

University of South Bohemia in České Budějovice
Faculty of Science

Bachelor thesis

2016

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**Diversity and geographical distribution of tapeworms of
the order Diphylobothriidea in Pinnipedia**

Bachelor thesis

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České Budějovice 2016

Morávková V. 2016: Diversity and geographical distribution of tapeworms of the order Diphyllbothriidea in Pinnipedia. Bc. Thesis, in English – 74pp., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation

The aim of the study was to obtain and elaborate information focused on tapeworms of the order Diphyllbothriidea and their hosts of marine environment (Pinnipedia). Faecal material of *Phoca vitulina* was obtained from the Seal Rehabilitation and Research Centre, Zeehondencrèche in Netherlands and and examined by two different coprological methods (flotation and sedimentation).

Declaration

I hereby declare that I have worked on my bachelor's thesis independently and used only the sources listed in the bibliography.

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Acknowledgements

I would like to express my sincere gratitude to my supervisor doc. RNDr. Oleg Ditrich, CSc. for his patience, knowledge and guidance of my bachelor thesis. My sincere thanks also goes to MVDr. Jana Kvičerová, Ph.D., Mgr. Eva Myšková and RNDr. Tomáš Týmľ for their help, assistance and pleasant working environment. I also wish to express my sincere thanks to RNDr. Roman Kuchta, Ph.D. for providing material for the literature review and sample collection of this work. Besides my advisors, I would also like to show gratitude to members of the SRRC, who provided me an opportunity to join their team as a volunteer. I am grateful to them for their patience, motivation and confidence but also for opportunity to collect samples from the seal patients. Last but not the least, I would like to thank my family for their enormous encouragement and unconditional love, which supported me the most.

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1. INTRODUCTION

Tapeworms (Cestoda) belong to the exclusively parasitic group, called Neodermata (Lophotrochozoa: Platyhelminthes) and include almost 6000 species with the adult stages inhabiting predominantly a digestive tract of vertebrates (Caira & Littlewood 2013). They are traditionally divided in two subgroups, the Cestodaria composed of two primitive orders Amphilinidea and Gyrocotylidea and the rest of “true cestodes” represented by Eucestoda, comprising 17 orders (Khalil et al. 1994; www.tapewormdb.uconn.edu¹). Phylogenetic relationships among the members of Eucestoda have not been clearly resolved so far, nevertheless they have been divided into “lower” bothriate cestodes (Bothriocephalidea, Caryophyllidea, Diphyllidea, Diphyllbothriidea, Haplobothriidea, Litobothriidea, Spathebothriidea, Trypanorhyncha) and “higher” acetabulate cestodes (Cathetocephalidea, Cyclophyllidea, Lecanicephalidea, Nippotaeniidea, Proteocephalidea, Phyllobothriidea, Rhinebothriidea, Tetrabothriidea and polyphyletic “Tetraphyllidea”) (www.tapewormdb.uconn.edu¹). The most speciose and derived order is Cyclophyllidea with around half of the known tapeworm species parasitizing mainly in birds and mammals. However, majority of the orders (9 out of 19) – Cathetocephalidea, Diphyllidea, Gyrocotylidea, Litobothriidea, Trypanorhyncha, Lecanicephalidea, Phyllobothriidea, Rhinebothriidea, “Tetraphyllidea” parasitize in Elasmobranchs (Caira & Littlewood 2013).

This study is focused on species composition and distribution of members of one of the less known orders, Diphyllbothriidea, which parasitizes mainly in marine mammals, namely seals. Diphyllbothriidean tapeworms parasitize in all groups of tetrapods (mammals, birds, reptiles and amphibians), including man (Bray et al. 1994; Delyamure et al. 1985). This group of cestodes is cosmopolite with 74 % of species living in the marine environment, especially in intestine of mammals as seals and cetaceans (Kuchta et al. 2008).

The basis of the thesis was focused on the diversity and geographical distribution of tapeworms of the order Diphyllbothriidea in Pinnipeds. Furthermore, the faecal material of *Phoca vitulina* L. was collected and examined from the Netherlands, during an volunteering work in Research and Rehabilitation Center of seals. Faeces samples were elaborated for the presence of endoparasites.

2. LITERATURE SURVEY

2.1. Cestoda

Cestodes are parasitic flatworms with complex life cycles including usually two or more hosts. Adult cestodes inhabit almost exclusively digestive system of their definitive host (DH), all groups of vertebrates. The larval forms are harboured in organs as well as in intestine of their intermediate host (IH), mainly invertebrates, but in some cases also vertebrates (Elsheikha & Khan 2011).

The body structure of the cestodes is generally composed off two basic parts: scolex, and strobila (Caira & Littlewood 2013). The scolex (“head”) is located anteriorly and is used to attach to the intestinal wall or spiral valve of its host. The attachment is often supported by additional attachment organs such as bothria or acetabulum (bothridia or suckers), or by additional specialized structures such as rostellum, apical organs, hooks or tentacles (Khalil et al. 1994). The cestode taxonomy is based mainly on the organisation and types of scoleces (Caira & Littlewood 2013; www.tapewormdb.uconn.edu¹). The neck, an undifferentiated narrow zone, is usually localized between the scolex and the strobila. The neck may be of various length and contains germ cells, responsible for production of new segments. If the neck is absent, the germ cells occur in the posterior part of the scolex (Roberts & Janovy 2009). The rest of the tapeworm body is called strobila. The most of the cestodes are known to be segmented or proglottized, but there are also species with just a single set of genital organs in a strobilus (i.e. monozoic) such as Caryophyllidea, or their strobilus is composed off several proglottids (i.e. polyzoic), but is not segmented as Spathebothriidea (Caira & Littlewood 2013; www.tapewormdb.uconn.edu¹). In case of segmented strobilus, the layout of segments is divided into two forms: craspedote (each segment is overlapped by the previous segment) or acraspedote (without overlapping segments).

Forming of segments is caused by asexual process known as strobilation. At this stage, segments increase in size and maturity, with the result of (usually) wider than long units carrying fully functional and active sexual organs (Elsheikha & Khan 2011). Mature proglottids situated at the end of strobila leave the body of oviparous tapeworm and migrate as independent, self- propelled segments (apolytic) or they pass in faeces out of the DH. Gravid segments leaving the body may disintegrate and release their eggs. In some species of tapeworms, proglottids are retained on the strobila (anapolytic) throughout the life of their host. In this case, eggs are released through uterine pores (Khalil et al. 1994; Elsheikha & Khan 2011).

The first embryonic form of the tapeworm develops within the tapeworm egg. These larvae may be divided into two groups based on the number of their embryonic hooks. Decatanths, also called lycophore, possesses 10 embryonic hooks and are present in Cestodaria. Six-hooked-larvae-hexacanth (or oncosphere) are known in all Eucestodes (Elsheikha & Khan 2011). The embryo possessing three pairs of hooks, also called coracidium, is covered by ciliated epithelium, intended for movement in water (Conn & Swiderski 2008).

The larvae (metacestodes) ingested by the specific IH hatch and develop into an immature stage. The stage called procercoid is always situated in the first IH. If the larval stage is harboured in invertebrate IH, tapeworm will be localized in haemocoel and develop to the procercoid form. The metacestodes harboured in the second IH, including both vertebrates and invertebrates, occur in different morphological types as plerocercus, cysticercus, plerocercoid or merocercoid (Chervy 2002).

As mentioned above, cestodes are usually harboured in two or more hosts. The two-host life cycle is typical for members of the genus *Taenia* Linnaeus, 1758 (Cyclophyllidea) or *Bothriocephalus* Rudolphi, 1808 (Bothriocephalidea), while the three-host life cycle is typical for members of the genus *Diphyllobothrium* Lühe, 1910 or *Spirometra* Faust, Campbell et Kellogg, 1929 (Diphyllobothriidea). Only few cestode species are able to develop in a single host, for example *Hymenolepis nana* (Siebold, 1852) (Cyclophyllidea) or *Archigetes* Leuckart, 1878 (Caryophyllidea).

Cestodes are almost exclusively hermaphrodites, usually in form of simultaneous hermaphroditism. The simultaneous hermaphrodites contain both male and female reproductive organs, mostly with faster ripening male organs (protandry). Few species (Cyclophyllidea: Anoplocephalidae, Schistotaeniidae, Hymenolepididae) are opposite, with the faster-growing female system (protogyny) (Warner 1975). It is considered that these two types of development prevent self-fertilization in the same segment (Khalil et al.1994). However, a few species are with a dioecious reproduction, such as *Infula macrophallus* Coil, 1955 (Cyclophyllidea).

Each segment of strobila usually contains one or rarely more sets of male and female reproductive systems (Khalil et al. 1994). The male reproductive organs include various amounts of testes linked to vas deferens carrying sperm to the terminal genitalia through a thin channel called vas efferens. Vas deferens opens into cirrus sac, in which the male copulatory organ called cirrus is localized. Female reproductive organs contain a single ovary which produces eggs. Formation of eggs is unconditionally supported by a vitellarium.

Vitellarium generates yolk-filled cells to nourish the developing eggs (embryos) and also compounds involving production of egg membrane. Vitellarium can also support forming of eggshell. Mature oocytes leave the ovary through the oviduct, often provided with a muscular orifice, known as sphincter or oocapt (Conn & Świdorski 2008). Fertilization occurs most frequently between two adjacent tapeworms when the cirrus of both of them is connected through the genital pore and sperm cells (spermatozoa) are exchanged. Spermatozoa travelling from the genital pore, move from base of the vagina into the ootype. Some groups contain a vagina constituting a seminal receptacle which stores these male reproductive cells. The male and female ducts usually open into a common genital atrium through a common genital pore or separately through the male and female genital pores. The developing embryo enters the uterus after leaving the ootype (Khalil et al. 1994; www.tapewormdb.uconn.edu¹).

The Eucestodes lack the digestive tract. Therefore they absorb nutrients through the specialized surface named tegument, an external cellular structure of the body (neodermis), covered by highly specialized microvilli, known as microtriches (Chervy 2009). The neodermis with its morphological variations of microtriches make up unique defining structures in cestodes. The external layer of microtriches consists of carbohydrate complex called glycocalyx. Microtriches are divided based on their size into two essential groups. The filitriches are specialized microtriches with the basal width ≤ 200 nm. Those with the basal width > 200 nm are known as spinitriches. There are three types of filitriches and 25 types of spinitriches (Chervy 2009).

The surface is responsible for absorption of bile salts, cations, for membrane transport of low molecular weight substances such as carbohydrates, amino acids, fatty acids, vitamins, and for pinocytosis (Cheng 1986). Tapeworms are unable to synthesize lipids which are significant for mechanism of reproduction. Therefore, the absorption of fatty acids is especially important (Mondal 2009).

However, at least one tapeworm species, termed as *Sanguilevator yearsleyi* Caira, Mega & Ruhnke, 2005 (Cathetocephalidea) is known to absorb blood cells. It is supposed, that they separate both leukocytes and erythrocytes within their scolex. They store white blood cells in spherical chambers and red blood cells in transverse channels. As mentioned before, cestodes are considered to absorb small molecules due to their lack of digestive tract. Therefore, it is improbable to consume these hematocytes with the aim of nutrition. The reason of consumption of blood cells by this parasite has not yet been established (Caira et al. 2005).

Additional function of tegument include protective cover to inhibit response from digestive enzymes of the external environment. The structure also acts as a sensory system for detection of the environmental conditions and target sites of anthelmintic drugs (Mansour 2002). At the level of morphological structures, it is supposed that microtriches help to prevent contact with host immune effector cells (Wedekind & Little 2004).

Process of absorbing of nutrients is as important as discarding waste materials. Cestodes use protonephridia, also termed “flame cells”, as a main functional unit of excretory system. They are attached to a tube cell, supported by microtriches, which help to move liquid through the tube. These “cup-shaped” flagellated cells regulate the osmotic pressure of tapeworm, and maintain its ionic balance (Ruppert et al.2004).

Cestodes belong to the group of acoelomates, which exhibit bilateral symmetry and have no body cavity. Therefore, the reproductive organs are supported by musculature. Muscles are located directly below the tegument in the form of several thin layers. There are three types of muscles: circular, oblique and longitudinal. Circular musculature occurs in periphery of tapeworm’s body with perpendicularly lying oblique tissues. Longitudinal muscles extend along the length of the cestodes body. Many tapeworms possess longitudinal muscle bundles located lengthwise from the scolex to the end of strobila, which separate the outer cortex and the inner center (medulla) of the body. Some cestodes contain a narrow, muscular enlargement (cephalic peduncle), supporting a posture of the scolex on the tapeworm’s body (www.tapewormdb.uconn.edu¹).

2.1.1. Diphylobothriidea Kuchta, Scholz, Brabec et Bray, 2008

The Diphylobothriidea is an order of bothriate eucestodes characterised by presence of unarmed scolex with two dorsoventrally localised bothria (Kuchta et al. 2008). The scolex is usually round, without apical disc, except the genus *Tetragonoporus* Skryabin, 1961.

The scolex is usually attached to the neck, from which the strobila grows (Khalil et al. 1994). The strobila is segmented with mostly wider than long, anapolytic craspedote segments. Lack of segmentation is rare (*Ligula*, Bloch 1782). Each segment generally contains one set of male and female reproductive organs, except of some genera with multiple reproduction sets in a single segment such as *Diplogonoporus* Lönnberg, 1892 or *Tetragonoporus* (Kuchta et al. 2008). The testes are numerous, and the cirrus sac is covered by a thick muscular wall, and the proximal part of the vas deferens forms muscular external seminal vesicle. The copulatory organ, cirrus, is unarmed. Female reproductive organs

contain a compact ovary and a ventral genital pore attached to a tubular uterus. The vitelline follicles are numerous, usually situated in cortical parenchyma (Kuchta et al. 2008).

The Diphyllbothriidea vary greatly in size. Most of them reach 1–2 m. One of the smallest species is *Diphyllbothrium wilsoni* Shipley, 1907 infecting leopard seal (*Hydrurga leptonyx* (de Blainville, 1820)) with high intensity, being approximately 10 mm long (Maltsev 2000). However, in less infected animals they could reach up to 5–9 cm (Markowski 1952a). The largest species is *Tetragonoporus calyptocephalus* Skryabin, 1961 infecting the bile ducts of the sperm whale (*Physeter catodon* L.), and reaching over 30 m (Yurakhno 1992). The longest cestode infecting humans, *Diphyllbothrium latum* (Linnaeus, 1758), may reach the total length up to 25 m, but most frequently reaches 3–10 m (Scholz et al. 2009).

The life cycle of Diphyllbothriidea usually involves three hosts. A ciliated free-swimming aquatic larva (coracidium) hatches from the thick-walled egg developing in water. Then, the coracidium is eaten by a copepod (Crustacea) and harboured in its body cavity. These hexacanth develop in copepods to another stage named proceroid, which is infective for another host, usually a vertebrate (fish or amphibian). In infected vertebrates, a next larval stage called plerocercoid, develops. The adult diphyllbothriids parasitize in the digestive tract of tetrapodes, mainly marine mammals and birds including humans (Kuchta et al. 2008). The members of the genus *Tetragonoporus* Skryabin, 1961 invade a biliary duct of cetaceans (Kuchta et al. 2008). The two-host-life cycle occurs only in *Cephalochlamys namaquensis* (Cohn, 1906), with a single intermediate copepod host (*Thermocyclops infrequens* (Kiefer, 1929)) and a single DH, known as African clawed frog (*Xenopus* Daudin, 1802) (Thurston 1967; Jackson & Tinsley 2001).

