is not a suitable host, the cercariae die within 24 h and are completely destroyed by the host's immune system after 72 h (GONZÁLEZ 1989, cited in TREMAINE et al. 2009). After one to two weeks, the disease disappears on its own and no consequential damages remain (SKÍRNISSON et al. 2009). The above applies to visceral schistosomes. However, humans can also contract the nasal schistosome *T. regenti*, which is known for its complicated migration through a host's peripheral nerves and central nervous system (SOLDÁNOVÁ et al. 2013). Experimental infections of mice have revealed that *T. regenti* may indeed manage to migrate into the spinal cord (figure 20); the same finding may also apply to other mammals, including humans (KOLÁŘOVÁ et al. 2013).

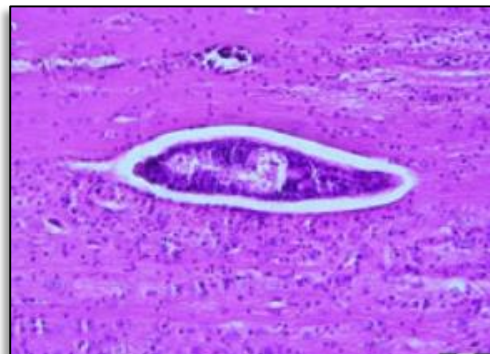


Figure 20. Schistosomulum of *T. regenti* in the mouse spinal cord on day 3 after infection (KOLÁŘOVÁ et al. 2013).

1.3.4.4. Diagnosis

Diagnosis is usually made solely based on clinical observation (TREMAINE et al. 2009). According to KOLÁŘOVÁ et al. 2013, three diagnostic criteria are most important for diagnosing cercarial dermatitis: contact with a natural waterbody within the previous 96 h, an emergence of itchy skin eruptions 12 to 24 h post exposure, and distribution of these lesions only on body parts that had contact with the particular waterbody. Skin biopsies of papules can also be performed to detect schistosomula, but only within 24 to 72 h post infection; thereafter they are destroyed by the host's immune system. It is also possible to detect specific antibodies, but this is not routinely done (KOLÁŘOVÁ et al. 2013).

1.3.4.5. Therapy

As no causative therapy is available, therapy only aims at relieving symptoms. Abirritant powders or gels may be applied, with systemic antihistaminics (such as hydroxyzine) or even corticosteroids (such as triamcinolone) being considered in serious cases (KOLÁŘOVÁ et al. 2013, TREMAINE et al. 2009).

1.3.4.6. Prevention and Control

As the most efficient control mechanism is to interrupt the schistosome life cycle, both the intermediate and definitive hosts can be targeted. The intermediate hosts can be controlled by either eliminating them or preventing their reproduction, which may be achieved using chemicals (e.g. niclosamide), mechanical means (i.e. removal of aquatic vegetation), or biological approaches (i.e. usage of bacterial products). With respect to the definitive hosts, birds may conceivably be treated using chemotherapy; their contact to the particular waterbodies should also be prevented as much as possible. In addition, humans can also modify their personal behaviour (e.g. by not swimming in burdened waterbodies and by using sun cream that contains niclosamide) to prevent infection (KOLÁŘOVÁ et al. 2013).

1.4. Echinostomatidae

The family Echinostomatidae exhibits a remarkable diversity; it comprises 355 heterogeneous species in 50 genera (CHOE et al. 2014, TOLEDO & ESTEBAN 2016), of which *Echinostoma* and *Echinoparyphium* are of particular medical importance (GRABDA-KAZUBSKA et al. 1998, KANEV 1990, cited in HUFFMAN & FRIED 2012, KOSTADINOVA et al. 2003). However, despite their relevance, their taxonomy is still controversial as it is based mainly on morphologies (SAIJUNTHA et al. 2011a), which are often very similar between biologically distinct taxa (KOSTADINOVA & GIBSON 2000, cited in KOSTADINOVA et al. 2003). The most conspicuous characteristic of echinostomatids is the head collar of spines that surrounds the oral sucker, either in one or in two circles (GONÇALVES et al. 2013, GRACZYK & FRIED 1998, TOLEDO & ESTEBAN 2016).

1.4.1. Geographical Distribution

Reports of echinostomatids stem from a variety of Asian countries (including India, Malaysia, Indonesia, China, the Republic of Korea (hereinafter Korea), Taiwan, Thailand, and the Philippines) which gives the impression that hotspots exist in Southeast Asia and the Far East (NOIKONG et al. 2014). However, the broad range of echinostomatids' intermediate and definitive hosts enables them to have a wide geographical distribution that extends beyond Asia (KOSTADINOVA et al. 2003). According to GEORGIEVA et al. (2014), they can also be found in Europe, North and South America, Australia, New Zealand, and Africa (RICHARD 1964, cited in GEORGIEVA et al. 2013).

1.4.2. Life Cycle

The life cycle of echinostomatids is depicted in figure 21. The eggs of an infected definitive host (1) are released through faeces (2). As these eggs are unembryonated, the miracidial development needs a watery environment. After approximately two to three weeks (22 °C), the miracidium hatches (3) and penetrates the head-foot region of the first intermediate host (4), which is a water snail (e.g. a planorbid, lymnaeid, or bulinid snail). It then migrates into the snail's heart, where it transforms into a mother sporocyst (4a). This sporocyst's germinal cells next develop into mother rediae (4b), which give rise to daughter rediae through asexual reproduction (4c) (CDC 2013, TOLEDO & ESTEBAN 2016). Echinostome cercariae then develop and are released into the aquatic environment (5), where they prospect a second intermediate host (6). This host could be a gastropod or one of several other aquatic animals, including a bivalve, crustacean, or vertebrate (such as a fish or amphibian) (CDC 2013, SAIJUNTHA et al. 2011a). The cercariae encyst in metacercariae, which wait to be taken up orally by the definitive host (7). In this case, the host could be a variety of vertebrates; (semi-) aquatic birds and mammals are most common (GONÇALVES et al. 2013, TOLEDO & ESTEBAN 2016), but fish and reptiles are also possibilities (STANEVIČIŪTĖ et al. 2015). The metacercariae excyst in the definitive host's duodenum and migrate to the small intestine, where they attach to the mucosa with their ventral sucker. These juvenile echinostomatids mature to the adult stage (1) and start to release eggs approximately 10 to 16 days after metacercarial ingestion (CDC 2013, TOLEDO & ESTEBAN 2016).

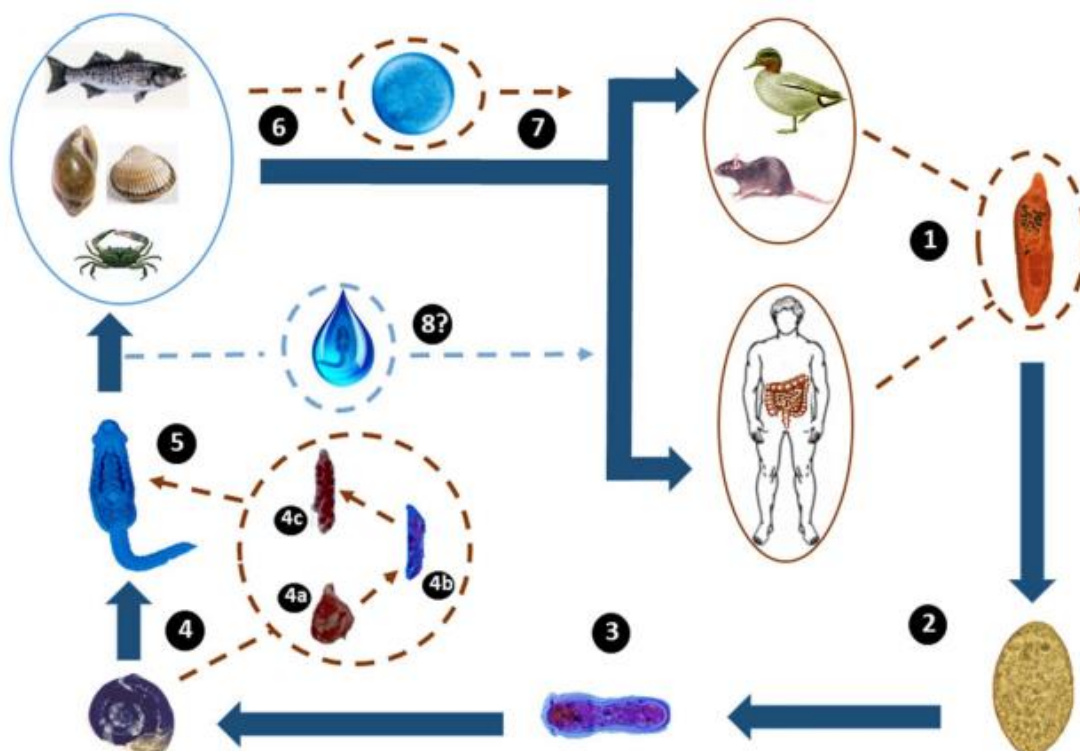


Figure 21. Life cycle of echinostomatids (TOLEDO & ESTEBAN 2016).

Humans usually become part of the life cycle when they ingest the metacercariae through eating an infected intermediate host (ASPÖCK & WALOCHNIK 2007). However, TOLEDO and ESTEBAN (2016) have found that infection also seems possible through drinking water that is contaminated with echinostome cercariae, which will encyst into metacercariae once they are in contact with the host's gastric juice. Their theory is supported by the study on *Echinochasmus liliputanus* conducted by XIAO et al. (1995, cited in TOLEDO & ESTEBAN 2016) in China, which revealed an infection rate of 20.1% in persons who drank unboiled water and 1.5% in persons who did not.

1.4.3. Morphology

1.4.3.1. Cercaria

Cercariae of the family Echinostomatidae have a characteristic head collar of spines that makes them resemble hedgehogs (figure 22) (LUCIUS & LOOS-FRANK 2008). The echinostomatid species are divided into groups based on the number of spines. The cercariae typically have a body length that is between 260 and 430 μm (AZIZI et al. 2015, GEORGIEVA et al. 2013).

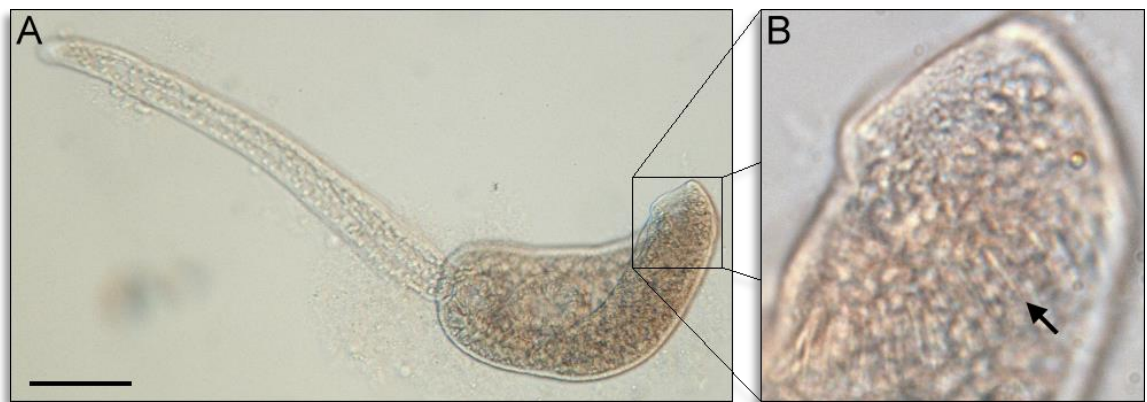


Figure 22. Unstained echinostome cercaria (A) with the head collar of spines (arrow) (B). Scale bar: 100 μm . Orig.

1.4.3.2. Metacercaria

The echinostomatid metacercariae are round to slightly elliptical with a typical diameter between 115 and 195 μm (figures 23 and 24) (AZIZI et al. 2015, CHANTIMA et al. 2013, SOHN et al. 2013). The cyst's wall consists of a transparent outer layer and an opaque inner layer that differ remarkably in total thickness between species; for instance, the cyst's wall is approximately 4.5 μm thick in *Echinostoma macrorchis* but 9.2 μm thick in *Echinostoma revolutum* (CHANTIMA et al. 2013, SOHN et al. 2013).



Figure 23. Unstained echinostomatid metacercaria. The double layer is visible. Scale bar: 50 μ m. Orig.



Figure 24. Unstained echinostomatid redia with several metacercariae. Scale bar: 100 μ m. Orig.

1.4.3.3. Adult

The length of adult echinostomatids varies between species; it is typically between 3 and 10 mm, but it may exceed these boundaries. Given their width of 1 to 3 mm, they are not as filiform as adult schistosomatids (figure 25). They possess a large ventral sucker (which is located close to the oral sucker), as well as a head collar of spines like their cercariae (GRACZYK & FRIED 1998). In addition, the tegument is also covered with scale-like spines (TOLEDO & ESTEBAN 2016).



Figure 25. *Echinostoma revolutum* adult isolated from an experimental host 20 days after infection. Scale bar: 2 mm. Modified from CHANTIMA et al. 2013.

1.4.4. Echinostomosis

1.4.4.1. Epidemiology

Echinostomosis is an important foodborne, intestinal, zoonotic trematodiosis that is caused by echinostomatids (GONÇALVES et al. 2013). Its highest incidence is in South-east Asia and the Far East (ASPÖCK & WALOCHNIK 2007, TOLEDO & ESTEBAN 2016), where it is most prevalent among low-wage earners in rural areas. Echinostomosis is associated with socioeconomic factors such as poverty, poor sanitation, poor economic

conditions, and malnutrition (PANIC et al. 2013, GRACZYK & FRIED 1998). Infections are strongly associated with dietary habits, namely eating raw or insufficiently cooked freshwater molluscs, crustaceans, amphibians, or fish, all of which may serve as second intermediate hosts for the parasites and may contain the infective stage (i.e. the metacercariae) (GRACZYK & FRIED 1998, SAIJUNTHA et al. 2011a, TOLEDO & ESTEBAN 2016).

The average prevalence of echinostome infections varies dramatically between countries and locations. Promiscuous defecation and the use of human excrement for fertilizing fishponds increase both the parasitic burden in the environment and the risk of infection (GRACZYK & FRIED 1998). According to GRACZYK and FRIED (1998), 1% of the Indonesian population excretes echinostome eggs, whereas an average prevalence of 5% was reported for China's Fujian and Guangdong provinces. In Korea, more than 50% of stool samples collected from high school students residing along the Namhan River contained echinostome eggs, while the prevalence was much lower (9.5%) in the country's Koje-myon, Kochang-gun, and Kyongsangnam-do provinces. In northern Thailand, more than 50% of the stool samples from residents contained echinostome eggs (GRACZYK & FRIED 1998). In contrast, few reports of echinostomosis have been made outside of Asia. According to TOLEDO and ESTEBAN (2016), a patient was diagnosed with *Isthmiophora melis* in Romania in 1916; a few cases of *Echinoparyphium recurvatum* and *Echinostoma revolutum* infections were also reported in Egypt and Russia, respectively.

No information on the total number of infected or at-risk people currently exists globally. However, the World Health Organization (WHO) has estimated that 1,000 people are infected with *Acanthoparyphium tyosenense*, 1,000 with *Echinostoma cinetorchis*, 5,000 with *Echinochasmus japonicus*, and as many as 50,000 with *Echinostoma hortense*. Geographical boundaries of echinostomatids have been expanding for a considerable time, due to improved transportation systems, growing international markets, and changing dietary practices in Western countries (TOLEDO & ESTEBAN 2016). Echinostomosis has already been reported in these countries (LELES et al. 2014).

1.4.4.2. Causative Agents

At least 24 species of 10 genera within the family Echinostomatidae are known to cause human echinostomosis (TOLEDO & ESTEBAN 2016); of these, the genus *Echinostoma* is the most important (GRACZYK & FRIED 1998). However, related genera may also cause this disease (CHAI et al. 2012, LUCIUS & LOOS-FRANK 2008). Table 4 provides an overview of the major causative agents.

Table 4. Major causative agents of echinostomosis and their geographical distribution and possible source of infection (TOLEDO & ESTEBAN 2016, supplemented by MORGAN & BLAIR 1998).

Echinostome species	Geographical distribution	Source of infection
<i>Acanthoparyphium tyosenense</i>	Japan, Korea	Bivalves
<i>Artyfechinostomum malayanum</i>	China, India, Indonesia, Lao PDR, Malaysia, Philippines, Singapore, Thailand	Snails
<i>Artyfechinostomum mehrai</i>	India	Snails
<i>Artyfechinostomum oraoni</i>	India, Thailand	Snails
<i>Artyfechinostomum sufrartifex</i>	India	Snail
<i>Echinochasmus fujianensis</i>	China	Not known
<i>Echinochasmus japonicus</i>	China, Japan, Korea, Lao PDR	Not known
<i>Echinochasmus jiufuensis</i>	China	Freshwater fish
<i>Echinochasmus liliputanus</i>	China, Egypt, Palestine, Syria	Freshwater fish, untreated water
<i>Echinochasmus perfoliatus</i>	China, Denmark, Egypt, Hungary, Italy, Japan, Korea, Romania, Russia, Taiwan	Freshwater fish
<i>Echinoparyphium recurvatum</i>	Egypt, Indonesia, Taiwan	Freshwater snails, tadpoles, frogs
<i>Echinostoma angustitestis</i>	China	Freshwater fish
<i>Echinostoma cinetorchis</i>	China, Japan, Korea, Taiwan	Freshwater fish
<i>Echinostoma echinatum</i>	Brazil, Europe, Indonesia, Japan, Lao PDR, Thailand	Mussels
<i>Echinostoma hortense</i>	China, Japan, Korea	Freshwater fish
<i>Echinostoma ilocanum</i>	Cambodia, China, India, Indonesia, Java, Malaysia, Philippines, Thailand	Snails
<i>Echinostoma macrorchis</i>	Indonesia, Japan, Korea, Lao PDR, Taiwan	Snails, frogs
<i>Echinostoma revolutum</i>	Australia, Cambodia, Egypt, Lao PDR, Russia, Taiwan, Thailand, Europe, North America	Snails, tadpoles, clams
<i>Episthmium caninum</i>	India, Thailand	Freshwater fish
<i>Euparyphium</i> sp.	Lao PDR	Not known
<i>Himasthla muelhensi</i>	Colombia, USA	Clams
<i>Hypoderaeum conoideum</i>	Thailand	Snails, tadpoles
<i>Isthmiophora melis</i>	China, Taiwan, Romania, USA	Tadpoles

1.4.4.3. Clinical Appearance

The symptoms of echinostomosis are very similar to those of other trematodioses. They may be more severe, but their manifestation depends on the parasite load. Common symptoms are diarrhoea, epigastric and abdominal pain, fatigue, and weight loss; however, eosinophilia, nausea, acid reflux, vomiting, headache, anorexia, and urinary incontinence can also occur (GRACZYK & FRIED 1998, TOLEDO & ESTEBAN 2016). These symptoms can be attributed to the parasites' head collar of spines, which damages the host's intestinal mucosa as the parasites attach to it. Endoscopic examination may reveal massive intestinal erosions, bleedings, chronic gastritis as well as infiltrating inflammatory cells (GRACZYK & FRIED 1998, TOLEDO & ESTEBAN 2016).

Despite these occasionally severe symptoms, morbidity and mortality are difficult to evaluate. This is because the prolonged latent phase, short acute phase, and frequent asymptomatic presentations lead to many cases remaining undiagnosed (GRACZYK & FRIED 1998).

1.4.4.4. Diagnosis

Echinostomosis is usually diagnosed using microscopy of faeces specimens, in which oval, unembryonated, yellow to brown or silver-white eggs can be observed (figure 26). The eggs also possess a small operculum and a slightly thickened abopercular end. The presence of wrinkles and a thickening at the abopercular end may help in the reliable differentiation between echinostomatid eggs and others, especially fasciolid eggs (GRACZYK & FRIED 1998, TOLEDO & ESTEBAN 2016).

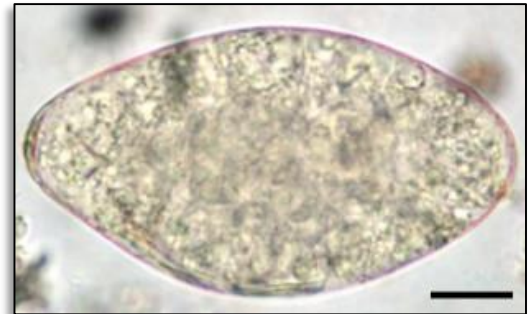


Figure 26. Egg of *Echinostoma hortense*. Scale bar: 20 μ m (TOLEDO & ESTEBAN 2016).



Figure 27. Bending echinostomatid worm observed during colonoscopy in the ascending colon (arrow) (JUNG et al. 2014).

However, species determination is more easily done with the help of adult worms, which can be isolated during gastroduodenal endoscopy or colonoscopy (figure 27) or received after anthelmintic treatment

(GRACZYK & FRIED 1998, TOLEDO & ESTEBAN 2016). The presence of a head collar of spines indicates an echinostome infection with certainty, and the number of spines can help to identify the actual species. While it is also conceivable to use molecular methods to determine the species, such methods are not routinely used (LELES et al. 2014, TANTRAWATPAN et al. 2016).

1.4.4.5. Therapy

The drug of choice for the treatment of echinostomosis is praziquantel, which is highly efficient, has only a few transient side effects, and is inexpensive (FERRAZ et al. 2012, GONÇALVES et al. 2013). It is also generally well tolerated and easy to administer, given that it can be taken orally (GONÇALVES et al. 2013). The recommended therapeutic scheme for trematodioses is a single dose of 25 mg/kg, while a single dose of 10 -

20 mg/kg is considered sufficient for echinostomosis. Praziquantel is also suitable during pregnancy (TOLEDO & ESTEBAN 2016).

As mebendazole shows high cure rates of approximately 85% when given in daily doses of 800 mg for 10 days, it can be considered as alternative. Albendazole is also effective, but no details about the therapeutic scheme have been reported (TOLEDO & ESTEBAN 2016).

1.4.4.6. Prevention and Control

While the prevalence of other foodborne trematodioses (such as paragonimosis, fasciolopsiosis, fasciolosis, clonorchiosis, and metagonomosis) has decreased over time in Asia, it has not changed for human echinostomosis – which remains a public health problem. Control programs initiated by the WHO have been successful for several foodborne trematodioses, but they have not worked for echinostomosis. The reason is the extremely broad specificity for the second intermediate host and the parasite's ability to complete its life cycle without the human being. In other foodborne trematodioses, the second intermediate host is either unnecessary (as is the case with fasciolosis) or a single group of organisms, such as fish (as is true with clonorchiosis, fasciolopsiosis, and metagonimosis) or crustaceans (as for paragonimosis). It is easier to interrupt the parasite's life cycle and eliminate the disease in humans if only a single group of animals serves as intermediate host. However, with echinostomosis the pathogens cannot be eradicated given that the sylvatic life cycle acts as a reservoir (GRACZYK & FRIED 1998). Although it is not possible to control echinostomatids, human infections can be prevented. Several possibilities for at least decreasing the risk of infection exist, such as protecting fish farms from the faeces of definitive hosts, providing thorough therapy to infected animals (including humans), handling and disposing of faeces in a proper manner, and conducting educational campaigns. The last of these aims at eliminating the consumption of raw seafood, which is the most practical and efficient prevention measure that can be undertaken. Another possibility is examining food for metacercariae, but this is not very feasible (GRACZYK & FRIED 1998, TOLEDO & ESTEBAN 2016).

1.5. Purpose of the Study

A detailed understanding of the prevalence of medically important digeneans in Austria is important for conducting a profound medical risk assessment. In this project 47 locations in the surroundings of Vienna were investigated with regard to the occurrence of freshwater snails, which are the intermediate hosts, and their trematode burden. These

investigations expand the data for Austria, which are to date incomplete (DVOŘÁK et al. 1999, KONEČNY et al. 1999).

The first aim was to gather detailed information on the occurrence of *Radix* spp. and *Lymnaea stagnalis*. These freshwater snails serve as intermediate host for a variety of trematodes, e.g. *Radix* spp. can harbour *Echinoparyphium recurvatum*, and *Trichobilharzia szidati* can develop in *L. stagnalis*. A monitoring of these intermediate hosts was therefor performed in different habitat types such as floodplains, rivers, ponds, and artificial waterbodies.

The second aim was to assess the overall infection rate of the snails with digenean trematodes. For this purpose the snails collected were examined microscopically and photos of trematode larvae were taken.

The third aim was the species determination of the schistosome and echinostome larvae. As morphological differentiation between closely related species is impossible, bio-molecular methods were applied. Whole cell DNA was therefor isolated from the larvae and specific fragments were amplified through PCR with suitable primers. For species identification, the amplicons were sequenced and compared to published sequences of trematode reference species from GenBank by multiple sequence alignments.

2. Material and Methods

2.1. Collection of Snail Intermediate Hosts

Snails of the genera *Radix* and *Lymnaea* were collected in Lower Austria and Burgenland, in locations that can be grouped into five clusters. Their geographical distribution within Austria is shown in figure 28.

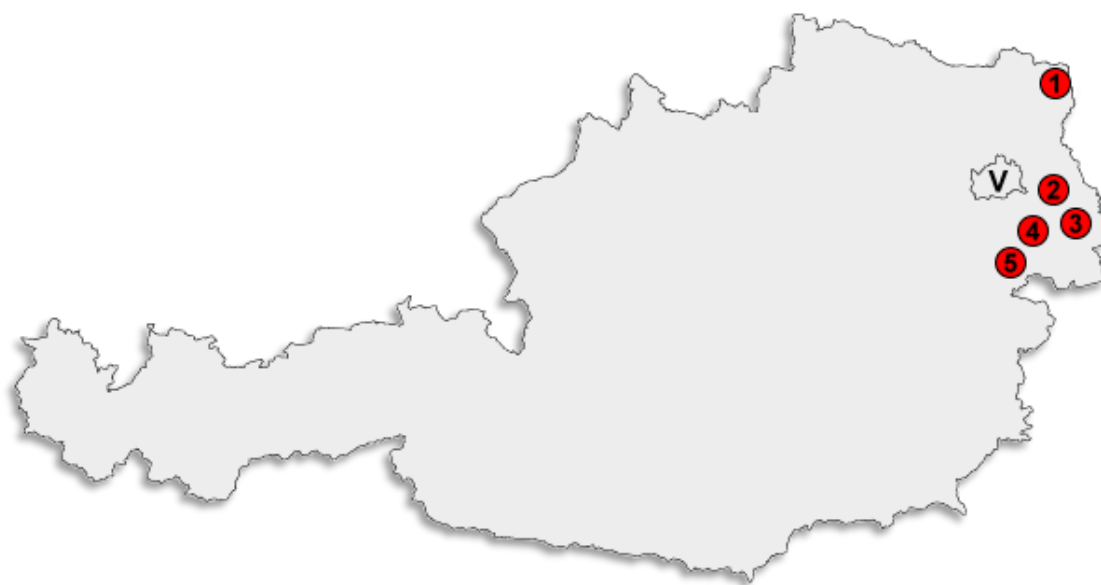


Figure 28. Clusters of sampling sites in Austria. V = Vienna. Circles 1 to 5 indicate clusters of visited locations, which are further presented in table 5 and figures 29 through 42. 1: Morava floodplains, 2: Danube floodplains, 3: Leitha floodplains, 4: Leitha Mountains, 5: Wulka River, and artificial waterbodies (such as swimming ponds). Modified from MEINBEZIRK 2016.

A total of 47 locations were visited, including sites in the Danube floodplains, the Leitha floodplains, the Leitha Mountains, the Morava floodplains, and the Wulka River. In order to ensure that a variety of habitats were covered, artificial waterbodies such as swimming ponds were also explored. Table 5 presents an overview of these locations, as well as their coordinates.

Table 5. Locations visited in Austria. The 47 sampling sites were either natural or man-made.

Abbreviation	Location	N°	E°	Kind of waterbody
MF01	Rabensburg	48,65153	16,90687	Morava floodplains
MF02	Rabensburg	48,64436	16,92278	
MF03	Rabensburg	48,64403	16,92314	
MF04	Hohenau	48,60102	16,93313	
MF05	Hohenau	48,60008	16,93256	
MF06	Ringelsdorf	48,56508	16,93822	
MF07	Ringelsdorf	48,56781	16,93684	
MF08	Ringelsdorf	48,56923	16,93059	
DF01	Fischamend	48,11756	16,65053	Danube floodplains
DF02	Orth	48,12517	16,69834	
DF03	Orth	48,123733	16,704947	
DF04	Orth	48,129347	16,706917	
DF05	Orth	48,129786	16,698881	
DF06	Fischamend	48,11501	16,65836	
DF07	Fischamend	48,12423	16,60568	
DF08	Regelsbrunn	48,11244	16,77998	
DF09	Regelsbrunn	48,11304	16,77917	
DF10	Fischamend	48,11942	16,62048	
LF01	Rohrau	48,06839	16,86828	Leitha floodplains
LF02	Rohrau	48,06791	16,86746	
LF03	Pachfurth	48,02846	16,82382	
LF04	Hollern	48,07316	16,89389	
LF05	Pachfurth	48,02866	16,82469	
LF06	Rohrau	48,06849	16,86640	
LF07	Rohrau	48,06129	16,85851	
LF08	Rohrau	48,06458	16,86017	
LF09	Prellenkirchen	48,06559	16,96307	
LF10	Pama	48,03435	17,03483	
LF11	Pama	48,04809	17,00839	
LM01	St. Anna in der Wüste	47,95266	16,60214	Leitha Mountains
LM02	Mannersdorf	47,97573	16,62483	
WR01	Hirm	47,78808	16,44192	Wulka River
WR02	Wulkaprodersdorf	47,79259	16,48074	
WR03	Wulkaprodersdorf	47,79275	16,48095	
WR04	Trausdorf	47,81126	16,53366	
WR05	Trausdorf	47,81586	16,56409	
WR06	Pöttelsdorf	47,74890	16,43317	
WR07	Pöttelsdorf	47,74837	16,43161	
WR08	Mattersburg	47,73855	16,40722	
WR09	Zemendorf	47,75272	16,45619	
WR10	Zemendorf	47,75886	16,45284	
WR11	Stöttera	47,76759	16,46247	
AW01	St. Margarethen	47,80036	16,62080	Artificial waterbodies
AW02	Hirm	47,78668	16,45002	
AW03	Hirm	47,78519	16,45413	
AW04	Antau	47,76765	16,47276	
AW05	Antau	47,76602	16,0247475	

2.1.1. Morava Floodplains

The Morava floodplains are located in the tri-state region of Austria, Slovakia, and the Czech Republic (figure 28, cluster 1). Figure 29 shows the eight sampling sites that span a 10 km-long section of the Morava River, which is a tributary to the Danube River. Figures 30 and 31 illustrate the typical appearance of the Morava floodplains, which are characterized by lush grasslands and forests that periodically flood, especially in the spring. The Morava floodplains are among the most species rich river basins in Austria and have been designated as one of the country's "Green Hearts" (WWF 2016a).



