

Opisthorchis

(platyhelminth: trematode)

Overview

Platyhelminths have triploblastic acoelomate soft bodies which are markedly flattened in profile (hence their common name as flatworms). They undergo protostomial embryonic development but do not moult during growth. On the basis of molecular evidence, they are classified within the Lophotrochozoa despite the absence of lophophore mouthparts and trochophore larvae. Three classes are composed entirely of parasitic flatworms (Cestoda, Trematoda and Monogenea), which have prominent attachment organs (suckers or bothria), syncytial teguments, shell glands and vitellaria involved in ectolecithal egg development, and life-cycles involving a variety of larval stages. Trematodes (flukes) have soft leaf-like bodies with oral and ventral suckers, a blind gut (mouth but no anus) and both male and female reproductive organs (hermaphroditic). Digeneans have indirect life-cycles involving alternation of sexual stages in vertebrates and asexual stages in molluscs. Miracidia released from eggs infect snails (obligate intermediate hosts) where they undergo massive asexual proliferation through sac-like sporocyst and redia stages eventually releasing larval cercariae into the water. Vertebrate (definitive) hosts become infected by penetration of the skin by cercariae or by eating encysted stages (metacercariae) on herbage or in second intermediate hosts. Adult opisthorchiideans are small to medium flukes, often spinose, living in the alimentary tracts of mammals, birds, reptiles and fish. They produce embryonated eggs which only hatch after ingestion by snails (first intermediate hosts) and metacercariae develop in fish (second intermediate hosts). Opisthorchids are parasitic in the bile ducts of fish-eating mammals and infections by *Opisthorchis*, *Clonorchis* and *Metorchis* spp. have been associated with liver disorders in dogs, cats and humans.

Classification:

Domain: Eukaryota (membrane-bound nucleus)
Supergroup: Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Kingdom: Metazoa (multicellular eukaryotes, heterotrophs, notably animals)
Group: Protostomia (triploblastic, spiral cleavage)
Subgroup: Lophotrochozoa (lophophore feeding structure or trochophore larva or neither)
Phylum: Platyhelminthes (flatworms, acoelomate, most hermaphroditic, prominent attachment organs)
Clade: Neodermata (syncytial tegument = neodermis)
Class: Trematoda (flukes, most with dorsoventrally-flattened bodies, sac-like gut)
Subclass: Digenea (heteroxenous, larval miracidium, sac-like sporocyst/redia stages in mollusc, cercariae/metacercariae)
Order: Plagiorchiida ('echinostomatids', plagiorchiids', mainly fish hosts, some tetrapods, infection by ingestion of cercariae or metacercariae)
Suborder: Opisthorchiata (egg eaten by gastropod IH, rediae formed, simple-tailed cercariae, encysts in second IH, metacercariae eaten by DH)
Superfamily: Opisthorchioidea (small-medium flukes, often spinose, piscivorous DH)
Family: Opisthorchiidae (medium leaf-shaped flukes, in bile ducts of mammals, rediae without appendages, cercariae with two eyespots, metacercariae in second IH)
Genus: *Opisthorchis* (parasitic in liver of piscivorous mammals)
Species: various species cause biliary obstruction, cholangitis and possibly cholangiocarcinoma in animals and humans

Parasite biodiversity and host range: Most Metazoa are multicellular triploblastic animals with differentiated tissues, many being bilaterally symmetrical with a body cavity. Most invertebrate animals are protostomes as their embryonic development involves spiral determinate cleavage. Those that do not moult during their life-cycles are grouped together in the enigmatic clade Lophotrochozoa, including the platyhelminths, rotifers, lophophorates, annelids and molluscs. Platyhelminths (flatworms) have soft acoelomate flat bodies with three-dimensional arrays of muscles that generate a typical writhing motion (cf. longitudinal muscles in nematodes producing a thrashing motion). Flatworms do not have a single unifying characteristic (synapomorphy) but comprise diverse free-living (most Turbellaria) and parasitic (Neodermata) assemblages. Neodermata have non-ciliated syncytial (multinucleate) teguments and 3 classes are recognized, all with prominent attachment organs, namely, Cestoda with anterior bothridia/bothria (true/false suckers), Trematoda with oral and ventral suckers (previously called acetabula), and Monogenea with posterior haptors (opisthaptors). All have shell glands surrounding the ootype, and most exhibit ectolecithal egg development (yolk not present in egg but secreted by accessory glands called vitellaria or yolk glands). Most have indirect life-cycles involving the development of adult worms in vertebrates and larval stages in intermediate hosts (usually invertebrates).

