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Mini-Review

Trematodes of the family Opisthorchiidae: a minireview

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Abstract: Examination of the original descriptions of genera placed within the family Opisthorchiidae has revealed that only 33 of the original 43 genera are valid members of this family. Further study of these descriptions should also reveal that many of the subfamilies are also invalid. With reference to the original descriptions of these genera, and subsequent literature, a brief survey of the family has been compiled. Information on the spectrum of definitive hosts that these helminths parasitize is provided, as well as information on the life cycles, geographic distribution, and socioeconomic impacts of the family. More in-depth information is given on those species that are of particular medical importance; namely, *Clonorchis sinensis*, *Opisthorchis viverrini*, and *O. felineus*. The final aims of this review are to provide information on the entire genera of the family Opisthorchiidae, which will aid understanding of the phylogenetic relationships not only within the family, but also within the Class Trematoda.

Key words: Opisthorchiidae, liver flukes, classification, survey of genera, host spectrum, distribution

INTRODUCTION

The liver flukes (Digenea) of the genera *Clonorchis* Looss, 1907 and *Opisthorchis* Blanchard, 1895 are notorious as causative agents of human infections transmitted by fish. The disease clonorchiasis, and the closely related opisthorchiasis, annually infect millions of people in regions where raw or undercooked fish harbouring opisthorchiid metacercariae are consumed, particularly in Southeast Asia and circumpolar parts of Eurasia and North America. However, the family Opisthorchiidae also includes numerous genera whose members rarely infect humans (Yamaguti, 1971).

The family Opisthorchiidae has been studied fairly exhaustively in a number of monographs or comprehensive volumes: Skrjabin and Petrov (1950), Yamaguti (1958, 1971, and 1975). In these works, the family was thought to include approximately 50 genera, encompassing several hundred species; however, the lack of more recent research has brought the taxonomic status and validity of these genera into question. Also, as no new data have been compiled recently as a specific condensed volume, very little is known about the family as a whole. The species composition, geographical distribution, life cycles and phylogenetic relationships within the family have all been insufficiently examined.

It is the purpose of this review, therefore, to give a brief summary of the family as a whole and to provide information concerning the taxonomy of the family in an attempt to

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eliminate invalidities and synonyms amongst genera. Information will also be provided on the host spectrum, life cycles, geographic distribution, and socioeconomic factors of the family.

CLASSIFICATION AND TAXONOMY

The main problem with classifying any group of digenetic trematodes is the fact that they have complex life cycles involving 2-4 hosts and many different larval stages. Thus, there are many morphologically different forms on which taxonomy can be based.

Yamaguti (1971) refers to his previous classifications and, indeed, those of others, when he maintains that the adult morphology of digeneans is the best method of classification. However, he also states: "We must be content for the present with an artificial classification based chiefly on adult morphology." It is with this statement in mind that another school of thought has arisen, which many believe is a much more accurate means of classification. It involves the examination of cercariae, as these are believed to be recapitulations of ancestral forms, and thus enable the production of a more phylogenetically accurate classification. It is considered that the use of cercarial forms also eliminates the possibility of misidentification through convergent evolution (Brooks et al. 1985).

Brooks et al. (1985) went some way towards addressing the issues concerned with the classification of digeneans and have carried out an extensive study on the phylogenetic relationships of the entire Digenea. Using both adult morphology and larval forms, they have attempted to provide a starting point for future phylogenetic studies on this diverse group, which, they hope, will grow into a comprehensive and increasingly accurate method of classification. However, the use of evidence from both adult and cercarial forms seems to further outline the prevalence of conflicting theories concerning the classification of this group. Until such inconsistencies can be overcome to a greater extent, the Digenea and methods used to classify them seem destined to remain under scrutiny. Indeed, Pearson (1992) argues that the methods of classification used by Brooks et al. (1985) are not reliable enough to base the phylogenetics of the digeaneans on. After his analysis of the characters used by Brooks et al. (1985), he states that 97 of the 212 characters used in the cladistic classification of the Digenea were invalidly used, resulting in the homoplasy increasing by 18% to 37%, with an accompanying decrease in resolution from 82% to 32%.

Cribb et al. (2001) have attempted to further unravel some of the mysteries surrounding digenean evolution and expand on such studies of Brooks et al. (1985) and Pearson (1992), among others, in order to resolve some of the many inconsistencies that can be found in this area of study. They have attempted to do this by combining many data sets, including those containing morphological and molecular characters, to provide a truer representation of the relationships between digenean families. The use of combined data sets can be controversial in itself, as outlined by de Queiroz et al. (1995) who have reviewed the arguments both for and against the use of combined data sets in phylogenetic analyses.