Figure 30. Typical appearance of the Morava floodplains. Lush grasslands and forests, which are flooded regularly, shape the appearance. (© HÖRWEG)



Figure 29. Overview of the eight locations of the Morava floodplains. Modified from GOOGLE MAPS 2016.



Figure 31. Typical appearance of the Morava floodplains. The muddy banks are ideal habitats for pond snails. (© HÖRWEG)

2.1.2. Danube Floodplains

The Danube crosses ten European countries on its way from the Black Forest in Germany to the Black Sea in Romania. It links different natural and artificial landscapes and plays an important role in reducing floods, improving water quality, and maintaining biodiversity (WWF-AUEN-INSTITUT). The Austrian Danube floodplains are located east of Vienna (figure 28, cluster 2). Their typical appearance (which is illustrated in figures 32

and 33) is similar to that of the Morava floodplains. Figure 34 depicts the ten sampling sites that span a 13 km-long section of the Danube River.



Figure 32. Typical appearance of the Danube floodplains. The river system and its distributaries look similar to the Morava floodplains. (© HÖRWEG)



Figure 33. Location DF06 in the Danube floodplains, which is a periodically flooded area. (© HÖRWEG)



Figure 34. Overview of the ten locations of the Danube floodplains. Modified from GOOGLE MAPS 2016.

2.1.3. Leitha Floodplains

The Leitha River, which is another important tributary to the Danube, is located southeast of Vienna (figure 28, cluster 3). Its typical appearance is shown in figure 35; however, as the Leitha has been regulated throughout almost its total length, flooding events are today rare (TRUXA 2012). The eleven sampling



Figure 35. Typical appearance of the Leitha River. Massive human interventions have resulted in only rare flooding events occurring today. (© HÖRWEG)

sites, which are located on the Leitha River and adjacent channels, are shown in figure 36.



Figure 36. Overview of the 11 locations in the Leitha floodplains and adjacent channels. Modified from GOOGLE MAPS 2016.

2.1.4. Leitha Mountains

The Leitha Mountains, which are southeast of Vienna (figure 28, cluster 4), are an offshoot of the Alps. In contrast to the floodplains, they consist of deep forests with isolated ponds (as illustrated in figure 37). These waterbodies have only a slight stream or are even standing; they also contain much more rotten vegetation and macrophytes than the locations in the floodplains. Figure 38 shows the two sampling sites in the Leitha Mountains, both of which are in the northern part in Lower Austria.



Figure 37. Pond in the Leitha Mountains (LM01), which comprises a lot of rotten material. (© HÖRWEIG)



Figure 38. Overview of the two locations in the Leitha Mountains. Modified from GOOGLE MAPS 2016.

2.1.5. Wulka River

The Wulka River, which is found south of Vienna (figure 28, cluster 5), is an important tributary to Lake Neusiedl in Burgenland. Its typical appearance is shown in figure 39; it is marked by a mostly low water depth. Figure 40 illustrates the eleven sampling sites on the river and its tributaries.



Figure 39. The Wulka River. The Wulka is a relatively small river with a low water level in most parts. (© HÖRWEG)



Figure 40. Overview of the locations in the south of Vienna. The red flags (WR01 to WR11) indicate the Wulka River and its tributaries, while the yellow (AW01 to AW05) show artificial waterbodies such as swimming ponds. Modified from GOOGLE MAPS 2016.

2.1.6. Artificial Waterbodies

The artificial waterbodies visited were also in the south of Vienna (figure 28, cluster 5); their exact localisations can be inferred from the yellow marks in figure 40. These locations were man-made and are being used as retention basins (figure 41) or for swimming (figure 42). As such, both the vegetation and biodiversity were less diverse than in some of the other locations visited.



Figure 41. Retention basin (AW04). Although it is completely man-made, a sandy ground has been deposited and grass-like vegetation is growing. (© HÖRWEG)



Figure 42. Private swimming pond (AW03) with reeds and algae growing on the ground. (© HÖRWEG)

The *Radix* species were determined with the help of the distinguishing characteristics identified by SCHNIEBS et al. (2011), which are summarized in table 6.

Table 6. Distinguishing characteristics of the European *Radix* species (SCHNIEBS et al. 2011).

Character	<i>Radix balthica</i>	<i>Radix lagotis</i>	<i>Radix labiata</i>	<i>Radix auricularia</i>
Shape of the line tangential to the whorls in adult shells	Usually convex, rarely concave	convex	Usually straight	Concave
Columella fold in adult shells	weak	weak	weak	Distinct
Typical mantle pigmentation above lung cavity	Many medium-sized light, distinct spots on dark background	A few light distinct or blurred medium-sized spots on dark background	Many small light blurred dots on dark background	A few large white spots on dark background
Presence of pigmentation reminding of "freckles" on tentacles, head and foot	no	no	no	Yes
Length of the bursa duct (if bursa is filled)	Between nearly not visible and half of the length of the bursa	From half to about 2/3 of the length of the bursa	From short to about nearly one third of the length of the bursa	Very long, usually as long as the provaginal duct
Position of bursa and bursa duct	Above vagina and provaginal duct	Above vagina, provaginal duct, uterus and prostate	Behind vagina and above provaginal duct	Near pericardium
Colour of praeputium	Usually uniform dark grey or dark bluish grey	Light to dark bluish grey	Uniform grey greenish or dark grey	With dorsal pigmentation reminding of "freckles"

Table 7 provides information on snails collected during the eight sampling trips we made to Lower Austria and Burgenland in 2014 and 2015. We collected 117 individuals of *Lymnaea stagnalis* and 505 *Radix* spp., for a total of 622 individuals.

Table 7. Overview of the sampling trips. Only locations where snails were found are mentioned. The sampling trip in April 2015 revealed no snails and is therefore not listed.

Date	Location	Snail species	Number
29.07.2014	LM01	<i>Radix labiata</i>	100
11.05.2015	LF07	<i>Lymnaea stagnalis</i>	7
		<i>Radix labiata</i>	4
24.07.2015	DF04	<i>Lymnaea stagnalis</i>	1
		<i>Radix auricularia</i>	11
	DF02	<i>Radix auricularia</i>	1
28.07.2015	LM01	<i>Radix labiata</i>	12
	AW04	<i>Radix auricularia</i>	8
	AW03	<i>Lymnaea stagnalis</i>	65
05.08.2015	DF06	<i>Radix auricularia</i>	14
	DF01	<i>Radix auricularia</i>	160
	DF10	<i>Radix auricularia</i>	14
	DF08	<i>Radix labiata</i>	4
		<i>Radix lagotis</i>	1
	DF09	<i>Lymnaea stagnalis</i>	20
14.08.2015	MF01	<i>Lymnaea stagnalis</i>	15
		<i>Radix auricularia</i>	5
	MF02	<i>Radix auricularia</i>	2
04.09.2015	MF07	<i>Radix balthica</i>	14
14.09.2015	LF05	<i>Lymnaea stagnalis</i>	9
	LM01	<i>Radix balthica</i>	147
	LF03	<i>Radix balthica</i>	8

2.2. Macro- and Microscopic Analyses

2.2.1. Measurement of Shell Size

A ruler was used to measure the shell size from the apex to the base (figure 43).

2.2.2. Dissection

The “cercariae release test” was performed first. Immediately after they were collected, the snails were separated in glasses of water and positioned in a place that is exposed to the sun for at least several hours per day. Diffuse water indicated the release of cercariae, which were then directly isolated and conserved in 70% ethanol. Snails that did not



Figure 43. Exemplary shell of *Radix labiata*. Modified from GALLEGO 2015.

release cercariae were dissected using tweezers and investigated with the help of a Leica Wild M3Z stereo microscope. Special attention was paid to the midgut gland. If parasitic life stages were visible, they were isolated using a dissecting needle and conserved in 70% ethanol. Some of the isolated parasites were used to make glycerol preparations for microscopy, while others were reserved for molecular analysis.

2.2.3. Glycerol Preparation

The parasites were incubated at room temperature with two to four drops borax carmine. After approximately one hour, they were transferred into ethanol. Four drops of a hydrochloric acid/ethanol mixture were added. This mixture was aspirated when the envelope of the parasites was decolorized and inner structures were still dyed. A glycerol/ethanol mixture was then added and the samples were slightly covered with a glass slide before being incubated o/n at 40 °C (which vaporized the ethanol slowly and evenly covered the parasites with the remaining glycerol). The samples were then mounted on object slides with pure, water-free glycerol. Two preparations of each sample were made: one with staining and one without. Samples without staining were immediately incubated with the glycerol/ethanol mixture.

The preparations were investigated using a Leitz Diaplan microscope and classified morphologically as far as possible with the help of the determination key published by Mikeš (2001) (figure 44). A Nikon DS-Fi 1 camera was used to photograph the samples.

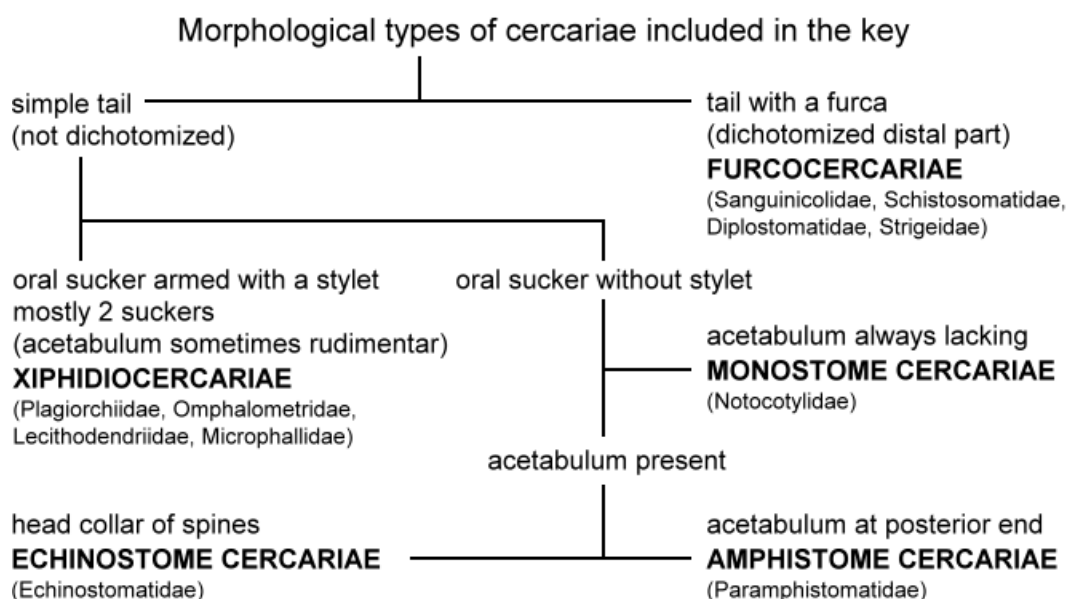


Figure 44. Determination key for cercariae. Morphological determination of cercariae depends on the structure of the tail as well as on the presence of a stylet and the number and position of suckers. Exemplary trematode families are given. Modified from Mikeš 2001.

2.3. Molecular Investigations

2.3.1. Samples

All samples with a possible trematode infection were investigated with a universal trematode Polymerase Chain Reaction (PCR). Parasites that were microscopically identified as echinostomatids or schistosomatids were further analysed using specific PCRs and selected samples were also sequenced in order to determine their species. Table 8 provides information on the 16 samples chosen for sequencing.

Table 8. Samples chosen for sequencing. A total of 16 samples were selected for sequencing in order to determine their species.

Date	Location	Sample	Microscopic result
29.07.2014	LM01	1	Echinostome cercariae
		7	Echinostome cercariae
		8	Echinostome cercariae
		100	Echinostome cercariae
24.07.2015	DF04	9	Echinostome cercariae
28.07.2015	LM01	1	Echinostome cercariae
		2	Echinostome cercariae
		3	Echinostome cercariae
	WR12	1	Echinostome cercariae
05.08.2015	AW03	1	Furcocercariae
	DF10	13	Echinostome cercariae
	DF09	6	Echinostome cercariae
14.09.2015	LM01	5	Echinostome cercariae
		9	Echinostome cercariae
		10	Echinostome cercariae
		12	Echinostome cercariae

2.3.2. Primer Selection

2.3.2.1. Universal Trematode Primers

In order to detect all different trematodes, it is necessary to use a primer pair that binds to a highly conserved region. The universal primer pair of TremF/TremR binds within the 18S rDNA and is therefore suitable for detecting trematodes (table 9).

Table 9. Sequences of the primer pair used for the detection of trematodes.

	TremF (forward)	TremR (reverse)	Amplicon length
Trem	GGTTCCTTAGATCGTACATGC	GTACTCATTCGAATTACGGAGC	~ 430 bp

In order to test specificity, the primer sequences were blasted against Digenea, Aspidogastrea, Nematoda, Cestoda, Ascomycota, and Bacteria as well as against their intermediate hosts (i.e. Lymnaeidae). Table 10 provides information on the maximum values of accordance with these different comparison groups.

Table 10. Maximum values of accordance of the Trem primers with the comparison groups.

Comparison Group	TremF	TremR
Digenea (Taxid: 6179)	21/21 (100%)¹⁾²⁾ <i>Austrodiplostomum ostrowskiae</i> (GenBank: KT728782.1)	22/22 (100%)¹⁾²⁾ <i>Diplostomidae</i> sp. (GenBank: KT728779.1)
Aspidogastrea (Taxid: 27867)	21/21 (100%) <i>Multicalyx elegans</i> (GenBank: DQ482610.1)	20/22 (91%)¹⁾²⁾ <i>Multicalyx elegans</i> (GenBank: DQ482610.1)
Nematoda (Taxid: 6231)	18/21 (86%) <i>Uncultured nematode</i> (GenBank: AF530550.1)	17/22 (77%)¹⁾ <i>Strongylus vulgaris</i> (GenBank: LM229732.1)
Cestoda (Taxid: 6199)	18/21 (86%) <i>Schistocephalus solidus</i> (GenBank: LL903846.1)	18/22 (82%)¹⁾ <i>Mesocostoides corti</i> (GenBank: LM531208.1)
Ascomycota (Taxid: 4890)	19/21 (90%)²⁾, 1 gap <i>Clavispora lusitaniae</i> (GenBank: JQ698972.1)	19/22 (86%)²⁾, 1 gap <i>Conioscypha</i> sp. (GenBank: HF937348.1)
Bacteria (Taxid: 2)	20/21 (95%), 1 gap <i>Campylobacter curvus</i> (GenBank: CP000767.1)	18/22 (82%) <i>Spirosoma radiotolerans</i> (GenBank: CP010429.1)
Lymnaeidae (Taxid: 6521)	12/21 (57%)¹⁾²⁾ <i>Lymnaea diaphana</i> (GenBank: JF909497.1)	19/22 (86%)¹⁾²⁾, 1 gap <i>Galba cubensis</i> (GenBank: JN614334.1)

Notes: Unless specified otherwise, all alignments had no gaps. 1) Several species with these values of accordance were found. 2) Several GenBank entries of this species with this value of accordance were found.

2.3.2.2. Schistosomatidae Primers

According to LOCKYER et al. (2003), the mitochondrial Cytochrome C Oxidase Subunit 1 gene (CO1) is suitable for discriminating between schistosome species. Primer sequences and approximate amplicon lengths are presented in table 11.

Table 11. Sequences of the primer pair used for the detection of schistosomes.

	CO1F (forward)	CO1R (reverse)	Amplicon length
CO1	TCTTTRGATCATAAGCG	TAATGCATMGGAACAAAAACA	~ 1,200 bp

In addition to the groups against which the trematode primers were blasted, the CO1 primer sequences were also blasted against the sister groups Sanguinicolidae, Diplostomatidae, and Strigeidae (table 12). Specificity runs were carried out with cercariae of *Trichobilharzia* sp., and the DNA was used in 1:10, 1:100, and 1:1000 dilutions as well as in an undiluted form (namely 1 µl, 3 µl, and 6 µl DNA).

Table 12. Maximum values of accordance of the CO1 gene primers with the comparison groups.

Comparison Group	CO1F	CO1R
Digenea (Taxid: 6179)	17/17 (100%)¹⁾²⁾ <i>Schistosoma mansoni</i> (GenBank: HE601612.1)	20/20 (100%)¹⁾²⁾ <i>Acanthoparyphium</i> sp. (GenBank: KJ956295.1)
Aspidogastrea (Taxid: 27867)	9/17 (53%) <i>Cotylogaster basiri</i> (GenBank: AY222164.1)	9/20 (45%) <i>Multicalyx elegans</i> (GenBank: DQ345325.1)
Nematoda (Taxid: 6231)	16/17 (94%)¹⁾ <i>Rhabditophanes</i> sp. (GenBank: LK995546.1)	18/20 (90%)¹⁾ <i>Strongylus vulgaris</i> (GenBank: LM271723.1)
Cestoda (Taxid: 6199)	17/17 (100%)¹⁾ <i>Diphyllbothrium latum</i> (GenBank: LL590800.1)	20/20 (100%)¹⁾²⁾ <i>Hymenolepis nana</i> (GenBank: KT951722.1)
Ascomycota (Taxid: 4890)	16/17 (94%)¹⁾²⁾ <i>Saccharomyces cerevisiae</i> (GenBank: CP005378.1)	17/20 (85%) <i>Schizosaccharomyces octosporus</i> (GenBank: XM_013161198.1)
Bacteria (Taxid: 2)	17/17 (100%)¹⁾²⁾ <i>Campylobacter jejuni</i> (GenBank: CP012149.1)	17/20 (85%)¹⁾ <i>Bacillus thuringiensis</i> (GenBank: CP009651.1)
Sanguinicolidae (Taxid: 45375)	14/17 (82%), 1 gap <i>Sanguinicolid</i> sp. (GenBank: AY465870.1)	10/20 (50%) <i>Chimaerohemecus trondheimensis</i> (GenBank: AY157239.1)
Diplostomatidae (Taxid: 57254)	9/17 (53%)¹⁾²⁾ <i>Alaria marcianae</i> (GenBank: KT254039.1)	14/20 (70%)²⁾, 1 gap <i>Posthodiplostomum</i> sp. (GenBank: HM064856.1)
Strigeidae (Taxid: 116872)	11/17 (65%) <i>Ichthyocotylurus pileatus</i> (GenBank: HM064721.1)	20/20 (100%)¹⁾²⁾ <i>Apatemon</i> sp. (GenBank: KT334182.1)
Lymnaeidae (Taxid: 6521)	14/17 (82%) <i>Lymnaea stagnalis</i> (GenBank: AF129397.1)	12/20 (60%)¹⁾²⁾ <i>Radix balthica</i> (GenBank: KT337605.1)

Notes: Unless specified otherwise, all alignments had no gaps. 1) Several species with these values of accordance were found. 2) Several GenBank entries of this species with this value of accordance were found.

2.3.2.3. Echinostomatidae Primers

According to GEORGIEVA et al. (2013), the mitochondrial Nicotinamid Adenin-Dinucleotide Dehydrogenase Subunit 1 gene (ND1) is well suited for distinguishing between closely related echinostomes. The primer pair is illustrated in table 13.

Table 13. Sequences of the primer pair used for the detection of echinostomes.

	NDJ11 (forward)	NDJ2A (reverse)	Amplicon length
ND1	AGATTCGTAAGGGGCCTAATA	CTTCAGCCTCAGCATAAT	~ 530 bp

The primer sequences were also blasted against Digenea, Aspidogastrea, Nematoda, Cestoda, Ascomycota, Bacteria, and the intermediate hosts (i.e. Lymnaeidae) (table 14). Similar specificity runs were carried out as for the schistosomes, but using echinostome cercariae.

Table 14. Maximum values of accordance of the ND1 gene primers with the comparison groups.

Comparison Group	NDJ11	NDJ2A
Digenea (Taxid: 6179)	21/21 (100%)¹⁾²⁾ <i>Echinostoma caproni</i> (GenBank: AJ564378.1)	18/18 (100%)¹⁾ <i>Echinostoma revolutum</i> (GenBank: KT726380.1)
Aspidogastrea (Taxid: 27867)	9/21 (43%)¹⁾ <i>Rohdella</i> sp. (GenBank: KC181852.1)	8/18 (44%)¹⁾ <i>Rohdella</i> sp. (GenBank: KC181852.1)
Nematoda (Taxid: 6231)	20/21 (95%), 1 gap <i>Ascaris lumbricoides</i> (GenBank: LK875972.1)	17/18 (94%)²⁾ <i>Angiostrongylus cantonensis</i> (GenBank: LK949792.1)
Cestoda (Taxid: 6199)	21/21 (100%)¹⁾²⁾ <i>Echinococcus granulosus</i> (GenBank: DQ080021.1)	18/18 (100%) <i>Schistocephalus solidus</i> (GenBank: LL901203.1)
Ascomycota (Taxid: 4890)	16/21 (76%) <i>Debaryomyces hansenii</i> (GenBank: CR382136.2)	17/18 (94%) <i>Bipolaris maydis</i> (GenBank: XM_014217976.1)
Bacteria (Taxid: 2)	16/21 (76%)¹⁾²⁾ <i>Burkholderia xenovorans</i> (GenBank: CP008760.1)	18/18 (100%) <i>Synechocystis</i> sp. (GenBank: CP007542.1)
Lymnaeidae (Taxid: 6521)	17/21 (81%)¹⁾²⁾, 1 gap <i>Radix auricularia</i> (GenBank: KP098540.1)	12/18 (67%) <i>Lymnaea stagnalis</i> (GenBank: X96933.1)

Notes: Unless specified otherwise, all alignments had no gaps. 1) Several species with these values of accordance were found. 2) Several GenBank entries of this species with this value of accordance were found.

2.3.3. DNA Isolation

The QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) was used for DNA isolation. The isolated parasites were centrifuged for 10 min at 6,000 x g with the 1-15 Microfuge (Sigma-Aldrich, St. Louis, USA). The ethanol was then removed and 200 µl 0.9% NaCl solution, 20 µl Proteinase K (Qiagen, Hilden, Germany), and 200 µl AL Buffer (Qiagen, Hilden, Germany) were added. After being vortexed, the samples were incubated in the Thermomixer comfort (Eppendorf, Hamburg, Germany) for 10 min (56 °C, 500 rpm). They were then centrifuged (30 sec, 6,000 x g). The next step was to add 200 µl 100% ethanol and vortex the samples again. After an additional centrifugation step was taken (30 sec, 6,000 x g), the samples were loaded onto spin columns and centrifuged for an additional 1 min at 6,000 x g. The flow-through was discarded and the spin columns were placed in new collection tubes. In order to wash the DNA, 500 µl AW1 Buffer (Qiagen, Hilden, Germany) was added onto the columns and the samples were centrifuged again (1 min, 6,000 x g). The flow-through was discarded. Thereafter 500 µl AW2 buffer (Qiagen, Hilden, Germany) was added onto the columns and they were centrifuged again (3 min, 18,000 x g). The spin columns were placed in new collection tubes and centrifuged (1 min, 18,000 x g). The spin columns were then placed in 1.5 ml microcentrifuge tubes. The DNA was eluted by adding 200 µl AE Buffer (Qiagen, Hilden, Germany) onto the columns and incubating for 1 min at room temperature. The samples were then centrifuged (1 min, 6,000 x g). The eluted DNA was measured using a NanoDrop ND-1000 spectrophotometer (peqlab, Erlangen, Germany) and then stored at -20 °C for further examination.

2.3.4. Polymerase Chain Reaction

The pipetting procedure was carried out on an HSP 12 sterile bench (Heraeus, Hanau, Germany). A mastermix that contained the following for each sample was prepared: 5 µl PCR Buffer (Solis Biodyne, Tartu, Estonia), 5 µl MgCl₂ (25 mM, Solis Biodyne, Tartu, Estonia), 11 µl DNase free water (Carl Roth, Karlsruhe, Germany), 1 µl dNTP mix (20 mM each, Solis Biodyne, Tartu, Estonia), 5 µl forward primer (10 pmol/µl, Microsynth, Balgach, Switzerland), 5 µl reverse primer (10 pmol/µl, Microsynth, Balgach, Switzerland), and 0.25 µl Hot FirePol DNA Polymerase (5 U/µl, Solis Biodyne, Tartu, Estonia). Trematode, Schistosomatidae, or Echinostomatidae primers were used. The amount of DNA added varied from 1 to 3 µl. The reaction mix was filled to 50 µl of volume with DNase free water (Carl Roth, Karlsruhe, Germany). All reagents were vortexed in advance, except for the DNA, which was mixed using a P1000 pipette (Eppendorf, Hamburg, Germany). In order to remove air bubbles, the tubes were centrifuged briefly

(30 sec, 6,000 x g). The Mastercycler ep gradient S (Eppendorf, Hamburg, Germany) was used to perform PCR, with the conditions that are summarized in table 15.

The Trem PCR program is a standard amplification program taken from GAST et al. (1996), but the annealing temperature used was increased. The CO1 and ND1 programs were taken respectively from LOCKYER et al. (2003) and GEORGIEVA et al. (2013), although phase lengths were extended.

Table 15. Overview of the different PCR programs.

	Trem PCR		CO1 PCR		ND1 PCR	
Initial denaturation	15 min 95 °C		15 min 95 °C		15 min 95 °C	
Denaturation	1 min 95 °C	30 cycles	1 min 95 °C	40 cycles	1 min 95 °C	35 cycles
Annealing	2 min 52 °C		1 min 52 °C		1 min 48 °C	
Extension	3 min 72 °C		2 min 72 °C		1 min 72 °C	
Final extension	7 min 72 °C		7 min 72 °C		7 min 72 °C	

2.3.5. Agarose Gel Electrophoresis

Generally, 2% agarose gels (Sigma-Aldrich, St. Louis, USA) containing 2.5 µl (50 ml gel), 5 µl (100 ml gel), or 7.5 µl (150 ml gel) 10,000x GelRed™ (Biotium, Hayward, USA) were prepared. Air bubbles were eliminated with the help of a pipette tip. 25 µl PCR product, and 5 µl 10x Loading Buffer (Sigma-Aldrich, St. Louis, USA) were mixed and loaded into the slots along with 30 µl of 50 bp DNA step ladder (Sigma-Aldrich, St. Louis, USA). The gels ran in a horizontal electrophoresis system (Bio-Rad, Berkeley, USA) at 100 to 140 V and 300 mA until the Loading Buffer reached the lower margin of the gel.

2.3.6. Purification of Gel Bands

The DNA bands were visualized by ultraviolet (UV) light in a gel imager (Intas, Göttingen, Germany). A scalpel was used to excise one band per sample. The DNA was purified with an Illustra™ GFX™ Gel Band Purification Kit (GE Healthcare, Little Chalfont, England). The excised gel bands were weighed in 1.5 ml microcentrifuge tubes. A total of 10 µl Capture buffer type 3 (GE Healthcare, Little Chalfont, England) was added for each 10 mg gel slice. If the gel slice weighed less than 300 mg, 300 µl Capture buffer type 3 was added. These mixtures were heated for approximately 15 min (60 °C, 500 rpm) in the Thermomixer comfort (Eppendorf, Hamburg, Germany) until the gel was dissolved. The solutions were loaded onto assembled GFX MicroSpin columns. The samples were incubated for 60 sec at room temperature and then centrifuged (30 sec, 16,000 x g). The

flow-through was discarded and the columns were replaced into the same collection tubes used previously. The samples were washed with 500 µl Wash buffer type 1 (GE Healthcare, Little Chalfont, England) and centrifuged again (30 sec, 16,000 x g). The flow-through was discarded and the columns were placed inside 1.5 ml microcentrifuge tubes. The samples were incubated with 10 to 50 µl Elution buffer type 6 (GE Healthcare, Little Chalfont, England) for 60 sec at room temperature. They were then centrifuged (60 sec, 16,000 x g). The eluted DNA was stored at -20 °C for further processing.

2.3.7. Sequencing

2.3.6.1. Sequencing PCR

A BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, USA) was used for the sequencing PCR. A combination of 2 µl Ready Reaction Mix (Applied Biosystems, Waltham, USA) and 1 µl 5x Sequencing Buffer (Applied Biosystems, Waltham, USA) were mixed in 0.2 ml PCR tubes. The added DNA volume varied between 1 and 3 µl. In addition, 2 µl primer (either forward or reverse) were also added. The reaction mix was filled to a 10 µl volume with DNase free water (Carl Roth, Karlsruhe, Germany). The tubes were briefly centrifuged (30 sec, 6,000 x g). A standard program was chosen for the sequencing PCR (table 16).

