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Diphyllobothrium, Diplogonoporus, *and* Spirometra

Roman Kuchta, Tomáš Scholz, Jan Brabec, and Barbara Narduzzi-Wicht

CONTENTS

17.1 Introduction

Most of the developed countries today are generally considered "dehelminthized," meaning that the prevalence (percentage of infected people) and medical impact of diseases caused by helminths, that is, flukes (Trematoda), tapeworms (Cestoda), parasitic roundworms (Nematoda), and spiny-headed worms (Acanthocephala), are negligible compared to other diseases. In contrast, helminthoses such as filariasis, schistosomiasis and infections with gastrointestinal nematodes, lung flukes, and many others represent serious medical and socioeconomic problems in tropical countries.[1]

However, globalization of food trade, climate change, and altered eating habits have contributed to the emergence of new foodborne helminthoses and recrudescence of those considered to be decreasingly important for human health. Among the so-called zoonoses, that is, diseases caused by parasites that can be transmitted between humans and animals, recent reemergence of human infections with fish tapeworms (*Diphyllobothrium*) and the resulting diseases diphyllobothriosis and diplogonoporosis (*Diplogonoporus*) represent examples of new challenge for public health services in developed countries. Due to the increasing popularity of eating dishes made of raw or undercooked fish, the number of cases of diphyllobothriosis in areas considered historically endemic has recently risen, where previously the disease had been in decline or almost disappeared.

Diphyllobothriosis is a human disease caused by adult stages of tapeworms of the genus *Diphyllobothrium* Cobbold, 1858 (Cestoda: Diphyllobothriidea). *Diphyllobothrium* species, commonly called broad or fish tapeworms, mature and produce eggs in the human intestine. Even though diphyllobothriosis itself is not a life-threatening disease, it is considered the most important fish-borne zoonosis caused by a cestode parasite with up to 20 million people estimated to be infected worldwide.[2] Dozens of nominal species have been described so far, but only 14 species currently recognized as valid have been reported to infect humans.[3] Out of these, only *Diphyllobothrium latum*, *Diphyllobothrium nihonkaiense*, *Diphyllobothrium dendriticum*, and *Diphyllobothrium pacificum* are considered potential human pathogens. Despite its long history (the most common broad tapeworm, *D. latum*, was described as *Taenia lata* in 1758 by C. Linné), numerous gaps persist in our understanding of the taxonomy, biology, and epidemiology of the causative agents of diphyllobothriosis. Many previously unresolved problems are now elucidated thanks to the application and continuous improvement of molecular genetic methods, which facilitate diagnostics of clinical cases and have provided explicit data on species identity. Being able to reliably distinguish among individual species of parasites improves considerably the current knowledge of their epidemiology, host specificity, and distribution.

Diplogonoporosis is a disease caused by adult tapeworms of the genus *Diplogonoporus* Lönnberg, 1892, but will be discussed along with diphyllobothriosis in this text, given the striking similarity of various aspects of these diseases. To prevent any confusion, the genus name of *Diplogonoporus* will be abbreviated to *Dipl.* throughout the text.

Another human disease, sparganosis (spirometrosis), is caused by tapeworms of the genus *Spirometra* Faust, Campbell, and Kellogg, 1929 (Cestoda: Diphyllobothriidea), which are close relatives of both *Diphyllobothrium* and *Diplogonoporus*. Infective agents of sparganosis are tapeworm larval stages (plerocercoids) that infect second intermediate hosts or, in very rare cases, adult tapeworms.[4] *Spirometra* infections may seriously impact the human health—particularly those caused by *Sparganum proliferum*.[1] More than 1400 human cases of sparganosis have been reported globally, including travel- and immigration-associated cases.[5] Since humans do not represent a natural definitive host of *Spirometra*, plerocercoids are usually located in the subcutaneous tissue and muscles or may invade a number of internal organs such as the eyes, abdominal cavity, pleura (lung), pericardium (heart), liver, lungs, kidney, urinary bladder, and brain or spine.[6]

Reliable identification of *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* tapeworms to the species level is difficult and requires correctly preserved parasite material and a high level of expertise, because numerous morphological characteristics of individual taxa tend to overlap and may be even difficult to observe (e.g., sagittal sections of proglottids are needed). Even though species identification is not considered necessary by clinicians and medical doctors (the same anthelmintics are used for treatment,

and only the drug dosage may differ according to parasite species), it is essential for the epidemiological analysis. The recent application of genetic methods has considerably improved the specificity and speed of detection of the causative agents of human infection and helped determine the sources of human cases. Molecular diagnostics thus represents a valuable epidemiological tool in the control of these human zoonoses.

In this chapter, basic data on the causative agents of diphyllobothriosis, diplogonoporosis, and sparganosis are summarized with a focus on the molecular diagnostics of human cases. Existing gaps in the current knowledge of the epidemiology of the most important species of *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* are discussed.

17.2 Morphology and Classification

17.2.1 Morphology

Diphyllobothrium, *Diplogonoporus*, and *Spirometra* are usually large worms whose total length spans from several centimeters (e.g., *Diphyllobothrium lanceolatum*) up to several meters in total length (most frequently 3–12 m, exceptionally up to 27 m in *Diplogonoporus balaenopterae*) (Figure 17.1). However, the body size may vary among individuals of conspecific worms according to the host species, body size, physiological state, infection intensity, and other factors.[7] The body (strobila) of these tapeworms consists of several hundred (up to 2000–4000) of segments (proglottids), each containing one or two sets of hermaphroditic genitalia that consist of both male and female reproductive organs. Individual proglottids are well separated from each other, usually have a slightly trapezoid shape, with the posterior margin being somewhat wider than the anterior end of the following one.[8] Internal morphology, as depicted in Figure 17.2, is difficult to observe on native samples using light microscopy techniques due to a relatively thick surface layer of subtegumental musculature.

The scolex (worm's anterior end that attaches to the intestinal wall of the definitive host) is variable in shape but always bears a paired slit-like attachment groove (bothrium) of different length and depth on the dorsal and ventral surface, dividing the scolex into two lips or leaves (Figure 17.3). At its posterior end, scolex transforms into a neck—a germinative zone from which strobila originates. The length and appearance of the neck are highly species specific but also strongly depend on the state of worm contraction during fixation.

The male genital system is formed by numerous spherical to widely oval testes located in the medulla (i.e., in the parenchyma surrounded by the inner longitudinal musculature) and *vasa efferentia* connecting individual testes with the common sperm duct (*vas deferens*). In its terminal (distal) end, *vas deferens* enlarges to form a muscular external seminal vesicle (*vesicula seminalis externa*). The size and position of the vesicle (viewed in sagittal sections) are some of the most important diagnostic characteristics used to distinguish individual species of diphyllobothriideans. The vesicle enters the muscular cirrus sac that

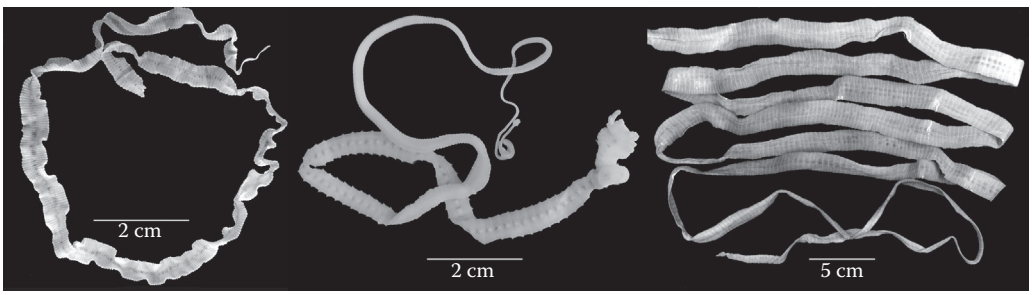

FIGURE 17.1 Photomicrographs of strobila: (a) *D. dendriticum* from hamster, (b) *D. latum* from hamster, and (c) *Dipl. balaenopterae* from whale (original).

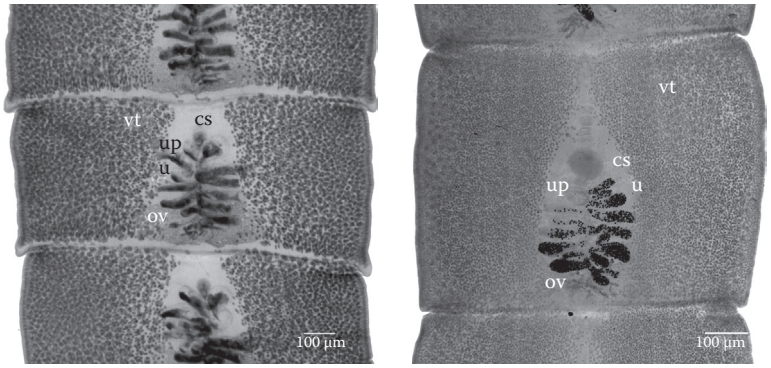

FIGURE 17.2 Photomicrogtaph of segments. (a) *Diphyllobothrium latum* from man, (b) *D. pacificum* from fur seal (original). cs, cirrus sac; ov, ovary; t, testes; u, uterus; up, uterine pore; v, viteline folicles.

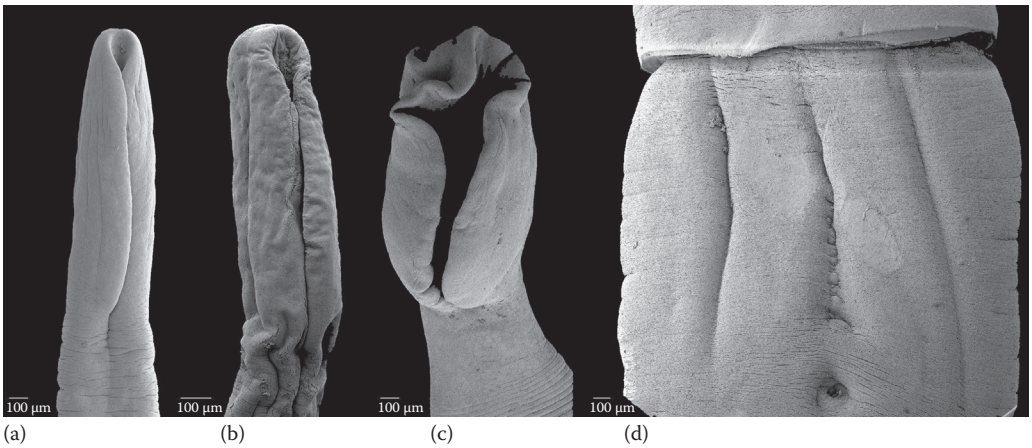

FIGURE 17.3 Photomicrographs (SEM) of scoleces: (a) *D. latum* from hamster, (b) *D. dendriticum* from dog, (c) *D. pacificum* from seal, and (d) strobila of *D. pacificum* from seal (original).

contains a muscular copulatory organ (cirrus). The cirrus sac opens medially, anterior to the uterine pore into a common genital atrium on the ventral surface of segments on an elevation densely covered with papillae (nipples).[9]

The female genital system consists of a bilobed ovary situated medially in the posterior third of a segment and small, numerous subspherical to spherical vitelline follicles situated in the cortex (i.e., external to the inner longitudinal musculature). Tubular vagina connects the ovarian complex (ovary terminating in a muscular sphincter called an oocapt, oviduct, common vitelline reservoir, ootype, and Mehlis' gland) with a common genital pore (in *Diphyllobothrium* and *Diplogonoporus*) or separated genital pores, that is, male and female gonopores (*Spirometra*), on the ventral side of the segments. The uterus is organized as a spiral of closely compressed coils (in *Spirometra*) or a rosette-type uterus (in *Diphyllobothrium* and *Diplogonoporus*), anterior to the ovary, and opens on the ventral side of the segment at its midline, posterior to the genital pore.

