University of South Bohemia in České Budějovice
Faculty of Science

Trematodes of Family Opecoelidae in Central Part of Svalbard

Bachelor thesis

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Annotation:

This study provides results of morphological analyses of material from the fish species *Myoxocephalus scorpius* and *Gymnocanthus tricuspis* from Svalbard. Adults of *Podocotyle atomon* were studied using carmine stain (inner structures) and scanning electron microscopy for viewing surface structures. Experimental infection of an intermediate host (Amphipoda) in the natural environment was documented during a field trip to Svalbard. Based on these findings, the life cycle of *Podocotyle atomon* was complemented.

Declaration:

Prohlašuji, že jsem svoji bakalářskou práci vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své bakalářské práce, a to v nezkrácené podobě – v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích, 24th of April, 2015.

Ondřej Otáhal……..

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1 Introduction

The unprecedented changes being experienced in the Arctic emphasize the importance and urgency of providing information to decision-makers in a timely manner (CAFF 2013). The extreme climate together with cold, seasonality and limited accessibility have kept human influence low, so ecological processes can be undisturbed. But climate changes and an increasing demand for Arctic sources are driving a new era of human activity with consequences for Arctic biodiversity (CAFF 2013).

The role of parasites in the polar areas is very closely connected to the issue of global changes since the problems associated with climate change are also connected with introduction of parasites to the polar areas. For example, although there are no feline beasts in the Svalbard ecosystem, the prevalence of *Toxoplasma gondii* in polar foxes (*Vulpes lagopus*) and polar bears (*Ursus maritimus*) is surprisingly high (more than 20% in both species) Also, how parasites influence their hosts and change host behaviour can be very interesting and useful in medicine and other fields.

Data for this thesis were collected during the summer course of Polar Ecology, which occurred in the Arctic in Svalbard in 2013. The main goal was to clarify the species of Opecoelid trematodes found in fish hosts using morphological methods.

Polar regions are typified by low temperatures, predominance of high pressure, low annual rainfall and snowfall, by polar days, polar nights, and permafrost. The ocean is mostly frozen, while the land has the character of an "arctic desert".

The Arctic is the polar region located in the northern part of the Earth with an area of 26.5 million of km². This area is defined as the region above the Arctic Circle, as the area bounded by latitude 66° 32"N. There are six months of daylight and six months of polar night at the same year. In the areas that are at lower latitude, the durations of daylight and polar night are shorter.

1.1 Svalbard

1.1.1 Geography

The Svalbard archipelago is located in the Arctic Ocean in the north western corner of the Barents Sea (Fig. 1). The archipelago lies between 74° and 81° northern latitude and 10° and 35° eastern longitude and is composed of 8 islands namely Spitsbergen, Nordaustlandet, Barentsøya, Edgeøya, King Karl's Land, Hopen, Prins Karls Forland and Bjørnøya. The land mass is about 61,020 km² and the sea area to the territorial border is about 90,700 km².

Ice cover is at 60% of the landmass and less than 10% has any vegetation. Norway's largest glacier**,** Austfonna, is located on Svalbard (Lydersen et al. 2010). Austfonna is the world's third-largest icecap after Antarctic and Greenland, with a glacier front of 200 kilometres. The fauna and flora, well as the geological wealth of the archipelago and its landscape, are protected (Lydersen et al. 2010).

Fig. 1: Location of Svalbard, Petuniabukta and research station.

Svalbard is surrounded by a shallow sea-shelf of the Arctic Ocean. To the west of Svalbard is the Greenland Sea and to the east is the Barents Sea. The average depth of the Barents Sea is 230 meters, and the shallowest areas lie between Bjørnøya and Edgeøya (Lydersen et al. 2010).

1.1.2 Climate

The climate of the Svalbard is milder than in other areas at the same latitude because of frequent passage of low-pressure system and the warm water of the Atlantic Ocean. The annual average temperature in Longyearbyen (administrative centre of Svalbard) is -4 °C.

The highest measured temperature in Svalbard was recorded in July 1979 at 21.3 °C and the lowest was in 1986 at -49.2 °C (Førland et al. 1997).

Svalbard may be described as an "arctic desert" with annual rainfall and snowfall at a mere 200 - 300 mm, the central part is drier than western part of Svalbard thanks to the influence of the ocean. The depth of permafrost on Svalbard was about 100 meters close to the ocean and 500 meters in the mountains (Liestøl 1980).

1.1.3 History

The archipelago of Svalbard was discovered in 1596 by the Dutch captain Willem Barents, but the first historical references are from 1191 from Iceland. In 1611, this area started to be very attractive for hunting bowhead whale mostly led by English and Dutch companies. The whaling was very intensive and very brutal and aggressive. Whaling stations started to be built in the 17th century - with the biggest one at Smeerenburg (Fig. 2) (Conway 1906).

Fig. 2: Location of Smeerenburg Source: wwww.ecowatch.se.

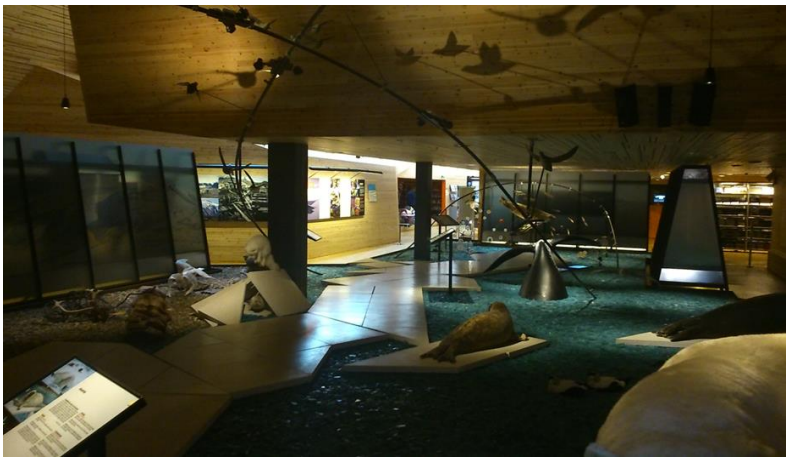

Fig. 3: Svalbard museum.

Pomors led the hunting until the 17th century, but by the 19th century, Russians and Norwegians dominated hunting. Swedish and Norwegian scientists led an international latitude measurement expedition at the end of the 19th century.

1.1.4 Svalbard Treaty

The Svalbard Treaty was signed in Paris on 9 February 1920 and establishes Norway's full and absolute sovereignty over Svalbard (Overrein 2011). The Treaty of Svalbard became effective on 14th of August 1925, and according to Norwegian law – Act of 17 July 1925, Svalbard became part of Norway. At the same time, the Treaty gives other countries including the Czech Republic

extensive rights (Overrein 2011). For example, citizens from the signatory states are granted equal rights to undertake industrial enterprises, mining, fishing, trapping, maritime and commercial activities. The Treaty stipulates that collected taxes in Svalbard may only benefit Svalbard. All military activities are prohibited, so it is not allowed to establish naval bases, build fortresses or use Svalbard in a war situation (Overrein 2011).

1.1.5 Animals and Marine Life of Svalbard

The Arctic tundra, freshwaters and seas are home to more than 21,000 known species of highly cold-adapted organisms. Such as metazoans, plants and fungi including lichens, as well as tens of thousands of protists and prokaryotes (CAFF 2013).

Svalbard is a important breeding area for birds. About 30 species nest annually in Svalbard, but only a few species such as the rock ptermigan (*Lagopus muta*) or black guillemot (*Cepphus grylle*) (Fig. 4) remain in winter (Kovacs and Lydersen 2006). The most known species that occur in Svalbard are the barnacle goose (*Branta leucopsis*), ivory gull (*Pagophila eburnea*), blacked-legged kittiwake (*Risa tridactyla*), or the Atlantic puffin (*Fratercula arctica*) and the Arctic tern (*Sterna paradisaea*).

Terrestrial mammalian species that live a hectic life during the summer months of June, July and August are represented by the arctic fox (*Vulpes lagopus*), the Svalbard reindeer (*Rangifer tarandus platyrhynchus)*, polar bear (*Ursus maritimus*) (Fig. 4) and accidentally introduced southern vole (*Microtus levis*).

Marine mammals are represented by 12 species of whales, dolphins, 5 species of seals, walruses and narwhals. The polar bear, the walrus (*Odobenus rosmarus*) and the harbor seal (*Phoca vitulina*) are Red Listed (Kovacs and Lydersen 2006).

Fig. 4: From left upper corner: Black guillemot (*Cepphus grylle*), polar fox (*Vulpes lagopus*), polar bear (*Ursus maritimus*) and the Atlantic puffin (*Fratercula artica*). Source: Jan Kavan.

The parasitofauna of the Svalbard archipelago is poorly known. Most of these studies focused on the ecology and distribution of helminths in Svalbard and are useful, but most of them are quite old such as Odhner´s Die Trematoden des arktischen Gebietes – Trematodes of the artic regions (1905).

The polar regions have been a bit neglected, unlike for example the Mediterranean that received much more attention in recent years (Bartoli and Gibson 1991; Jousson et al. 1999; Jousson and Bartoli 2000, 2001).

Recently, several studies on helminthofauna of sea birds occurring in Western Svalbard or parasites of ringed seals have been published (Kuklin et al. 2004; Johansen et al. 2010). This thesis aims to determine the species of *Podocotyle* Dujardin, 1845 trematodes found in marine organisms caught and dissected during our field trip in Svalbard in 2013. The main parts of this thesis are: Trematoda, trematodes lifecycles, and description of the family Opecoelidae and genus *Podocotyle*.

1.2 Trematoda Rudolphi, 1808

Trematoda is a very successful class of invertebrates belonging to the phylum Platyhelminthes, with tapeworms (Cestoda) and Monogenea forming the monophyletic group Neodermata.

This group is characterised by the presence of a syncytial surface called the neodermis, which replaced the epidermis during their ontogenetic development. The class of trematodes is represented by more than 25 000 described species, divided into two subclasses: Aspidogastrea and Digenea (Esch et al. 2002; Roberts and Janovy 2005).

Trematodes are parasites of all classes of vertebrates, especially marine fishes, and nearly every organ of the vertebrate body can be parasitized by some kind of trematode (Schmidt and Roberts 2000).

Adults have a dorsoventrally flattened body with metabolically active tegument with the distal part being of the syncytial type (Roberts and Janovy 2005). Typical organs of trematodes are two suckers, with an oral sucker placed on the front end of the body and the ventral sucker placed on the ventral side. However, these suckers may be modified in some groups (Esch et al. 2002).

Body size ranges from micrometres up to several centimetres such as *Fasciola gigantica* Cobbold, 1856. Species can also differ by the localization of infection in hosts, by shape of the body and by localization of suckers (Roberts and Janovy 2005).

The space between organs in the skin-muscular sac is filled by parenchymal binder which has a supporting function and participates in the distribution of nutrients received by the *gastrodermis* (epithelium of the digestive system) into the various parts of the trematode body.

Trematodes have a very well developed digestive system, beginning with an oral opening mounted on the bottom of the oral sucker and continuing with the prepharynx, muscular pharynx, oesophagus and two blindly ending branches of the intestine (Fig. 5).

Due to the fact, that they are parasites of the digestive system, their diet consists of blood, tissue fluid and content of the gut (Smyth and Halton 1983).Tapeworms anchor to the host´s tissue by two suckers, the oral sucker and well developed ventral sucker (acetabulum).

The reproductive system of hermaphroditic trematodes includes both male and female sexual organs. The male reproductive system consists of testes, which may be a different number (usually 2). The testes are connected by thin channels (*vasa efferentia*) to the vas deferens. The vas deferens passes into the cirrus sack. In some trematodes, the cirrus sack can be completely absent and replaced by other organs such as the gonotyl in Heterophyidae.

The cirrus sack contains the seminal vesicle (*vesicula seminal interna*), prostate gland and cirrus (or in some cases penis) and the cirrus sack opens into a common genital atrium. The testes sperm are stored in the seminal vesicle until the cirrus or penis is placed in the uterine or vaginal opening of the partner, or during self-fertilisation, when it is inserted into its own female opening. During copulation sperm leave by the *ductus ejaculatorius.*

In the female reproductive system there are seperate organs for the production of the ovum, and for the production of the yolk cells and shell material. The female reproductive system consists of almost always an unpaired ovary (*ovarium*). The seminal reservoir (*receptaculum seminis*) is near the ovary. From the ovary the ova are released via a short oviduct that opens into the ootype. In The ootype ova are surrounded with vitelline cells, coming to the proximal part of the ootype from the common vitelline duct – vitelline reservoir (Galaktinov and Dobrovolskij 2003).

The ootype is surrounded by the Mehlis gland. The function of the Mehlis gland is still unclear (Galaktinov and Dobrovolskij 2003; Świderski et al. 2011). It may help nourish the developing egg and provide lubricating fluid that helps with the movement of the eggs in the ootype and uterus. The Mehlis gland also may takes part in shell tanning and/or hardening as the eggs pass through the uterus (Świderski et al. 2011).

Fertilization takes place in the oviduct or the ootype. Sperm enter the female reproductive system via a specialized structure called Laurer's canal (an opening from the dorsal surface of the worm) which enter into the oviduct. The formed and fertilised eggs, covered with a thin shell then enter the uterus. The uterus is a highly coiled, walled structure. The terminal part (metraterm) is equipped with a specialized muscular wall (Smyth and Halton 1983). The eggs then leave the parasite through the genital pore and can continue the parasite lifecycle.

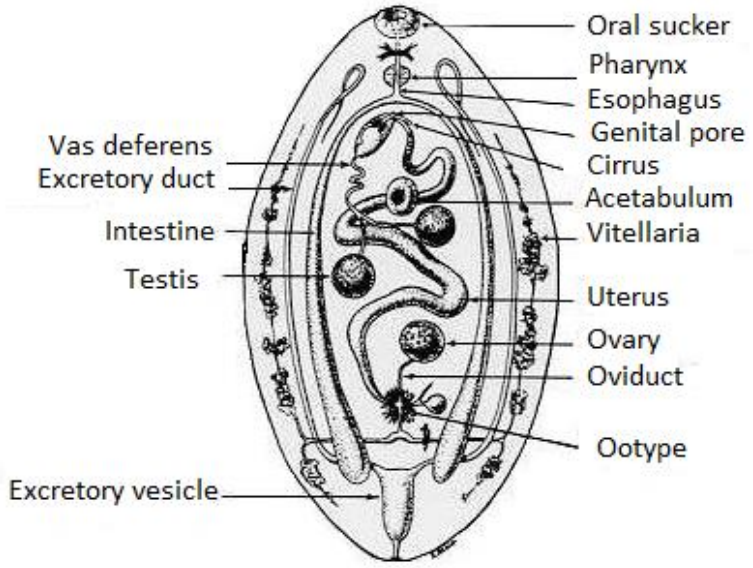

Fig. 5: Structure of hermaphroditic trematode (modified from Hunter et al. 1976).