The trematodes (flukes) and monogeneans have blind sac-like guts (lacking an anus) while the cestodes (tapeworms) lack digestive tracts. Trematodes have leaf-like bodies well adapted to living in confined spaces in tubular organs of vertebrate hosts. Two trematode subclasses are recognized: the Aspidogastrea with relatively few species (obligate external parasites of molluscs,

fish and turtles, adults possessing a large ventral disc divided with numerous alveoli (suckerlets) or rows of suckers and the tegument having short protrusions (microtubercles)); and the speciose Digenea (obligate endoparasites of vertebrates, adults bearing undivided ventral suckers (when present) and life-cycles involving alternation of sexual stages in vertebrates and asexual stages in molluscs). The success of digeneans as widespread parasites has been attributed to their ability to proliferate at 2 separate parts of their life-cycles. Adults worms in vertebrate definitive hosts produce numerous eggs which are excreted and release free-swimming miracidia which seek molluscan intermediate hosts. Massive asexual proliferation occurs in molluscs involving unique sporocysts and rediae. Both stages are sac-like structures with almost no anatomical features (no suckers, no reproductive organs). The difference is that sporocysts lack a gut (they absorb their food), whereas rediae have a mouth, a muscular pharynx and a sac-like gut (they browse on molluscan tissues). Sequential development of these stages varies considerably, with mother sporocysts producing daughter sporocysts or rediae over multiple generations, culminating in the production of cercariae. The infected molluscs are typically rendered sterile ('castrated') with parasites replacing their gonads and producing dozens to thousands of infective cercariae every day. The cercariae are larval forms, almost always with tails, and they actively emerge from molluscs and swim around in water. There is enormous variation in cercarial behaviour, but the 3 most important routes of infection for definitive hosts are by penetration of the skin by cercariae (e.g. blood flukes), by ingestion of encysted stages (metacercariae) on vegetation (e.g. sheep liver flukes), or ingestion of encysted metacercariae in the tissues of a second intermediate host (e.g. human liver flukes). Some 6,700 digenean species belonging to 22 superfamilies have been described in fish and tetrapods. The subclass Digenea is divided into 2 orders: Diplostomida characterized by furcocercous cercariae that penetrate definitive hosts; and Plagiorchiida with variable life-cycles but often involving cercariae being ingested by definitive hosts.

Superfamily (+ no. families)	No. spp.	DH ^a	Egg ^b	IH1 ^c	Asexual ^d	Cercaria ^e	IH2 ^f	Mode ^g
Subclass: Aspidogastrea (large ventral disc with numerous alveoli (suckerlets) or rows of suckers, tegument with short protrusions (microtubercles), obligate ectoparasites on molluscs, turtles, fish)								
Aspidogastroidea (4)	65	M,F,C,T	A	G,B	-	-	-	8
Subclass: Digenea (oral and ventral sucker; syncytial tegument; obligate endoparasites of vertebrates)								
Order: Diplostomida (blood flukes, 'strigeids') ~1,480 species								
Brachylaimoidea (2)	250	T	E	G	S	S,F	M	6,7
Diplostomoidea (5)	800	T	P	G	S	F	C,M,A	6
Schistosomatoidea (5)	430	F,C,T	P	G,B,A	R,S	F	-	1,6
Order: Plagiorchiida ('echinostomatids', 'plagiorchiids') ~5,200 species								
Allocreadioidea (6)	1,118	F,T	P	G,B	R,S	S,Y	C,M,R,A	6
Apocreadioidea (1)	94	F	P	G	R	S	M,A	6
Azygioidea (1)	43	F,C	E	G	R	F	C	3,4
Bivesiculoidea (1)	28	F	P	G	R	F	C	3,4
Bucephaloidea (2)	410	F	P	B	S	F	C	4
Echinostomatoidea (10)	112	F,T	P	G	R	S	C,M,R	5,6,7
Gorgoderoidea (10)	106	F,C,T	P	G,B	R,S	S,Y	C,M,R	5,6,7
Gymnophalloidea (4)	200	F,T	P	B	S	F	C,M,R,A,E,N	3,4,6
Haplospalchnoidea (1)	51	F	P	G	S	S	-	5
Hemiuroidea (15)	1,160	F,C,T	E	G,B,S	R,S	F	C,M,R,N	4
Heronimoidea (1)	1	T	P	G	S	S	-	7
Lepocreadioidea (8)	473	F	P	G	R	S	C,M,R,A,E,N	6
Microphalloidea (12)	414	F,T	P	G,B	S	S,Y	C,M,R,A,E	6,7
Monorchioidea (3)	270	F	E	G,B	R,S	S	C,R,A,E	6
Opisthorchioidea (3)	436	F,T	E	G	R	S	C	6
Paramphistomoidea (5)	74	F,T	P	G	R	S	-	5
Plagiorchioidea (16)	47	F,T	P	G	R,S	S,Y	C,M,R,A	6
Pronocephaloidea (6)	131	F,T	E	G	R	S	-	5
Transversotrematoidea (1)	27	F	P	G	R	F	-	2
LEGEND								
^a DH = definitive host: F = teleost fish; C = chondrichthyan fish; T = tetrapod; M = mollusc								
^b Fate of egg: A = larva hatches and attaches to IH1, E = eaten by IH1, P = hatches releasing miracidium which penetrates IH1								
^c IH1 = first intermediate host: G = gastropod, B = bivalve, A = annelid, S = scaphopod								
^d Asexual reproduction involves formation of secondary: R = redia, S = sporocyst								
^e F = fork-tailed cercaria, S = simple tailed cercaria, Y = cercaria with stylet								
^f IH2 = second intermediate host: C = chordate, M = mollusc, R = arthropod, A = annelid, E = echinoderm, N = cnidaria, ctenophore								
^g Mode of infection for DH: 1 = cercaria penetrates DH; 2 = cercaria attaches to DH; 3 = cercaria eaten by DH; 4 = cercaria eaten by IH2; 5 = cercaria emerges, encysts in open and eaten by DH; 6 = cercaria emerges, penetrates IH2, encysts and eaten by DH; 7 = cercaria remains in IH1, encysts and eaten by DH; 8 = no cercarial stage, infected IH1 eaten by DH.								