The eggs are polylecithal (see Ref. [10] for terminology of cestode eggs), thick-shelled, operculate, and variable in size (Table 17.1, Figure 17.4). Eggs are laid from gravid segments not fully embryonated, that is, they do not contain formed larvae (oncospheres) that bear three pairs of embryonic hooks (hexacanths). The size of eggs varies considerably even within a parasite species, depending mainly on the definitive host and intensity of infection. The high intraspecific variability and overlapping ranges of egg size in individual species make accurate diagnostics based on egg morphometrics almost impossible.[11,12]

TABLE 17.1

Morphometry of Eggs of Human-Infectious Diphyllobothriids

D. latum	*D. dendriticum*	*D. nihonkaiense*	*D. pacificum*	*Diplogonoporus*
Rausch and Hilliard[131]: 62–76 × 42–51	Serdyukov[137]: 59–68 × 39–42	Yamane et al.[139]: 53.9–56.4 × 36.7–39.7	Baer et al.[141]: 50–60 × 36–40	Kamo et al.[147]: 61.6–70.2 × 43.2–48.6
	Delyamure et al.[8]: 53–61 × 37–45	Yoshida[140]: 56–66 × 38–46	Kamo et al.[142]: 48.8–60.4 × 38.6–45	Kamo and Miyazaki[148] (*Diplogonoporus fukuokaensis*): 62–75.6 × 43.2–48.6
Wicht et al.[136]: 70.7–73.2 × 54.7–59.3	Wicht et al.[138]: 49.6–63.9 × 35.7–43.4	Wicht et al.[136]: 56,6–57.8 × 43.6–45.4	Makiya et al.[145]: 52–59 × 42–50	Delyamure et al.[8]: 57–61 × 38–42
Knoff et al.[143] [a]: 51–56.4 × 38.2–45.5	de Marval et al.[71]: 62.5–70 × 48.5–52.5	Wicht et al.[138]: 53.2–58.5 ×35.4–40.2	Mercado et al.[41]: 53.5–58 × 41.8–43.6	Chung et al.[87]: 57–80 × 34–45
	Kuchta et al.[68]: 49–50 × 30	Shimizu et al.[144]: 57.5–65 × 40–42.5	Rausch et al.[146]: 48.7–56 × 38.9–48.7	Clavel et al.[43]: 57–71.2 × 38.4–42.7
Total: 62–76 × 42–51	49–70 × 30–52.5	53–66 × 35.5–46	48.5–60 × 36–50	57–80 × 34–48.5

[a] Most probably *D. dendriticum* or *D. nihonkaiense*.

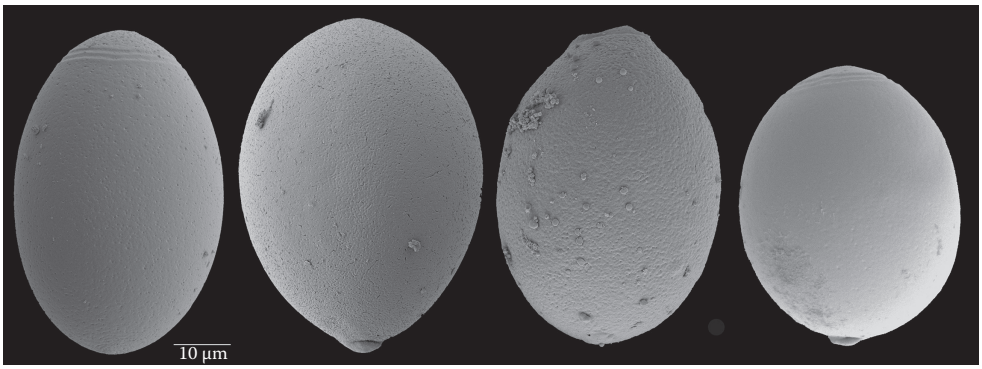

FIGURE 17.4 Photomicrographs (SEM) of eggs: (a) *D. dendriticum* from dog, (b) *D. latum* from dog, (c) *D. nihonkaiense* from man, and (d) *D. pacificum* from man (original).

17.2.2 Classification

The taxonomy of diphyllobothriideans has been always complicated, and generic placement of many species as well as opinions on their validity has changed frequently. The genus *Diphyllobothrium* was proposed by Cobbold[13] to accommodate *D. stemmacephalum* Cobbold, 1858, described from the harbor porpoise (*Phocoena phocoena*) as the type species of the genus. However, *D. latum* and *D. dendriticum* were placed in various genera, such as *Bothriocephalus* Rudolphi, 1808, *Dibothriocephalus* Lühe, 1899, whereas *D. pacificum* was originally described as *Adenocephalus pacificus* Nybelin, 1931. A reasonable estimate of the number of valid species would be ~40 out of nearly 100 nominal taxa placed in the genus.[14]

The complicated and yet unsatisfactorily resolved taxonomy of the genus has only been reviewed by Russian[8] and Japanese[14] authors. Some other important taxonomic contributions are represented by papers by G. Bylund, T.A. Dick, F. Kuhlow, J.F. Mueller, R.L. Rausch, Y. Yamane, and many others. K. Andersen, B. von Bonsdorff, I.V. Muratov, R. Vik, R.A. Wardle, and others have provided important data on the life cycles and ecology of the most common species (see Refs. [3,15,16]).

The genus *Diplogonoporus* Lönnberg, 1892, which probably includes only two or three valid species, is characterized by the presence of double (exceptionally one or even more than two) sets of genital organs per segment. Adult tapeworms are parasites of marine mammals (whales and seals), rarely humans, dogs, or otters.[14] Second intermediate hosts are marine fish (probably Japanese anchovy, *Engraulis japonica*, and Japanese sardine, *Sardinops melanostictus*, and others) (Table 17.2, Figure 17.5).

TABLE 17.2

Fish Intermediate Hosts of Human-Infectious *Diphyllobothrium* Species

D. latum	*D. dendriticum*	*D. nihonkaiense*	*D. pacificum*	*Diplogonoporus*
Esox lucius (E, N)	*Coregonus autumnalis* (A, E, N)	*Oncorhynchus gorbuscha* (A, N)	*Sciaena deliciosa* (S)	*Engraulis japonicus* (A)
Gymnocephalus cernuus (E)	*Gasterosteus aculeatus* (E, N)	*Oncorhynchus keta* (A, N)	*Paralonchurus peruanus* (S)	*Sardinops melanostictus* (A)
Lota lota (E)	*Oncorhynchus mykiss* (E, N, S)	*Oncorhynchus masou* (A, N)	*Seriolella violacea* (S)	
Perca flavescens (N)	*Oncorhynchus* spp. (N, A)	*Oncorhynchus nerka* (A, N)	*Trachinotus paitensis* (S)	
Perca fluviatilis (E)	*Salmo trutta* (E, N, S)		*Ariopsis seemanni* (S)	
Sander vitreus (N)	*Salvelinus alpinus* (E, N)	*Liza haematocheila* (A)?	*Paralichthys adspersus* (S)	
Sander canadensis (N)	*Salvelinus fontinalis* (N)		*Cynoscion analis* (S)	
Oncorhynchus mykiss (S)			*Merluccius gayi peruanus* (S)	

A, Asia; E, Europe; N, North America; and S, South America.

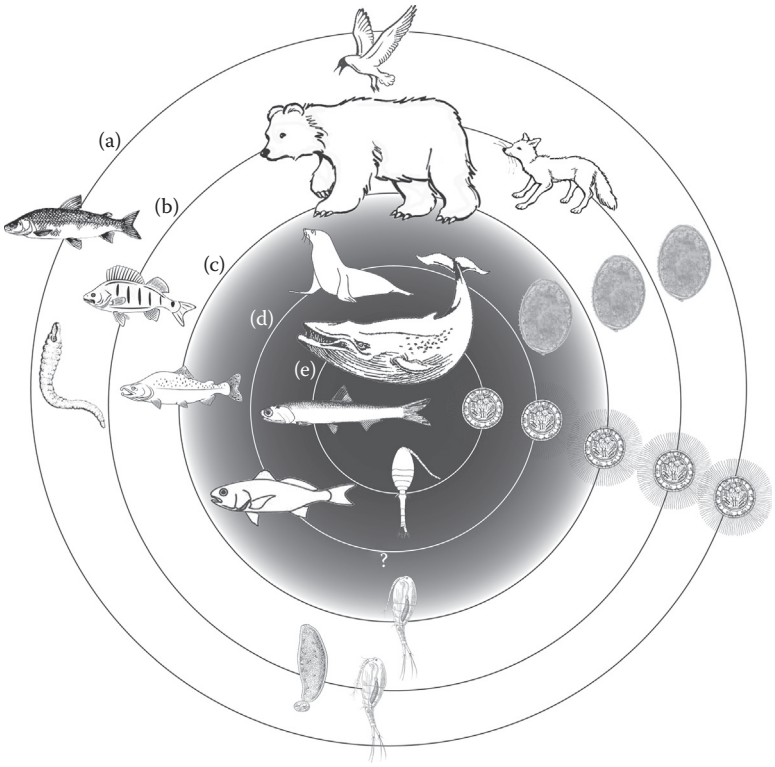

FIGURE 17.5 Life cycles of diphyllobothriids. (a) *D. dendriticum*, adult in seagull, egg, coracidium, procercoid in copepod, plerocercoid in whitefish. (b) *D. latum*, adult in bear or fox, egg, coracidium, procercoid in copepod, plerocercoid in perch. (c) *D. nihonkaiense*, adult in bear, egg, coracidium, procercoid in copepod, plerocercoids in Pacific salmon. (d) *D. pacificum*, adult in seal, egg, coracidium, first intermediate host is unknown, plerocercoid in lorna drum. (e) *Dipl. balaenopterae*, adult in whale, egg, coracidium, procercoid in oithoniid copepod, plerocercoid in anchovy (original).