Many species of trematodes are medically important, such as *Fasciola hepatica* Linnaeus, 1758, *Paragonimus westermani* (Kerbert, 1878) Braun, 1899, *Opisthorchis viverrini* (Poirier, 1886) Stiles et Hassall, 1896 and representatives of the genus *Schistosoma* Weinland, 1858 (Cribb et al. 2003; Roberts and Janovy 2005). Trematodes are also characterized by having very complex life cycles.

1.3 Life cycle

Development cycles usually involve several larval stages (except for Aspidogastrea) with at least one intermediate host (Fig. 6). The first intermediate hosts are usually molluscs, in which asexual reproduction takes place. Sexual reproduction takes place in the final host (usually a vertebrate).

Parthenogenetic generations are represented by a mother sporocyst, a larva (miracidium) and rediae or daughter sporocysts (Galaktinov and Dobrovolskij 2003).

These stages are called intramolluscan stages, because most of the parthenitae are obligate parasites of molluscs (Galaktinov and Dobrovolskij 2003).

The free-living, ciliated larva (miracidium) and the mother sporocyst represent the first parthenogenetic generation.

Mature adults produce eggs that pass to the environment. From the egg hatches a motile, short-living, non-feeding, ciliated larva (miracidium). Most eggs have an operculum, through

which the miracidium escapes, but in the case of inoperculate eggs, the miracidium hatches after rupture of the wall of the shell.

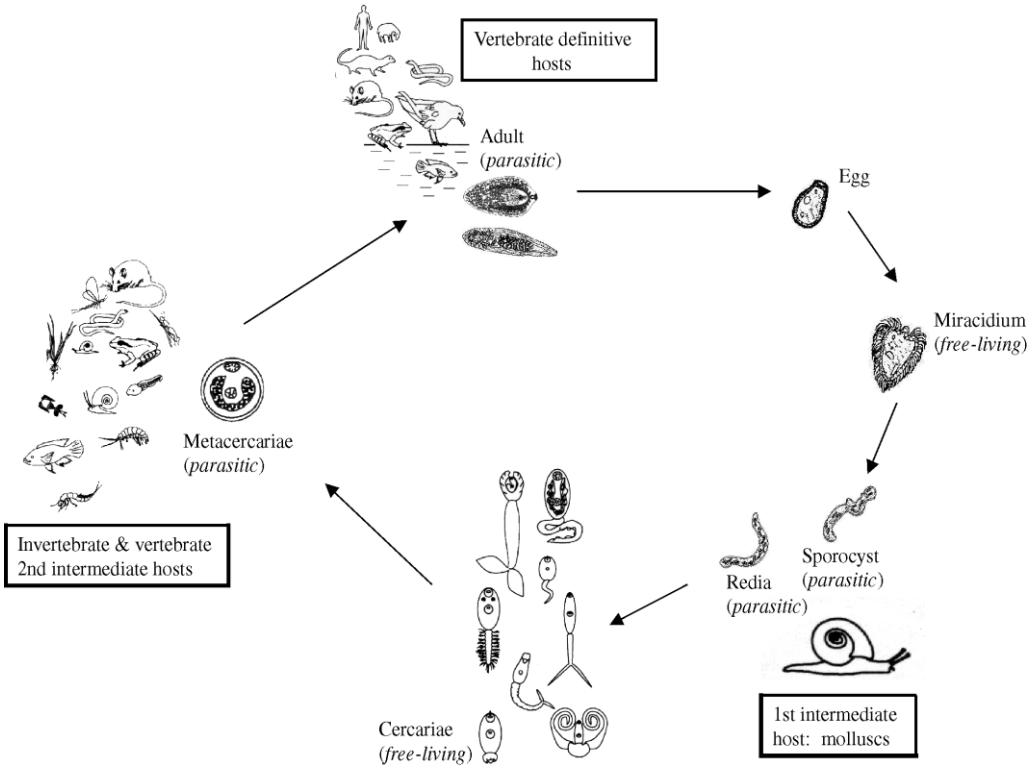

Fig. 6: Scheme of typical trematode life cycle (Sukhdeo and Sukhdeo 2004).

In some species light, oxygen, osmotic pressure and temperature appear to be important signals (Smyth and Halton 1983). The miracidium relies on stored energy, so it is imperative that it quickly locate and infect a first intermediate host - mollusc.

Wright (1959) proposed that, host finding is an active process that occurs in three distinct steps: (1) location of the host habitat, (2) random search for the host, and (3) specific attraction for the host. For orientation in the environment, the miracidium probably uses different receptors, which recognize molecules (glykoproteins) secreted by a snail.

The miracidium swims (no longer than 24 hours) and penetrates the first intermediate host. At the time of penetration or soon after, the larva discards its ciliated epithelium and metamorphoses into the mother sporocyst (Sukhdeo and Sukhdeo 2004).

The mother sporocysts produce the second parthenogenetic generation that is represented by: daughter sporocysts or rediae. Trematode mother sporocysts producing rediae are normally

localized in the haemocoel of the mollusc palium complex or in the mollusc heart (Galaktinov and Dobrovolskij 2003).

The rediae and sporocysts differ from each other in morphology as well as biology.

Young individuals of the second parthenogenetic generation actively migrate along the haemocoel (rarely directly through the host tissues) to the hepatopancreas and/or gonads (Køie 1982; Galaktinov and Dobrovolskij 2003).

Rediae are normally characteristic of more primitive trematodes for example Echinostomatidae, Fasciolidae, Psilostomatidae or Lepocreadiidae, but can also be found in specialized groups such as Heterophyidae or Opisthorchiidae (Galaktinov and Dobrovolskij 2003).

Rediae have an elongated cylinder shaped body with three conic locomotory extensions.

While the mother and daughter sporocysts lack any trace of feeding structures, redia has a mouth, pharynx, oesophagus and sac-like intestine (Cribb 2005; Galaktinov and Dobrovolskij 2003). The mouth is located at the anterior end of the rediae. A small opening called a birth pore is located sub terminally and serves to give rise to individuals of the next generation (Galaktinov and Dobrovolskij 2003).

Variability of rediae (often age-dependent), is observed in the shape of the body as well as in the degree of development of different structures (Galaktinov and Dobrovolskij 2003).

Daughter sporocysts are typical for more specialized groups such as Strigeidida and Plagiorchiida (Galaktinov and Dobrovolskij 2003). Daughter sporocysts of Gymnophallidae, Schistosomatidae, Cyathocotylidae, Strigeidae and Diplostomidae are capable of producing a next generation of sporocysts alongside of cercariae (Galaktinov and Dobrovolskij 2003). The mobility of daughter sporocysts is limited compared to that of rediae.

Daughter sporocysts and rediae can asexually produce the generation represented by cercariae or metacercariae.

Cercariae mostly leave the body of the first intermediate host actively or passively by secretion. In general it is accepted that host finding by cercariae is strategically similar to that of host finding by miracidia, with the same three steps: (1) movement to the environment, (2) energy efficient search, and (3) attachment to the specific host (Sukhdeo and Sukhdeo 2004; Haas 1994).

Emergence from the snail host can depend on the physiology or behaviour of the snail host and physicochemical factors such as temperature, light and pH of water can stimulate this emergence of cercaria. A mature cercaria leaves the mollusc and can live an active life in the external, usually aquatic environment. In the past, when there was no connection with life cycles, cercariae were described as individual organisms with their own zoological system.

The cercarial body is formed by body part and a tail. The body often has similar characteristics as the adult trematode (such as suckers and thorns) (Galaktinov and Dobrovolskij 2003). An oral sucker is localized sub terminally at the anterior end and a ventral sucker is always located in the middle or posterior part of the body. The tail is the mechanism of cercarial locomotion, which brings the parasite to the next host. The morphology varies in larvae of different species and reflects the functional needs of each cercaria (locomotion and adherence or crawling on the substrate, swimming in water or floating (Galaktinov and Dobrovolskij 2003; Sukhdeo and Sukhdeo 2004; Schell 1970).

Cercariae that infect mobile hosts (mostly fish) may have structures and behaviours that often mimic a host's prey items (Shell 1970; Combes 1994; Sukhdeo and Sukhdeo 2004).

Cercaria can encysts on objects (in the external environment such as blades of grass or shells of molluscs) to an adolescarie (*Fasciolidae*, *Paramphistomidae*) and waits for ingestion by the final host. Another strategy is penetration of second intermediate host, in which cercariae develop into metacercariae (*Strigeidae*, *Echinostomidae*) and wait for ingestion of the second intermediate host by the final host (vertebrate) (Cribb 2005; Galaktinov and Dobrovolskij 2003; Sukhdeo and Sukhdeo 2004). Cercariae of *Schistosomidae* and *Sanguinicolidae* penetrate directly to the final host, skip the phase of metacercariae in the final host and develop directly to the adult (Kirk and Lewis 1993). After development has been completed, a sexually mature individual (marita) of the hermaphroditic generation can starts reproducing. Adults release their eggs, usually in host faeces. The eggs can be ingested by a suitable mollusc or from eggs hatch miracidia (Leung 2009). The cycle is closed.

1.4 Opecoelidae Ozaki, 1925

The Opecoelidae represent one of the largest cosmopolitan family of trematodes infecting both marine and freshwater fishes, with over 800 species recognized in 85 genera (Cribb 2005; Jousson et al. 1999). The taxonomy within the family is difficult due to its size and that the

extents of the characters, which have been used for taxonomic purposes, are of equivocal value (Cribb 2005).

Ozaki (1925) developed the concept of this family, where he proposed the type-genus *Opecoelus* Ozaki, 1925 and its type species *Opecoelus sphaericus* Ozaki, 1925.

In literature Opecoelidae can be found also as synonyms Coltocaecidae Ozaki, 1929; Notoporidae Yamaguti, 1938 and Podocotylidae Dollfus, 1960 (Cribb 2005).

Opecoelids are not specialized, so there are no characters that allow the immediate recognition of the family (Cribb 2005). Typical characters are a smooth tegument, oral and ventral sucker, I-shaped excretory vesicle and the presence of a prepharynx, pharynx, two caeca and a preovarian uterus (Jousson and Bartoli 2000; Cribb 2005).

The male reproductive system consists of 2 to 10 testes, which are intercecal, diagonal, tandem or side by side in short bodied forms (Hoffman 1967; Cribb 2005).

The female reproductive system consists of an ovary, sperm receptor (uterine or canalicular) and Laurer's canal (Cribb 2005). The uterus may be in the area between the ovary and the genital pore or may fill the hindbody (Cribb 2005). The genital pore is always in the forebody.

The eggs are usually coloured gold, operculate and 40-80 micrometres long (Jousson and Bartoli 2000).

Typically, unembryonated eggs are passed to the environment in the faeces (Køie 1981; Jousson and Bartoli 2000; Cribb 2005). From the egg hatchs the miracidium which penetrates a wide range of prosobranch snails (Jousson and Bartoli 2000; Cribb 2005).

The body of miracidium is covered with flattened ciliated epithelial cells, so called epithelial plates, which form several circular rows, separated by ridges of hypodermis. In most trematode families with the actively infecting miracidium the number of rows is four in Opecoelidae family (Galaktinov and Dobrovolskij 2003).

Within the snail, the miracidium develops to the mother sporocyst, which produces daughter sporocysts that produce cercariae (Jousson and Bartoli 2000; Cribb 2005).

Most opecoelid cercariae are cotylocercouse forms with reduced tail to a small stump (Cribb 2005). Presence of the stylet in the oral sucker, cotylocercouse tail and aspinose tegument represent distinguishing features at the family level, but species identification of opecoelid cercariae using morphological characters seems to be difficult (Born-Torrijos et al. 2012; Jousson

et al. 1999). The cercariae lack eye spots, but have well developed oral and ventral suckers, penetrations glands and the stylet embedded in the oral sucker.

The unexceptional morphology of the Opecoelidae has led to considerable confusion in their delineation from a number of other families. Preeminent among these is the Allocreadiidae Looss, 1902, in which many opecoelids were originally described due to morphological similarities (Cribb 2005). For example, Dawes (1966) placed *Podocotyle atomon* Dujardin, 1845 into the family Allocreadiidae (Fig. 7). The main characters which distinguish Allocreadiidae from Opecoelidae are that the allocreadiid life cycle involves an opthalmoxiphidocercariae usually produced in freshwater bivalves, but sporadically in gastropods, and the fact that the allocreadiid cercariae typically have a legacy of eye-spot pigment scattered in the forebody (opecoelid cercariae lack eye-spots) (Cribb 2005).

Opecoelids have also been confused with the lapocreadiids. Members of the family Lapocreadiidae Odhner, 1905 have prominent tegumental spines. In addition, lapocreadiid cercariae have eye-spots that leaves eye-spot pigment in the forebody as in the Allocreadiidae (Cribb 2005).

Members of the family Fellodistomidae Nicoll, 1909 can be distinguished from members of the family Opecoelidae by Y-shaped excretory vesicles and vitelline follicles that are far more restricted in the latter family than those of the Opecoelidae (Cribb 2005).

Subfamily level classification within the Opecoelidae is complex and still unsatisfactory, because no phylogenetic analysis has been performed for most of the family. In addition to the type-subfamily Opecoelinae Ozaki, 1925, ten further subfamilies have been proposed (Cribb 2005).

The current taxonomy of opecoelids is influenced by the classification proposed by Gibson and Bray (1984) which recognized four subfamilies: Opecoelinae Ozaki, 1925, Plagioporinae Manter, 1947, Stenakrinae Yamaguti, 1970 and Opecoelininae Gibson et Bray, 1984 (Cribb 2005; Gibson and Bray 1984).

The main characters used to distinguish the subfamilies are combinations of the form of the male terminal genitalia, sperm reception in the female system and the distribution of the uterus (Cribb 2005; Gibson and Bray 1984).

1.5 Genus *Podocotyle* Dujardin, 1845

The genus *Podocotyle* (the second oldest opecoelid genus) was named as a subgenus by Dujardin (1845) and raised to generic rank by Strossich (1892), but was first well characterized by Odehner (1905) as subgenus for *Distoma angulatum* Dujardin, 1845 (Gibson and Bray 1982).

In older literature *Podocotyle* was a synonyms of *Sinistoparus* Stafford, 1904, *Podocotyloides* Yamaguti, 1934 and *Neopodocotyloides* Pritchard, 1966 (Dawes 1968). *Psilolintum* Oshmarin, 1964 was proposed by Oshmarin (1964) for *Psilostomum lineatum* Linton, 1928, described originally in the Atlantic seagulls, but Kostadinova (2001) shows that its presence in the Atlantic seagulls was an accidental infection and that species is an opecoelid (a synonymum of *Podocotyle reflexa* (Creplin, 1825), Odhner, 1905). *Podocotyle reflexa* has been considered as synonym of *P. atomon* (Rudolphi, 1802) Odhner, 1905, but Køie (1981) shows that both ale valid species.

