

Clonorchis

(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: *Clonorchis* (parasitic in liver of piscivorous mammals)
Species: *C. sinensis* causes biliary obstruction, cholangitis and possibly cholangiocarcinoma in 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*, *Metamatorchis*, *Metorchis*, *Paramatorchis*); Oesophagicolinae (*Oesophagicola*); Opisthorchiinae (*Agrawalotrema* (syn. *Thaparotrema*), *Amphimerus*, *Cladocystis*, *Clonorchis*, *Cyclorchis*, *Evranchis*, *Nigerina*, *Opisthorchis* (syn. *Hepatiarius*), *Paropisthorchis*, *Pseudogomtiotrema* (syn. *Gomtia*), *Trionychotrema*); Pachytrematinae (*Pachytrema*); Plotnikoviinae (*Plotnikovia*); Pseudamphimerinae (*Erschoviorchis*, *Euamphimerus*, *Pseudamphimerus*); Pseudamphistominae (*Microtrema*, *Pseudamphistomum*); Ratzinae (*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 *Clonorchis* is monotypic and contains only one species (the Chinese liver fluke *C. sinensis*) found in fish-eating mammals throughout Asia (Far-East, South-East Asia, Japan, Siberia), mostly humans, pigs, dogs and cats. It is estimated that 20-30 million people are currently infected and that 600 million are at risk of infection. *C. sinensis* is very similar in its biological characteristics to other Oriental liver flukes commonly infecting humans in Asia; namely *Opisthorchis felineus* and *Opisthorchis viverrini*. The genera *Clonorchis* and *Opisthorchis* are conventionally differentiated on the basis of adult worm morphology, particularly in the position and shape of testes and vitellaria (*Clonorchis* having highly branched testes positioned tandemly and vitellaria distributed continuously along both lateral margins, while *Opisthorchis* spp. have lobular testes and vitelline glands arranged in clusters). Nonetheless, some workers consider the genera to be synonymous and have transferred the species *C. sinensis* to the genus *Opisthorchis*. Molecular studies have confirmed their close identity but most often depict them as closely-related sister clades.

<i>Clonorchis</i> species	Definitive hosts [adults in bile ducts]	First intermediate hosts [sporocysts/rediae in tissues]	Second intermediate hosts [metacercariae in tissues]	Distribution
<i>C. sinensis</i> (syn. <i>C. endemicum</i> , <i>Opisthorchis sinensis</i>) (Chinese liver fluke, Oriental liver fluke)	Primates: hominid (human); Carnivora: canid (dog), felid (cat), mustelid (mink, badger); Artiodactyla: suid (pig), camelid (camel), bovid (cattle); Lagomorpha: leporid (rabbit); Rodentia: murid (rats, mice), caviid (guinea pig), cricetid (muskrat)	freshwater Gastropoda: bithyniid (<i>Parafossarulus anomalospiralis</i> , <i>manchouricus</i> , <i>sinensis</i> , <i>striatulus</i> , <i>Bithynia fuchsianus</i> , <i>longicornis</i> , <i>misella</i> , <i>robustus</i>); assimineid (<i>Assimineea lutea</i>), thiarid (<i>Melanooides tuberculata</i>), semisulcospirid (<i>Semisulcospira amurensis</i> , <i>cancellate</i> , <i>libertine</i> , <i>Koreoleptoxis amurensis</i>)	freshwater Cypriniformes: cyprinids (common carp, crucian carp, predatory carp, grass carp, black carp, silver carp, mud carp, bighead carp, goldfish, dark chub, pale chub, sharpbelly, stone moroko, Korai moroko, ito moroko, Amur false gudgeon, topmouth gudgeon, Korean southern gudgeon, Manchurian gudgeon, three-lips, pike gudgeon, mugitsuku, Chinese lake gudgeon, Chinese lizard gudgeon, rainbow gudgeon, variegated gudgeon, rosy bitterling, Khanka spiny bitterling, Amur bitterling, slender bitterling, Korean bitterling, kanehira, Yamatsuta paljasloug, Chinese bleak, Korean splendid dace, common sawbelly, spotted steed, barbel steed, barbel chub, zunaga-nigo, Chinese minnow, big-scaled redfin, wataka, common dace, black Amur bream, white Amur bream, hiina laikviidikas, hiina morukas, bangana, kamatsuka, mugitsuku, lake minnow), cobitid (pond loach); Siluriformes: bagrid (Korean stumpy bullhead, yellowhead catfish, orca-whip, Brazhnikov's catfish), silurid (Amur catfish, Chinese large-mouth catfish); Gobiformes: oxudercid	Asia, India, Eastern Russia