The first human case was described by Ijima and Kurimoto (1894) in Japan.[17] Blanchard[18] established a new genus to accommodate his new species *Krabbea grandis* for human tapeworms that were considered to be the causative agent of diplogonoporosis. However, later authors synonymized *Diplogonoporus grandis* (Blanchard, 1894) with *Dipl. balaenopterae* Lönnberg, 1892, the species originally described from whales.[8] This synonymy of diplogonoporid tapeworms from humans and whales has been recently supported by molecular data.[19] The majority of human cases of diplogonoporosis (more than 200) have been reported from Japan.[20]

The taxonomy of *Spirometra* also remains unsatisfactorily resolved despite the fact that the genus encompasses a significantly lower number of nominal species than *Diphyllobothrium*. The biggest complication lies in the nomenclature and systematics of human-infecting species—the causative agents of sparganosis, because identical tapeworm species have likely been described under different names. The genus was erected (as subgenus) by Faust et al.[21] to accommodate *Spirometra erinaceieuropaei* (Rudolphi, 1819), the oldest nominal species described from larvae found in the body cavity of the European hedgehog (*Erinaceus europaeus*).

The validity of *Spirometra* has been questioned repeatedly because of its morphological similarity to *Diphyllobothrium*. However, there is a convincing morphological and biological evidence that *Diphyllobothrium* and *Spirometra* represent separate genera,[14] which has been further supported by molecular data.[22,23] The generic name *Spirometra* remains valid, as a result of the recommendation of the International Commission on Zoological Nomenclature,[24] even though the genera *Gatesius* Stiles, 1908, and *Lueheella* Baer, 1924, were proposed earlier. The most commonly used names of *Spirometra* species have been *S. erinacei* (=*S. erinaceieuropaei*), *Sparganum mansoni*, and *Spirometra mansonoides*. The causative agent of the proliferative form of sparganosis called *Sparganum proliferum* has not yet been clarified because the adults have never been found and experimental infections of potential definitive hosts failed. It is assumed that this worm is an aberrant form of *S. erinaceieuropaei* or *S. mansonoides*.[25]

Diphyllobothrium, *Diplogonoporus*, and *Spirometra* were for a long time placed within the family Diphyllobothriidae of the order Pseudophyllidea van Beneden in Carus, 1863. The members of this historical order shared several characteristics, such as the presence of bothria on the scolex, extensive vitelline follicles, and usually operculate eggs and copepods serving as intermediate hosts.[26] However, the order Pseudophyllidea has been eventually recognized as an artificial assemblage of two unrelated clades, and two new orders, Diphyllobothriidea and Bothriocephalidea, were established in its place.[27] The validity of Diphyllobothriidea and Bothriocephalidea has been unequivocally supported by all relevant morphological and molecular characterization and is widely accepted among cestodologists (see http://tapeworms.uconn.edu/).

17.3 Biology, Genetics, and Genomics

17.3.1 Biology

Although broad fish tapeworms have been notoriously known as human parasites for a long time, their life cycle was not elucidated until 1917 by F. Rosen in Switzerland,[28] who experimentally infected copepods with coracidia of *D. latum* and obtained procercoid larvae infective for further intermediate fish hosts. However, the embryonic development, that is, formation of the coracidium within the egg shell, was previously described by Knoch,[29] who implanted coracidia in the brain, eyes, and under the skin of a dog and cat. He also unsuccessfully tried to experimentally infect fish, frogs, birds, cats, and dogs with coracidia. The complete life cycle of *Spirometra* was elucidated by T. Okumura in Japan in 1919.[30]

Life cycles of species of *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* are mutually analogous, except for the spectrum of second intermediate hosts, and involve a total of three hosts (one definitive and two intermediate) (Figure 17.5). Planktonic crustaceans (cyclopids, diaptomid, and other copepods) serve as the first intermediate host, whereas a wide spectrum of poikilothermic vertebrates harbor plerocercoids. Fish serve exclusively as the second intermediate hosts of *Diphyllobothrium* and *Diplogonoporus* and as such represent the only source of human infection. Typically, *Diphyllobothrium* and *Diplogonoporus* adults reach sexual maturity and produce eggs in the intestine of piscivorous mammals and birds. In

contrast, plerocercoids of *Spirometra* infect tetrapods (mainly frogs and other amphibians, but also reptiles, birds, and mammals) and thus definitive hosts are not exclusively fish-eating vertebrates, but also other predatory mammals such as felines and canines, rarely also humans.[31,32]

Ripe eggs, which possess an operculum on the narrower end of the eggshell, are released from gravid segments via the uterine pore into the intestinal lumen of the definitive hosts and then to the outer environment with stool (Figure 17.4). The development of the first-stage larva (oncosphere) continues in the water until it develops three pairs of embryonic hooks (hexacanth) and is covered with a ciliated outer layer, thus becoming a coracidium (see Conn and Świderski[10] for terminology). Coracidium actively hatches from the eggshell via the operculum and swims in the water where its movement attracts the first intermediate hosts, planktonic crustaceans.[33]

The coracidium, when ingested by a suitable intermediate host, detaches the ciliated envelope and penetrates through the intestinal wall to the copepod's body cavity, where it grows and transforms into a larva (metacestode) called a procercoid (see Chervy[34] for terminology of cestode larvae or metacestodes). The fully formed procercoid is infective for the next intermediate host. At this stage, it does not possess a fully formed scolex at the anterior end; instead, it bears a small posterior appendage (cercomer) where embryonic hooks are located.[28]

Diaptomids (Copepoda: Diaptomidae) of several genera, such as *Acanthodiaptomus* Kiefer, 1932; *Arctodiaptomus* Kiefer, 1932, *Diaptomus* Westwood, 1836, and *Eudiaptomus* Kiefer, 1932; centropagids (Centropagidae) of the genus *Boeckella* de Guerne et Richard, 1889; cyclopoids (Cyclopidae), for example, *Cyclops* Müller, 1785, *Eucyclops* Claus, 1893, and *Mesocyclops* Sars, 1914; and temorids (Temoridae) of the genus *Eurytemora* Giesbrecht, 1881, have been reported as suitable first intermediate hosts under natural and laboratory conditions.[31,33] The only known first intermediate hosts of marine diphyllobothriideans (*Diphyllobothrium macroovatum* and *Dipl. balaenopterae*) are *Acartia clausi* Giesbrecht, 1889 (Acartiidae), *Oithona nana* Giesbrecht, 1893 (Oithonidae), and *Labidocera japonica* Mori, 1935 (Pontellidae).[35,36]

Second intermediate hosts include fish (Table 17.2, Figure 17.5) or tetrapods that eat or swallow planktonic crustaceans. After the ingestion of an infected copepod, the procercoid penetrates the intestinal wall to get into the parenteral site of infection or the musculature of other internal organs and develops into a plerocercoid, which often remains free in the abdominal cavity. Most plerocercoids are encapsulated in the host tissue, but some may be free in the body cavity. The presence of the larvae in the muscles, gonads, or liver is important for human epidemiology, even though some plerocercoids may migrate from the viscera to the muscles after the death of the host or can be attached on the ventral abdominal flap to the fillet and thus not removed from fillets when the fish is gutted.[37,38]

In freshwater environments, predatory fish such as perch (*Perca fluviatilis*), pike (*Esox lucius*), ruff (*Gymnocephalus cernua*), and burbot (*Lota lota*) in Europe and Asia and also yellow perch (*Perca flavescens*), walleye (*Sander vitreus*), and sauger (*Sander canadensis*) in North America represent the most important source of human infection with *D. latum* (Table 17.2, Figure 17.6). The prevalence of

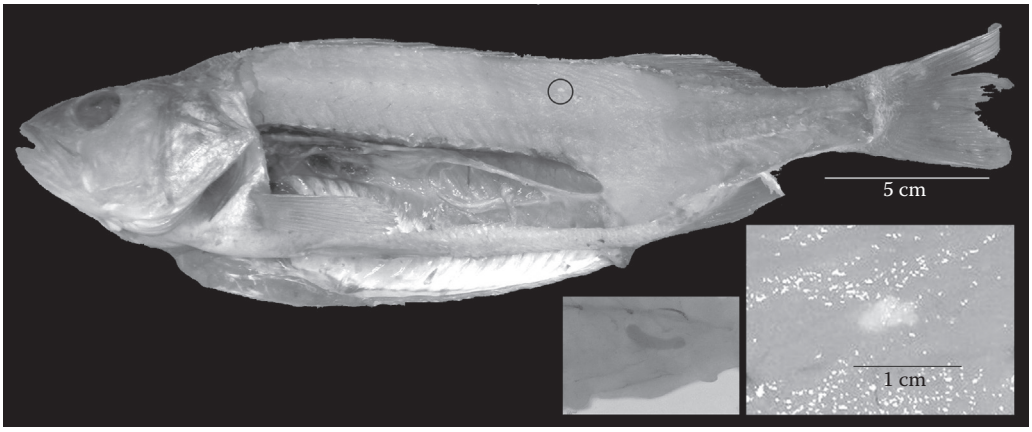

FIGURE 17.6 Plerocercoids of *D. latum* in *P. fluviatilis* from Italy, with details of the plerocercoid (original).

infection in some of these hosts can reach high levels, such as perch in Alpine regions, where 4%–10% of perch fillets from Lake Geneva examined in 2003–2005 and up to 14% of perch from Lake Maggiore contained plerocercoids, respectively.[39,40] The prevalence of walleye in some Canadian lakes (Manitoba) may exceed 50%.[16]

Other species, such as *D. dendriticum* and *D. nihonkaiense*, are transmitted by salmonid or coregonid fish (Table 17.2, Figure 17.5). Pacific salmons (*Oncorhynchus* spp.) are the principal second intermediate hosts of *D. nihonkaiense* in the northern Pacific Ocean, whereas whitefish (Coregoninae) are frequently infected with larvae of other *Diphyllobothrium* species, especially *D. dendriticum* and *D. ditremum*. Records of *D. latum* from salmonids (as *Salmo trutta* or *Oncorhynchus mykiss*) are frequently reported from South America (Argentina and Chile), but just three human cases from Chile were confirmed as *D. latum* using molecular tools.[41]

Much less is known about the role of marine and brackish-water fish in the transmission of the broad fish tapeworms. Existing records indicate that mullets such as so-iny mullet (*Liza haematocheila*), common snook (*Centropomus undecimalis*), Japanese anchovy (*E. japonica*), Japanese sardine (*S. melanostictus*), or lorna drum (*S. deliciosa*) may serve as the source of human infection of *D. pacificum* and *Dipl. balaenopterae*.[42,43] However, the full spectrum of fish intermediate hosts remains to be more satisfactorily determined (Table 17.2, Figure 17.5).

Plerocercoids ingested by suitable definitive hosts attach with their scoleces to the intestinal epithelium and develop rapidly into adult worms. The growth rate may be very high (up to 1 cm/h in *D. latum*), and the prepatent period (which depends on the parasite and host species, the infection intensity, host size and physiological state, as well as other abiotic and biotic factors) may be as short as 1–6 weeks.[7,44] Longevity (survival in the definitive host) is variable, with extreme cases of survival for up to 20 years. However, some authors[45] questioned such a long longevity of tapeworm adults and assumed that these patients rather went through several consecutive infections. The longevity of adult cestodes is supposed to be much shorter in mammals and birds, depending mainly on host size.[7] The eggs of *Spirometra* were found 1–4 weeks after infection of definitive hosts[32,46] and the longest survival was 4 years in a cat.[31]

At the level of the definitive host, most species of *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* exhibit rather wide host specificity (euryxenous) and the multiple definitive hosts may contribute to the perpetuation of the parasite population in a given locality. As a result of this low host specificity, humans can occasionally get infected with several species of broad fish tapeworms that naturally utilize carnivorous and fish-eating mammals and birds as definitive hosts.