The type-species has at times been thought to be synonymous with *P. atomon* but Gibson and Bray (1982) proposed that *P. angulatum* Dujardin, 1845 should continue to be the type-species.

Many species were described in *Podocotyle* and then later transferred to other genera (Cribb 2005).

The genus Podocotyle is represented probably by more than 100 recognized species, but only about 26 valid species (Cribb, 2005; Gibson 2015). Pritchard (1966) listed 17 Podocotyle species from fishes from the Pacific and Atlantic Oceans. Some of these species have already been listed as synonyms, and the number will

undoubtedlv be further reduced in the future. Species found in Europe are *Podocotyle atomon* (Rudolphi, 1802) Odhner, 1905 found in cods and flounders (Køie 1981). *Podocotyle olssoni* Odhner, 1905 (synonym to *Distoma simplex* Olsson, 1868) found in *Gadus melanostomus*, accepted as *Podocotyle reflexa* (Creplin, 1825) Odhner, 1905. *Podocotyle angulata* Dujardin, 1845 found in the brook trout (*Salvelinus fontinalis*) synonymous to *Podocotyle staffordi* Miller, 1941 and *Podocotyle scarpaenae* (Rudolphi, 1919) Bartoli et Gibson, 1991 (Gibson 2001). Species found in USA are *Podocotyle congeri* (Yamaguti, 1970) Bartoli, Bray et Gibson, 2003 or *Podocotyle caithnessi* Manter, 1954 and *Podocotyle bathyhelminthos* Blend et Dronen, 2015 found in the cusk eel (*Luciobrotula corethromycter*) (Blend and Dronen 2015).

The flukes have an elongated, flattened or subcylindrical, unspined body and are colourless. Their size is 1.9 – 3.5 mm. The oral sucker and pharynx are well developed (Pritchard 1966). Acetabulum is much larger than the oral sucker (Køie 1981). The pharynx is short and

twice smaller as the oesophagus. The cirrus sac is club-shaped and extends behind the ventral sucker. The testes are behind each other, placed in the middle of the body. The three or four-lobed ovary is placed in front of the testes, vitellaria are generally from the acetabulum to the posterior. The genital pore is sinistral at the level of the oesophagus. Laurer´s canal is present (Pritchard 1966). The follicles are small and compact and usually are not entering the forebody but extending posteriorly beyond the testes to the posterior end (Cribb 2005; Dawes 1968). The uterus is restricted to the area between the ovary or the anterior and the genital pore (these characters distinguish *Podocotyle* from other genera such as *Allopodocotyle* Pritchard, 1966, *Macvicaria* Gibson et Bray, 1982, and *Neolebouria* Gibson, 1976. Eggs are brownish yellow, variable in size (0.06-0.08 mm) and the number does not exceed 60 (Hunninen and Cable 1943; Køie 1981). Most authors have accepted the absence of vitellaria in the forebody as important characteristic of *Podocotyle* (Pritchard 1966). Dawes (1968) separated the European species by characters to the following key:

1) Testes are very close to each other.

a. Cirrus sack is extending back to the mid-point of the ventral sucker.

P. levinseni

b. Cirrus sack is extending back to the posterior end of the ventral sucker.

P. odhneri

2) Testes considerably apart, or separated by vitelline follicles.

a. Cirrus sack is not extending behind the ventral sucker.

b. Cirrus sack is extending behind the ventral sucker.

i. Oesophagus is twice as long as the pharynx.

1. Vitteline follicles are not reaching the ventral sucker. *P. atomon*
2. A few vitteline follicles are extending in front of the ventral sucker.

P. atomon var *dispar*

ii. Oesophagus is as long as the pharynx.

1. Body is flattened *P. olsoni*
2. Body is cylindrical *P. reflexa*

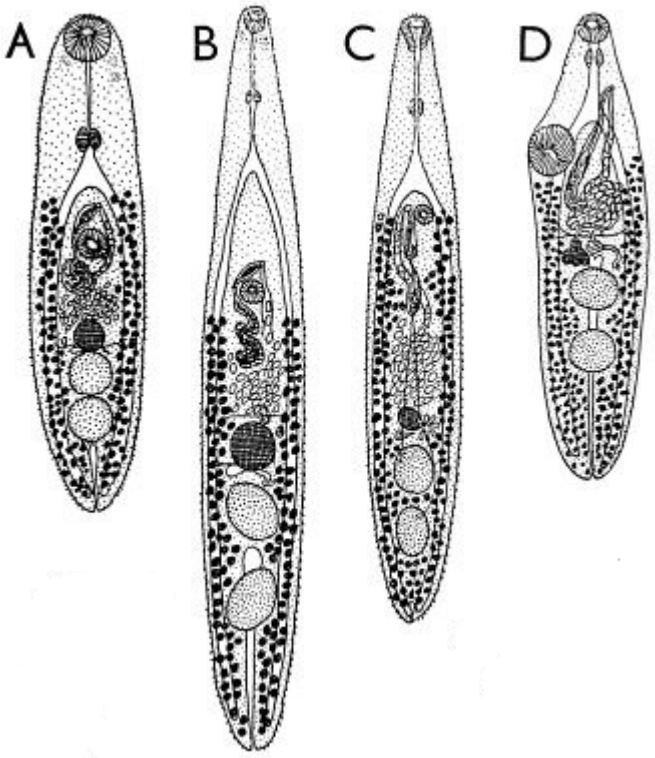

Fig. 7: Incorretly classified *Podocotyle atomon* (D) (to family Allocreadiidae) from Dawes 1968. (A) *Lepidopedon rachion*, (B) *L. elongatum*, (C) *Opechona bacillaris.*

Podocotyle reflexa (Creplin, 1825) Odhner, 1905 has been recorded from fish occurring in the arctic-boreal area. The cercariae of *P. reflexa*, *Cercaria buccini* Lebour, 1911, has been recorded from British waters, Danish waters and the Barents Sea (Køie 1969; Lebour 1911). According to Køie (1981) *Podocotyle reflexa* occurs in fish which live sublittorally while *Podocotyle atomon* dominates in fish that live littorally.

Cercariae of both species are cotylomicrocercouse (Hunninen and Cable 1943). The main differences between the cercariae of *P. atomon* and *P. reflexa* (Fig. 8) is that the double pointed stillet of *P. atomon* is more narrow and the external sufrace of *P. atomon* contains about 5 micrometre long thin mocrovilli, whereas the surface of *P. refelxa* is smooth.

Also *Podocotyle atomon* has only three pairs of cephalis glands (*Podocotyle reflexa* has at least five pairs) (Køie 1981).

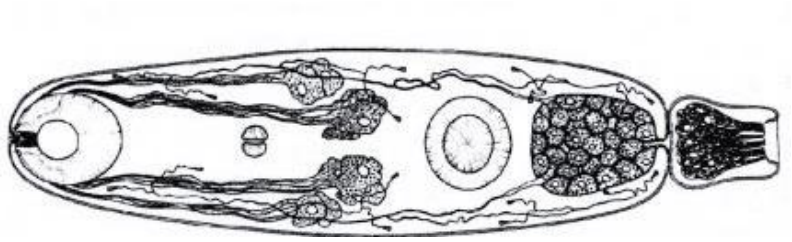

Fig. 8: Cercariae of *Podocotyle reflexa* (Opecoelidae) Køie (1981).

It may be very difficult to distinguish between *P. atomon* and *P. reflexa* (Fig. 9) if the worms are immature or have only a few eggs and are less than 1 mm long (Køie 1981).

The following are the main differences between the two species as listed by Brinkmann (1975): The body of *P. atomon* is flattened, whereas the body of *P. reflexa* is cylindrical.

The distance between suckers is about 1/4 of the body length in *P. atomon* and about 1/7 in *P. reflexa*. The vitellaria of *P. atomon* are unbroken (covering the intestinal caeca) and do not come together between the testes, whereas the vitellaria of *P. reflexa* are broken and come together between the testes. In *Podocotyle atomon*, the body is not constricted level with the testes, while it is in *P. reflexa*. The testes of *P. atomon* are relatively small and do not occupy more than half of the cross section of the body. The testes of *P. reflexa* are large and occupy the greater part of the body in cross section.

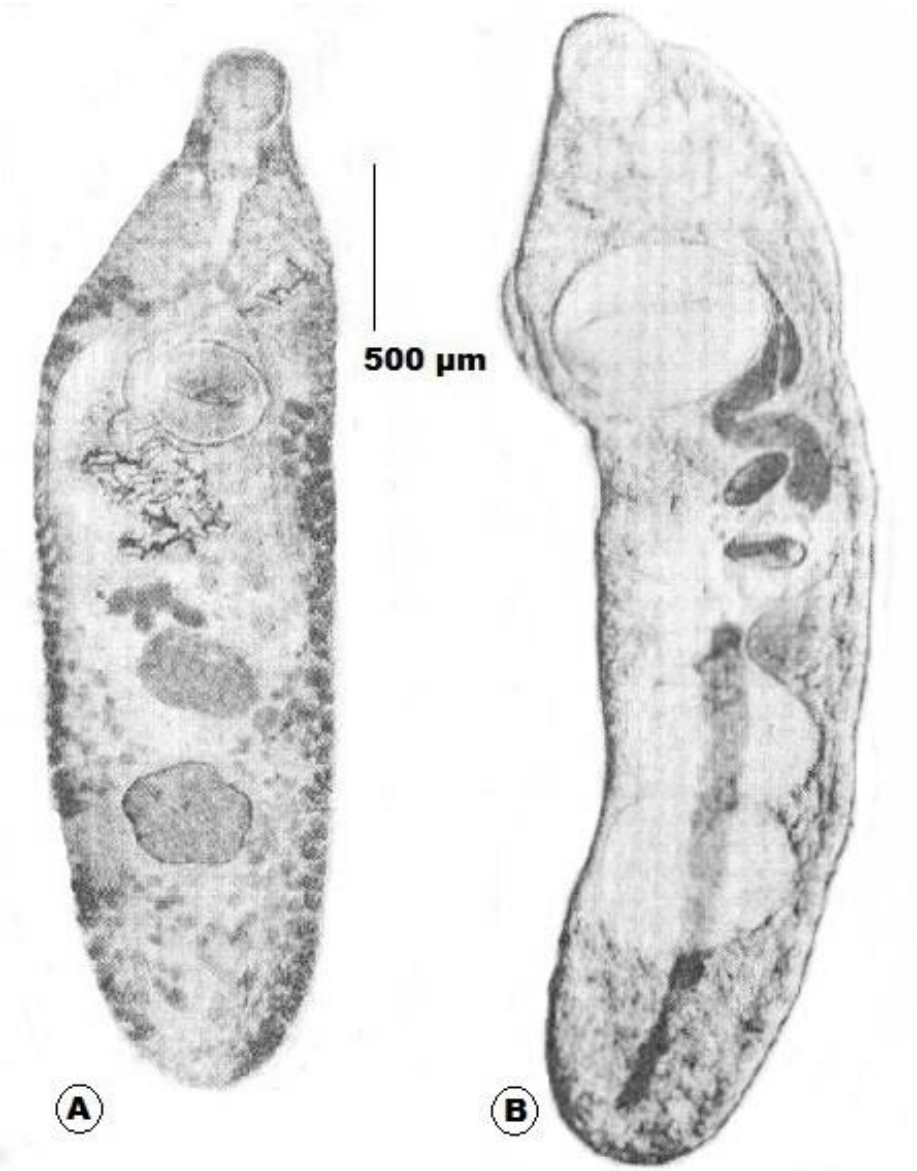

Fig. 9: Stained, flattened specimens of *Podocotyle atomon* (A) and *Podocotyle reflexa* (B) (from Køie 1981).

However these character are not always valid according to Køie (1981), because of possible variability between smaller and larger speciemens.

2 Aims and Objectives

An important goal of this study is to investigate literary sources focused on trematodes of family the Opecoelidae family, their morphology and lifecycles. In the field part of my bachelor work I and my colleagues have collected samples from marine hosts. Collected material will be used in future molecular analysis. I intend to process samples from a Svalbard field trip in 2013 and use morphological methods for morphological identification and differentiation of the found trematodes.

Objectives:

1. To review literature on the theme.
2. Morphologically and morphometrically analyse Opecoelid trematodes found in *Gymnocanthus tricuspis* and *Myoxocephalus scorpius* in year 2013.
3. Compare findings from experimental infection with previous life-cycle literature data.

3 Materials and Methods

3.1 Study area

Samples were collected during a course of Polar ecology in year 2013 in the central part of Svalbard. Petuniabukta station is in northernmost part of the Billefjorden, Isflorden and located in a non-protected area. Nearby are three national parks-Sassen-Bunsow Land NP, Norde Isfjorden NP and Indre Wijdefjorden NP. The research station is located on the shore of Billefjorden, surrounded by mountainous terrain. Petuniabukta is divided into four bays: Adolfabukta, Skansbukta, Petuniabuktra and Mimerbukta. The highest points are De Geerfjellet (1023 meters above sea level) and Pyramiden (935 meters above sea level).

Petuniabukta is more continental and drier than the western part of Svalbard. Many various biotopes are within walking distance and close to research station, such as sandy beaches, waterlogged tundra, bird cliffs and glaciers. Fauna is represented by reindeers, arctic foxes, gulls, barnacle geese and polar bears.

During our stay in Petuniabukta from 1th to 16th of August 2013, marine fish were caught by me and other course participants using gill nets laid in the littoral zone close to Petuniabukta. Other underwater organisms were also collected during scuba dives by members of the parasitological group in Billefjorden. Set fishnets were checked with a rubber boat (Fig. 10) every day (sometimes twice a day). Species of fish that occur in Petuniabukta were determined during previous fieldtrips by zoologists that visited Svalbard in previous years.

Data from parasitological dissections were noted for later processing. Those dissections were led by Oleg Ditrich with the assistance of Tomáš Tyml and every student of the parasitological group participated. An Olympus BX 53 with DP 73 camera was used for microscopy.

In every individual under parasitological dissection sex and the standard body length was recorded. In cases, that were infected with trematodes from the family Opecoelidae (determined by Oleg Ditrich) we attempted to localize the most infected part of the body and count the number of parasites in the entire individual

Trematodes found during dissections were fixed in 97% EtOH (ethanol) for molecular analysis and in FA (formaldehyde) for SEM and carmine staining. Samples for morphological analyses were fixed to hot FA or fixed by pressure.

Fish hosts were divided by species and sex. From collected data prevalence and intensity of infection was calculated.

Fig. 10: Rubber boat and beach with prepared fish nets.

