Chapter 9

Histological Change of Aquatic Animals by Parasitic Infection

Watchariya Purivirojkul

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52769

1. Introduction

The aquatic parasite usually classified in six groups include Protozoa (Phylum Rhizopoda, Phylum Mastigophora, Phylum Ciliophora, Phylum Myxozoa, Phylum Microspora, Phylum Apicomplexa), Phylum Platyhelminthes (Turbellaria, Monogene, Digenia, Cestoda), Phylum Nematoda, Phylum Acanthocephala, Phylum Annelida and Phylum Arthropoda (Branchiura, Copepoda, Malacostraca (order Isopoda)). Because of their size are vary from microscopic size (1 μm) to macroscopic type (more than 1 meter in some species), more techniques will need to help scientist to classified them. Histological methods are becoming quite common in diagnostic methodology in aquatic animals for a long time (Klontz, 1985). From 1999 to 2012, the author collected more than 120 species of parasites from wild and cultured fish from freshwater and marine fish in many parts of Thailand; North of Thailand (Chiang Rai province, Payao province), North-east of Thailand (Nakornratchasima province, Kalasin province, Sakonnakorn province), East of Thailand (Chachoengsao province, Chonburi province, Rayong province, Trat province), Middle of Thailand (Suphan Buri province, Ratchaburi province, Phra Nakhon Si Ayutthaya province). The specimens from this study were fixed by fixative, dehydrated through a graded ethanol series and embedded in paraffin. Five micrometer thick sections were prepared and stained with Harris' hematoxylin and eosin (H&E). This method advantage to classify the disease because in some situation parasites hide deep in the organ that can not observe by the simple diagnosis such as brain, vertebrae and heart etc.. Moreover, histological study can explain the effect of parasite in the host and predict for chemical and drug therapy for aquatic animals more precisely.

All groups of aquatic parasite are explained at below:

2. Protozoa

The protozoa is a vast assemblage of essentially single celled eukaryotic organisms [2]. Protozoans can be ectoparasites or endoparasites depending on their species. Protozoan ectoparasites are the most common parasites encountered in cultured fish [3]. They are also frequently found in wild fish [4]. Ciliates and flagellates feed on the most superficial skin layer [4]. Parasites cause a reactive hyperplasia of the epithelium and increased mucus production. Hyperplasia appears as a cloundiness to the skin and leads to hypoxia if occur on the gills [4]. Microspora are intracellular and affect a wide variety of vertebrates and invertebrates, Myxosporea are largely intercellular and infect mainly fish [5].

2.1. Phylum Rhizopoda (amoeba)

Amoeba can infect on the external surface such as gill, skin and internal organ as intestine. *Paramoeba pemaquidensis* has caused chronic mortality [4]. This parasite causes proliferative gill lesions. Histopathological change includes focal lamellar hypertrophy with epithelial hyperplasia and metaplasia [4]. *Neoparamoeba perurans* causes amoebic gill disease (AGD) [6]. This parasite affects some species of fish farmed in the marine environment such as Atlantic salmon [7]. The gills of infected fish are pale, multifocal with diffused white AGD-like lesions. Histological changes in the gills are characterized by hyperplasia of epithelial like cells across the gill filaments. This resulted in the fusion of lamellae and the development of round to oval interlamellar vesicles. Hyperplasia of mucous cells are also observed in the gills [6].

2.2. Phylum Mastigophora (flagellated protozoa)

Aquatic parasites in this subphylum separated in two classes; Phytomastigophora and Zoomastigophora

Phytomastigophora contain chloroplasts in their cytoplasm [2]. Common type of this group was dinoflagellate parasites *Amyloodinium* sp. and *Piscinoodinium* sp. [2]. *Amyloodinium* sp. and *Piscinoodinium* sp. cause necrotic dermatitis to their host. *Amyloodinium ocellatum* is marine fish parasites that can infect both elasmobranchs and teleosts [4]. They attach to the host's epithelium by rhizoids (root-like structures) and feed on the host [4]. The gills are the primary site of infection. Heavy infestations can cause gill hyperplasia which include epithelium to severe hyperplasia of the entire gill filament, inflammation, hemorrhage and necrosis. In freshwater, *Piscinoodinium* is reported, histopathological changes are similar to *Amyloodinium* sp. Filament degeneration and necrosis may also occur [4].

Zoomastigophora do not contain chloroplasts in their cytoplasm [2]. Three orders; Kinetoplastida, Retortamonodida and Diplomonadida are usually reported as fish parasites [2].

Parasites of order Kinetoplastida have one or two flagella such as *Cryptobia* sp. [2]. *Cryptobia* spp. are reported to invade the gastric epithelium of European cyprinids and flounders, they may cause gastric dilation, lesions include submucosal granuloma, gastric perforation, peritonitis and full thickness necrosis of the body wall musculature (Ferguson, 1989).

Parasites of order Retortamonodida are possessing two to four flagella, one turned posteriorly such as genus *Ichthyobodo* [2]. *Ichthyobodo* is especially dangerous to young fish and can attack healthy fry and even eggs [4]. *Ichthyobodo necatrix* induces severe erosion and ulcerative dermatitis following epithelial hyperplasia and increased mucus production [4].

Parasites of order Diplomonadida have one to four flagella and two-fold rotational or bilateral symmetry such as *Hexamita* spp. and *Spironucleus* spp. [2]. *Hexamita* spp. are reported in the intestine of many fish such as siamese fighting fish, oscar, discus fish and rainbow trout, cause hexamitiasis [5]. This parasites are associated with a full-thickness gastritis and may penetrate blood vessels leading to dissemination [5]. *Spironucleus* sp. cause of hole-in-the-head disease of oscars, discus and other aquarium cichlids. This parasite ia associated with large erosions in the cranial cartilages, may ulcerate, resulting often in bilaterally symmetrical lesions [5].

2.3. Phylum Ciliophora

Members of this phylum have cilia in at least one stage of the life cycle. They always have two types of nucleus; micronucleus and macronucleus. Most of fish parasites can separate in three classes; Kinetofragminophorea, Oligohymenophorea and Prostomatea [2].

Members of class Kinetofragminophorea have oral ciliature slightly distinct from body ciliature such as *Chilodonella* spp. [2]. Only two species *C. piscicola* and *C. hexasticha* are pathogenic for fish [4]. *Chilodonella* spp. attach on fish skin and gills, this parasite causes infection in fish especially in ornamental fish (Koi, goldfish). *Chilodonella* sp. may feed directly on epithelium, appears to feed by penetrating the host cells with its cytostome and sucking their the contents [4, 8]. After infection, fish secrete excessive mucus, with acute to subacute dermatitis with hyperplasia [4].

Oral apparatus of the members of class Oligohymenophorea usually well defined and oral ciliature is distinct from somatic ciliature such as *Ichthyophthirius multifilis*, *Trichodina* sp. *Tetrahymena* sp. and *Epistylis* sp. *I. multifilis* parasitizing many species of wide and culture freshwater fish, cause Ich or white spot disease. This parasite is large in size (about 0.1-1.0 mm) contains a horseshoe-shaped macronucleus. Infected fish have the presence of small white spots on the skin or gills. *I. multifilis* cause acute to sub acute dermatitis with hyperplasia, they present within epidermis [5]. The epithelial erosion and ulceration that result from the parasites' entrance into and exit from the host are probably as damaging as its feeding activity. Lesions produced by the parasites may also lead to secondary microbial infections [4].

Trichodinid species (*Trichodina*, *Trichodinella*, *Tripartiella*, *Paratrichodina*, *Hemitrichodina* and *Vauchomia* sp.) especilly *Trichodina* sp. (Figure 1) are reported from infected skin and gills of freshwater and marine fish. They inhabit the surface of fish, adhere through the suction on the epithelium may cause damage [4,9]. They cause subacute dermatitis with hyperplasia [5]. Mortalities can be much higher, especially in young fish [4]. In incidental case, some species of Trichodina may infect the urinary bladder, oviducts or gastrointestinal tract [4]. In this case, unilateral aplasia of a ureter or chronic inflammation may result in cystic dilation of that portion proximal to the obstruction [5].

Tetrahymena sp. (Figure 1) is the parasite of freshwater fish, cause erosion of the cranium in Atlantic salmon [5]. Tetrahymena pyriformis are usually reported in the parasite of guppy, capable of disseminated infections with dermal ulceration. In some cases, this parasite may invade various internal organs, kidney or brain [4].

The peritrichous ciliate, *Epistylis* sp. and *Scyphidia* sp. are widely spread on fresh water fish such as cichlid and cyprinid. They may infect skin, fins, oral cavity and gills. *Epistylis* sp. is the most common and pathogenic type of sessile, colonial ectocommensal ciliate [4]. Host response for this infection occur by hemorrhagic lesions, necrotic dermatitis with ulceration [5]. In some case, *Vorticella* sp. was found on the thoracic appendages and the cercopods of fairy shrimps [10] (Figure 2). Although in much literature, *Vorticella* sp. is reported as a free-living organism, in some case, when aquatic animals are stressed by adverse environmental conditions and they are completely debilitated and moribund, these free-living ciliophorans become facultative parasites externally [11].