Thirteen plagiorchidan suborders have been recognized containing 19 superfamilies. The suborder Opisthorchiata contains one superfamily Opisthorchioidea comprising tiny to medium-sized flukes which form rediae without appendages, then simple-tailed cercariae that encyst in second intermediate hosts forming metacercariae. Over 400 species have been described in 90 genera in 3 families (Opisthorchiidae, Cryptogonimidae, Heterophyidae). The family Opisthorchiidae is found primarily in the bile ducts of piscivorous mammals and some 30 genera have been recognized in 13 subfamilies: Allogomtiotrematinae (*Allogomtiotrema*, *Satyapalia*); Aphallinae syn. Witenbergiinae (*Witenbergia*); Delphinicolinae (*Delphinicola*); Diasiellinae (*Diasiella*); Metorchiinae (*Holometra*, *Metametorchis*, *Metorchis*, *Parametorchis*); Oesophagicolinae (*Oesophagicola*); Opisthorchiinae (*Agrawalotrema* (syn. *Thaparotrema*), *Amphimerus*, *Cladocystis*, *Clonorchis*, *Cyclorchis*, *Evranchorchis*, *Nigerina*, *Opisthorchis* (syn. *Hepatiarius*), *Paropisthorchis*, *Pseudogomtiotrema* (syn. *Gomtia*), *Trionychotrema*); Pachytrematinae (*Pachytrema*); Plotnikoviinae (*Plotnikovia*); Pseudamphimerinae (*Erschoviorchis*, *Euamphimerus*, *Pseudamphimerus*); Pseudamphistominae (*Microtrema*, *Pseudamphistomum*); Ratziainae (*Ratzia*); and Tubangorchiinae (*Tubangorchis*). The subfamily Opisthorchiinae is characterized by small-medium elongate flukes whose uterus and vitellaria do not extend anteriorly beyond the level of the ventral sucker. The genus *Opisthorchis* contains over 50 species which have been found in a wide range of fish-eating animals (9 species in mammals, 20 in birds, 17 in fish (most reassigned to genus *Agrawalotrema* (syn. *Thaparotrema*), 2 in turtles, 1 each in snakes and frogs), mostly in Indochina and eastern Europe. Several species infect humans and it is estimated that 17-30 million people are currently infected and that over 400 million are at risk of infection. Molecular and biological studies suggest that the species *Opisthorchis viverrini* may actually be a species complex predominantly infecting humans inhabiting different watersheds throughout Asia, while the species *Opisthorchis felineus* infects a broader range of carnivores and only occasionally causes disease in humans. Another Oriental liver fluke similar to *Opisthorchis felineus* and *Opisthorchis viverrini* in its biological characteristics is *Clonorchis sinensis* (considered separately in detail in next section). The genera *Opisthorchis* and *Clonorchis* are conventionally differentiated on the basis of adult worm morphology, particularly in the position and shape of testes and vitellaria (*Opisthorchis* spp. have lobular testes and vitelline glands arranged in clusters, while *C. sinensis* has highly-branched testes positioned tandemly and vitellaria distributed continuously along both lateral margins). Nonetheless, some workers consider the genera to be synonymous, and molecular studies have indeed shown them to be very closely-related (forming sister clades).

<i>Opisthorchis</i> species	Definitive hosts [adults in hepatic bile ducts]	First intermediate hosts [sporocysts/rediae in tissues]	Second intermediate hosts [metacercariae in tissues]	Distribution
Mammalian definitive hosts				
<i>O. chabaudi</i>	Carnivora: felid (cat)	freshwater Gastropoda: bithyniid (<i>Gabbia neumannii</i>)	tadpoles of Anura: bufonid (African toad), ranid (white-lipped frog), conrauid (Togo slippery frog)	Europe
<i>O. felineus</i> (cat liver fluke)	Carnivora: felid (cat), canid (dog, red fox, silver fox, Arctic fox, corsac fox, wolf, raccoon dog), pinniped (Caspian seal, bearded seal, grey seal), mustelid (marten, wolverine, European polecat, Steppe polecat, American mink, European mink, ermine, weasel, Siberian weasel, sable, European badger, European otter), procyonid (raccoon); Artiodactyla: suid (pig), phocoenid (porpoise); Eulipotyphla: erinaceid (European hedgehog); Primates: hominid (human); Rodentia: murid (brown rat), cricetid (muskrat, European water vole, golden hamster, black-bellied hamster), castorid (beaver), sciurid (chipmunk), caviid (guinea pig); Lagomorpha: leporid (European rabbit)	freshwater Gastropoda: bithyniid (<i>Bithynia siamensis</i> , <i>B. inflata</i> , <i>B. leachi</i> , <i>B. troscheli</i> , <i>B. tentaculata</i>)	freshwater Cypriniformes: cyprinid (bream, ide, asp, Eurasian dace, common roach, tench, minnows)	Asia, Europe, Russia, Canada
<i>O. noverca</i> (syn <i>O. caninus</i>)	Carnivora: canid (dog); Artiodactyla: suid (pig); Primates: hominid (human)		Cypriniformes: cyprinid (carp)	India