3.2 Hosts

First intermediate hosts

During scuba dives collected mollusc were parasitologically dissected. Opecoelid trematodes were found in *Buccinum undatum* Linnaeus, 1761, *Buccinum glaciale* Linnaeus, 1761 and *Plicifusus kroeyeri* (Möller, 1842).

Buccinum undatum is marine gastropod living from 0-1200m. It occurs on both hard and soft bottoms (rock, gravel, coarse and muddy sand) in the sublittoral zones and sometimes as far up as the tidal zone (Moen and Svensen 2004). The species can live in brackish water to a salinity of 14%. Temperature higher than 29 °C is deadly for this species (Ten Hallers-Tjabbes 1996).

Buccinum undatum (Fig. 11) can grow up to 110 high, and 68 mm wide (Moen and Svensen 2004). The shell is broadly oval, solid, pale and its surface is rough with a checkered appearance due to spiral striations and crossing growth lines (Moen and Svensen 2004). The outer lip of the shell opening is evenly curved towards a short siphon canal. The inner and outer lips are white, while the main shell is grey white. It is difficult to distinguish *Buccinum undatum* from other species in some areas where the range of the species overlap.

Fig. 11: *Buccinum undatum*. Source: marinespecies.org

They spawn large aggregations of egg capsules and attached them to hard substrates (Moen and Svensen 2004). *B. undatum* is a pray of a range of species as cod, dogfish, crabs and starfish (Hancock 1967). The eggs are eaten by starfish and sea urchins.

Since the 1970s there is disappearing or decreasing populations of whelks in North Sea and Wadden Sea (Ten Hallers-Tjabbes 1996). The presence of male gonads on females (so called imposex) and viability of whelk population has been detected since the 1990s, possibly due to activities of the shipping industry and using TBT (tributiltyn) as anti-fouling paint of the ships (Ten Hallers-Tjabbes 1996; Mensink 1996).

Trematodes of the family Opecoelidae were found in *Buccinum glaciale* Linnaeus, 1761 sometimes also synonymous to *Buccinum groenlandicum* Hancock, 1846, *B. hancocki* Mörch, 1857 or *B. donovani* Gray, 1839, is a highly variable species (which has resulted in a number of synonyms) of the marine snail of family Buccinidae. This species has a circum-arctic distribution and can be found from intertidal to subtidal zone in the Northwest Atlantic Ocean (European waters around Svalbard), in arctic zone of Canada and around Japan.

The size of the shell (Fig. 12) reaches 90mm, with the colour varying from cream to brown to bluish-grey. The shell is roundish with many spiral lines, ovate-oblong and obliquely contracted at the base. This species has two or three spiral cords and some axial ribbing . The body whirl is slightly keeled, while the siphonal canal is short. The thick outer lip is flaring and turns back (Brunel et al. 1998).

Fig. 12: *Buccinum glaciale.* Source: marinespecies.org

The last mollusc in which trematodes of the family Opecoelidae were found is *Plicifusus kroeyeri* (Möller, 1842).

This species is also synonymous to *Colus kroeyeri* (Möller, 1842), *Fusus arcticus* Phillippi, 1850, *Colus cretaceus* (Reeve, 1847) or *Fusus kroeyeri* Möller, 1842 is a marine gastropod mollusc in the family Buccinidae.

Plicifusus kroeyeri (Fig. 13) has a circumpolar distribution, according to Kosyan and Kantor (2012) it is not present in Norway and Iceland. The species is recorded from the Bering Sea, the Sea of Okhotsk, the eastern coast of Kamchatka and in the western and northern parts of the Sea of Japan from 0-225m (Kosyan and Kantor 2012).

The shell is variable from narrowly fusiform to widely-oval, small to medium-sized (Kosyan and Kantor 2012). The colour is greyish-pink to brownish. The siphonal canal is narrow to broad and slightly curved to the left. The shell has a spiral sculpture and very thin inconspicuous riblets. The periostracum is thin, easily peeling and lightly-brown coloured (Kosyan and Kantor 2012).

The head is very short and broad, with long thick tentacles. The foot is folded transversely, with narrow propodium and deep propodial groove (Kosyan and Kantor 2012).

Fig. 13: *Plicifusus kroeyeri. Source: gastropods.com*

Except those species of molluscs describe aboved, other species were also collected.
For example *Euspira pallida, Mya truncata* or *Hiatella arctica.*

Second intermediate hosts

Gammarus Fabricius, 1775 is a key genus in the structure and function of the aquatic ecosystems and constitute an important food source for a variety of animals (Costa et al. 2009; Costa and Costa 2000). *Gammarus* is used in ecotoxological research (Clason and Zauke 2000) and are also important model organisms for studying host-parasite interactions (Kostadinova and Mavrodieva 2005; Rolbiecki and Normant 2005) since they are the intermediate hosts of the family Opecoelidae.

Their body is flattened from side to side and they are defined by the presence of three pairs of uropods and usually by having the gnathopods modified to grasp food (Vihtakari 2008). The head carries two pairs of antennae, the stalkless eyes, and the mouthparts (Schram 1986; Barnard and Karaman 1991).

Found species were determined by molecular analysis from samples collected in Svalbard during the field trip in 2014 by Jindra Šíchová her colleagues (Šíchová et al. 2012). *Gammarus setosus* Dementivea, 1931 has a circumpolar distribution so we can find them in nearly all of the world´s oceans. They live in the seafloor in near shore intertidal waters especially beneath loose stones. The ideal water temperature is around 15 °C, but they can survive in temperatures 10 °C above or below this value (Kruschwitz 1978). The size of its body is about 20 to 30 millimetres with juveniles being 2-10 mm long (Šíchová et al. 2012). Their characteristic colour is yellowish to brown. This species has dark kidneys shaped eyes and long *setae* on a telson (Fig. 14). *Gammarus setosus* is more active at night, but can survive most intensities of light that can appear in their nature environment.

Fig. 14: *Gammarus setosus*.. Source: Jindra Šíchová

Their feeding behaviour can be described as opportunistic. They are scavengerous, carnivorous, detritovous or herbivorous (Steele and Steele 1970) and consume animal material that settles in the sea floor. Scavenger activity is important for the study of host-parasite interactions (Kostadinova and Mavrodieva 2005). In few observed cases *Gammarus* species have preyed upon other small, weak or stressed marine organisms.

Gammarus setosus plays an important role in the diet of invertebrates especially fishes and in some cases also whales (Wisenden et al. 1999). They are also eaten by shorebirds. During dives in Petuniabukta were also collected amphipods *Onisimus caricus* Hansen, 1887.

Onisimus caricus has a circum-arctic distribution and lives on soft bottoms of glacial bays and fjords in Svalbard. It is also recorded from the Barents Sea and Canadian Arctic, occurs in depth deeper than 5 metres and is mainly found at 30 to 60 metres depth (Legezynska et al. 2000).

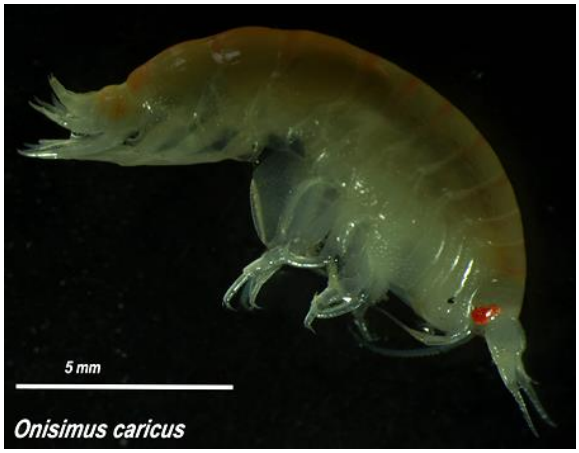

Fig. 15: *Onisimu caricus.* Foto: Jindra Šíchová

The colour of the body is pale pinkish to yellowish, while the eyes are red (Fig. 15).
The dactylus is powerful and hook-like, shorter than palm. The diet of this species consists of dead zooplankton during the melting season (due to osmotic shock when glacial bays are in the melting period), and uses other carrions for the rest of the year (Zajaczkowski and Legezynska 2001; Nygard et al. 2009). Genus *Onisimus* Boeck, 1871 has been reported to be opportunistic scavenger and predator (Murdoch 1885; Dahl 1953).

Fish hosts

Trematodes were found in fish *Myoxocephalus scorpius* (Linnaeus, 1758) and *Gymnocanthus tricuspis* (Reinhardt, 1830) caught to set gill nets.

Myoxocephalus scorpius (Fig. 16) mostly known as the shorthorn sculpin, bull-head, and the father-lasher, is a moderately sized species of scorpion fish occasionally reaching as much as 60 cm in length (Froese and Pauly 2014). The common length is 24 cm SL male/unsexed. The etymology of the name *Myoxocephalus* comes from Greek, myos means muscle and kephale is head (Froese and Pauly 2014). It is a common and widespread benthic fish of the North Atlantic and adjacent subarctic shallow offshore habitats, living on rocky bottoms with sand or mud in depth range 0-451 metres (Froese and Pauly 2014).

They are generalists feeding on fishes (cod, smelts, flounders), large crustaceans (*Hyas, Leander*), occasionally polychaetes or amphipods (Moore and Moore 1974; Norderhaug et al. 2005).

Adults are without swim bladder (typical for *Cottidae* Bonaparte, 1832) (Nelson 2006).

Fig. 16: *Myoxocephalus scorpius* *Source: Tomáš Tyml*

Except *Myoxocephalus scorpius* one other species of sculpins, *Gymnocanthus tricuspis*, was caught during the field trip in Svalbard and infected by trematodes.

The Arctic staghorn sculpin *(Gymnocanthus tricuspis)* has typical sculpin body with large head, wide mouth and large pectoral fin. The etymology of the name is from Greek, gymnos means naked and akantha is thorn.

It is a marine, demersal fish living in a depth range 0-450 m., but it is the most common at depths below 18 m. (Parin et al. 2002). This species has a circumpolar distribution (Froese and Kesner-Rayes 2014) and occurs from the Northwest to Northeast Atlantic (Eastern coasts of Greenland and Iceland), the Arctic, near shores of Norway throughout the Barents Sea to Spitzbergen and Novaya Zemlya (Fedorov 1986).

Common length is about 25 cm. The preferred water temperature is from 1.5 °C to 5 °C, although some sources states -2 °C to 13 °C (Froese and Kesner-Rayes 2014).

This species is distinguishable due to the shape of the first dorsal fin, which is wide and flat with dark and light stripes, at the end of thorn is branched into three short ends (other *Cottidae* have a simple, except *Icelus bicornis* Reinhard, 1840) (Bigelow and Schroeder 1953; Fedorov 1986). The second dorsal fin is soft-rayed (Froese and Kesner-Rayes 2014). The caudal fin is rounded. *Gymnocanthus tricuspis* (Fig. 17) is missing spines at the upper part of the head or the spines are short. The body is almost scaleless and smooth.

This species is brown or grey coloured with dark stripes and light or dark green stains. There is an irregular line of dark spots below the lateral line. The belly is light, yellowish in females, while males have white spots. Adult are without a swim bladder (Nelson 2006).

It is a benthic fish, and burrows into sand and sand-mud bottoms. This species feeds on small benthic amphipods, polychaetes and gastropods (Fedorov 1986).

The spawning season is in fall, producing eggs that are over 2 mm in diameter (Froese and Kesner-Rayes 2014).

Fig. 17: *Gymnocanthus tricuspis.* Source: Tomáš Tyml

Other species of fish were also caught to set fish nets and dissected. For example the Arctic cod, (*Boreogadus saida*), the Atlantic cod (*Gadus morhua*), the Atlantic herring (*Clupea harengus*) or snakeblenny (*Lumpenus lampretaeformis*).

3.3 Experimental infection of intermediate host (Gammarus)

One of our goals during the field trip in Svalbard was to clarify the life history of trematodes of the Opecoelidae family with regard to the earlier unpublished research of Andrea Bednářová. During dissections of collected molluscs (*Buccinum undatum*, *B. glaciale* and *Plicifisus kroeyeri*) we found a large number of sporocysts with cercariae of trematodes of the Opecoelidae family located in their hepatopancreas.

We collected 45 individuals of *Gammarus setosus* (determined by Jindra Šíchová). Fifteen were dissected for the presence of parasites as a control. None were infected. Then we put infected hepatopancreas from *B. undatum* into a plastic bottle with holes inside and placed it along with living *Gammarus* to a littoral zone of the sea. Every day we took 3 individuals from the bottle, dissected and examined them with a microscope for the presence of developmental stages (metacercariae).

3.4 Scanning electron microscopy (SEM)

Samples were processed at the Laboratory of Electron Microscopy in the Biology Centre of ASCR (Academy of Science in Czech Republic) – Institute of Parasitology. The fixed samples were purified for 15 minutes in a washing buffer (3x), then cleaned with 4%

osmium solution, again purified in washing buffer and dehydrated in an acetone series (50%, 70%, 80%, 90%, 100%; for 15 minutes each).

Samples were dried by critical point drying with CO_2 and then stacked to targets and gilded. Targets were imaged using a SEM JOEL 740-F.

We measured the length of samples, width at the widest part of the worm body and distance between the oral and ventral suckers, as well as calculating the ratio between the distance of the suckers and length of the body. These data were used for calculation of the prevalence of *P. atomon* in *M. scorpius* and *G. tricuspis*. In some images, SEM length data were missing so these data were assessed using the ImageJ (Fig. 18) program for Windows 7.

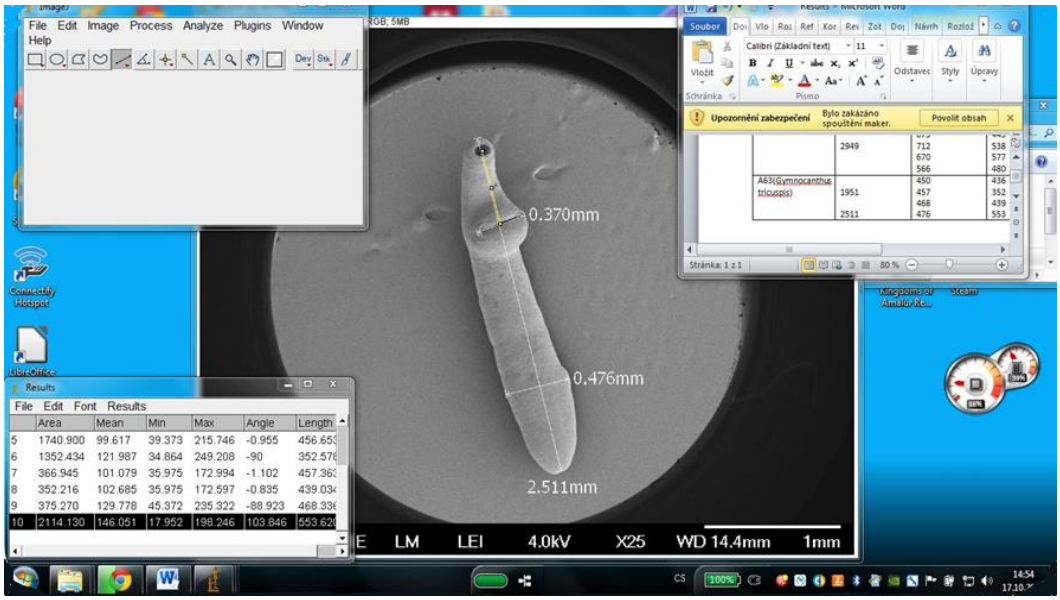

Fig. 18: ImageJ used for measures of length and width.