			(pseudogoby), eleotrid (sleeper); Mugiliformes: mugilid (mullet); Perciformes: percichthyid (oriental perch, golden mandarin fish), ehippid (orbfish), odontobutid (Chinese sleeper); Clupeiformes: pristigasterid (slender shad); Anabantiformes: osphronemid (paradise fish, paradise gourami); Osmeriformes: osmerid (pond smelt); Cichliformes: cichlid (Mozambique tilapia); Beloniformes: adrianichthyid (Japanese rice fish)	
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Parasite morphology: *Clonorchis sinensis* forms 7 different developmental stages: eggs, miracidia, sporocysts, rediae, cercariae, metacercariae and adult flukes. Embryonated eggs passed in faeces are small and ovoid to flask-shaped, with dimensions ranging from 25-35 x 12-19 μm . They are surrounded by a thick tanned (yellow-brown) eggshell with a distinct operculum sporting prominent opercular shoulders at one end and a small knob-like protuberance at the other end. The eggs contain a fully-developed pyriform miracidium with 2 simple eyespots, a ciliated covering, lateral sensory papillae and 8-25 germinal cells. Sporocysts are elongate pleomorphic sacs measuring up to 0.5 mm long with thin walls containing balls of germinal cells that give rise to small rediae (embryos). Rediae grow into large elongate stages measuring over 1mm in length and possess a mouth, pharynx and simple digestive tract. They contain balls of germ cells which develop into more rediae or cercariae. Cercariae are long brown pleurolophocercous stages with elliptical bodies (around 200 μm long) and elongate unforked tails (up to 500 μm long) with long dorsal fins and shorter ventral fins. They have a pair of eyespots, well-developed anterior glands (penetration and cystogenous), a rudimentary ventral sucker, without stylets but with 2-3 short transverse rows of oral spines, and their cuticle is covered with delicate bristles and tiny spines. Metacercariae are essentially tail-less cercariae whose bodies have become folded and encapsulated in a thin cyst wall. Cysts are elliptical, ranging from 120-280 x 85-220 μm , and are usually white with brown pigment granules. Metacercariae have oral and ventral suckers (of approx. equal size) and a sac-like excretory bladder. Adult flukes have long flat narrow lanceolate bodies that are tapered anteriorly and rounded posteriorly. Their size ranges from 4-28 x 1-7 x 1 mm, but most are 8-15 x 1.5-4.0 x 1 mm. They are covered by a delicate translucent aspinose tegument and are whitish-brown. They have an anterior oral sucker and a ventral sucker located in the upper mid-body. The digestive tract comprises a mouth, pharynx, oesophagus and simple bifurcate caeca that are blind-ended (no anus). Mature adult flukes are hermaphroditic, each worm containing both male and female reproductive organs. The generic name *Clon-orchis* (meaning 'rear'-'testis') refers to 2 large branched testes located in tandem near the posterior end (*C. sinensis* has branched rather than lobed testes like *Opisthorchis*). The testes are connected to a large, serpentine seminal vesicle leading to the ejaculatory duct which opens into the genital pore (both cirrus and cirrus sac are absent). The single pretesticular ovary has several lobes and is connected to a broad looped uterus with numerous folds and numerous small dense vitelline follicles continuously distributed in both lateral fields (cf. *Opisthorchis* spp. have clustered vitelline glands). The uterus opens into the common genital duct and is normally filled with many small embryonated eggs.

Site of infection: Adult *C. sinensis* flukes usually infect the intrahepatic bile ducts in the livers of their definitive hosts (mammals), although in heavy infections flukes may also occur in extrahepatic bile ducts, the gall bladder and sometimes in pancreatic ducts. Asexual developmental stages (sporocysts and rediae) are found associated with the digestive tracts and haemolymph spaces in their first intermediate hosts (snails), while metacercariae encyst within the muscles and connective tissues of their second intermediate hosts (fish).