17.3.2 Genetics

Most of the genetic studies on broad fish tapeworms employing some sort of molecular approach have focused either on phylogeny and systematics or differential diagnostics of these parasites. The field began to develop with the use of various biochemical techniques (isoenzymatic assay, immunoelectrophoresis) to distinguish between *Diphyllobothrium* spp. (e.g., see Refs. [47–49]) but more recently has been based on DNA sequence data, particularly nuclear ribosomal RNA (rDNA) and mitochondrial protein-coding genes.[50]

The first characterized sequences of diphyllobothriideans were the small subunit nuclear rDNA (ssrDNA) and the mitochondrial NADH dehydrogenase subunit 3 (*nad3*) of *Spirometra* (Kokaze et al.[149]; Liu et al.[150]). Olson and Tkach[51] reviewed the early molecular phylogenetic studies on the interrelationships of major cestode lineages including representatives of diphyllobothriidean human parasites based on various data (the ssrDNA, the large subunit rDNA—lsrDNA, and the elongation factor 1α gene). Ando et al.[151] and Miyadera et al.[60] then added cytochrome *c* oxidase subunit 1 (*cox1*) gene sequences to the pool of mitochondrial markers characterized and thus basically launched a specific line of genetic studies that mostly aimed to determine the correct pathogen identity or cryptic diversity of the infectious agents of recorded human disease.

Over the last 15 years, numerous studies on the topic have accumulated, often driven by newly appearing human cases of diphyllobothriosis, diplogonoporosis, or sparganosis. The studies of Yera et al.,[52] Arizono et al.,[53] Yera et al.,[54] Yamasaki et al.,[55] Mercado et al.,[41] Wicht et al.,[56] and Yamasaki et al.[57] represent just a few examples that dealt with diagnosis and taxonomical status of one or several *Diphyllobothrium* species

using molecular tools; Yera et al.[54] and Wicht et al.[56] also evaluated the utility of individual molecular markers used in their studies. Arizono et al.[152] undertook a molecular survey of the diversity of *Diplogonoporus* spp., and Dai et al.[58] and Liu et al.[23] contributed to important molecular insights into the genetic variability of *S. erinaceieuropaei* from various geographical regions. Most of the mentioned authors relied on a single or several molecular markers, with *cox1* being almost always part of the data set. Other sequences characterized include nuclear internal transcribed spacers 1 and 2 (ITS1 and ITS2)[52–54,56] and/or mitochondrial protein-coding genes such as cytochrome *b* (*cob*),[56] cytochrome *c* oxidase subunit 3 (*cox3*),[23] and NADH dehydrogenase subunit 1, 3, and 4 (*nad1*, *nad3*, and *nad4*).[23,52,53,58]

When jointly evaluated, all the studies unequivocally agree on the supreme utility of the well-established mitochondrial genes, especially *cox1*, for both systematic and diagnostic purposes. *cox1* currently represents the most densely sampled gene of the species discussed and also the most straightforward one to choose for differentiating between individual congeneric diphyllobothriidean species, given its relatively rapid mutation rate. In contrast, none of the rDNA markers are able to unambiguously distinguish among *D. nihonkaiense*, *D. latum*, *D. dendriticum*, and *D. ditremum*. From the phylogenetic point of view, those four species form a stable evolutionary lineage of closely related organisms whose relationships remain to be clarified, most likely on the basis of additional molecular targets because the internal branching pattern of this clade cannot be considered stable. In other words, based on the mitochondrial molecular data, we currently possess only a single, weekly supported hypothesis about the interrelationships of *D. nihonkaiense*, *D. latum*, *D. dendriticum*, and *D. ditremum*. Since nuclear ribosomal genes, which form the second source of molecular data today, lack resolution, another phylogenetic marker is needed to clearly resolve these relationships.

Molecular data also indicate a close relationship of *Dipl. balaenopterae* and *D. stemmacephalum*. This phylogenetic placement would imply an invalidity of *Diplogonoporus*, a genus that in fact only differs from *Diphyllobothrium* morphologically by a doubled set of genitalia per proglottid. An analogous morphological scenario could be observed in the case of *Digramma* Cholodkovsky, 1915, a diphyllobothriidean genus that is distinguished from *Ligula* Bloch, 1782, only by a doubled set of genitalia per segment.[59] The phylogenetic relationships of the rest of *Diphyllobothrium* and *Spirometra* species remain insufficiently known mainly due to limited access to specimens suitable for molecular studies. *D. pacificum* seems to steadily form the most basal lineage of the entire *Diphyllobothrium* clade. *Spirometra* then forms a sister lineage of this clade. From the estimated number of a few valid *Spirometra* species (no more than four according to Kamo[14]), only *S. erinaceieuropaei* representatives have been sequenced, and multiple isolates of the parasite from Asia display relatively low levels of *cox1* sequence divergence. On the other hand, the enigmatic and life-threatening human disease, proliferative sparganosis (see Section 17.6.5), is most likely caused by a different *Spirometra* species than *S. erinaceieuropaei*, as the causative agents sequenced form a well-separated sister lineage rather than clustering within the numerous sequences of *S. erinaceieuropaei*.[60]

17.3.3 Genomics

The second decade of the twenty-first century will undoubtedly witness a rapid increase in the number of fully characterized parasite genomes, mainly thanks to the advent of next generation sequencing techniques, which have already become largely affordable even for medium-sized parasitological laboratories. Given the fact that well-annotated full genome assemblies of the most important cestode human pathogens (*Taenia* and *Echinococcus*) have been published just recently,[61] genome sequencing of the remaining medically important cestodes, among which species of *Diphyllobothrium* and *Spirometra* undoubtedly belong, will be underway in the close future. *Diphyllobothrium* and *Spirometra* representatives already are among the 50 draft genomes that are currently being characterized under the scope of 50 Helminth Genomes project led by the Wellcome Trust Sanger Institute (see http://www.sanger.ac.uk/research/initiatives/globalhealth/research/helminthgenomes for more details). Contiguous genomic sequences of *D. latum* and *S. erinaceieuropaei* are also available at the website. Knowledge of the genomic sequences will not only help us to better deal with the medically problematic cestode cases, but mainly it will allow us to better understand cestode evolution, development, and other molecular peculiarities of their life cycles.[61]

Currently, however, diphyllobothriidean genomics is limited to a few complete sequences of mitochondrial genomes. *D. latum* and *D. nihonkaiense*[62–64] were the first species to have their mitochondrial genomes characterized, followed by the mitochondrial genome of *Dipl. balanopterae*. Liu et al.[65] then characterized the whole mitochondrial genome of *S. erinaceieuropaei* from China and compared it with an unpublished genome of the same species from Japan. Mitochondrial genomes of the four diphyllobothriidean cestode species characterized so far have a comparable size (13,641–13,747 bp) and display a uniform gene content, each encoding 12 proteins (*atp6*, *cob*, *cox1–cox3*, *nad1–nad6*), 22 tRNAs, and 2 rRNAs. This is in general agreement with the features of the mitochondrial genomes of known cyclophyllidean cestodes and other flatworms, which also lack the ATPase subunit 8 gene (*atp8*). The cestode mitochondrial genes are transcribed in the same direction and retain an identical linear order. Comparative analysis of these genomes then allowed to estimate the relative mutation rate of individual genes. Whereas *cox1* and *cox2* seem to belong to the most conserved of the mitochondrial protein-coding genes, *nad5* and *nad6* occupy the other end of scale, displaying the highest mutation rates.

17.4 Diagnosis and Typing

17.4.1 Morphology-Based Diagnostics

Morphology-based diagnostics still represents the most common method of detection of the causative agents of human infections with species of the genera *Diphyllobothrium* and *Diplogonoporus*. Diagnosis is largely based on finding ovoid eggs with an operculum on a narrowed pole, or pieces of the strobila with segments that possess medially situated genital pores (common male and female genital pores and uterine pore). Segments of broad fish tapeworms may have a similar shape to species of *Taenia* (*Taenia saginata* and *Taenia solium*), which differ by possessing lateral (marginal) genital pores.

The egg size of *Diphyllobothrium* and *Diplogonoporus* species varies from 40 to 80 μm in length and from 25 to 65 μm in width and roughly resembles those of flukes (or trematodes—Digenea), which often possess operculate eggs of a similar size (Table 17.1). The eggs of *Spirometra* tend to be elongate and oval (55–66 μm × 27–41 μm) with somewhat pointed ends; this helps in their differentiation from eggs of *Diphyllobothrium* tapeworms, which are slightly larger and more round.[46] The eggs of the diphyllobothriids could be confused with eggs of the digenean *Nanophyetus salmincola* Chapin, 1926 (size 62–72 μm × 43–48 μm), which may infect humans in the North Pacific.[66]

The main advantage of morphology-based diagnostics is the fact that it is cheap and relatively easy. However, it usually does not allow a reliable identification of individual species because some of them can be differentiated only on the basis of the scolex shape and size, which often are not found in clinical samples. Moreover, tapeworm tissue obtained after anthelmintic-treated patients is mostly damaged or decomposed (macerated) and thus is not suitable for morphological evaluation.

Difficulties with reliable morphological species identification of clinical cases (egg size, morphology of segments, etc.) may have resulted in numerous misidentifications. The majority of records were assigned to *D. latum*, which was originally believed to represent the principal causative agent of diphyllobothriosis. However, in North America, especially the Northwest and in Siberia, *Diphyllobothrium alascense*, *Diphyllobothrium dalliae*, or *D. nihonkaiense* may have been misidentified as *D. latum*,[3] and in the near Arctic (Northern Canada and Alaska), *D. dendriticum* instead of *D. latum* may represent the only species involved in human infections.[67,68] Molecular confirmation of human cases of diphyllobothriosis is thus needed to provide more reliable data on the current distribution of individual human-infecting taxa in endemic areas.

Larvae in fish (plerocercoids) do not possess developed genital organs and thus provide only a limited number of morphological characteristics suitable for species differentiation. Despite this limitation, identification of plerocercoids of some species is possible and a simple key to plerocercoids of the three common species of *Diphyllobothrium* in the Holarctic region, *D. dendriticum*, *D. ditremum*, and *D. latum*, was provided by Andersen et al.[69] This key is based on gross morphology (size and shape of the body, presence/absence of transverse wrinkles on the body, and degree of retraction of the scolex), the number of the longitudinal muscles (seen in histological sections), and the length and density of

TABLE 17.3

Morphological and Morphometrical Characters of Plerocercoids of Human-Infectious *Diphyllobothrium* Species

Character	*D. latum*	*D. dendriticum*	*D. ditremum*	*D. nihonkaiense*
Transverse wrinkles	Present	Present	Absent	?
Scolex (cold fixative)	Fully retracted	Partly retracted	Fully extruded	Fully extruded
Microtriches (size) (μm)	1–2	7–13	13–30	3
Second intermediate host (family/FW or B)	Esocidae, Lotidae, Percidae (FW)	Coregonidae, Gasterosteidae, Salmonidae (FW)	Coregonidae, Osmeridae, Salmonidae (FW)	Salmonidae (*Oncorhynchus*) (B)
Site of infection	Free in muscles or body cavity, rarely encysted in viscera	Encysted in viscera or in body cavity, rarely free	Encysted in viscera or free in body cavity	Usually encysted in muscles

Source: Modified from Andersen and Gibson.[153] B - brackishwater, FW - freshwater.

microtriches (hair- or spine-like structures on the surface of tapeworms, visible using scanning electron microscopy—SEM) (Table 17.3). However, the identification of plerocercoids of other species, including human-infecting *D. nihonkaiense* and *D. pacificum*, is difficult or impossible because limited data on their morphology are available. Application of molecular markers is thus necessary in future epidemiological studies.