Table 16. Sequencing PCR program.

	Sequencing PCR	
Initial denaturation	30 sec 96 °C	
Denaturation	10 sec 96 °C	40 cycles
Annealing	5 sec 50 °C	
Extension	4 min 60 °C	

2.3.6.2. Purification of PCR Products

The samples were centrifuged (30 sec, 6,000 x g) and transferred in 0.5 ml tubes. A combination of 1 µl 3 M NaAc and 33 µl 100% ethanol were added. The samples were vortexed, incubated on ice for 17 min, and then centrifuged at 4 °C (30 min, 13,800 x g) in a Heraeus Fresco 17 Centrifuge (Thermo Electron Corporation, Waltham, USA). The pellets were washed with 90 µl 70% ethanol and centrifuged again at 4 °C (10 min, 13,800 x g). The supernatant was removed and the pellets were air-dried with open lids for 3 min. After 20 µl Hi-Di™ Formamide (Applied Biosystems, Waltham, USA) were added, the samples were incubated with open lids for 5 min. They were then incubated

in a 95 °C water bath for 5 min. The samples were cooled on ice for 10 min and sequenced with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Waltham, USA).

2.3.8. Data Analysis

The sequences of each sample were aligned with the help of the alignment program ClustalX (THOMPSON et al. 1997). GeneDoc (NICHOLAS et al. 1997) was used to determine a consensus sequence, which was then aligned with reference sequences of the Schistosomatidae or Echinostomatidae family, and corrected if a definite nucleotide was found to be missing. The final consensus sequence was used for determining the species (or genus) with the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

3. Results

3.1. Collection of Snail Intermediate Hosts

3.1.1. Seasonal Distribution of the Collected Snails

The seasonal distribution of the collected snails is illustrated in figure 45. No snails were found in April and no sampling trip was undertaken in June. Only a few snails were found in May, but the number of both *Radix* and *Lymnaea* snails rose thereafter. The number of *Radix* snails collected was high throughout the entire summer, ranging from 132 individuals in July to 200 in August and 169 in September. Fewer *L. stagnalis* than *Radix* were found, with a peak in July (66 *L. stagnalis*). The number of *L. stagnalis* collected decreased from August (35 individuals) to September (9). In total, 505 *Radix* and 117 *L. stagnalis* snails were collected.

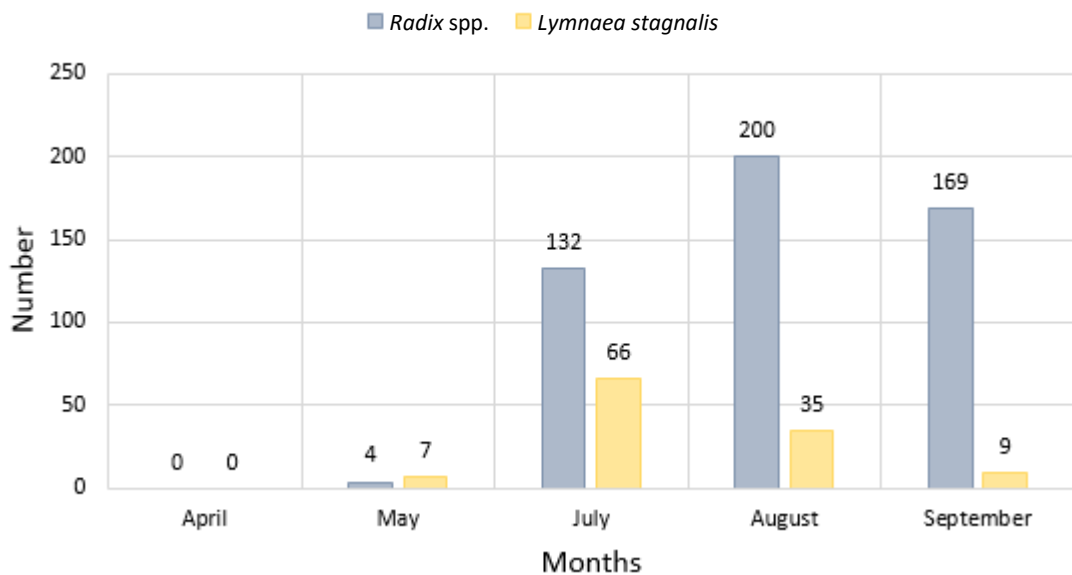


Figure 45. Seasonal distribution of collected snails. No sampling trip was undertaken in June.

3.1.2. Geographical Distribution of the Collected Snails

Lymnaeid snails were found in 16 of the 47 locations. Of the 505 *Radix* spp. collected, most were found in the Leitha Mountains (259) and the Danube floodplains (205) (figure 46). Conspicuously fewer were found in the Morava floodplains (21), Leitha floodplains (12), and artificial waterbodies (8).

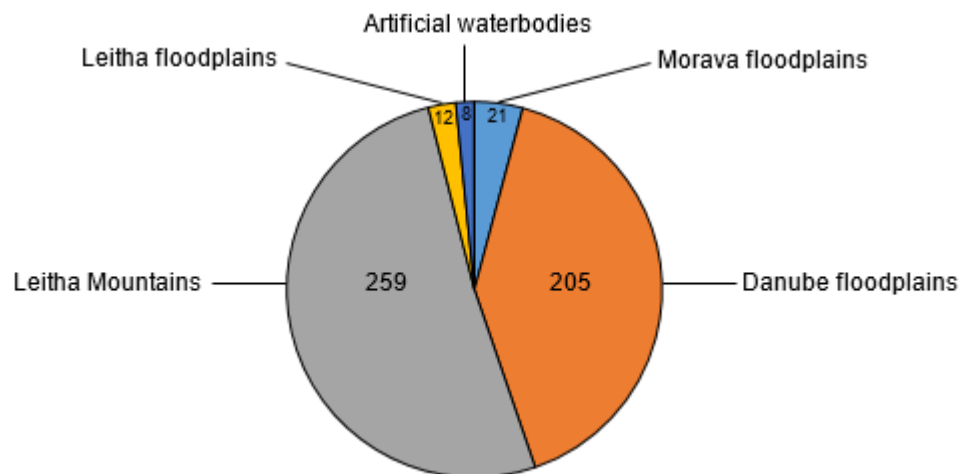


Figure 46. *Radix* spp. in different locations.

Of the 117 *L. stagnalis* collected, more than half (65 individuals) were found in a private swimming pond (i.e. artificial waterbody) (figure 47). Further *L. stagnalis* were collected in the Danube floodplains (21), Leitha floodplains (16), and Morava floodplains (15).

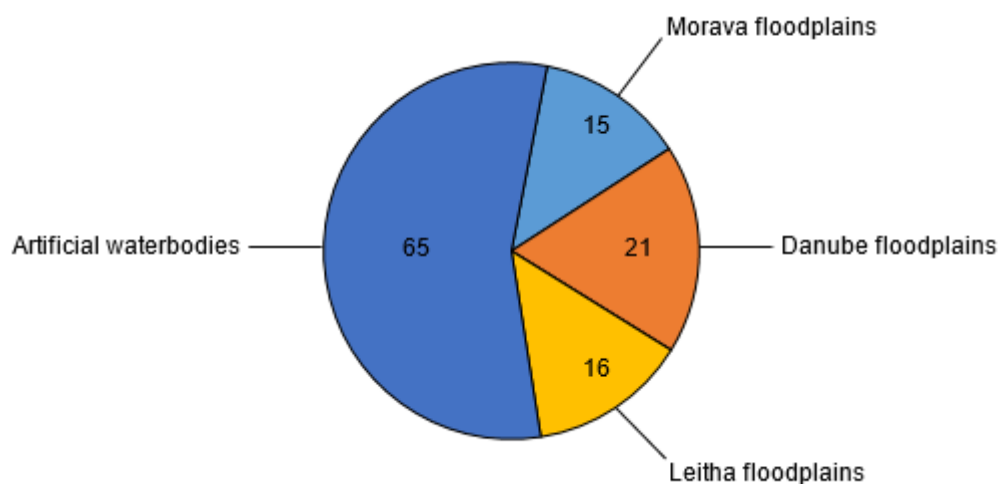


Figure 47. *Lymnaea stagnalis* in different locations.

In summary, lymnaeid snails could be found in all of the habitat types explored, with the exception of the Wulka River. However, only three of these habitats (namely the Leitha Mountains, Danube floodplains, and artificial waterbodies) provided 90% of all of the lymnaeid snails collected (figure 48).

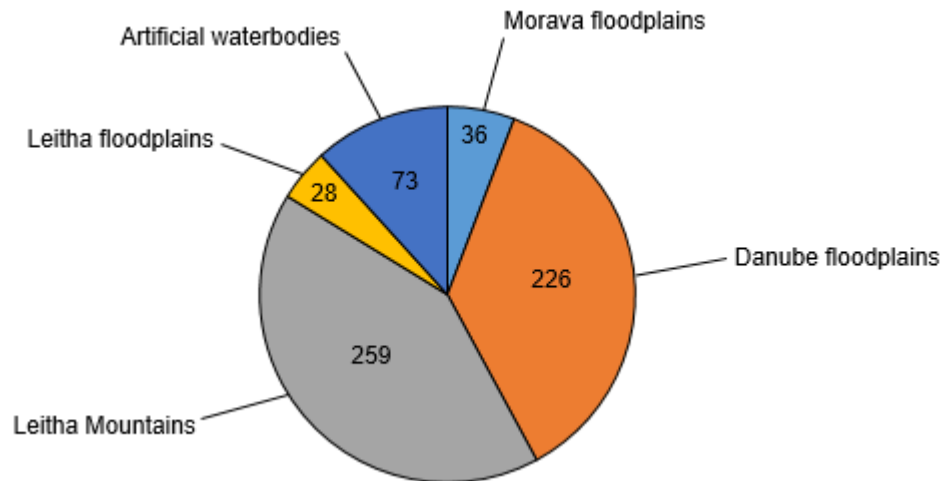


Figure 48. Lymnaeids in different locations.

3.2. Macro- and Microscopic Analyses

3.2.1. Shell Sizes

The shell size distribution of the *Radix* spp. and *Lymnaea stagnalis* collected is presented in figure 49. Most of the 505 collected *Radix* individuals were between 10 mm and 15 mm long. The smallest were only 4 mm, while the largest was 24 mm. The 117 *Lymnaea stagnalis* collected varied between 16 mm and 58 mm in length, although most exceeded 26 mm. However, it should be taken into account that several *Radix* species, which may differ distinctly in size, are pooled in figures 49 through 51.

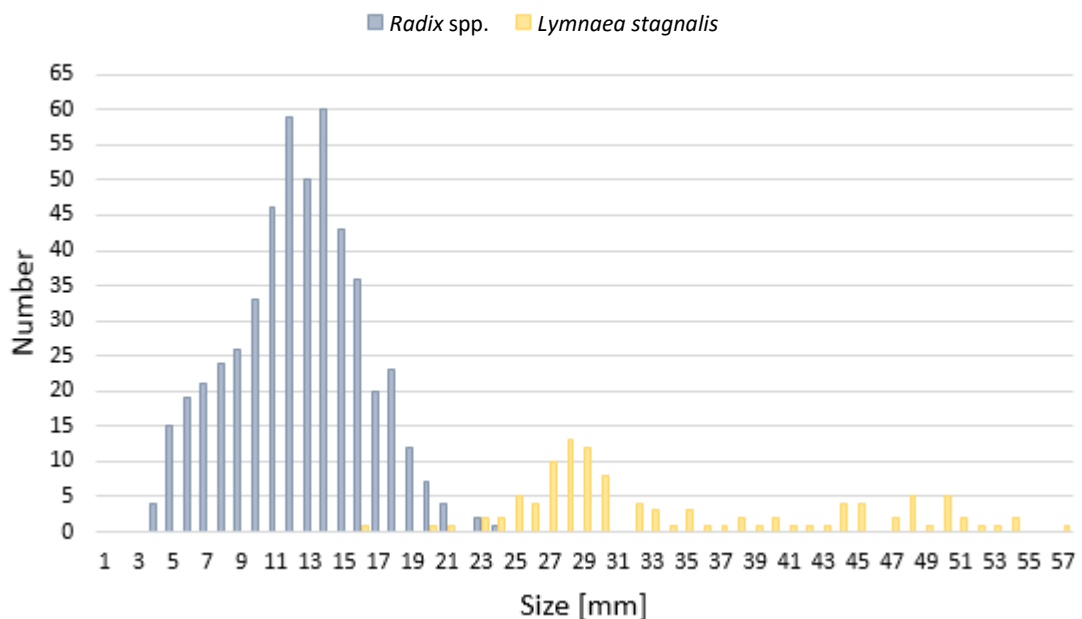


Figure 49. Distribution of shell sizes of *Radix* spp. and *Lymnaea stagnalis*.

Figure 50 illustrates the shell size distribution of both *Radix* and *Lymnaea stagnalis* individuals in a boxplot. The *Radix* individuals collected were much smaller than the *Lymnaea stagnalis* collected: 50% of the *Radix* individuals had a length between 10 mm and 15 mm (with a median length of 13 mm), while 50% of the *Lymnaea stagnalis* individuals were between 28 mm and 44 mm long (with a median length of 30 mm). The latter also showed a much greater variety in size than the former.

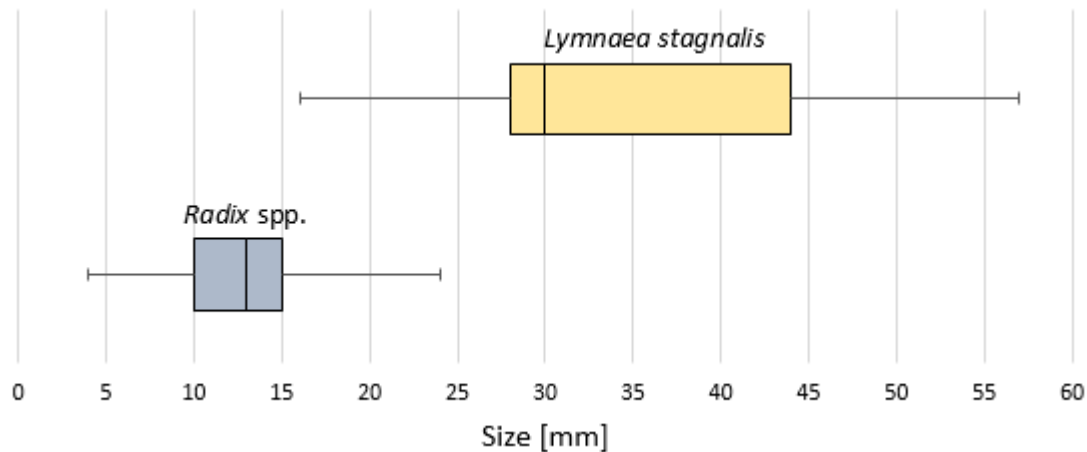


Figure 50. Boxplot illustrating the shell sizes of 50% of *Radix* and *Lymnaea stagnalis* individuals.

The shell sizes of *Radix* spp. showed seasonal variation. Figure 51 depicts four size classes and their occurrence rates from May to September. As no sampling trip was undertaken in June, no size information is available for that month.

Only a few snails were found in May. Those snails belonged to the medium and larger classes, and no class 1 (4 mm – 8 mm) snails were observed. During the summer months, markedly more individuals were found. While the numbers found in all classes rose in May to July, class 2 (9 mm – 13 mm) discoveries increased the most. The numbers of class 3 (14 mm – 18 mm) and class 4 (19 mm – 24 mm) snails found then further increased from July to August, although class 1 and class 2 numbers decreased. From August to September, class 1 and class 2 discoveries rose again, while class 3 and class 4 levels declined.

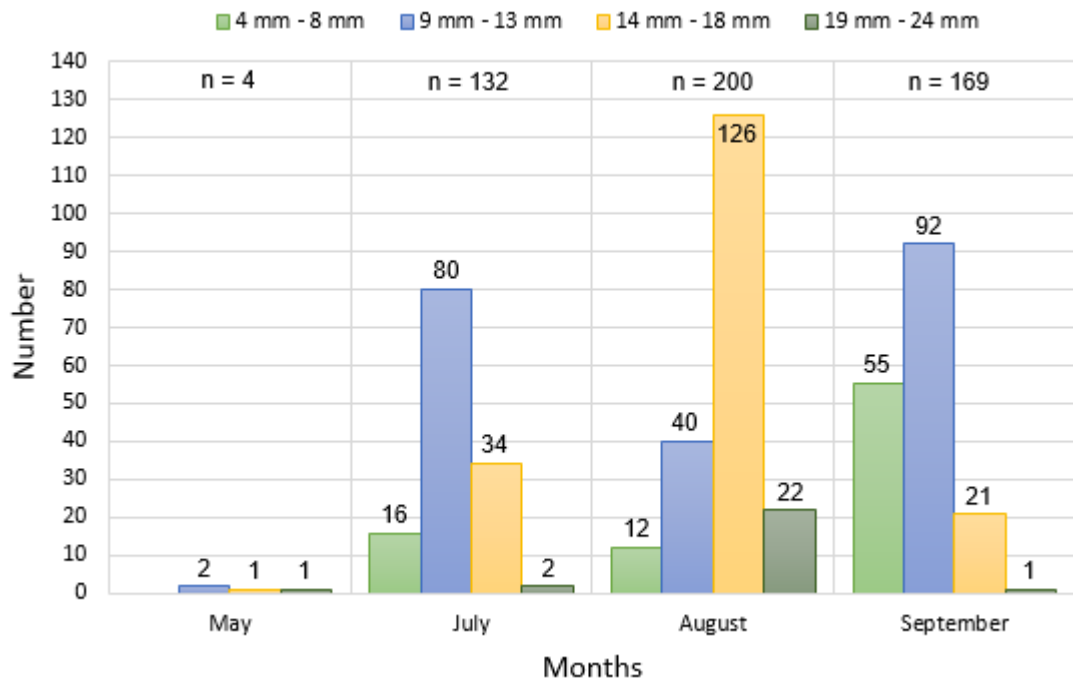


Figure 51. Seasonal changes in shell size of *Radix* spp. As no sampling trip was undertaken in June, no size data are available for that month.

The shell sizes of *Lymnaea stagnalis* also varied by season. The four size classes and their occurrence rates are illustrated in figure 52. In May, few individuals were found in all four classes. From May to July, the number of class 1 (16 mm – 25 mm) and class 2 (26 mm – 35 mm) snails increased, while the number of class 3 (36 mm – 45 mm) snails remained low. No class 4 (46 mm – 57 mm) snail was observed. From July to August, the levels of collected class 3 and class 4 snails increased, whereas only a few class 2 and no class 1 snails were observed.

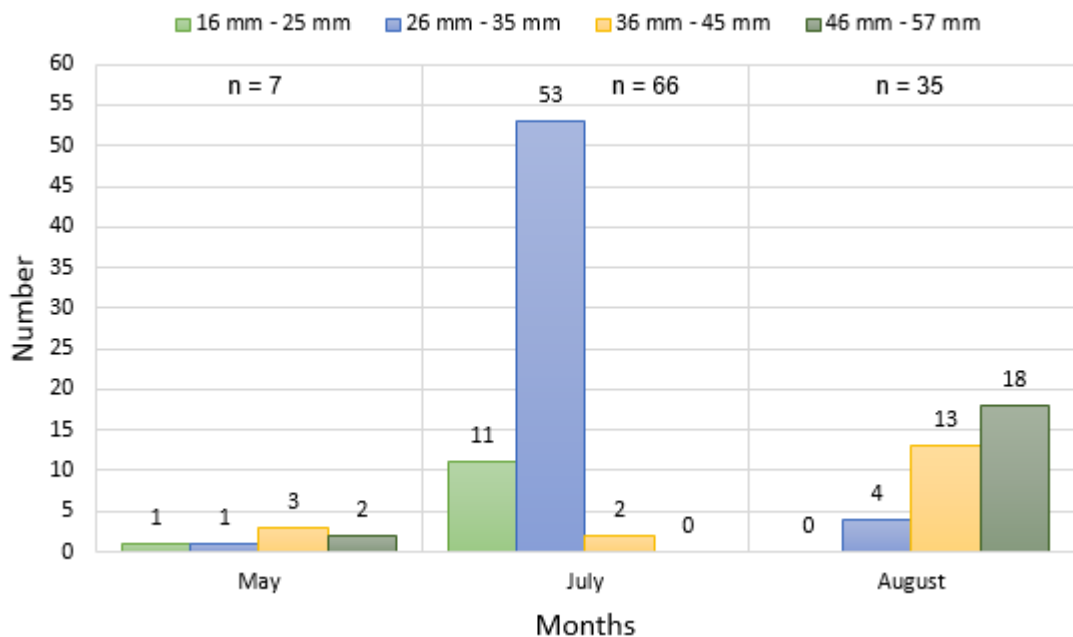


Figure 52. Seasonal changes in shell size of *Lymnaea stagnalis*. As no sampling trip was undertaken in June, no size data are available for that month.

3.2.2. Snail Examinations

Figure 53 illustrates the results of the microscopical snail examinations. Of the total of 622 collected snails, 473 were not infected (76%) and 149 had trematode infections (24%; figure 53 A). More than the half of the infected snails harboured echinostomes (81), while the residual half had furcocercariae (7), amphistome cercariae (9), or xiphidiocercariae (25). One snail possessed monostome cercariae and 26 snails had only other trematode larvae, such as rediae or sporocysts (figure 53 B). Two of the seven snails with furcocercariae had a trichobilharzian infection (figure 53 C).

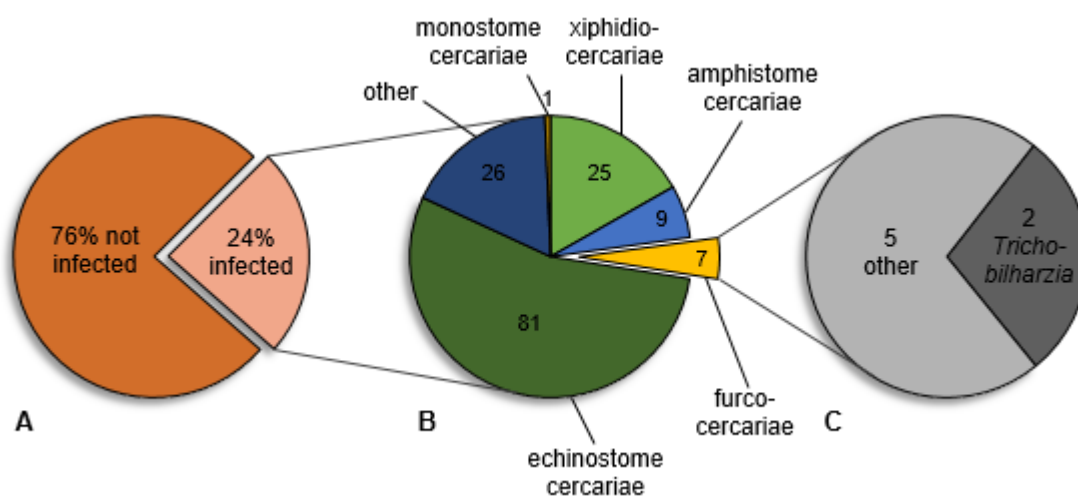


Figure 53. Results of snail examinations. **A.** Infection rate of collected snails. **B.** Kind of infections. **C.** Kind of furcocercariae.

The infected snails were found in 13 different locations (figures 54 and 55). Furcocercariae (yellow) were observed in an artificial waterbody (AW03) and in two locations in the Danube floodplains (DF01, DF06). The echinostome samples (green) originated from the Leitha Mountains (LM01), four locations in the Danube floodplains (DF01, DF04, DF09, DF10), the Morava floodplains (MF07), and an artificial waterbody (AW04). In AW04, we also found a monostome infection (brown). The DF10 and LF03 locations in the Danube floodplains and Leitha floodplains, respectively, revealed amphistome infections (blue). Snails infected with xiphidiocercariae (red) were found in the Leitha Mountains (LM01), the Danube floodplains (DF01, DF09), the Leitha floodplains (LF05, LF07), and in the Morava floodplains (MF01).



Figure 54. Locations with infected snails in the south and east of Vienna. Yellow: Furcocercariae. Green: Echinostome infections. Red: Infections with xiphidiocercariae. Blue: Amphistome infections. Brown: Monostome infections. Modified from GOOGLE MAPS 2016.



Figure 55. Locations with infected snails in the tri-state region of Austria, Slovakia, and the Czech Republic. Green: Echinostome infections. Red: Infections with xiphidiocercariae. Modified from GOOGLE MAPS 2016.

The seasonal distribution of infections and the respective temperatures in the sampling area are illustrated in figure 56. The mean temperature rose from 11.6 °C in April (ZAMG 2016d) to 24 °C in July (which was the warmest month in 2015) (ZAMG 2016f). However, it was only slightly lower in August (23.7 °C) (ZAMG 2016g). In September, the mean temperature was only 16.5 °C (ZAMG 2016h).

In May, 2 of the 11 snails found (18.2%) were infected. The infection rate rose in July, when 53 of 198 snails (26.8%) harboured trematodes. In August, a conspicuously lower infection rate of 14% was observed (33 of 235 snails). September revealed the highest infection rate: 34.3% of collected snails were infected (61 of 178 snails).

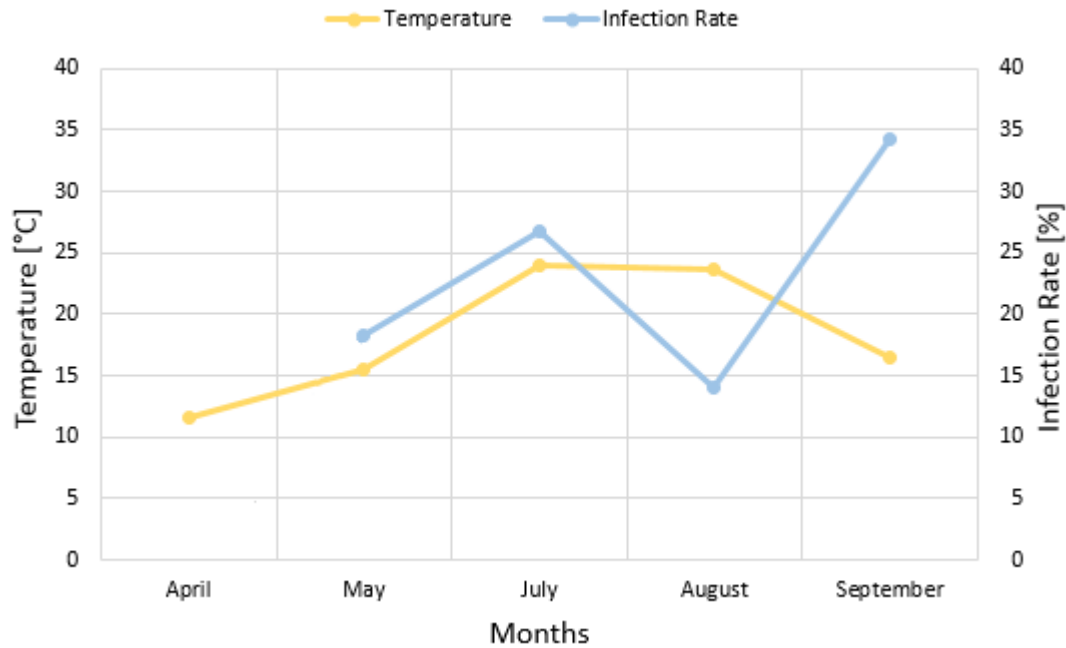


Figure 56. Temperatures and seasonal distribution of trematode infections in snails in the sampling period. As no snails were collected in April and June, no data on the infection status are available.

3.2.3. Microscopy

Exemplary microscopy results are illustrated in five plates that demonstrate the diversity within the respective larval stages. Figure 57 depicts a sporocyst that contains germinal cells (A) and three different rediae (B-D). While (B) reveals visible germinal cells, (C) and (D) show rediae that have already developed cercariae. Figure 58 presents examples of xiphidiocercariae, which differ mainly in the appearance of their stylet. Figure 59 illustrates monostome cercariae with three eye-spots (A) and two eye-spots (B), as well as an amphistome cercaria (C); (D) provides a detailed view of an excretory system. Figure 60 depicts three different furcocercariae, the last of which is a trichobilharzian (C). Figure 61 shows echinostome cercariae (B-C) and a detailed view of their typical head collar of spines (A).