<i>O. pricei</i>	Didelphimorphia: didelphid (woolly opossum)			South America
<i>O. pseudofelineus</i> (syn. <i>O. guayaquilensis</i> , <i>Amphimerus</i>)	Carnivora: felid (cat), canid (coyote, dog); Didelphimorphia: didelphid (opossum); Primates: hominid (human)		Cypriniformes: catostomid (suckers)	Americas
<i>O. starkovi</i>	Carnivora: felid (serval)			Africa
<i>O. tonkae</i>	Rodentia: cricetid (muskrat), caviid (guinea pig), murid (white rat); Carnivora: felid (cat), canid (dog)		Cypriniformes: cyprinid (minnow)	North America
<i>O. viverrini</i> (Southeast Asian liver fluke)	Carnivora: felid (cat, civet cat, Indian fishing cat, leopard cat), canid (dog); Artiodactyla: suid (pig); Rodentia: murid (rat); Primates: hominid (human)	freshwater Gastropoda: bithyniid (<i>Bithynia siamensis siamensis</i> , <i>B. siamensis goniompharus</i> , <i>B. funiculata</i> , <i>Codiella (B.) inflata</i> , <i>C. troscheli</i> , <i>C. leachi</i>)	freshwater Cypriniformes: cyprinid (silver barb, Java barb, Javean barb, Smith's barb, beardless barb, swamp barb, Siamese mud carp, Hampala barb, spotted Hampala barb, sa, hard-lipped barb, thin-lipped barb, mrigal, saitan takhao, pa sou, soi pik daeng, dok niew, sicklefin barb, striped flying barb, blunt-snouted barb, shovel-jaw carp, common carp, grass carp, silver carp, topmouth gudgeon, Amur bream, goldfish)	South-East Asia
<i>O. wardi</i>	Carnivora: felid (cat)			Philippines
Avian definitive hosts				
<i>O. ahingii</i>	Suliformes: anhingid (Oriental darter)			India
<i>O. allahabadii</i>	Accipitriformes: accipitrid (red-headed vulture)			India
<i>O. brahmini</i>	Anseriformes: anatid (Brahminy duck)			India
<i>O. choledochum</i>	Anseriformes: anatid (duck)			Europe
<i>O. dendriticus</i>	Gruiformes: gruid (sarus crane)			India
<i>O. entzi</i>	Pelecaniformes: ardeid (purple heron)			
<i>O. geminus</i> (syn. <i>O. altaevi</i> , <i>O. allahabadii</i> , <i>O. tenuicollis</i> , <i>O. cheelia</i> , <i>O. milvusensis</i>)	Anseriformes: anatid (duck, white-faced whistling duck, spur-winged goose); Pelecaniformes: ardeid (grey heron, purple heron, squacco heron); Accipitriformes: accipitrid (greater spotted eagle, tawny eagle, western marsh harrier, eastern marsh harrier, hen harrier, pied harrier, black kite, yellow-billed kite, red-headed vulture); Gruiformes: rallid (Eurasian coot)			Europe, Asia, India, Africa
<i>O. giddhis</i>	Accipitriformes: accipitrid (red-headed vulture)			India
<i>O. hardoiensis</i>	Suliformes: phalacrocoracid (little cormorant)			India
<i>O. interruptus</i>	Coraciiformes: alcedinid (green kingfisher)			
<i>O. jonesae</i>	Accipitriformes: accipitrid (black kite)			Pakistan
<i>O. kashmirensis</i>	Anseriformes: anatid (duck)			India
<i>O. longissimus</i>	Gruiformes: gruid (sarus crane);			Europe, Asia,