Diagnostics of sparganosis is complicated because of the localization of larvae in various tissues/organs and their migration. Spargana do not have adequate morphological characteristics for differentiation. They are flat, ribbon-like worms that vary from a few millimeters to 50 cm or more in length (Figure 17.7). The anterior end of the worm, which does not represent a fully formed scolex, is flattened and possesses a cleft-like invagination called rudimentary ventral groove (bothrium). However, the causative agent of the most serious form of sparganosis, *Sparganum proliferum*, is unusual among the diphyllobothriidean metacestodes in having continuous branches and buds. These larvae are present in two morphological forms: (1) large, motile, vermiform structures with irregular branches and (2) small forms with vesicular budding.

Morphological uniformity of diphyllobothriidean procercoids in copepods prevents their identification to the species level. The prevalence of infection of copepods with *Diphyllobothrium* larvae is generally very low (usually less than 1% in natural conditions, even though Dörücü[70] reported a prevalence of 2.6% in Loch Lomond, Scotland), and thus the use of data on infection rate in first intermediate hosts in epidemiological studies is limited.

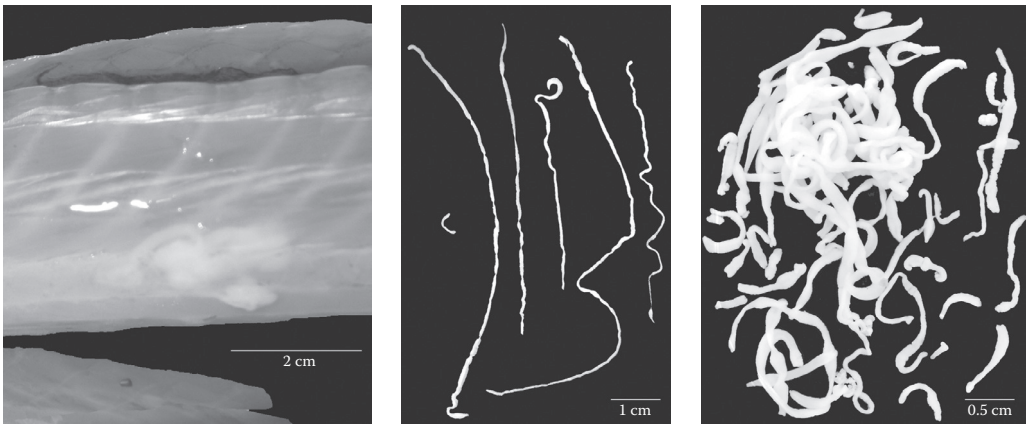

FIGURE 17.7 Plerocercoids (sparganum) of *Spirometra* spp. (a and b) *S. mansonoides* from a snake (*Thamnophis proximus*), Mississippi, United States, and (c) *S. erinaceieuropaei* from a snake (*Xenochrophis flavipunctatus*) from Vietnam (original).

17.4.2 Molecular Diagnostics

Application of molecular genetic methods for the detection of the causative agents of diphyllobothriosis has greatly improved diagnostic specificity and made it possible to evaluate a high number of samples in a short time. Genetic markers currently used are species–specific and represent the most suitable tool to identify clinical samples of *Diphyllobothrium* and *Spirometra* to the species level. In fact, molecular analysis by polymerase chain reaction (PCR) amplification and sequencing of a selected marker represents the only reliable tool to identify these parasites to the species level. Molecular diagnostics has also enabled the unequivocal identification of individual ontogenetic parasite stages, including eggs, which has important implications for epidemiological surveys. Due to the recent spread of human diphyllobothriosis in several countries, a correct diagnosis has become crucial for a better understanding of the distribution and the life cycle of human-infecting species, as well as to prevent the introduction of parasites to disease-free water systems.[71]

D. latum and *D. nihonkaiense* were originally distinguished with the help of restriction fragment length polymorphism (RFLP)[49] and the same technique was used to compare *Spirometra erinaceieuropaei* and *S. mansonoides* by Lee et al.[72] However, soon after that, the first sequences of nuclear ribosomal and mitochondrial genes were characterized and begun to be employed for species identification. Since then, numerous diagnostic studies have accumulated a reasonable pool of sequence data that allowed us to compare the utility of individual molecular loci for successful differentiation among closely related species of diphyllobothriideans.[56]

It is somewhat unfortunate that rDNA sequences fail to distinguish among the closely related species of *D. nihonkaiense*, *D. latum*, *D. dendriticum*, and *D. ditremum*, three of which represent the most common human parasites, as already discussed in the text earlier. However, ssrDNA and lsrDNA sequences are still useful in assigning organisms to higher taxonomical ranks such as families or orders. This is mainly due to their relatively slower rate of evolution and a very large number of rDNA sequences known from virtually all groups of organisms, which is a limiting factor for mitochondrial molecular markers in numerous cestode orders other than diphyllobothriideans. In other words, if there is no adequate reference sequence, then a given sequence cannot be assigned to the correct organism. This can be illustrated by a recent case of finding *Diphyllobothrium*-like eggs in human stool, which could only be assigned to the correct parasite, bothriocephalidean *Bothriocephalus acheilognathi* Yamaguti, 1934, with the use of lsrDNA sequences (the most densely sampled locus among bothriocephalideans).[73] However, among diphyllobothriideans, the *cox1* gene represents the most abundantly sampled molecular marker. Until a completely new molecular approach appears, *cox1* and possibly other mitochondrial genes such as *cob* and *nad3* will represent a decently working and most densely characterized target of choice for specific diagnosis of either *Diphyllobothrium*, *Diplogonoporus*, or *Spirometra*.

For the purposes of medical diagnostic laboratories, a quick and cheap differential method for routine species-specific diagnostics of parasites has been developed.[74] The method is based on a multiplex PCR of *cox1*, does not require sequencing, and allows for a rapid differential diagnostics of the four most commonly detected *Diphyllobothrium* species infecting man (*D. nihonkaiense*, *D. latum*, *D. dendriticum*, and *D. pacificum*). An analogous method was developed earlier by Kim et al.[62] to differentiate between *D. nihonkaiense* and *D. latum*, but the method of Wicht et al.[74] offers a more broadly applicable test that is better suited for the current epidemiology of diphyllobothriosis worldwide.

DNA extraction for PCR can be achieved either with the use of a commercial kit or with the traditional phenol–chloroform extraction method, which seems to yield higher amounts of DNA, especially from smaller-sized procercoid larvae. Eluting DNA in water, instead of elution buffer, can be useful in the case of low DNA yields as the diluted DNA can be further concentrated using a vacuum centrifuge (e.g., SpeedVac). However, the most serious obstacle in the diagnostics of diphyllobothriosis is the absence of good-quality, properly fixed reference material from clinical samples. For morphological evaluation, samples should not be deformed or digested (decomposed), whereas samples for genetic analysis should be fixed solely with DNA-grade ethanol. Other routinely used fixatives such as formalin, AFA (a mixture of ethanol, formalin, and acetic acid), or denatured ethanol prevent isolation of intact DNA and frequently inhibit PCR.

17.5 Epidemiology

Diphyllobothriosis, diplogonoporosis, and sparganosis are typical zoonoses, that is, parasitic diseases in which humans do not represent the principal host but can occasionally become infected. The parasite population is maintained in the environment by other definitive hosts, which are not dependent on human final hosts. This is because most species of *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* that can infect humans do not exhibit strict host specificity at the level of the definitive host and can mature and survive also in nonhuman hosts. These animal hosts thus represent reservoirs in which parasite populations are maintained in the environment, even in the absence of human populations. This also explains the existence of endemic foci of diphyllobothriosis in sparsely inhabited areas, such as Siberia, Far East of Russia, Alaska, and northern Canada.

Fourteen species of *Diphyllobothrium*, which are currently recognized as valid, have been reported from humans,[3] but the medical importance of individual taxa and their frequency in human infections markedly differ from each other.[3] Available estimates indicate that up to 20 million persons can be infected with *Diphyllobothrium* worldwide.[2] However, these numbers are inaccurate, because diphyllobothriosis is generally regarded as a mild illness and thus is not systematically reported even in developed countries. The majority of cases have been reported from the Palaearctic (Europe and northern and eastern Asia) and Nearctic (Canada and United States) geographical regions[3] (Figures 17.8 through 17.12).

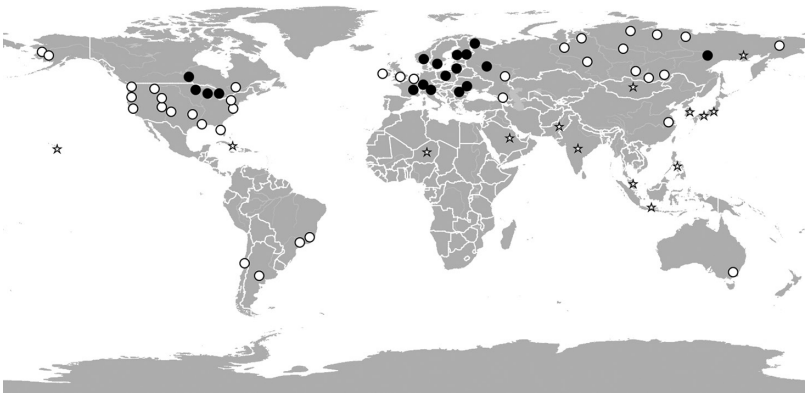

FIGURE 17.8 The distribution of human cases of *D. latum*. Solid circles, cases confirmed by molecular methods; empty circles, cases not confirmed by molecular data; and asterisks, sporadic cases.