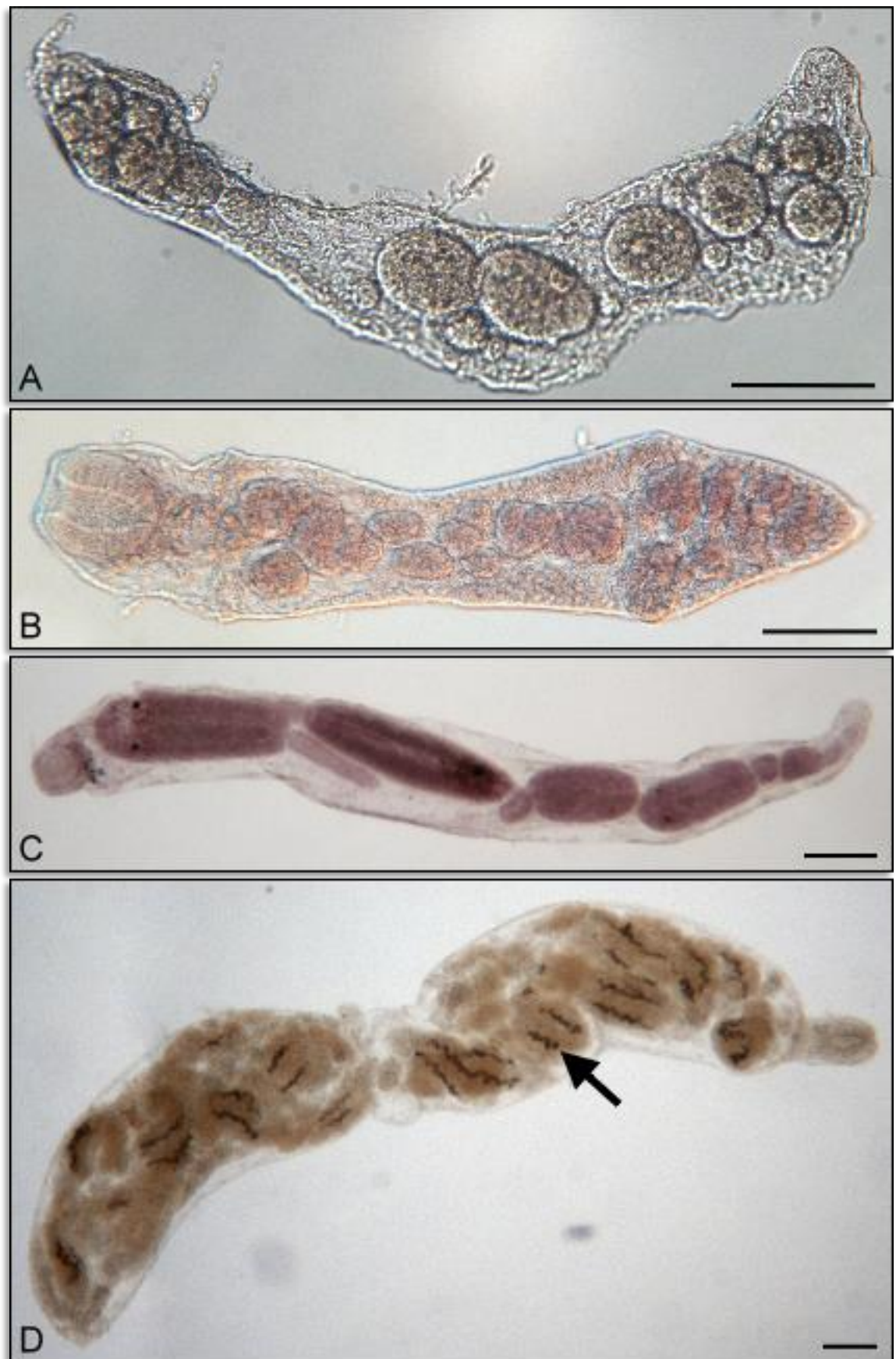


Figure 57. Sporocyst and rediae. **A.** Sporocyst with germinal cells (5 Aug. 2015, DF01, *R. auricularia*). **B.** Stained rediae with germinal cells (29 Jul. 2014, LM01, *R. labiata*). **C.** Stained rediae with ocellate amphistome cercariae (28 Jul. 2015, AW04, *R. auricularia*). **D.** Rediae with echinostome cercariae, identifiable through the dark granules in their excretory system (arrow) (14 Sept. 2015, LM01, *R. balthica*). Scale bars: 100 μ m. Orig.

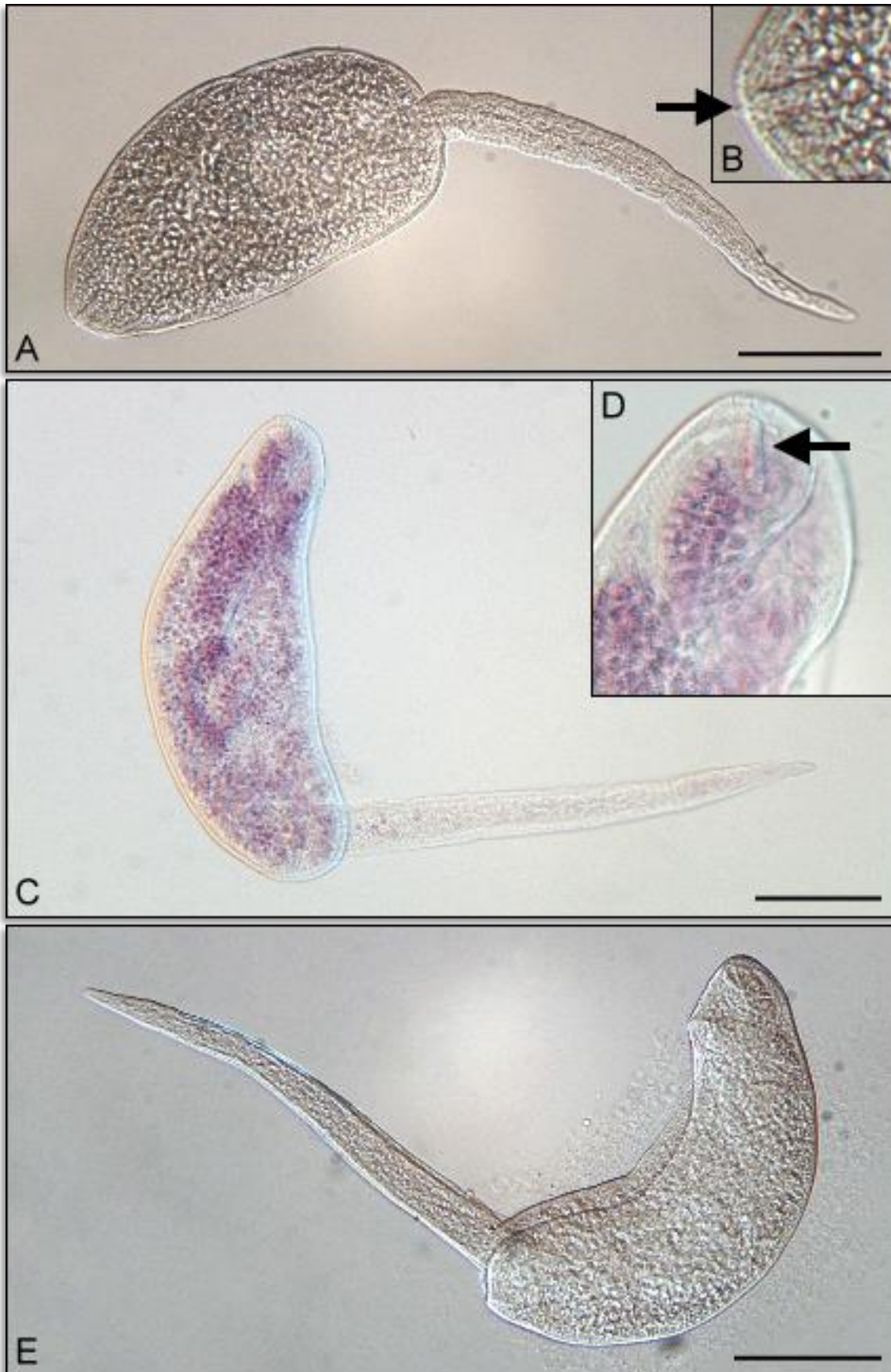


Figure 58. Xiphidiocercariae. **A.** Ventral view of a xiphidiocercaria with a sharp stylet (arrow in **B**) (14 Sept. 2015, LF05, *L. stagnalis*). **C.** Ventrolateral view of a stained xiphidiocercaria with a sharp stylet (arrow in **D**) (11 May 2015, LF07, *R. labiata*). **E.** Lateral view of a xiphidiocercaria. The stylet is hardly visible (05 Aug. 2015, DF09, *L. stagnalis*). Scale bars: 100 μ m. Orig.

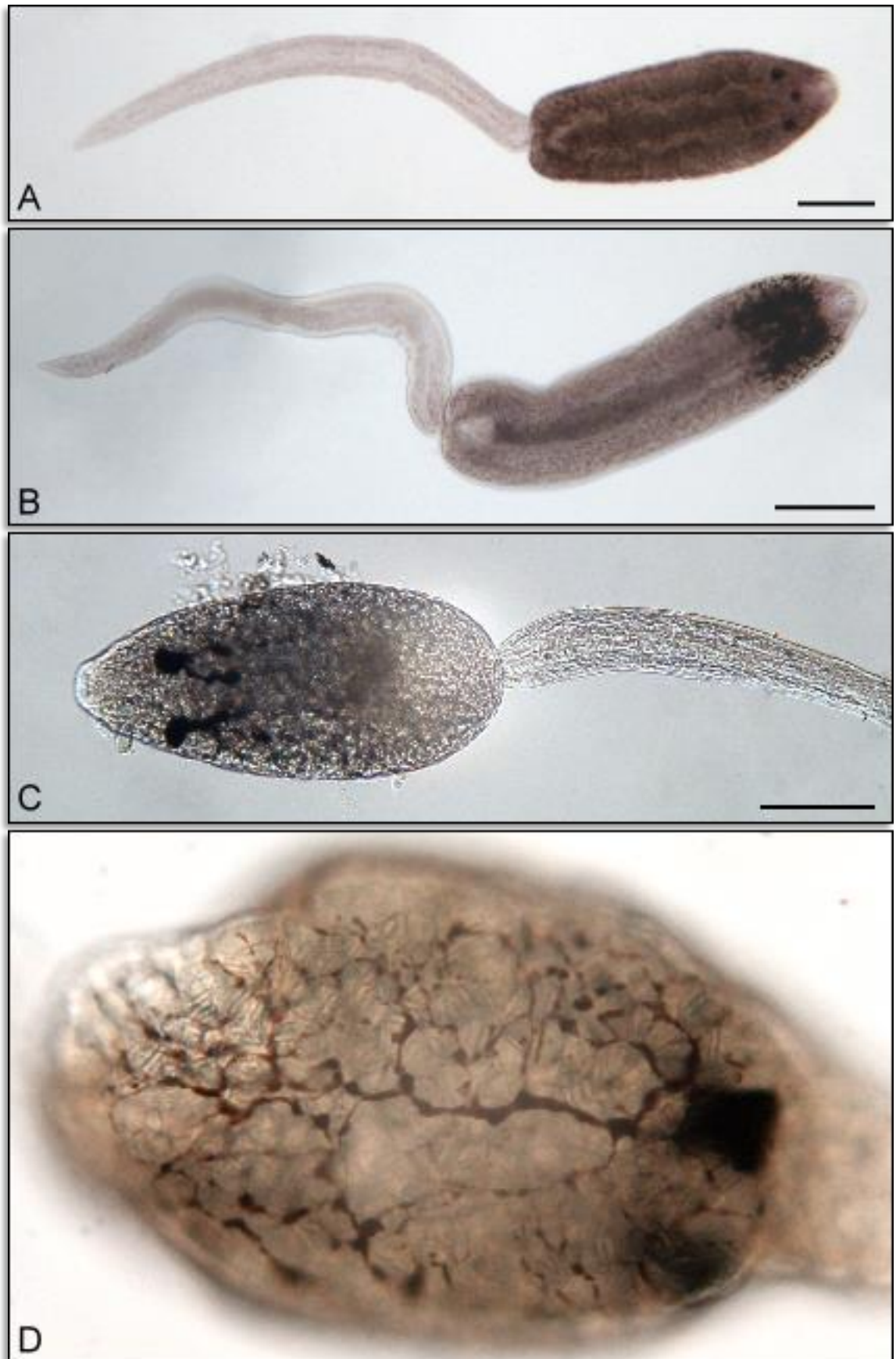


Figure 59. Monostome and amphistome cercariae. **A.** Dorsal view of a mature monostome cercaria with three eye-spots (28 Jul. 2015, AW04, *R. auricularia*). **B.** Ventral view of an immature monostome cercaria with only two eye-spots (28 Jul. 2015, AW04, *R. auricularia*). **C.** Dorsal view of an amphistome cercaria with two eye-spots (14 Sept. 2015, LF03, *R. balthica*). **D.** Detail view of the dorsal head region of an amphistome cercaria. The dark lines might be the excretory system (05 Aug. 2015, DF10, *Physella*). Scale bars: 100 μ m. Orig.

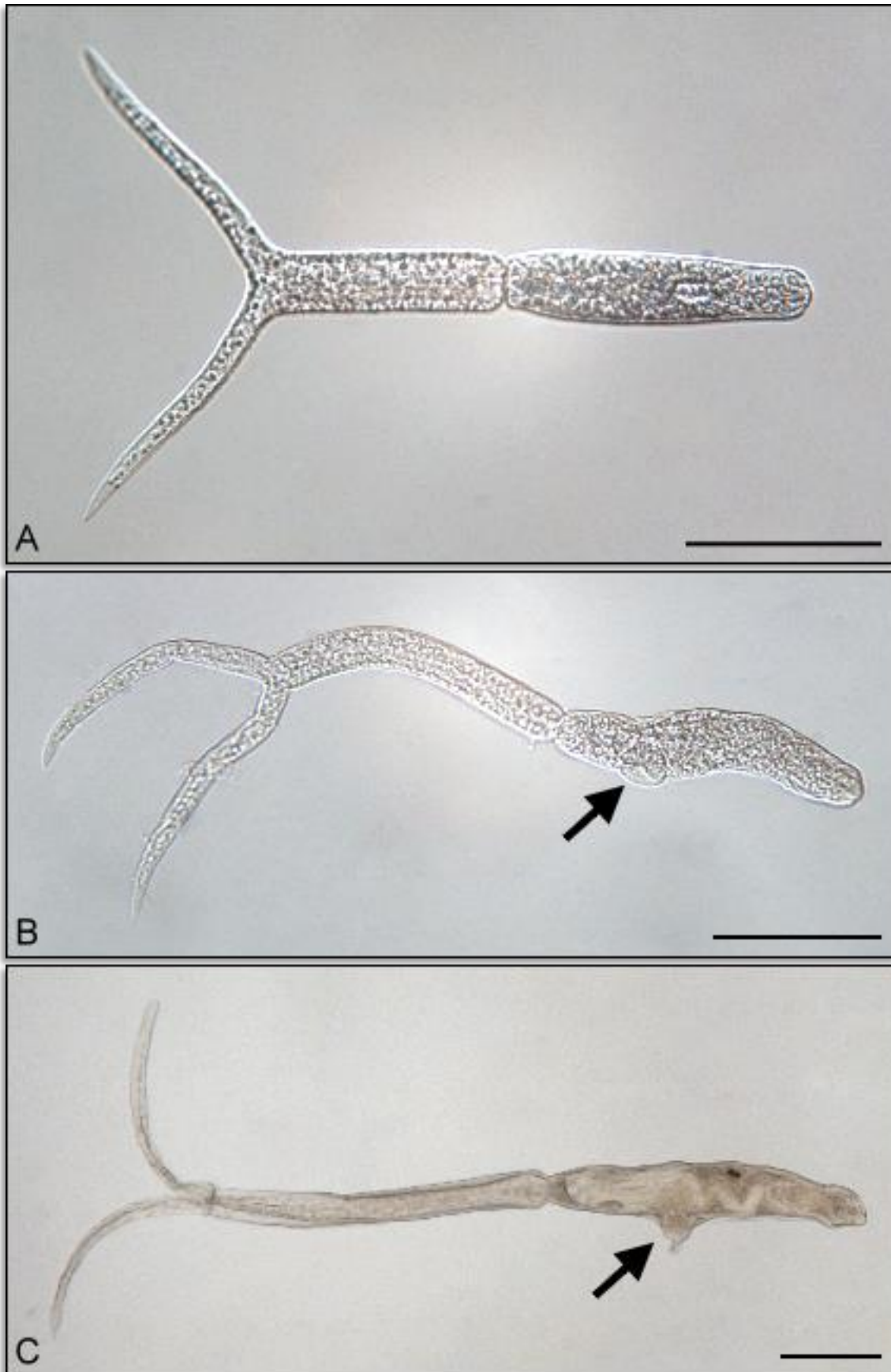


Figure 60. Furcocercariae. **A.** Ventral view of a furcocercaria (05 Aug. 2015, DF06, *R. auricularia*). **B.** Ventrolateral view of a furcocercaria with a visible acetabulum (arrow) (05 Aug. 2015, DF01, *R. auricularia*). **C.** Lateral view of a trichobilharzian furcocercaria. The acetabulum is protruded (arrow) and an eye-spot is visible (28 Jul. 2015, AW03, *L. stagnalis*). Scale bars: 100 μ m. Orig.



Figure 61. Echinostome cercariae. **A.** Detail view of the head region of a stained echinostome cercaria with the head collar of spines (arrows) (29 Jul. 2014, LM01, *R. labiata*). **B.** Lateral view of a stained echinostome cercaria. The acetabulum is protruded (29 Jul. 2014, LM01, *R. labiata*). **C.** Ventrolateral view of an echinostome cercaria (29 Jul. 2014, LM01, *R. labiata*). Echinostome cercariae have a blunt tail end. Scale bars: 100 μ m. Orig.

3.3. Molecular Investigations

3.3.1. PCR Results

3.3.1.1. PCR Test Runs

3.3.1.1.1. Test Runs with Schistosomatidae Primers

The Cytochrome C Oxidase Subunit 1 gene was successfully amplified by the CO1 primer pair (figure 62). The gel bands were of expected size of approximately 1,300 bp and the primer pair was able to detect the DNA up to a 1/100 dilution.

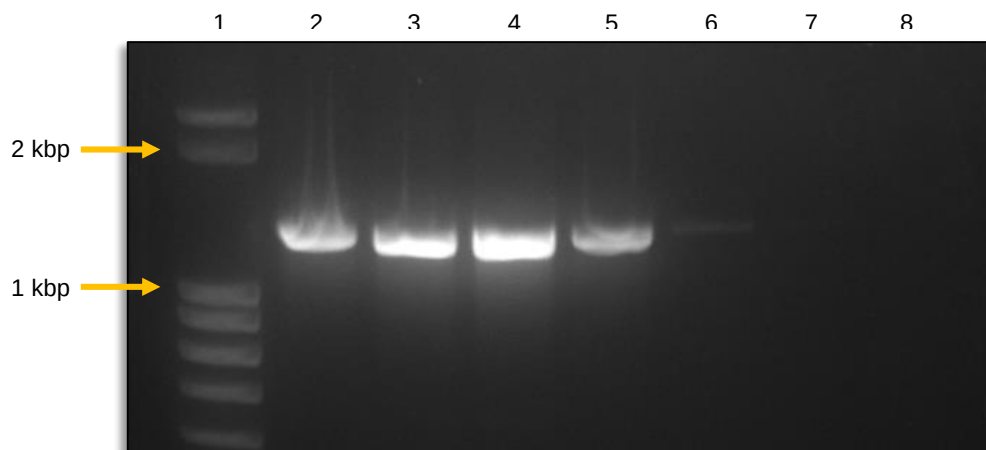


Figure 62. Test PCR with the CO1 primer pair. Slot 1: Step marker. Slot 2: 1 μ l DNA. Slot 3: 3 μ l DNA. Slot 4: 6 μ l DNA. Slot 5: 2 μ l of 1/10 dilution. Slot 6: 2 μ l of 1/100 dilution. Slot 7: 2 μ l of 1/1000 dilution. Slot 8: Negative control.

3.3.1.1.2. Test Runs with Echinostomatidae Primers

The ND1 primer pair was able to amplify the Nicotinamid Adenin-Dinucleotide Dehydrogenase Subunit 1 gene in all tested DNA concentrations. The gel bands were of expected size of approximately 500 bp (figure 63).

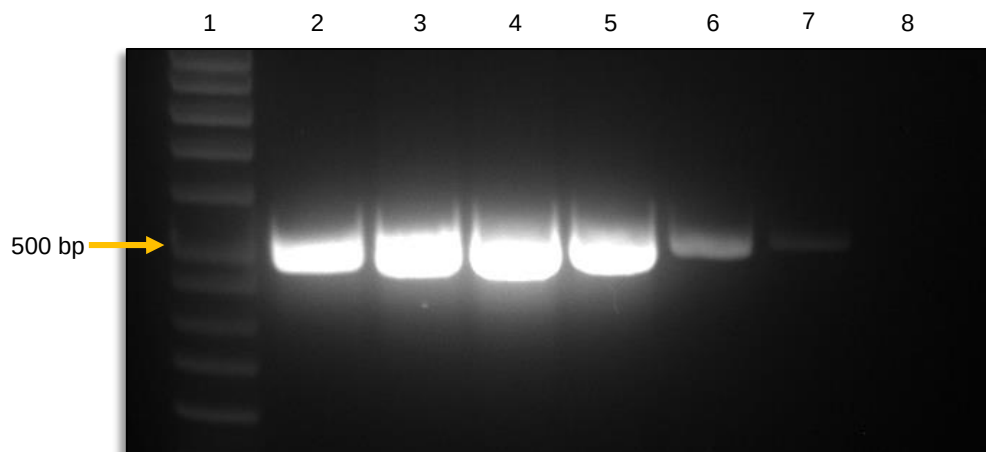


Figure 63. Test PCR with the ND1 primer pair. Slot 1: Step marker. Slot 2: 1 μ l DNA. Slot 3: 3 μ l DNA. Slot 4: 6 μ l DNA. Slot 5: 2 μ l of 1/10 dilution. Slot 6: 2 μ l of 1/100 dilution. Slot 7: 2 μ l of 1/1000 dilution. Slot 8: Negative control.

3.3.1.2. Trematode PCR

All collected samples with a possible trematode infection were tested for trematodes. Figure 64 shows exemplarily five different of those. The samples 63, 71, 85, 89, and 100 (29.07.2014, LM01) were positive and showed bands of expected size of approximately 430 bp.

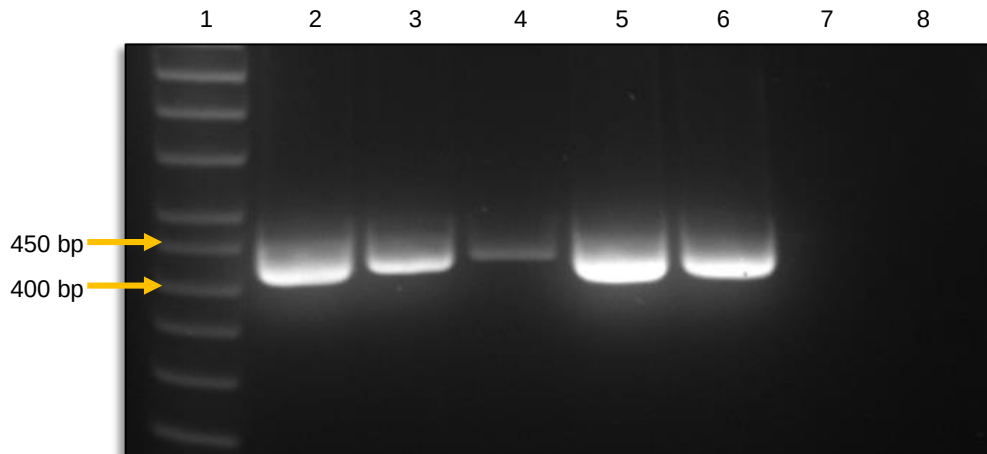


Figure 64. Trem primer pair. Slot 1: Step marker. Slot 2: 63. Slot 3: 71. Slot 4: 85. Slot 5: 89. Slot 6: 100 (29.07.2014, LM01). Slot 7: Empty. Slot 8: Negative control.

3.3.1.3. Schistosomatidae PCR

All trematodes that could be schistosomatids were further analysed with the specific CO1 primer pair. Exemplary samples are illustrated in figure 65. The samples 1 (28.07.2015, AW03), 1 (05.08.2015, DF06), 1, and 122 (05.08.2015, DF01) were positive and showed bands of the expected size of approximately 1,300 bp.

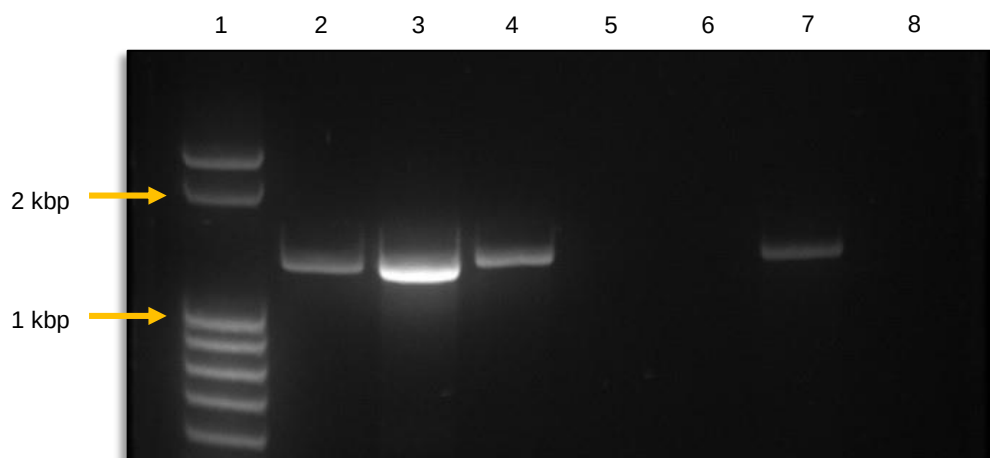


Figure 65. CO1 primer pair. Slot 1: Step marker. Slot 2: 1 (28.07.2015, AW03). Slot 3: 1 (05.08.2015, DF06). Slot 4: 1 (05.08.2015, DF01). Slot 5: 100 (05.08.2015, DF01). Slot 6: 118 (05.08.2015, DF01). Slot 7: 122 (05.08.2015, DF01). Slot 8: Negative control.

3.3.1.4. Echinostomatidae PCR

All trematodes that had been microscopically identified as echinostomatids were further investigated with the specific ND1 primer pair. Figure 66 depicts exemplary samples. The samples 89, 93, 97, and 100 (29.07.2014, LM01) were positive and showed bands of the expected size of approximately 500 bp.

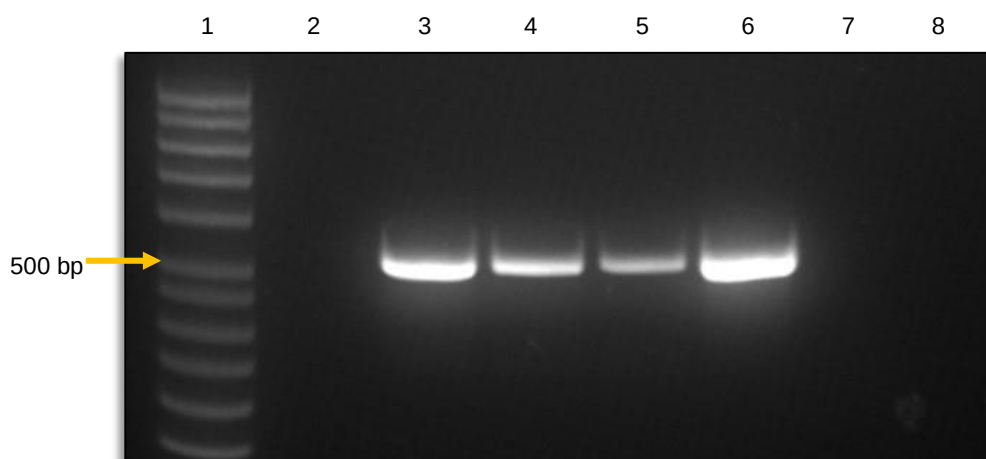


Figure 66. ND1 primer pair. Slot 1: Step marker. Slot 2: Empty. Slot 3: 89. Slot 4: 93. Slot 5: 97. Slot 6: 100 (29.07.2014, LM01). Slot 7: Empty. Slot 8: Negative control.

3.3.2. Sequencing Results

3.3.2.1. Schistosomatidae

The sequencing result of sample 1 of the private swimming pond (28.07.2015, AW03) is shown in table 17 and revealed *Trichobilharzia szidati*.

Table 17. Sequencing result of schistosomatid sample.

Date	Location	Species	Sample	Microscopic result	Sequencing result
28.07.2015	AW03	<i>L. stagnalis</i>	1	Furcocercariae	<i>Trichobilharzia szidati</i>

Figure 67 illustrates the alignment of a suitable part of the sample's sequence with the most similar sequences from GenBank. BRANT and LOKER (2009) stated an intraspecific variability in the CO1 gene of 3.3% between *T. szidati* populations, while *T. szidati*'s CO1 gene differs between 9.8% and 11.7% from other *Trichobilharzia* species. The 371 bp fragment of the sample's sequence is to 98% (362/371 bp) identical to the *T. szidati* isolate Lsm2 from Russia (GenBank: JF838198.1). In contrast, the fragment is only to 89% (332/371 bp) identical to the *T. regenti* isolate ACI25 from Iceland (GenBank: HM439504.1) and to 88% (327/371 bp) identical to the *T. anseri* isolate ANS40 from France (GenBank: KP901384.1).