Diphyllbothriidea is actually divided into three families (Kuchta et al. 2008):

I. Cephalochlamydidae Yamaguti, 1959

Genus: *Cephalochlamys* Jackson & Tinsley, 2001

Genus: *Paracephalochlamys* Jackson & Tinsley, 2001

II. Solenophoridae Monticelli et Crety, 1981

Genus: *Scyphocephalus* Riegenbach, 1898

Genus: *Bothridium* Blainville, 1824

Genus: *Duthiersia* Perrier, 1873

III. Diphyllbothriidae, Lühe, 1910

Genus: *Adenocephalus* Nybelin, 1931

Genus: *Baylisia* Markowski, 1952

Genus: *Baylisiella* Markowski, 1952
Genus: *Diphyllobothrium* Cobbold, 1858
Genus: *Diplogonoporus* Lönnberg, 1892
Genus: *Flexobothrium* Yurakhno, 1979
Genus: *Glandicephalus* Fuhrmann, 1921
Genus: *Ligula* Bloch, 1782
Genus: *Plicobothrium* Rausch & Margolis, 1969
Genus: *Pyramicocephalus* Monticelli, 1890
Genus: *Schistocephalus* Creplin, 1829
Genus: *Spirometra* Faust, Campbell & Kellog, 1929
Genus: *Tetragonoporus* Skryabin, 1961

The family Cephalochlamydidae parasitizes African amphibians of the genus *Xenopus*. Tapeworms of the family Solenophoridae invade reptiles of Africa, Asia, Australia and South America and the members of the family Diphyllobothriidae colonize a wide range of birds and mammals worldwide (Kuchta et al. 2008). The majority of cestodes (including Diphyllobothriidean tapeworms) are invading animals living in the aquatic environment (Caira & Pickering 2013). The following scheme (Fig. 1.) shows tapeworm orders with three various categories of their regular hosts. These hosts are also common for the order Diphyllobothriidea.

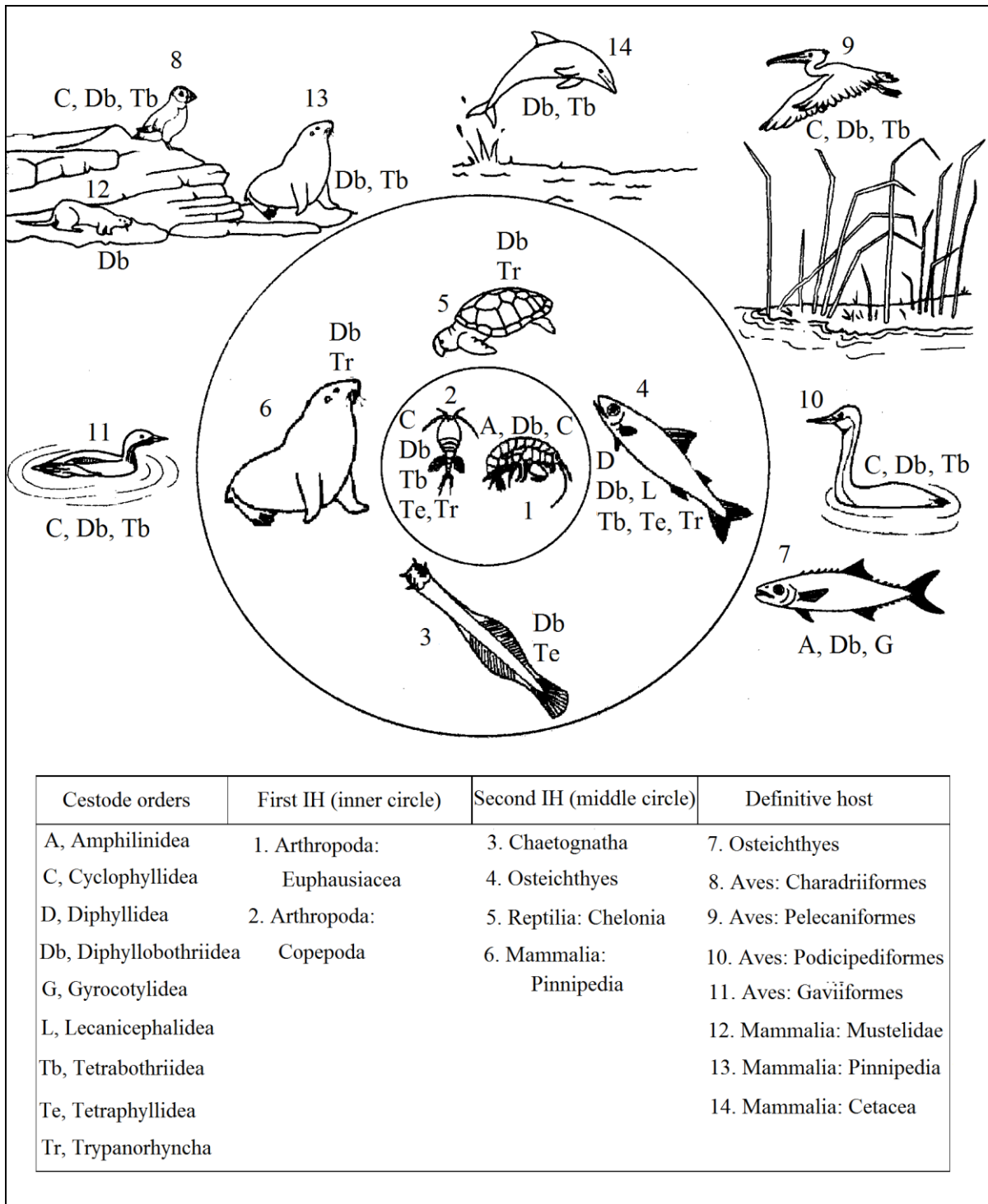


Fig. 1. Aquatic vertebrates and invertebrates serving as an IH (inner circle), second IH (middle circle) and DH (outside of circle) for cestodes (including Diphyllobothriidea) (adapted from Énumération des cestodes du plankton et des invertébrés marins by Dollfus R.P. 1976, Annales de Parasitologie Humaine et Comparée, 51, 207-22.)

2.2. Classification and evolution of Pinnipedia

Members of Pinnipedia are semi-aquatic, fin-footed marine mammals belonging to the order Carnivora, with sister groups of terrestrial carnivorous mammals (Yonezawa et al. 2009). Pinnipeds are divided into three monophyletic families: Phocidae, Otariidae and Odobenidae (Perrin et al. 2009). Phocidae consists of two monophyletic subfamilies Phocinae (Tab. 1.) and Monachinae (Tab. 2.), with 12 genera and 17 species described so far, while Otariidae comprises 7 genera and 14 species (Tab. 3.). In Odobenidae, the only living species is *Odobenus rosmarus* L. (Perrin et al. 2009; Yonezawa et al. 2009; Berta & Churchill 2012). Walruses are divided into two living subspecies: Atlantic walrus (*Odobenus r. rosmarus* L.) and Pacific walrus (*Odobenus r. divergens* (Illiger, 1811)), while both of them are distributed in northern hemisphere.

Tab. 1. List of the family Phocidae of the Phocinae Subfamily with their geographic distribution (Rice 1988; Wilson & Reeder 2005; Yonezawa et al. 2009; Berta & Churchill 2012).

Genus	Species	Geographic distribution
<i>Cystophora</i>	<i>Cystophora cristata</i> (Erxleben, 1777)	Arctic, North Atlantic North America (Canada), Iceland, Greenland
<i>Erignathus</i>	<i>Erignathus barbatus</i> (Erxleben, 1777)	Arctic- North America (Canada, Greenland), central Eurasia
<i>Halichoerus</i>	<i>Halichoerus grypus</i> (Fabricius, 1791)	Atlantic - North America, Europe (from Estonia to Denmark), Baltic Sea
<i>Pagophilus</i>	<i>Pagophilus groenlandicus</i> (Erxleben, 1777)	Arctic (Eastern Canada, Greenland, Iceland, Norway) North Atlantic
<i>Phoca</i>	<i>Phoca largha</i> Pallas, 1811	North Pacific (from Alaska to Japan, excluding China)

Tab. 1. (Continued).

Genus	Species	Geographic distribution
<i>Phoca</i>	<i>Phoca vitulina</i>	Northern Hemisphere
	Linnaeus, 1758	North Atlantic - from James & Hudson Bays (Canada) to Southern Greenland, USA (Massachusetts) East Atlantic - from Barents Sea to Portugal Pacific - west coastal area of North America, Eastern Asia- Hokaido (Japan)
<i>Pusa</i>	<i>Pusa caspica</i> Gmelin, 1788	Caspian Sea
	<i>Pusa hispida</i> (Schreber, 1775)	Arctic Ocean, Bering Sea, Northern Europe (Finland), Northern Baltic Sea Pacific Ocean (Kamchatka, Hokkaido) Northern Asia - Lake Ladoga (Russia)
	<i>Pusa sibirica</i> (Gmelin, 1788)	Lake Baikal (Russia)

Tab. 2. Species of the family Phocidae with the subgroup Monachinae and their geographic distribution (Rice 1988; Wilson & Reeder 2005; Yonezawa et al. 2009; Berta & Churchill 2012).

Genus	Species	Geographic distribution
<i>Hydrurga</i>	<i>Hydrurga leptonyx</i> (de Blainville, 1820)	Southern Ocean - South America, South Africa, Australia, New Zealand, Antarctica
<i>Leptonychotes</i>	<i>Leptonychotes weddellii</i> (Lesson, 1826)	Southern Ocean - Antarctica

Tab. 2. (Continued).

Genus	Species	Geographic distribution
<i>Lobodon</i>	<i>Lobodon carcinophaga</i> (Hombron & Jacquinot, 1842)	Southern Ocean - Antarctica
<i>Mirounga</i>	<i>Mirounga angustirostris</i> Gill, 1866	North Pacific - North America
	<i>Mirounga leonina</i> Linnaeus, 1758	Southern Ocean- Macquarie; Pacific - Chatham Islands; Atlantic - Falkland Islands, Valdez Peninsula
<i>Monachus</i>	<i>Monachus monachus</i> (Hermann, 1779)	Atlantic - Canary Islands Mediterranean, Black Sea
	<i>Monachus schauinslandi</i> Matschie, 1905	Pacific - Hawaiian Islands
<i>Ommatophoca</i>	<i>Ommatophoca rossii</i> Gray, 1844	Southern Ocean - Antarctica

Tab. 3. Geographic distribution of the family Otariidae (Brunner 2004; Berta & Churchill 2012; Higdon et al. 2007; Maloney et al. 2008; Reppenning 1971; Wilson & Reeder 2005; Yonezawa et al. 2009; Waerebeek & Würsig 2008).

Genus	Species	Geographic distribution
<i>Arctocephalus</i>	<i>Arctocephalus australis</i> (Zimmermann, 1783)	South Ocean - Falkland Islands East Pacific - South America
	<i>Arctocephalus forsteri</i> (Lesson, 1828)	Pacific - New Zealand, Australia, Sub – Antarctic islands
	<i>Arctocephalus gazella</i> (Peters, 1875)	Southern Ocean - Antarctic
	<i>Arctocephalus philippii</i> (Peters, 1866)	East Pacific - The Juan Fernández Islands (Chile)

Tab. 3. (Continued).

Genus	Species	Geographic distribution
	<i>Arctocephalus pusillus</i> (Schreber, 1775)	Indian - South Africa Pacific - Australia, Tasmania; Atlantic Ocean, African coastal regions from Namibia to Algoa Bay (South Africa)
	<i>Arctocephalus townsendi</i> Merriam, 1897	East Pacific - Guadalupe Island (Mexico), Channel Islands (California)
	<i>Arctocephalus tropicalis</i> (Gray 1872)	Indian- Amsterdam, Crozet, Marion; Pacific - Macquarie; Atlantic - Gough, Tristan
<i>Callorhinus</i>	<i>Callorhinus ursinus</i> Linnaeus, 1758	Pacific (Canada, Mexico, Japan, USA, Russia) Bering Sea, Sea of Okhotsk
<i>Eumetopias</i>	<i>Eumetopias jubatus</i> (Schreber, 1776)	Pacific (Canada, China, Japan, Russia, USA)
<i>Neophoca</i>	<i>Neophoca cinerea</i> (Péron, 1816)	Australia
<i>Otaria</i>	<i>Otaria flavescens</i> Shaw, 1800	Coast of South America (Argentina, Brazil, Chile, Peru, Uruguay, Panama, Ecuador (Galapagos Islands)
<i>Phocarcos</i>	<i>Phocarcos hookeri</i> (Gray, 1844)	Southern Ocean - Auckland, Campbell (New Zealand subantarctic islands)

Tab. 3. (Continued).

Genus	Species	Geographic distribution
<i>Zalophus</i>	<i>Zalophus californianus</i> (Lesson 1828)	Pacific - western North America
	<i>Zalophus wolfebaeki</i> Sivertsen, 1953	Pacific - Galapagos Islands (Equador), Columbia

2.2.1. General characteristics

Pinnipeds differ from other marine mammals like cetaceans or sirenians in their ability of terrestrial locomotion. These carnivorous, amphibious mammals need to mate, give birth, suckle their young, moult and rest on land (Geraci & Lounsbury 2005). However, they obtain food mainly from marine environments, less frequently also from inland or tropical freshwater systems (www.britannica.com²).

The members of Phocoidea have torpedo-shaped bodies with a broad middle and tapered at the head and hindquarters. They use four limbs modified into webbed flippers for the movement. Pinnipeds swim by paddling their flippers, compared to sirenians and cetaceans moving their tails or flukes up and down. They tend to be slower swimmers than cetaceans (Shirihai & Jarrett 2006). On the other hand, pinnipeds are more flexible and agile, typically swimming at 9–28 km/h (Riedman 1990). Pinnipeds reach depths on average over 200 metres for not more than 10 minutes during diving (Stirling & Kooyman 1971; MacDonald 1984; Georges, et al. 2000). Elephant seals (genus *Mirounga* Gray, 1827) can reach depth of 1.5 km and can also dive regularly for more than an hour (Riedman 1990).

The body size varies from 1 to 5 m, reaching the weight from about 45 kg to 3000 kg (Berta 2009). Males and females differ in size on the basis of sexual dimorphism. The adult males in otariids such as southern elephant seals (*Mirounga leonina* L.) are significantly larger than females. They can reach the mass up to 4000 kg, compared to females weighing not more than 800 kg. Adult females of odobenids weigh generally two-thirds as much as males. In phocines, the males are generally little smaller than females. Sexual dimorphism also comprises differences in colour, development of appendages, thickness of fur or vocalization (Ralls & Mesnick 2009; Le Boeuf & Campagna 2013). These traits are present mostly in males, used in defense of females as well as defending of territories during breeding season. Most differences of secondary sex characteristics in males occur in polygynous species (Ralls & Mesnick 2009). The mating system of pinnipeds is also related

to breeding on land or ice. Land- breeding otariids tend to be polygynous, as females gather to groups (Riedman 1990). Phocids and walruses use to be monogamous and include mostly ice- or water- breeding species. While otariids tend to return to the same place for many years, the ice- breeding seals use to change their breeding sites every season (Riedman 1990; Ralls & Mesnick 2009).

The lifespan of pinnipeds is generally 20–30 years, when females typically mature faster and live longer than males (Fay 1960; Berta 2012). The sexual maturity of these marine mammals varies among species, mostly attaining within 2–12 years (Riedman 1990).