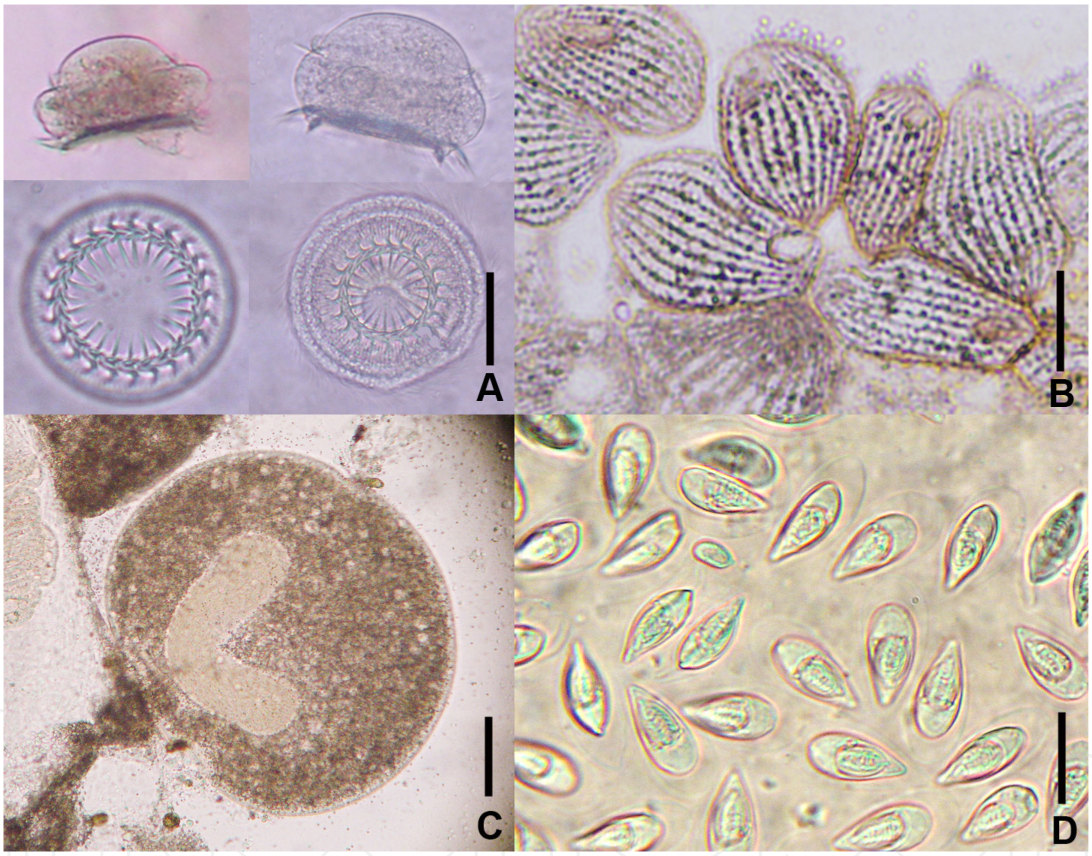

Figure 1. Some parasitic protozoa from aquatic animals.
(A) *Trichodina* spp. from *Pangasianodon gigas*. (scale bar = 50 µm) (source: Purivirojkul W, Areechon N, Purukkiate C. Parasites and bacteria of giant catfishes (*Pangasianodon gigas* Chevey) larva. Thai science and technology journal 2004; 12(3) 1-11.)
(B) *Tetrahymena* sp. from *Poecilia reticulata*. (scale bar = 50 µm)
(C) *Ichthyophthirius multifilis* from *Pangasianodon gigas*. (scale bar = 100 µm)
(D) Myxozoa (*Thelohanellus* sp.) from *Barbonymus gonionotus*. (scale bar = 20 µm)

Cryptocaryon irritans was the member of class Prostomatea, which cause marine white spot disease also present within epidermis as *I. multifilis*. This parasite cause acute to subacute dermatitis with hyperplasia [4].

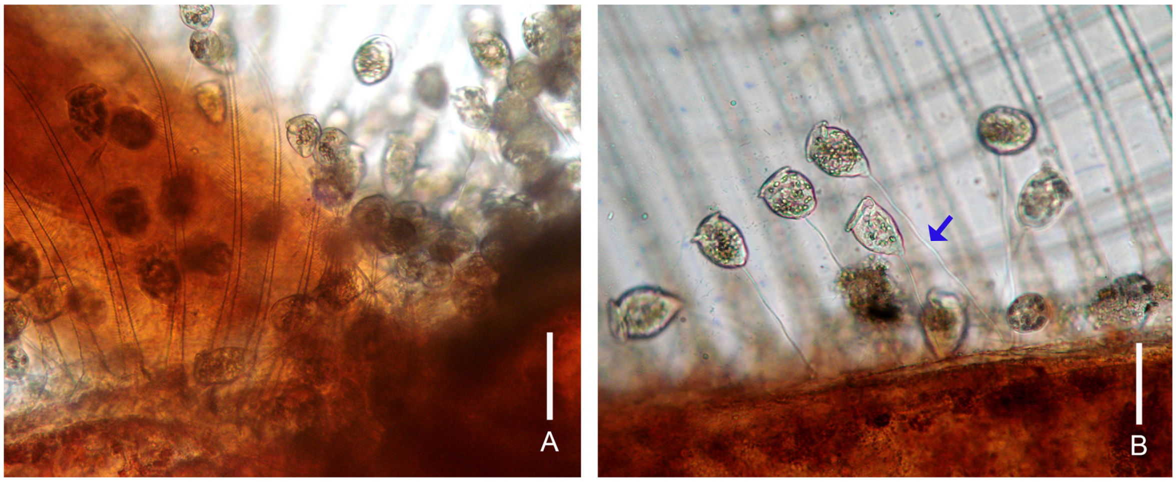

Figure 2. *Vorticella* sp.
(A) Clusters of *Vorticella* sp. infected on the cercopod of fairy shrimp.
(B) Myoneme of *Vorticella* sp. (arrow). (scale bar = 50 μm)

2.4. Phylum Myxozoa

Members of this phylum have spores of multicellular origin, with one or more polar capsules and valves [2]. Classification is based on spore morphology [5]. They are divided into two orders; Bivalvulida and Multivalvulida [2]. Spore of members of order Bivalvulida have two valves such as *Myxidium* sp., *Sphaerospora* sp., *Ceratomyxa* sp., *Thelohanellus* sp. (Figure 1) and *Myxobolus* sp. While spore of members of order Multivalvulida have three or more valves such as *Kudoa* sp. [2].

Parasites in this group settle in muscles and bones of fish hosts lead to fibroplasia and vertebral deformities. In muscle, dermal or sub-dermal cysts are reported [5]. A few myxosporean genera develop intracellularly in skeletal muscle, while others have some development stages that are intracellular [5]. *Myxobolus* spp., infect bone causes vertebral deformities especially *M. cerebralis*, causes whirling disease in farmed salmon and trout. This parasite develops within cartilage, causing lysis and may lead to skeletal abnormalities [5]. The parasite may distort the vertebrae and cause compression lesions [5].

Myxobolus sp. occurred as intact plasmodia between muscle fibers, often adjacent to vertebral spines. The inflammatory response consisted of melanin-laden macrophages and associated with fibroplasia [12]. Many species of *Myxobolus* spp. are reported in fish such as *M. hakyi* in *Pangasianodon hypophthalmus* [13]. *M. hendricksoni* is found in the optic lobes, cerebellum, ventricles and meninges of the fathead minnow. Host response is minimal even though large areas of the brain may be occupied by the parasites [5]. In minnows, *Myxobolus* are found in dorsal root ganglia and spinal canal [5].

The pathology of *Henneguya* spp. is related to the site of infection in the gill (lamellae, filaments or arch) [14]. *H. tunisiensis* infecting the gill arches of *Symphodus tinca*, the parasites develop plasmodia in the connective tissue elements of the gill arch, under the mucosal epithelium. Large plasmodia are usually situated at the ends of the gill arch and induce the compression of the capillaries and retraction of the neighbouring tissue [14]. *H. creplini* infecting the gills of *Stizostedion lucioperca*. The plasmodia located in gill lamellae cause epithelial hyperplasia, and the formation of a thick layer of granular tissue [15]. *H. salminicola* infect pacific salmon muscle cause grossly visible opaque milky white blotches or streaks [5].

Myxosporidiosis associated with the genera *Mitrospora* sp. and *Sphaerospora* sp. found in the kidney caused degenerative changes within tubular epithelium and cast. *Ceratomyxa shasta* is parasite of salmonids that induces granulomatous lesions throughout the viscera, including the kidney [5]. This parasite causes granulomatous peritonitis and sometime total loss of the gall bladder [5]. *Myxidium oviformis* in Atlantic salmon causes severe cholangiohepatitis and inflammation of the gall bladder with emaciation of the host [5]. *Kudoa* sp. release myolytic enzymes after the death of the fish thereby resulting in rapid liquefaction [5].

2.5. Phylum microspora

The phylum Microspora is comprised of unicellular organisms living as intracellular parasites in a variety of invertebrates and in all five classes of vertebrate hosts [16]. Their extrusion apparatus, always with a polar tube and cap [2]. Microsporidia are currently classified on the basis of their ultrastructural features, including size and morphology of the spores, number of coils of the polar tube, developmental life cycle and host-parasite relationship [17].

Some species infect the muscle, dermal or subdermal cysts are seen and often rupture through the skin [5]. Some species form cysts within the intestinal wall such as *Thelohanellus kitauei*, cause intestinal giant cystic disease of carp [5]. The intracellular parasite, *Ichthyosporidium*, *Glugea* and *Spraguea* replicate and cause cytomegaly, leads to atrophy and encapsulating fibroplasia. A large xenoma with a refractile wall and filled with refractile spores may elicit the formation of granulation tissue [5]. Moreover, *Glugea* spp. cause serious pathological changes in a variety of tissues. It infects macrophages and other mesenchymal cells that subsequently undergo massive hypertrophy, causing space-occupying compression and deformations [5].

Many species of microsporidians do not induce hypertrophy of the myocyte but rather replicates within the sarcoplasm, eventually occupying and destroying it such as *Pleistophora* [5]. *P. macrozorcidis* infect the ocean pouts *Macrozoarces americanus*, their cyst within the muscle give the gross appearance of neoplasms [5]. These may cause pressure necrosis of the overlying skin with release of spores into the water as a consequence [5]. *P. longifilis* is recorded from the testis of *Barbus flaviatilis* in Germany [5]. In some species such as *P. ovariae*, ovaries are the prime target of this parasite in golden shiner *Notemigonus crysoleucas*. The large cysts are considered to reduce fecundity, cause pressure atrophy of adjacent uninfected ova and parenchyma and lead to sterility [5].

The genera *Heterosporis*, *Kabatana*, *Microsporidium*, *Pleistophora* and *Thelohania* have been found to infect muscle tissue [16]. Some microsporidian may infect brain and heart for example, *Spraguea lophii* is found in the cerebrospinal ganglia of angle fish, *Lophius* spp.

where it evokes grossly visible xenoma formation [5]. *Loma* sp. cause endarteritis of epizootic proportions in juvenile Chinook salmon [5].

The aquatic parasite microsporideans, such as *Pleistophora* sp. infect muscle of fairy shrimp *Branchinella thailandensis* [10]. In host' muscle, white tubular masses are seen which are indicative of microsporidian infection. Close contact between the sporophorous vesicle and degenerating muscle fibers are observed, but there is no evidence of xenoma or a host response to the parasite (Figure 3). In another case, *Thelohania* (*Agmasoma*) infect both the hepatopancreas and abdominal muscle of Pacific white shrimp (*Litopenaeus vannamei*). Affected shrimp show a whitish or milky appearance in various parts of the body. As the shrimp grow larger, this clinical signs are more easily observed especially dorsally from the hepatopancreas to the middle of the body. Histological changes found in microsporidian infection of most of the hepatopancreas. Hepatopancreatic tubules of the heavily infected shrimp are dilated and necrotic. Gills and striated abdominal muscle are mostly infected by microsporidia [18] (Figure 4).