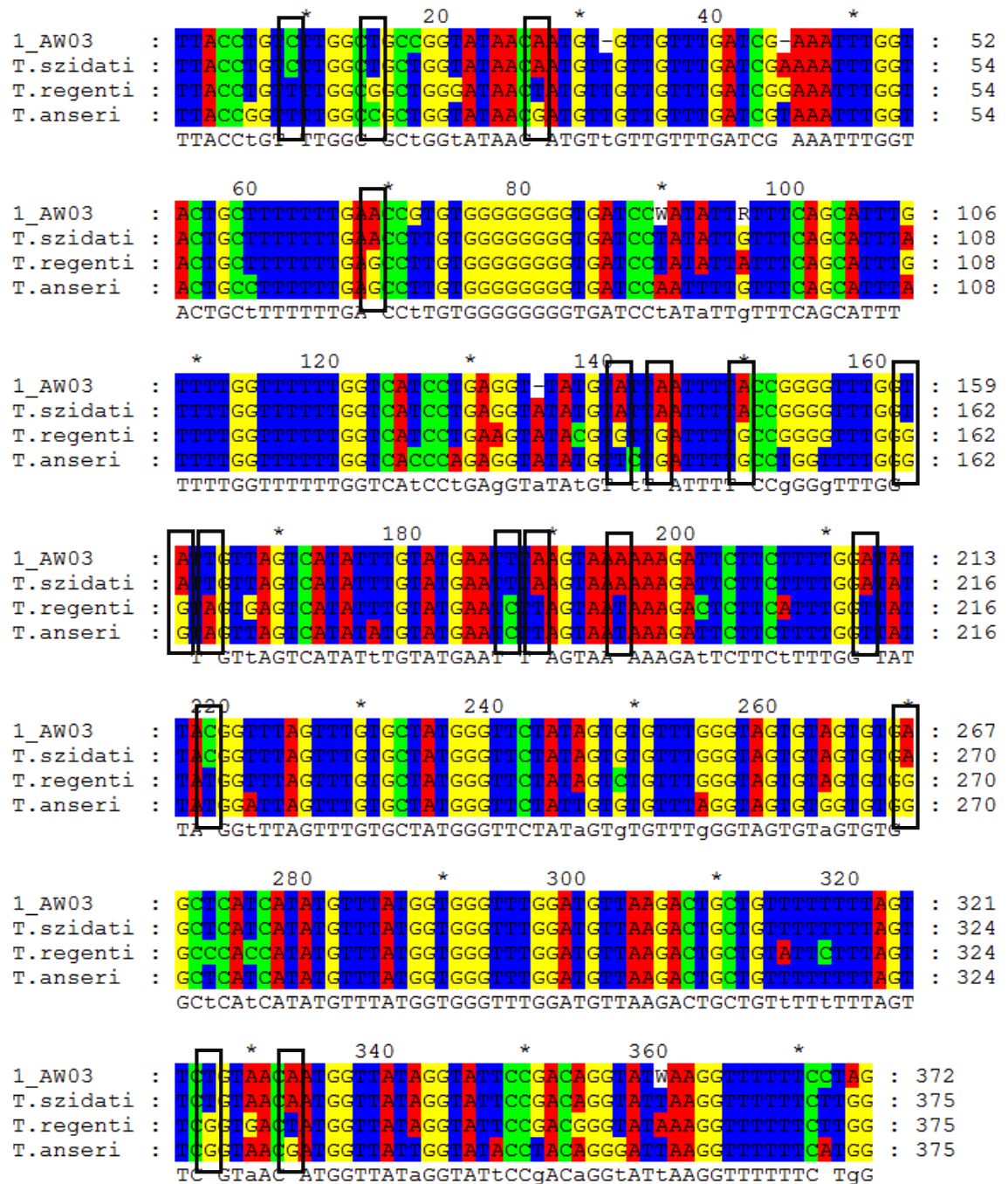


Figure 67. Alignment of the 371 bp fragment of sample 1 (28.07.2015, AW03) with the most similar sequences from GenBank. The black rectangles indicate sequence positions where the sample's sequence and the GenBank sequence of *T. szidati* are identical and differ together from the other sequences.

3.3.2.3. Echinostomatidae

The sequencing results of the 15 echinostomatid samples are summarized in table 18. Of sample 10 (14.09.2015, LM01) only the reverse sequence was suitable, no consensus could thus be made and the reverse sequence was blasted directly.

Table 18. Sequencing results of echinostomatid samples.

Date	Location	Species	Sample	Microscopic result	Sequencing result
29.07.2014	LM01	<i>R. labiata</i>	1	Echinostome cercariae	<i>Echinoparyphium recurvatum</i>
			7	Echinostome cercariae	
			8	Echinostome cercariae	
			100	Echinostome cercariae	
24.07.2015	DF04	<i>R. auricularia</i>	9	Echinostome cercariae	<i>Echinoparyphium recurvatum</i>
28.07.2015	LM01	<i>R. labiata</i>	1	Echinostome cercariae	
			2	Echinostome cercariae	
			3	Echinostome cercariae	
	AW04	<i>R. auricularia</i>	1	Echinostome cercariae	<i>Echinostoma revolutum</i>
05.08.2015	DF10	<i>R. auricularia</i>	13	Echinostome cercariae	<i>Echinoparyphium recurvatum</i>
	DF09	<i>L. stagnalis</i>	6	Echinostome cercariae	<i>Hypoderaeum conoideum</i>
14.09.2015	LM01	<i>R. balthica</i>	5	Echinostome cercariae	<i>Echinoparyphium recurvatum</i>
			9	Echinostome cercariae	
			10	Echinostome cercariae	
			12	Echinostome cercariae	

Figure 68 depicts the distribution of the different echinostomatid species revealed by sequencing of a 530 bp fragment of the ND1 gene. Thirteen of 15 samples were *Echinoparyphium recurvatum*, whereas the other two species (i.e. *Hypoderaeum conoideum*, *Echinostoma revolutum*) were found only once.

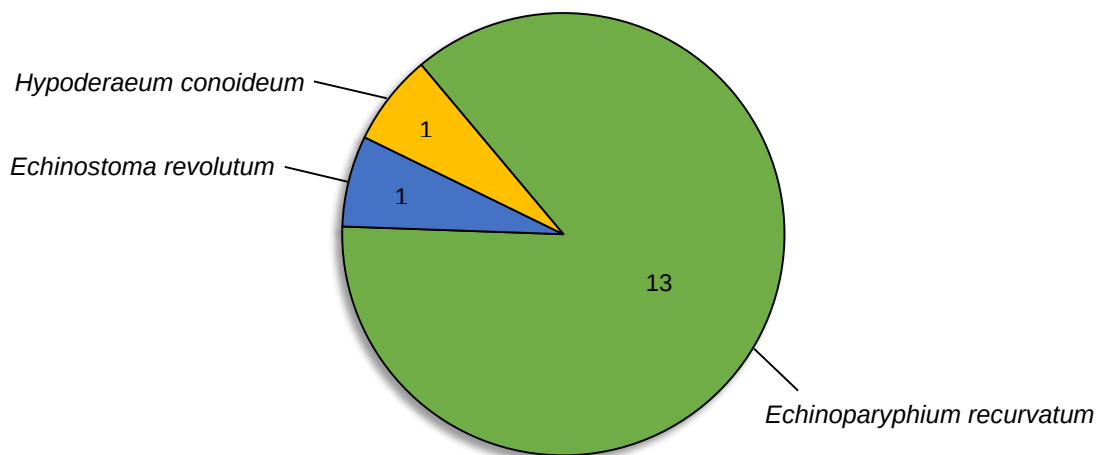


Figure 68. Species revealed by sequencing of echinostomatid samples. Thirteen snails harboured *Echinoparyphium recurvatum*. *Hypoderaeum conoideum* and *Echinostoma revolutum* occurred only in one snail each.

An exemplary alignment of a suitable part of sample 9 (14.09.2015, LM01) with the most similar sequences from GenBank is shown in figure 69. MORGAN and BLAIR (1998, cited in MORGAN & BLAIR 2000) stated an intraspecific variability in the ND1 gene of up to 2.5% between populations, while this gene differs to 12.5% between echinostome species. The 457 bp fragment of the sample's sequence is to 99% (453/457 bp) identical to the

Echinoparyphium recurvatum isolate W18 from Wales, UK (GenBank: AY168944.1). In contrast, the fragment is only to 84% (275/328 bp) identical to the *Euparyphium albufere* isolate from Spain (GenBank: AJ564380.2) and to 78% (277/353 bp) identical to the *Echinostoma caproni* isolate from Egypt (GenBank: AJ564378.1).

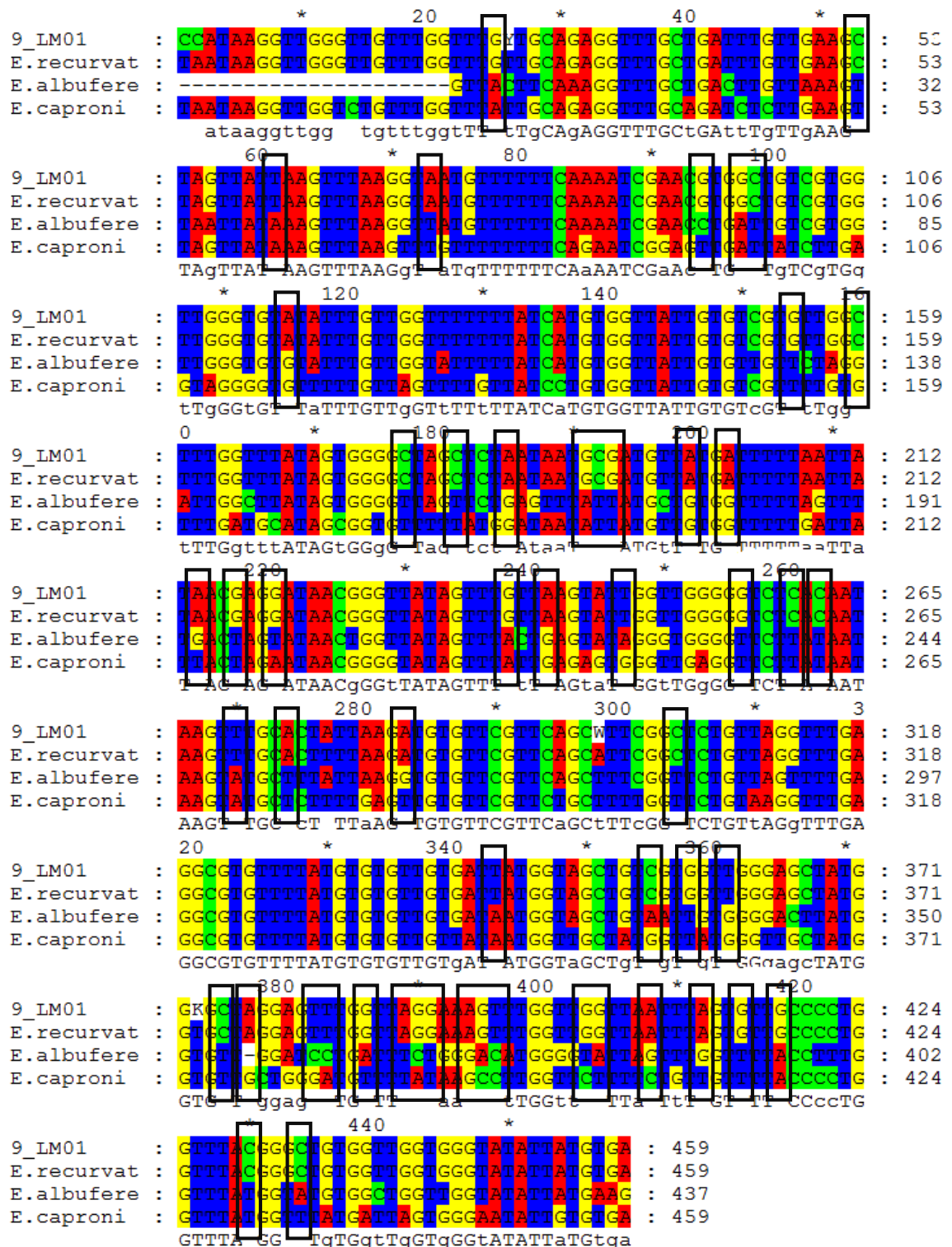


Figure 69. Alignment of the 457 bp fragment of sample 9 (14.09.2015, LM01) with the most similar sequences from GenBank. The black rectangles indicate sequence positions where the sample's sequence and the GenBank sequence of *E. recurvatum* are identical and differ together from the other sequences.

4. Discussion

4.1. Distribution of Snail Intermediate Hosts

4.1.1. Habitat and Frequency of Snails

Lymnaeid snails prefer calcium-rich waterbodies with a muddy ground and either a slow flow rate or no stream at all (ADW 2000, GRUNER et al. 2000, WHITE-MCLEAN 2011). Several *Radix* snails were found in the Leitha Mountains and many *L. stagnalis* in a private swimming pond. The St. Anna in der Wüste location (LM01) in the Leitha Mountains is a standing pond with a muddy ground and submerged vegetation. In comparison to other locations, several visits to LM01 almost always resulted in a high number of *Radix* snails. This observation conforms to the findings of ALI et al. (2007) who concluded that macrophytes provide microhabitats that enhance invertebrate colonization. In general, the fauna of different invertebrates observed in this pond was highly diverse. HANN (1995) also commented on submersed vegetation being beneficial for invertebrates as it provides shelter from predators, spawning sites, organic material as feed, and attachment sites.

Although the private swimming pond (AW03) had no muddy ground, it comprised many plants and algae. This vegetation explains why high numbers of *L. stagnalis* were observed in this location, as was the case in LM01.

A further hotspot for *Radix* sp., more specifically *R. auricularia*, was DF01 in the Danube floodplains. This location is a green, more or less muddy meadow that is occasionally flooded. Such dynamic water systems with changing water levels proved to be suitable habitats for various lymnaeids (not only for *Radix* spp., but also for *G. truncatula*) (RECKENDORFER & GROISS 2006). Although the Danube floodplains are today a nature sanctuary, only a few areas are still in their natural state (ICPDR 2016). Nonetheless, *R. auricularia* is known for its ability to adapt to anthropogenic habitats (SEDDON et al. 2014a), which explains its high prevalence at DF01.

Location LF04 on the Leitha River is an example of a site where no *Radix* or *Lymnaea* snails were found at all. GAUB (2014) visited this location several times and also found no *L. stagnalis*, although its prevalence was much higher at sites other than the Leitha River. While we did find some *Radix* and *L. stagnalis* in three locations in the Leitha floodplains (LF03, LF05, LF07), the numbers were small; furthermore, only LF03 was a tributary of the Leitha River (LF05 and LF07 were instead small waterbodies without an actual connection to the Leitha). In summary, few to no snails could be collected from

the Leitha River and its floodplains. According to TRUXA (2012), many human interventions have occurred in the Leitha River. That interventions could have resulted in an environment that is unsuitable for *Radix* spp. and *L. stagnalis*.

4.1.2. Season and Frequency of Snails

Despite a relatively short sampling period that lasted only from May to September (with no sampling in June), obvious trends emerge. The snail density varied dramatically, increasing from the spring to the summer and then starting to decrease in September. This is in agreement with ROZENDAAL (1997), who described seasonal variations in snail population densities. It is known that climate factors such as temperature, rainfall, and water level have an effect on snail densities and can cause distinct fluctuations (ROZENDAAL 1997). As a general rule, however, freshwater snails are highly flexible with regard to environmental conditions and tolerate a wide variety of ecological factors better than terrestrial or marine animals do (HUBENDICK 1958). Nevertheless, AZIZ and RAUT (1996) designate temperature as an especially critical ecological factor. Low temperatures cause snails to hibernate, during which time their movement, feeding, growth, and reproduction are inhibited and respiration is reduced to a minimum (HUBENDICK 1958); this indicates that temperature plays a crucial role in regulating a snail's physiology (AZIZ & RAUT 1996). The years 2014 and 2015 were the warmest and the second warmest year, respectively, since records began being kept in 1768. The deviation from the mean was +1.7 °C in 2014 and +1.5 °C in 2015, with particularly high temperatures being recorded during the summer of 2015 (ZAMG 2016a,b). Warm conditions lead to denser freshwater snail populations, which explains why the most snails were found during the summer and very few in May. This conforms to the results of GAUB (2014), who found the most *G. truncatula* and *L. stagnalis* respectively in June and June to August while sampling in 2012, and HAIDER (2010, 2012), who observed the highest densities from June to September during her 2008 sampling trips in the same region.

The reproduction cycle of freshwater snails also contributes to the frequency of snails in relation to the season. The smaller size classes 1 (4 mm – 8 mm) and 2 (9 mm – 13 mm) of *Radix* spp. showed peaks in both July and September, while the larger size classes 3 (14 mm – 18 mm) and 4 (19 mm – 24 mm) had a peak in August. This shell size distribution indicates that the *Radix* spp. has a two-peak reproduction cycle, with a first peak in the spring/early summer and a second in the late summer/autumn. A similar pattern has also been observed in the lymnaeid snail *G. truncatula* by GAUB (2014), HAIDER (2010, 2012), and HÖRWEIG et al. (2011).

A bimodal reproduction cycle is not as obvious in *L. stagnalis* as in *Radix* spp., as we have size information for only three months (which are not even consecutive). However, the smaller size classes 1 (16 mm – 25 mm) and 2 (26 mm – 35 mm) showed a conspicuous first peak in July, while the larger classes 3 (36 mm – 45 mm) and 4 (46 mm – 57 mm) increased slowly until August. The *L. stagnalis* thus seems to have a two-peak reproduction cycle, which has more clearly been observed by GAUB (2014).

Snails of the larger size classes that were found in the spring/early summer are usually hibernated individuals from previous years. In May, no *Radix* spp. and only one *L. stagnalis* of class 1 were found. Instead the following was found: two class 2, one class 3, and one class 4 *Radix* spp.; one class 2, three class 3, and two class 4 *L. stagnalis*. The larger sizes were thus overrepresented. The individuals found hibernated during the winter and returned to active life once the temperatures rose (HUBENDICK 1958), which led to the first reproduction peak in the spring/early summer. In contrast, the second reproduction peak in the late summer/autumn can be traced back to the new generation of snails that sexually matured during the summer's warm conditions (AZIZ & RAUT 1996).

It is evident that the physiology of freshwater snails is crucially affected by temperature, thus also by the seasons. Altered lengths of winter may thus have a severe impact on snail populations.

4.2. Digenean Infections in Snails

4.2.1. Infection Rates and Snail Sizes

In this project, a total trematode prevalence of 24% was observed in lymnaeid snails. This prevalence is in accordance with the average prevalence of 23% that was noted by KURIS and LAFFERTY (1994, cited in LOY & HAAS 2001) on the basis of combined data on 296,180 snails from 62 studies. The infection rates of *Radix* spp. and *L. stagnalis* were only slightly different (respectively 24.8% and 20.5%). GAUB (2014) observed a similar infection rate (19.5%) in *L. stagnalis* collected at the Leitha River, while FALTÝNKOVÁ et al. (2007) discovered a remarkably lower rate (10.8%) among 203 *L. stagnalis* collected from the Austrian Danube. Infection rates can significantly vary both locally and annually, depending on the vertebrate and invertebrate diversity (LOY & HAAS 2001). For instance, LOY and HAAS (2001) observed a distinctly higher infection rate in *L. stagnalis* in southern Germany (35% in 15,809 individuals). However, we observed a clear difference in infection rates between smaller and larger individuals. In *Lymnaea stagnalis*, 3% of individuals

35 mm or smaller were infected with trematodes, whereas the infection rate in those 36 mm or greater was 37%. A positive correlation between shell size and infection rate thus obviously exists in *L. stagnalis*. This observation, which is referred to as “gigantism”, is known from numerous invertebrate taxa (especially gastropods infected with trematodes) (POULIN 1998, cited in SORENSEN & MINCHELLA 2001). This phenomenon observed by many researchers led to the general assumption that larger snails usually have a higher infection rate than smaller snails. MCCLELLAND and BOURNS (1969, cited in BROWN et al. 1988) were also able to observe this relation in *L. stagnalis* infected with *Trichobilharzia ocellata*, which not only showed increased growth rates, but also infecundity.

In contrast, the correlation between infection rate and shell size could not be observed in *Radix* spp., as 28% of smaller snails (≤ 13 mm) and 20% of larger individuals were infected. The inability to observe the correlation could be due to the influence of trematodes on their host. As previously explained, larger snails observed in the spring or early summer were born in previous years and hibernated during the winter. Since trematodes have a negative effect on their first intermediate host, they can decrease its survivability significantly (SOSENSEN & MINCHELLA 2001). A possible explanation for the missing correlation between size and infection rate is thus that numerous infected snails died during hibernation. This would also explain why we found such few *Radix* snails in May. However, the effect of trematodes on *Radix* spp. must be further analysed to test the gigantism hypothesis (POULIN 1998, cited in SORENSEN & MINCHELLA 2001).

4.2.2. Digeneans in *Radix* spp. and *Lymnaea stagnalis*

Echinostome infections were found with a prevalence of 15.6% in *Radix* spp., which makes it the most common infection within this genus in this survey; in contrast, only 1.7% of *L. stagnalis* harboured echinostomes. GAUB (2014) noticed a similarly low prevalence of echinostomes in *L. stagnalis* (1.8%). However, BROWN et al. (1988) observed echinostomes as the dominant infection in *Lymnaea elodes* collected in Indiana, USA. Members of the family Echinostomatidae are known to be common trematodes in lymnaeid snails (BARGUES et al. 2001). In this study, the echinostomatids *Echinostoma revolutum* and *Echinoparyphium recurvatum* were observed in 0.2% (1), and 2.6% (13) of 505 *Radix* spp., respectively, and *Hypoderaeum conoideum* in 0.9% (1) of 117 *L. stagnalis*. All of these species are known to occur in European lymnaeid snails and belong to the common fauna (FALTÝNKOVÁ et al. 2015, FALTÝNKOVÁ et al. 2016, TOLEDO et al. 1999). *E. revolutum* has even been observed in a similar prevalence in France (i.e. 0.9% of 206 *Omphiscola glabra*) (RONDELAUD et al. 2015). However, no further data

concerning the prevalences of these species in snails that could be used for comparative purposes were found, as most surveys engaged in echinostome infections in definitive hosts. For instance, SAIJUNTHA et al. (2013) examined 90 free-gazing ducks in northern, central, and north-eastern regions of Thailand with regard to echinostome infections and detected *Echinostoma revolutum*, *Echinoparyphium recurvatum*, and *Hypoderaeum conoideum* in 51 (56.7%) of them. High prevalences of these echinostomes in domestic ducks have also been observed in Bangladesh (YOUSUF et al. 2009, cited in SAIJUNTHA et al. 2013), while *Hypoderaeum conoideum* has been observed in Vietnam (ANH et al. 2010, cited in SAIJUNTHA et al. 2013). In Korea, SOHN et al. (2015) detected metacercariae of *Echinostoma* sp. in 50 of 594 (8.4%) and in 94 of 283 (33.2%) freshwater fish in Hantangan and Imjingang, respectively. Also in Korea, SHIN et al. (2015) analysed stray cats with regard to trematode infections and observed *Echinochasmus* spp. in 9.4%, *Echinostoma hortense* in 1%, and *Echinoparyphium* sp. in 0.5% of 203 individuals in Seomjingang. In Europe, *Echinostoma* sp. was found in 10.6% of 99 foxes in Lithuania (BRUZINSKAITE-SCHMIDHALTER et al. 2012, cited in AL-SABI et al. 2014), in 1% of 100 foxes in Denmark (GLUIDAL & CLAUSEN 1973, cited in AL-SABI et al. 2014), and in 0.9% of 639 foxes in Poland (BORECKA et al. 2009, cited in AL-SABI et al. 2014). All of these data indicate a generally cosmopolitan occurrence of echinostomes in the wildlife in both Asia and Europe, which conforms to the high prevalence of echinostomes observed in *Radix* snails in this study.

In this survey, xiphidiocercariae were found in 1.2% of *Radix* snails and in 16.2% of *L. stagnalis*. A similarly high prevalence in *L. stagnalis* was also observed by GAUB (2014), who found xiphidiocercariae in 13.3% of 339 collected individuals. Xiphidiocercariae were thus the most common trematode infection in *L. stagnalis* in both of these studies. This is not surprising, as xiphidiocercariae are recognized as being the most common trematodes in *L. stagnalis*, especially those of *Plagiorchis* genus (which is regarded not only as cosmopolitan, but also as ecologically important in European freshwater snail populations) (FALTÝNKOVÁ et al. 2007, KONEČNY et al. 1999, SOLDÁNOVÁ et al. 2011, cited in ZIKMUNDOVÁ 2014). The member *P. elegans* is one of the larvae that has been recorded most frequently (ZBIKOWSKA 2007, cited in ZIKMUNDOVÁ 2014); in the Czech Republic, it is the most common larva in snails of eutrophic ponds (SOLDÁNOVÁ & KOSTADINOVA 2011, cited in ZIKMUNDOVÁ 2014). Furthermore, FALTÝNKOVÁ et al. (2007) observed *P. elegans* and *Opisthioglyphe ranae* – the latter also developing xiphidiocercariae – as the most dominant cercariae in their study on 3,628 *L. stagnalis* collected in Austria, the Czech Republic, Germany, Poland, and Slovakia. An exemplary photo of a xiphidiocercaria that we isolated from *L. stagnalis* is provided in figure 58 A. As the stylet

is approximately 30 µm in length and has a distinct arrow-like triangular tip and the acetabulum is very large, this xiphidiocercaria most likely is *Plagiorchis* sp. or possibly even *P. elegans* (FALTÝNKOVÁ et al. 2007).

Amphistome cercariae occurred in 1.8% of the *Radix* snails found but in no *L. stagnalis*. GAUB (2014) also did not find any amphistome cercariae in *L. stagnalis*, although she did in *G. truncatula*. HÖRWEIG et al. (2011) also observed amphistome cercariae of *Paramphistomum* in *G. truncatula*. Lymnaeid snails are known to transmit, inter alia, *Paramphistomum* spp. (BARGUES et al. 2001) and further members of the family Paramphistomatidae, which are digeneans developing amphistome cercariae with characteristic eye-spots and a terminal acetabulum. The observed amphistome cercariae could belong to this trematode family (SATTMANN 2015, pers. comm., December). As only nine snails infected with amphistomes (1.5% of 622 snails) were found, they seem to be a rare taxon. This is in agreement with SEY (2001).

Monostome cercariae occurred even more rarely; it was found in only one *Radix* snail, which indicates a prevalence of 0.2% within this genus. No *L. stagnalis* with monostome cercariae was found, although GAUB (2014) observed monostome cercariae of *Notocotylus* sp. in 2.1% in the Leitha surroundings and KONEČNÝ et al. (1999) observed them in one of six (16.7%) *L. stagnalis* in the Lobau. Exemplary photos of the monostome cercariae observed in this study are provided in figure 59 A and B. A morphological comparison with monostome cercariae isolated by SKÁLA et al. (2014) and FALTÝNKOVÁ et al. (2007) leads to the conclusion that this specimen is *Notocotylus* sp. Mature notocotylid cercariae possess a dark pigmentation and three eye-spots, whereas those that are immature are brighter and have only two eye-spots (SKÁLA et al. 2014). Beyond the work of GAUB (2014) and KONEČNÝ et al. (1999), this genus has also been found through many other surveys in Europe, including those conducted by FALTÝNKOVÁ (2005, cited in FALTÝNKOVÁ et al. 2007) and NASINCOVÁ (1992, cited in FALTÝNKOVÁ et al. 2007).

Two *L. stagnalis* (1.7%) possessed trichobilharzian cercariae, namely of *T. szidati*. DVOŘÁK et al. (1999) found *T. szidati* in higher prevalences, namely 5.2% (n = 96) and 6.3% (n = 32) in two ponds in the Vienna Woods. In contrast, GAUB (2014) observed a prevalence of only 0.9% of *Trichobilharzia* in the Leitha River. LOY and HAAS (2001) observed an even lower prevalence of *T. ocellata* (0.17%) in 43,441 *L. stagnalis*. It is known that infection rates are not stable and may change over years (or even more rapidly), which leads to very different prevalences locally (LOY & HAAS 2001). This is also true for *Trichobilharzia* in *Radix* spp. In this study, no *Radix* snails harbouring bird schistosomes

were found. Instead, 1% possessed other furcocercariae, possibly belonging to the family Diplostomidae or Strigeidae (figure 60 A, B). However, *Radix* spp. is known to transmit bird schistosomes (CHRISTIANSEN et al. 2016, DVOŘÁK et al. 1999, HUNOVA et al. 2012, cited in LAWTON et al. 2015). For example, CHRISTIANSEN et al. (2016) found avian schistosomes in 1.7% of the *Radix* sp. (n = 116) they collected in the surroundings of Copenhagen, Denmark, whereas LOY and HAAS (2001) observed *Trichobilharzia* spp. in 0.24% of 4,245 *Radix* snails in southern Germany and KONEČNÝ et al. (1999) found *Trichobilharzia* sp. in 1 of 45 (2.2%) *R. balthica* in Schönau/Donau, Austria.

Lymnaeid snails are obviously favoured intermediate hosts for many kinds of trematodes, with variable infection rates that change from time to time and depend on the location. To ensure that up-to-date information is available, regular investigations are therefore necessary.

4.2.3. Distribution of Infected Snails and Risk Sites

Although 47 locations were visited, only 16 harboured lymnaeid snails and 13 revealed snails infected by trematodes. In total, 149 trematode infections were found. Almost half of the locations with infections (5) were situated in the Danube floodplains. However, most positive snails were found at the LM01 location in the Leitha Mountains (64%). Positive samples found in the Danube floodplains account for 24% of all collected positive samples. The remaining 12% are apportioned to the Leitha floodplains (8%), artificial waterbodies (3%), and the Morava floodplains (1%). However, it should be taken into account that conspicuously more snails were collected in the Leitha Mountains (259) and Danube floodplains (220) than in the artificial waterbodies (73), the Morava floodplains (34), or the Leitha floodplains (28).