Pathogenesis: Adult liver flukes live in the bile ducts and feed on epithelia which may result in profound inflammatory responses due to mechanical irritation (worm attachment, feeding and movement) and chemical irritation (release of toxic and/or allergenic worm metabolites invoking molecular and cellular host immune responses with immunopathological consequences). Many infections are light and benign (involving < 100 worms), but the risk of symptomatic infection and complications increases with heavier and more persistent infections. Nevertheless, only around 10% of patients develop clinical disease, manifest usually by diarrhoea, obstructive jaundice, and even cancer formation. Infections do not lead to the acquisition of any solid protective immunity, so repeated infections in endemic areas result in increasing worm burdens with age. Symptomatic infections are more common in aging adults and disease onset may be delayed for years following primary infection. Three stages of disease progression have been observed for heavier infections. An acute phase occurs around 1 week after infection, with relatively nonspecific clinical signs including anorexia, weight loss, diarrhoea, flatulence, fever, rigors and chills, colic abdominal pain, indigestion, slight icterus, rash, liver enlargement, swollen abdomen, oedema, fatigue, leucocytosis and eosinophilia. A prolonged chronic phase then ensues, with progressive gall bladder enlargement, oedema, meteorism (intestinal gas accumulation), cachexia, recurrent pyogenic liver cholangitis, diarrhoea, hepatomegalia, growth retardation and neuropsychiatric symptoms. Various complications may then arise,

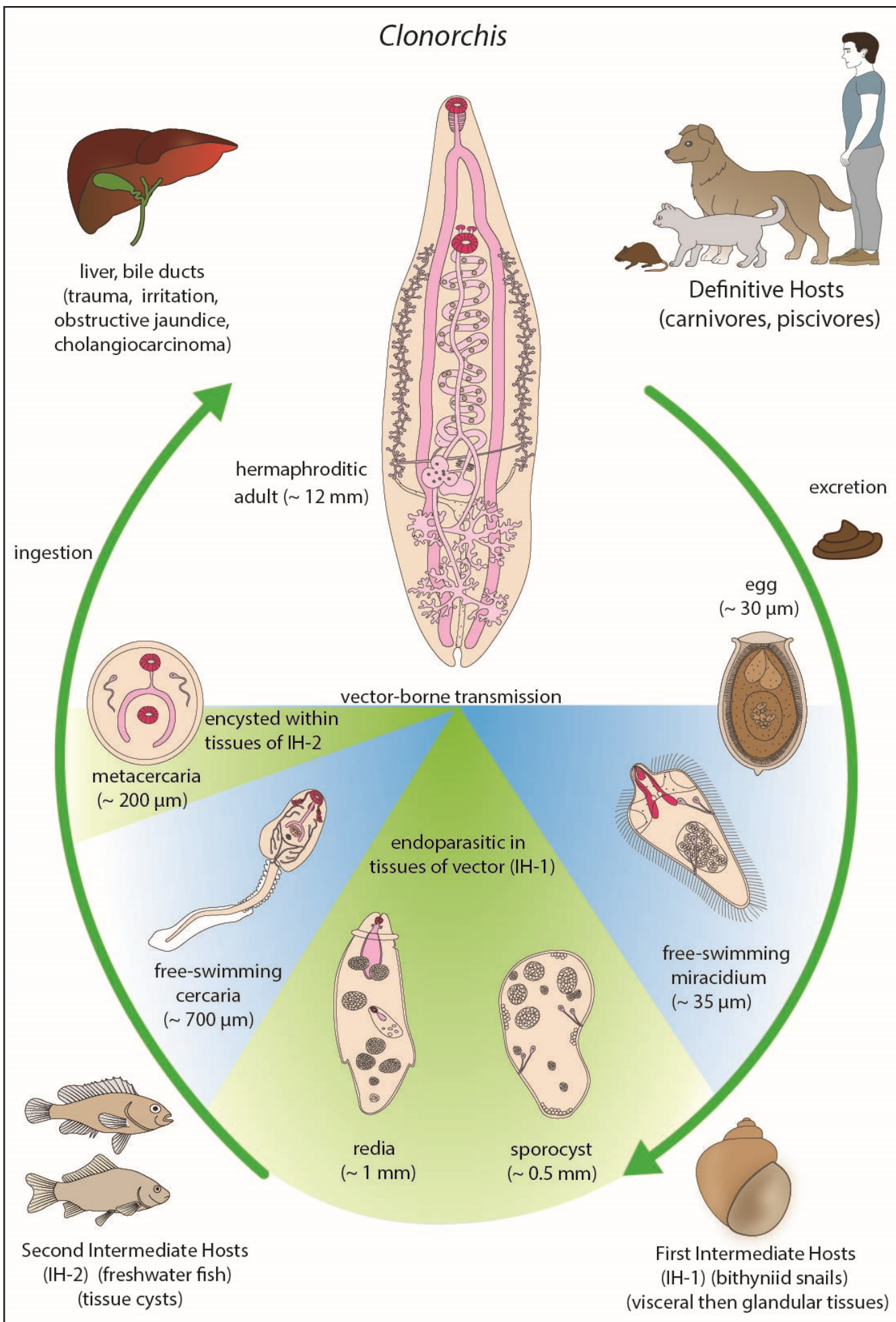
ranging from obstructive jaundice, cholangitis, cholestasis, liver cirrhosis, hepatolithiasis, pancreatitis, ascites, portal hypertension, night blindness (lack of vitamin A) and hepatic tumours. Adult flukes cause erosion of the biliary epithelial lining, adenomatous epithelial hyperplasia, mucin-secreting metaplasia and sometimes the formation of crypts. Profuse leucocytic infiltrates (eosinophils and mononuclear cells) occur around ducts, with necrosis and atrophy of adjacent hepatocytes. The ducts become fibrotic, thickened and obstructed, with cholangitis, stenosis and bile retention. The gall bladder often becomes enlarged and nonfunctional with worms causing cholecystitis, cholelithiasis (gallstones), intrahepatic pigment stones and pancreatitis. Infiltrating eggs may also result in granuloma formation further interfering with liver function. Recurrent infection and inflammation of the bile duct by *C. sinensis* can lead to dysplasia or neoplasia of biliary epithelial cells, particularly cholangiocarcinoma (CCA) which is malignant and invariably fatal unless a liver transplant is performed. *C. sinensis* has recently been classified as a group 1 biocarcinogen with causative mechanisms involving chronic irritation and inflammation, nitric oxide formation, protein dysregulation, and parasite excretory-secretory (ES) products.