Although the studies mentioned above have used extensive phylogenetic analysis in order to try and resolve the relationships between all the families within the Digenea, such work is important when individual families are re-examined in order to validate subfamilies and genera. In essence, when classifying trematodes within a particular family, in this case the Opisthorchiidae, it is the species that are closely related enough to be included within the same family, subfamily, and genus that are being identified. The taxonomic state of the entire class or subclass being studied plays an important role in such a re-examination as closely related families can provide information on the composition of the family of interest.

Perhaps still considered as the most extensive attempt to form a complete classification of digenetic trematodes was that of Yamaguti (1971) (with the life cycles treated separately in 1975). Although three decades old, the material in these volumes is still referred to as one of the most comprehensive studies of digeneans, and for this review, these volumes were a starting point from which to source the original descriptions of members of the Opisthorchiidae. Subsequently, it was these original descriptions which formed the basis of the reclassification reported in this study.

Firstly, by referring to papers containing the original descriptions of each genus, 11 genera can be omitted from the family on the basis of being invalid or not belonging to the Opisthorchiidae at all:

Invalid genera

Allometorchis Baer, 1943
Synonym of *Metametorchis* Morozov, 1939 — see Skrjabin & Petrov (1950)
Assamia Gupta, 1955
Preoccupied by *Assamia* Sorensen, 1884 (Arachnida) and *Assamia* Buckton, 1896 (Insecta) — Lakshminarayana & Hafeezullah (1974)
Satyapalia nom. nov. proposed by Lakshminarayana & Hafeezullah (1974)
Brachymetra Stossich, 1904
Preoccupied by *Brachymetra* Mayr, 1865 (Insecta) — Poche (1926)
Synonym of *Ratzia* Poche, 1926
Diasia Travassos, 1922
Preoccupied by *Diasia* Travassos, 1923 — Yamaguti (1958)
Synonym of *Diasiella* Travassos, 1949
Gomtiotrema Gupta, 1955
Preoccupied (Yamaguti 1958, 1971)
Allogomtiotrema nom. nov. Proposed by Yamaguti (1958)
Minuthorchis Linton, 1928
Synonym of *Pachytrema* Looss, 1907 — Yamaguti (1958, 1971)
Multivitellaria Phadke & Gulati, 1929
Synonym of *Pachytrema* Looss, 1907 — Yamaguti (1958, 1971)
Notaulus Skrjabin, 1913
Synonym of *Opisthorchis* Blanchard, 1895 — Yamaguti (1958, 1971)

Genera not belonging to Opisthorchiidae

Neotropicotrema Caballero & Caballero, 1975
(Family Acanthostomidae)
Perezitrema Baruš & Moravec, 1967
(Family Acanthostomidae)

The two genera found not to belong to the Opisthorchiidae are thought to be synonymous with one another, and, as indicated by their morphology, belong to the family Acanthostomidae (subfamily Acanthostominae of the Cryptogominidae according to some authors) as originally reported. Thus, with all of the above genera omitted from the family, it is proposed that the family Opisthorchiidae contain only 33 genera, as listed below:

Valid genera of Opisthorchiidae

Agrawalotrema Sahay & Sahay, 1988
Allogomtiotrema Yamaguti, 1958
Amphimerus Barker, 1911
Cladocystis Poche, 1926
Clonorchis Looss, 1907
Cyclorchis Luhe, 1908
Delphinicola Yamaguti, 1933
Diasiella Travassos, 1949
Erschoviorchis Skrjabin, 1945
Euamphimerus Yamaguti, 1941
Evranorchis Skrjabin, 1944
Gomtia Thapar, 1930
Hepatiarius Fedzullaev, 1961
Holometra Looss, 1899
Metametorchis Morozov, 1939
Metorchis Looss, 1899
Microtrema Kobayashi, 1915
Nigerina Baugh, 1958
Oesophagicola Yamaguti, 1933
Opisthorchis Blanchard, 1895
Pachytrema Looss, 1907
Parametorchis Skrjabin, 1913
Paropisthorchis Stephens, 1912
Plotnikovia Skrjabin, 1945
Pseudamphimerus Gower, 1940
Pseudamphistomum Luhe, 1908
Pseudogomtiotrema Gupta & Jain, 1991
Ratzia Poche, 1926
Satyapalia Lakshminarayana & Hafeezullah, 1974
Thaparotrema Gupta, 1955
Trionychotrema Chin & Zhang, 1981
Tubangorchis Skrjabin, 1944
Witenbergia Vaz, 1932