(syn. <i>O. (Notaulua) asiaticus</i>)	Anseriformes: anatid (duck, northern shoveler, Eurasian teal, ferruginous duck); Pelecaniformes: ardeid (grey heron, purple heron, Chinese pond heron, Eurasian bittern, black-crowned night heron); Accipitriformes: accipitrid (eastern imperial eagle, western marsh harrier, cinereous harrier, black kite)			India, North America
<i>O. obsequens</i> (syn. <i>O. indicus</i>)	Accipitriformes: accipitrid (goshawk); Anseriformes: anatid (duck, red-crested pochard)			India, Asia, Australia
<i>O. parageminus</i>	Anseriformes: anatid (duck)			Vietnam
<i>O. pelecani</i>	Pelecaniformes: pelecanid (rosy pelican)			India
<i>O. simulans</i> (syn. <i>O. anatis</i>)	Anseriformes: anatid (greylag goose, greater white-fronted goose, duck, tufted duck, greater scaup, Eurasian wigeon); Pelecaniformes: ardeid (little egret); Accipitriformes: accipitrid (western marsh harrier, European honey buzzard); Gruiformes: rallid (Eurasian coot)			Europe, Asia, Africa
<i>O. skrjabini</i>	Anseriformes: anatid (duck)			Asia
<i>O. tsingkiapuensis</i>	Anseriformes: anatid (duck)			
<i>O. vitellatus</i>	Suliformes: phalacrocoracid (double-crested cormorant)			China
Piscine definitive hosts				
<i>O. attui</i>	Anabantiformes: channid (great snakehead) [intestines]			India
<i>O. attuai</i>	Siluriformes: silurid (wallago catfish)			India
<i>O. bagarius</i>	Siluriformes: sisorid (goonch)			India
<i>O. caudalspinutum</i>	Siluriformes: silurid (wallago catfish)			India
<i>O. dayali</i>	Siluriformes: bagrid (catfish)			India
<i>O. elongatum</i>	Siluriformes: sisorid (goonch) [intestines]			India
<i>O. gomtii</i>	Siluriformes: silurid (catfish) [intestines]			India
<i>O. gorakhpurensis</i>	Siluriformes: bagrid (striped dwarf catfish)			India
<i>O. gurdaspurensis</i>	Cypriniformes: cyprinid (rohu)			India
<i>O. gwaliorensis</i>	Siluriformes: sisorid (goonch)			India
<i>O. parasiluri</i> (syn. <i>Thaparotrema</i>)	Siluriformes: bagrid (yellowhead catfish)			China
<i>O. pedicellata</i> (syn. <i>Thaparotrema</i> , <i>O. mehrai</i> , <i>O. thaparae</i> , <i>O. thapari</i>)	Siluriformes: bagrid (rita catfish, tangan), sisorid (goonch), silurid (wallago catfish); Synbranchiformes: mastacembelid (tire track eel)			India
<i>O. piscicola</i> (syn. <i>Thaparotrema</i>)	Osteoglossiformes: gymnarchid (frankfish); Siluriformes: clariid (African sharptooth catfish)			Africa
<i>O. raptii</i>	Siluriformes: bagrid (catfish) [intestines]			India
<i>O. ritai</i>	Siluriformes: bagrid (rita)			India

	catfish)			
<i>O. thapari</i> (syn. <i>Thaparotrema</i>)	Siluriformes: bagrid (catfish), sisorid (goonch)			India
Other definitive hosts				
<i>O. lomeensis</i>	Anura: pyxicephalid (brown ball frog)			Africa
<i>O. ophidiarum</i>	Serpentes: elapid (spine-bellied sea snake)			Philippines
<i>O. ovalis</i>	Testudines: trionychid (smooth softshell turtle, spiny softshell turtle)			North America
<i>O. wuhanensis</i>	Testudines: trionychid (Chinese softshell turtle)			China

Parasite morphology: *Opisthorchis* spp. form 7 different stages in their developmental cycles: eggs, miracidia, sporocysts, rediae, cercariae, metacercariae, and adult flukes. Eggs are small and oval, measuring 21-36 µm in length by 11-22 µm in width. They appear bile-stained (yellow-brown colour) and are surrounded by an eggshell with a roughened surface (musk-melon pattern). The eggs have an operculum with a distinct shoulder and some species also have a small knob-like abopercular appendage. Eggs voided by the host are embryonated and contain a ciliated miracidium with conspicuous germinal cells and flame cells. Sporocysts are elongated sac-like structures measuring up to 1.1 x 0.6 mm. They are thin-walled and contain little other than balls of germinal cells. Rediae are elongate stages measuring around 500 x 90 µm without lateral ambulatory appendages. They are surrounded by an aspinose tegument and possess a mouth, a thick-walled pharynx, a short saccular gut, ciliated flame cells and tubular cells (protonephridial system). Cercariae are pleurolophocercous with tubular shapes comprising elongate bodies (150 x 75 µm) surmounting long powerful tails (400 x 25 µm) with dorsal and ventral fins. Cercariae have spinose teguments with sensory hairs, rudimentary ventral suckers, without stylets but with 2-3 short transverse rows of oral spines, and developing organ systems. Metacercariae are tail-less cercarial bodies that have encysted and become surrounded by several cyst wall layers (derived from both parasite and host); their final morphology tends to be intermediate between that of the cercaria and the sexual adult. Adult flukes are lancet-shaped with dorsoventrally flattened bodies measuring from 7-12 mm long by 1-3 mm wide. They are covered with a translucent tegument and appear red-yellow in colour. They possess a subterminal oral sucker, pharynx, oesophagus, bifurcate caeca, mid-body ventral sucker and a tubular excretory bladder. Adult worms are hermaphroditic and possess both male and female reproductive organs. The generic name *Opith-orchis* actually means 'behind'-'testis', referring to the posterior location of the testes with respect to the ovary. Worms have 2 testes located in diagonal or tandem apposition and they may be lobular or branched (*O. viverrini* has deeply lobed testes cf. *O. felineus*). The testes are connected to a muscular seminal vesicle that ends in the ejaculatory duct (no cirrus sac) opening into the genital pore. Worms possess a single multilobed ovary connected to lateral vitelline follicles (both *O. viverrini* and *O. felineus* have vitelline clusters, but those of the latter species have transversely compressed patterns) and a coiled uterus opening into the genital pore. Internal fertilization occurs resulting in the production of numerous small and embryonated eggs.