Developmental cycle and mode of transmission: *Clonorchis* flukes have indirect life cycles involving 3 different types of hosts: asexual larval stages occurring in first intermediate hosts (snails), encysted infective stages in second intermediate hosts (fish), and adult worms in piscivorous definitive hosts (mammals). Transmission between the two intermediate hosts involves aquatic developmental stages, while final hosts become infected by predator-prey transmission when they consume infected fish. Embryonated eggs are excreted with definitive host faeces into the external environment, some ending up in water where they are ingested by freshwater snails (first intermediate hosts). The most suitable snails are bithyniid species living in slow-moving water bodies such as rice paddies, aquaculture ponds, and lakes used for commercial fishing. Reports in assimineid, thiarid and semisulcospirid snail species may represent mis-identifications and remain to be confirmed by molecular characterization studies. The eggs hatch in the snail digestive tract releasing their enclosed miracidia which penetrate the gut wall and develop into sporocysts in haemolymph spaces within 4 hours of infection. Sporocysts feed by absorption (lack mouths) and their germ balls undergo asexual reproduction forming 20-50 rediae over several weeks. Rediae have functional digestive tracts and they feed actively on snail tissues. At least 2 generations of rediae are produced; mother rediae forming daughter rediae which eventually produce up to 50 cercariae which emerge from the snail into water. The prepatent period in snails (time from infection to first release of cercariae) is around 95 days. Cercariae use their tails to swim around in water for 1-2 days actively seeking a suitable second intermediate host (freshwater fish, mainly cyprinids). Chinese publications have recorded over 130 fish species (and 4 shrimp species?) as second intermediate hosts for *C. sinensis*. When cercariae find a fish host, they invade the skin shedding their tails and then encyst as metacercariae in the muscles and subcutis where they may survive for up to 1 year. Young small fish are particularly susceptible to heavy infections due to their thinner skins and naive immune status. Infections are transmitted to piscivorous definitive hosts when they eat fish containing infective metacercariae. Humans become infected by eating raw, undercooked, salted, smoked or slightly pickled fish. Wine, vinegar, mustard, wasabi, and spices do not destroy encysted flukes. In Asia, many traditional dishes include raw freshwater fish (yu sang, sushi), lightly pickled fish (koi pla), fermented fish several days old (pla som), fermented and salted fish several weeks old (pla ra, jaewbhong), and lightly cooked fish (yusheng zhou, fish congee, hotpot, thick fillets). Ingested metacercariae excyst in the duodenum releasing immature flukes which quickly migrate to the bile ducts where they develop into adult flukes. The prepatent period in mammals (time from infection to first release of eggs) is around 4 weeks and adult worms may produce from 2,500-4,000 eggs per day for at least 6 months. Infections have been recorded to persist for decades and there is circumstantial evidence to suggest that adult worms may live for 25-30 year.