Secondly, on the basis that characteristics of a less refined nature can be common to one or more genera, each genus can be allocated to a specific subfamily. Under Yamaguti's (1971) classification, the above genera would be accommodated by no less than 13 subfamilies, many of which are represented by only one or two genera (number of genera in parentheses):

Subfamilies of Opisthorchiidae

Allogomtiotrematinae (Gupta, 1955) Yamaguti, 1958 (2)
Aphallinae Yamaguti, 1958 (1)
Delphinicolinae Yamaguti, 1933 (1)
Diasiellinae Yamaguti, 1958 (1)
Metorchiinae Luhe, 1909 (4)
Oesophagicolinae Yamaguti, 1933 (1)
Opisthorchiinae Yamaguti, 1899 (14)
Pachytrematinae (Railliet, 1919) Ejsmont, 1931 (1)
Plotnikoviinae (Skrjabin, 1945) Skrjabin et Petrov, 1950 (1)
Pseudamphimerinae Skrjabin et Petrov, 1950 (3)
Pseudamphistominae Yamaguti, 1958 (2)
Ratziinae (Dollfus, 1929) Price, 1940 (1)
Tubangorchiinae Yamaguti, 1958 (1)

The number of subfamilies supposedly housed within the Opisthorchiidae is inconsistent with the number of genera they contain, especially when over half of the above mentioned subfamilies contain only one genus. Although not having been re-examined for this study, we suggest that future revisions of the family will result in the subfamilies being reduced considerably in number to leave a truer representation.

Thirdly, the overall classification of the Opisthorchiidae family, as well as being covered in Yamaguti's volumes, was extensively covered by Brooks et al. (1985), with specific regard to the phylogeny and adaptive radiation of phylum Platyhelminthes as a whole.

Position of Family Opisthorchiidae according to Brooks et al. (1985):

Order Opisthorchiiformes La Rue, 1957

Diagnosis: Digenea with oculate cercariae; secondary excretory pore in cercariae terminal; acetabulum in adults midventral; spinose adult body; rediae without appendages; cercariae encyst in next host; mesostomate cercarial excretory system; cercarial tail not furcate; cercarial excretory bladder lined with epithelium; oviduct seminal receptacle present; primary excretory pore in cercariae extending short distance into tail; dorsoventral fin fold present on cercarial tail; eggs small, generally less than 40 μm long; eggs ingested and hatch in molluscan host; no cirrus sac; no cirrus.

Order Opisthorchiiformes also includes family Cryptogonimidae Ward, 1917 and family Heterophyidae Odhner, 1914.

Family Opisthorchiidae Looss, 1899

Diagnosis: as above. Parasitic in pulmonate and prosobranch gastropods, in fish, and in liver, gall bladder, bile duct, and rarely intestine of fish, amphibians, reptiles, birds and mammals. Cosmopolitan.

Clearly, Brooks et al. (1985) have primarily based their classification on the examination of cercarial stages, whereas Yamaguti (1971) relied chiefly on adult morphology. As mentioned in the previous section on classification and taxonomy, much controversy still surrounds which of these methods is more reliable, and whether or not the use of combined data sets would provide a truer representation. Indeed, Wykoff et al. (1965) reported that they were unable to distinguish between *Opisthorchis viverrini* and *O. felineus* using adult or egg morphology alone, and that the only means of separating the two species was to use the flame cell patterns of cercaria or metacercaria.

SURVEY OF GENERA

On the basis of the revision of the pertinent literature of family Opisthorchiidae, we propose that the following genera (housed in the subfamilies suggested by Yamaguti (1971)) constitute a true representation of the family with all invalidities and synonyms removed. Clearly, the family has been greatly reduced in size, now compromising of only 33 genera; however, it is still a cosmopolitan family with the species inhabiting various geographical areas and having many different hosts. It should also be noted that this reduction represents the first step in a critical re-examination of the validity of opisthorchiid genera, which should be expanded upon to include the examination of specimens.