All of pinnipeds, whether old or young, must be aware of predators both on land or underwater. Whereas they spend most of their time in water, they are hunted by killer whales (*Orcinus orca* L.) and few species of sharks, as a great white shark (*Carcharodon carcharias* L.). Their natural predators on land are polar bears (*Ursus maritimus* Phipps, 1774) or terrestrial predators such as canids (Nowak 2005; Weller 2009; Brown et al. 2010).

As noted above, pinnipeds are widespread, mostly living in cold and nutrient-rich waters of Northern and Southern Hemispheres. Their natural habitat includes waters of Polar regions with temperatures below 20 °C. The average air temperature is generally lower than 10°C (Longton 1988). While most species live in coastal areas, several members inhabit freshwater systems. The only exclusively freshwater species is the Baikal seal (*Pusa sibirica* (Gmelin, 1788)), endemic to the Lake Baikal (Reeves et al. 2002). Other seals, like the monk seals (genus *Monachus*) and few species of otariids, live in tropical and subtropical areas. Only two species have been reported from both, marine and freshwater ecosystems, the harbor seals (*Phoca vitulina* L.) and the ringed seal (*Pusa hispida* (Schreber, 1775)), respectively (Riedman 1990).

The digestive system of seals usually include enormously long small intestine compared to common carnivorous mammals. The length of small intestine of Southern Elephant Seal is 25–42 times the body length (Laws 1953). The length of the gut and content of water affect the passage of food, which usually runs about less than 5 hours (Helm 1984).

The diet of pinnipeds includes variety of fishes, cephalopods and other marine invertebrates (Riedman 1990; Hobson et al. 1997). The leopard seal represents an exception, feeding on penguins or other seals, especially pups of crabeater seals (*Lobodon carcinophaga* (Hombron & Jacquinot, 1842)) (Riedman 1990; Siniff & Bengtson 1977). There are also other feeding specialists such as pacific walrus (*Odobenus rosmarus divergens* (Illiger, 1815)) or atlantic walrus (*Odobenus rosmarus rosmarus* L.), which are main predators of bivalve mollusks in the Arctic (Fukuyamaa & Olivera 1985). Pinnipeds

are generally known to prey and feed underwater. The pattern of consumption depends on the species of seal and size of their prey. Too heavy seal catches are pulled out of the water and processed on land (Roffe & Mate 1984). Walrus typically ingest their prey directly in water by suction feeding (Berta 2012).

3. MATERIAL AND METHODS

3.1. Literature review

Information material for this work was obtained from majority of articles including data of the order Diphylobothriidea related to Carnivoran families of Pinnipedia. The resources were obtained from databases as NHM, CiNii, BHL, BioMedSearch, CJO, GoogleBooks, HathiTrust, JSTOR, NRC Research Press, PubMed, ScienceDirect, SpringerLink, Taylor & Francis, WOS. The keywords of the publications were processed using Endnote Basic software. The original version of the data has been reduced because some records were duplicated or did not contain the necessary information. The literature survey included study of over 150 publications focused on geographical distribution and prevalence of tapeworms infecting seals. The relationships between seals and tapeworms of the order Diphylobothriidea were possible to determine due to the obtained data compared to the information of pinnipeds.

3.2. Collection of material

Due to the possibility to work as a volunteer at the Seal Rehabilitation and Research Centre (SRRC), Zeehondencrèche located in Pieterburen, the Netherlands (www.zeehondencreche.nl³), for two months, I also had an opportunity to gather material in the field for this work.

The fieldwork included fresh faecal material collection during an internship in the SRRC. In the agreement with the veterinarians in the SRRC, the faecal sampling from seals placed at the Centre was approved. Samples were transferred to the Faculty of Science, University of South Bohemia in České Budějovice (Czech Republic) after finishing the work, where they were analyzed under supervision of specialists.

The Center works to save injured, weakened or sick wild seals and release them back to the nature for over 40 years (<http://www.zeehondencreche.nl/historie>). The internship lasted 2 months (from 16.8. to 10.10.), when members of the SRRC mostly took care of juveniles of Common seal (*Phoca vitulina*). In order to keep all important aims of the Centre (to rescue, cure and release the seals in to the wild), it was necessary to maintain strict hygiene protocols, nutritional and medical schemas with the seal patients. To keep the seals wild and stress free, it was important to avoid human interaction as much as possible.

My work concerned the Seal Care Department, where direct contact with seals was necessary. This work comprised mostly 2 or more feedings of seal patients per day, and extensive morning sanitation of all areas in contact with seals and people working with them. Before entering the enclosure where the seal is housed, visual check of the health status was necessary. When considering negative status of the seal patient, it was necessary to adapt to the situation and take action, which usually involved closer contact with the animal (measurement of body temperature, giving medication and wound cleaning). Due to these facts, it was possible to collect samples during labour. The collection of the samples was discussed and coordinated by veterinary experts of the SRRC, and it was always personally agreed by a nurse in a given situation.

At first, faecal samples were gathered from new seals, which arrived into the SRRC during a period of my internship. All patients of *Phoca vitulina* were captured from the locality of Wadden Sea (Zuid Holland, Friesland, Vlieland, Noord Holland, Schiermonnikoog and Terschelling), due to their poor health condition. Their age was estimated under one year (juveniles), except one case of adult harbour seal. Faecal material was collected immediately after intake, and then after 24-48 hours or later (if possible). During intake were given anti-parasitics (Praziquantel, Mebendazole) to seals, to treat cestodes, nematodes, trematodes or other diseases. For sampling, nitrile powder-free gloves were used. Faecal material was placed in sampling bottles filled up with pure ethanol at room temperature (20-23 °C / 68-73.4 °F). After the internship, a total of 60 faecal samples from 20 individuals (70% males, 30% females) were coprologically analysed by two qualitative coprological concentration techniques (Flotation, Sedimentation) for the presence of endoparasites of the order Diphyllbothriidea.

3.3. Coprological examination

Faeces were examined by two different coprological methods, flotation and sedimentation, to examine the parasitofauna of the digestive tract of seals, focusing on parasites of the order Diphylobothriidea. During this research the attention was paid especially on sensitivity and efficiency of both methods focused on the above mentioned helminths.

3.3.1. Flotation

Cestodes of the order Diphylobothriidea can be diagnosed by identifying of their eggs or proglottids from faeces. Flotation is one of the standard parasitological methods for separation components of stool with different buoyancy. Less dense material as helminth eggs, cysts, oocysts, proglottids or larval forms are concentrated on the surface of the faecal float solution (with an appropriate specific gravity), while the heavier parts of the faecal material are located at the bottom (Dryden et al. 2005). We used Sheather's sucrose solution of the specific gravity 1.30 as a flotation fluid.

Sheather's sucrose solution of the specific gravity 1.30 was prepared by boiling 1 kg of granulated sugar dissolved in 700 ml of tap water. After cooling down, the mixture was enriched with 10 ml of liquid phenol for stabilization and durability.

Flotation apparatus was composed of a stand, nylon tea strainer, laboratory clamp holder, ring clamp and glass test tubes without cap. Faeces were homogenized in the original homeopathic bottle by shaking or with tweezers. Approximately 2 g of the mixture was poured through a tea strainer into the test tube. The rest of faecal material stuck on the nylon sieve was poured through with tap water to fill the tube ca. 1.5 cm below the rim. Such prepared samples were centrifuged for 10 minutes at 1106, 82 g. The supernate was poured. The sediment was mixed with a small amount of Sheather's sugar solution and subsequently filled with it ca. 1 cm below the rim of the test tube. Samples were then centrifuged for another 10 minutes at 1106, 82 g and then were prepared for light microscopy.

For the microscopy, the following equipment was required: test tubes with samples processed by flotation, test tube rack, light microscope (Olympus CX31), microslides, coverslips, inoculation loop, cotton, flask and tap water. From the test tube, a drop of the membrane from the top of the floated liquid was picked with an inoculation loop and transferred on a microslide. This process was repeated with another drop and then the microslide was covered with a coverslip. Such a native mount was microscoped and the results consulted with specialists. Eggs were measured and photographed by the specialists

using an Olympus BX53 light microscope equipped with digital camera and OLYMPUS cellSens Standard 1.13 imaging software. All measurements were given in μm . Prevalence was estimated as the percentage of infected seals.

3.3.2.Sedimentation

The sedimentation technique is based on removing light unintended fragments from the faecal material. Heavy components as eggs of trematodes (e.g. *Fasciola hepatica* Linnaeus, 1758), oocysts of Conoidasida (e.g. *Eimeria leuckarti* Flesch, 1883), or larvae of nematodes (e.g. *Trichinella spiralis* Owen, 1835) fall to the bottom of a faecal suspension (Leiper 1949; Bauer 1988; Kaufmann 1996; Baker 2007). This coprological method is also commonly used to diagnose eggs of cestodes (e.g. *Diphyllobothrium latum* (Linnaeus, 1758)) (Thienpont et al. 1979; Zajac & Conboy 2012).

For the sedimentation technique, following equipment was used: glass test tubes, cork stoppers, test tube rack, glass funnel, gauze, wooden spatulas, 3 ml plastic pipettes, laboratory hood, AMS III solution (SG 1.080), Triton solution, and ether (Hunter et al. 1948).

The AMS solution was prepared by dissolving of 115.2 g anhydrous Na_2SO_4 in a medium consisting of 540 ml HCl and 660 ml H_2O . The Triton solution consisted of 16.5 ml Triton X-100 and 33.5 ml H_2O .

Faeces were homogenized in the original sampling bottle by shaking or with a wooden spatula. The test tube was filled up with approximately 3 g of faeces samples fixed by ethanol and 6 ml of AMS solution. The compound was poured through the funnel with gauze to another clean test tube. The mixture was filled up with 3 drops of the Triton solution and 3 ml of diethylether inside the safety hood. Such prepared samples were closed with cork stoppers, homogenized by shaking and centrifuged for 2 minutes at 600 g. The supernatant was poured off. The sediment was used for light microscopy; after being slightly stirred, several drops were put on a microslide and examined using 40x10 and 60x10 magnification.

The results were consulted with specialists. Eggs were measured and photographed with an Olympus Camedia C-5060, light microscope equipped with digital camera and Quick PHOTO MICRO 2.3 imaging software. All measurements are given in μm . Prevalence was calculated as the percentage of infected seals.

4. RESULTS

4.1. Literature review

The publications, containing information of the order Diphyllbothriidea invading the digestive tract of Phocidae, Otariidae and Odobenidae, were elaborated and reduced due to unclear, false or duplicated the same data. Relevant information was identified from over 150 publications and modified to required categories. The following table (Tab. 4.) is showing specific species of Diphyllbothriidean tapeworms invading seals (Phocidae) and sea lions (Otariidae).

Almost all species of the family Diphyllbothriidae infect Phocids and Otariids, except the genera *Plicobothrium* and *Spirometra*. The Otariids species are predominantly infected by *Adenocephalus pacificus* (Nybelin, 1931), which is not invading any member of Phocids. More than a half of the given species of Diphyllbothriideans invade only one species of seal or sea lion. The species *Baylisia baylisi* Markowski, 1952, *B. supergonoporis* Yurakhno, 1989 and *D. lobodoni* Yurakhno & Maltsev, 1994 infect only *Lobodon carcinophagus*. Other member of Phocidae, *Mirounga leonina* is the only host within Phocids and Otariids for *Baylisiella tecta* (Linstow, 1892) and *Flexobothrium microovatum* Yurakhno, 1989. *D. archeri* Leiper & Atkinson, 1914 and *Glandicephalus perfoliatus* (Rennie & Reid, 1912) are invading only *Leptonychotes weddellii*. The Hawaiian monk Seal (*Monachus schauinslandi*) is the only host for *D. cameroni* Rausch, 1969, *D. minutus* Andersen, 1987 and *D. rauschi* Andersen, 1987. Other species of the genus *Glandicephalus* invading seals and sea lions, *G. antarcticus* (Baird, 1853), has the only pinniped host, *Ommatophoca rossii*. *Diphyllbothrium pterocephalum* Delyamure & Skryabin, 1966 parasitizes only *Cystophora cristata*. The only tapeworm representing the genus *Ligula* in Phocids is *L. colymbi* Zeder, 1803 harboured by *Phoca caspica*. This endemic seal to the Caspian Sea is only pinniped host also for *D. phocarum* Delyamure, Kurochkin & Skryabin, 1964 (Berta et al. 2006). The leopard seal (*Hydrurga leptonyx*) is the only pinniped host to *D. pseudowilsoni* Wojciechowska & Zdzitowiecki, 1995. Other species of diphyllbothriidean tapeworms invade more than one species of Phocidae or Otariidae. A detailed description of the geographical distribution of the Phocidae and Otariidae host species is given below (Tab. 5.). In publications occur unspecified species of parasite, *D. sp.* Cobbold, 1858, which are mentioned in both lists only in case of new locations of tapeworm (genus: *Diphyllbothrium*) in a host.

Due to odobenids are hosts probably only for 4 species of the order Diphyllbothriidea, the next table (Tab. 6.) was made separately.

The only known genus of the family Diphyllbothriidea, which infect walruses (Odobenidae), is *Diphyllbothrium*. The following species are mentioned: *Diphyllbothrium cordatum*, *D. latum*, *D. fayi* n. sp. Rausch 2005 and *D. roemeri* Zschokke 1903. Common diphyllbothriidean parasites in walruses are *D. cordatum* and *D. fayi*, while *D. fayi* invades only subspecies *Odobenus rosmarus divergens*. Hilliard and Douglas (1972) studied unspecified species of the genus *Diphyllbothrium* which was localized in walrus at Kodiak Island. Species *D. roemeri*, *D. latum* in walrus were mentioned by Dailey (1975) with unknown locality. Another case of no locality of *D. roemeri* in intestine of walrus was written by Lauckner (1985).

Tab. 4. Tapeworms of the order Diphylobothriidea invading Phocidae and Otariidae.

Host \ Tapeworm	<i>Hydrurga leptonyx</i>	<i>Leptonychotes weddellii</i>	<i>Lobodon carcinophaga</i>	<i>Mirounga angustirostris</i>	<i>Mirounga leonina</i>	<i>Monachus monachus</i>	<i>Monachus schauinslandi</i>	<i>Ommatophoca rossii</i>	<i>Cystophora cristata</i>	<i>Erignathus barbatus</i>	<i>Pagophilus groenlandicus</i>	<i>Phoca largha</i>	<i>Phoca vitulina</i>	<i>Pusa caspica</i>	<i>Pusa hispida</i>	<i>Arctocephalus australis</i>	<i>Arctocephalus gazella</i>	<i>Arctocephalus philippii</i>	<i>Arctocephalus pusillus</i>	<i>Arctocephalus tropicalis</i>	<i>Callorhinus ursinus</i>	<i>Eumetopias jubatus</i>	<i>Neophoca cinerea</i>	<i>Otaria flavescens</i>	<i>Zalophus californianus</i>	<i>Zalophus wollebaeki</i>	
<i>Adenocephalus pacificus</i>																+	+	+	+	+	+	+	+	+	+	+	
<i>Baylisia baylisi</i>			+																								
<i>B. supergonoporis</i>			+																								
<i>Baylisiella tecta</i>					+																						
<i>Diphylobothrium archeri</i>		+																									
<i>D. cameroni</i>							+																				
<i>D. cordatum</i>										+	+	+	+														+
<i>D. ditremum</i>		+											+		+												
<i>D. elegans</i>						+			+																		
<i>D. hians</i>						+				+			+		+												
<i>D. lanceolatum</i>										+	+		+		+												+
<i>D. lashleyi</i>		+						+																			
<i>D. lobodoni</i>			+																								
<i>D. minutus</i>							+																				
<i>D. mobile</i>		+						+																			
<i>D. phocarum</i>														+													
<i>D. pseudowilsoni</i>	+																										
<i>D. pterocephalum</i>									+																		
<i>D. rauschi</i>							+																				
<i>D. quadratum</i>	+	+	+																								

Tab. 4. Continued.