The prerequisite for a trematode infection being established in a waterbody is the availability of a definitive host that feeds on the snails and leaves its faeces near or directly in the waterbody. The highest average infection rate was observed in the Leitha floodplains (42.9%), with the highest focal prevalence at the LF05 site (where 88.9% of *L. stagnalis* collected were infected). This high percentage at LF05 needs some reconsideration seeing as so few snails were found here (9). GAUB (2014) observed a noticeably lower infection rate of 19.6% (n = 311) at this location. These high prevalences of trematode infections result from the habitat's structure, which is very attractive for avian definitive hosts given that it is part of a nature sanctuary and provides numerous nesting and stopover possibilities for migrating birds. The floodplains in the Danube-Auen National Park are also a nature sanctuary. Since the calculated average prevalence is

15.9%, the trematode burden in the Danube floodplains is also high. This is in agreement with Liesinger (2011), who observed many faecal deer samples that tested positive for trematode infections in the Danube floodplains (especially in Orth/Donau). The prevalences she calculated for three locations in the surroundings of Orth/Donau ranged between 11.8% ($n = 34$) and 55% ($n = 20$). The infection rates observed in this study along the 13 km-long section of the Danube ranged between 6.9% at DF01 ($n = 160$) and 64.3% at DF10 ($n = 14$), although the sample size of the latter was very small. Due to these high prevalences observed as part of both of these studies, it can be concluded that the Danube plays an important role in spreading snail populations and their inhabitants (LIESINGER 2011) and thus should be recognized as a risk site for infection.

Another site with a high trematode burden was LM01 in the Leitha Mountains. Three visits to this pond in two summers revealed constantly high infection rates that ranged from 34% ($n = 147$) to 50% ($n = 12$), with an average of 36.7%. These high prevalences were likely due to the pond's location in a broadleaf forest of the Mannersdorf-Wüste nature park, which has a great Pannonian faunal biodiversity (WWF 2016b). Furthermore, it is near a farm with free-range chickens, which makes repeated contact between the snails in the waterbody and a variety of potential definitive hosts likely (and may have led to a constantly high infection status).

The AW04 location stands out among the artificial waterbody sites. As it is a completely man-made construction (namely a retention basin with only little grass-like vegetation), the snail population illustrates its great adaptability to artificial habitats. Only eight snails were found here, but two of them harboured trematodes (25%). Since this basin has little to no contact to other waterbodies, it is most likely that this infection was introduced by an infected vertebrate that left its faeces nearby. This explanation might also be applicable to the second artificial waterbody (AW03) that possessed trematode infected snails, albeit with a lower prevalence (4.6%, $n = 65$). However, as this man-made waterbody was built as a natural swimming pond (with reeds on its waterfront and algae on the ground), it offers a suitable habitat for freshwater snails. This location was chosen as sampling site because its owner contracted cercarial dermatitis after swimming there. As the pond had not had trematodes before, the relevant species must have been recently introduced by an avian host.

It is striking that 13 of 16 locations where snails were found also had verifiable infections, which means that 81% of the locations inhabited by snails were also inhabited by trem-

atodes. One possible explanation is that snails serve as food for several vertebrate animals, including not only amphibians, reptiles, and birds (e.g. songbirds, waterfowl, and poultry), but also mammals such as hedgehogs and rodents (NORDSIECK n.d.). If snails occur at one location, it is thus likely that their predators will also occasionally visit the site and possibly stay for more time. A cycle of repeating infections and trematode population can thereby emerge. In conclusion, nearly every location inhabited by freshwater snails should be considered to be a risk site for trematode infections.

4.2.4. Seasonality of Infections

The highest infection rates were observed in September (34.3%) and July (26.8%). The development of trematode larvae and release of cercariae are influenced by various ecological factors, such as season and water temperature (FARAHNAK 2006, cited in IMANI-BARAN 2013). However, these influences are species-specific and may vary (IMANI-BARAN 2013). According to RAPSCH et al. (2008), trematode eggs are not able to develop below 10 °C. In March, the mean temperature in Lower Austria and Burgenland was below 10 °C (ZAMG 2016c). In April, which is when the first sampling trip was undertaken, the mean temperature was about 12 °C (ZAMG 2016d); in May it rose to 15 °C (ZAMG 2016e). The development of trematode eggs and the infection of an intermediate host were thus possible and potentially increasing. An infection rate of 18.2% was observed in May, but as the sampling size was very small ($n = 11$), this high trematode prevalence could be a statistical outlier. This is supported by the fact that only one sampling trip was undertaken in May and all snails collected during that trip were found in one location. The temperatures rose throughout June and the mean temperature in July was about 24 °C (ZAMG 2016f), which is when the second highest infection rate (namely 26.8%) was observed. Miracidial development within the egg needs two to three months between 12 and 16 °C, but only two to three weeks between 23 and 26 °C. The latter is thus the optimal temperature range for infections, although the ideal temperatures may vary slightly between different species (RAPSCH et al. 2008). The rising temperatures from May to July thus offered good conditions for larvae development and led to many infection events in July. This is in agreement with SATTMANN and HÖRWEIG (2006), who assumed that the spring and early summer, when rainfall and therefore water levels are usually high and eggs easily come into contact with water, provide good conditions for the infection of snails by *Fascioloides* miracidia.

In August, a remarkably low infection rate (14%) was observed that was even lower than that of May. That month had several days with temperatures far above 25 °C (ZAMG

2016g). Although such temperatures are usually too high for snails to survive and reproduce (KENDALL & MCCULLOUGH 1951, ARMOUR 1975, both cited in RAPSCH et al. 2008), August was the most productive month in regard to snail numbers. The explanation for the low infection rate in August should thus not be sought in the temperature profile, but rather in rainfall. Snails need moisture to both survive and reproduce (FRÖMMING 1956, cited in RAPSCH et al. 2008), and free-living trematode stages need rainfall in order to be transmitted to the next host. The optimal rainfall is approximately 90 to 210 mm per month (RAPSCH et al. 2008). July already had dry phases, but August had even more: Compared to the mean, rainfall was 36% in Lower Austria and 41% lower in Burgenland. Total rainfall for August was 54 mm in Eisenstadt and 44 mm in Vienna (ZAMG 2016g). This drought may explain the lower infection rate observed in August.

The highest infection rate (34.3%) was observed in September. Although this month had only slightly more rainfall than August (Eisenstadt had 63 mm while Vienna had 50 mm), the temperature was around 17 °C in the sampling area and several days were between 20 and 25 °C (ZAMG 2016h). These temperatures created conditions that were suitable for both snail reproduction and trematode larvae development. SOLDÁNOVÁ et al. (2016) observed even a seven-fold increase in cercarial emission in September. They suggest that sporocysts producing cercariae alternate with those producing daughter sporocysts. It thus takes 35 to 40 days in schistosomes until a new cercariae generation emerges. As a consequence, the infection rate may differ monthly due to varying cercarial emission and, as a result, varying infection events. It is assumed that the pattern of cercarial emergence coincides with the activity rhythms of subsequent hosts (COMBES et al. 1994, cited in SOLDÁNOVÁ et al. 2016).

Larvae development within the snails and cercarial shedding are also both temperature-dependent. No development takes place below 10 °C, and the development rate quickens with rising temperature; at the optimum, cercariae may be released as early as five weeks post infection (HANSEN & PERRY 1994). Cercariae can be shed for weeks or even months (ECKERT et al. 2008). HÖRWEG et al. (2006) observed cercarial dermatitis in Austria between May and August, but found most cases in August. Furthermore, they recognized swimming pond owners as a new at-risk segment of the public. The case of cercarial dermatitis observed during this study occurred in a private swimming pond in the mid of June 2015, which means the infection of the snails by miracidia could have happened in May. However, as the larvae may survive in the snails during the winter, the infection could also have occurred in 2014. HORÁK and KOLÁŘOVÁ (2011) also observed that bird schistosomes are indeed capable of surviving in overwintering snails

and thereby serve as a source of infection in spring. This ability demonstrates how infections often persist from season to season (HANSEN & PERRY 1994).

Another fact contributing to the infection status is the survivability of free metacercariae, which essentially depends on temperature. Metacercariae of *Fasciola hepatica* can survive for six months when the temperature is between 12 and 14 °C. Between 2 and 5 °C, life spans of up to one year have been reported. Survivability decreases with increasing temperature, but is still eight weeks at 20 °C (RAPSCH et al. 2008). Metacercariae produced in the spring and summer may thus remain infective during the following weeks and months, thereby increasing the infection rate of snails until autumn. However, it is very unlikely that metacercariae will survive during the winter, as they cannot resist prolonged periods of freezing and drought (HANSEN & PERRY 1994). The situation may be different for metacercariae that reside in a homeothermic second intermediate host, where conditions are constant.

While recognizing a real seasonality in the overall data is difficult given the different locations considered, the influence of temperature, drought, and rainfall are clear.

4.3. Medical Importance of the Findings

4.3.1. Schistosomatid Digeneans

Trichobilharzia szidati was observed in two *L. stagnalis* snails of the private swimming pond (AW03). The cercariae of the genus *Trichobilharzia* are the most important causative agents of cercarial dermatitis in Europe and North America (SOLDÁNOVÁ et al. 2013, BRANT & LOKER 2013, both cited in SOLDÁNOVÁ et al. 2016), and *L. stagnalis* is its only intermediate host known so far (BRANT & LOKER 2009, JOUET et al. 2010, SEMYENOVA et al. 2015). *T. szidati* was molecular biologically proven for the first time in Austria in 2014 by GAUB (2014), and it is, besides *Bilharziella polonica* – another literary confirmed causative agent of human cercarial dermatitis (KOLÁŘOVÁ 2007) – the only bird schistosome that is proven to occur in Austria (AUER et al. 1999, HÖRWEG et al. 2006). However, cercarial dermatitis is known in Austria since 1969, when the first cases of this disease appeared (GRAEFE 1971, GRAEFE et al. 1973, both cited in HÖRWEG et al. 2006). Further cases in Austria were summarized by KONEČNÝ and SATTMANN (1996, cited in SATTMANN et al. 2004). Since 1969 the disease has occurred almost every summer (ASPÖCK et al. 2002, HÖRWEG et al. 2006). In contrast to *Trichobilharzia* spp., which use lymnaeid snails as intermediate hosts (KOLÁŘOVÁ 2007), *B. polonica* is transmitted by planorbid snails

(ALDHOUN et al. 2012, BRANT & LOKER 2009) and most commonly by *Planorbarius corneus* (ALDHOUN et al. 2012). However, KHALIFA (1972, cited in BAYSSADE-DUFOUR et al. 2006) isolated cercariae of *Bilharziella* also from *Planorbis planorbis*, and *Bathymophalus contortus*. Although *P. planorbis* and *B. contortus* also occur in Austria and the Czech Republic (SEDDON & VAN DAMME 2014a,b), only *P. corneus* is known from these countries to transmit *B. polonica* (ALDHOUN et al. 2012, HÖRWEIG et al. 2006), and even though *B. polonica* has already been reported as causative agent of human cercarial dermatitis in Germany (BAYSSADE-DUFOUR et al. 2006), the infection is most frequently associated with the genus *Trichobilharzia* (KOLÁŘOVÁ 2007). *B. polonica* is considered to play a minor role as causative agent of this disease (GRAEFE 1971, cited in SATTMANN et al. 2004).

Further causative agents of human cercarial dermatitis in Europe might be the trichobilharzian species *T. franki* and *T. regenti*, both of which use lymnaeid snails of the genus *Radix* as intermediate hosts (ALDHOUN et al. 2012). *T. franki* was observed in *R. auricularia* and *R. labiata*, while *T. regenti* was found in *R. labiata* and *R. balthica* (BRANT & LOKER 2009, FERTÉ et al. 2005, JOUET et al. 2010). Although so far *T. franki* and *T. regenti* have not been proven in Austria, it is likely that they also occur in Austria given the easy spreading of schistosomes by migrating waterfowl (KOLÁŘOVÁ 2007).

The genus *Trichobilharzia* is closely related to the African blood flukes that cause bilharziosis, namely *Schistosoma* spp. (SEMYENOVA et al. 2015). Invasion in human skin is similar, but *Trichobilharzia* spp. appear to be more efficient than *Schistosoma* spp. The efficiency of *Trichobilharzia* spp. might be an adaptation to duck hosts, whose skin contains much fewer specific penetration signals than human skin (HAAS & HAEBERLEIN 2009). *Trichobilharzia* spp. are attracted by stimuli such as unsaturated fatty acids (KOLÁŘOVÁ 2007), ceramides, cholesterol, shadowing, movements, and warmth (FEILER & HAAS 1988a,b, cited in HAAS & HAEBERLEIN 2009), with a particular affinity for linolenic acid. The skin of ducks also possesses these components, but the cercariae are much more stimulated and attracted by human skin given its higher content of fatty acids (HAAS & HAEBERLEIN 2009) – which is why humans can be mistaken for waterfowl by *Trichobilharzia* species. HAAS and HAEBERLEIN (2009) observed that *T. szidati* prefers to penetrate in wrinkles and hair follicles; no single cercaria entered through smooth skin. The cercariae managed penetration within 4.0 min on average, although the fastest succeeded after only 83 sec. This indicates that short contacts to a burdened waterbody can be sufficient for infection, which agrees with the findings of HÖRWEIG et al. (2006). These researchers' investigation revealed that 3 of the 34 interviewed individuals who suffered from cercarial dermatitis had been in the water for only 5 min. Although the remaining 31

individuals stayed in the water for up to several hours, this long exposure did not necessarily alter the intensity of disease. For instance, one person bathed for 4 h but did not develop symptoms that were more severe than those experienced by others.

In comparison, the owner of the swimming pond (AW03) bathed twice for totally 30 min and developed severe symptoms.

As cercarial dermatitis is caused by the cercariae, it might be interesting to look at the cercarial shedding of the snails. SOLDÁNOVÁ et al. (2016) analysed *L. stagnalis* snails infected with *T. szidati* and observed a daily emission rate of 1,117 cercariae per snail. However, this rate was highly variable between different individuals and ranged from 40 to 4,560 cercariae. That investigation highlights that one single snail might be enough to lead to cercarial dermatitis, especially during prolonged water activities. In comparison, in this survey 65 *L. stagnalis* were collected from the private swimming pond and only two were found to be infected, but they shed enough cercariae to evoke massive itchy papules distributed over the owner's whole body.

Further causes of human cercarial dermatitis might be species of the genera *Dendritobilharzia*, *Austrotilharzia*, and *Gigantobilharzia* (FREW et al. 2016, HÖRWEG et al. 2006, KOLÁŘOVÁ 2007), which are also bird schistosomes and known to occur in Europe (HÖRWEG et al. 2006, KOLÁŘOVÁ 2007). *Gigantobilharzia* and *Austrotilharzia* are genera that occur in salt as well as brackish waters (KOLÁŘOVÁ 2007), and the former shows also considerable diversity in the intermediate hosts: In marine environments, the genus *Haminoea* (McGeachin 1979) is used whereas in freshwater cycles members of the families Physidae (BRANT et al. 2011, BRANT & LOKER 2009) and Planorbidae (ALDHOUN et al. 2012) are used as intermediate hosts. Planorbid snails are also the intermediate hosts of *Dendritobilharzia* spp. (BRANT et al. 2011, BRANT & LOKER 2009), which were reported as causative agents of human cercarial dermatitis in Germany and New Zealand (BAYSSADE-DUFOUR 2006). *Austrotilharzia* spp. were reported as causative agents of this condition from several regions in Australia (FREW et al. 2016).

However, avian schistosomes are not the only species that might cause human cercarial dermatitis. Mammalian schistosomes such as *Orientobilharzia*, transmitted by *Lymnaea* species, are also capable of causing the condition in humans (KOLÁŘOVÁ 2007, MORLEY 2016b). *Orientobilharzia* is considered the major cause of this disease in the Caspian Sea area of Iran and several provinces in China (WANG et al. 2009).

Several snail families and schistosome genera obviously contribute to the risk of contracting cercarial dermatitis. This disease is a recurring problem not only in Austria, but also many other European countries, and *T. szidati* has once again been proven to be a potential cause in Austria. Both regular investigations by researchers and personal measures are necessary in order to reduce the risk of infection.

4.3.2. Echinostomatid Digeneans

Thirteen *Radix* snails harboured larvae of *Echinoparyphium recurvatum*, and one was infected with *Echinostoma revolutum*. *Hypoderaeum conoideum* was also found once in *L. stagnalis*. TOLEDO and ESTEBAN (2016) stated that these three trematode species belong to the major causative agents of human echinostomosis. However, *E. revolutum*, which is the type species of the so-called “37 collar-spined” or “revolutum” group, is the most widespread species and responsible for most cases in Southeast Asia (NAGATAKI et al. 2015). Infections with this particular species are contracted through the eating of raw snails and frogs (TAYLOR et al. 2016) and have been reported from Cambodia (SOHN et al. 2011), Lao PDR (CHAI et al. 2012), Taiwan (ANAZAWA 1929, CARNEY 1991, both cited in TOLEDO & ESTEBAN 2016), Indonesia (EVELAND & HASEEB 2003), and Thailand (BHAIBULAYA et al. 1966, cited in NAGATAKI et al. 2015), but also from Africa and sporadically from Russia (CHAI 2009, cited in TOLEDO & ESTEBAN 2016). However, the question of whether the European strain is as pathogenic as the Asian strain remains unanswered. NAGATAKI et al. (2015) studied mitochondrial DNA sequences of *E. revolutum* specimens and observed that those from both Southeast Asia and Europe cluster in a monophyletic clade that is genetically distinct from American specimens. It is thus likely that both the European and Asian *E. revolutum* strain can cause echinostomosis. According to TOLEDO and ESTEBAN (2016), there were in fact cases of human echinostomosis due to *E. revolutum* in Europe.

Human infections with *E. recurvatum* have been reported from Taiwan, Indonesia, and sporadically Egypt (BEAVER et al. 1984, cited in SOHN 1998, CHAI 2009, EVELAND & HASEEB 2003), in contrast, infections with *H. conoideum* were observed only in Thailand, in whose northeast region it is a common causative agent of echinostomosis (EVELAND & HASEEB 2003, GRACZYK & FRIED 1998, YOKOGAWA et al. 1965, cited in TOLEDO & ESTEBAN 2016). Infectious metacercariae of both species can be contracted through the eating of raw snails, fish, shellfish, and tadpoles (TAYLOR et al. 2016).

Echinostomes generally exhibit a great variety in their molluscan intermediate hosts: They were found in planorbids (i.e. *Indoplanorbis* spp., *Gyraulus* spp., *Hippeutis* spp., *Polypylis* spp.), lymnaeids (i.e. *Lymnaea* spp., *Austropeplea* spp., *Radix* spp.), bithyniids (i.e. *Parafossarulus* spp.), members of the family Ampullariidae (i.e. *Pila* spp.), and in those of the family Viviparidae (i.e. *Viviparus* spp., *Filopaludina* spp.) (DETWILER et al. 2010, PARIYANONDA & TESANA 1990, cited in SAIJUNTHA et al. 2011b, WHO 1995). Species diversity is even greater when it comes to the second intermediate host, which can be a variety of fish, molluscs, and amphibians (WHO 1995). Many animal species thus contribute to the abundance of echinostome pathogens, and many echinostome species are not specific to one single intermediate host species (DETWILER et al. 2010). For example, SOHN (1998), who analysed the life cycle of *E. recurvatum* in Korea, found this trematode in *R. auricularia*, while ESTEBAN and MUÑOZ-ANTOLI (2009) mention *R. labiata* as intermediate host of *E. recurvatum*. In this study, *E. recurvatum* was found in *R. auricularia*, *R. labiata*, and *R. balthica*.

Knowledge concerning the appearance of echinostomes in Austria is important for two reasons. First, eating habits in Europe are predicted to change in the future and will include new animal taxa. Second, although humans usually become infected from eating snails that harbour metacercariae, TOLEDO and ESTEBAN (2016) reported on cases where the infection may have resulted from drinking water that was contaminated with cercariae. This form of transmission is much harder to control, as the causative agents are ubiquitous and a simple bath in a burdened waterbody might lead to an infection through swallowed water. Furthermore, knowledge on the occurrence of echinostomatids is also important for veterinary medicine, as domestic animals such as dogs, cats, and pigs can also suffer from echinostomosis (ASPÖCK & WALOCHNIK 2007, OKANISHI et al. 2013).

5. Abbreviations

µl	-	microlitre
µm	-	micrometre
ADW	-	Animal Diversity Web
APO	-	Aquatic Parasite Observatory
AW	.	artificial waterbody
BLAST	-	Basic Local Alignment Search Tool
bp	-	base pairs
CABI	-	Centre for Agriculture and Bioscience International
CDC	-	Centers for Disease Control and Prevention
cm	-	centimetre
CO1	-	Cytochrome C Oxidase Subunit 1
DF	-	Danube floodplains
DMG	-	Deutsche Malakozoologische Gesellschaft
DNA	-	deoxyribonucleic acid
dNTP	-	deoxyribonucleotide
g (force)	-	gravitational
GBIF	-	Global Biodiversity Information Facility
ICPDR	-	International Commission for the Protection of the Danube River
ITIS	-	Integrated Taxonomic Information System
IUCN	-	International Union for Conservation of Nature
kg	-	kilogram
km	-	kilometre
LF	-	Leitha floodplains
LM	-	Leitha Mountains
M	-	molar
mA	-	milliampere
MF	-	Morava floodplains
mg	-	milligram
MgCl ₂	-	magnesium chloride
min	-	minute(s)
ml	-	millilitre
mM	-	millimolar
mm	-	millimetre
NaAc	-	sodium acetate
NaCl	-	sodium chloride
NCBI	-	National Center for Biotechnology Information

ND1	-	Nicotinamid Adenin-Dinucleotide Dehydrogenase Subunit 1
PCR	-	Polymerase Chain Reaction
PDR	-	People's Democratic Republic
pmol	-	pikomole
rDNA	-	ribosomal DNA
rpm	-	rounds per minute
sec	-	second(s)
sp.	-	species (singular)
spp.	-	species (plural)
U	-	unit(s)
USF	-	University of South Florida
UV	-	ultraviolet
V	-	volt
WHO	-	World Health Organization
WR	-	Wulka River
WWF	-	World Wide Fund For Nature
ZAMG	-	Zentralanstalt für Meteorologie und Geodynamik

6. Glossary

Acetabulum: A muscular organ of attachment that is commonly called “ventral sucker”.

Adenine: A purine derivate that is one of the four nucleotides of nucleic acids.

Agarose: A polysaccharide used in molecular sciences, e.g. gel electrophoresis.

Alignment: Arrangement of two or more DNA sequences. Homology and evolutionary relationships can be inferred from the output.

Allergic reaction: Hypersensitivity against a foreign substance.

Alternation of generations: A species' life cycle in which two or more distinct forms occur in successive generations, one sexual, the other asexual.

Amphistome: A type of trematode cercariae that possesses suckers on both anterior and posterior end.

Amplicon: A nucleic acid segment revealed by amplification, e.g. PCR.

Amplification: Multiplication of nucleic acid segments with the help of enzymes.

Anterior: An anatomical term of location that refers to the frontal end.

Anthropogenic: Changes caused by humans.

Apex: Tip of the snail's shell.

Axoneme(s): Axonemes are a cytoskeletal structure that compose the inner core of cilia.

Barcode: A barcode is a short DNA segment that is often used in molecular phylogeny.

Base: Four bases exist in nucleic acids, namely adenine, cytosine, guanine, and thymine/uracil.

Base pair (bp): DNA strands bind to each other by complementary base pairing. Of the four bases of DNA guanine pairs with cytosine and adenine with thymine.

Benign: Harmless.

Bilateral (symmetry): Synonymous with mirror symmetry. Organisms with bilateral symmetry can be divided in mirror image halves.

Bilharziosis: See schistosomosis.

Bird schistosome: Refers to species of the family Schistosomatidae that use birds as definitive host. Bird schistosomes can cause cercarial dermatitis.

Birth pore: A pore through which cercariae are released from sporocysts and rediae.

Bursa: An organ of the reproductive system of gastropods.

Cephalic: An anatomical term of location that refers to the head region.

Ceramide: A lipid molecule that can be found in cell membranes.

Cercaria(e): Free-swimming larval stage of trematodes that are released from rediae or sporocysts within the intermediate molluscan host.

Cercariae release test: The cercariae release test is the first step in the examination of collected snails. The snails are therefore separated in glasses of water and positioned in a sunny place for 24 hours. Diffuse water indicates the release of cercariae.

Cercarial dermatitis: See swimmer's itch.

Cestoda/cestodes: Tapeworms, a class of flatworms living in the gut of vertebrates.

Cholesterol: A lipid molecule that can be found in cell membranes.

Chronic: A chronic disease is a persistent, long-lasting or recurring disease.

Clade: See monophylum.

Cladistics: A method of classification of species within a tree diagram according to their evolutionary relationships.

Class: A category consisting of similar or related orders.

Classification: The hierarchic arrangement of species to higher taxa based on relationships.

Clonorchiosis: A disease caused by *Clonorchis sinensis*, the Chinese liver fluke.

Coelom: A body cavity of various animals between ectoderm and endoderm.

Columella: An anatomical term that refers to a certain fold in adult gastropod shells.

Cyst: Quiescent stage of microorganisms, which is resistant to chemical and physical influences.

Cytochrome C Oxidase Subunit 1 (CO1): A subunit of a protein complex that is encoded mitochondrially and often used as DNA barcode.

Cytosine: A pyrimidine derivative that is one of the four nucleotides of nucleic acids.

Dead-end host: Intermediate or definitive host in which the parasite is not able to complete its life cycle.

Definitive host: A host in which the parasite reaches sexual maturity and undergoes sexual reproduction.

Digenea/digenean trematodes: A subclass of trematodes that is characterized by an alternation of generations.

Dioecious: A species of which the individuals are either female or male.

DNA (deoxyribonucleic acid): Carrier of genetic information for cells and organisms, which is composed of nucleotides.

Dorsal: An anatomical term of location that refers to the upper side of a parasite.

Dorsoventral: An anatomical term of location that refers to the side of a parasite.

Echinostomatid/echinostome: Refers to species of the family Echinostomatidae. The cercariae and rediae possess an eponymous head collar of spines.

Echinostomida: One of the four orders within the Digenea.

Echinostomosis: A gastrointestinal zoonotic disease caused by a variety of species of the family Echinostomatidae.

Ectoderm: One of the three germinal sheets in an early embryo. The other two sheets are endoderm and mesoderm.

Endoderm: One of the three germinal sheets in an early embryo. The other two sheets are ectoderm and mesoderm.

Ectoparasite/ectoparasitic: Parasites that parasitize on the host's body surface.

Electrophoresis: Method used for separating proteins and nucleic acids with the help of their electrical charge, size and shape.

Embryonated: An egg that contains a fully developed miracidium.

Encystation: The process in which a cercaria is encased in a cyst wall.

Endemic: Indigenous.

Endoparasite: Parasites that parasitize within the host's body.

Eosinophilia: Increased incidence of eosinophils in the blood.

Eosinophils: A special type of white blood cells.

Epidemiology: The study of distribution, causes and consequences of communicable and non-communicable diseases.

Epithelium/epithelial: Specialized cells that cover the body surface and internal cavities.

Eutrophic/eutrophication/eutrophized: An aquatic ecosystem in which artificial or natural nutrients were introduced by humans.

Evolution: Phylogenetic development of organisms, which is driven by mutations and selection.

Excretion: Elimination of waste products of the metabolism and other non-useful materials from an organism with the faeces.

Excystation: The process in which the parasite escapes from the cyst for further development.

Faeces: The waste matter evacuated from the digestive tract.

Family: A taxonomic unit that comprises genera.

Fasciolopsiosis: A disease caused by *Fasciolopsis buski*, which is the largest intestinal fluke of humans.

Fasciolosis: A disease caused by species of the genus *Fasciola*, for instance *F. hepatica*.

Fatty acid: A carboxylic acid that is an important source of energy.

Fauna/faunal: The totality of the animal species of a given area or a given period of time.

Final host: See definitive host.

Flatworm: Flatworms are characterized by bilateral symmetry, a hermaphroditic way of life, and usually a dorsoventrally flattened body. The latter characteristic is eponymous.

Fluke: See Trematoda.

Furcocercaria(e): Cercariae with a forked tail.

Gastritis: Inflammation of the gastric mucosa.

Gastropoda: Snails, a class of the phylum Mollusca.

Gel electrophoresis: A method to separate proteins or DNA fragments according to their size.

GenBank: A database that contains a collection of cloned gene fragments.

Gene: Smallest, hereditary unit of DNA that contains the information for the synthesis of a protein or RNA.

Genus: A category that contains species due to their similarity or relationship. The genus is in the hierarchy below the family.

Germ(inal) cells: Cells in an egg, redia or sporocyst from which the larvae grow.

Germinal sheet: A layer of cells formed during embryogenesis. Flatworms possess three germinal sheets, namely ectoderm, mesoderm and endoderm.

Gigantism: A phenomenon that refers to an increased body size and that is frequently observed in gastropods infected with trematodes.

Gonopore: A genital pore in the female adult through which the eggs are released.

Guanine: A purine derivative that is one of the four nucleotides of nucleic acids.

Habitat: A location in which a species occurs regularly.

Helminth: This term means “worm” and originally referred to intestinal worms. It includes nematodes, trematodes, and cestodes.

Herbal: Herbal food consists of plants and vegetables.

Herbivorous: Herbivorous animals feed only on herbal food.

Hermaphrodite/hermaphroditic: Individuals that possess both male and female reproductive systems.

Hibernation: A process during which movement, feeding, growth, and reproduction of the respective animal are inhibited and respiration is reduced to a minimum. Low temperatures cause snails to hibernate.

Homology: Term for characteristics of organisms that derived from a common ancestor.

Host specificity: The degree to which a parasite can use a certain species as a host.

Infection: The attack on a host by a pathogen that is characterized by invasion, proliferation, and immune response.

Infestation: A form of parasitism in which at least one of the three criteria of infections is not fulfilled.

Intermediate host: A host in which larval development occurs. These larvae are then infectious for the definitive host or a second intermediate host.