Differential diagnosis: The disease clonorchiasis manifests with relatively nonspecific biliary or hepatic signs, but should be suspected in symptomatic patients with a history of residence in, or travel to, endemic areas and a dietary intake of raw freshwater fish. Infections are usually diagnosed by the microscopic detection of fluke eggs in faecal samples, either in smears (Kato-Katz technique) or concentrates (formalin-ether technique), although their differentiation from other liver flukes (esp. *Opisthorchis*) is difficult due to their morphological similarity. Eggs may also be detected in duodenal aspirates or bile specimens collected when there is complete obstructive jaundice. Adult flukes may also be recovered from host faecal samples following anthelmintic therapy, or from fluids collected by endoscopy (esp. retrograde cholangiopancreatography). Modern imaging techniques have been used to gauge the extent and severity of infections by assessing tissue/organ changes, although it is difficult to distinguish between pathology caused by current infection or prior exposure. Ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and cholangiography may reveal intrahepatic bile duct inflammation and periductal fibrosis with dilatation, stenosis or wall irregularities, gall bladder enlargement and 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. Various immunoserological tests have been developed to detect host antibodies against whole worm extracts, excretory-secretory (ES) products or recombinant antigens, including intradermal tests, complement fixation, haemagglutination, fluorescence antibody tests and enzyme immunoassays, but problems with specificity and cross-reactivity have been encountered. Molecular biological techniques have been developed to detect and characterize parasites in mammals, fish and snails, mostly by polymerase chain reaction (PCR, including realtime-PCR) or loop-mediated isothermal (LAMP) amplification of random amplified polymorphic DNA (RAPD), mobile genetic elements (MGE), nuclear DNA (internal transcribed spacer regions of ribosomal RNA) or mitochondrial DNA (cytochrome c oxidase subunits 1 and 2, nicotinamide adenine dinucleotide dehydrogenase subunits 1 and 2).

Treatment and control: The isoquinoline anthelmintic praziquantel is currently the drug of choice for the chemotherapeutic treatment of *C. sinensis* infections in clinical patients as well as for preventive chemoprophylaxis in high-risk communities. Cure rates up to 100% have been reported with minimal side-effects (headache, dizziness, insomnia, nausea and vomiting). Acute and chronic infections have also been treated successfully with the broad-spectrum benzimidazoles, albendazole and mebendazole. The concurrent administration of antibiotics is used to treat recurrent pyogenic cholangitis. Other interventions may also be required in cases of obstructive jaundice, including biliary drainage and/or surgery. If cholangiocarcinoma (CCA) develops, however, a complete liver resection with clear margins gives the greatest chance for long-term survival because CCA responds poorly to chemotherapy. Many regions have implemented control programs based around mass population treatments with praziquantel, but re-infection is quite common in endemic areas. Other strategies have been integrated into preventive programs to reduce transmission cycles between piscivorous and aquatic hosts. Much can be done to prevent the faecal contamination of freshwater bodies, such as preventing promiscuous defaecation, installing sewers or septic tanks, treating sewage, fencing out feral and wild mammals which may be reservoir hosts, banning night-soil fertilizers, and especially by preventing the ingress of faeces into aquaculture ponds (some communities actually build toilets over carp ponds). Parasites infect and multiply in snail intermediate hosts but attempts to control their population numbers in domestic, peridomestic and wild situations have met with limited success. Various molluscicides have been used to kill snails but their indiscriminate use has been associated with serious environmental and public health problems because snails are integral to healthy aquatic ecosystems. Some aquaculture ventures regularly drain ponds to control aquatic biota, but this is difficult to do in regions with wet seasons involving monsoons and periodic flooding. The greatest impact for control in endemic regions has been to reduce transmission to humans by encouraging them not to eat raw freshwater fish. Cooking fish at temperatures above 63°C, or freezing them at -20°C for at least 7 days, has been shown to kill metacercariae. Proactive public health educational campaigns are required to get communities to change entrenched culinary practices perceived to be normal. Food hygiene regulations should also be upgraded to check the exportation of potentially contaminated fish products (frozen, dried, salted or pickled) from endemic areas. Lastly, some preliminary research on vaccine development has shown promising results, despite the apparent lack of acquired protective immunity in natural infections. Two DNA vaccines, one encoding a cysteine proteinase and another encoding a fatty-acid binding protein, and an oral vaccine, using tegumental protein fused with bacterial spore coat, have provided some protection (30-45%) in experimental animal models.

Clonorchis





Clonorchis adult worm



Clonorchis section through worm in liver



Clonorchis eggs



Clonorchis cercaria