<i>D. scoticum</i>	+																								
<i>D. schistochilos</i>									+	+		+													
<i>D. sp*</i>				+			+					+	+												
<i>D. wilsoni</i>	+	+	+				+																		
<i>Diplogonoporus tetrapterus</i>									+	+	+	+	+		+							+	+		
<i>Flexobothrium microovatum</i>					+																				
<i>Glandicephalus antarcticus</i>							+																		
<i>G. perfoliatus</i>		+																							
<i>Pyramicocephalus phocarum</i>									+	+		+	+		+							+	+		
<i>Ligula colymbi</i>														+											
<i>Schistocephalus solidus</i>															+										

* Unspecified *Diphyllobothrium* with previously not mentioned location of infecting the given Phocid.

Tab. 5. List of diphyllbothriidean parasites invading Phocidae and Otariidae with their geographical distribution.

Parasite Species	Host		Locality		References	
	Subfamily	Species	Ocean	Land/ Island/ Archipelago/Sea		
<i>Adenocephalus pacificus</i>	Otariinae	<i>Arctocephalus australis</i>	Atlantic Ocean	Isla Arce	Hernández-Orts et al. 2013 Morgades et al. 2006 Hernández-Orts et al. 2013	
				Isla de Lobos		
				Northern Patagonia		
		<i>Arctocephalus gazella</i>	Pacific Ocean	Southern Ocean	Galapagos Islands	Dailey 1975
					Robinson Crusoe Island	Nybelin 1931
					Avian Island	Rengifo-Herrera 2013
South Shetland	Rengifo-Herrera 2013					
King George Island	Rengifo-Herrera 2013					

Tab. 5. Continued.

<i>A. pacificus</i>	Otariinae	<i>Arctocephalus philippii</i>	Pacific Ocean	Alejandro Selkirk Island/ Juan Fernández Islands	Cattan et al. 1980, Sepulveda & Alcaino 1993
		<i>Arctocephalus pusillus</i>	Atlantic Ocean	Namibia	Pansegrouw 1990
				South Africa	Delyamure & Parukhin 1968
			Indian Ocean	Lady Julia Percy Island	Drummond 1937
		<i>Arctocephalus tropicalis</i>	Atlantic Ocean	Cape Town	Shaughnessy & Ross 1980
				Gough Island	Bester 1989
				Richards Bay-Natal	Shaughnessy & Ross 1980
		<i>Callorhinus ursinus</i>	Pacific Ocean	California Coast /Año Nuevo Island	Gerber et al. 1993
				Kamchatka	Cholodkovsky 1914
				Hokaido	Maejima et al. 1981
		Honshu	Machida 1969,		
		Russian Far East	Yamaguti 1951		
			Afanassjew 1941		
		Pacific Ocean,	St. George Island/ Pribilof Islands	Stiles 1899	
			St. Paul's Island	Wardle et al. 1947,	
		Pacific Ocean,	Tuleniy Island	Kuzmina et al. 2015	
				Chupakhina 1971,	
				Krotov & Delyamure 1952	

Tab. 5. Continued.

<i>A. pacificus</i>	Otariinae	<i>Eumetopias jubatus</i>	Pacific Ocean	Aleutian Islands Alaska California Coast/ Año Nuevo Island Oregon Coast Vancouver Island Bering Sea Sea of Okhotsk Pearson Islands Isla de Lobos Northern Patagonia (Islotes Los Leones) Guañape Islands Isla Santa Maria Juan de Marcona Falkland Islands California Coast/ Año Nuevo Island Galapagos Islands	Dailey 1975 Fay et al. 1978 Dailey & Hill 1970 Stroud 1978 Margolis 1956 Shults 1986 Dailey 1975 Johnston 1937 Cattan et al. 1977. Morgades et al. 2006 Hernández-Orts et al. 2013 Baer 1969, Miranda et al. 1968 George-Nascimento & Carvajal 1981 Tantalean 1993 Baylis & Hamilton 1934 Dailey & Hill 1970 Dailey 1975
		<i>Neophoca cinerea</i> <i>Otaria flavescens</i>	Indian Ocean Atlantic Ocean		
		<i>Zalophus californianus</i> <i>Zalophus wollebaeki</i>	Pacific Ocean Southern Ocean		
<i>Baylisia baylisi</i>	Monachinae	<i>Lobodon carcinophaga</i>	Southern Ocean	South Shetland Islands / Graham Land	Markowski 1952a

Tab. 5. Continued.

<i>B. baylisi</i>	Monachinae	<i>Lobodon carcinophaga</i>	Southern Ocean	King George Island/ South Shetland Islands	Wojciechowska & Zdzitowiecki 1995
				Balleny Islands (D'Urville Sea)	Yurakhno & Maltsev 1997
<i>Baylisia supergonoporis</i>		<i>L. carcinophaga</i>		Balleny Islands (D'Urville Sea)	Yurakhno 1989a, Yurakhno & Maltsev 1997
<i>Baylisiella tecta</i>		<i>Mirounga leonina</i>	Southern Ocean	South Georgia	Linstow 1892, Markowski 1952b
				King George V Land	Johnston 1937
				Adelie Land	Johnston 1937
				Queen Mary Land	Johnston 1937
<i>Diphyllobothrium archeri</i>		<i>Leptonychotes weddellii</i>	Southern Ocean	Balleny Islands (D'Urville Sea)	Maltsev & Zhdamirov 1995
				Cape Denison	McEwin 1957
				Commonwealth bay	Johnston 1937
				Falkland Islands	Maltsev & Zhdamirov 1995
				Graham Land	Markowski 1952b
				King George V Land	McEwin 1957, Wojciechowska & Zdzitowiecki 1995
				McMurdo Sound	Beverley-Burton 1971
				Ross Sea	Maltsev & Zhdamirov 1995
				South Georgia	Maltsev & Zhdamirov 1995

Tab. 5. Continued.

<i>D. archeri</i>	Monachinae	<i>Leptonychotes weddellii</i>	Southern Ocean	South Shetland	Markowski 1952b, Maltsev & Zhdamirov 1995, Wojciechowska & Zdzitowiecki 1995
<i>D. cameroni</i>	Phocinae	<i>Monachus schauinslandi</i>	Pacific Ocean	Palmer Archipelago	Markowski 1952b
<i>D. cordatum</i>		<i>Erignathus barbatus</i>	Arctic Ocean	Midway Atoll/Hawaii	Andersen 1987, Rausch 1969
	Phocinae	<i>Pagophilus groenlandicus</i>	Pacific Ocean	Bernard Harbour	Cooper 1921
		<i>Phoca largha</i>	Arctic Ocean	Novaya Zemlya (west coast)	Vagin 1933
		<i>Phoca vitulina</i>	Atlantic Ocean	Disko Island	Krabbe 1868
			Arctic Ocean	Svalbard	Markowski 1952a
	Otariinae		Pacific Ocean	St. Lawrence Island	Hilliard 1960, Fiscus et al. 1976
		<i>Eumetopias jubatus</i>	Arctic Ocean	Disko Island	Ariola 1899
			Pacific Ocean	Svalbard	Markowski 1952a
<i>D. ditremum</i>	Monachinae	<i>L. weddellii</i>	Southern Ocean	Alaska	Shults 1982
				Kattegat-Skagerrak/ Baltic Sea	Heide-Jorgensen 1992
				Wadden Sea	Strauss et al. 1991
				Svalbard	Zschokke 1903
				Oregon Coast	Stroud 1978
				McMurdo Sound	Nieland 1962

Tab. 5. Continued.

<i>D. ditremum</i>	Phocinae	<i>Phoca vitulina</i>	Pacific Ocean	Alaska	Margolis & Dailey 1972
		<i>Pusa hispida</i>	Atlantic Ocean	Lake Saimaa	Sinisalo et al. 2003
<i>D. elegans</i>	Monachinae	<i>Monachus monachus</i>		St. George Arm (Black Sea)	Schnapp et al. 1962
	Phocinae	<i>Cystophora cristata</i>	Arctic Ocean	Disko Island	Krabbe 1868
<i>D. hians</i>	Monachinae	<i>M. monachus</i>	Atlantic ocean	Genoa (Italy)	Ariola 1900
	Phocinae	<i>Erignathus barbatus</i>		Tunis Island	Stossich 1895 Diesing 1850
			Arctic Ocean	Svalbard	Markowski 1952a
		<i>Phoca vitulina</i>	Atlantic Ocean	Mecklenburg (Baltic Sea)	Braun 1891
		<i>Pusa hispida</i>		Warnemünde (Baltic Sea)	Matz 1892
<i>D. lanceolatum</i>		<i>E. barbatus</i>	Arctic Ocean	Gryphiae (Baltic Sea)	Diesing 1850
				Disko Island	Krabbe 1868
				Chukchi Sea	Cooper 1921
				Kara Sea	Stunkard & Schoenborn 1936
				Kotelny Island	Linstow 1905
				Novaya Zemlya	Vagin 1933
				Taymyr Island	Linstow 1905

Tab. 5. Continued.

<i>D. lanceolatum</i>	Phocinae	<i>Erignathus barbatus</i>	Arctic Ocean	Svalbard	Fiscus et al. 1976, Guiart 1935, Markowski 1952a, Zschokke 1903, Cooper 1921. Lyster 1940 Cooper 1921, Hilliard 1960, Shulman & Popov 1982 Hilliard 1960, Popov 1975, Delyamure & Popov 1975, Delyamure et al. 1976, Stunkard & Schoenborn 1936
			Pacific Ocean	Bering Sea	
				Sea of Okhotsk	
				St. Lawrence Island	
<i>D. lashleyi</i>	Otariinae	<i>Pagophilus groenlandicus</i> <i>Phoca vitulina</i>	Arctic Ocean	Baffin Island	Lyster 1940
			Pacific Ocean	Kvichak River	Rausch & Hilliard 1970
			Arctic Ocean	Russian Coast	Popov 1982
Monachinae	<i>Pusa hispida</i>	Pacific Ocean	Arctic Ocean	Novaya Zemlya	Vagin 1933, Delyamure & Alekseev 1965
			Pacific Ocean	Sea of Okhotsk	Krotov & Delyamure 1952
		<i>Eumetopias jubatus</i>		Kuril Islands/ Sea of Okhotsk	Kovalenko 1975
		<i>Leptonychotes weddellii</i>	Southern Ocean	Balleny Islands (D'Urville Sea)	Leiper & Atkinson 1914, Maltsev 1995

Tab. 5. Continued.

<i>D. lashleyi</i>	Monachinae	<i>Leptonychotes weddellii</i>	Southern Ocean	Bellingshausen Sea	Leiper & Atkinson 1914, Maltsev & Zhdamirov 1995
				Graham Land	Leiper & Atkinson 1914, Maltsev & Zhdamirov 1995
				Ross Sea	Leiper & Atkinson 1914, Maltsev & Zhdamirov 1995
				South Shetland	Leiper & Atkinson 1914, Maltsev & Zhdamirov 1995
				Weddell Sea	Leiper & Atkinson 1914, Maltsev & Zhdamirov 1995
		<i>Ommatophoca rossii</i>		Balleny Islands (D'Urville Sea)	Maltsev & Zhdamirov 1995
				Bellingshausen Sea	Maltsev & Zhdamirov 1995
				Graham Land	Maltsev & Zhdamirov 1995
				Ross Sea	Maltsev & Zhdamirov 1995
				South Shetland	Maltsev & Zhdamirov 1995
<i>D. lobodoni</i>		<i>Lobodon carcinophaga</i>		Weddell Sea	Maltsev & Zhdamirov 1995
				Balleny Islands (D'Urville Sea)	Yurakhno & Maltsev 1994

Tab. 5. Continued.

<i>D. minutus</i>	Monachinae	<i>Monachus schauinslandi</i>	Pacific Ocean	Midway Atoll (Hawaii)	Andersen 1987, Rausch 1969
<i>D. mobile</i>		<i>Leptonychotes weddellii</i>	Southern Ocean	Balleny Islands (D'Urville Sea)	Maltsev 2000
				Graham Land	Maltsev 2000, Markowski 1952b
				McMurdo Sound	Beverley-Burton 1971
				Petermann Island	Maltsev 2000
				Ross Sea	Maltsev 2000
		<i>Ommatophoca rossii</i>		Balleny Islands (D'Urville Sea)	Maltsev 2000
				Drygalski Island off Queen Mary Land	Johnston 1937
				Graham Land	Maltsev 2000
				Petermann Island	Maltsev 2000
				Ross Sea	Maltsev 2000
<i>D. phocarum</i>	Phocinae	<i>Pusa caspica</i>	-	Caspian Sea	Delyamure et al. 1964
<i>D. pseudowilsoni</i>	Monachinae	<i>Hydrurga leptonyx</i>		South Shetland	Wojciechowska & Zdzitowiecki 1995
<i>D. pterocephalum</i>	Phocinae	<i>Cystophora cristata</i>	Arctic Ocean	Disko Island	Delyamure & Skryabin 1966
<i>D. rauschi</i>	Monachinae	<i>Monachus schauinslandi</i>	Pacific Ocean	Midway Atoll (Hawaii)	Chapin 1927, Rausch 1969, Andersen 1987
<i>D. quadratum</i>		<i>H. leptonyx</i>	Indian Ocean	Adelaide	Maltsev 2000
			Southern Ocean	Amundsen Sea	Maltsev 2000
				Argentine Islands	Maltsev 2000

Tab. 5. Continued.

<i>D. quadratum</i>	Monachinae	<i>Hydrurga leptonyx</i>	Southern Ocean	Balleny Islands (D'Urville Sea)	Maltsev 2000
		<i>Leptonychotes weddellii</i>		Bellinghausen Sea	Maltsev 2000
	Coronation Island		Maltsev 2000		
	Graham Land	Maltsev 2000			
	Kerguelen Islands	Joyeux & Baer 1954			
	Macquarie Island	Johnston 1937			
		Maltsev 2000,			
	McDonald Islands	Markowski 1952b,			
		McEwin 1957			
	Petermann Island	Maltsev 2000, Railliet & Henry 1912			
	Ross Sea	Maltsev 2000			
	South Georgia	Fuhrmann 1921,			
		Linstow 1892, Maltsev 2000			
	South Shetland	Maltsev 2000,			
		Wojciechowska & Zdzitowiecki 1995			
	Adelaide	Maltsev 2000			
	Balleny Islands (D'Urville Sea)	Maltsev 2000			
	Coronation Island	Maltsev 2000			
	Graham Land	Maltsev 2000			
	McDonald Islands	Maltsev 2000			
	Petermann Islands	Maltsev 2000			
	Ross Sea	Maltsev 2000			
	South Georgia	Maltsev 2000			

Tab. 5. Continued.