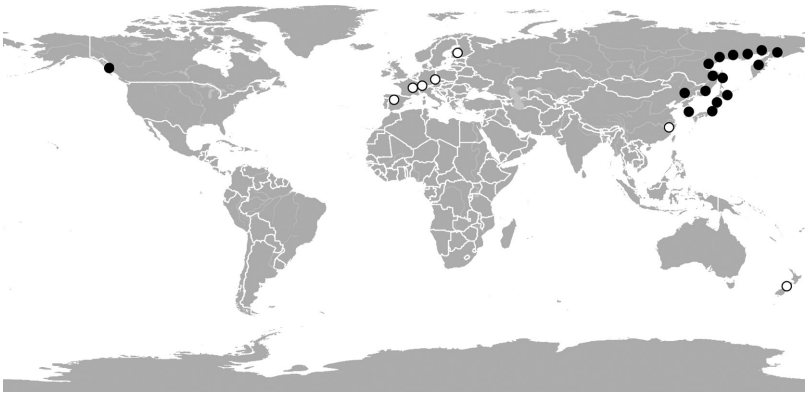

FIGURE 17.9 The distribution of human cases of *D. nihonkaiense*. Solid circles, autochthonous cases; empty circles, imported cases.

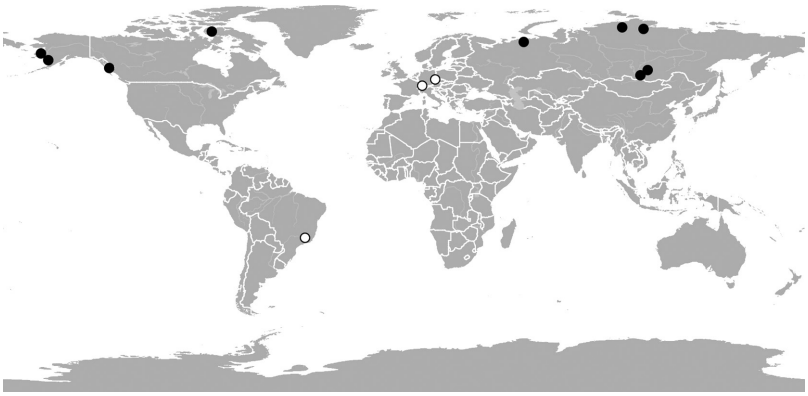

FIGURE 17.10 The distribution of human cases of *D. dendriticum*. Solid circles, autochthonous cases; empty circles, imported cases.

FIGURE 17.11 The distribution of human cases of *D. pacificum*. Solid circles, autochthonous cases; empty circles, imported cases.

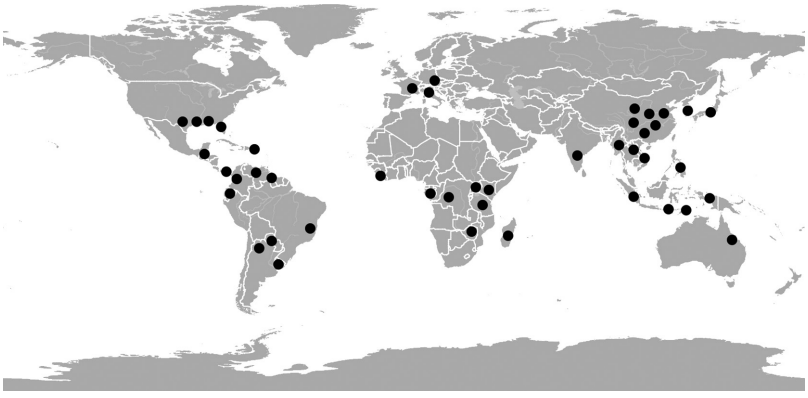

FIGURE 17.12 The distribution of human cases of sparganosis (*Spirometra* spp.).

Only one species of *Diplogonoporus*, *Dipl. balaenopterae*, is responsible for human diplogonoporosis.[19] More than 200 cases of human diplogonoporosis are known from Japan,[20] whereas only 3 human cases have been reported outside Japan (Korea and Spain).[43,75]

There has been a considerable decline in reported human diphyllobothriosis cases over the last decades in several endemic areas, especially in North America (Alaska, northern United States, and

southern Canada) and northern Europe.[3,16] In sharp contrast to this trend, diphyllobothriosis seems to be reemerging in other regions such as Alpine lakes in Switzerland, northern Italy, and eastern France (Haute-Savoie), where it had not represented a public health problem for decades.[76] An increase in human cases has also been reported from some Asian countries (Japan, Korea, Russia) and South America.[3,20]

As already mentioned earlier, reliable species identification of adult worms and plerocercoids is necessary for a better understanding of the epidemiology of the disease and current geographical distribution of individual taxa. As a result of worldwide transport of fresh fish and higher mobility and migration of people, the number of imported cases has increased and exotic (imported) causative agents of diphyllobothriosis can be found far from their native areas. In regions where diphyllobothriosis was not previously detected, the number of human cases may be underestimated unless outbreaks appear.[3,68,71,75,154]

In the case of sparganosis, humans can become infected in the following ways: (1) ingestion of raw or undercooked flesh of snakes, frogs, or other tetrapods; (2) drinking untreated water containing *Spirometra* larvae (procercoids) in copepods; or (3) using raw snake or frog flesh in traditional poultices (*Spirometra* larvae have been shown to penetrate intact human skin and cause infection in this way). In Asia, raw snake or frog meat is eaten as a remedy for various morbid states in uneducated people due to superstition and traditional misbelieve.

17.5.1 Geographical Distribution

Diphyllobothriosis is a cosmopolitan disease, but human infections are generally associated with cold waters, because most cases were reported from the Palaearctic region and some parts of North America. Clinical cases have also been reported from the Pacific coast of Asia (Japan) and South America (Peru, Chile). Unlike diphyllobothriosis, most cases of sparganosis are currently reported from China, Japan, and South East Asia, while diplogonoporosis is limited to Japan, with one record from Korea and two from Spain.[3,5,20,75] However, knowledge of the actual distribution of the causative agents of diphyllobothriosis, diplogonoporosis, and sparganosis is incomplete, because only a limited number of large-scale surveys have been carried out.

The absence of reliable and updated surveys also impedes presentation of detailed data on the current distribution of causative agents of diphyllobothriosis in this text. Figures 17.8 through 17.12 provide a general outline of the geographic distribution of the most frequent human-infecting species. In addition, the occurrence of the most important species has been briefly surveyed for individual continents (see Scholz et al.[3] for more data).

17.5.1.1 Europe

D. latum has been considered the principal species infecting humans in Europe, whereas *D. dendriticum* was supposed to be limited to northern Europe.[3,68] Human diphyllobothriosis is still present in western and northern Europe, but it shows a marked decrease in historical endemic areas, such as Baltic countries, Poland and Scandinavia, including Finland, where the number of human cases used to be very high.[76]

The disease caused by *D. latum* seems to reemerge in the subalpine region of France, Italy, and Switzerland, especially around big lakes such Geneva Lake, Lago Maggiore, and Lago di Como, with more than 500 cases reported from 1987 to 2007.[56,76] Raw fillets of perch (*P. fluviatilis*) served as "carpaccio" represent the principal source of human infection. Sporadic cases have also been reported in some countries of Central and western Europe, which were previously considered disease free. It is apparently linked to the increased consumption of raw imported fish. Most of these records include imported (exotic) species such as *D. nihonkaiense* or recently *D. pacificum*, which originally occurred in the Pacific region.[3,75,154] To date, 11 cases of sparganosis (7 subcutaneous, 2 ocular, 2 cerebral) have been reported from Europe, namely the Czech Republic, France, and Italy.[77,78]

17.5.1.2 North America

In North America, the number of reports assigned to *D. latum* declined drastically over the last 100 years,[16] with most cases reported from the Great Lakes region, central Canada (Manitoba), and Alaska. Seven species of *Diphyllobothrium* were reported as causative agents of human infection in

North America: *D. alascense* Rausch et Williamson, 1958, *D. dalliae* Rausch, 1956, *D. dendriticum*, *D. lanceolatum* Krabbe, 1865, *D. latum*, *D. nihonkaiense*, and *D. ursi* Rausch, 1954,[3,16] but some of them are only occasionally found in humans in Alaska.[66] In the near Arctic, *D. dendriticum* seems to dominate, but precise data on its actual prevalence are not available.[66–68]

Regarding sparganosis, most of the patients in the United States are men from the eastern seaboard and Gulf coast, from Texas to New York. About 70 cases of all kinds of sparganosis have been reported.[79] Some cases of sparganosis have been reported from Central America and the Caribbean such as Belize and Puerto Rico.[79]

17.5.1.3 South America

Diphyllobothriosis caused by *D. pacificum* is endemic on the Pacific coast (in Peru, Chile, and Ecuador); the principal definitive hosts of this species are sea lions or eared seals (Pinnipedia: Otariidae). The spectrum of its second (fish) intermediate hosts is not well known,[42] but numerous marine fishes that are eaten raw as "ceviche" may serve as a source of human infection (Tables 17.2 and 17.4, Figure 17.5).[3]

Two species, *D. dendriticum* and *D. latum*, are supposed to have been introduced to Argentina and Chile.[80] However, no convincing evidence to support this hypothesis is available, and other explanations of the occurrence of these species in South America are possible. For example, *D. dendriticum* may have been imported with migratory birds such as *Sterna hirundo*, *Sterna paradisea*, and *Larus pipixcan* on their visits to South America.[81] It should also be noted that native fish, such as *Galaxias maculatus*, *Galaxias platei*, *Diplomystes composensis*, *Percichthys trucha*, and several others, are heavily infected with *Diphyllobothrium* plerocercoids.[82] Import of rainbow trout or salmon infected with *Diphyllobothrium* plerocercoids to Chile does not seem to be a possible explanation of the current occurrence of *D. dendriticum* and *D. latum* in South America because only salmonid eggs were imported from Germany.[83] Recently, outbreaks of diphyllobothriosis have been reported from Brazil (Rio de Janeiro and São Paulo),[3,68] with individuals likely becoming infected by consuming Pacific salmon (*Oncorhynchus*) brought from Chile, Alaska or Canada.

Around 16 cases of sparganosis have been described from South America (Argentina, Brazil, Columbia, Ecuador, Guyana, Panama, Paraguay, Uruguay, and Venezuela), including several cases of proliferative sparganosis.[84,85]

17.5.1.4 Asia

Diphyllobothriosis is widely distributed in the northern and eastern parts of the continent. Numerous human infections have been reported from Japan, with the average of about 100 cases/year.[20] Until 1986, when Yamane et al. described *D. nihonkaiense*, most human cases were attributed to *D. latum*, but its occurrence in Japan, Korea, and Far East of Russia was not confirmed. Although as many as 11 species of human-infecting diphyllobothriidean cestodes have been reported from Japan,[14,20] recent data using

TABLE 17.4

Fish Dishes Responsible for Diphyllobothriosis and Their Distribution

Salted fish fillet in marinade for 2–3 days (Finland).
Fish stroganina or fish "shashlik"—pieces of raw fish (Russia, Amur River).
Salted fish egg in pike (Karelia and St. Petersburg).
Raw, dry, or salted fish (North Europe, Far East—Baikal Lake).
Slightly roasted fish on stick in fire (Yakutia, Krasnoyarskiy Region).
Raw liver of burbot (Russia and Lithuania—Curonian Lagoon; Switzerland).
Gefüllter Fisch (*gefilte fish*)—The belly of big fish (as pike) is cut, and the fish is skinned; entrails are removed, and the meat is chopped and seasoned with various spices and cooked. The risk is with tasting before cooking (Jewish population in both Europe and United States).
Sashimi—Pieces of raw fish (Japan, Korea, worldwide).
Sushi—Pieces of raw fish in rice (Japan, Korea, worldwide).
Ceviche—Pieces of raw fish with lemon and chilli (Latin America).

molecular diagnostics strongly suggest that the majority of cases were caused by *D. nihonkaiense* (>2000 cases) and *Dipl. balaenopterae* (>200 cases).[20,86] Human cases of diphyllobothriosis and diplogonoporiasis have also been reported recently from Korea, Taiwan, China, and Far East of Russia.[86–89]

Infections with *Diphyllobothrium* are common in Russia, and *D. latum* and *D. dendriticum* are widespread in all major river basins east of the Ural up to the Far East including Lake Baikal and the Amur River region.[68,90] In the latter region, *Diphyllobothrium klebanovskii* is regarded as an important zoonotic species,[90] but that species was synonymized with *D. nihonkaiense* and this synonymy was confirmed by molecular methods.[53] The numbers of autochthonous cases of diphyllobothriosis in other countries south of Russia (China, India, Indonesia, Malaysia, the Middle East, or Saudi Arabia) are very low, and they may have been imported from endemic areas, especially Japan and Russia. In addition, species of *Spirometra* may have been misidentified as *Diphyllobothrium*, because adults of both genera are similar in their gross morphology.