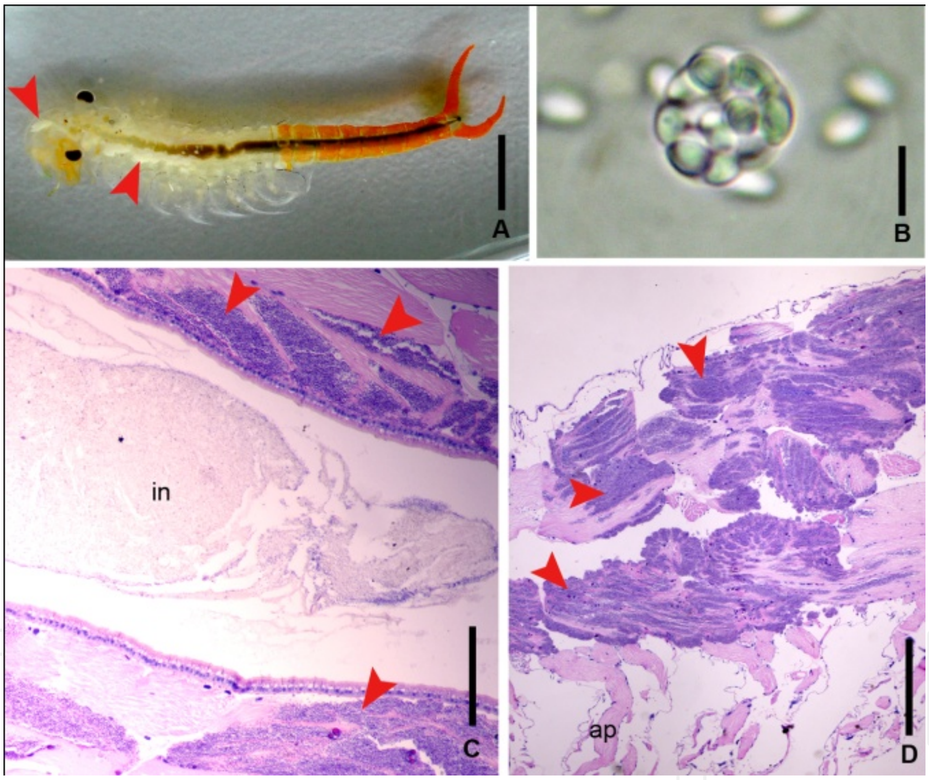

Figure 3. Fairy shrimp *Branchinella thailandensis* infected with microsporidia.
(A) *B. thailandensis* infected with microsporidia, white tubular masses (arrow) indicative of microsporidia infection. (scale bar = 3 mm)
(B) Fresh spores of *Pleistophora* sp. showing one group of spores in a sporophorous vesicle. (scale bar = 6 µm) (source: Purivirojkul W., Khidprasert S. First report of microsporidiosis in fairy shrimp *Branchinella thailandensis* (Sanoamuang, Saengphan and Murugan, 2002). Aquaculture 2009; 289 (1-2) 185-190)
(C) Longitudinal section of *B. thailandensis* at intestine region, the muscle fibers were damaged by microsporidia (arrow). (scale bar = 100 µm) (in = intestine)
(D) Longitudinal section of muscle at the dorsal part of *B. thailandensis* shows degenerating muscle fibers (H&E). (scale bar = 500 µm) (ap = appendage of fairy shrimp)

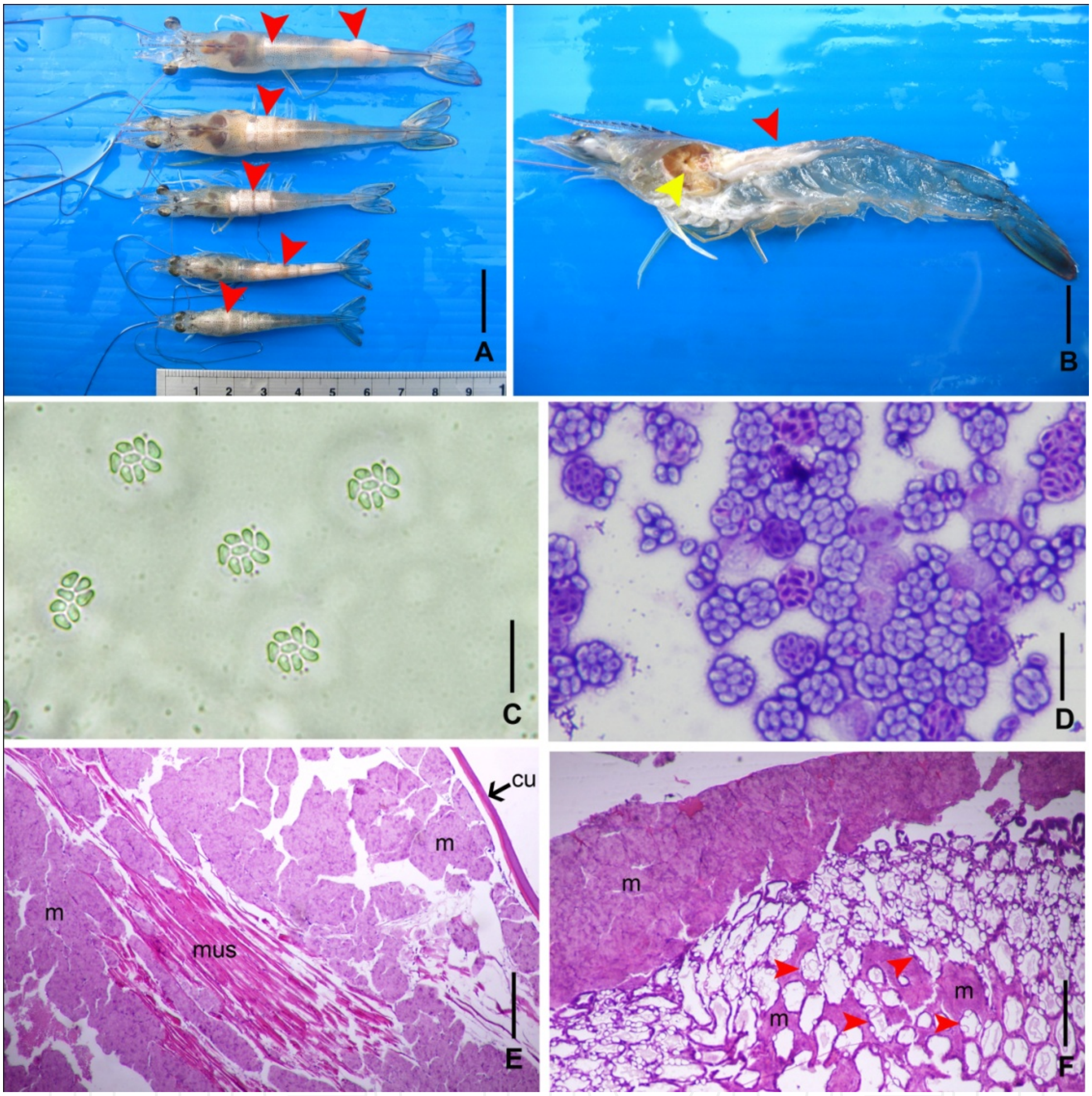

Figure 4. Microsporidian (*Thelohania*) infection in Pacific white shrimp (*Litopenaeus vannamei*) (Photo from Associated Professor Dr. Chalor Limsuwan).
(A) Various size Pacific white shrimp were infected by microsporidia (arrow). (scale bar = 1.5 cm)
(B) Long section of Pacific white shrimp, showed white masses of microsporidian in infested striated muscle (red arrow) and hepatopancreas (yellow arrow). (scale bar = 1 cm)
(C) Fresh spores of *Thelohania* sp. showing 8 spores in a sporophorous vesicle.
(D) Giemsa's strain of *Thelohania* sp. (C,D scale bar = 15 μm)
(E) Striated muscles of infected shrimp, the muscle fibers were damaged and replaced by microsporidia. (scale bar = 200 μm)
(F) Hepatopancreatic tubule epithelium of heavily microsporidian-infected shrimp were dilated and necrotic (arrows). (scale bar = 200 μm)
(cu = cuticle, m = microspore, mus = striated muscles)

2.6. Phylum Apicomplexa

Members of this phylum have a unique organelle, the apical complex, visible only with the electron microscope [2]. They are intracellular parasites, the apical complex serves to assist in penetrating the host cell [2]. The genus *Eimeria* is often reported as fish parasite both marine and freshwater fish. *Eimeria subepithelialis* is the cause of nodular coccidiosis in European carp, the mucosa is damaged when the parasites are released [5]. *Eimeria brevoortiana* are present in the testis of menhaden and get shedded with the milt [5]. In some case, this group of parasite can destroy host tissue such as *Calyptospora* (*Eimeria*) *fundulil* can damage 85% of the liver and pancreas of killifish *Fundalus grandis* when infect in large number [5].

3. Phylum platyhelminthes

Members of phylum Platyhelminthes have a dorsoventrally flattened, bilaterally symmetrical body. This phylum is the first phylum which exhibit three primary germ layers; ectoderm, mesoderm and endoderm. Aquatic parasitology in this phylum includes four classes; Turbellaria, Monogene, Digenia and Cestoda.

3.1. Turbellaria

Although, most of turbellarian are free-living worms but *Ichthyophaga* and *Paravortex* are reported as fish parasites. *Paravortex* spp. infest a wide variety of fishes and molluscs [19-20]. They induce a hypermelanization reaction, resulting in dark foci on the skin, there may be acute, focal dermatitis and hemorrhage [4]. *Piscinquilinus* (*Ichthyophaga*) sp. is reported in marine fish such as a parrotfish (*Scarus ribulatus*). This parasite induces a proliferative epithelial response [4].

3.2. Monogenea

Monogenes are common parasites of the skin and gills of both marine and freshwater fish [4]. Monogenes feed on the superficial layers of the skin and gills, causes skin cloudiness or focal reddening resulting from excess mucus production [4]. Large numbers of monogenes can kill small fish [4]. Taxonomic identification of monogenes is based on the morphology of opishaptor (posterior attachment organ), mode of reproduction and presence of eye spot, etc. [4]. However, *Dactylogyrus* spp. (gill flukes) usually attaches to the gil*ls* of freshwater fish (Figure 5). *Gyrodactylus* spp. (skin flukes) usually attached to the skin of freshwater fish. Monogenes have the simple life cycle without having intermediate hosts.

In skin area, epithelial hyperplasia or hemorrhage are found in monogene infected area [21]. Heavy monogenean infections by their attachment and feeding can induce a range of histopathological changes to the epithelium [22], sometimes severe dermatitis with hyperplasia [5]. *Gyrodactylus* sp. parasite on fish skin may cause superficial lesions of the epidermis. This parasite attaches to and feeds on the epidermis, causes an increase in production of mucus. The excess mucus disturbs the respiratory function of the skin [5].