<i>D. quadratum</i>	Monachinae	<i>Lobodon carcinophaga</i>	Indian Ocean Southern Ocean	Adelaide Balleny Islands (D'Urville Sea) Coronation Island Graham Land McDonald Islands Petermann Islands Ross Sea South Georgia	Maltsev 2000 Maltsev 2000 Maltsev 2000 Maltsev 2000 Maltsev 2000 Maltsev 2000
<i>D. scoticum</i>		<i>Hydrurga leptonyx</i>		Graham Land Kerguelen Islands Macquarie Island McDonald Islands	Markowski 1952b Joyeux & Baer 1954 Johnston 1937 Maltsev 2000
<i>D. schistochilos</i>	Phocinae	<i>Erignathus barbatus</i>	Arctic Ocean	Chukchi Sea Novaya Zemlya (west coast) Svalbard	Delyamure 1955 Vagin 1933 Germanos 1896, Guiart 1935, Zschokke 1903
		<i>Pagophilus groenlandicus Phoca vitulina</i>		Svalbard Siberia Svalbard	Guiart 1935 Cholodkovsky 1914 Guiart 1935
<i>D. sp.*</i>	Monachinae	<i>Mirounga angustirostris Monachus schauinslandi</i>	Pacific Ocean	California Coast French Frigate Shoals, Laysan Island (Hawaii Islands)	Gerber et al. 1993 Dailey et al. 1988

Tab. 5. Continued.

<i>D. sp.*</i>	Phocinae	<i>Phoca vitulina</i>	Atlantic Ocean	Netherlands	Borgsteede et al. 1991
			Pacific Ocean	Gray's Harbor, Washington California Coast	Dailey & Fallace, 1989 Gerber et al. 1993
		<i>Pusa caspica</i>		Kulaly Island (Mangyshlak Peninsula)	Kurochkin & Zablotzky 1985
<i>D. wilsoni</i>	Monachinae	<i>Hydrurga leptonyx</i>	Southern Ocean	Antarctic/ King George Island	Fuhrmann 1921, Maltsev 2000, Wojciechowska & Zdzitowiecki 1995
		<i>Leptonychotes weddellii</i>		South Shetland	Wojciechowska & Zdzitowiecki 1995
		<i>Lobodon carcinophaga</i>		Petermann Island	Fuhrmann 1921, Railliet & Henry 1912
				Amundsen Sea	Maltsev 2000
				Argentine Islands	Maltsev 2000
				Balleny Islands (D'Urville Sea)	Maltsev 2000
				Bellinghausen Sea	Maltsev 2000
				Graham Land	Maltsev 2000
		<i>Ommatophoca rossii</i>		Antarctic	Fuhrmann 1921, Rennie & Reid 1912
<i>Diplogonoporus tetrapterus</i>	Phocinae	<i>Cystophora cristata</i>	Arctic Ocean	Greenland Sea	Delyamure 1966
		<i>Erignathus barbatus</i>		Iceland	Baer 1962, Krabbe 1868
				Iceland	Baer 1962

Tab. 5. Continued.

<i>D. tetrapterus</i>	Phocinae	<i>Pagophilus groenlandicus</i>	Arctic Ocean	Arctic Greenland Sea	Delyamure 1966 Treshchev 1982
		<i>Phoca largha</i>	Pacific Ocean	Bering Sea Navarin-Anadyr Karaginsky Gulf (Bering Sea)	Shults 1982 Delyamure et al. 1984 Delyamure et al. 1984, Fiscus et al. 1976
		<i>Phoca vitulina</i>		Pribilof Islands, Bristol Bay Glacier Bay, Prince William Sound (Alaska) Sea of Japan (Kit Bay) Sea of Okhotsk	Delyamure et al. 1984 Herreman et al. 2011 Belopolskaya 1960 Popov 1975
	Otariinae	<i>Pusa hispida</i>	Atlantic Ocean Arctic Ocean	Disko Island Kolokolkova Bay (Barents Sea)	Krabbe 1868 Treshchev & Popov 1975 Measures & Gosselin 1994
		<i>Callorhinus ursinus</i>	Pacific Ocean	Salluit (Canada) Alaska	Fiscus et al. 1976 Keyes 1965, Kuzmina 2015, Margolis 1954, Stunkard 1948, Rausch 1964
		<i>Eumetopias jubatus</i>		Russian Far East Bering Sea	Afanassjew 1941 Shults 1986

Tab. 5. Continued.

<i>D. tetrapterus</i>	Otariinae	<i>Eumetopias jubatus</i>	Pacific Ocean	Gulf of Alaska Karaginsky Gulf (Bering Sea) Montague Island	Shults 1986 Yurakhno 1986 Rausch 1964 Delyamure 1976, Kovalenko 1975, Krotov & Delyamure 1952, Yamaguchi 1978
<i>Flexobothrium microovatum</i>	Monachinae	<i>Mirounga angustirostris</i>	Southern Ocean	Sea of Okhotsk	Rausch 1964 Krotov & Delyamure 1952, Yamaguchi 1978
<i>Glandicephalus antarcticus</i>		<i>Ommatophoca rossii</i>		St. Lawrence Island Antarctic	Rausch 1964 Maltsev 2000, Yurakhno 1989b, Baird 1853, Railliet & Henry 1912, Rennie & Reid 1912, Shimpley 1907
<i>G. perfoliatus</i>		<i>Leptonychotes weddellii</i>		Antarctic	Yurakhno & Maltsev 1995 Johnston 1937 Yurakhno & Maltsev 1995
<i>Pyramicocephalus phocarum</i>	Phocinae	<i>Cystophora cristata</i>	Arctic Ocean	Balleny Islands (D'Urville Sea) Queen Mary Land Balleny Islands (D'Urville Sea) Commonwealth Bay McMurdo Sound Petermann Island South Shetland Island	Johnston 1937 Beverley-Burton 1971 Fuhrmann 1921, Railliet & Henry 1912 Wojciechowska & Zdzitowiecki 1995
				Iceland	Zschokke 1903

Tab. 5. Continued.

<i>P. phocarum</i>	Phocinae	<i>Erignathus barbatus</i>	Arctic Ocean	Baffin's Bay (Greenland) Bernard Harbour Iceland Karaginsky Gulf/ Bering Sea Kotelny Island/ New Siberian Islands Novaya Zemlya (west coast)	Clarke 1958 Cooper 1921 Baer 1962 Delyamure et al. 1976 Linstow 1905 Vagin 1933 Guiart 1935, Markowski 1952a, Zschokke 1903 Popov 1975, Delyamure & Popov 1975, Maejima et al. 1983
			Pacific Ocean	Svalbard Sea of Okhotsk Kivalina, Chukchi Sea (Alaska)	Fiscus et al. 1976, Johnson et al. 1966, Rice 1963 Hilliard 1960 Popov 1975 Delyamure et al. 1984 Popov 1975, Popov 1982
	Otariinae	<i>Phoca largha</i> <i>Phoca vitulina</i> <i>Pusa hispida</i> <i>Eumetopias jubatus</i>	Pacific Ocean	St. Lawrence Island Sea of Okhotsk Pribilof Islands Sea of Okhotsk Alaska Kamchatka Oregon Coast	Fiscus et al. 1976 Yurakhno 1986 Stroud 1978

Tab. 5. Continued.

<i>P. phocarum</i>	Otariinae	<i>Eumetopias jubatus</i>	Pacific Ocean	Sakhalin/ Kuril Islands/ Sea of Okhotsk	Kovalenko 1975, Krotov & Delyamure 1952
		<i>Callorhinus ursinus</i>		Sea of Okhotsk	Chupakhina 1971
<i>Ligula colymbi</i>	Phocinae	<i>Pusa caspica</i>		Sea of Okhotsk	Chupakhina 1971
<i>Schistocephalus solidus</i>		<i>Pusa hispida</i>		Caspian Sea	Delyamure et al. 1964
				Baltic Sea	Delyamure et al. 1980

* Unspecified *Diphyllobothrium* with previously not mentioned location of infecting the given Phocid.

Tab. 6. List of diphyllbothriidean parasites invading Odobenidae with their geographical distribution .

Parasite Species	Host		Locality		References	
	Subfamily	Species	Ocean	Land/ Island/ Archipelago/Sea		
<i>Diphyllobothrium cordatum</i>	Odobenidae	<i>Odobenus rosmarus</i>	Arctic Ocean	Disko Island	Ariola 1899	
					Siberia	Cholodkovsky 1914
					Chukchi Sea	Protasova 2006
<i>D. fayi</i>				Arctic Ocean	Skull Cliff, Beaufort Sea	Rausch 2005
			Pacific Ocean	St. Lawrence Island, Bering Sea	Rausch 2005	
<i>D. sp.**</i>				Kodiak Island, Alaska	Hilliard 1972	

** Unspecified *Diphyllobothrium* in the subfamily Odobenidae.

4.1.1. Maps of the geographical distribution of Pinnipedia and their parasites of the order Diphylobothriidea

For more pronounced illustration of the relationships among diphylobothriidean tapeworms, their marine hosts and their geographical distribution, the obtained data were transferred from the Table 5. and Table 6. to maps of geographical distribution of individual species of phocids (Fig. 2. - Fig.14.), otariids (Fig. 15. - Fig. 25.) and odobenids (Fig. 26.) and their diphylobothriid cestodes with the so far described occurrence.

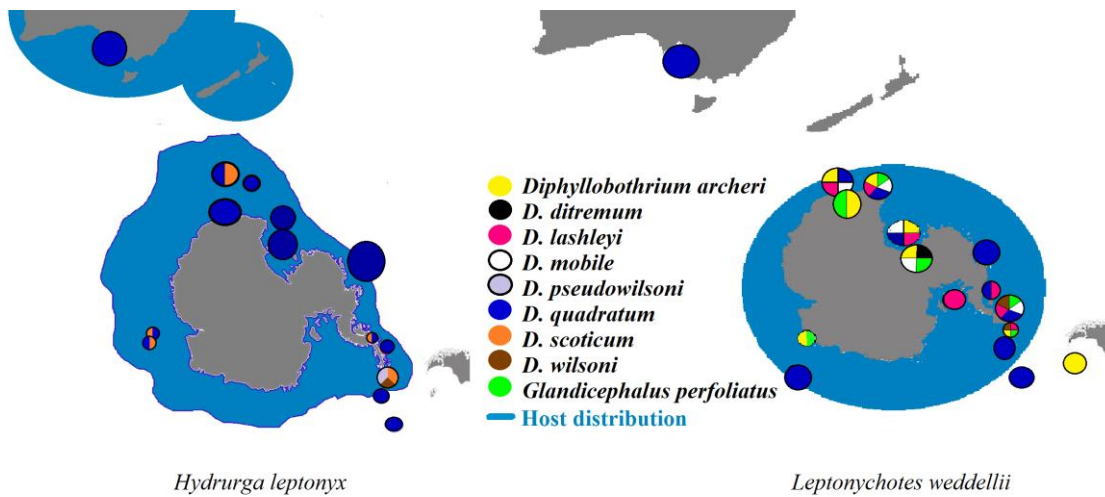


Fig. 2. Occurrence of diphylobothriidean tapeworms in *Hydrurga leptonyx* and *Leptonychotes weddellii*.

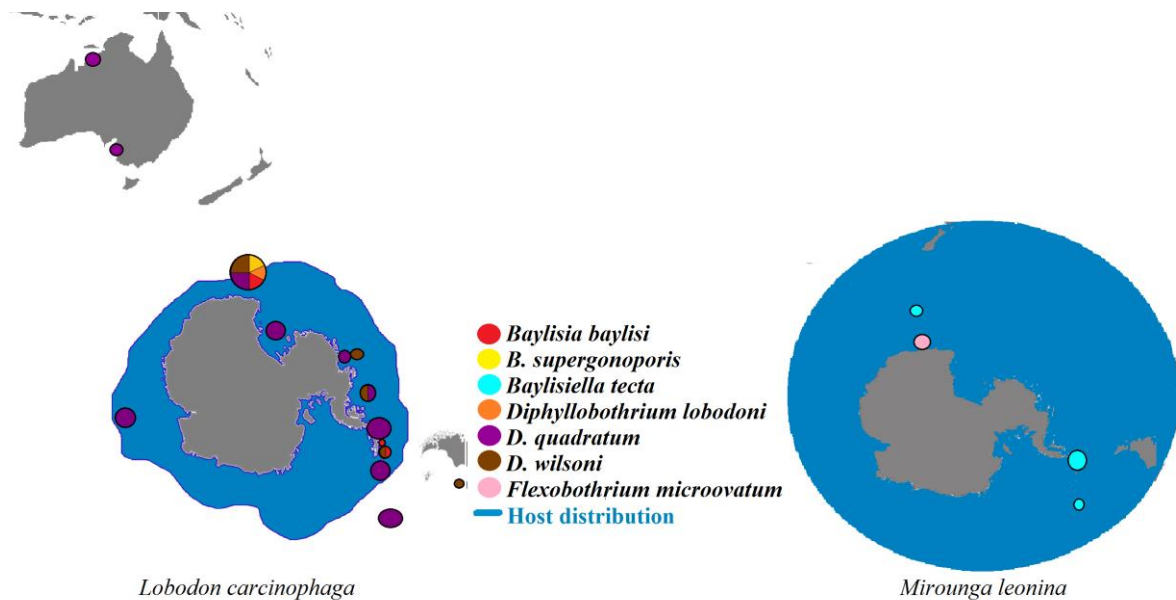


Fig. 3. Occurrence of diphylobothriidean tapeworms in *Lobodon carcinophaga* and *Mirounga leonina*.

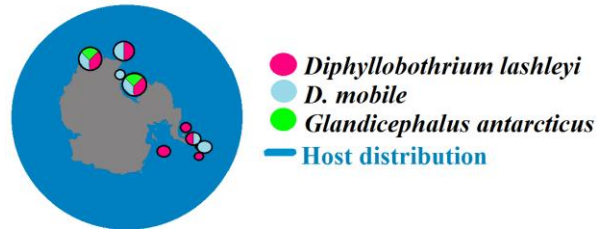


Fig. 4. Occurrence of diphyllbothriidean tapeworms in *Ommatophoca rossii*.

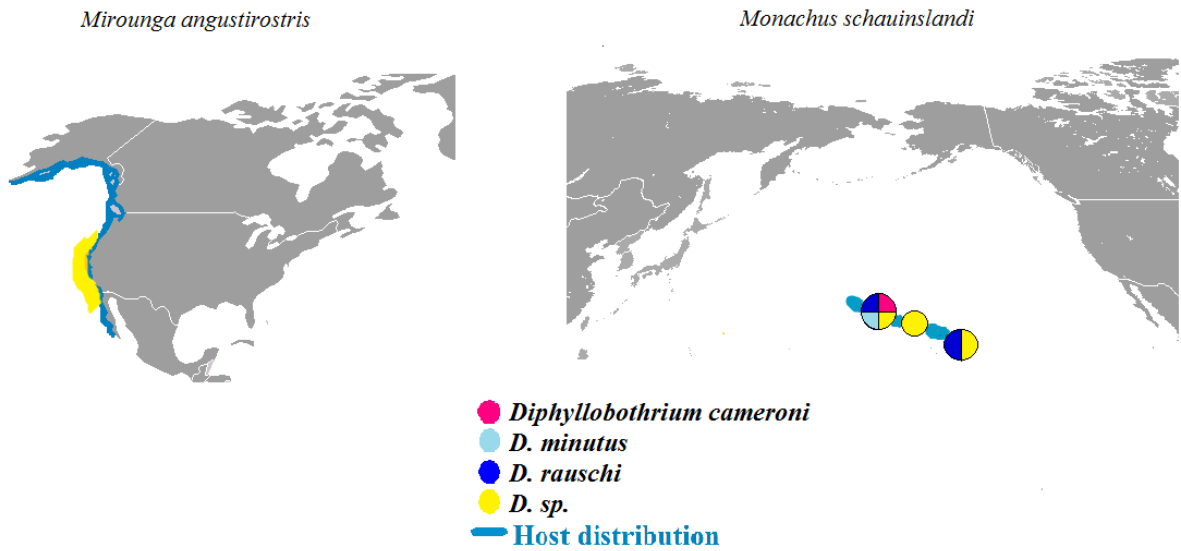


Fig. 5. Occurrence of diphyllbothriidean tapeworms in *Mirounga angustirostris* and *Monachus schauinslandi*.