Site of infection: Adult *Opisthorchis* worms infect the biliary tract of their vertebrate definitive hosts (mammals, birds, fish, reptiles), being found predominantly in intrahepatic bile ducts, sometimes in extrahepatic bile ducts, the gall bladder or pancreas, and a few species in fish even occur in the intestines. Asexual developmental stages (sporocysts, rediae) are found within various tissues of their first intermediate hosts (snails), while metacercariae develop in the muscles of their second intermediate hosts (fish).

Pathogenesis: Adult liver flukes cause tissue damage by mechanical irritation (when attaching, feeding and moving), by chemical irritation (releasing parasite metabolic products that are toxigenic and/or antigenic) and by immune-mediated reactions (provoking chemical and cellular inflammatory responses that have cytotoxic, proliferative and mutagenic effects). The flukes live in the bile ducts and while light infections may be asymptomatic, heavier infections (> 100 worms) may cause mild, moderate or severe disease manifestations due to tissue trauma, persistent inflammation and obstruction. A range of acute and chronic clinical symptoms/signs have been reported, including diarrhoea, abdominal pain, flatulence, indigestion, anorexia, malaise, fatigue, fever, eosinophilia, tender hepatomegaly, cholangitis, cholecystitis and biliary obstruction with jaundice and mild scleral icterus. In acute infections caused by *O. felineus*, symptoms may also include urticaria, nausea, vomiting, arthralgia, sometimes lymphadenopathy and rarely an acute serum sickness-like presentation. Chronic infections cause cumulative damage to bile ducts as epithelial cells undergo adenomatous hyperplasia, goblet cell metaplasia increases mucus production and periductal areas become fibrotic and sclerotic (esp. left hepatic lobe). Persistent inflammation and granuloma formation may occur throughout tissues in association with adult flukes and eggs, but cholelithiasis is uncommon even though eggs and worm fragments may be found in gallstones. Obstructive jaundice, intrahepatic duct stones and recurrent suppurative cholangitis (occasionally with liver abscesses) are considered to be strongly indicative of opisthorchiasis. Both children and adults may be clinically affected, although symptomatic

infection is more common in adults as worm burdens accumulate due to repeated infection and the lack of any acquired protective immunity (possibly due to immunosuppressive factors). Serious complications may also develop in adults with progressive hepatic cirrhosis, cholecystitis and pancreatitis. Heavy *O. viverrini* infections have also been implicated in the development of bile duct cancer, cholangiocarcinoma (CCA), due to the constant irritation, hypersecretion and extensive fibrosis making the biliary epithelium more susceptible to malignant transformation (chronic inflammation induces oxidative and nitrate stress with consequential DNA damage). *O. viverrini* is now classified as a class 1 carcinogen for CCA in humans (together with *Clonorchis sinensis*) and risk factors include geographic location, high alcohol consumption, advancing age, male gender, a specific GST-M1 (glutathione S-transferase enzyme) genetic polymorphism, and exposure to nitrosamines in foods and betel nut tobacco.