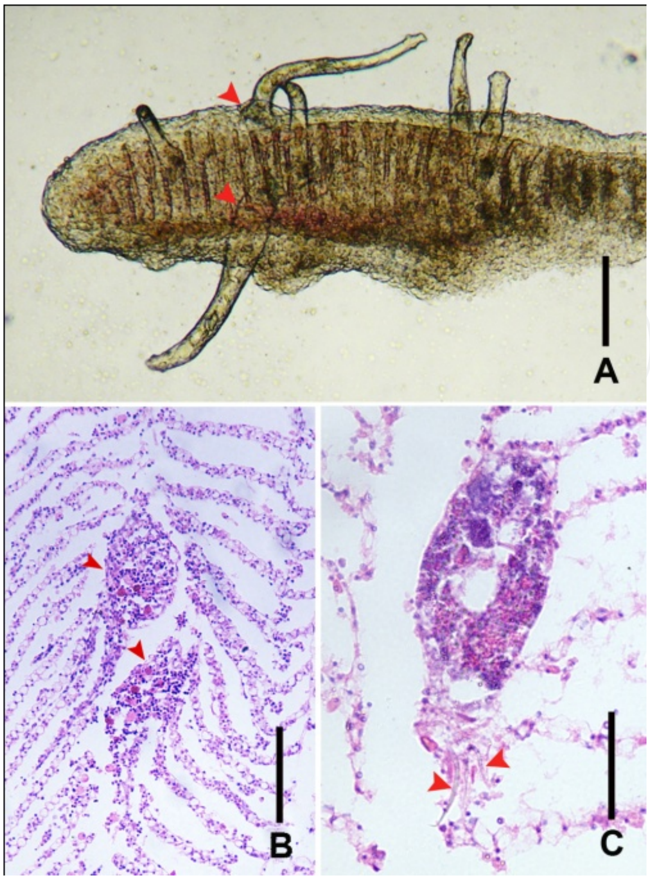

Figure 5. Monogene infested on gill of fish.
(A) Fresh sample, opishaptor (arrows) attach to gill filament of fish. (scale bar = 70 μm)
(B) *Dactylogyrus* sp. infested on gill filaments of *Barbonymus gonionotus*. Hyperplasia of gill filament were observed (arrows). (scale bar = 70 μm)
(C) Anchor of *Dactylogyrus* sp. (arrows) attached to the gill filaments of *Barbonymus gonionotus*. (scale bar = 50 μm) (Photo by Ms.Tanawan Leeboonngam).

Most of monogene infect the gill area (Figure 5), a proliferation of epithelial tissue resulting in the fusion of the secondary lamellae by the attach of the opisthaptor. In some gills, this latter pathology was accompanied by the presence of blood vessel aneurysms (telangiectasis) [22]. Histopathological change by monogene on fish gill was also studied by [22]. In the histological sections of cultured European sea bass, *Dicentrarchus labrax*, the opisthaptors of the parasites *Diplectanum aequans* were observed to penetrate in the basal membrane of primary lamella where they induced a hyperplastic response. Disruption and fusion of the secondary lamellae are common in all infected specimens where several individuals are with erosion and inflammation of the epithelium of the primary and secondary lamellae. The infection of monogenes in fish gills have an impact on the host's ability to regulate its ion balance. This parasite can reduce the number of chloride cells which are the main site of ion absorption and secretion [23].

Another site of infection are reported in *Neobenedenia melleni* which causes serious skin damage and has a predilection for the eye [4]. The hooks of parasite cause ophthalmic lesions leading to blindness [24].

3.3. Digenea

Digeneans have two suckers to attach their host; oral sucker surrounding the mouth and ventral sucker (acetabulum) on the ventral surface. The life cycle of this group consists of intermediate hosts (one or two types) and final host. They form seven different developmental stages; egg, miracidium, sporocyst, rediae, cercariae, metacercariae and adult. In Aquatic animals, sporocyst stage usually found in infected gastropod (Mollusca: Gastropoda) (Figure 6A). Metacercariae (Figure 6B) and adult may be found as parasites of fish. Metacercariae encyst Metacercariae are found in many sites throughout the host such as gill, fin, bone, muscle, eyeballs, brain, spinal cord, nervous system, intestinal peritoneum, liver, gall bladder, heart ventricle and kidney [4]. Adult trematodes are found in skin, gill, intestine, stomach, pyloric caeca. Sometime other larval stages are reported to effect the aquatic animals such as *Sanguinicola klamathensis* miracidium cause acute hemorrhage of the gills accompanies the mass exodus of this parasite [5].

Many species of freshwater fishes serve as second intermediate host of digenetic trematode [25]. Five families of digenetic trematodes metacercariae are reported from fresh water fish namely, Clinostomidae, Diplostomidae, Isoparorchiidae, Strigeidae and Heterophyidae [26-27]. Trematodes metacercariae encyst in different organs of fish such as fin, skin, gill, branchial chamber, body cavity and other internal organs (intestinal peritoneum, liver, gall bladder, heart ventricle, on the bulbus arteriosus, kidney) [28-30]. Invasions of metacercariae of numerous trematode species result in pathogenic changes occurring in different organs of the intermediate hosts affected [31].

Metacercariae of *Apophallus* sp. are encapsulated by new bone or present within vertebral spines. The metacercariae are associated with prominent dysplastic and cartilage proliferation, often resulting in complete encapsulation of the parasite [12]. Proliferation of branchial cartilage can occur in response to encysted digenetic metacercariae [5]. Some metacercariae encyst within filamental cartilage of goldfish gill. The hyperplastic response has distorted the normal architecture [5]. Metacercariae of heterophyid digeneans cause prominent dysplastic and proliferative changes in the gill cartilage of freshwater fishes [32-33]. Histopathological examination of the infected fish gills reveals the cartilage proliferation around metacercarial cysts, hyperplasia, hypertrophy and fusion in the affected gill filaments [34]. Lesions of the gills associated with parasitic infection vary with the agent, host and density of infection [2]. Metacercariae of *Centrocestus* sp. located in gill cartilage cause similar respiratory stress in cichlids and carp and mortality in farmed Japanese elvers [35].

Digeneans metacercariae are frequently found in the skin. Metacercaria may or may not evoke a melanin response, if they do, the parasites become a grossly visible as black spot such as *Cryptocotyle lingua* which cause black spot disease [5]. Also in other species, *Diplostomulum* sp. cause of black spot disease, they invade in the epidermis. The black spots are due to melanophore infiltration of the dermis in response to the presence of the metacercariae [5]. Muscle infections by *Bucephalus polymorphus* causes vertebral deformities in cyprinid fishes [36]. *Euclinostomum heterostomum* is very widespread in many regions in Europe, Asia and Africa [37]. Metacercariae of this parasite infect in the muscular tissues of freshwater fish.

Histopathological section has shown metacercariae invaded into the muscular tissue and was encapsulated by a sheet of connective tissue in host musculature (Figure 7).

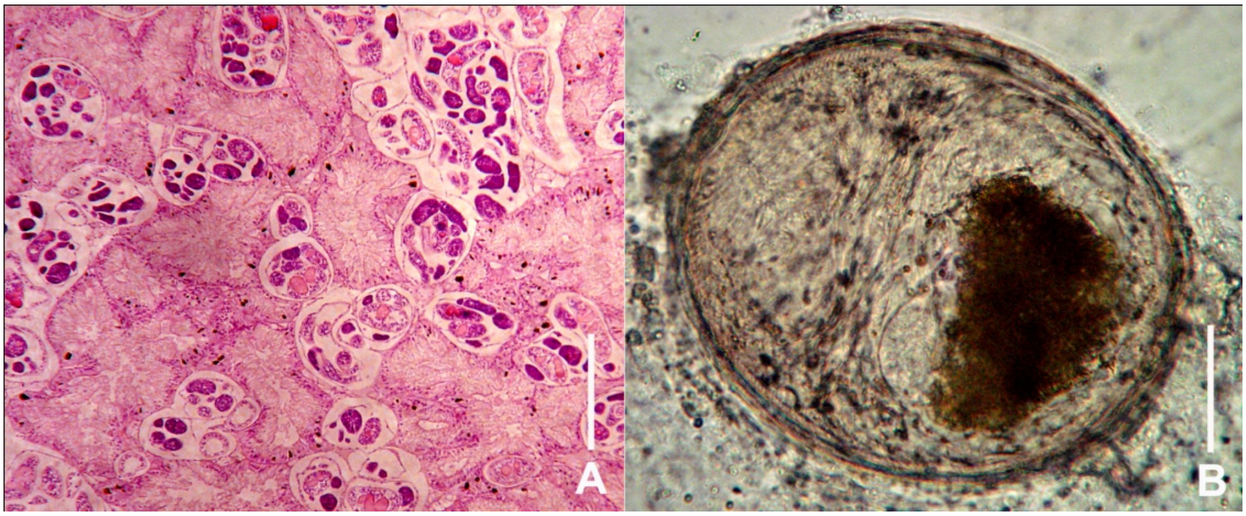

Figure 6. Some larval stage of digeneans.
(A) Sporocyst stage infected gastropod. (scale bar = 200 μm)
(B) Metacercaria infected fish muscle. (scale bar = 40 μm)

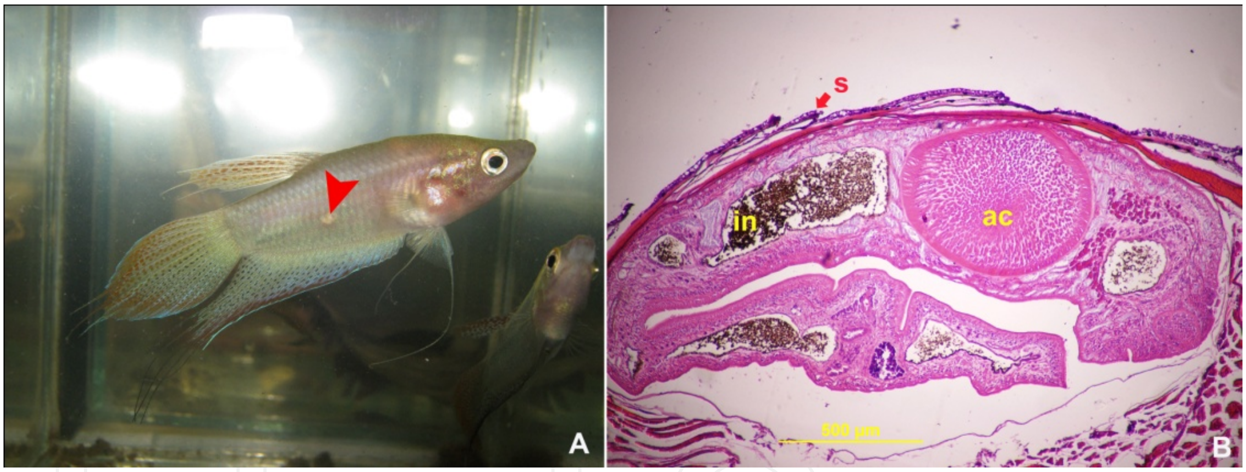

Figure 7. *Euclinostomum* sp. metacercariae infected in the muscle of *Trichopsis vittata*.
(A) *Trichopsis vittata* infested with metacercaria of *Euclinostomum* sp. (arrow) (Photo from Mr. Montri Sumontha)
(B) Histopathological section of fish muscle infected by *Euclinostomum* sp. metacercariae showed metacercariae invaded into the muscular tissue and was encapsulated by sheet of connective tissue in host musculature. (ac = acetabulum, in = intestine, s = scale)