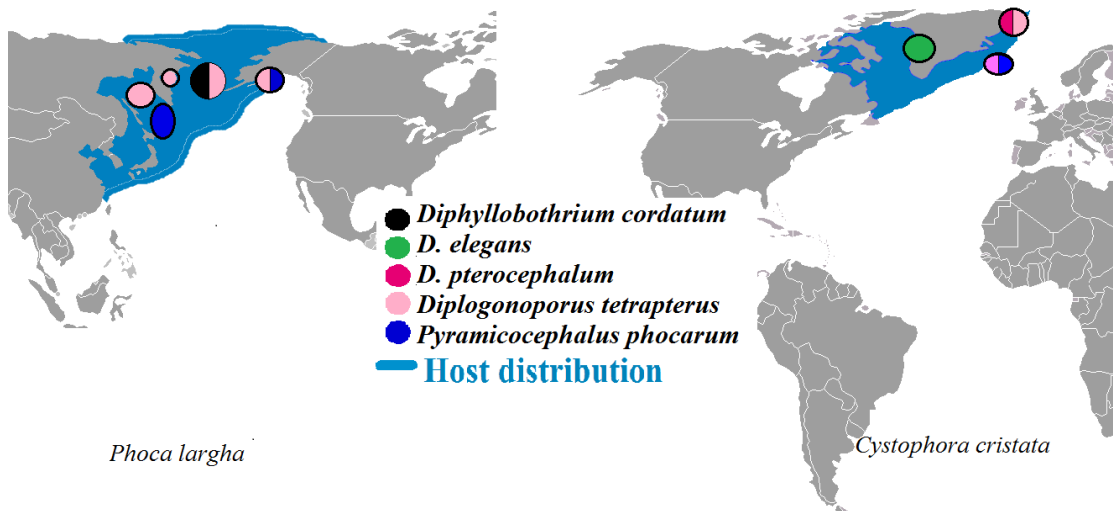


Fig. 6. Occurrence of diphyllbothriidean tapeworms in *Phoca largha* and *Cystophora cristata*.

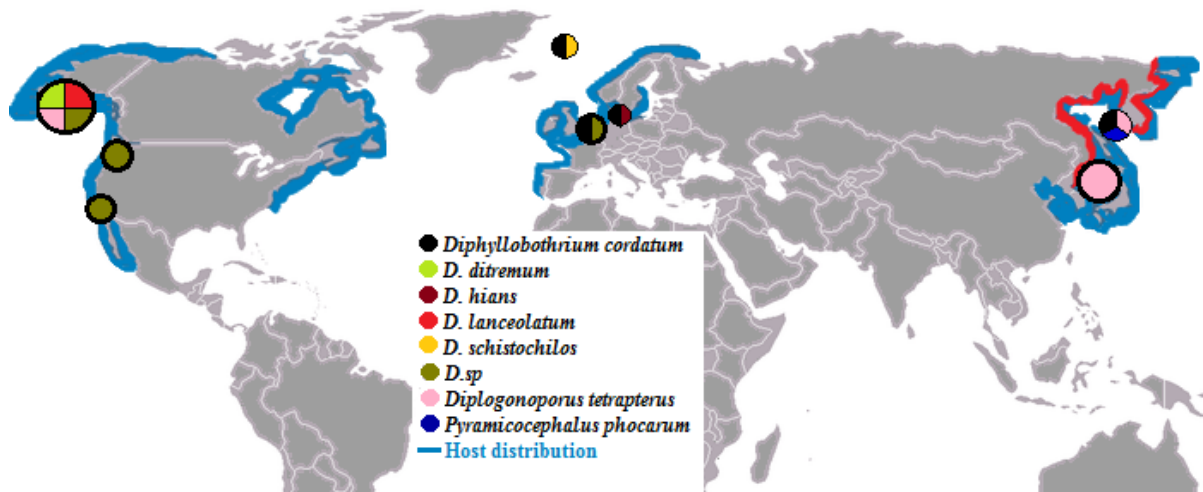


Fig. 7. Occurrence of diphyllobothriidean tapeworms in *Phoca vitulina*.

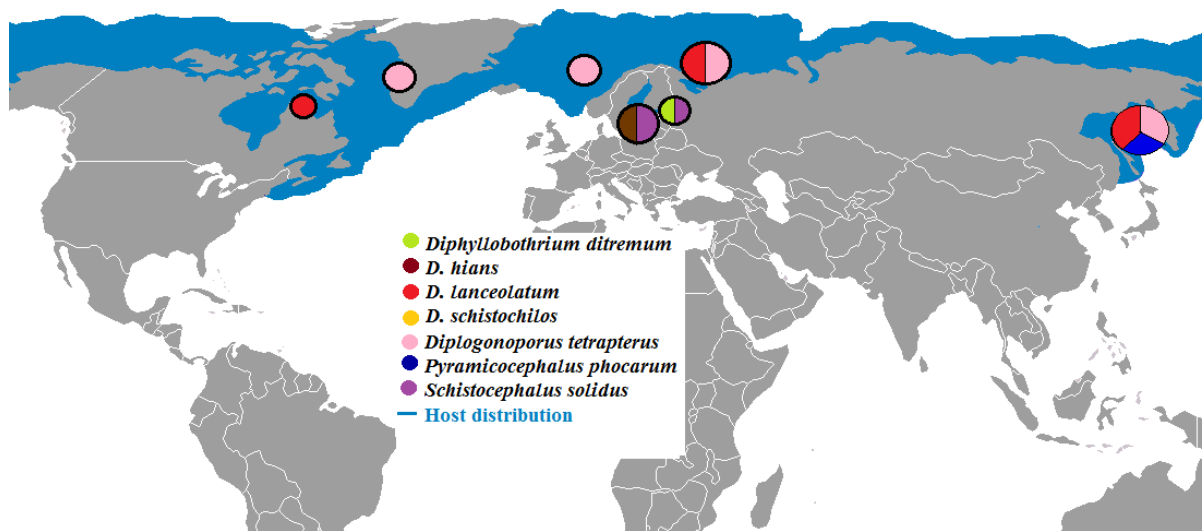


Fig. 8. Occurrence of diphyllobothriidean tapeworms in *Pusa hispida*.

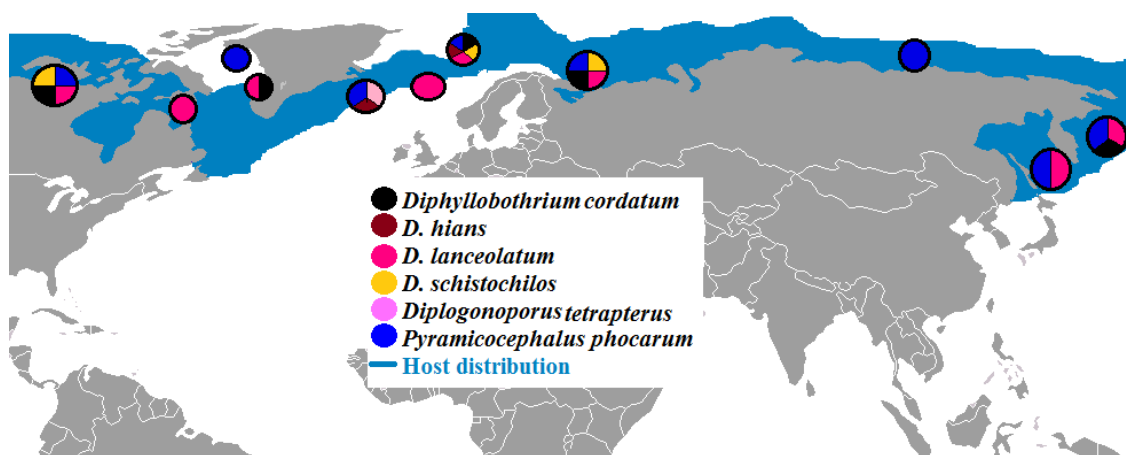


Fig. 9. Occurrence of diphyllobothriidean tapeworms in *Erignathus barbatus*.

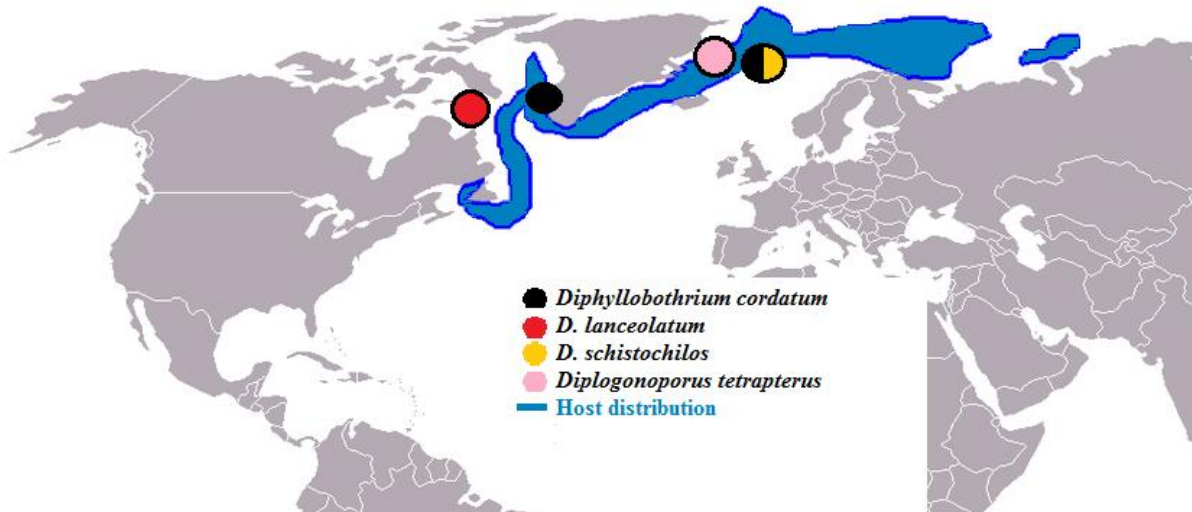


Fig. 10. Occurrence of diphyllobothriidean tapeworms in *Pagophilus groenlandicus*.

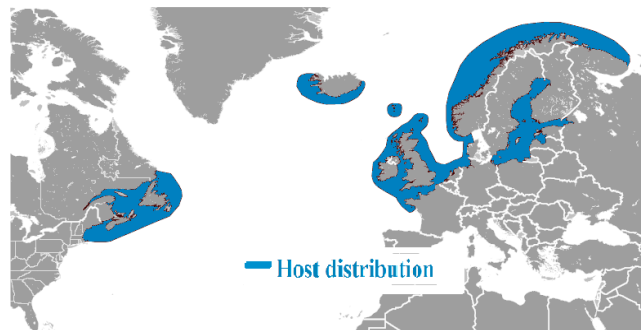


Fig. 11. Occurrence of diphyllobothriidean tapeworms in *Halichoerus grypus*.

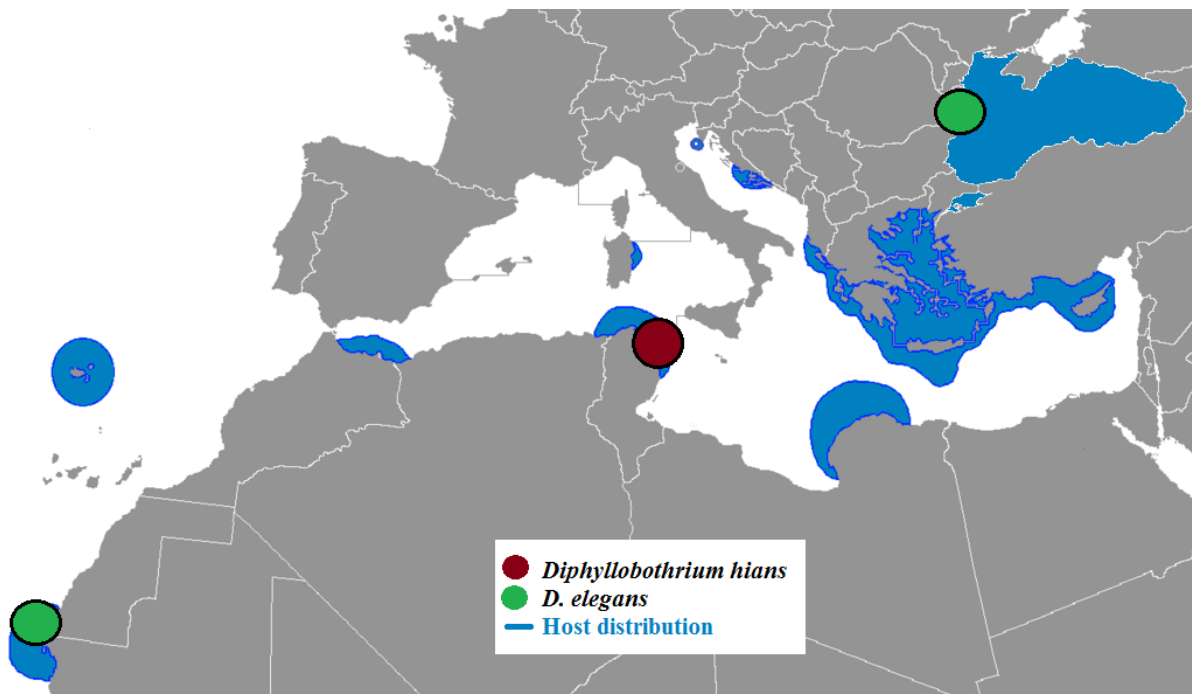


Fig. 12. Occurrence of diphyllobothriidean tapeworms in *Monachus monachus*.

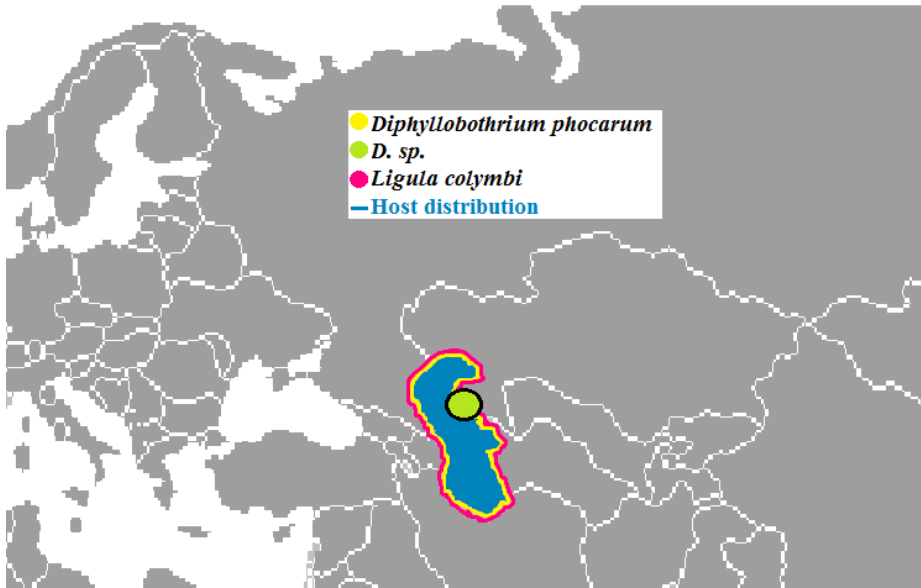


Fig. 13. Occurrence of diphyllobothriidean tapeworms in *Pusa caspica*.