3.5 Carmine staining

The collected Opecoelids were prepared for morphological and morphometric comparison. The fixed digeneans were transfered into 70% ethanol. The specimens for morphological study were stained for several hours with Schuberg hydrochloric carmine solution, distained in acid ethanol (70% ethanol with HCl), dehydrated in an alcohol series (80%, 90%, 96%, twice 100%; for 15 minutes at each) and mounted as permanent slides in Canadian balsam. These steps were recommend by the Laboratory of Helminthology.

3.6 Microscopy and drawing

An Olympus BX53 microscope (max. magnification 100x) with an optical device *camera lucida* (Fig. 19), was used for examining and drawing of the stained and fixed samples, so we could draw trematodes of the family Opecoelidae for illustrations of their main

morphological features. Measurement of morphological structures was done by QuickPHOTO MICRO 2.3. For samples suitable for morphological analyses we measured: length, distance between the oral and ventral suckers and noted the position of the testes and follicles in the body of the fluke. The data obtained were put together with data from SEM and processed by basic statistical analyses.

3.7 Data processing

From data collected from the field trip we calculated mean standard length (SL) and mean height of shells of trapped uninfected/infected intermediate hosts and final hosts.

Data of length, width and distance between suckers of trematodes were divided by hosts, where they were found and after determination of the species of trematodes we were able to calculate the prevalence of *Podocotyle* species in the final hosts. The measured data were processed via MATLAB and Statistica 12. Equal groups from the final hosts were compared (length x length, width x width and distance between suckers x distance between suckers) and the measured data were visualized in 3D.

Based on the ratio of body length and the distance between suckers we determined the species of flukes found in *M. scorpius* and *G. tricuspis*. If the ratio was between 0.306 to 0.196, the fluke was classified as *Podocotyle atomon* and if the ratio was between 0.197 to 0.090, the fluke was classified as *Podocotyle reflexa*. The border value was half of the distance between 1/4 and 1/7.

Fig. 19: Drawing and measurement with BX53 with optical device DP 72 and *camera lucida*.

Samples were displayed in 3D model and results were displayed by a dendrogram. The distance between clusters of samples was computed using Euclidean distance

4 Results

Our records, pictures from light microscopy and SEM, measurements, statistics and visualizations of the data are presented here.

4.1 Records from dissections

In 2013 we dissected 136 vertebrates and 99 invertebrates (Tabs. 1, 2), caught to set fish nets and collected during scuba dives.

Table 1: Records from dissections of vertebrates caught to fish nets.

Dissected vertebrates during field trip in Svalbard 2013	
Name	**Number of dissected individuals**
Black quillemot (*Cepphus grylle*)	1
Shorthorn sculpin (*Myoxocephalus scorpius*)	48
Arctic staghorn sculpin (*Gymnocanthus tricuspis*)	13
Arctic cod (*Boreogadus saida*)	23
Atlantic herring (*Clupea harengus*)	22
Atlantic cod (*Gadus morhua*)	8
American plaice (*Hippoglossoides platessoides*)	2
Capelin (*Mallotus villosus*)	6
Snakeblenny (*Lumpenus lampretaeformis*)	13

Together we have dissected 136 vertebrates including black quillemot (*Cepphus grylle*). Most of the dissected fish were *Myoxocephalus scorpius*, *Boreogadus saida* and *Clupea harengus*. Only two individuals of *Hippoglossoides platessoides* were dissected.

Table 2: Records from dissections of invertebrates collected during scuba dives.

Dissected invertebrates during field trip in Svalbard 2013	
Name	**Number of dissected individuals**
Buccinum undatum	45
Buccinum glaciale	18
Plicifusus kroeyeri	6
Euspira pallida	1
Serripes groenlandicus	2
Mya truncata	16
Hiatella arctica	10
Chlamys islandica	1

Most of the dissected molluscs were *Buccinum undatum*, *Buccinum glaciale* and *Mya truncata*.

In 2013, forty eight individuals of *Myoxocephalus scorpius* (23 males, 25 females) and 13 individuals of *Gymnocanthus tricuspis* (1 male, 12 females) were dissected. The data were divided by sex, recorded maximum and minimum standard length (SL), calculated mean SL, mean SL of infected and uninfected specimens, the calculated prevalence of *Podocotyle atomon* and calculated mean intensity of infection.

Table 3: Records from dissections of final hosts.

Occurrence of *Podocotyle atomon* in *Myoxocephalus scorpius*								
Host	**No. Dissected Fish**	**SL mean/max/min (mm)**			**Mean SL of infected (mm)**	**Mean SL of uninfected (mm)**	**Prevalence (%)**	**Mean intensity of infection**
M. scorpius males	23	152,6	185	110	154,5	160,5	39,1	8,2
M. scorpius females	25	169,7	200	122	168,1	170,1	48	2,6
Total	48	161,5	200	110	162,3	160	43,7	5,5

The mean SL of infected females is higher than mean SL of infected males and the prevalence of *Podocotyle atomon* is higher in females than in males. Also, the mean SL of uninfected females is higher than the SL of uninfected males by almost 10 mm (Table 3).

The mean intensity of infection is more than 3 times higher in males than in females.

The smallest fish was a male with 110 mm and the biggest fish was a female with 200 mm.

The overall mean SL of infected individuals of *M. scorpius* is greater than the mean SL of uninfected individuals of *M. scorpius.*

The records of dissected *G. tricuspis* were divided by sex, from which were recorded maximum and minimum SL, calculated mean SL, mean SL of infected and uninfected, calculated prevalence of *Podocotyle atomon* and calculated mean intensity of infection (Table 4).

Table 4: Records from dissections of final hosts.

Occurrence of *Podocotyle atomon* in *Gymnocanthus tricuspis*								
Host	**Number of Dissected Fish**	**SL mean/max/min (mm)**			**Mean SL of infected (mm)**	**Mean SL of uninfected (mm)**	**Prevalence (%)**	**Mean intensity of infection**
G. tricuspis males	1	136	136	136	136	0	100	2
G. tricuspis females	12	163,4	210	115	171,6	160,6	25	3
Total	13	161,3	210	115	162,7	160,6	30,7	2,75

Only one male of *G. tricuspis* was dissected and infected. The mean SL of infected females is higher than the SL of uninfected females by 10 mm. A quarter of females were infected by trematodes of the family Opecoelidae. Two trematodes were found in the dissected male. Overall the mean SL of infected *G. tricuspis* is longer than the mean SL of uninfected ones (Table 4).

Results of the dissections of the intermediate hosts *Buccinum undatum*, *Buccinum glaciale* and *Plicifusus kroeyeri* found in Petuniabukta are shown in the table (Tab. 5). We noted maximum and minimum height of shells and calculated the mean height of infected and uninfected molluscs.

Table 5: Records from dissections of *Buccinum undatum, Buccinum glaciale* and *Plicifusus kroeyeri* from Petuniabukta.

Occurrence of *Podocotyle atomon* in intermediate hosts							
Host	**Number of dissected molluscs**	**Height of shell mean/max/min (mm)**			**Prevalence (%)**	**Mean height of shell of infected (mm)**	**Mean height of shell of uninfected (mm)**
B. undatum	45	47,3	74	31	24%	52,3	45,7
B. glaciale	18	46,3	64	21	27,7%	45,6	46,6
P. kroeyeri	3	42	48	32	33,3%	46	40
Total	66	X	X	X	X	X	X

Forty five individuals of *Buccinum undatum*, 18 individuals of *Buccinum glaciale* and three individuals of *Plicifusus kroeyeri* were dissected. The largest measured shell of *B. undatum* was 74 mm high and the largest measured shell of *B. glacial* was 64 mm high.
The prevalence of *Podocotyle atomon* was 24 % in *B. undatum*, 27.7 % in *B. glaciale* and 33.3% in *Plicifusus kroeyeri.*

Shells of infected individuals of *B. undatum* and *P. kroeyeri* were higher than uninfected ones. On the other hand, uninfected individuals of *B. glaciale* were higher than infected ones (Table 5).

Results of carmine staining and scanning electron microscopy are shown (Figs. 20, 21).

4.2 Adults of *Podocotyle* stained with Schuberg hydrochloric carmine

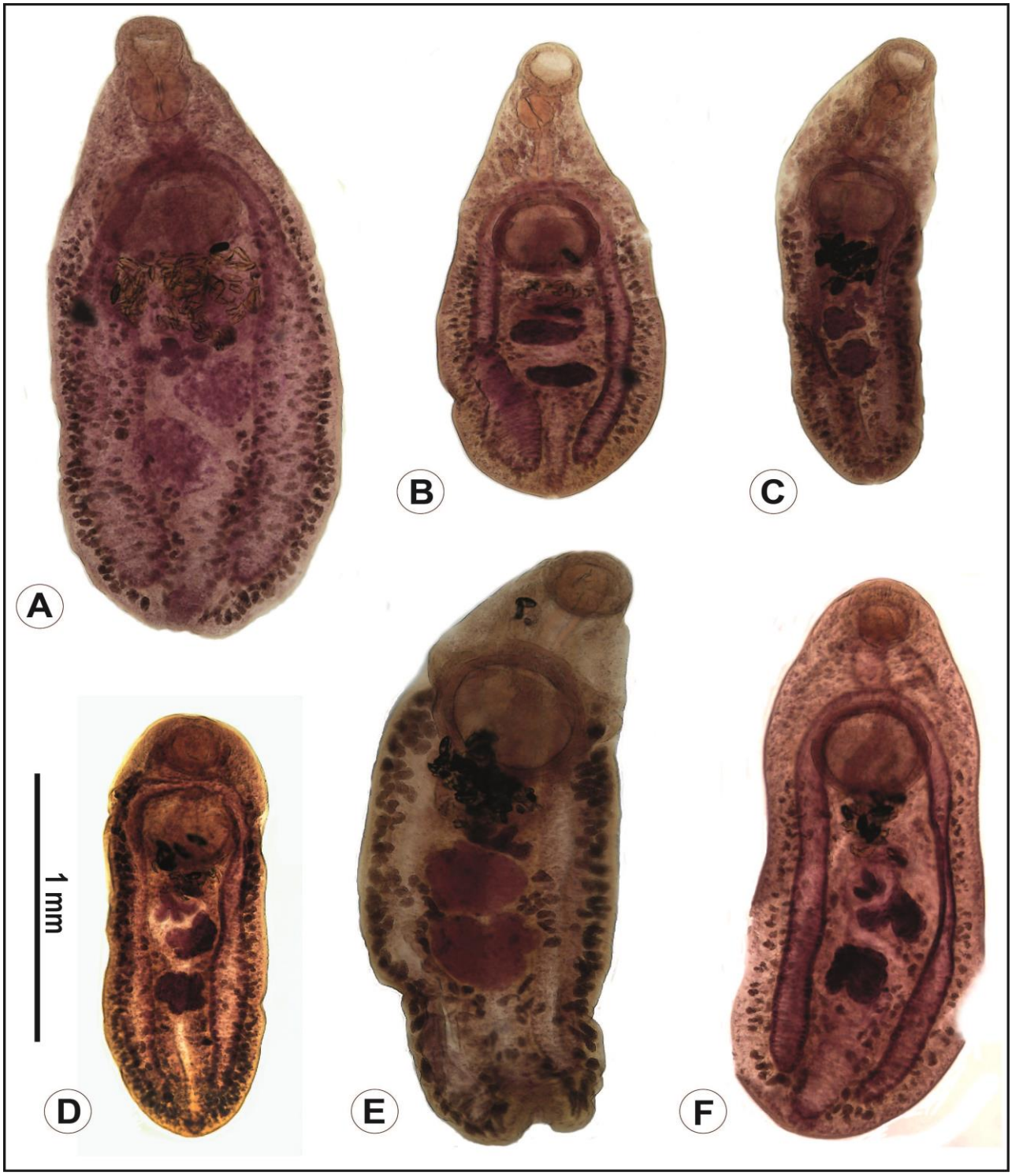

Fig. 20: Pictures of carmine stained *Podocotyle atomon*. **A-C** were found in *Myoxocephalus scorpius*. **D-E** were found in *Gymnocanthus tricuspis*.