Examples of some metacercariae such as *Diplostomum spathaceum*, *Cotylurus erraticus* parasite in eyeballs and brain of numerous cyprinids, destroy the structure of the eye and cause disappearance of rods following blindness [31, 38-39]. *Bucephalus polymorphus* and *Rhipidocotyle illense* are occurred in eyeballs, brain, spinal cord and nervous system coats. Histopathological changes are found as cornea delamination and retina damage leading to blindness [40-41]. Metacecariae of *Apophallus brevis* (sand-grain grub) found in yellow perch causing bony cysts within the lumen of the peripheral vasculature, especially in the axial musculature, but also of the extrinsic muscles of the eye [5]. Some metacercariae are occasionally found within extralenticular sites such as cornea and retina, usually with minimal inflammatory reaction [5]. Immature *Diplostomum* can be found in many host fish tissues including all portions of the eye, often causing very little host reaction [5].

In kidney, vast necrosis of the endoparenchymal tissue and hemorrhage from the main kidney vein near metacercaria cyst are found [31]. In kidney parenchyma, complex granulation of connective tissue cells and blood morphotic elements are seen. Metacercaria cyst wall with connective tissue fiber layer results in congested and necrotically changed kidney parenchyma . Degeneration necrosis of the kidney tissue are reported. [31].

In pericardium, inflammational infiltration, destroyed structure and fragmentation of muscle fibers, necrotic lesion, extravasation of erythrocytes, cytolysis of muscle fibers and karyolysis or heteropyknosis of nuclei were found in pericardium when fish are infected by metacercariae this also include losses of muscle tissue [31]. The degenerative and inflammational processes are associated with disturbed circulation [31]. For more cases, metacercariae of digenean *Stephanostomum baccatum* infected rainbow trout pericardium. A constrictive pericarditis is occasionally seen in severe fibrogranulomatous responses [5].

In some case, adult *Sanguinicola inermis* infected in the branchial and other vessels of fish, released eggs that hatched in the gill capillaries. These then migrated out of the vessels, causing hemorrhage and possibly death [5].

3.4. Cestoda

Cestode (tapeworms) body consists of three regions; scolex, neck and strobila. Cestodes do not have a digestive system, for this reason adult forms are usually found in host intestine to absorb host nutrients through the integument. The attachment of cestode in fish intestine cause necrosis, hemorrhage and inflammation at attachment points, space occupying distension of the intestine and possible perforation for example pseudophylliidean tapeworm, *Bothriocephalus gowkongensis* found to infect the cyprinids [5].

Larval stage of cestodes are also reported as parasite in fish. The larval cestode *Diphyllobothrium dentriticum* can cause severe granulomatous enteritis in trout and lead to peritonitis with visceral adhesions and death [5]. Larval tapeworm, *Ligula* is found in the peritoneal cavity, will cause pressure atrophy of ovaries [5].

4. Phylum Nematoda

Nematodes are invertebrate round worms, they are elongated, cylindrical form with unsegmented body. The diseases occur due to the adult and larval nematodes which are very common in marine fishes. The nematode parasites infect various tissues and organs of fish such as stomach, intestine, liver, gonads, visceral mesenteries, peritoneum body cavity, blood vessels, swim bladder, and connective tissues, fin, orbits of the eye and brain [42]. Most species of nematodes in adult stage live in the alimentary canal except the family Philometridae which are found in body cavity, liver and gonads [42]. *Philometra* spp. and

Philometroides spp. cause chronic active ulcerative dermatitis [5]. *Philometra* sp. may encyst within the testes cause trauma from migrating of this parasite [5]. Moreover, *Philometra* is found in the ovaries (Figure 8A) as well as testis and although there may be little effect on egg production, in some cases there is hemorrhage, fibrosis, and increased number of melanomacrophages [5].

Nematode larvae are more harmful than adults and can penetrate into the tissues of various organs, causing severe tissue damage and destruction of the cells of the organ [42]. Ascarididans (Order Ascaridida) are reported to parasitize fish and cephalopods [43]. Their larval stage occurs in the internal organ or maybe digestive tracts of marine fish (Figure 8B). Pathogenesis is a result of their mode of feeding, attachment and movement or migration within the host [42]. Ascarididans genera *Phocanema* (*Pseudoterranova*) and *Contracaecum* are found within the muscle, the effect on the fish is usually minimal [5]. Another genus of Ascarididans, *Anisakis simplex* causes infection of cod, where gastritis, craterous granulomatous lesions can be found [5]. Larval stage of *Anisakis* can be present in number which is large enough to represent a space occupying threat to normal functioning [5]. The infected intestine by the nematode parasites causes destruction, atrophy of intestinal mucosa, necrosis and degeneration of the intestinal tissue, causing damage to the whole thickness of the bowel wall due to nematode larvae [42]. *Capillaria* spp. may cause hepatic destruction if the infection is massive, either by virtue of the parasites themselves or their eggs [5].

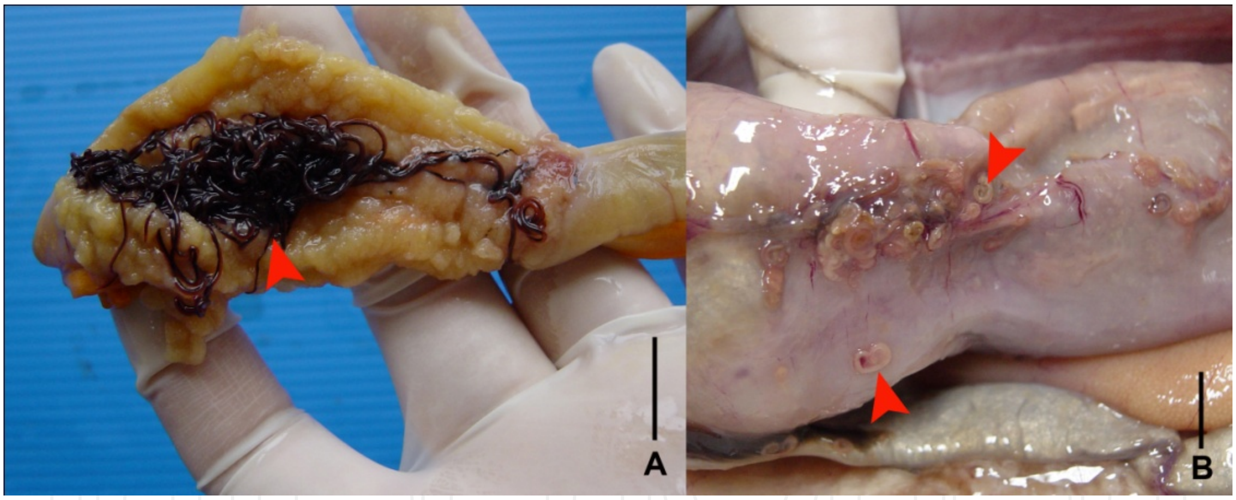

Figure 8. Nematode infest marine fish.
(A) *Philometra* sp. (arrow) is found in the ovary of marine fish *Lutianus johni*.
(B) Larval stage of ascarididans (arrow) occur in the internal wall of marine fish *Trichiurus haumela*.
(scale bar = 1 cm)

In reference [42] reported the histological change of the intestinal wall of *Pomadasys maculatum* with nematode where erosion of intestinal wall with loss of villous epithelial lining was usually seen. If the fish get infected with nematode, all the intestinal morphology become lost. Villious structures are not differentiated and appear as string of tissues. The mucosa of infected intestine appeared congested and edematous. The deformation of mucosa and submucosa results in separation of muscle fibers. The shape of villi are changed as compared with the normal shapes [42].

5. Phylum Acanthocephala

Acanthocephala is a phylum of parasitic worms which have the presence of an evertable proboscis (Figure 9). Body of acanthocephalan consist of two part presoma (proboscis, anterior neck, and posterior neck) and metasoma (trunk). The proboscis extend through the host epithelium into the submucosa with limited hemorrhaging at the point of attachment. The lumen of the host intestine was obstructed, and compressed villi were present [44]. Attachment point of acanthocephalans may be necrotic and can be even perforated, leading to peritonitis [5]. In reference [45] reported the histopathological changes when the presoma and the anterior part of the metasoma of *Longicollum pagrosomi* passed through the intestinal wall and infect the intestinal tissue, perforating the loose connective tissue. In the inflammatory connective tissue, collagen and muscle fibers were fragmented and revealed partial necrosis. Lipid drops and eosinophilic granular cells aggregated in the connective tissue of the tissue capsule.

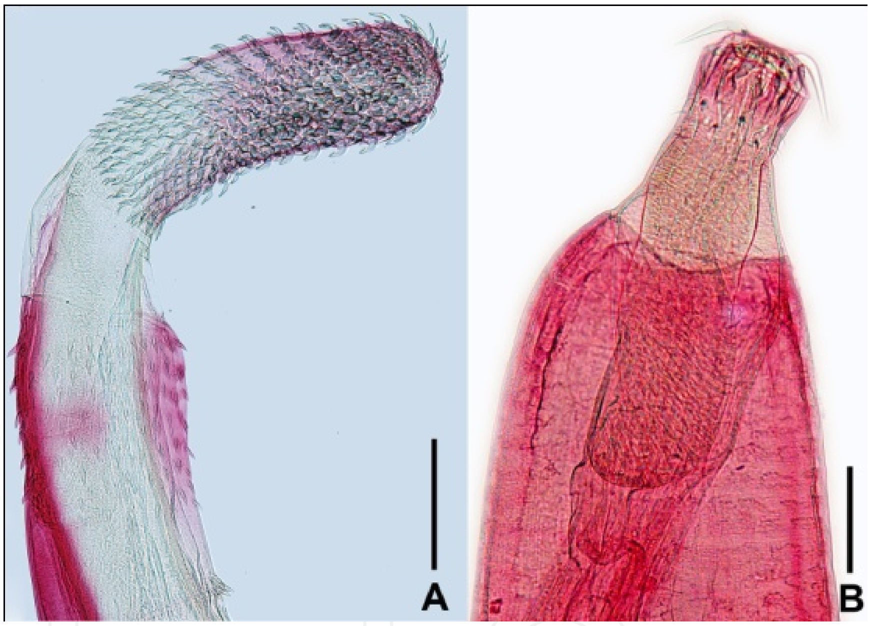

Figure 9. Anterior part of some acanthocephalans from marine fish.
(A) *Serrasentis* sp.
(B) *Neoechinorhynchus* sp. (scale bar = 130 μm)

In reference [46] observed heavy infections of acanthocephalan parasite, *Tenuiproboscis* sp. in the mangrove red snapper (*Lutjanus argentimaculatus*). They found that the large number of acanthocephalans in the posterior region of the intestine, almost blocking the lumen. At the site of parasite attachment, the surface of the intestine appeared thickened and the mucosal epithelium showed compression and abrasion. Intestinal folds were eroded along with the thickening of lamina propria. The presoma of the parasites pierced the mucosal epithelium, lamina propria, muscle layers and serosa, reaching the peritoneal cavity, surrounded by a tunnel with collagenous fibers and granulocytes. Inflammation, granular tissue formation, connective tissue proliferation and associated host immune reactions were evident.