Most of the cases of sparganosis have been reported from China (>1000 cases reported from 25 provinces from the mainland, with 164 recorded cases from 2000 to 2010[91]), Korea (119 cases),[92] Japan (by 1996 nearly 400 cases),[20] Thailand (52 cases),[93] India, Indonesia, Laos, the Philippines, and Vietnam. There are also 13 cases of parasitism caused by adult *Spirometra* (i.e., spirometrosis) in humans in Japan and Korea.[20]

17.5.1.5 Africa

Diphyllobothriosis is probably not present in Africa, even though some uncertain records exist in the literature.[45] In contrast, the occurrence of sparganosis in Africa is well documented. Schmid and Watschinger[94] reported a total of 30 cases in this continent, 24 of which were mainly from eastern or equatorial Africa (Democratic Republic of the Congo, Gabon, Kenya, Liberia, Madagascar, Tanzania, Uganda, and Zimbabwe). Most of the cases were subcutaneous, with only three visceral and two ocular cases. The causative agent of sparganosis in Africa is probably not *S. erinaceieuropaei*, but a different species, for example, *Spirometra theileri* (Baer, 1925) or *Spirometra pretoriensis* (Baer, 1925), whose life cycle involves big mammals (e.g., antelopes), without amphibians and reptiles serving as intermediate hosts.[32]

17.5.1.6 Australia

Diphyllobothriosis is absent in Australia, but several immigrants have been infected (unpublished records—Queensland Museum—Woolongong Hospital 2. v. 1956; Melbourne 26. ix. 1956). One well-documented case of diphyllobothriosis caused by *D. nihonkaiense* has been reported from New Zealand, which most likely originated from ingestion of raw salmon by a Japanese tourist.[55] Four cases of autochthonous cases of subcutaneous sparganosis are reported from Australians who had never left the country.[95]

17.5.2 Foodborne Transmission

Diphyllobothriosis is a typical foodborne disease because humans get infected via consuming larvae (plerocercoids) with food. Since heat kills infective larvae quickly and safely, the main problem lies with the human habit of eating raw or poorly cooked fish, which is distributed worldwide as is obvious from Table 17.4. However, transmission is successful only in the endemic areas where the parasite is able to complete its three-host life cycle. Parasitological inspections of fish sold in restaurants, fisheries, and supermarkets may help detect potential sources of human infection,[3] whereas parasitological examination of muscle samples of fish in salmon-processing factories may prevent exportation of infected fish.[96]

A better awareness by the human population of the risk of eating raw or undercooked fish, together with improved hygienic conditions, which prevent contamination of water with parasite eggs in feces, has contributed to a considerable decline in human cases of diphyllobothriosis in many countries, especially in northern Europe and North America. In contrast, the increasing popularity of eating raw fish, which seems to be worldwide fashion mainly in developed countries, represents the key factor that has

contributed to the recent reemergence of diphyllobothriosis in the sub-Alpine regions of Europe but also to the emergence of new foci of diphyllobothriosis, such as those in the biggest cities of Brazil or China.[3,97] Salmon and the growing popularity of salmon meat consumption are probably the most common causes of the increase in the number of human cases of diphyllobothriosis. In some countries with high-level medical care such as Japan and Korea, the habit of consuming raw fish dishes is so ingrained that it will not change despite good awareness of the risk of infection with fish-borne parasites such as broad fish tapeworm, anisakid nematodes, or small intestinal flukes.[2]

From an epidemiological point of view, the site of infection (localization) of plerocercoids of *Diphyllobothrium* and *Diplogonoporus* in fish and those of *Spirometra* in amphibians and reptiles is of key importance, because the larvae of species that occur in muscles, such as *D. latum* and *D. nihonkaiense*, represent a more important source of human infection than species with plerocercoids in the body cavity, on the mesenteries, or on the surface of the stomach or duodenal wall (e.g., *D. dendriticum* and *D. ditremum*) (Table 17.3). Unlike the aforementioned species, limited information exists on site preference of plerocercoids of marine species, especially *D. pacificum*, and the spectrum of their fish hosts. Similarly, data on hosts of plerocercoids of rare human-infecting species (e.g., *D. cameroni* Rausch, 1969, *D. cordatum* Leuckart, 1863, or *D. hians* Diesing, 1850) and their localization are scarce or completely missing.[3]

17.5.3 Environmental Contamination and Reservoirs

The existence of foci of diphyllobothriosis infection depends on numerous factors, such as the fecundity of adult worms, survival of larvae (plerocercoids) in fish, and the spectrum of potential definitive hosts. Migration of people, who retain their dietary habits, also represents an important aspect of the epidemiology of the disease. The reproductive potential of large-sized species of *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* is extraordinarily high, with estimates that some species can produce up to 1 million eggs/day.[3] This implies that small numbers of definitive hosts can heavily contaminate water bodies with eggs. The presence of first intermediate hosts (copepods, which are available almost everywhere) and the long survival of plerocercoids in fish and adults in a wide spectrum of nonhuman definitive hosts also facilitate the perpetuation of the life cycle.[16]

Since *Diphyllobothrium*, *Diplogonoporus*, and *Spirometra* tapeworms can mature in nonhuman hosts, treatment of the human population does not necessarily eliminate the parasite from concerned areas. Sylvatic cycles involving bears, foxes, seals, whales, gulls, and other fish-eating birds and mammals probably play a crucial role in maintaining the parasite even in uninhabited regions. However, the actual role of individual definitive hosts as reservoirs of the parasite is insufficiently known.

Another important aspect that may contribute to the dissemination of diphyllobothriosis to new regions is the fact that animals serving as reservoirs of adult tapeworms have often high vagility (capacity to move about or disperse in a given environment) and thus can disseminate the parasite to new geographical areas. Similarly, the importation of fish intermediate hosts, such as Pacific salmon, rainbow trout, or whitefish, also contributes to the dissemination of diphyllobothriosis to new regions.[3,71] Fish represent an important reservoir of parasite larvae because plerocercoids may survive in their body from several months up to a few years.[3,16]

17.6 Pathogenesis and Clinical Features

Broad fish tapeworms are usually large sized and thus mechanically affect their hosts, but many infections with these parasites are asymptomatic.[6] The severity of the disease depends on worm burden and the by-products produced by tapeworms. Symptoms of diphyllobothriosis include diarrhea or loose bowels (14%) and abdominal pain or discomfort (13%), but they appear only in ~20% of infections.[86] In most cases, evacuation of the worm (83%) is the only symptom of the infection.[86] Other nonspecific symptoms may include fatigue, constipation, headache, and allergic reactions.[98] Massive infections may result in intestinal obstruction, and migrating segments can cause cholecystitis or cholangitis, but these cases are exceptional. Although the symptoms of diphyllobothriosis are generally mild, the infections can have a

substantial emotional impact on patients and their families, because segments are evacuated over a long period of time. More severe cases may require specialized consultations and complementary analyses, which are costly.[99]

Prolonged or heavy *D. latum* infections are reported to cause megaloblastic anemia due to parasite-mediated dissociation of the vitamin B_{12}–intrinsic factor complex within the gut lumen, making B_{12} unavailable to the host.[100] About 40% of *D. latum*–infected individuals may show low B_{12} levels, but only 2% or less develop clinical anemia, which is rare or nonexistent in diphyllobothriosis caused by *D. pacificum*.[101] After successful treatment, B_{12} levels come back to normal ranges over a period of several months. *Diphyllobothrium*-associated pernicious anemia has been reported only in Finland after the Second World War.[102]

Pathogenesis of sparganosis is much more variable. Two forms of the organism have been described: nonproliferative and proliferative. The nonproliferative form is slow growing and does not reproduce, whereas the proliferative form reproduces in intermediate or paratenic hosts. The proliferative form is thought to be caused by *Sparganum proliferum*, but the taxonomic status of its causative agent remains to be clarified.

The first case of sparganosis was described by Manson in 1881 from a Chinese man and described by Cobbold[103] as *Ligula mansoni*. However, the first human case was reported as early as in 1596.[5] The majority of plerocercoids penetrate the stomach or duodenal wall, distribute in the abdominal cavity, and finally migrate to the skeletal muscles or subcutaneous tissues, where they typically develop into a nodular mass. The characteristic host response includes an inflammatory cell infiltration in the first week after the inoculation into the soft tissue, tunnel-like structures (an elongated tract-like cavity through which a larva has migrated) 2 weeks later, and a fibroblast proliferation between 4 weeks and 6 months postinfection.[104] The rate of growth in humans is about 2 cm/month, and the rate of migration was observed to be 4–5 cm/month.[105]

Sparganosis usually manifests in the form of a painful, inflamed, subcutaneous nodule, which may have a history of migration, disappearance, and reappearance due to the movements of the worm. The sparganum can live up to 20 years in human hosts, but it is rare to find living worm after 5 years.[106] Most of the patients with sparganosis harbor a single plerocercoid larva (sparganum), which can migrate, causing various symptoms depending on its final localization. Most larvae lodge and die in subcutaneous tissue or muscles, but they may locate anywhere. Symptoms do not usually develop until the sparganum lodges or dies in tissue.

Although the most common and natural localization of sparganum is subcutaneous tissue, the plerocercoids have been found in many areas of the human body. The sparganosis is divided into five groups on the basis of localization of the sparganum.