Fig. 14. Distribution of *Pusa sibirica*.

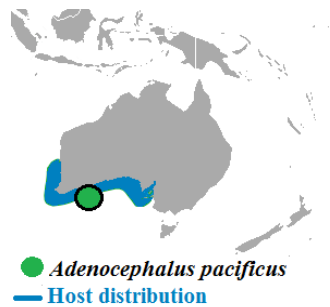


Fig. 15. Occurrence of diphyllobothriidean tapeworms in *Neophoca cinerea*.

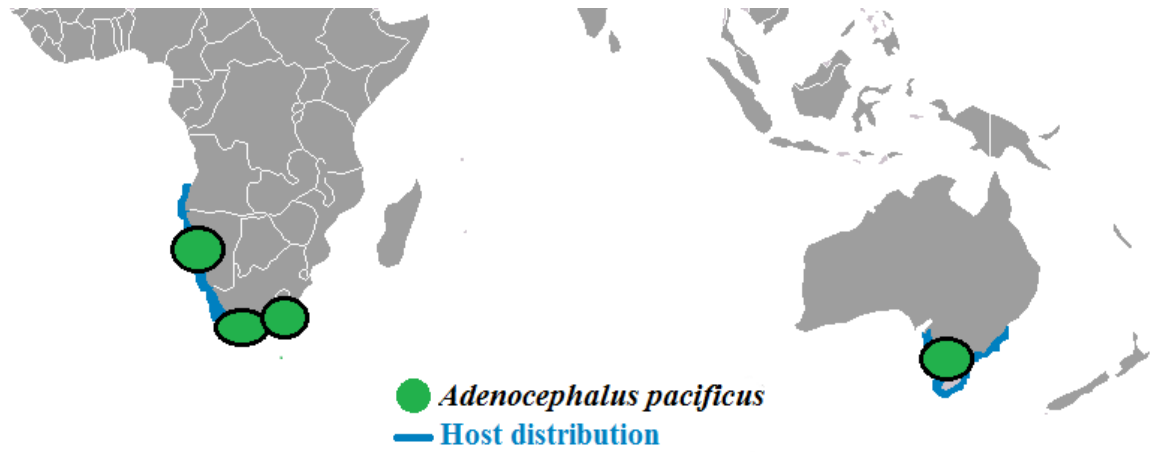


Fig. 16. Occurrence of diphyllobothriidean tapeworms in *Arctocephalus pusillus*.



Fig. 17. Distribution of *Phocarcetos hookeri*. Fig. 18. Distribution of *A. forsteri*.

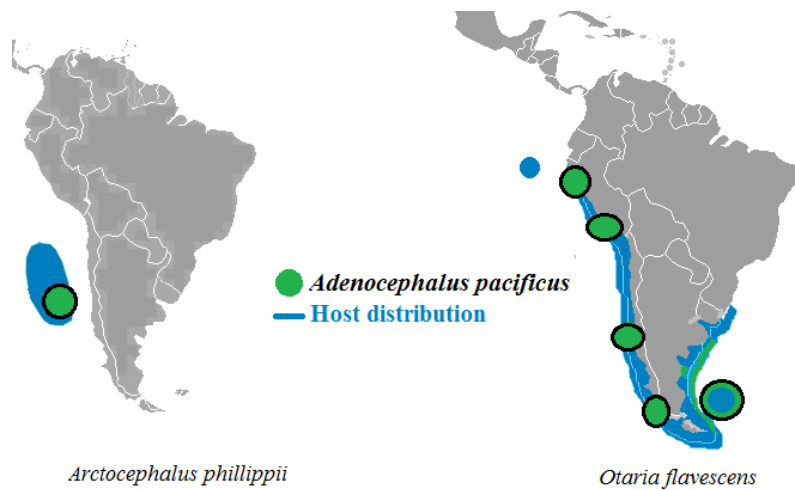


Fig. 19. Occurrence of diphyllobothriidean tapeworms in *A. phillippii* and *Otaria flavescens*.

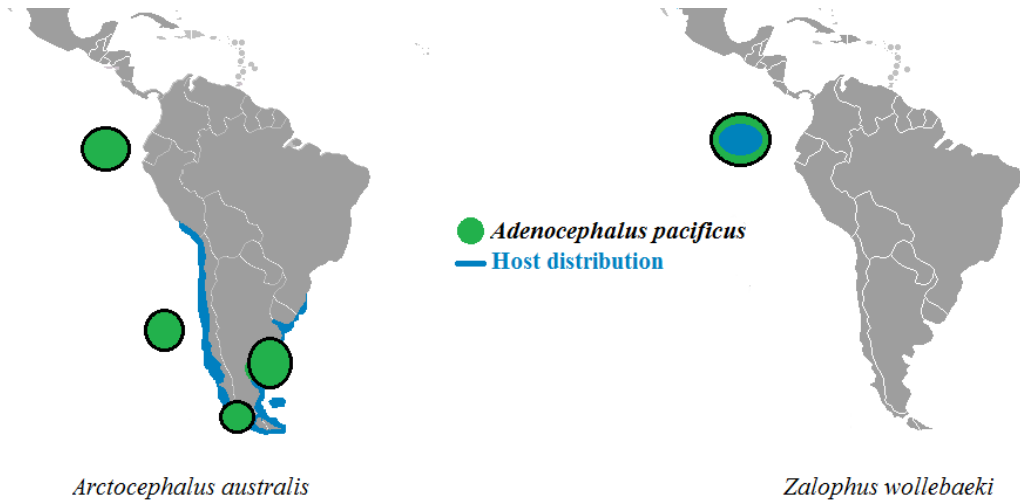


Fig. 20. Occurrence of diphyllobothriidean tapeworms in *A. australis* and *Zalophus wollebaeki*.

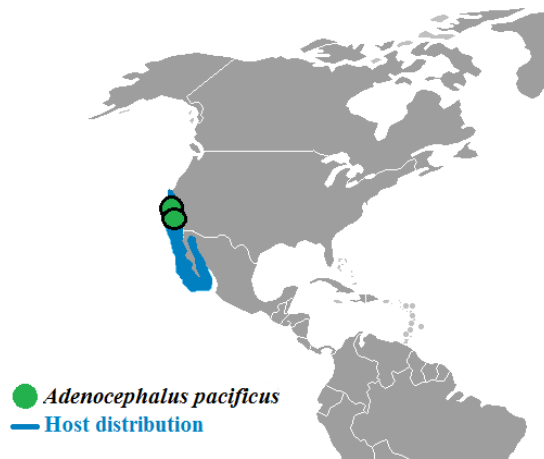


Fig. 21. Occurrence of diphyllobothriidean tapeworms in *Z. californianus*.

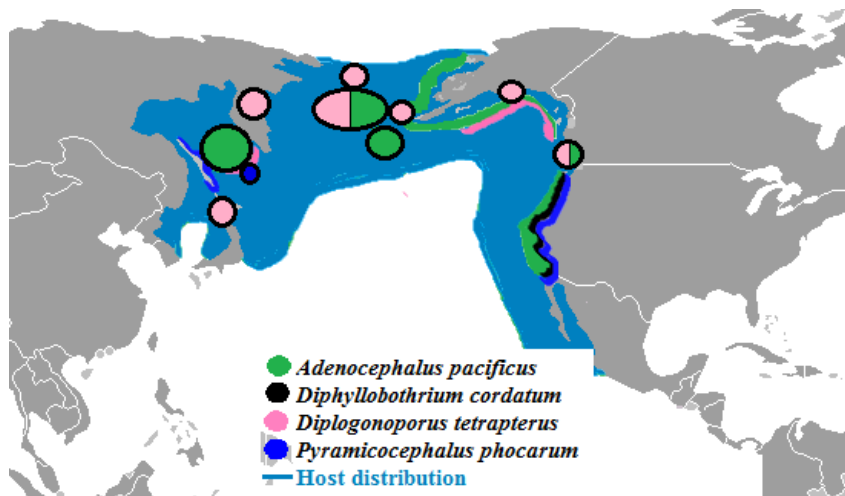


Fig. 22. Occurrence of diphyllobothriidean tapeworms in *Eumetopias jubatus*.

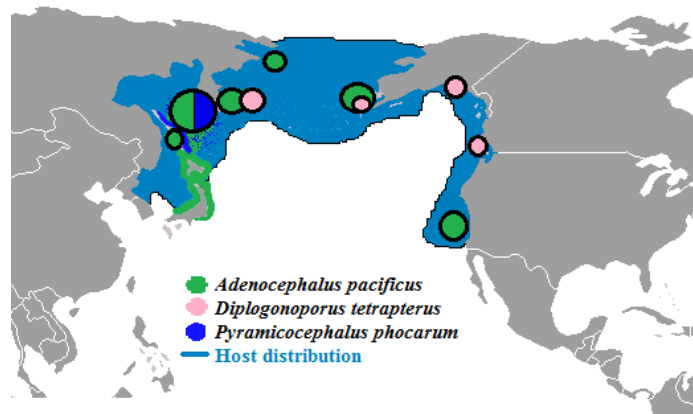


Fig. 23. Occurrence of diphyllobothriidean tapeworms in *Callorhinus ursinus*.

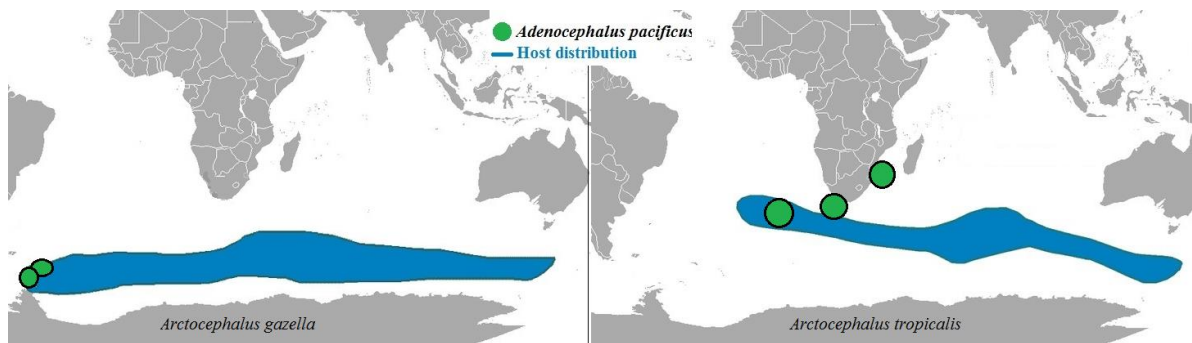


Fig. 24. Occurrence of diphyllobothriidean tapeworms in *A. gazella* and *A. tropicalis*.



Fig. 25. Distribution of *A. townsendi*.

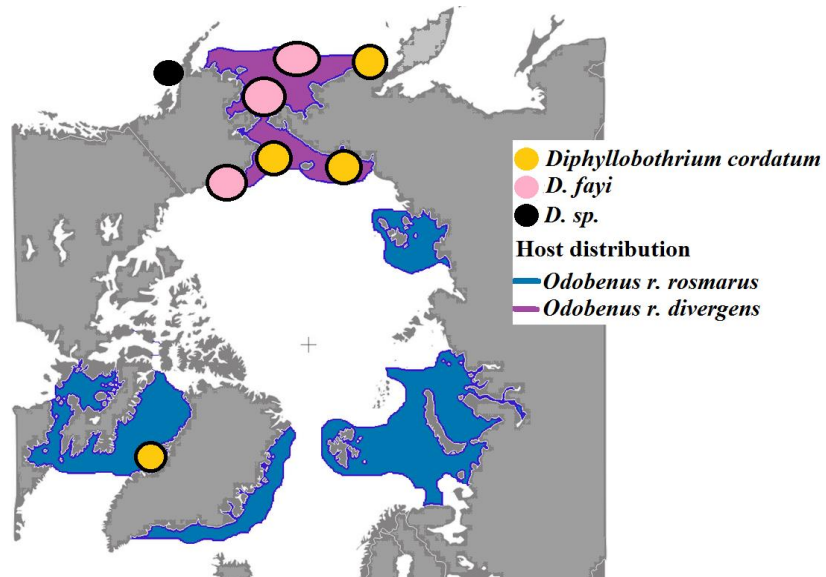


Fig. 26. Occurrence of diphyllbothriidean tapeworms in *Odobenus rosmarus*.

Five members of Phocids belonging to group Monachinae have the same range of distribution. *Hydrurga leptonyx*, *Leptonychotes weddellii* (Fig. 2.), *Lobodon carcinophaga*, *Mirounga leonina* (Fig. 3.) and *Ommatophoca rossii* (Fig. 4.) can be generally localized in the realm of the Arctic Ocean ((Rice 1988; Wilson & Reeder 2005; Yonezawa et al. 2009; Berta & Churchill 2012). Types of their diphyllbothriidean cestodes differ across the phocid species. The Weddell seal (*L. weddellii*) is a host for 7 species of the order Diphyllbothriidea: *Diphyllobothrium archeri*, *D. ditremum*, *D. lashleyi*, *D. mobile*, *D. quadratum*, *D. wilsoni* and *Glandicephalus perfoliatus*.

Diphyllobothrium lashleyi is a common tapeworm of the Weddell seal and the Ross seal (*O. rossii*) with the same localities of infection (Tab. 5.). The Leopard seal, Weddel seal and Crabeater seal (*L. carcinophaga*) harbour *D. quadratum*. The same group of seals, with the addition of Ross seal, are hosts for *D. wilsoni*, with the similar localities as mentioned above. The Crabeater seal is a host for *D. wilsoni* in more areas, as Amundsen Sea, Argentine Islands, Balleny Islands, Bellinghausen Sea and Graham Land (Maltsev 2000). Southern elephant seal (*M. leonina*) is the only member across the Phocidae and Otariidae infected by *Baylisiella tecta* and *Flexobothrium microovatum*. Other cestodes, with hosts of the phocids living close to Antarctic, include *Baylisia baylisi*, *B. supergonoporis*, *D. cameroni*, *D. elegans*, *D. hians*, *D. lobodoni*, *D. minutus*, *D. scoticum*, *D. rauschi*, *D. pseudowilsoni*, *Glandicephalus antarcticus* and *Schistochilos perfoliatus*.

In *Mirounga angustirostris* (Fig. 5.) and *Phoca vitulina* (Fig. 7.) were found unidentified species of the genus *Diphyllobothrium* along the California coast (Gerber et al.

1993). They belong to the group of seals living in the northern hemisphere. Seals inhabiting North Pacific Ocean, North Atlantic Ocean and Arctic Ocean are hosts for the following diphyllbothriidean parasites: *D. cordatum*, *D. ditremum*, *D. elegans*, *D. hians*, *D. lanceolatum*, *D. pterocephalum*, *D. schistochilos*, *Diplogonoporus tetrapterus*, *Ligula colymbi*, *Pyramicocephalus phocarum* and *Schistocephalus solidus*, while the parasites of *Diphyllbothrium cameroni*, *D. minutus* and *D. rauschi* belong to the endemic species of Hawaiian monk seal (Fig. 5.), inhabiting only Hawaii Islands (Rice 1998).

The Baikal seal (*Pusa sibirica*) is endemic to the Baikal Sea with no diphyllbothriidean cestodes (Fig. 14.).