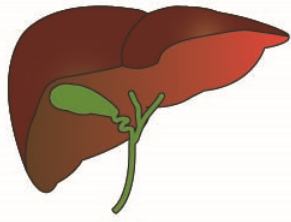
Developmental cycle and mode of transmission: *Opisthorchis* spp. have indirect life cycles involving 3 hosts: definitive hosts (vertebrates) infected with adult flukes; first intermediate hosts (gastropods) in which asexual reproduction of larval stages occurs; and second intermediate hosts (fish) infected with encysted metacercariae. Transmission between hosts involves motile aquatic developmental stages that actively seek hosts. Embryonated eggs produced by fertile worms (up to 300 eggs per day) are passed in host faeces into the external environment. Those contaminating freshwater sources may be ingested by bithyniid snails which act as first intermediate hosts. The eggs hatch in the snail digestive tract releasing their enclosed miracidia which penetrate the gut wall and form sac-like sporocysts. Germ balls within the sporocysts form rediae which are released into adjacent tissues. Germ balls within the rediae then form cercariae which are released into lymph spaces to undergo maturation. Eventually, the cercariae emerge from the snails, typically with diurnal periodicity (peaking in early morning). Cercariae may emerge from snails for up to 2 months. They swim about in the water column, particularly in stagnant or slow-moving waters (including ponds, streams, rivers, swamps, rice fields), and penetrate the skin of freshwater fish, mostly cyprinids, which act as second intermediate hosts. In particular, infections have been associated with rural communities that have established rice paddies for agriculture and carp ponds for aquaculture, with the boundaries between these pursuits often becoming blurred. The cercariae penetrate the fish skin dropping their tails and encyst as metacercariae in the skin, fins and muscles within 3 weeks. Piscivorous hosts become infected by eating infective metacercariae in raw, undercooked, dried, smoked, salted or pickled fish. Freshwater fish dishes are very common in the cuisine of Laos and Thailand as koi-pla (raw fish), larb-pla (raw fish in spicy salad), pla-ra, pla-som and som-fak (salted semi-fermented fish). Infections may also be exported outside of endemic regions with frozen, dried and pickled fish. Following ingestion, metacercariae excyst in the small intestines releasing the juvenile worms which migrate through the common bile duct to reach the intrahepatic bile ducts where they mature into adult worms 3-4 weeks later. Gravid worms release embryonated eggs that are carried with bile into the gut to be passed with faeces. The prepatent period (time from infection to first egg release) ranges from 4-6 weeks in definitive hosts, and infections have been shown to persist for 10-30 years.

Differential diagnosis: Infections are routinely diagnosed by the microscopic detection of fluke eggs in faecal samples or in bile or duodenal fluid biopsy samples. Coprological examination may require multiple stool samples due to irregular egg excretion. While eggs may be detected in wet faecal preparations or faecal smears (Kato-Katz thick smears), they are better concentrated from stool samples by sedimentation (e.g. formalin-ethyl acetate technique) rather than floatation (eggs do not float readily in saturated salt or sugar solutions). Many fluke eggs resemble each other so specific diagnosis based on egg morphology alone is difficult. Adult flukes may be recovered from hosts ante-mortem by surgery, endoscopy (such as gastroscopy or endoscopic retrograde cholangiopancreatography (ERCP)) or following chemotherapy, or at post-mortem by dissection. Key anatomical features of adult worms may be examined by microscopy to facilitate differential diagnosis. Infections may also be revealed by modern medical imaging techniques, including ultrasonic or X-ray (cholecystocholangiography) examination, or computed tomography (CT) or magnetic resonance imaging (MRI) scans. The severity and extent of associated pathological changes (inflammation, fibrosis) may be indicated by enlargements, thickenings, dilations, strictures or filling defects. Infections by metacercariae in fish may be detected by microscopic examination of dissected muscles, usually in squash preparations or following pepsin-acid digestion. A range of immunodiagnostic tests (including immunoelectrophoresis, haemagglutination, fluorescent-antibody tests and enzyme immunoassays) have been developed to detect host antibodies in serum samples, but they are unable to differentiate between recent infections and prior exposure. An enzyme immunoassay has recently been developed to detect parasite copro-antigens in faecal samples, although some cross-reactivity problems were encountered. Research laboratories have recently used molecular techniques to detect and characterize DNA from adult worms and eggs in mammals and from metacercariae in fish, via DNA hybridization assays or by polymerase chain reaction (PCR) amplification of repetitive DNA elements or by random amplified polymorphic DNA (RAPD) analyses.

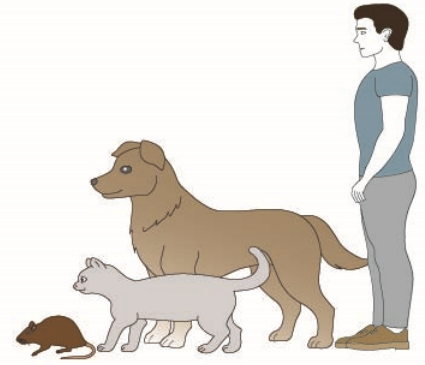
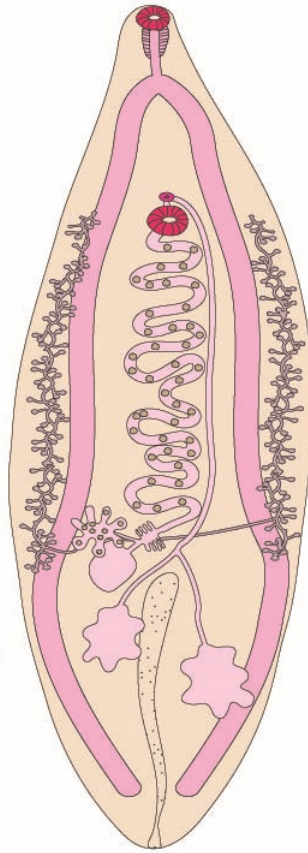
Treatment and control: Anthelmintics have been used for the chemotherapeutic treatment of individual patients as well as for the chemoprophylactic treatment of cohorts/communities most at-risk of infection. The isoquinoline (praziquantel) has proven most efficacious in eradicating adult worms, despite some minor side-effects such as headache, dizziness, insomnia, nausea and vomiting. Good results have also been obtained using the broad-spectrum benzimidazoles (mebendazole and albendazole) as well as the new diamidine derivative (tribendimidine). However, prognosis is poor once cholangiocarcinoma (CCA) develops. Surgical resection may be attempted in early cases of CCA, while bile drainage may be carried out as palliative treatment to relieve symptoms of