17.6.1 Subcutaneous Sparganosis

This is the most common form of sparganosis in humans. The sparganum migrates to subcutaneous connective tissues and superficial muscles, for example, abdominal wall, scrotum, lower extremities, and chest wall. The lesion (sparganum) under the skin appears as a palpable, rubber-like, irregular nodule or lump (1–2 cm in diameter), resembling a lipoma, fibroma, or sebaceous cyst that is slow growing, itchy, inflamed, painful, and moveable. Some patients have slowly growing, painful, sometimes migratory subcutaneous nodules.[107]

17.6.2 Ocular Sparganosis

This form is associated with larval migration to the subconjunctiva, conjunctiva, and the orbit. Located in the posterior pole, the larva induces periorbital edema, exophthalmos (protrusion of the eyeball), and lagophthalmia, which in turn causes eye pain, itching, epiphora (excessive lacrimation or watering of the eye), ptosis (drooping of the upper eyelid), irritation, orbital cellulitis, and marked swelling of the eyelids and cornea ulcer.[108] If untreated, ocular sparganosis can lead to blindness.[109] It is common in Asia and is usually, but not always, attributed to application of infected frog flesh to eyes. The sparganum often invades only the conjunctiva but can enter periorbital tissues as well. In the subconjunctival tissue,

spargana provoke pruritus, pain, edema, and lacrimation.[110] Rarely, a sparganum may enter the globe and cause endophthalmitis. Ocular sparganosis was described inside the ocular globe in India and inside the anterior chamber in France and the Czech Republic.[77,111] The largest sparganum removed from the eye was 44 cm long from a man in Thailand.[112] Surgery is the only effective treatment.[108]

17.6.3 Visceral Sparganosis

This form relates to the migration and growth of the larvae in the intestinal wall, the breast, abdominal cavity, pleura (lung), and the pericardium (heart),[113] resulting in damage to or malfunction of the particular organ or tissue. Sparganosis can cause intestinal obstruction or peritonitis in the case of intestinal perforation. Sparganosis of the urinary bladder sometimes leads to cystitis.[114]

17.6.4 Cerebral Sparganosis

This form involves the growth of the larvae in the cerebral hemispheres or spine, especially the frontoparietal lobes, which in some cases may extend to the cerebellum. The disease may appear as a cerebral hemorrhage. When spargana settle in the brain or spine, various neurological symptoms may appear, including fatigue, motor weakness, confusion, headache, seizure, memory loss, coma, fever, paresthesias, hemiparesis, brain abscesses, and abnormal skin sensations (e.g., numbness or tingling).[115–117] Intraspinal sparganosis can induce paraparesis and autonomic function deficits,[118] and inner ear involvement can provoke vertigo or deafness.[119] Seizures, hemiparesis, and headache are present in most patients.[120] A dead or degenerated sparganum may appear as a calcified mass in the brain and can produce intraventricular hemorrhage and obstructive hydrocephalus.[121] CNS involvement is common, more often with proliferative disease, but the mechanism of dissemination is unknown. In China, 82 cases of cerebral sparganosis have been reported so far, which represent around 8% of all reported cases of sparganosis.[117]

17.6.5 Proliferative Sparganosis

This is caused by the so-called *Sparganum proliferum*, which invades the subcutaneous region and other tissues of the human body including bones and undergoes continuous branching and budding, producing vast numbers of asexually multiplying plerocercoids in a single site, which finally leads to fatal human sparganosis. It often begins with an open, tumor-like subcutaneous nodule (due to ulceration or scarification) in the thigh, shoulder, or neck and spreads to other parts of the skin, muscles, and internal organs (e.g., lungs, abdomen, and brain) over 5–25 years, usually with a very poor outcome.[122] *Sparganum proliferum* usually forms gradually expanding and ulcerating cutaneous and subcutaneous papules and nodules.[122] *Sparganum proliferum* was reported as a cause of a disseminated and proliferative infection with CNS involvement.[123] In total, only 16 cases of proliferative sparganosis have been reported—7 cases from Japan, 3 cases from Taiwan, 2 cases from Florida (United States), 2 from Venezuela, 1 from Paraguay, and 1 from the Czech Republic.[124,125] Most of these cases were lethal.

17.7 Treatment and Prevention

17.7.1 Treatment

Adult *Diphyllobothrium* and *Diplogonoporus* tapeworms in the human intestine are easily treated with praziquantel in a single oral dose. However, the recommended dose to be used for the treatment of individual species of *Diphyllobothrium* may differ. For example, a dose of 10 mg/kg was effective against human infections with *D. pacificum*, but it was not effective against *D. latum* in experimentally infected golden hamsters.[126,127] In contrast, a dose of 25 mg/kg was highly effective against human *D. latum* infections.[128] *D. nihonkaiense* seems to be more sensitive to praziquantel than *D. latum* and equally or more sensitive to praziquantel than *D. pacificum*. In some studies, oral administration of a single dose of praziquantel at 5–10 mg/kg was reported to be effective,[129] but a single administration of a 25–50 mg/kg

dose is usually applied to ensure complete expulsion of the worm. Side effects of praziquantel are usually mild and do not require treatment but may be more serious in patients with heavy worm burdens.[128]

Other anthelmintic drugs are also effective against *Diphyllobothrium* or *Diplogonoporus* tapeworms, but they have some limitations compared to praziquantel. Niclosamide (a single dose of 2 g in adults) is effective for diphyllobothriosis, and its side effects are infrequent because it is not absorbed from the gastrointestinal tract.[87] However, it is now not permitted for treatment of human cases in several countries because of its potential negative effects to humans. Intraduodenal gastrografin is efficacious in the treatment of large cestodes, including species of *Diphyllobothrium* and *Diplogonoporus* occurring in Japan.[130] It also enables complete discharge of living worms with the scolex intact and is thus suitable for species identification, but the insertion of the duodenal tube is painful, the therapy is expensive, and fluoroscopic images are needed.[3]

To expel intact worms, Rausch and Hilliard[131] proposed a fat-free diet for 24 h, after which the patients are given 30 g magnesium sulfate dissolved in water in the evening. The next morning, 100 mg of phenobarbital is administered, then 1 g of quinacrine with an equal amount of sodium bicarbonate. Two hours later, 30 g magnesium sulfate is again administered. No food is allowed until defecation.

Treatment of peripheral infection of sparganosis with praziquantel has produced limited success.[132] Cerebral infection responds best to surgical excision of the parasite, as praziquantel has no effect on adult worms in the CNS.[133] Most spargana respond to medical or surgical therapy, but attempts at surgical removal of *Sparganum proliferum* are usually unsuccessful due to the widespread dissemination of this species.[123] Although various treatments have been used, none has been effective for proliferative disease.[125]

17.7.2 Prevention

The most effective and cheapest individual prophylaxis of diphyllobothriosis and diplogonoporosis is avoiding the consumption of unsafe fish, that is, raw, undercooked, smoked, or pickled. Thoroughly cooking fish and/or freezing them kill infective larvae (plerocercoids) in the muscles or viscera. Cooking fish at a temperature of 55 °C kills plerocercoids in 5 min,[16] whereas freezing needs much more time and the recommended temperature and time necessary to destroy the larvae differ from each other (Table 17.5).

Fish helminths are usually introduced into new areas via salmon transported worldwide fresh, nonfrozen on ice. They are shipped mainly from the Pacific Northwest, Alaska, or Chile. It is, therefore, necessary to inform consumers about the risks linked to some culinary habits. They should also be aware that cold-smoking of fish does not kill the parasite.[134] Salting of fish results in reduced infectivity of *Diphyllobothrium* plerocercoids, but it may take several days or weeks depending on the size of the fish and the volume of salt used.[135]

Effective control in a large scale, that is, in endemic foci, should include breaking the life cycle of the parasite. Due to the complex nature of these life cycles (three different hosts involved), elimination of any ontogenetic stage can prevent completing the developmental cycle. Theoretically, the simplest way to prevent human infection is to avoid consumption of raw fish, but traditional habits or increasing

TABLE 17.5

Recommended Procedures for Fish Freezing

Temperature (°C)	Time	Reference	Remarks
− 8	12 h	Eguchi[155]	
−10	6 h	Eguchi[155]	
−10	8–72 h	Feachem et al.[156]	Depends on thickness of fish flesh
−10	24 h	Raethel and Hanel[157]	
−11	24 h	Nyberg[158]	Depends on size of fish
−18	8–24 h	Salminen[159]	Fish up 1 kg (8 h); up to 5 kg (24 h)
−20	>24 h	EU–Hazard Analysis and Critical Control Points	
−20	7 days	U.S. Food and Drug Administration	For food products
−35	15 h	U.S. Food and Drug Administration	For food products

popularity of this "fashion" make this control measure very difficult. Treatment of infected persons is effective but does not help eliminate the parasite in an ecosystem, because other definitive hosts may play a role as reservoirs of the parasite. Prevention of water contamination is also important, but it has limited impact if other definitive hosts occur in the area. The existence of reservoir hosts (piscivorous mammals and birds) represents a serious obstacle in programs to control diphyllobothriosis or sparganosis.

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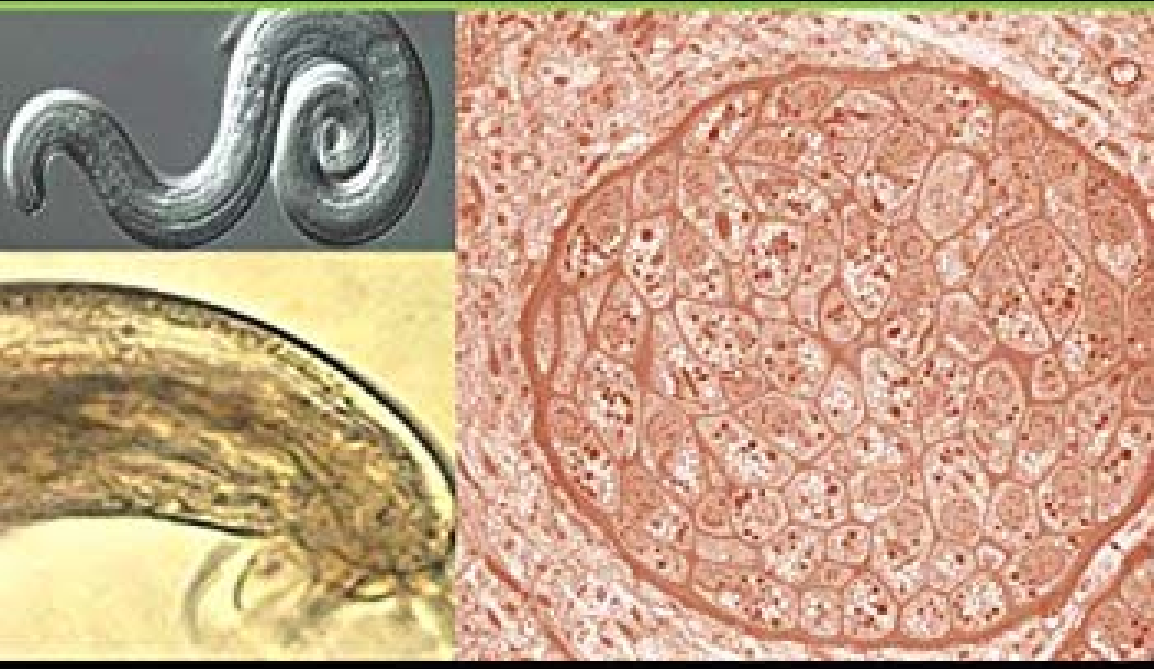

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