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Scanning electron micrograph of *Podocotyle atomon* from *Myoxocephalus scorpius* (Fig. 21)

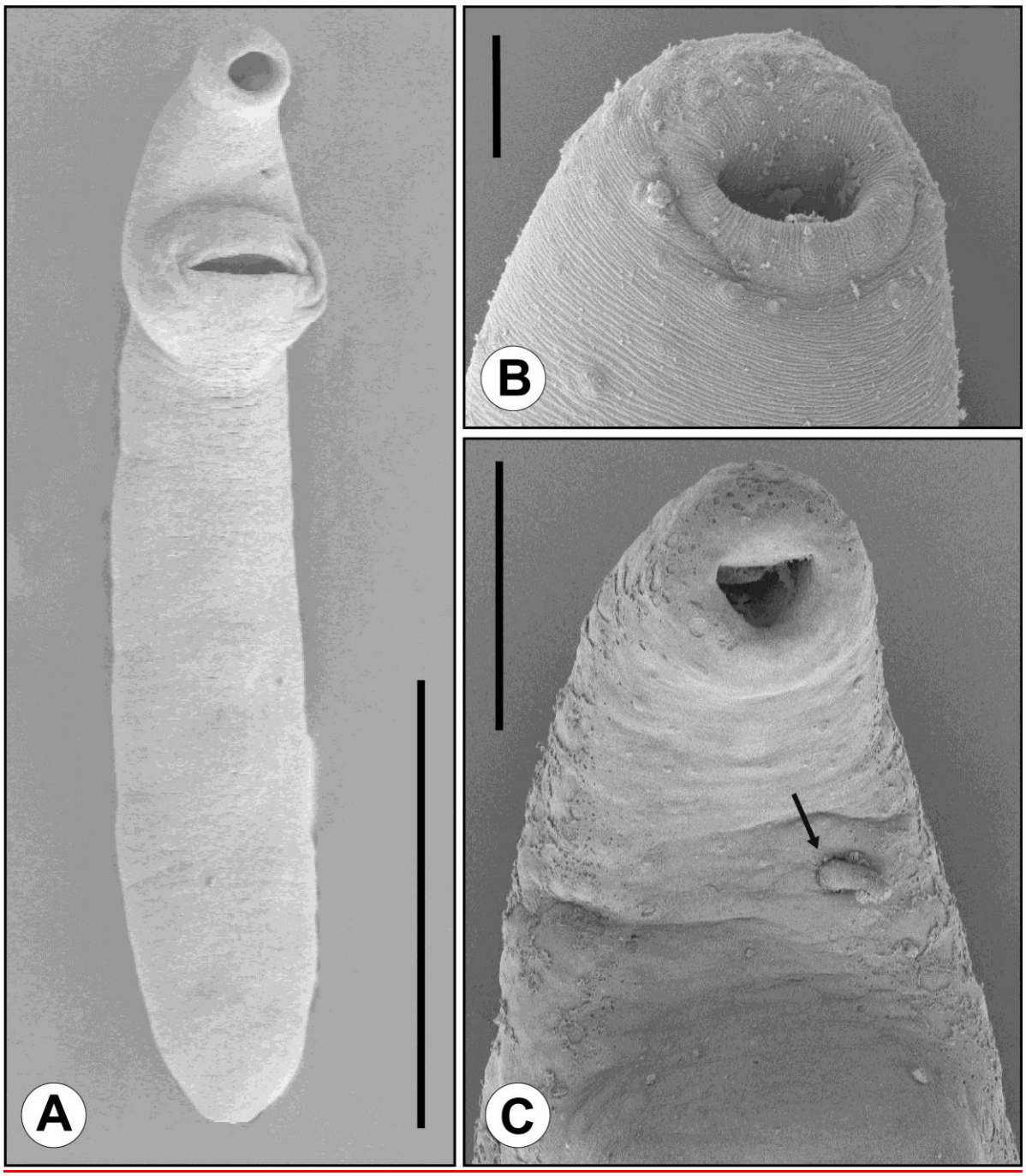

Fig. 21: A - Adult of *Podocotyle atomon* found in *Myoxocephalus scorpius* (scale is 1 mm). **B -** Detail of the surface of the oral sucker (scale is 25 μm). **C** – Ventral view with penis (scale is 400 μm).

4.3 Measured data from carmine stained samples and SEM

Table 6: Measured data from the carmine stained samples and SEM of *Podocotyle atomon* found in the final hosts.

Host	No. of Trematode	Sample	Length (µm)	Width (µm)	Distance between suckers (µm)	Length/Distance between suckers ratio	Species
Myoxocephalus scorpius	1	A55	1204	393	300	0,249	*P. atomon*
	2	A55	1484	401	346	0,233	*P. atomon*
	3	A55	1246	292	448	0,360	*P. atomon*
	4	A55	1425	466	376	0,264	*P. atomon*
	5	A366	2109	815	451	0,214	*P. atomon*
	6	A366	2255	612	555	0,246	*P. atomon*
	7	A366	2390	624	493	0,206	*P. atomon*
	8	A366	2450	465	589	0,240	*P. atomon*
	9	A366	2141	620	506	0,236	*P. atomon*
	10	A366	2701	510	624	0,231	*P. atomon*
	11	A366	1764	678	395	0,224	*P. atomon*
	12	A366	2762	759	590	0,214	*P. atomon*
	13	A366	2446	603	517	0,211	*P. atomon*
	14	A366	2648	781	573	0,216	*P. atomon*
	15	A366	2579	801	603	0,234	*P. atomon*
	16	A366	2413	513	820	0,340	*P. atomon*
	17	E17	2459	440	601	0,244	*P. atomon*
	18	E17	2508	502	593	0,236	*P. atomon*
	19	E17	2650	510	688	0,260	*P. atomon*
	20	E17	2186	439	605	0,277	*P. atomon*
	21	E17	2499	715	730	0,292	*P. atomon*
	22	E17	2355	530	619	0,263	*P. atomon*
	23	E18	2133	598	470	0,220	*P. atomon*
	24	E18	1343	402	320	0,238	*P. atomon*
	25	E19	1730	602	451	0,261	*P. atomon*
	26	E20	1420	458	378	0,266	*P. atomon*
	27	E21	2058	715	561	0,273	*P. atomon*
	28	E22	2487	421	548	0,220	*P. atomon*
	29	E22	1487	449	400	0,269	*P. atomon*
	30	E27	1198	278	342	0,285	*P. atomon*
	31	E29	1977	721	503	0,254	*P. atomon*
	32	E29	2639	605	748	0,283	*P. atomon*
	33	E30	2549	487	622	0,244	*P. atomon*
	34	E31	1855	659	487	0,263	*P. atomon*
	35	E32	1872	674	454	0,243	*P. atomon*

The first 17 trematodes were measured by SEM, while the other 18 trematodes were measured by carmine staining.

Table 7: Measured data from carmine stained samples and SEM of *Podocotyle atomon* found in the final hosts.

Host	No. of Trematode	Sample	Length (µm)	Width (µm)	Distance between suckers (µm)	Length/Distance between suckers ratio	Species
Gymnocanthus tricuspis	36	A58	3263	818	670	0,205	*P. atomon*
	37	A59	2200	595	652	0,296	*P. atomon*
	38	A59	2547	694	537	0,211	*P. atomon*
	39	A60	2350	488	530	0,226	*P. atomon*
	40	A60	2586	690	747	0,289	*P. atomon*
	41	A61	2465	695	600	0,243	*P. atomon*
	42	A61	1890	673	443	0,234	*P. atomon*
	43	A61	2949	712	680	0,231	*P. atomon*
	44	A62	2560	670	577	0,225	*P. atomon*
	45	A62	2733	606	578	0,211	*P. atomon*
	46	A62	2240	450	558	0,249	*P. atomon*
	47	A62	1951	457	411	0,211	*P. atomon*
	48	A62	2132	468	539	0,253	*P. atomon*
	49	A62	2511	476	553	0,220	*P. atomon*
	50	A62	2419	424	578	0,239	*P. atomon*
	51	A63	2460	518	622	0,253	*P. atomon*
	52	A63	2128	482	461	0,217	*P. atomon*
	53	A63	1783	446	375	0,210	*P. atomon*
	54	A63	1950	515	506	0,259	*P. atomon*
	55	A63	2294	413	562	0,245	*P. atomon*
	56	A63	2100	554	509	0,242	*P. atomon*
	57	A63	2520	423	564	0,224	*P. atomon*
	58	A63	1790	525	397	0,222	*P. atomon*
	59	A63	1956	454	483	0,247	*P. atomon*
	60	A63	2256	466	612	0,271	*P. atomon*
	61	A63	2659	614	565	0,212	*P. atomon*
	62	A63	2029	325	571	0,281	*P. atomon*
	63	A63	1986	680	509	0,256	*P. atomon*
	64	A63	2612	752	573	0,219	*P. atomon*
	65	A69	2078	524	529	0,255	*P. atomon*
	66	A69	2740	589	658	0,240	*P. atomon*
	67	A69	2180	564	563	0,258	*P. atomon*
	68	A70	2556	646	725	0,284	*P. atomon*
	69	A70	2012	495	558	0,277	*P. atomon*
	70	A70	2719	694	724	0,266	*P. atomon*

The first 19 trematodes were measured by SEM, while the other 16 trematodes were measured by carmine staining.

The species of flukes found in *M. scorpius* and *G. tricuspis* were determined based on the ratio of length and distance between suckers (Tabs. 6, 7). If the ratio was between 0.306 to 0.196, the fluke was classified as *Podocotly atomon* and if the ratio was between values 0.197 to 0.090, fluke was classified as *Podocotyle reflexa*. The border value was half of the distance between 1/4 and 1/7 (Figs. 22, 23).

We were interested in the length and width of the trematodes and distance between suckers (Table 8).

Table 8: Measured data of *Podocotyle atomon* in *Myoxocephalus scorpius* and *Gymnocanthus tricuspis* from SEM and measured data of carmine stained adults.

Measured data of length, width and distance between suckers of *P. atomon* from SEM and carmine staining					
Host	**No. of trematodes**	**Species**	**Length (µm) min – max (mean)**	**Width (µm) min – max (mean)**	**Distance between suckers (µm) min – max (mean)**
M. scorpius	35	*P. atomon*	1204 – 2946 (2097)	292 – 815 (558)	300 – 820 (519)
G. tricuspis	35	*P. atomon*	1955 – 3263 (2331)	325 – 818 (560)	365 – 747 (537)

For better visualization, we displayed the results and the individual ratios of each individual (Figs. 22, 23, 25).

Ratios of body length to distance between the suckers of each measured individual that I found, were compared with the ratio of body length and distance between the suckers of *P. atomon* and *P. reflexa* listed by Brinkmann (1975) (Figs. 22, 23).

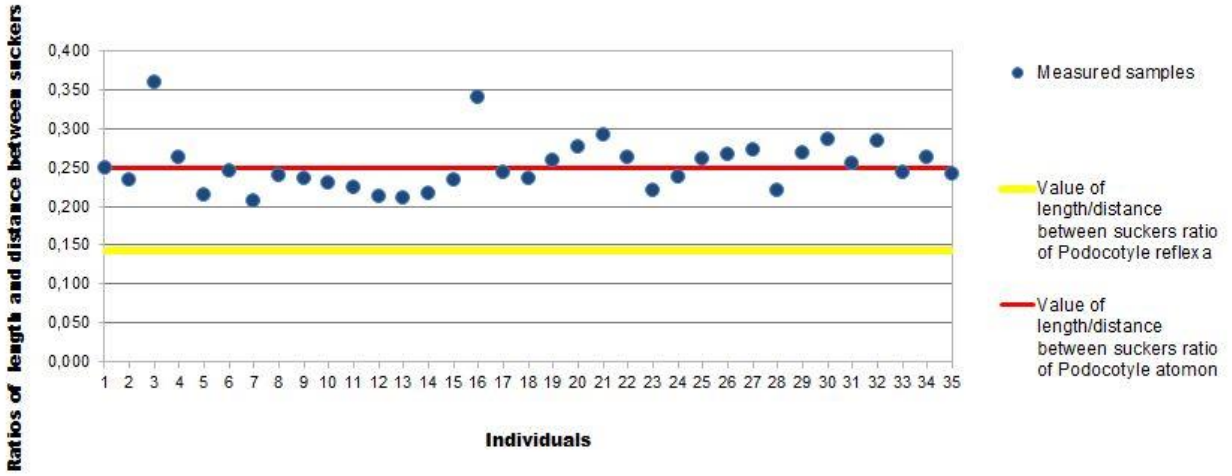

Fig. 22: Length and distance between suckers ratio of *Podocotyle atomon* from *Myoxocephalus scorpius* with ratios of *P. atomon* and *P. reflexa* listed by Brinkmann (1975).

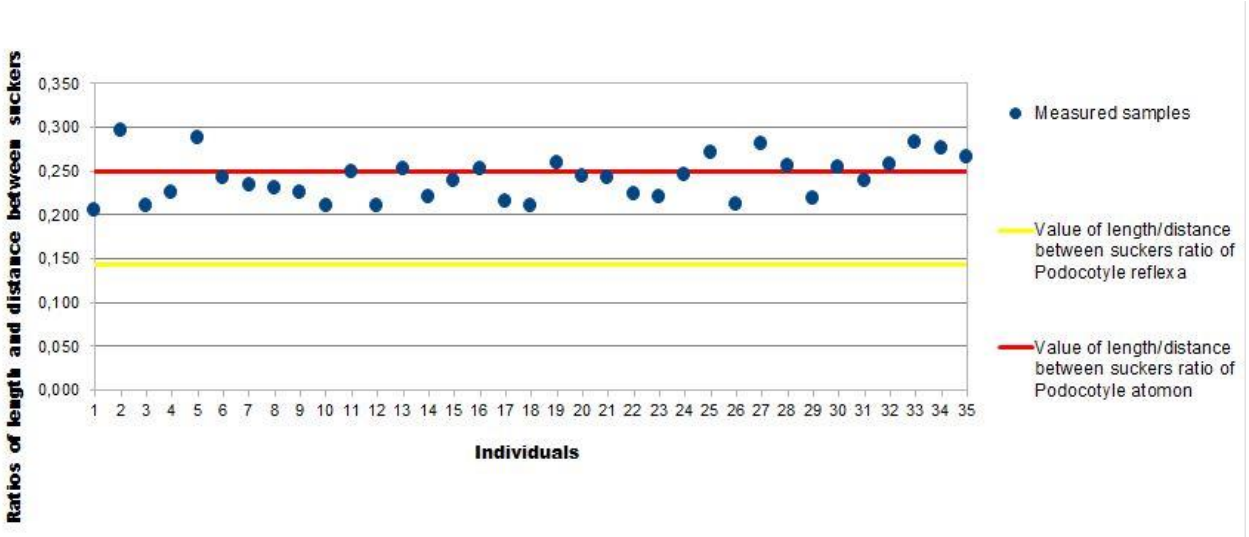

Fig. 23: Length and distance between suckers ratio of *Podocotyle atomon* from *Gymnocanthus tricuspis* with ratios of *P. atomon* and *P. reflexa* listed by Brinkmann (1975).

The measured data were displayed via Matlab and processed by Statistica 12 for Windows 7. A dendrogram (Fig. 24) represents each step of a cluster analysis centred by PCA (Principal Component Analysis) (Fig. 25).

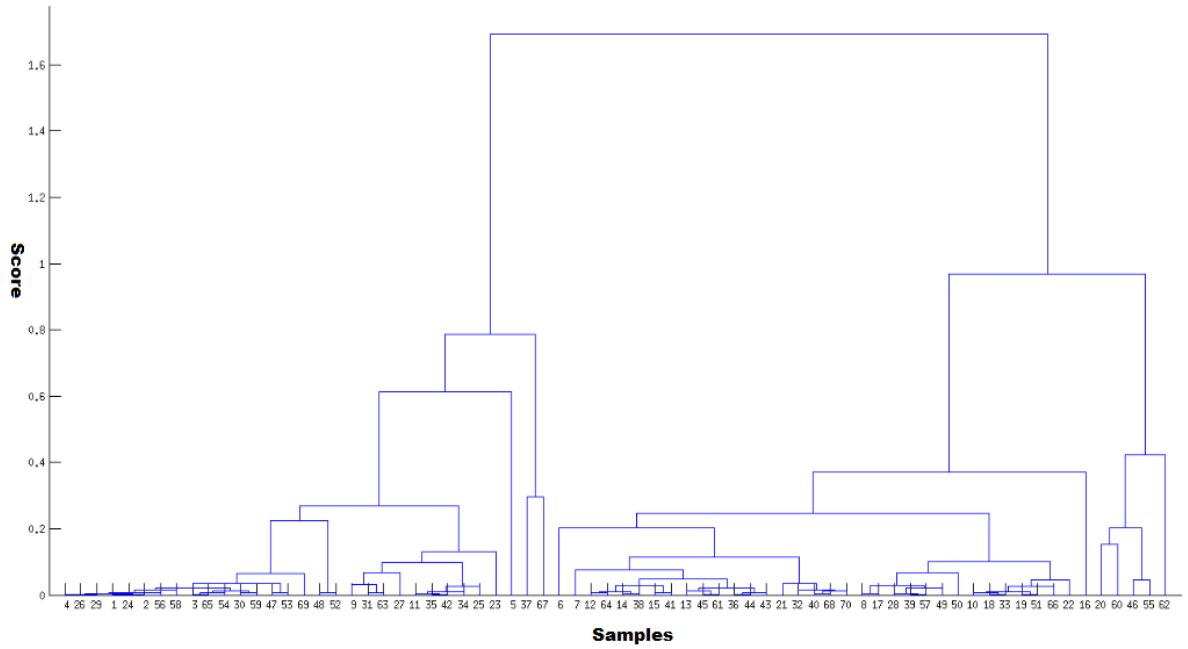

Fig. 24: Dendrogram showing the hierarchical clustering of samples, where each sample is represented in 3D space by length, width and distance between suckers.