obstructive jaundice in later cases. Regrettably, re-infection commonly occurs in endemic areas due to the high frequency of eating raw or improperly cooked fish in Asia. Preventive measures developed to break transmission patterns centre around 3 strategies: limiting faecal contamination of water (avoiding promiscuous defaecation and night-soil fertilizers, improving sanitation, sewage treatment, treating humans and companion animals (dogs and cats are important reservoirs of infection)); reducing snail intermediate host populations (using barriers or molluscicides); and killing metacercariae in fish products (cooking above 63°C, freezing at -20°C for > 7 days). Public health education campaigns are mandatory to raise awareness of the consequences of acute and chronic infections (particularly cancer development), to promote community treatment programs, to improve food and water hygiene, and to advocate for changes in culinary customs (cook fish).

Opisthorchis



liver, bile ducts
(trauma, irritation,
inflammation, granulomas,
fibrosis, obstruction)



Definitive Hosts
(carnivores, piscivores)

hermaphroditic
adult (~ 15 mm)

excretion



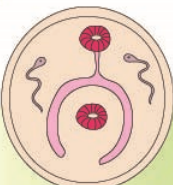
egg
(~ 30 μm)



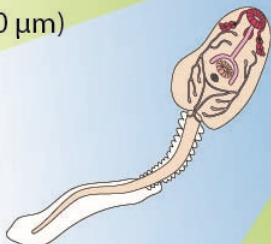
vector-borne transmission

encysted within
tissues of IH-2

metacercaria
(~ 200 μm)



endoparasitic in
tissues of vector (IH-1)



free-swimming
cercaria
(~ 550 μm)



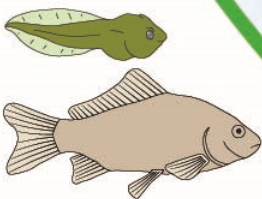
free-swimming
miracidium
(~ 35 μm)



redia
(~ 500 μm)



sporocyst
(~ 1 mm)



Second Intermediate Hosts
(IH-2) (fish, tadpoles)
(muscle cysts)

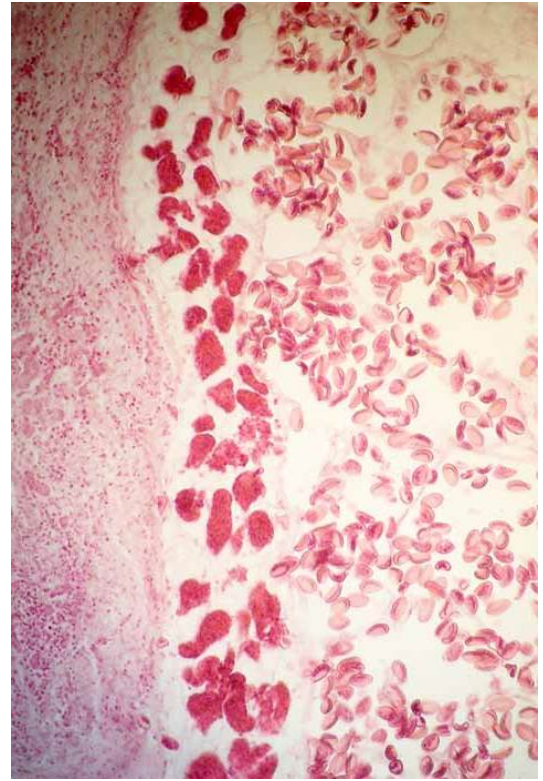


First Intermediate Hosts
(IH-1) (bithyniid snails)
(viscera then glandular tissue)

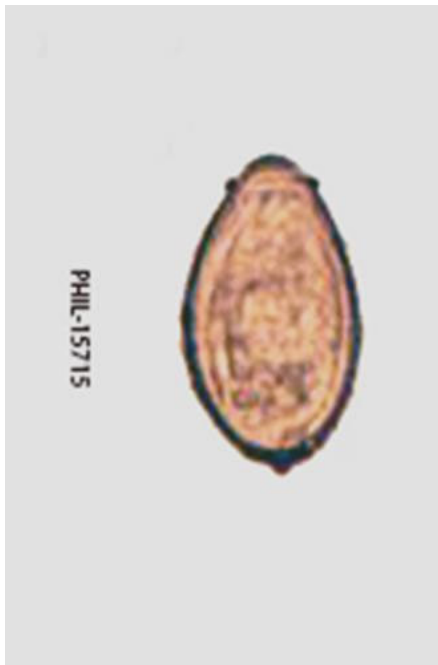
ingestion



Opisthorchis adult worm



Opisthorchis section through worm in liver



Opisthorchis egg