Allogomtiotrematinae

Allogomtiotrema Yamaguti, 1958
(Synonym *Gomtiotrema* Gupta, 1955)
Type species: *Allogomtiotrema attu* (Gupta, 1955) (syn. *Gomtiotrema attu* Gupta, 1955)
Type host: *Wallagonia attu* (Actinopterygii: Siluriformes: Siluridae)
Type locality: Gomti River, Lucknow, India (Asia)

Satyapalia Lakshminarayana & Hafeezullah, 1974
(Synonym *Assamia* Gupta, 1955)
Type species: *Satyapalia gauhatiensis* (Gupta, 1955) (syn. *Assamia gauhatiensis* Gupta, 1955)
Type host: *Rita rita* (Actinopterygii: Siluriformes: Bagridae)
Type locality: Brahmaputra River at Gauhati, Assam, India

Aphallinae

Witenbergia Vaz, 1932
Type species: *Witenbergia witenbergi* Vaz, 1932
Type host: *Pseudoplatystoma tigrinum* (Actinopterygii: Siluriformes: Pimelodidae)
Type locality: Rio Piracicaba, Estado de Sao Paolo, Brazil

Delphinicolinae

Delphinicola Yamaguti, 1933
Type species: *Delphinicola tenuis* Yamaguti, 1933
Type host: *Delphinus dusumieri* (Mammalia: Cetacea: Delphinidae)
Type locality: Pacific coast of Japan

Diasiellinae

Diasiella Travassos, 1949
(Synonym *Diasia* Travassos, 1922)
Type species: *Diasiella diasi* (Travassos, 1922)
Type host: *Plotus anhinga* (syn. of *Anhinga anhinga*) (Aves: Pelecaniformes: Anhingidae)
Type locality: Brazil

Metorchiinae

Holometra Looss, 1899
Type species: *Holometra exigua* (Muhling, 1898) (syn. *Distomum exiguum* Muhling, 1898)
Type host: *Circus rufus* (Aves: Falconiformes: Falconidae)
Type locality: East Prussia (now Poland) and Europe

Metametorchis Morozov, 1939
(Synonym *Allometorchis* Baer, 1943)
Type species: *Metametorchis skrjabini* (Morozov, 1939) (syn. *Parametorchis* (*Metametorchis*) *skrjabini* Morozov, 1939)
Type host: *Putorius putorius* (Mammalia: Carnivora: Mustelidae)
Type locality: Gorkov Region, Russia

Metorchis Looss, 1899
Type species: *Metorchis albidus* (Braun, 1893) (syn. *Distomum albidum* Braun, 1893)
Type host: *Felis catus domesticus* (Mammalia: Carnivora: Felidae)
Type locality: Europe, Palestine, Alaska, France, and Volga delta

Parametorchis Skrjabin, 1913
Type species: *Parametorchis complexus* (Stiles et Hassall, 1894) Skrjabin, 1913 (syn. *Distoma* (*Dicrocoelium*) *complexus* Stiles & Hassall, 1894)
Type host: cats
Type locality: not designated (New York, Maryland and D.C.)

Oesophagicolinae

Oesophagicola Yamaguti, 1933
Type species: *Oesophagicola laticaudae* Yamaguti, 1933
Type host: *Laticauda laticaudata* (Reptilia: Squamata: Elapidae)
Type locality: Isigaki-zima, Okinawa, Japan

Opisthorchiinae

Agrawalotrema Sahay & Sahay, 1988
Type species: *Agrawalotrema ritai* Sahay & Sahay, 1988
Type host: *Rita rita* (Actinopterygii: Siluriformes: Bagridae).
Type locality: Patna, Bihar (India)

Amphimerus Barker, 1911
Type species: *Amphimerus ovalis* Barker, 1911
Type host: soft-shell turtles *Trionyx spinifer* Lesson or *T. muticus* Lesueur (Reptilia: Testudines: Trionychidae)
Type locality: Mississippi River in Minnesota and Iowa, North America

Clonorchis Looss, 1907
Type species: *Clonorchis sinensis* (Cobbold, 1875), syn. *Distoma sinense* Cobbold, 1875
Type host: man, dog, rat, pig, rabbit, hare, *Mustela* (Mammalia: Carnivora: Mustelidae), *Vulpes* (Mammalia: Carnivora: Canidae)
Type locality: Japan, Korea, Siberia, China, Indochina, and India

Cladocystis Poche, 1926
Type species: *Cladocystis trifolium* (Braun, 1901) (syn. *Distomum trifolium* Braun, 1901)
Type host: *Ardea cocoi* (Aves: Ciconiiformes: Ardeidae), *Salminus maxillosus* (Actinopterygii: Characiformes: Characidae)
Type locality: Brazil

Cyclorchis Lühe, 1908
Type species: *Cyclorchis amphileucus* (Looss, 1896) (syn. *Distomum amphileucum* Looss, 1896)
Type host: *Naja haje* (Reptilia: Squamata: Elapidae)
Type locality: Alexandria (Egypt)