Invertebrate: Spineless animals.

Juvenile: Immature stage of an organism that has not yet reached sexual maturity.

Kingdom: A category that contains phyla due to their similarity or relationship.

- Larva(e):** Juvenile, immature stage of many animal groups that is marked by characteristics that are missing in adults.
- Life cycle:** The development of individuals of a particular species from the fertilized egg to sexual maturity.
- Linolenic acid:** A type of fatty acid.
- Lipid:** A molecule that is a structural component of cell membranes, but also involved in energy storage.
- Macrophyte:** A type of aquatic vegetation.
- Maculopapular:** A type of rash that is characterized by red bumps.
- Mesoderm/mesodermal:** One of the three germinal sheets in an early embryo. The other two sheets are endoderm and ectoderm.
- Metacercaria(e):** A developmental stage of digeneans that occurs after the encystation of cercariae.
- Metagonomosis:** A disease caused by *Metagonimus yokogawai*.
- Miracidium:** The first larval stage of trematodes that hatches from the egg.
- Mitochondrion/mitochondrial:** A cell organelle that provides the cell with energy and possesses its own DNA, which is a circular ring.
- Mollusca:** A phylum of soft-bodied invertebrates with an open blood circulation. This phylum includes, inter alia, the Gastropoda.
- Monogenea:** A subclass of the Trematoda that comprises ectoparasites of fish and amphibians. The Monogenea have no alternation of hosts or generations.
- Monophylum/monophyletic:** The totality of descendants of a single common ancestor.
- Monostome:** A type of trematode cercariae that lack a ventral sucker.
- Morbidity:** Number of cases of a particular disease.
- Morphology:** The study of the appearance, form and structure of organisms and organs.
- Mortality:** Number of deaths due to a particular disease in a particular population.
- Mutation:** Heritable changes in the nucleic acid sequence. Mutations are one of the drivers of evolution.
- Nausea:** Urge to vomit.
- Nematoda:** Free-living and parasitic roundworms with a thick cuticle and a pharynx, which functions as a sucker.
- Neodermata:** A subphylum of parasitic flatworms that produce a neodermis once the larvae reach their host.
- Neodermis:** A syncytial epidermis that emerges from mesodermal cells once the larvae reach their host. The neodermis is important for the uptake and secretion of material.
- Nicotinamid Adenin-Dinucleotide Dehydrogenase Subunit 1 (ND1):** A subunit of a protein complex that is encoded mitochondrially and often used as DNA barcode.

Nucleic acid: Long unbranched chain of nucleotides (DNA, RNA).

Nucleotide: Organic building block of DNA and RNA. A nucleotide consists of a base, sugar and phosphate.

Oligonucleotide: Short single-stranded DNA, e.g. a primer.

Operculum: Lid that covers an aperture, such as the opening of a trematode egg.

Opisthorchiida: One of the four orders within the Digenea.

Oral sucker: A muscular organ of attachment that surrounds the pharynx.

Order: A category that contains families due to their similarity or relationship.

Pannonian: The Pannonian basin is an area in eastern Central Europe.

Paragonimosis: A disease caused by the lung fluke *Paragonimus westermani*.

Parasite: An organism that lives in or on another organism (host) and feeds on this host.

Parasitism: Interrelationship between different species. One partner (parasite) feeds on the second (host). This interrelationship is thus unfavourable for the host.

Parenchyma: The totality of specific cells of an organ that are responsible for the function of the particular organ.

Pathogen/pathogenic: An organism that is able to cause a disease.

Pharynx: Mouth, opening of the digestive tract.

Phylogeny: The evolutionary history of related organisms and the process through which new species emerge.

Phylum: A category within the animal kingdom that contains classes due to their similarity or relationships.

Plagiorchiida: One of the four orders within the Digenea.

Platyhelminthes: See flatworm.

Polymerase: An enzyme that synthesizes DNA from a DNA template.

Polymerase chain reaction: Enzymatic method for the exponential amplification of a specific DNA sequence.

Posterior: An anatomical term of location that refers to the rear end.

Praeputium: Male copulation organ of gastropods.

Prevalence: Percentage of individuals in a population that are infected or sick at a particular time point.

Primer: Single-stranded synthetic oligonucleotide with a typical length between 17 and 30 bp. Primers are used for PCR to flank the DNA section that should be amplified.

Proliferation: Reproduction, multiplication.

Protonephridia: A primitive excretory system for the elimination of liquid wastes in Platyhelminthes.

Pulmonata: Subclass of non-marine Mollusca with a lung.

Redia(e): Larval stage of certain trematodes that produces cercariae within the intermediate molluscan host. Rediae possess a mouth, gut, central nervous system, salivary glands, and a birth pore.

Ribosome: Complex machine that is composed of protein and ribosomal RNA for the synthesis of proteins.

Ribosomal DNA: A DNA sequence that encodes ribosomal RNA.

RNA (ribonucleic acid): Single-stranded chain-like molecule that carries genetic information transcribed from DNA. RNA is involved in protein synthesis.

Schistosomatid/schistosome: Refers to species of the family Schistosomatidae.

Schistosomosis: A parasitic tropical disease caused by species of the genus *Schistosoma*.

Schistosomulum: Acaudal cercaria; larval stage of schistosomes that emerges after a cercaria penetrated the skin of a definitive host.

Selection: Selection leads to a greater reproduction of individuals that are best adapted to the particular environmental conditions. Selection is thus one of the drivers of evolution.

Sequencing: The study of the order (sequence) of the DNA bases.

Species: A species comprises all individuals that are capable of producing fertile offspring among themselves. Individuals of one species are reproductively isolated from members of other species.

Spectrophotometer: An instrument that is used for the quantitative measurement of DNA or RNA with the help of light reflection.

Sporocyst: Larval stage of trematodes that produces either rediae or cercariae within the intermediate molluscan host. In contrast to rediae, sporocysts lack a locomotor system, mouth and digestive tract. Some sporocysts possess a birth pore.

Strigeatida: One of the four orders within the Digenea.

Stylet: A dagger-like structure at the anterior end of xiphidiocercariae, which is used for the penetration into a second intermediate host.

Swimmer's itch: An allergic reaction that is characterized by itchy maculopapular skin eruptions. This reaction is caused by the penetration of bird schistosomes into the skin.

Sylvatic: A term that refers to wild animals. A sylvatic live cycle of a parasite can act as an infectious reservoir.

Syncytial: Multiple cell fusions lead to cytoplasmic bridges that join the cells together. Although the cells remain discrete, a multinucleated structure is built.

Systematics: Aims to capture and present the diversity of organisms and their relationships.

Tapeworm: See Cestoda.

Taxon/taxa: A homogenous group of organisms that is distinguishable from other organisms.

Taxonomy: Theory and praxis of the classification of organisms.

Tegument: The outer body border of trematodes and cestodes. The tegument has a complex structure and provides for mechanical protection and absorption of nutrients.

Temperate zone: A geographical zone between the polar region and the tropics. It is marked by moderate temperatures and temperature changes in summer and winter.

Thymine: A pyrimidine derivate that is one of the four nucleotides of DNA.

Transmission: Communication of a disease.

Trematoda/trematodes: Flukes, a class of flatworms that are obligate parasites of vertebrates. The Trematoda comprises two subclasses: Digenea and Monogenea.

Trichobilharzia: Most important genus of bird schistosomes in Europe.

Unembryonated: An egg that does not yet contain a fully developed miracidium.

Vector: A carrier of pathogens that transmits parasites from one host to another.

Ventral: An anatomical term of location that refers to the underside of a parasite.

Ventral sucker: See acetabulum.

Vertebrate: Animals with a chondroitic or bony endoskeleton including a spine.

Xiphidiocercaria(e): A type of trematode cercariae that possesses a stylet at the anterior end.

Zoonosis/Zoonotic: Diseases and infections that can be transmitted from animals to humans.

Definitions largely following:

Fried B 1997: *An Overview of the Biology of Trematodes*. In: Fried B, Graczyk TK (eds). *Advances in Trematode Biology*. CRC Press, Boca Raton, New York.

Haseeb MA, Fried B: *Modes of Transmission of Trematode Infections and Their Control*. In: Fried B, Graczyk TK (eds). *Advances in Trematode Biology*. CRC Press, Boca Raton, New York.

Kaushansky K 1996: *Glossary of Molecular Biology Terminology*. *Biologicals*, 24:157-175.

USF Health 2016: *Parasitology Glossary*. Available from: <<http://health.usf.edu/NR/rdonlyres/DEA73306-A309-40FC-B97D-0F6AA7D4CF3B/0/glossary.pdf>>. [18 March 2016]

Walochnik J, Aeschl E 2002: *Glossar medizinischer und biologischer Fachbegriffe*. In: Aspöck H. Amöben, Bandwürmer, Zecken... Parasiten und parasitäre Erkrankungen des Menschen in Mitteleuropa. Biologiezentrum des OÖ. Landesmuseums. Denisia 0006, 184:573-596.

7. References

- ADW 2000: *Lymnaeidae*. Available from: <<http://animaldiversity.org/accounts/Lymnaeidae>>. [14 February 2016]
- Alba A, Vázquez AA, Sánchez J, Fraga J, Hernández H, Martínez E, Marcet R, Figueredo M, Sarracent J 2016: *Assessment of the FasciMol-ELISA in the detection of the trematode Fasciola hepatica in field-collected Galba cubensis: a novel tool for the malacological survey of fasciolosis transmission*. Parasites & Vectors, 9:22.
- Aldhoun JA, Podhorský M, Holická M, Horák P 2012: *Bird schistosomes in planorbid snails in the Czech Republic*. Parasitology International, 61(2):250-259.
- Ali MM, Mageed AA, Heikal M 2007: *Importance of aquatic macrophyte for invertebrate diversity in large subtropical reservoir*. Limnologia, 37:155-169.
- Al-Sabi MNS, Halasa T, Kapel CMO 2014: *Infections with cardiopulmonary and intestinal helminths and sarcoptic mange in red foxes from two different localities in Denmark*. Acta Parasitologica, 59(1):98-107.
- APO 2013a: *Lymnaea spp. (Lamarck, 1799)*. Available from: <http://aquaticparasites.org/hosts/gastropoda/basommatophora/lymnaeidae/lymnaea/Lymnaea_spp.php>. [14 February 2016]
- APO 2013b: *Radix spp. (Montfort, 1810)*. Available from: <http://aquaticparasites.org/hosts/gastropoda/basommatophora/lymnaeidae/radix/Radix_spp.php>. [14 February 2016]
- Arya LK, Rathinam SR, Lalitha P, Kim UR, Ghatani S, Tandon V 2016: *Trematode Fluke Procerovum varium as Cause of Ocular Inflammation in Children, South India*. Emerging infectious diseases, 22(2):192-200.
- Aspöck H, Auer H, Walochnik J 2002: *Parasiten und parasitäre Erkrankungen des Menschen in Mitteleuropa im Überblick*. In: Aspöck H. Amöben, Bandwürmer, Zecken... Parasiten und parasitäre Erkrankungen des Menschen in Mitteleuropa. Biologiezentrum des OÖ. Landesmuseums. Denisia 0006, 184:33-74.
- Aspöck H, Walochnik J 2002: *Was sind Parasiten?* In: Aspöck H. Amöben, Bandwürmer, Zecken... Parasiten und parasitäre Erkrankungen des Menschen in Mitteleuropa. Biologiezentrum des OÖ. Landesmuseums. Denisia 0006, 184:1-12.
- Aspöck H, Walochnik J 2007: *Die Parasiten des Menschen aus der Sicht der Koevolution*. Biologiezentrum des OÖ. Landesmuseums. Denisia 20, 66:179-254.
- Auer H, Aspöck H 2002: *„Vogelbilharzien“ als Erreger einer Hautkrankheit: die Zerkarien-Dermatitis*. In: Aspöck H. Amöben, Bandwürmer, Zecken... Parasiten und parasitäre Erkrankungen des Menschen in Mitteleuropa. Biologiezentrum des OÖ. Landesmuseums. Denisia 0006, 184:321-331.
- Auer H, Cejka R, Aspöck H 1999: *Die Zerkariendermatitis in Österreich – Eine Übersicht*. Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie, 21:57-68.
- Aziz A, Raut SK 1996: *Thermal Effect on the Life-cycle Parameters of the Medically Important Freshwater Snail Species Lymnaea (Radix) luteola (Lamarck)*. Memórias do Instituto Oswaldo Cruz, 91(1):119-128.
- Azizi H, Farahnak A, Mobedi I, Molaei Rad MB 2015: *Experimental Life Cycle of Hypoderaeum conoideum (Block, 1872) Diez, 1909 (Trematoda: Echinostomatidae) Parasite from the North of Iran*. Iranian Journal of Parasitology, 10(1):102-109
- Barber I, Poulin R 2002: *Interactions between Fish, Parasites and Disease*. In: Hart PJB, Reynolds JD (eds). Handbook of Fish Biology and Fisheries. Volume 1: Fish Biology. Blackwell Publishing.

- Bargues MD, Vigo M, Horák P, Dvořák J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S 2001: *European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiasis, based on nuclear ribosomal DNA ITS-2 sequences*. Infection, Genetics and Evolution, 1(2):85-107.
- Bayssade-Dufour C, Jouet D, Rudolfova J, Horák P, Ferté H 2006: *Seasonal morphological variations in bird schistosomes*. Parasite, 13(3):205-214.
- Brant SV, Bochte CA, Loker ES 2011: *New intermediate host records for the avian schistosomes Dendrobilharzia pulverulenta, Gigantobilharzia huronensis, and Trichobilharzia querquedulae from North America*. The Journal of Parasitology, 97(5):946-949.
- Brant SV, Loker ES 2009: *Molecular Systematics Of The Avian Schistosome Genus Trichobilharzia (Trematoda: Schistosomatidae) In North America*. The Journal of Parasitology, 95(4):941-963.
- Brown KM, Leathers BK, Minchella DJ 1988: *Trematode Prevalence and the Population Dynamics of Freshwater Pond Snails*. The American Midland Naturalist, 120(2):289-301.
- Budha PB, Dutta J, Daniel BA 2010: *The IUCN Red List of Threatened Species: Lymnaea stagnalis*. Available from: <<http://dx.doi.org/10.2305/IUCN.UK.2010-4.RLTS.T155475A4782225.en>>. [16 February 2016]
- CDC 2012: *Parasites - Cercarial Dermatitis (also known as Swimmer's Itch): Biology*. Available from: <<http://www.cdc.gov/parasites/swimmersitch/biology.html>>. [17 February 2016]
- CDC 2013: *Echinostomiasis: Parasite Biology*. Available from: <<http://www.cdc.gov/dpdx/echinostomiasis/index.html>>. [20 February 2016]
- Cejka R 1998: *Untersuchungen über das Vorkommen dermatitisserregender Zerkarien in Ost-österreich (Trematoda: Schistosomatidae: Bilharziellinae)*. Diploma thesis, University of Vienna.
- Chai JY 2009: *Echinostomes in Humans*. In: Fried B, Toledo R (eds). The Biology of Echinostomes – From the Molecule to the Community. Springer Science+Business Media, New York.
- Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Hoang EH, Phammasack B, Insisiengmay B, Rim HJ 2012: *Echinostome Flukes Recovered from Humans in Khammouane Province, Lao PDR*. The Korean Journal of Parasitology, 50(3):269-272.
- Chanová M, Lichtenbergová L, Bulantová J, Mikeš L, Horák P 2012: *Trichobilharzia regenti: Antigenic structures of intravertebrate stages*. Central European Journal of Biology, 7(1):83-90.
- Chantima K, Chai JY, Wongsawad C 2013: *Echinostoma revolutum: Freshwater Snails as the Second Intermediate Hosts in Chiang Mai, Thailand*. The Korean Journal of Parasitology, 51(2): 183-189.
- Choe S, Lee D, Park H, Oh M, Jeon HK, Lee Y, Na KJ, Kim Y, Lee H, Eom KS 2014: *Three echinostome species from wild birds in the Republic of Korea*. The Korean Journal of Parasitology, 52(5):513-520.
- Christiansen AØ, Olsen A, Buchmann K, Kania PW, Nejsum P, Vennervald BJ 2016: *Molecular diversity of avian schistosomes in Danish freshwater snails*. Parasitology Research, 115(3):1027-1037.
- Conn DB 1993: *The Biology of Flatworms (Platyhelminthes): Parenchyma Cells and Extracellular Matrices*. Transactions of the American Microscopical Society, 112(4):241-261.
- Cumming J (n.d.): *Isle of Harris: Freshwater Invertebrates*. Available from: <<http://www.isleofharris.com/discover-harris/wild-harris/freshwater-invertebrates>>. [15 January 2016]
- Detwiler JT, Bos DH, Minchella DJ 2010: *Revealing the secret lives of cryptic species: Examining the phylogenetic relationships of echinostome parasites in North America*. Molecular Phylogenetics and Evolution, 55(2):611-620.

- DMG 2016: *Molluscs of central Europe: Radix*. Available from: <http://www.mollbase.de/list/index.php?aktion=zeige_taxon&id=221>. [15 February 2016]
- Dvořák J, Sattmann H, Horák P, Konečný R (1999): Bird schistosomes from freshwater snails in Austria, with some notes on current problems (Digenea, Schistosomatidae). *Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie*, **21**: 69-76.
- Dvořák J, Vanáčová S, Hampl V, Flegr J, Horák P 2002: *Comparison of European Trichobilharzia species based on ITS1 and ITS2 sequences*. *Parasitology*, **124**:307-313.
- Eckert J, Friedhoff KT, Zahner H, Deplazes P 2008: *Lehrbuch der Parasitologie für die Tiermedizin*. 2th edition, Enke Verlag, Stuttgart.
- Esteban JG, Muñoz-Antoli C 2009: *Echinostomes: Systematics and Life Cycles*. In: Fried B, Toledo R (eds). *The Biology of Echinostomes – From the Molecule to the Community*. Springer Science+Business Media, New York.
- Esteban JG, Muñoz-Antoli C, Toledo R, Ash LR 2014: *Diagnosis of Human Trematode Infections*. In: Toledo R, Fried B (eds). *Digenetic Trematodes*. Springer Science+Business Media, New York.
- Eveland LK, Haseeb MA 2003: *Echinostoma Species*. In: Miliotis MD, Bier JW (eds). *International Handbook Of Foodborne Pathogens*. Marcel Dekker, Inc., New York, Basel.
- Faltýnková A, Georgieva S, Soldánová M, Kostadinova A 2015: *A re-assessment of species diversity within the 'revolutum' group of Echinostoma Rudolphi, 1809 (Digenea: Echinostomatidae) in Europe*. *Systematic Parasitology*, **90**(1):1-25.
- Faltýnková A, Nasincová V, Kablášková L 2007: *Larval trematodes (Digenea) of the great pond snail, Lymnaea stagnalis (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification*. *Parasite*, **14**(1):39-51.
- Faltýnková A, Sures B, Kostadinova A 2016: *Biodiversity of trematodes in their intermediate mollusc and fish hosts in the freshwater ecosystems of Europe*. *Systematic Parasitology*, **93**(3):283-93.
- Fechter R, Falkner G 1989: *Die farbigen Naturführer: Weichtiere*. Mosaik Verlag, München.
- Feliu C, Torres J, Miquel J, Segovia JM, Fons R 2006: *Digenean trematodes*. In: Morand S, Krasnov BR, Poulin R (eds). *Micromammals and Macroparasites. From Evolutionary Ecology to Management*. Springer-Verlag, Tokyo.
- Ferraz J, Souza J, Costa-Silva M, Torres E, Santana A, Lanfredi R, Maldonado A, Garcia J 2012: *Effect of praziquantel on adult Echinostoma paraensei worms in experimentally infected mice*. *Parasitology Research*, **111**:143-148.
- Ferté H, Depaquit J, Carré S, Villena I, Léger N 2005: *Presence of Trichobilharzia szidati in Lymnaea stagnalis and T. franki in Radix auricularia in northeastern France: molecular evidence*. *Parasitology Research*, **95**:150-154.
- Frew JW, Henderson CJA, McCrossin ID 2016: *The early bird and the worm: a case of cercarial dermatitis*. *The Medical Journal Of Australia*, **204**(3):122-3.
- Fried B 1997: *An Overview of the Biology of Trematodes*. In: Fried B, Graczyk TK (eds). *Advances in Trematode Biology*. CRC Press, Boca Raton, New York.
- Fuchs C 2002: *Cercarien aus Radix ovata und Radix auricularia*. Available from: <http://www.uni-bielefeld.de/biologie/Didaktik/Zoologie/html_deutsch/galerie_Ra.html>. [14 February 2016]
- Gallego JL 2015: *Familia Lymnaeidae: Radix labiata*. Available from: <<http://www.elrincondelmalacologo.com/Web%20fotos%20agua%20dulce/Fotos%20coleccion/Lymnaeidae/Radix%20labiata.jpg>>. [21 January 2016]
- Gast RJ, Ledee DR, Fuerst PA, Byers TJ 1996: *Subgenus Systematics of Acanthamoeba: Four Nuclear 18S rDNA Sequence Types*. *Journal of Eukaryotic Microbiology*, **43**(6):498-504.

- Gaub L 2014: *Molecular species determination of larval Digenea as basis for reliable epidemiological analyses, enhanced biodiversity data and significant medical risk assessments*. Master's thesis, University of Vienna.
- GBIF 2016: *Radix auricularia* (Linnaeus, 1758). Available from: <<http://www.gbif.org/species/113519095>>. [15 February 2016]
- Georgieva S, Faltýnková A, Brown R, Blasco-Costa I, Soldánová M, Sitko J, Scholz T, Kostadinova A 2014: *Echinostoma 'revolutum' (Digenea: Echinostomatidae) species complex revisited: species delimitation based on novel molecular and morphological data gathered in Europe*. Parasites & Vectors, 7:520.
- Georgieva S, Selbach C, Faltýnková A, Soldánová M, Sures B, Skírnisson K, Kostadinova A 2013: *New cryptic species of the 'revolutum' group of Echinostoma (Digenea: Echinostomatidae) revealed by molecular and morphological data*. Parasites & Vectors, 6:64.
- Gibson DI 1996: *Trematoda*. In: Margolis L, Kabata Z (eds). Guide to the parasites of fishes of Canada. Part IV. Canadian Special Publication of Fisheries and Aquatic Sciences.
- Gibson DI 2002: *Class Trematoda Rudolphi, 1808*. In: Gibson DI, Jones A, Bray RA (eds). Keys to the Trematoda. Volume 1. CABI Publishing, Wallingford.
- Gibson DI, Bray RA, Hunt D, Georgiev BB, Scholz T, Harris PD, Bakke TA, Pojmanska T, Niewiadomska K, Kostadinova A, Tkach V, Bain O, Durette-Desset MC, Gibbons L, Moravec F, Petter A, Dimitrova ZM, Buchmann K, Tellervo Valtonen E, de Jong Y 2014: *Fauna Europaea: Helminths (Animal Parasitic)*. Biodiversity Data Journal, doi: 10.3897/BDJ.2.e1060.
- Gonçalves JP, Oliveira-Menezes A, Maldonado Junior A, Carvalho TMU, de Souza W 2013: *Evaluation of Praziquantel effects on Echinostoma paraensei ultrastructure*. Veterinary Parasitology, 194:16-25.
- Grabda-Kazubská B, Borsuk P, Laskowski Z, Moné H 1998: *A phylogenetic analysis of trematodes of the genus Echinoparyphium and related genera based on sequencing of Internal Transcribed Spacer region of rDNA*. Acta Parasitologica, 43(3):116-121.
- Graczyk TK, Fried B 1998: *Echinostomiasis: a common but forgotten food-borne disease*. The American Journal of Tropical Medicine and Hygiene, 58(4):501-504.
- Gruner HE, Hannemann HJ, Hartwich G, Kilias R 2000: *Wirbellose Tiere 1*. In: Die große farbige Enzyklopädie Urania-Tierreich. Urania Verlag, Berlin.
- Haas W n.d.: *Life cycle of Trichobilharzia ocellata*. Available from: <http://www.apps.nlga.niedersachsen.de/eu/batlas/fotos/zerkarienzklus_w498.jpg>. [17 February 2016]
- Haas W, Haeberlein S 2009: *Penetration of cercariae into the living human skin: Schistosoma mansoni vs. Trichobilharzia szidati*. Parasitology Research, 105(4):1061-6.
- Haider M 2010: *Bestimmung der Prävalenz von Fascioloides magna und anderen digenen Trematoden in Galba truncatula an drei ausgewählten Standorten im Bereich Orth/Donau*. Diploma thesis, University of Vienna.
- Haider M, Hörweg C, Liesinger K, Sattmann H, Walochnik J 2012: *Recovery of Fascioloides magna (Digenea) population in spite of treatment programme? Screening of Galba truncatula (Gastropoda, Lymnaeidae) from Lower Austria*. Veterinary Parasitology, 187:445-451.
- Hann BJ 1995: *Invertebrate associations with submersed aquatic plants in a prairie wetland*. UFS (Delta Marsh) Annual Report, 30:78-84.
- Hansen J, Perry B 1994: *The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants*. International Laboratory for Research on Animal Diseases, Nairobi, Kenya.
- Haseeb MA, Fried B 1997: *Modes of Transmission of Trematode Infections and Their Control*. In: Fried B, Graczyk TK (eds). Advances in Trematode Biology. CRC Press, Boca Raton, New York.

- Hayward A 2010: *Cryptic diversity and patterns of host specificity in trematode flatworms*. Molecular Ecology, 19:2602-2604.
- Hof H, Dörries R 2009: *Medizinische Mikrobiologie*. 4 ed., Georg Thieme Verlag KG, Stuttgart.
- Hong NT, Seo BS 1969: *Study on Metagonimus yokogawai (Katsurada, 1912) in Korea I. On the metacercaria, its distribution in the second intermediate host and the development in the final host*. The Korean Journal of Parasitology, 7(3):129-142.
- Horák P, Kolářová L 1997: *Zerkarien-Dermatitis in Mitteleuropa – Überblick und aktuelle Probleme*. Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie, 19:59-64.
- Horák P, Kolářová L 2011: *Snails, waterfowl and cercarial dermatitis*. Freshwater Biology, 56:779-790.
- Horák P, Kolářová L, Adema CM 2002: *Biology of the schistosome genus Trichobilharzia*. Advances in Parasitology, 52:155-233.
- Horák P, Kolářová L, Dvořák J 1998: *Trichobilharzia regenti n. sp. (Schistosomatidae, Bilharziellinae), a new nasal Schistosome from Europe*. Parasite, 5:349-357.
- Horák P, Mikeš L, Lichtenbergová L, Skála V, Soldánová M, Brant SV 2015: *Avian Schistosomes and Outbreaks of Cercarial Dermatitis*. Clinical Microbiology Reviews, 28(1):165-190.
- Hörweg C, Prosl H, Will-Piazzai W, Joachim A, Sattmann H 2011: *Prevalence of Fascioloides magna in Galba truncatula in the Danube backwater area east of Vienna, Austria*. Wiener Tierärztliche Monatsschrift, 98:261-267.
- Hörweg C, Sattmann H, Auer H 2006: *Cercarial dermatitis in Austria: Questionnaires as useful tools to estimate risk factors?* Wiener klinische Wochenschrift, 118(19-20 Suppl 3):77-80.
- Hubendick B 1958: *Factors conditioning the habitat of freshwater snails*. Bulletin of the World Health Organization, 18(5-6): 1072–1080.
- Huffman JE, Fried B 2012: *The biology of Echinoparyphium (Trematoda, Echinostomatidae)*. Acta Parasitologica, 57(3):199-210.
- Huňová K, Kašný M, Hampel V, Leontovyč R, Kuběna A, Mikeš L, Horák P 2012: *Radix spp.: Identification of trematode intermediate hosts in the Czech Republic*. Acta Parasitologica, 57(3):273-284.
- ICPDR 2016: *Wetlands*. Available from: <<https://www.icpdr.org/main/issues/wetlands>>. [22 February 2016]
- Imani-Baran A, Yakhchali M, Malekzadeh-Viayeh R, Farahnak A 2013: *Seasonal and Geographic Distribution of Cercarial Infection in Lymnaea gedrosiana (Pulmonata: Lymnaeidae) In North West Iran*. Iranian Journal of Parasitology, 8(3): 423–429.
- ITIS Report 2013: *Trematoda*. Available from: <http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=54556>. [20 March 2015]
- IUCN 2010: *Range map of Lymnaea stagnalis*. Available from: <<http://maps.iucnredlist.org/map.html?id=155475>>. [10 April 2016]
- IUCN 2014: *Range map of Radix auricularia*. Available from: <<http://maps.iucnredlist.org/map.html?id=155568>>. [10 April 2016]
- Jouet D, Skírnisson K, Kolářová L, Ferté H 2010: *Molecular diversity of Trichobilharzia franki in two intermediate hosts (Radix auricularia and Radix peregra): A complex of species*. Infection, Genetics and Evolution, 10(8):1218-1227.
- Jung WT, Lee KJ, Kim HJ, Kim TH, Na BK, Sohn WM 2014: *A Case of Echinostoma cinetorchis (Trematoda: Echinostomatidae) Infection Diagnosed by Colonoscopy*. The Korean Journal of Parasitology, 52(3):287-290.
- Kearn GC 1998: *Parasitism and the Platyhelminths*. Chapman & Hall, London.