Members of Otariidae predominantly inhabit southern hemisphere, including Australia and South America with adjacent islands, and South Africa. Almost all species of sea lions are hosts of *Adenocephalus pacificus*. Sea lions living in the northern hemisphere (except *Arctocephalus townsendi* and *Zalophus californianus*) are infected by more species of diphyllbothriidean tapeworms. Records from the Steller sea lion (*Eumetopias jubatus*) (Fig. 22.) and the northern fur seal (*Callorhinus ursinus*) (Fig. 23.) are showing occurrence of *A. pacificus*, *Diplogonoporus tetrapterus* and *P. phocarum* in the North Pacific Ocean. In the Steller sea lion was also detected *D. cordatum*. The New Zealand sea lion (*Phocarctos hookeri*) (Fig. 17.), New Zealand fur seal (*Arctocephalus forsteri*) (Fig. 18.) and Guadalupe fur seal (*A. townsendi*) (Fig. 25.) were found negative for cestodes of the order Diphyllbothriidea.

Both subspecies of walrus are distributed in northern hemisphere (Fig. 26.). The Pacific walrus is a host for at least two species of the order Diphyllbothriidea (*D. cordactum*, *D. fayi*), while the only one known species of the Atlantic walrus is *D. cordatum* from the Kodiak Island near Alaska. Diphyllbothriidean parasites of the Pacific walrus are distributed both in Pacific Ocean and Arctic Ocean.

4.2. Coprological examination of *Phoca vitulina*

The examined material was negative for the diphyllbothriidean tapeworms (Cestoda), but positive for several trematode and nematode species. Eggs of parasites were measured and photographed by OLYMPUS cellSens Standard 1.13 imaging software and Quick PHOTO MICRO 2.3 imaging software. Eggs were divided into three different groups based on their size. From the total number of 107 eggs, 43 eggs measured on average $23 \times 47 \mu\text{m}$ (width 19—27, length 42—51), 44 eggs measured on average $18 \times 35 \mu\text{m}$ (width 15—22, length 30—39) and 20 eggs measured on average $11 \times 21 \mu\text{m}$ (width 10—13, length 18—25).

Faecal samples contained definitely at least three species of nematodes. From 20 seals, 14 patients harboured larvae (Fig. 27.) of the *Anisakis* Dujardin, 1845 complex, which sizes varied from 60 to 299 μm . Samples of another three seals contained eggs of *Anisakis* with average size $47 \times 45 \mu\text{m}$ (Fig. 30.). Eggs of the average size $11 \times 21 \mu\text{m}$ (Fig. 28.) probably belonged to lungworms of the species *Parafilaroides gymnurus* (Railliet, 1899). Eggs of the genus *Capillaria* Zeder, 1800 (Fig. 31.) occurred only in two seal patients (1 adult, 1 juvenile). The size of eggs reached approximately $63 \times 30 \mu\text{m}$ and the species is recognized as *Capillaria delamurei*, Zablotskii, 1971.

Trematode eggs (Fig. 29.) belonged to the class of Heterophyidae Odhner, 1914 and very probably to the species *Ascocotyle septentrionalis* (van den Broek, 1967).



Fig. 27. Microphotograph of the *Anisakis* complex larva.



Fig. 28. Egg of Nematoda, *Parafilaroides* cf. *gymnurus*.

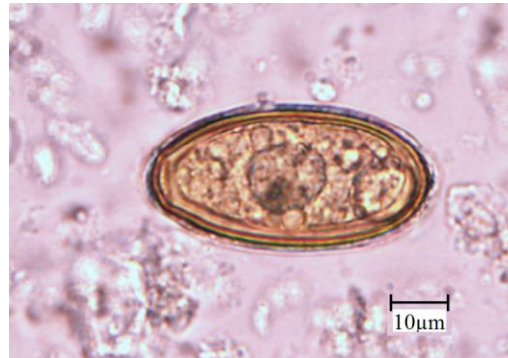


Fig. 29. Egg of Trematoda, *Ascocotyle septentrionalis*.

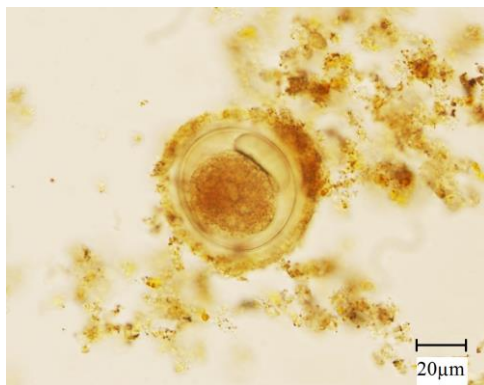


Fig. 30. Egg of Anisakidae (Nematoda). Fig. 31. Egg of *Capillaria* (Nematoda).

Comparing of sedimentation and flotation methods based on the endoparasites mentioned above, have shown following results: larvae of Anisakiidae were more often recognized using the sedimentation technique with 77% success rate (flotation - 48%), while the flotation method was more efficient (with 74% success rate) in occurrence of nematode and trematode eggs (sedimentation - 59%).

Due to combination of these two coprological methods, it was possible to conclude that 95% of examined seal patients had trematode eggs and 70 % of mentioned seals were infected by larvae of Anisakiidae. The sedimentation method also revealed the presence of fungi *Alternaria* Nees ex Wallroth, 1816 and digested remains of crustaceans.

The periods between sampling (i.e. from arrival of the patient to 24-48 hours (or more) after giving a medication) was established to determine the effectiveness of medicaments attacking endoparasites of harbour seal. The results showed the reduction of parasites even after 24 hours of taking medicine.

5. DISCUSSION

5.1. Literature review

The first occurrence of diphyllbothriidean tapeworm infecting pinnipeds is dated to 1848, when Siebold described *Diplogonoporus tetraapterus* for the first time in *Phoca vitulina* (Phocidae) (Siebold 1848). At this time, it has not yet been possible to identify species by using molecular-biology techniques, which can provide further information. Until then, the authors of the publications could have been mistaken in determinations of species. In some cases, species of tapeworms or marine hosts were not mentioned at all. Based on the publications, any references on parasites of *Pusa sibirica* (Phocidae) and three members of Otariids, called *Arctocephalus forsteri*, *Arctocephalus townsendi* and *Phocarctos hookeri*, as hosts of Diphyllbothriids, were missing. In *P. sibirica* were previously present species of Anoplura and Nematoda (Felix 2013). The Russian publications, focused on parasites of marine mammals, were precise from the 19th century. It is very unlikely to consider, that the authors overlooked the order Diphyllbothriidea in elaborated seals. The parasitofauna of above-mentioned otariids is probably less known due to small number of studies.

Linstow (1901) mentioned the existence of *Pyramicocephalus phocarum* infecting the genus *Phoca*, however, with any identification of the species. It is difficult to determine which species of the genus *Phoca* served as the host for the above mentioned tapeworm, because both species (*P. vitulina* (Fig. 7.) and *P. largha* (Fig. 6.) are distributed worldwide in the northern hemisphere, and both of them were described to be associated with *Pyramicocephalus phocarum* (Popov 1975; Popov 1982; Delyamure 1984). All available data related to the given hosts and parasite are located in the Pacific Ocean, while the information from Linstow (1901) describes the locality of Iceland. Due to this fact, the locality was kept in the database for possible verifying in the future.

In the southern hemisphere, in area of the Antarctic Ocean, most of individuals of same phocid and otariid species originated from same localities (Fig. 2 – 4, Fig. 24.). It was probably due to existence of research stations, as Arctowski Station localized on the King George Island of the South Shetland Archipelago, where was easier to elaborate fresh-collected material compared to unknown, uninhabited areas of Antarctic (Wojciechowska & Zdzitowiecki 1995).

The diet of *Odobenus rosmarus* generally consists of benthic invertebrates, while they exceptionally harbour cestode plerocercoids after eating fish (Yurakhno 1971). The species *Diphyllbothrium fayi* is strictly host-specific to *Odobenus rosmarus divergens*. It is hard to

identify specific host-subspecies of *D. latum* and *D. roemeri* present in *O. rosmarus*, because they were mentioned in studies with unknown locality. Other parasite, *D. cordatum* is (beside the genus *Odobenus*) also known from another 3 genera of phocids and one genus of otariids. The generalist, *D. cordatum*, invades species *Erignathus barbatus*, *Pagophilus groenlandicus*, *Phoca largha*, *Phoca vitulina*, *Eumetopias jubatus* and the above-mentioned *Odobenus rosmarus*. The occurrence of *D. cordatum* is common in phocids, but rare in odobenids. *Adenocephalus pacificus* is family-specific to Otariidae. Another three generalists invade digestive tract, at least, of 4 phocids and 1 otariid. *Diphyllobothrium lanceolatum* infects *E. barbatus*, *P. groenlandicus*, *P. vitulina* and *Pusa hispida* from the family Phocidae. The only one otariid host for *D. lanceolatum* is *E. jubatus*. The generalist *Diplogonoporus tetraapterus* invades members of pinnipeds as for *D. lanceolatum*, including phocids *Cystophora cristata*, *Phoca largha* and one extra otariid member *Callorhinus ursinus*. *Pyramicocephalus phocarum* parasitizes the same range of pinniped hosts as *D. tetraapterus*, except for *Pagophilus groenlandicus*. Other 29 diphyllbothriids are host-specific to the family Phocidae, while 17 tapeworm species of the order Diphyllbothriidea are identified as strict specialists. *Diphyllobothrium pseudowilsoni* and *D. scoticum* are specialists for *Hydrurga leptonyx*. *Diphyllobothrium archeri* and *Glandicephalus perfoliatus* are specialist for *Leptonychotes weddellii*. *Baylisia baylisi*, *B. supergonoporis* and *D. lobodoni* are specialists for *Lobodon carcinophaga*. *Mirounga angustirostris* is distributed along California coast and harbours cestode of the genus *Diphyllobothrium*, but the species of the parasite is unknown (Gerber et al. 1993). *Diphyllobothrium cordatum*, *P. phocarum* and *Diplogonoporus tetraapterus* share the same range of distribution (Fig. 6 – 9) of their phocids hosts. Another common tapeworm of this locality (Fig. 21 – 23) is *Adenocephalus pacificus* (cosmopolite), which is a strict specialist to otariids. Other phocids harbour from 2 to 8 strict specialists. Another 12 species of diphyllbothriidean tapeworm in phocids are generalists. According to Rausch (2005), the host-specificity of cestodes in marine mammals is low, while the results of this study shows that the host-specificity of the order Diphyllbothriidea in pinnipeds is relatively high (51 % of diphyllbothriidean tapeworms are specialists).

Diphyllbothriidean specialists of *Lobodon carcinophaga* are limited in distribution by range of localities of their host (Fig. 3.). *B. baylisi*, *B. supergonoporis*, *Baylisiella tecta* and *D. lobodoni* are distributed near the Balleny Islands and few other localities, while the generalist *D. quadratum* is distributed along the entire Antarctic (Wojciechowska & Zdzitowiecki 1995; Yurakhno & Maltsev 1997). *Diphyllobothrium scoticum* has in

Hydrurga leptonyx and *G. perfoliatus* in *L. weddellii* the similar range of distribution (Fig. 2.) as *D. quadratum*. *Flexobothrium microovatum* is limited only to one place (St. Lawrence Island, Antarctic) of the *M. leonina* distribution (Fig. 3.) (Rausch 1964). The location (Fig. 5.) of *Monachus schauinslandi* limits area of distribution of its specialists: *D. cameroni*, *D. minutus*, *D. rauschi* near the Midway Atoll in the Pacific Ocean (Rausch 1969). The generalists, *D. cordatum* and *Diplogonoporus tetrapterus*, are distributed exclusively in the northern hemisphere. Specialists of species *D. phocarum* and *Ligula colymbi*, invading endemic species *Pusa caspica* (Fig. 13.), are limited to Caspian Sea (Delyamure et al. 1964). The opposite case is already above-mentioned *Adenocephalus pacificus*, which is widely distributed and its occurrence may be limited by distribution of its intermediate hosts.

In order to maintain timeliness of the information, it is useful to repeat the study. In case of *Pusa caspica*, the examination of parasites of this host was done only few times, one mentioning *D. phocarum* and *Ligula colymbi*, and two mentioning unidentified species of the genus *Diphyllobothrium* (Delyamure et al. 1964; Kurochkin 1958).

According to The IUCN Red List of Threatened Species, the Caspian seal is currently classified as endangered species, so the studies of his endoparasites may be difficult (www.iucnredlist.org⁴). In this and many other cases, the coprological examination of faeces is the method of choice. Because the collection of material can be challenging, a great advantage is the existence of rescue, rehabilitation and research centers, such as SRRC in Netherlands or Pacific Marine Mammal Center in California, USA. In these centers it is easier to collect faecal samples than in nature because members (nurses) of the organisation have to be in direct contact with seals and sea lions, if necessary.

5.2. Material from coprology of *Phoca vitulina*

The samples were negative for the cestode parasites. On the other hand, several species of nematodes and trematodes were detected in the samples.

The sedimentation technique is more efficient to prove heavy eggs of trematodes or acathocephalans in the faecal material, while the flotation technique can show presence of lighter elements of sample as larvae, oocysts and eggs of Protozoa, Cestoda and Nematoda.

The reason of no occurrence of protozoa or any diphyllbothriidean stages in the faecal samples can be the age of seal patients. The majority of seal patients of my study were juveniles (around 3 months old). Cestodes of the order Diphyllbothriidea could be present in bodies of seal patients, but their demonstrable stages in faeces did not exist yet, due to low

age of the seal. It is also possible, that they were not very efficient in feeding at this period. The probability of infection were lower due to lack of food.

From the previous studies, *Phoca vitulina* harbours a wide range of tapeworms of the order Diphylobothriidea in compare to other pinnipeds. According to elaborated data, *P. vitulina* is a host for *D. cordatum*, *D. ditremum*, *D. hians*, *D. lanceolatum*, *D. schistochilos*, *Diplogonoporus tetrapterus*, *Pyramicocephalus phocarum*, while *D. cordatum* and *D. hians* are probably common for the locality near the Netherlands in seals. Borgsteede (1991) studied 94 seals, which died during the epidemic of the phocine distemper virus. His study revealed, that only 8.5 % of examined seals, had parasites of the order Diphylobothriidea. The prevalence of these tapeworms increased in direct proportion with the age of examined seals.

The trematode *Ascocotyle septentrionalis*, present in examined samples, is in most publications known as *Phagicola* cf. *septentrionalis* van den Broek, 1967 (Gibson 2001).

Since the identification of parasites was based only on light microscopy and measurements of eggs and larvae, the endoparasites could not be determined to species with certainty. Combination of coprological methods with molecular analyses are noninvasive and perspective for the future study of seals and sea lions, which is the aim of my master thesis.

6. CONCLUSIONS

- 1) The elaborated data were summarized to gain a view of the host specificity and geographical distribution of the order Diphylobothriidea invading Pinnipeds.
- 2) From 33 species of diphylobothriids, 29 are family-specific to Phocidae, while *Diphylobothrium cordatum* is host-specific to Pinnipedia. *Adenocephalus pacificum* is family-specific to Otariidae. The species *D. fayi* is strictly host-specific to *Odobenus rosmarus divergens*.
- 3) Diphylobothriidean cestodes appear to be low host-specific, with the exception of few species, which are probably strict on related to intermediate hosts.
- 4) The faecal material from predominantly young seal patients (juveniles) of *Phoca vitulina*, was positive for following endoparasites: *Anisakis* complex, *Parafilaroides* cf. *gymnurus*, *Capillaria delamurei*, belonging to Nematoda and *Ascocotyle septentrionalis* (Trematoda). Tapeworms of the order Diphylobothriidea were not found.

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