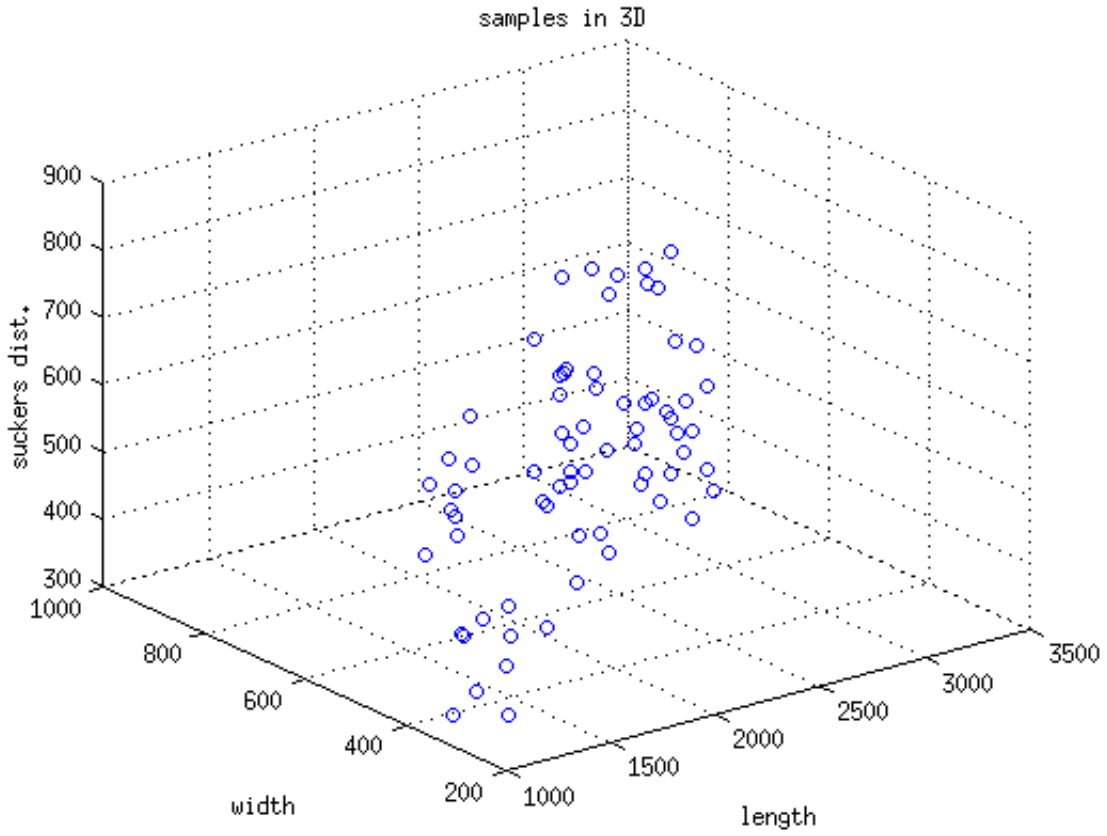

Fig. 25: 3D model of the measured data of length, width and distance of suckers (µm) of trematodes from *M. scorpius* and *G. tricuspis* after PCA.

The measured data of SEM and carmine stained adults (Tabs. 6, 7) were processed via Statistica 12 (Fig. 26). We applied Student´s t-test, independent samples by variables.

The null hypothesis (H_0) was: The measured data are not significantly different.

Measured data of flukes found in *Myoxocephalus scorpius* vs. Measured data of flukes found in *Gymnocanthus tricuspis*	Value of t	df	p	No. of groups 1	No. of groups 2	Standard deviation Gr. 1	Standard deviation Gr. 2	F-ratio variances	p variances
Length vs. Length	-2,3043	68	**0,02426552**	35	35	491,7636	344,0734	2,04273	0,040754
Width vs. Width	-0,0528	68	**0,95807022**	35	35	140,8842	116,1366	1,47159	0,264980
Distance between suckers vs. Distance between suckers	-1,2712	68	**0,20798655**	35	35	125,0312	94,5733	1,74783	0,108325

Fig. 26: Results of Student´s t-test applied to measured data of length, width and distance of suckers (µm) of trematodes from *M. scorpius* and *G. tricuspis.* The significance level of the test was set to: 0.05.

In case of width vs. width and distance between suckers vs. distance between suckers, was $p > 0.05$. In case of length vs. length, p-value was smaller than 0.05.

The null hypothesis was rejected in case of comparison measured data of length from *M. scorpius* and data of length from *G. tricuspis*.

The null hypothesis (H_0) cannot be rejected in case of comparisons of measured ratios and widths.

4.4 Drawings of adults *Podocotyle* from *Myoxocephalus scorpius* and *Gymnocanthus tricuspis* according to stained adults

Twenty individuals from *G. tricuspis* and 20 individuals from *M. scorpius* were stained and examined with a microscope. Figure 27 shows the positions and shapes of the oral sucker and acetabulum (OS, AC); pharynx (PH); cephalic glands (CG); oesophagus (EP); cirrus sac (CS); uterus (UT); ovaries and testes (OV, TE); vitellaria (VIT); intestinal caecum (IN); excretory bladder (EV),

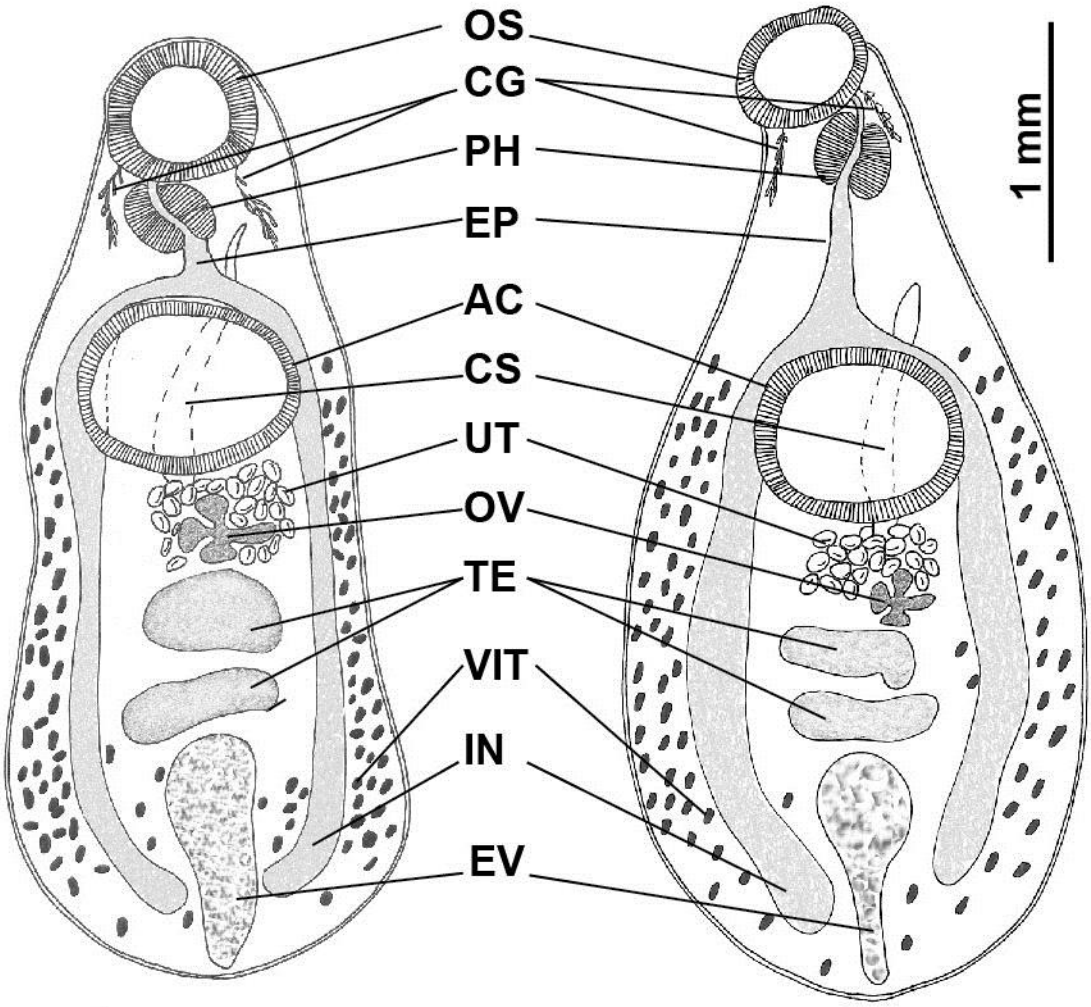

Fig. 27: Drawings of *P. atomon* found in *M. scorpius* and *G. tricuspis.*

In stained adults, the genital pore was not found, but the genital pore is always in the forebody, anywhere from median to marginal, and anywhere from the oral sucker to the anterior margin of the ventral sucker.

4.5 Observation of living sporocysts, cercariae and metacercariae

Daughter sporocysts (Fig. 28) full of cercariae were found in *Plicifusus kroeyeri* during the dissection of molluscs of *Buccinum undatum, B. glaciale* and *Plicifusus kroeyeri*.

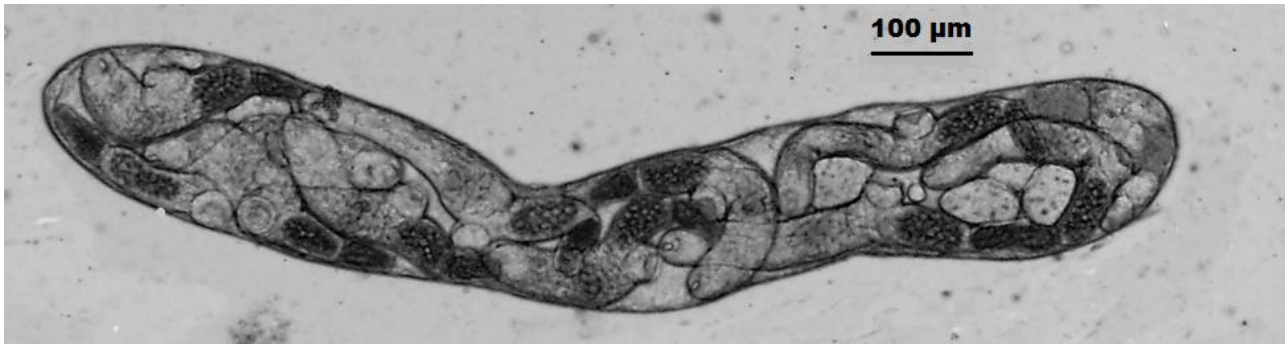

Fig. 28: Sporocyst of *Podocotyle atomon* from the digestive gland of *Plicifusus kroeyeri,* containing germ balls, immature and mature cercariae.

Cercariae (Fig. 29) were found during the dissection of molluscs of *Buccinum undatum, B. glaciale* and *Plicifusus kroeyeri*.

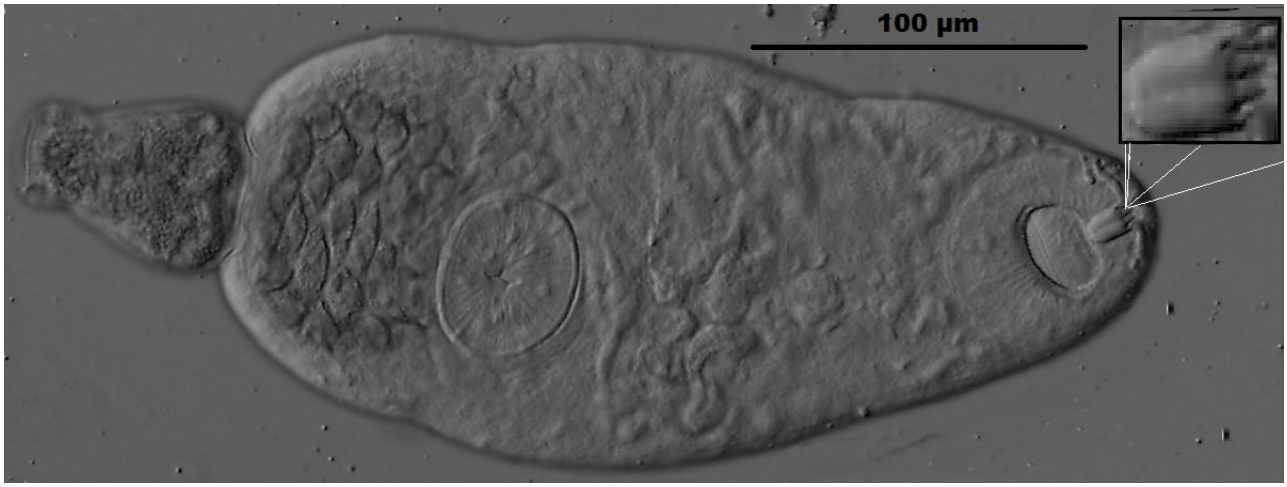

Fig. 29: Living cercaria found in *Plicifusus kroeyeri*, with well-developed oral and ventral suckers and a stylet embedded in the oral sucker.

Cotylomicrocercouse, xiphidocercous cercaria is 347 µm long and 129 µm wide. The double pointed stylet is 15 µm long and 11 µm wide. The stylet is found in the stylet pocket, and only the distal ends of the two points protrude above the surface (detail figure of the stylet). Cercaria lacks eye spots.

Infected amphipods were dissected every day since experimental infection, when amphipods were exposure to infected hepatopancreas of *Buccinum undatum.* Durring these dissections of *Gammarus setosus* metacercariae were found (Figs. 30, 31).