- Kolářová L 2007: *Schistosomes causing cercarial dermatitis: a mini-review of current trends in systematics and of host specificity and pathogenicity*. Folia Parasitologica, 54(2):81-87.
- Kolářová L, Horák P, Skírnisson K, Marečková H, Doenhoff M 2013: *Cercarial Dermatitis, a Neglected Allergic Disease*. Clinical Reviews in Allergy & Immunology, 45(1): 63-74.
- Kolářová L, Skírnisson K, Horák P 1999: *Schistosome cercariae as the causative agent of swimmer's itch in Iceland*. Journal of Helminthology, 73(3):215-220.
- Konečný R, Dvořák J, Horák P, Sattmann H 1999: *Zerkarien von Süßwasserschnecken in Ost-Österreich*. Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie, 21:77-82.
- Korsunen AV, Chrisanfova GG, Ryskov AP, Movsessian SO, Vasilyev VA, Semyenova SK 2010: *Detection of European Trichobilharzia schistosomes (T. franki, T. szidati, and T. regenti) based on novel genome sequences*. The Journal of Parasitology, 96(4):802-806.
- Kostadinova A, Herniou EA, Barrett J, Littlewood DTJ 2003: *Phylogenetic relationships of Echinostoma Rudolphi, 1809 (Digenea: Echinostomatidae) and related genera re-assessed via DNA and morphological analyses*. Systematic Parasitology, 54(3):159-176.
- Kumar V 1999: *Trematode Infections and Diseases of Man and Animals*. Springer Science+Business Media, Dordrecht.
- Lafferty KD, Hechinger RF, Lorda J, Soler L 2005: *Trematodes associated with mangrove habitat in Puerto Rican salt marshes*. The Journal of Parasitology, 91(3):697-699.
- Lawton SP, Lim RM, Dukes JP, Kett SM, Cook RT, Walker AJ, Kirk RS 2015: *Unravelling the riddle of Radix: DNA barcoding for species identification of freshwater snail intermediate hosts of zoonotic digeneans and estimating their inter-population evolutionary relationships*. Infection, Genetics and Evolution, 35:63-67.
- Leles D, Cascardo P, dos Santos Freire A, Maldonado Jr. A, Sianto L, Araújo A 2014: *Insights about echinostomiasis by paleomolecular diagnosis*. Parasitology International, 63:646-649.
- Liesinger K 2011: *Microscopic and molecular analyses on digenean trematodes in red deer (Cervus elaphus)*. Diploma thesis, University of Vienna.
- Lim YAL, Vythilingam I 2013: *Southeast Asia: Hotspot for Parasitic Infections*. In: Lim YAL, Vythilingam I (eds). Parasites and their vectors: A special focus on Southeast Asia. Springer-Verlag, Wien.
- Littlewood DTJ 2006: *The Evolution of Parasitism in Flatworms*. In: Maule AG, Marks NJ (eds). Parasitic flatworms – molecular biology, biochemistry, immunology and physiology. CABI Publishing, Wallingford.
- Littlewood DTJ, Bray RA, Clough KA 1998: *A phylogeny of the Platyhelminthes: towards a total-evidence solution*. Hydrobiologica, 383:155-160.
- Lockyer AE, Olson PD, Østergaard P, Rollinson D, Johnston DA, Attwood SW, Southgate VR, Horák P, Snyder SD, Le TH, Agatsuma T, McManus DP, Carmichael AC, Naem S, Littlewood TJ 2003: *The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of Schistosoma Weinland, 1858*. Parasitology, 126:203-224.
- Loos-Frank B, Gottstein B 2006: *Helminthen*. In: Hiepe T, Lucius R, Gottstein B (eds). Allgemeine Parasitologie mit den Grundzügen der Immunologie, Diagnostik und Bekämpfung. Parey, Stuttgart.
- Loy C, Haas W 2001: *Prevalence of cercariae from Lymnaea stagnalis snails in a pond system in Southern Germany*. Parasitology Research, 87:878-882.
- Lucius R, Loos-Frank B 1997: *Parasitologie: Grundlagen für Biologen, Mediziner und Veterinärmediziner*. Spektrum Akademischer Verlag, Heidelberg, Berlin.

- Lucius R, Loos-Frank B 2008: *Biologie von Parasiten*. 2th edition, Springer-Verlag, Berlin Heidelberg.
- Mas-Coma S, Valero MA, Bargues MD 2009: *Climate change effects on trematodiasis, with emphasis on zoonotic fascioliasis and schistosomiasis*. *Veterinary Parasitology*, 163(4):264-280.
- McGeachin WT 1979: *Emergence and penetration by cercariae of Gigantobilharzia huronensis (Trematoda: Schistosomatidae)*. Retrospective Theses and Dissertations. Paper 7232. Available from: <<http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=8231&context=rtd>>. [09 April 2016]
- MeinBezirk 2016: *Austria Map*. Available from: <<http://kleinanzeigen-old.meinbezirk.at/map/oes-terreich.gif>>. [10 January 2016]
- Mikeš L 2001: *Cercariae – Simplified Determination Key*. 1st Workshop on Bird Schistosomes & Cercarial Dermatitis, 10th – 14th September 2001, Dolní Věstonice, Czech Republic.
- Mohamed-Nor Z 2013: *Epidemiology of Cestode and Trematode in Southeast Asian Countries*. In: Lim YAL, Vythilingam I (eds). *Parasites and their vectors: A special focus on Southeast Asia*. Springer-Verlag, Wien.
- Morgan JAT, Blair D 1998: *Mitochondrial ND1 gene sequences used to identify echinostome isolates from Australia and New Zealand*. *International Journal for Parasitology*, 28:493-502.
- Morgan JAT, Blair D 2000: *Molecular Biology Of Echinostomes*. In: Fried B, Graczyk TK (eds). *Echinostomes as Experimental Models for Biological Research*. Springer Science+Business Media, Dordrecht.
- Morley NJ 2016a: *Cercarial Dermatitis or Cercariosis: What's in a Name?* *Trends in Parasitology*, 32(2):92-93.
- Morley NJ 2016b: *Cercarial Dermatitis or Cercariosis: Defining the Condition*. *Trends in Parasitology*, doi: 10.1016/j.pt.2016.03.004.
- Nagataki M, Tantrawatpan C, Agatsuma T, Sugiura T, Duenngai K, Sithithaworn P, Andrews RH, Petney TN, Saijuntha W 2015: *Mitochondrial DNA sequences of 37 collar-spined echinostomes (Digenea: Echinostomatidae) in Thailand and Lao PDR reveals presence of two species: Echinostoma revolutum and E. miyagawai*. *Infection, Genetics and Evolution*, 35:56-62.
- Naturbiotop 2015: *Radix labiata*. Available from: <<http://www.naturbiotop.de/html/wiw/mol/radix/wiwmolradixlab1.html>>. [15 February 2016]
- Nicholas KB, Nicholas HB Jr., Deerfield DW 1997: *GeneDoc: analysis and visualization of genetic variation*. *Embnet.news*, 4:14.
- Noikong W, Wongsawad C, Chai JY, Saenphet S, Trudgett A 2014: *Molecular Analysis of Echinostome Metacercariae from Their Second Intermediate Host Found in a Localised Geographic Region Reveals Genetic Heterogeneity and Possible Cryptic Speciation*. *PLOS Neglected Tropical Diseases*, 8(4):e2778.
- Nolan M, Cribb TH 2005: *The use and implications of ribosomal DNA sequencing for the discrimination of digenean species*. *Advances in Parasitology*, 60:101-163.
- Nordsieck R n.d.: *Feinde der Landschnecken*. Available from: <<http://www.weichtiere.at/Schnecken/land.html?/Schnecken/land/feinde.html>>. [01.03.2016]
- Okanishi H, Matsumoto J, Nogami S, Kagawa Y, Watari T 2013: *Echinostoma hortense infection with enteritis diagnosed by upper gastrointestinal endoscopy in a dog*. *The Journal of Veterinary Medical Science*, 75(7):991-4.
- OpenStax College 2013: *Concepts of Biology*. Available from: <<https://www.openstaxcollege.org/textbooks/concepts-of-biology/get>>. [14 February 2016]

- Panic G, Ingram K, Keiser J 2013: *Development of an in vitro drug sensitivity assay based on newly excysted larvae of Echinostoma caproni*. Parasites & Vectors, 6:237.
- Paull SH, Johnson PT 2011: *High temperature enhances host pathology in a snail-trematode system: possible consequences of climate change for the emergence of disease*. Freshwater Biology, 56:767-778.
- Peters L n.d.: *Photograph of the great pond snail Lymnaea stagnalis*. Available from <http://www.oikosjournal.org/sites/oikosjournal.org/files/files/lymnea-stagnalis_04_1200px.jpg>. [16 February 2016]
- Poulin R 2006: *Global warming and temperature-mediated increases in cercarial emergence in trematode parasites*. Parasitology, 132:143-151.
- Proschwitz von T 2011: *The IUCN Red List of Threatened Species: Radix lagotis*. Available from: <<http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T155502A4788468.en>>. [15 February 2016]
- Rapsch C, Dahinden T, Heinzmann D, Torgerson PR, Braun U, Deplazes P, Hurni L, Bär H, Knubben-Schweizer G 2008: *An interactive map to assess the potential spread of Lymnaea truncatula and the free-living stages of Fasciola hepatica in Switzerland*. Veterinary Parasitology, 154(3-4):242-9.
- Reckendorfer W, Groiss M 2006: *Der amerikanische Riesenleberegel in den Donau-Auen östlich von Wien. Eine GIS-basierte Risikoanalyse*. Available from: <http://www.donauauen.at/dateien/437_LeberegelRisikoanalyse.pdf>. [16 February 2016]
- Rondelaud D, Vignoles P, Dreyfuss G 2015: *Larval trematode infections in Lymnaea glabra populations living in the Brenne Regional Natural Park, central France*. Parasite, 22:38.
- Rozendaal JA 1997: *Vector control – Methods for use by individuals and communities*. World Health Organization Geneva, Switzerland.
- Saijuntha W, Duenngai K, Tantrawatpan C 2013: *Zoonotic Echinostome Infections in Free-Grazing Ducks in Thailand*. The Korean Journal of Parasitology, 51(6): 663–667.
- Saijuntha W, Sithithaworn P, Duenngai K, Kiatsopit N, Andrews RH, Petney TN 2011b: *Genetic variation and relationships of four species of medically important echinostomes (Trematoda: Echinostomatidae) in South-East Asia*. Infection, Genetics and Evolution, 11(2):375-381.
- Saijuntha W, Tantrawatpan C, Sithithaworn P, Andrews RH, Petney T 2011a: *Genetic characterization of Echinostoma revolutum and Echinoparyphium recurvatum (Trematoda: Echinostomatidae) in Thailand and phylogenetic relationships with other isolates inferred by ITS1 sequence*. Parasitology Research, 108:751-755.
- Sandhall Å 1973: *BLV Bestimmungsbuch - Insekten + Weichtiere*. BLV Verlagsgesellschaft, München.
- Sattmann H, Hörweg C 2006: *Untersuchung zur Messung der Befallsrate der Leberegelschnecke Galba truncatula mit dem Amerikanischen Riesenleberegel Fascioloides magna im Nationalpark Donau-Auen*. Available from: <http://www.donauauen.at/dateien/436_FascioloidesEndberichtNHMFeb06.pdf>. [01 March 2016]
- Sattmann H, Hörweg C, Konečný R 2004: *Zerkariendermatitis in Österreich – Rückblick und Perspektiven*. Denisia 0013, 457-461.
- Schets FM, Lodder WJ, Duynhoven van YTHP, Roda Husman de AM 2008: *Cercarial dermatitis in the Netherlands caused by Trichobilharzia spp.* Journal of Water and Health, 6(2):187-195.
- Schniebs K, Glöer P, Vinarski MV, Hundsdoerfer AK 2011: *Intraspecific morphological and genetic variability in Radix balthica (Linnaeus 1758) (Gastropoda: Basommatophora: Lymnaeidae) with morphological comparison to other European Radix species*. Journal of Conchology, 40(6):657-678.

- Seddon MB, Van Damme D 2014a: *The IUCN Red List of Threatened Species: Planorbis planorbis*. Available from: <<http://www.iucnredlist.org/details/156175/0>>. [08 April 2016]
- Seddon MB, Van Damme D 2014b: *The IUCN Red List of Threatened Species: Bathymophalus contortus*. Available from: <<http://www.iucnredlist.org/details/155930/0>>. [08 April 2016]
- Seddon MB, Kebapçı U, Van Damme D, Prie V 2014b: *The IUCN Red List of Threatened Species: Radix balthica*. Available from: <<http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T155647A42430553.en>>. [15 February 2016]
- Seddon MB, Van Damme D, Proschwitz von T, Madhyastha A 2014a: *The IUCN Red List of Threatened Species: Radix auricularia*. Available from: <<http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T155568A42429613.en>>. [15 February 2016]
- Semyenova SK, Chisanfova GG, Guliaev AS, Yesakova AP, Ryskov AP 2015: *Structural and Population Polymorphism of RT-Like Sequences in Avian Schistosomes Trichobilharzia szidati (Platyhelminthes: Digenea: Schistosomatidae)*. BioMed Research International, 2015:315312.
- Sey O 2001: *Amphistomes of the World - A check-list of the amphistomes of vertebrates*. Hungarian Natural History Museum & University of Pécs.
- Shin SS, Oh DS, Ahn KS, Cho SH, Lee WJ, Na BK, Sohn WM 2015: *Zoonotic Intestinal Trematodes in Stray Cats (Felis catus) from Riverside Areas of the Republic of Korea*. The Korean Journal of Parasitology, 53(2):209-13.
- Skála V, Bulantová J, Walker AJ, Horák P 2014: *Insights into the development of Notocotylus attenuatus (Digenea: Notocotylidae) in Lymnaea stagnalis: from mother sporocyst to cercariae*. Parasitology International, 63:94–99.
- Skírnisson K, Aldhoun JA, Kolářová L 2009: *A review on swimmer's itch and the occurrence of bird schistosomes in Iceland*. Journal of Helminthology, 83:165-171.
- Smith NF, Ruiz GM, Reed SA 2007: *Habitat and Host Specificity of Trematode Metacercariae in Fiddler Crabs from Mangrove Habitats in Florida*. The Journal of Parasitology, 93(5):999-1005.
- Sohn WM 1998: *Life history of Echinoparyphium recurvatum (Trematoda: Echinostomatidae) in Korea*. The Korean Journal of Parasitology, 36(2):91-98.
- Sohn WM, Chai JY, Na BK, Yong TS, Eom KS, Park H, Min DY, Rim HJ 2013: *Echinostoma macrorchis in Lao PDR: Metacercariae in Cipangopaludina Snails and Adults from Experimentally Infected Animals*. The Korean Journal of Parasitology, 51(2): 191-196.
- Sohn WM, Chai JY, Yong TS, Eom KS, Yoon CH, Sinuon M, Socheat D, Lee SH 2011: *Echinostoma revolutum infection in children, Pursat Province, Cambodia*. Emerging Infectious Diseases, 17(1):117-9.
- Sohn WM, Na BK, Cho SH, Lee SW, Choi SB, Seok WS 2015: *Trematode Metacercariae in Freshwater Fish from Water Systems of Hantangang and Imjingang in Republic of Korea*. The Korean Journal of Parasitology, 53(3):289-98.
- Soldánová M, Selbach C, Kalbe M, Kostadinova A, Sures B 2013: *Swimmer's itch: etiology, impact, and risk factors in Europe*. Trends in Parasitology, 29(2):65-74.
- Soldánová M, Selbach C, Sures B 2016: *The Early Worm Catches the Bird? Productivity and Patterns of Trichobilharzia szidati Cercarial Emission from Lymnaea stagnalis*. PLoS One, 11(2):e0149678.
- Sorensen RE, Minchella DJ 2001: *Snail-trematode life history interactions: past trends and future directions*. Parasitology, 123:S3-S18.
- Sousa WP, Grosholz ED 1991: *The influence of habitat structure on the transmission of parasites*. In: Bell SS, McCoy ED, Muskinsky HR (eds). Habitat Structure - The Physical Arrangement of Objects in Space. Springer Science+Business Media, Dordrecht.

- Stanevičiūtė G, Stunžėnas V, Petkevičiūtė R (2015): *Phylogenetic relationships of some species of the family Echinostomatidae Odner, 1910 (Trematoda), inferred from nuclear rDNA sequences and karyological analysis*. Comparative Cytogenetics, 9(2): 257-270.
- Tantrawatpan C, Saijuntha W, Manochantr S, Kheolamai P, Thanchomnang T, Sadaow L, Intapan PM, Maleewong W 2016: *A singleplex real-time fluorescence resonance energy transfer PCR with melting curve analysis for the differential detection of Paragonimus heterotremus, Echinostoma malayanum and Fasciola gigantica eggs in faeces*. Transactions of the Royal Society of Tropical Medicine and Hygiene, 110:74-83.
- Tatem AJ, Rogers DJ, Hay SI 2006: *Global Transport Networks and Infectious Disease Spread*. Advances in Parasitology, 62:293-343.
- Taylor MA, Coop RL, Wall RL 2016: *Veterinary Parasitology*. 4th edition, Wiley Blackwell, West Sussex.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG 1997: *The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools*. Nucleic Acids Research, 25(24):4876–4882.
- Toledo R, Esteban JG 2016: *An update on human echinostomiasis*. Transactions of the Royal Society of Tropical Medicine and Hygiene, 110(1):37-45.
- Toledo R, Muñoz-Antoli C, Pérez M, Esteban JG 1999: *Survival and infectivity of Hypoderaeum conoideum and Euparyphium albuferensis cercariae under laboratory conditions*. Journal of Helminthology, 73(2):177-82.
- Tremaine AM, Whittemore DE, Gewirtzman AJ, Bartlett BL, Mendoza N, Rapini RP, Tyring SK 2009: *An unusual case of swimmer's itch*. Journal of the American Academy of Dermatology, 60(1):174-176.
- Truxa C 2012: *Community ecology of moths in floodplain forests of Eastern Austria*. Ph.D thesis, University of Vienna.
- Wang CR, Chen J, Zhao JP, Chen AH, Zhai YQ, Li L, Zhu XQ 2009: *Orientobilharzia species: Neglected parasitic zoonotic agents*. Acta Tropica, 109(3):171-175.
- Webb RA 2004: *Studies on platyhelminths: yesterday, today, and tomorrow*. Canadian Journal of Zoology, 82:161-167.
- Welter Schultes F 2013a: *Species summary for Radix auricularia*. Available from: <<http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/species?id=1881>>. [15 February 2016]
- Welter Schultes F 2013b: *Species summary for Radix labiata*. Available from: <<http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/species?id=3173>>. [15 February 2016]
- Welter Schultes F 2013c: *Species summary for Radix balthica*. Available from: <<http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/species?id=1882>>. [15 February 2016]
- Welter Schultes F 2013d: *Picture of Radix lagotis by Dilian Georgiev*. Available from: <<http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/picture?id=12999>>. [15 February 2016]
- Welter Schultes F 2015: *Species summary for Radix lagotis*. Available from: <<http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/species?id=2064>>. [15 February 2016]
- Westheide W, Rieger R 1996: *Spezielle Zoologie. Teil 1: Einzeller und wirbellose Tiere*. Gustav Fischer Verlag, Stuttgart.
- White-McLean JA 2011: *Terrestrial Mollusc Tool: Lymnaeidae*. Available from: <<http://idtools.org/id/mollusc/factsheet.php?name=lymnaeidae>>. [15 February 2016]

- WHO 1995: *Control of foodborne trematode infections: report of a WHO Study Group*. WHO Technical Report Series, 849.
- WWF 2016a: *The Morava-Dyje floodplains*. Available from: <<http://www.wwf.at/de/menu465/subartikel1541>>. [15 January 2016]
- WWF 2016b: *Temperate broadleaf and mixed forests: Eastern Europe*. Available from: <<http://www.worldwildlife.org/ecoregions/pa0431>>. [01 March 2016]
- WWF Auen Institut 1999: *Evaluation of wetlands and floodplain areas in the Danube river basin. Final report. May 1999*. Available from: <<https://www.icpdr.org/main/sites/default/files/EVALUATIONWETLANDSFLOODPLAINAREAS.pdf>>. [15 January 2016]
- ZAMG 2016a: *Weterrückblick für 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/jahresrueckblick/weterrueckblick/?jahr=2015&monat=GJ>>. [25 February 2016]
- ZAMG 2016b: *Weterrückblick für 2014*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/jahresrueckblick/weterrueckblick?monat=GJ&jahr=2014>>. [25 February 2016]
- ZAMG 2016c: *Weterrückblick für März 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/monatsrueckblick/weterrueckblick/?jahr=2015&monat=03>>. [01 March 2016]
- ZAMG 2016d: *Weterrückblick für April 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/monatsrueckblick/weterrueckblick/?jahr=2015&monat=04>>. [01 March 2016]
- ZAMG 2016e: *Weterrückblick für Mai 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/monatsrueckblick/weterrueckblick/?jahr=2015&monat=05>>. [01 March 2016]
- ZAMG 2016f: *Weterrückblick für Juli 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/monatsrueckblick/weterrueckblick/?jahr=2015&monat=07>>. [01 March 2016]
- ZAMG 2016g: *Weterrückblick für August 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/monatsrueckblick/weterrueckblick?monat=08&jahr=2015>>. [01 March 2016]
- ZAMG 2016h: *Weterrückblick für September 2015*. Available from: <<https://www.zamg.ac.at/cms/de/klima/klima-aktuell/monatsrueckblick/weterrueckblick?monat=09&jahr=2015>>. [01 March 2016]
- Zikmundová J, Georgieva S, Faltýnková A, Soldánová M, Kostadinova A 2014: *Species diversity of Plagiorchis Lühe, 1899 (Digenea: Plagiorchiidae) in lymnaeid snails from freshwater ecosystems in central Europe revealed by molecules and morphology*. Systematic Parasitology, 88(1):37-54.

8. Appendix

8.1. Abstract

8.1.1. English

Digenean trematodes in freshwater snails in the surroundings of Vienna with a focus on species involved in human infections.

Digenean trematodes are a group of parasitic flatworms that are characterized by a complex life cycle including molluscs as intermediate hosts (particularly freshwater snails) and vertebrates as definitive hosts. The aim of this study was to determine the trematode burden in *Radix* spp. and *Lymnaea stagnalis* collected in the surroundings of Vienna. Particular emphasis was given on bird schistosomes and echinostomes, both of which are of medical as well as of veterinary interest. While bird schistosomes lead to annually recurring outbreaks of cercarial dermatitis in summer, the gastrointestinal disease caused by echinostomes (i.e. echinostomosis) is mainly known from Southeast Asia.

Snails were collected in 47 locations in Lower Austria and Burgenland between April and September 2015. Different habitat types (i.e. floodplains, ponds, rivers, and artificial waterbodies) were included. Altogether, 622 snails were found in 16 locations. All samples were investigated microscopically and screened for trematode larvae. Infected snails were found in 13 locations and subjected to molecular analysis. To achieve this, DNA was isolated and three different PCRs were performed. The general trematode PCR amplifies a fragment of the 18S rDNA, while the ND1 gene was used for identification of echinostomes and the CO1 gene for bird schistosomes. Sixteen samples were chosen for species identification by DNA sequencing.

In total, 149 of the 622 collected snails (24%) were positive for trematode larvae. The majority (81 snails, 13%) was infected with echinostome larvae. Xiphidocercariae occurred second most (25 snails, 4%). Nine snails (1.5%) harboured amphistome cercariae, seven (1.1%) furcocercariae, and one snail (0.2%) monostome cercariae. The remaining 26 snails (4.2%) had infections with not identifiable trematode larvae.

Two of the seven furcocercariae samples were identified as *Trichobilharzia szidati*, which had an overall prevalence of 0.3%. *T. szidati* is known to be a frequent causative agent of cercarial dermatitis. The remaining five furcocercariae samples were tentatively identified as members of the families Diplostomidae and Strigeidae, respectively, which both do not cause cercarial dermatitis. Among the echinostome samples, *Echinoparyphium*

recurvatum was found 13 times (2.1% overall prevalence), and once each *Echinostoma revolutum* (0.2%), and *Hypoderaeum conoideum* (0.2%), all of which can cause echinostomosis. These are the first molecular data on the occurrence of schistosomatid and echinostomatid trematodes in Austria. A detailed knowledge on the distribution of medically important digeneans in Austria is important for a profound risk assessment.

8.1.2. German

Digene Trematoden in Süßwasserschnecken der Wiener Umgebung mit Hauptaugenmerk auf humanpathogene Spezies.

Die digenen Trematoden sind eine Gruppe parasitischer Plattwürmer, die durch einen komplexen Lebenszyklus gekennzeichnet sind. Dieser beinhaltet Mollusken, insbesondere Süßwasserschnecken, als Zwischenwirt und Wirbeltiere als Endwirt. Das Ziel dieser Studie war, die Infektionsrate mit Trematoden bei in der Wiener Umgebung gesammelten Süßwasserschnecken der Arten *Radix* spp. und *Lymnaea stagnalis* zu bestimmen. Dabei lag der Schwerpunkt dieses Projektes auf Vogelschistosomen und echinostomen Trematoden, die sowohl von humanmedizinischer als auch veterinärmedizinischer Bedeutung sind. Während Vogelschistosomen jedes Jahr im Sommer wiederholt zu kleineren Ausbrüchen von Zerkariendermatitis führen, ist die Echinostomose, die durch echinostome Trematoden hervorgerufen wird, bisher nur aus Südostasien bekannt.

Zwischen April und September 2015 wurden Schnecken an insgesamt 47 verschiedenen Standorten in Niederösterreich und dem Burgenland gesammelt. Dabei wurde darauf geachtet, möglichst viele verschiedene Habitattypen wie Überschwemmungsgebiete/Auen, Teiche, Flüsse und auch künstliche Gewässer einzubeziehen. Insgesamt wurden 622 Schnecken an 16 Standorten gefunden. Alle Proben wurden mikroskopisch auf Trematodenlarven untersucht. Positive Schnecken fanden sich an insgesamt 13 Standorten. Die isolierten Trematoden wurden morphologisch bestimmt und anschließend molekularbiologisch analysiert. Dazu wurde die DNA isoliert und drei verschiedene PCRs durchgeführt. Die allgemeine Trematoden-PCR amplifiziert ein Stück der 18S rDNA und eignet sich zum Nachweis und zur groben Klassifizierung von Trematoden. Für alle echinostomen Proben wurde das ND1-Gen zur weiteren Bestimmung genutzt und für die Vogelschistosomen das CO1-Gen. Schließlich wurden 16 Proben für die Artbestimmung durch DNA-Sequenzierung ausgewählt.

Insgesamt waren 149 der 622 gesammelten Schnecken mit Trematoden infiziert (24%), dabei handelte es sich in der Mehrheit um echinostome Larven (81 Schnecken, 13%). Xiphidiozerkarien wurden in 25 Schnecken (4%) und damit am zweithäufigsten nachgewiesen. Neun Schnecken enthielten amphistome Zerkarien (1,5%), sieben Furkozerkarien (1,1%) und eine Schnecke war mit monostomen Zerkarien infiziert (0,2%). Die restlichen 26 Schnecken enthielten nicht weiter identifizierbare Trematodenlarven (4,2%). Bei zwei der sieben Furkozerkarienfunde handelte es sich um *Trichobilharzia szidati* (0,3% Gesamtprävalenz). *T. szidati* ist als häufiger Erreger der Zerkariendermatitis bekannt. Die anderen fünf Furkozerkarienfunde lassen sich am ehesten den Familien Diplostomidae oder Strigeidae zuordnen, die keine Zerkariendermatitis verursachen. Unter den echinostomen Proben war *Echinoparyphium recurvatum* 13 Mal (2.1% Gesamtprävalenz) und *Echinostoma revolutum* (0.2%) und *Hypoderaeum conoideum* (0.2%) je einmal vertreten, die alle Echinostomose verursachen können. Diese Studie liefert die ersten molekularen Daten zum Vorkommen von schistosomatiden und echinostomatiden Trematoden in Österreich. Die genaue Kenntnis über die Verbreitung medizinisch relevanter Digenea in Österreich ist essentiell für eine profunde Risikoabschätzung.

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8.4. Acknowledgements

I would like to give special thanks to Prof. Dr. Julia Walochnik. She has not only provided me with this thesis project but has also given me the opportunity and privilege to work in a professional laboratory with pleasant colleagues as well as learn extensively about tropical medicine and parasitology. I feel I have and could further profit from her impressive knowledge. She has not only allowed me to tutor during her practical course at the Medical University Vienna, she has also given me amazing opportunities to present my project at several national and international congresses.

I am also very thankful to Dr. Helmut Sattmann and Mag. Christoph Hörweg from the Museum of Natural History Vienna, who co-supervised my master thesis. They supported me in my project in any way they could, whether it was with regular sampling trips, throughout the microscopy process, or with literature and knowledge. I would not have managed to find suitable collecting sites and be able to distinguish between the different snail species without their excellent zoological skills. In addition, I want to thank Stefan Szeiler, who has given up time to teach me how to make good glycerol preparations of the samples. I would also like to give thanks and a special mention to Anna Feix, who has spent hours and days with me conducting snail dissections and with whom I have had nice conversations.

Furthermore, I would like to thank my dear colleagues Iveta Häfeli, Dr. Martina Köhler, Mag. Ute Scheikl, Mag. Elisabeth Dietersdorfer, Susanne Glöckl, Jacek Pietrzak, Verena Mündler BSc and Christian Husch BSc not only for the support, but also for the conversation and laughter. I particularly want to highlight Iveta Häfeli who did a great job in incorporating me in common lab techniques.

I am also very thankful to my family for both their mental and financial support throughout my whole study and last but not least I am also deeply grateful to my partner Felix who always stood behind me, supported me, and encouraged me to go my way.



Left to right: Helmut Sattmann, Anna Feix, Christoph Hörweg and I during a sampling trip in the Mannersdorf-Wüste nature park. (© HÖRWEG)