6. Phylum Annelida

Annelids are eucoelomate, exhibit metamerism. They are known as segmented worms. The phylum is divided into three classes; Polychaeta, Oligochaeta and Hirudinea. Only class Hirudinea (leeches) are parasites, with a fixed number of body segments 34. They have anterior and posterior suckers, leeches can attach to hosts by using this sucker. Leeches are rare in cultured fish but are occasionally seen in wild or pond-raised fish [4]. Heavily infected fish often have a chronic anaemia [4]. This group of parasite cause skin lesions of fish. They damages the skin and small round wounds are found. In other site of infections, destruction of the fins and erosion of epithelium of the isthmus region of fish are also reported. *Piscicola* spp. cause ulcerative dermatitis with hyperemia, hemorrhage and epidermal hyperplasia [5].

7. Phylum Arthropoda

The body of arthropods consists of head, thorax and abdomen. They have exoskeleton and have jointed appendages, one pair to each somite, but in parasitic species, the appendages usually reduce or lost. All aquatic parasites are the members of Subphylum Crustacea. Class Branchiura, Copepoda and Malacostraca (order Isopoda) are usually found as parasites in aquatic animals.

7.1. Class Branchiura

The branchiurans are flattened crustaceans. the eyes are sessile, the antennae are very small, the abdomen is small and unsegmented. They do not have gills, most of branchiurans are parasites of fish. A known genus of fish parasite in this group is *Argulus* (Figure 10).

Argulus sp. has a preoral stylet causes local mechanical injury and especially the release of digestive enzymes, giving rise to systemic as well as local effects [5]. They scrape the epithelium while feeding, causing erosions and at times deep ulcers extending to bone. They attach to the host by pressing their shield-like cephalothorax onto the skin like a sucker. The second antennae and maxillipeds are used as clamps [4]. It may cause small petechial hemorrhages at feeding sites and also necrotizing and ulcerative dermatitis [4, 47].

The copepod body is short and cylindrical. The head and thorax are fused to form a cephalothorax. Parasitic copepods are increasingly serious problems in cultured fish and can also impact wild populations. Most parasites affect marine fish [4]. Parasitic copepods attached to the host by frontal filament of chalimus larvae [4]. Most sites of infections are gill, fin or skin but sometimes they can attach to oral cavity or eye. Such as Greenland sharks had copepods embedded in both corneas [5]. Histopathological change of fish infected by copepod are epithelial erosions, ulceration around the site of attachment of the parasite's mouth organs, hemorrhages and around the penetration sites of the claws there occur tissue necrosis, proliferation happens around the site of penetration of antennae [4]. Some copepods may be deeply embedded within the skin and elicit host response, mainly localized in mild dermal fibrosis and epidermal hyperplasia [5].

Figure 10. *Argulus* sp. (scale bar = 1 mm)

7.2. Copepoda

Lernaea spp. (anchor worms) insert part of their body, burrow deeply into tissues and evokes severe acute inflammation [4, 47]. Necrotizing and ulcerative dermatitis are seen [5]. Hemorrhage at the site of attachment, or hyperplasia or fibroblast may develop at the attachment site [4]. Heavy infections can lead to debilitation, secondary infection by bacterial or fungal infection will follow [48].

Ergasilus spp. have antennae modified for gasping the host and large trunk for reproductive products [49] mostly infected the gills of freshwater fish and marine fish [4]. Ergasilid copepods have extrabuccal digestion that leads to lysis of tissue and hyperplasia at the point of attachment [5]. In reference [50] study the host (*Abramis brama*) response when attach by *E. sieboldi* on their gill. The parasite elicits an intense host cellular reaction at the site of attachment with a high number of inflammatory cells, eosinophilic granular cells and neutrophilic granular cells.

Lepeophtheirus causes severe ulcerative dematitis with loss of substantial areas of the skin [5]. In some case, this copepod only superficially attach to the skin such as in *L. pectoralis*, the slight penetration of this parasite elicits massive dermal fibroplasia with hemorrhage in severe cases and a chronic inflammatory infiltrate [5].

In elasmobranch, *Eudactylina* sp. found from gill of shark *Chiloscyllium punctatum*. Histological examination of gill tissue has shown hyperplasia of the gill filaments and fusion of secondary lamellae and finally loss of respiratory surface area (Figure 11).

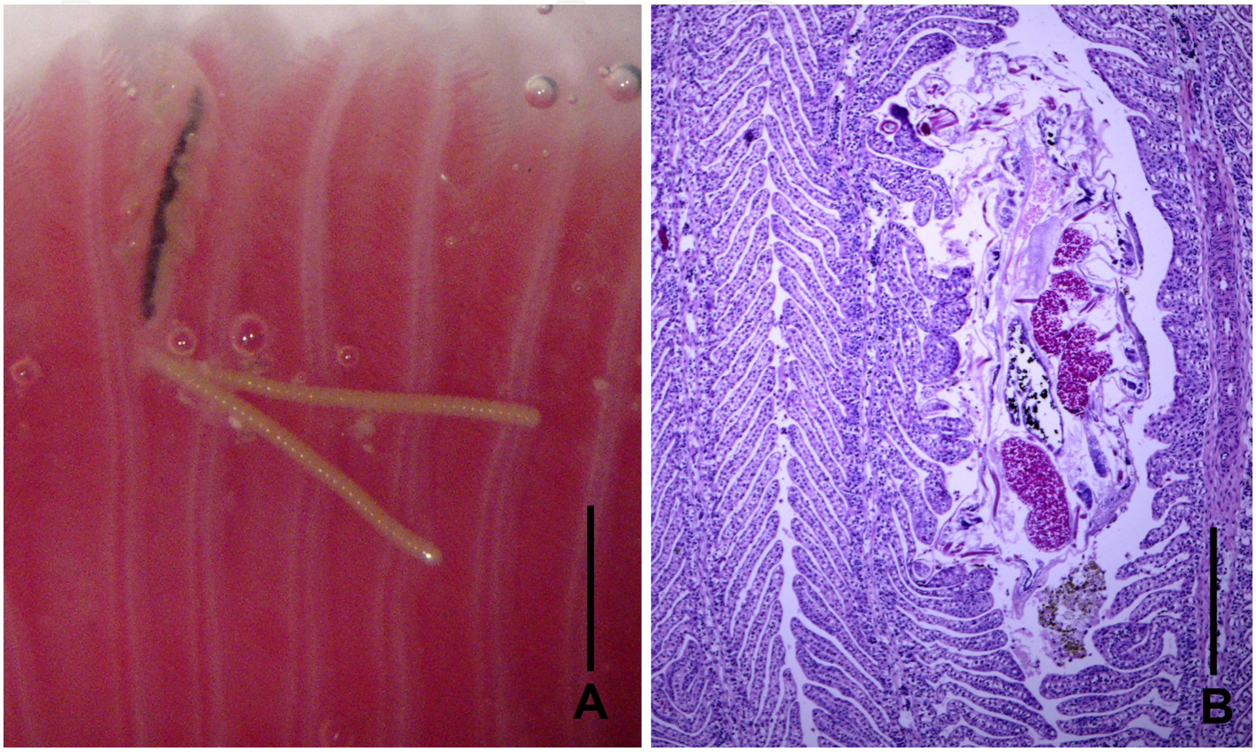

Figure 11. *Eudactylina* sp. from gill of *Chiloscyllium punctatum*.
(A) Fresh sample.
(B) Gill filament, sagittal section. The histological structures of the secondary lamellae pathology, gill lamellae were damaged at attach site. (scale bar = 1 mm)

7.3. Order Isopoda

Isopod predominantly attach to the body or fins of the fish, although some have been discovered inside gill chambers, buccal cavities and body pouches [51-53]. They occur in marine, estuarine and freshwater habitats, especially in the near shore coastal environment [52,54]. Single isopod can cause damage with their biting and sucking mouth parts. Heavy infestations of parasitic juveniles can kill small fish when they first attach [4].

Many genera of isopods are reported as parasite of fish both teleost and elasmobranch such as *Livoneca*, *Alitropus*, *Nerocila*, *Gnathia*, *Cirolana*, *Rocinela* and *Aega*. Recently, *Nerocila depressa* is found to attach to the bodies of *Sardinella albella* [55]. The hooks of the pereopods penetrate into the skin and anchor the isopod to the fish host. At the mouth part or pereopod site of attachment, the skin (epidermis and dermis) are eroded and exposed to the underlying tissue (Figure 12).