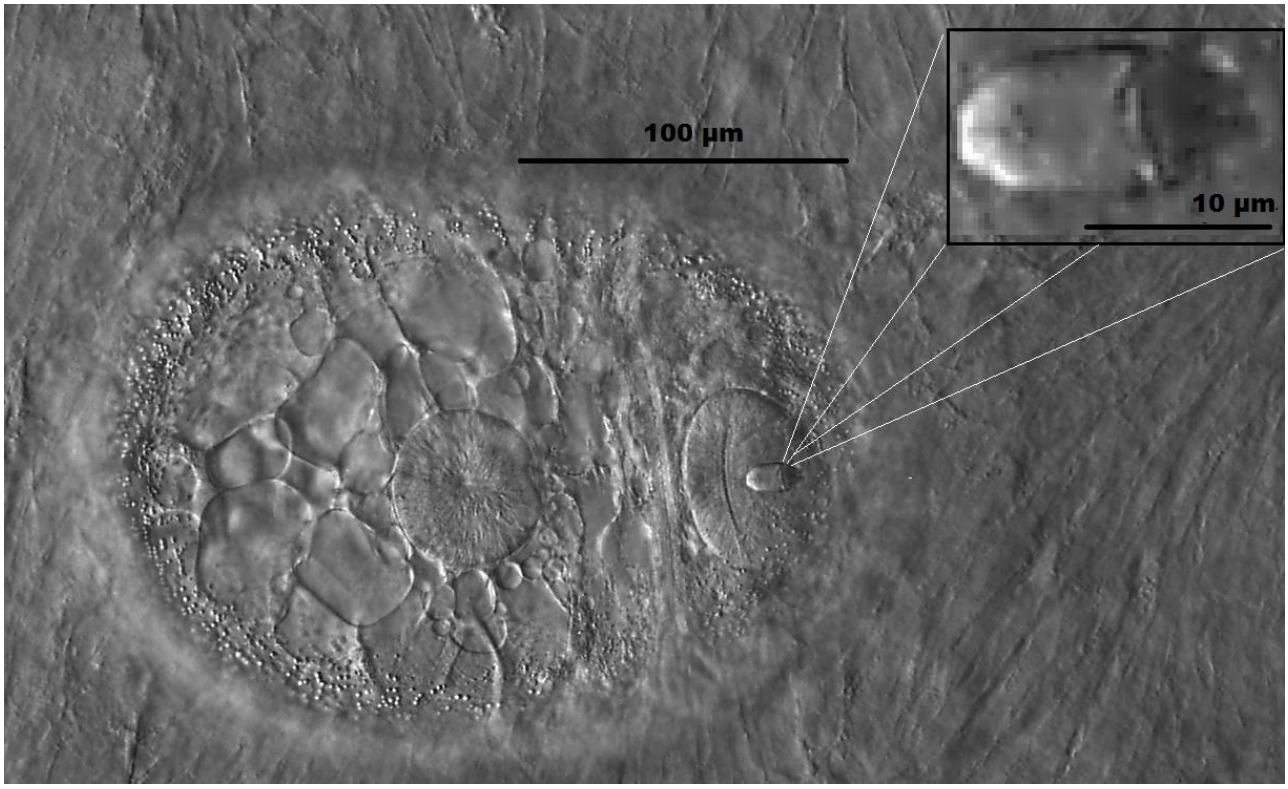

Fig. 30: Metacercaria found in *Gammarus setosus* 72 hours after experimental infection.

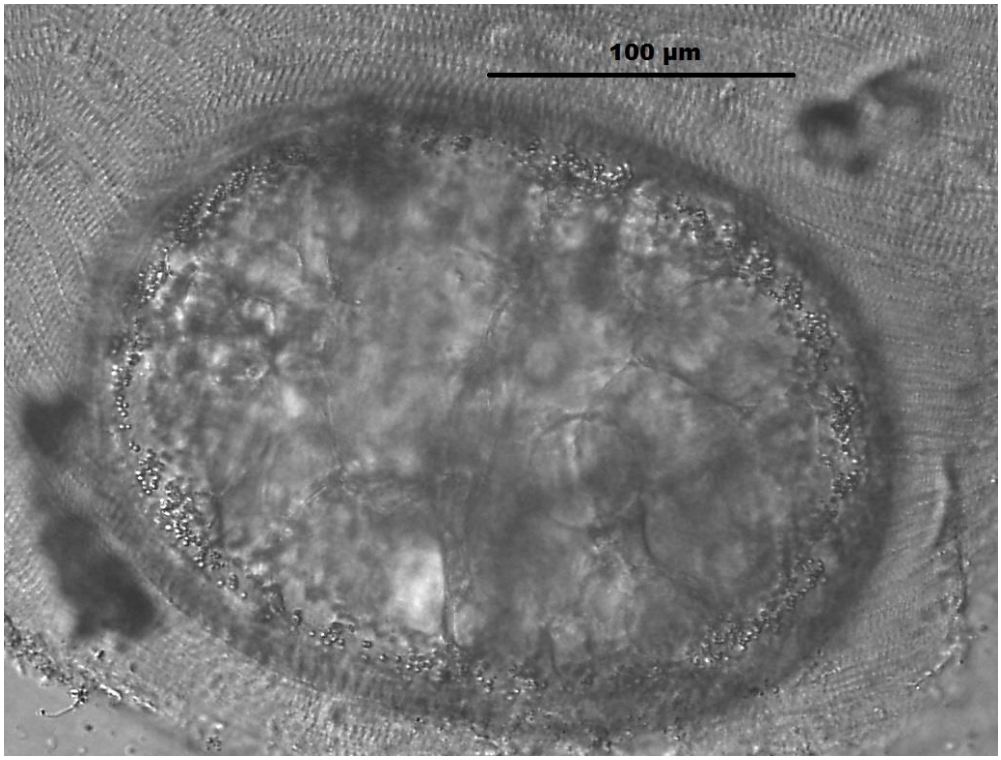

Fig. 31: Metacercaria found in *Gammarus setosus* 144 hours after experimental infection.

5 Discussion

Results of SEM and carmine staining together with morphological descriptions of the family Opecoelidae by Yamaguti (1971), Køie (1981), Cribb (2005) and Galanktinov and Dobrovolskij (2003) confirmed that the trematodes found in *Myoxocephalus scorpius* and *Gymnocanthus tricuspis* were from the family Opecoelidae and genus *Podocotyle*.

Since *Podocotyle atomon* and *Podocotyle reflexa* have parallel life histories (Køie 1981; Hunninen and Cable 1943), the studies of Brinkmann (1975) and Køie (1981) suggests that sexual adults can be distinguished by the shape of the body, the ratios between the length of an individual and distance between the suckers, size and position of the testes and vitellaria. However, Køie (1981) showed that these characters can be very variable.

The measured data of the body shape, length of the body and distance between suckers from carmine stained individuals and from SEM (Tabs. 4, 5) put together with the listed differences, confirmed that all found flukes in *M. scorpius* and *G. tricuspis* are individuals of *Podocotyle atomon*. A disadvantage of this method of distinguishing based on the ratios of length and distance between suckers could be the border area between ratios, when measures based on ratios could be counted to either *P. atomon* or *P. reflexa.* We set the decision threshold as half of the distance between 1/4 and 1/7 as listed by Brinkmann (1975), but we have no evidence for confirming the correctness of this value.

Another disadvantage is the inaccuracy of measurements of length and distance between suckers in SEM, because some flukes were bent after the process of preparation for SEM bended towards the observer and it may cause inaccuracies in the measurements that may lead to a different ratio of length and distance between the suckers and hence to a wrong determination of the species. Carmine staining and fixation to Canadian balsam is a much more suitable method for morphological comparisons.

The results of the Student´s t-test independent samples by variables could disprove the null hypothesis, that the measured data were not significantly different in case of the comparison of measured lengths, but we cannot reject the null hypothesis in case of other measured data including comparison of widths and distances between suckers from both final hosts (Fig. 26). Partial components analyses (PCA) was also applied, together with cluster analyses. These methods showed a certain possible distribution, but the differences between the two hosts were still inconclusive. Even if they were conclusive, it would not be evidence that found specimens were two different species of parasite. It could be that the different hosts are acting on the morphology of the parasite in various of ways.

With higher number of representative samples and more balanced data of trematodes from the final hosts, tests could be more conclusive. For a better understanding of the results, we displayed the measured data via a dendrogram and in a 3D model (Figs. 24, 25).

Køie (1981) recorded that the testes of *P. atomon* are relatively small, not occupying more than half of the cross-section, whereas those of *P. reflexa* are large, occupying the greater part of the body in cross-section. Also, the vitellaria of *P. atomon* are unbroken and do not come together between the testes, whereas the vitellaria of *P. reflexa* are broken and come together to each testis. In carmine stained individuals from Svalbard we did not find any differences that could lead to distinguishing between the two species. These findings (Fig. 27) are consistent with those findings of Køie (1981) and confirmed that found flukes are only species *Podocotyle atomon*.

The dissections showed that infected individuals of *B. undatum* had greater mean height of shells than uninfected ones. It is known that parasites alter the distribution and abundance of their hosts through changes in behaviour, morphology and life history (Dobson 1988; Moore and Gotelli 1990; Moore 1995, 2002).

One likely explanation is that older, thus larger individuals had more time to be infected by miracidium. Another option is the fact that digeneans typically seriously affect the health of their first intermediate host (in our case *B. undatum*) (Cribb 2005) and infected individuals can be castrated, which can lead to parasitic gigantism, where the infected individuals grows larger than uninfected ones. That could explain the outputs of the dissections of *B. undatum* (Tab. 5). Uspenkaya (1963) noted that the reproductive system of the metacercariae of *P. reflexa* is undeveloped, whereas metacercariae of *P. atomon* may contain several eggs. Our observed metacercariae from experimentally infected amphipods were too young for recognition of these structures.

Previous fieldtrips to Svalbard showed the presence of sporocysts and cercariae of *Podocotyle* in *Buccinum undatum, B. glaciale, Plicifusus kroeyeri* and the presence of adults in *Myoxocephalus scorpius* and *Gymnocanthus tricuspis* (Figs. 28, 29).

Experimental infection should verify the connection between the presence of cercariae in molluscs and mature individuals of *Podocotyle* in the fish hosts. Cercaria behaves in one of seven distinct behaviours (Cribb et al. 2003) that lead to the infection of the definitive vertebrate host. The cercaria morphology is related to its infection behaviour.

The ways of cercaria behaviour in which leads to infection of the definitive host are as follows (Cribb et al. 2003; Cribb 2005).

1. The cercaria may penetrate the definitive host directly (occurs only and in all blood flukes (Ogawa et al. 1994)
2. The cercaria is eaten directly by the definitive host.
3. The cercaria attaches to the surface of the definitive host.
4. The cercaria is eaten by a second intermediate host, and then frequently penetrates the gut. A metacercaria forms and waits for the definitive host to eat the second intermediate host.
5. The cercaria emerges from the mollusc, encysts in the open as a metacercaria on a potential food source of the definitive host and wait to be eaten.
6. The cercaria emerges from the mollusc and externally penetrates a second intermediate host. A metacercaria forms and waits for the definitive host to eat the second intermediate host (most common life cycle in the Digenea).
7. The cercaria remains in the first intermediate host, which is eaten directly by the definitive host.

Ditrich and Bednářová (Ditrich, personal communication) tried to clarify the shedding of cercariae when they kept in captivity infected individuals *B. undatum* in different conditions of water (salinity, depth, solar radiation, temperature) that could influence the emergence from the snail and during this experiment no cercaria of *P. atomon* left the mollusc *B. undatum* to water.

Successful infection a of second intermediate host (*Gammarus)* by ingestion of an infected hepatopancreas proved by metacercariae found during dissection of second intermediate hosts (*Gammarus*) confirmed the idea that cercariae are affecting fitness of first intermediate host (*B. undatum, B. glaciale, and P. kroeyeri)* and do not leave the first intermediate host to open space. Køie (1981) also found most infected *B. undatum* seriously affected by the parasite. Køie (1981) recorded, that other infected individuals of *Neptunea antiqua* from the family Buccinidae that were also infected as intermediate hosts, were not as affected as individuals of *B. undatum.*

These findings of metacercariae in experimentally infected Amphipods, together with the found dead individuals of *B. undatum* during scuba dives in the Svalbard fieldtrip (Ditrich, personal communication, observation during dissections) led to the hypothesis that cercariae negatively affect the health of *B. undatum,* which is very uncommon behaviour.

When an individual of *B. undatum* infected with cercariae dies, the next amphipod intermediate host (*Gammarus*) due its scavenger diet, feeds on it and the cercariae become metacercaria in the second intermediate host (*Gammarus*). This could be a new behaviour which leads to the killing of the first intermediate host, because the cercariae avoid leaving the intermediate host to open space, for example because of unsuitable conditions.

The positive result of our experimental infection, when we found metacercaria in *Gammarus* fed by the infected hepatopancreata of *B. undatum* (Figs. 30, 31), corresponds with this hypothesis. *Gammarus* is eaten by the definitive host (*M. scorpius*, *G. tricuspis*) and the life cycle is completed. In order to confirming this hypothesis, more specimens of the second intermediate host (*Gammarus*) should be dissected and any found metacercariae compared with existing studies.

The results showed that adults from both species of the final hosts are morphologically identical. Future study should include molecular analyses. Only one 18S rDNA sequence of *Podocotyle* was found in the NCBI database, so it will be useful to sequence collected trematodes and compare sequence similarity among various samples. Discriminant analyses should be applied together with the results of molecular analyses.

6 Conclusions

1. *Podocotyle atomon* from the family Opecoelidae was recorded from *Myoxocephalus scorpius* (prevalence 43.7%) and *Gymnocanthus tricuspis* (prevalence 30.7%).

2. *Podocotyle atomon* from the family Opecoelidae was also recorded from *Buccinum undatum* (prevalence 24%), *Buccinum glaciale* (prevalence 27.7%) and *Plicifusus kroeyeri* (prevalence 33.3%).

3. Morphological analyses of trematodes from *Gymnocanthus tricuspis* and *Myoxocephalus scorpius* from the central part of Svalbard confirmed only the presence of *Podocotyle atomon* based on measured data with scanning electron microscopy and carmine staining and compared with the findings of Køie (1981) and Brinkmann (1975) and Hunninen and Cable (1943).

4. The results of an experimental infection of a second intermediate host (Amphipoda) confirmed the hypothesis of uncommon behaviour of cercariae of *Podocotyle atomon* and the absence of cercariae shedding the first intermediate host (Buccinidae). We documented the developmental stages in the first intermediate host (cercariae) and second intermediate hosts (young metacercariae).

5. Materials from 63 infected intermediate hosts (Buccinidae) and 30 fish hosts for molecular analyses have been collected.

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