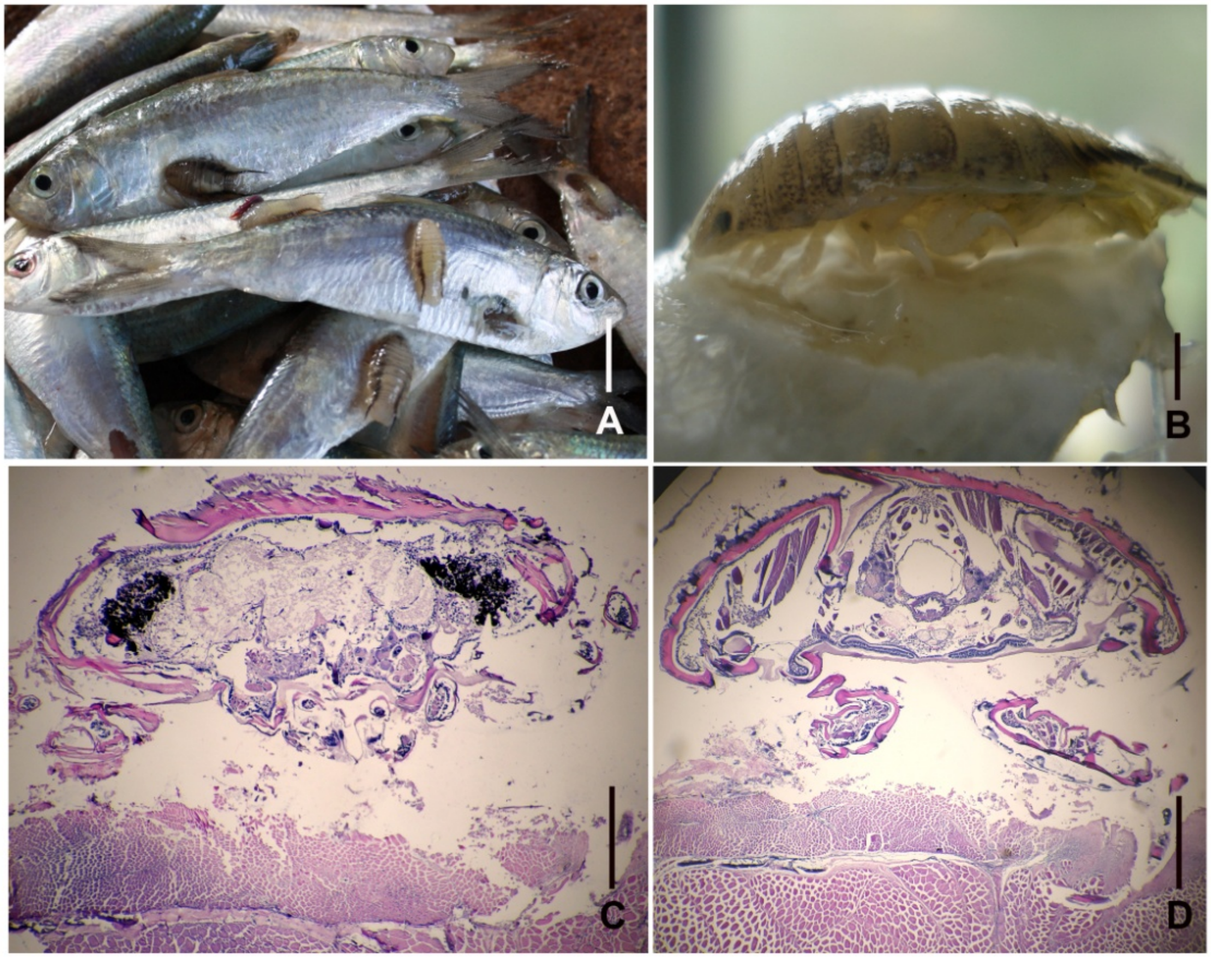

Figure 12. *Nerocila depressa* attached to the bodies of *Sardinella albella*.
(source: Printrakoon C, Purivirojkul W. Prevalence of *Nerocila depressa* (Isopoda, Cymothoidae) on *Sardinella albella* from a Thai estuary. Journal of Sea Research 2011; 65(2) 322–326.)
(A) *Nerocila depressa* attached *Sardinela albella*. (scale bar = 2 cm)
(B) *N. depressa* using hook-like legs penetrated in the skin of *S. albella*.
(scale bar = 250 μm)
(C) Pathology at the skin caused by biting mouthparts (arrow) of *N. depressa*.
(scale bar = 300 μm)
(D) Pathology of the skin caused by piercing of the pereopods (arrow). (scale bar = 300 μm)

8. Phylum Mollusca

The larvae of fresh water bivalve molluscs (Bivalvia: Unionoida) are often found attached to the gills and outer surfaces of fish. The larvae have thin bivalve shells often with little hooks on their inner edge [2]. This larva stage call "glochidia", usually found from gill both wide and cultured freshwater fish. Histopathology change of host by glochidia infection found hyperplasia of the epithelium, granulomatous dermatitis [5]. After glochidia attached the gills or outer surfaces of fish, epidermal or branchial epithelial cells of the host ultimately encapsulate the larva, completely enveloping it to form a cyst [56] (Figure 13). However, the glochidia must attach to a suitable host fish, if a glochidium attaches to a fish that is unsuitable as a host, an "abnormal" cyst may form and finally glochidium may death [57-58].

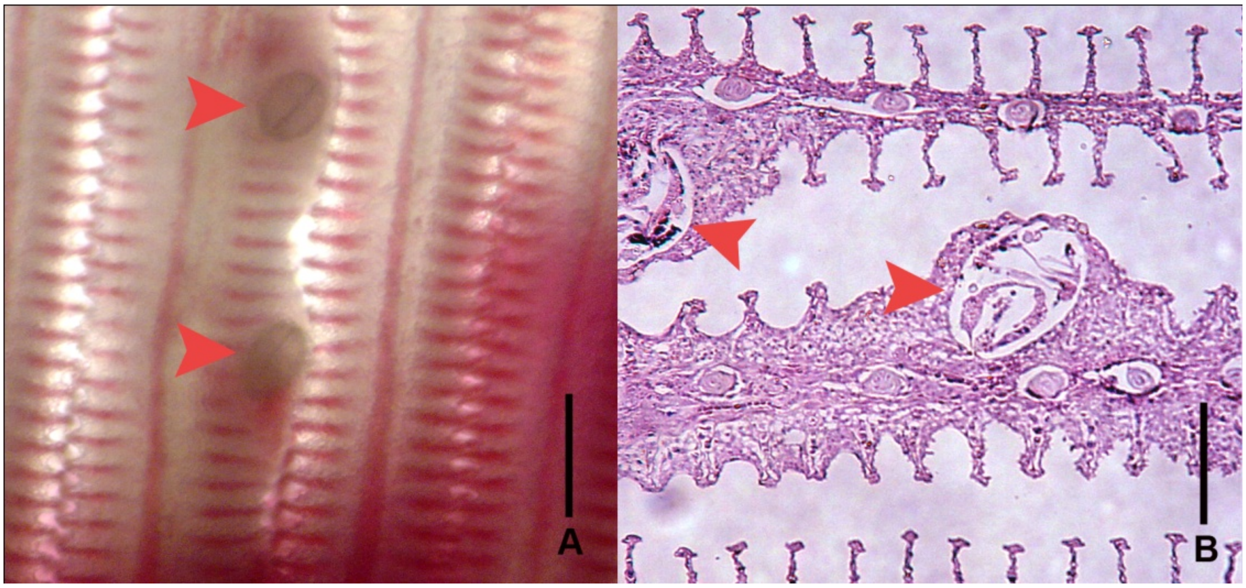

Figure 13. Glochidia of freshwater mollusc from the gill of *Pangasianodon gigas*.
(source: Purivirojkul W, Areechon N, Purukkiate C. Parasites and bacteria of giant catfishes (*Pangasianodon gigas* Chevey) larva. Thai science and technology journal 2004; 12(3) 1-11.)
(A) glochidia attached to the gills of a giant catfish (arrows). (scale bar = 400 μm)
(B) Host tissue has completely surrounded the glochidia (arrow). (scale bar = 250 μm)

9. Conclusion

Although the first step of identifying parasitic specimen is the direct wet mount but in some case the parasites are difficult to distinguish grossly, histology has a role in helping to identify intracellular parasite. Tissue stains not only use for the identification of the parasite but also in the visualization of cellular morphology [59]. Moreover, histopathology can use for examining each organ system for tells tale changes due to pathogenic agents and confirm the diagnosis [60].

Author details

Watchariya Purivirojkul
Department of Zoology, Faculty of Science, Kasetsart University, Thailand

Acknowledgement

The author would like to thank Associated Professor Dr. Chalor Limsuwan, Mr. Montri Sumontha and Dr. Cheewarut Printrakool for provide their own picture for this chapter and also thanks to Assistant Professor Siriwan Khidprasert for provide fairy shrimp specimens.

10. References

[1] Klontz GW. Diagnostic methods in fish diseases: Present status and needs. In: Ellis AE. (ed.) Fish and shellfish pathology. London: Academic press; 1985. p1-10.

[2] Roberts RJ. Fish pathology, 3rd edition. London: W.B. Saunders publishing; 2001.
[3] MacMillan JR. 1991 Biological factors impinging upon control of external protozoan fishparasites. Annual Review of Fish Disease 1 119-131.
[4] Noga EJ. Fish disease: diagnosis and treatment. Iowa: Iowa state university press; 2000.
[5] Ferguson H. Systemic pathology of fish: a text and atlas of comparative tissue responses in diseases of teleosts. Ames: Iowa state university Press; 1989.
[6] Bustos PA, Young ND, Rozas MA, Bohle HM, Ildefonso RS, Morrison RN, Nowak BF. Amoebic gill disease (AGD) in Atlantic salmon (Salmo salar) farmed in Chile. Aquaculture 2011; 310 281–288.
[7] Young ND, Crosbie PBB, Adams MB, Nowak BF, Morrison RN. *Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar* L.). International Journal for Parasitology 2007; 37 1469–1481.
[8] Wiles M, Cone D, Odense PH. Studies on Chilodonella cyprinid and C. hexasticha (Protozoa: Ciliata) by scanning electron microscope. Canadian Journal of Zoology-Revue Canadienne de Zoologie 1985; 63 2483-2487.
[9] Lom J. Adhesive disc of Trichodinella epizootica – ultrastructure and injury to the host tissue. Folia Parasitologica 1973; 20 193-202.
[10] Purivirojkul W., Khidprasert S. First report of microsporidiosis in fairy shrimp *Branchinella thailandensis* (Sanoamuang, Saengphan and Murugan, 2002). Aquaculture 2009; 289 (1-2) 185-190.
[11] Basson L, Van As J. Trichodinidae and other Ciliophorans (Phylum Ciliophora). In: Woo PTK. (ed.) Fish diseases and disorders Volume 1: protozoan and metazoan infections 2nd edition. London: CAB international; 2006. p154-182.
[12] Kent ML, Watral VG, Whipps CM, Cunningham ME, Criscione CD, Heidel JR., Curtis L R, Spitsbergen J, Markle DF. A Digenean Metacercaria (*Apophallus* sp.) and a Myxozoan (*Myxobolus* sp.). Associated with Vertebral Deformities in Cyprinid Fishes from the Willamette River, Oregon. Journal of Aquatic Animal Health 2004; 16 116–129.
[13] Baska F, Voronin VN, Eszterbauer E, Müller L, Marton S, Molnár K. Occurrence of two myxosporean species, *Myxobolus hakyi* sp. n. and *Hoferellus pulvinatus* sp. n., in *Pangasianodon hypophthalmus* fry imported from Thailand to Europe as ornamental fish. Parasitology Research 2009; 105 1391–1398.
[14] Bahri S, Marton S, Marques A, Eszterbauer E. *Henneguya tunisiensis* n. sp. (Myxosporea: Bivalvulida), a new gill parasite of *Symphodus tinca* (L.) (Teleostei: Labridae) off Tunisia. Systematic Parasitology 2010; 76 93–101.
[15] Molnár K. Taxonomic problems, seasonality and histopathology of *Henneguya creplini* (Myxosporea) infection of the pikeperch *Stizostedion lucioperca* in Lake Balaton. Folia Parasitologica 1998; 45 261–269.
[16] Lom J, Dyková I, Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitologica 2006; 53 1-36.
[17] Sprague VV, Becnel JJ, Hazard EI. Taxonomy of phylum Microspora. Critical Reviews in Microbiology 1992; 18 285–395.

[18] Prasertsri S, *Limsuwan C*, Chuchird N. The Effects of Microsporidian (*Thelohania*) Infection on the growth and histopathological changes in pond-reared pacific white shrimp (*Litopenaeus vannamei*). Kasetsart Journal Natural Science 2009; 43 680 - 688.
[19] Cannon LRG. Turbellaria of the world. Brisbane: Queensland Museum; 1986.
[20] Kent ML, Olson AC. Interrelationships of a parasitic turbellarian (*Paravortex* sp.) (Graffillidae, Rhabdocoela) and its marine fish hosts. Fish Pathology 1986; 21 65–72.
[21] Kabata Z. Parasites and diseases of fish cultured in the tropics. London: Taylor and Francis Ltd.; *1985*.
[22] Dezfuli BS, Giari L, Simoni E, Menegatti R, Shinn AP, Manera M. Gill histopathology of cultured European sea bass, *Dicentrarchus labrax* (L.), infected with *Diplectanum aequans* (Wagener 1857) Diesing 1958 (Diplectanidae: Monogenea). Parasitology Research 2007; 100 707–713.
[23] Pritchard JB. The gill and homeostasis: transport under stress. The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2003; 285 1269–1271.
[24] Nigrelli RF. Mortality statistics for specimen in New York Aquarium 1939 1940; Zoologica, scientific contributions of the New York Zoological Society 25 525-552.
[25] Purivirojkul W. A survey of fish species infected with trematode metacercariae from some areas in northeast Thailand. Journal of Fisheries Technology Research 2011; 5(2) 75-86.
[26] Han ET, Shin EH, Phommakorn S, Sengvilaykham B, Kim JP, Rim HJ, Chai JY. *Centrocestus formosanus* (Digenea: Heterophyidae) Encysted in the Freshwater Fish, *Puntius brevis*, from Lao PDR. Korean Journal of Parasitology 2008; 46 49–53.
[27] Vankara AP, Mani G, Vijayalakshmi C. A report on various digenetic metacercariae from the freshwater fishes of River Godavari, Rajahmundry. Journal of Parasitic Diseases 2011; 35 177-185.
[28] Odening K, Mattheis T, Bockhardt I. 1970. The life cycle of *Cotylurus c. cucullus* (Thoss) (Trematoda, Strigeida) in the Berlin area. Zoological yearbooks, Department of Systematics, 97 125-198. (In German)
[29] Scholz T, Aguirre-Macedo ML, Salgado-Maldonado G. Trematodes of the family Heterophyidae (Digenea) in Mexico: a review of species and new host and geographical records. Journal of Natural History 2001; 35 1733-1772.
[30] Steele E, Hicks T. Histological effect of *Ascocotyle tenuicollis* (Digenea: Heterophyidae) metacercarial infection on the heart of *Fundulus heteroclitus* (Teleostei: Cyprinodontidae). Journal of the South Carolina Academy of Science 2003; 1 10-18.
[31] Orecka-Grabda T. Hemato-and histopathological changes in the whitefish (*Coregonus albula* (L.) invaded by metacercariae of *Cotylurus erraticus* (Syn. Ichthyocotylurus) (Rudolphi, 1809). Acta Ichthyologica et Piscatorial 1991; 21 3-19.
[32] Blazer VS, Gratzek JB. Cartilage proliferation in response to metacercarial infections of fish gills. Journal of Comparative Pathology 1985; 95(2) 273-280.
[33] Olson RE, Pierce JR. A Trematode metacercaria causing gill cartilage proliferation in steelhead trout from Oregon. Journal of Wildlife Diseases 1997; 33(4) 886-890.

[34] Shoaibi Omrani B, Ebrahimzadeh Mousavi HA, Sharifpour I. Occurrence and histopathology of *Ascocotyle tenuicollis* metacercaria in gill of platyfish (*Xiphophorus maculatus*) imported to Iran. Iranian Journal of Fisheries Sciences 2010; 9(3) 472-477.

[35] Paperna I. Diseases caused by parasites in the aquaculture of warm water fish. Annual Review of Fish Disease 1991; 1 155–194.

[36] Baturo B. Pathological changes in cyprinid fry infected by *Bucephalus polymorphos* Baer, 1827 and *Rhipidocotyle illensis* (Ziegler, 1883) metacercariae (Trematoda, Bucephalidae). Acta Parasitologica Polonica 1980; 27 241–246.

[37] Yamaguti S. Systema Helminthum Vol 1. The digenetic trematodes of vertebrates. Part I & II. New York: Interscience Pub Inc; 1958.

[38] Kozicka J. Diseases of fishes Drużno lake (Parasitofauna of the biocoenosis of Drużno lake. Part VIII). Acta Parasitologica Polonica 1958; 6(20) 393-432.

[39] Kozicka J. Diseases of fishes Drużno lake (Parasitofauna of the biocoenosis of Drużno lake. Part VIII) Acta Parasitologica Polonica 1959; 7(1) 1-72.

[40] Grabda E, Grabda J. Mass invasion of metacercariae of *Bucephalus polymorphus* Bear, 1827, in the eye of bream, *Abramis brama* (L.). Wiadomości parazytologiczne 1967; 13(6) 733-735. (in Polish)

[41] Baturo-Warszawska B. Pathological changes in cyprinid fry infected by Bucephalus polymorphus Bear, 1827 and Rhipidocotyle illensis (Ziegler, 1883) metacercariae (Trematoda, Bucephalidae). Acta Parasitologica Polonica 1980; 27 241-246.

[42] Akhtar Y. Feeding habits and nematode parasites of some fishes of Karachi coast. PhD thesis. Jinnah university for women for fulfillment; 2008.

[43] Purivirojkul W. An investigation of larval Ascaridoid nematodes in some marine fish from the Gulf of Thailand. Kasetsart Journal Natural Science 2009; 43(5) 85-92.

[44] Amin OM, Heckmann RA. Description and pathology of *Neoechinorhynchus idahoensis* n. sp. (Acanthocephala: Neoechinorhynchidae) in *Catostomus columbianus* from Idaho. Journal of Parasitology 1992; 78(1) 34-39.

[45] Kim SR, Lee JS, Kim JH, Oh MJ, Kim CS, Park MA, Park JJ. Fine structure of *Longicollum pagrosomi* (Acanthocephala: Pomphorhynchidae) and intestinal histopathology of the red sea bream, *Pagrus major*, infected with acanthocephalans. Parasitology Research 2011; 109(1) 175-84.

[46] Sanil NK, Asokan PK, John L, Vijayan KK. Pathological manifestations of the acanthocephalan parasite, *Tenuiproboscis* sp. in the mangrove red snapper (*Lutjanus argentimaculatus*) (Forsskål, 1775), a candidate species for aquaculture from Southern India. Aquaculture 2011; 310(3-4) 259–266.

[47] Ferguson HW. Systemic Pathology of Fish. Ames: Iowa State University Press; 1989.

[48] Noga EJ. The importance of *Lernaea cruciata* (Le Sueur) in the initiation of skin lesions in largemouth bass, *Micropterus salmoides* (Lacepede), in the Chowan River, North Carolina, USA. Journal of Fish Disease 1986; 9 295-302.

[49] Kabata Z. Parasites and diseases of fish cultured in tropics. London: Taylor and Francis; 1985.

[50] Dezfuli BS, Giari L, Konecny R, Jaeger P, Manera M. Immunohistochemistry, ultrastructure and pathology of gills of *Abramis brama* from Lake Mondsee, Austria, infected with *Ergasilus sieboldi* (Copepoda). Disease of Aquatic Organism 2003; 53 257–262.

[51] Brusca RC. A monograph on the Isopoda: Cymothoidae (Crustacea) of the eastern Pacific. Zoological Journal of the Linnean Society 1981; 73 117–199.

[52] Lester RJG, Hayward CJ. Phylum Arthropoda. In: Woo PTK. (ed.) Fish Diseases and Disorders Vol 1: Protozoan and Metazoan Infections. 2nd edition. London: CAB international; 2006. p466-565.

[53] Alas A, Öktener A, Iscimen A, Trilles JP. New host record, *Parablennius sanguinolentus* (Teleostei, Perciformes, Blenniidae) for *Nerocila bivittata* (Crustacea, Isopoda, Cymothoidae). Parasitology Research 2008; 102 645–646.

[54] Sullivan M, Stimmelmayr R. Cymothoid isopods on coral reef fishes in the near shore marine environment of St.Kitts, Lesser Autilles: Proceedings of the 11th International Coal Reef Symposium, July 7–11, 2008, Ft. Lauderdale, Florida; 2008.

[55] Printrakoon C, Purivirojkul W. Prevalence of *Nerocila depressa* (Isopoda, Cymothoidae) on *Sardinella albella* from a Thai estuary. Journal of Sea Research 2011; 65(2) 322–326.

[56] Rogers-Lowery CL, Dimock Jr RV. Encapsulation of Attached Ectoparasitic Glochidia Larvae of Freshwater Mussels by Epithelial Tissue on Fins of Naïve and Resistant Host Fish. Biological Bulletin 2006; 210 51–63.

[57] Meyers TR, Millemann RE, Fustish CA. Glochidiosis of salmonid fishes. IV. Humoral and tissue responses of coho and Chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). Journal of Parasitology 1980; 66 274–281

[58] Waller DL, Mitchell LG. Gill tissue reactions in walleye *Stizostedion vitreum vitreum* and common carp *Cyprinus carpio* to glochidia of the freshwater mussel *Lampsilis radiata siliquoidea*. Diseases of Aquatic Organisms 1989; 6 81–87.

[59] Woods GL, Walker DH. Detection of infection or infectious agents by the use of cytologic and histologic stains. Clinical Microbiology Reviews 1996; 9 382-404.

[60] Gupta E, Bhalla P, Khurana N, Singh T. Histopathology for the diagnosis of infectious diseases. Indian Journal of Medical Microbiology 2009; 27(2) 100-106.