Evranorchis Skrjabin, 1944
Type species: *Evranorchis ophidiarum* (Tugangui & Masilungan, 1935) (syn. *Opisthorchis ophidiarum* Tubangui & Masilungan, 1935)
Type host: *Lapemis hardwickii* (Hardwick's Sea Snake) Gray, 1836 (Reptilia: Squamata: Hydrophiidae)
Type locality: Paombong, Bulacan, Luzon, the Philippines

Gomtia Thapar, 1930
Type species: *Gomtia piscicola* Thapar, 1930
Type host: Siluroid fish
Type locality: River Gomti, Lucknow, India

Hepatiarius Feizullaev, 1961
Type species: *Hepatiarius longissimus* (syn. *Distomum longissimus* Linstow, 1883) Feizullaev, 1961
Type host: *Ardea stellaris* (Aves: Ciconiiformes: Ardeidae)
Type locality: Turkestan, Asia

Nigerina Baugh, 1958
Type species: *Nigerina hardoiensis* Baugh, 1958
Type host: *Phalacrocorax niger* (Aves: Ciconiiformes: Phalacrocoracidae)
Type locality: Hardoi District, Uttar Pradesh, India

Opisthorchis Blanchard, 1895
(Synonym *Notaulus* Skrjabin, 1913)
Type species: *Opisthorchis felineus* (Rivolta, 1884) (syn. *Distomum felineum* Rivolta, 1884)
Type host: not designated
Type locality: not designated

Paropisthorchis Stephens, 1912
Type species: *Paropisthorchis canius* (Barker, 1911) (syn. *Opisthorchis caninus* Barker, 1911)
Type host: dog
Type locality: India

Pseudogomtiotrema Gupta & Jain, 1991
Type species: *Pseudogomtiotrema caranxi* Gupta & Jain, 1991
Type host: *Caranx malabaricus* (Cuvier & Valenciennes) (Actinopterygii: Perciformes: Carangidae)
Type locality: Puri, Orissa, Bay of Bengal, India

Thaparotrema Gupta, 1955
Type species: *Thaparotrema vittalani* Gupta, 1955
Type-host: *Rita rita* (Hamilton) (Actinopterygii: Siluriformes: Bagridae)
Type locality: Brahmaputra River at Gauhati, Assam, India

Trionychotrema Chin & Zhang, 1981
Type species: *Trionychotrema taenioidea* Jin & Zhang, 1981
Type host: Chinese turtle, *Trionyx sinensis* (Wiegmann) (Reptilia: Testudines: Trionychidae)
Type locality: Hubei Province, China

Pachytrematinae

Pachytrema Looss, 1907
(Synonym *Minuthorchis* Linton, 1928 and *Multivitellaria* Phadke & Gulati, 1929)
Type species: *Pachytrema calculus* Looss, 1907
Type host: *Larus ridibundus* (Aves: Lariformes: Laridae)
Type locality: Trieste, Italy

Plotnikoviinae

Plotnikovia Skrjabin, 1945
Type species: *Plotnikovia podilymbae* (Olsen, 1938) (syn. *Diasia podilymbae* Olsen, 1938) (Original specific name *podilymbae* corrected to *podilymbi* Olsen, 1939 (Yamaguti, 1971))
Type host: *Podiceps podiceps* (Aves:

Ciconiiformes: Podicipedidae), *Gavia immer* (Aves: Ciconiiformes: Gaviidae)
Type locality: Minnesota, USA

Pseudamphimerinae

Erschoviorchis Skrjabin, 1945
Type species: *Erschoviorchis lintoni* (Gower, 1939) Skrjabin, 1945 (syn. *Amphimerus lintoni* Gower, 1939; *Erschoviorchis lintoni* Skrjabin, 1945)
Type host: *Gavia immer* (Aves: Gaviiformes: Gaviidae)
Type locality: Woods Hole, Massachussets, USA

Euamphimerus Yamaguti, 1941
Type species: *Euamphimerus nipponicus* Yamaguti, 1941
Type host: *Luscinia calliope calliope* (Aves: Passeriformes: Muscicapidae)
Type locality: Siriyazaki, Aomori Prefecture, Japan

Pseudamphimerus Gower, 1940
Type species: *Pseudamphimerus sterni* Gower, 1940
Type host: common tern (*Sterna hirundo* Linnaeus) (Aves: Ciconiiformes: Laridae)
Type locality: Michigan, USA

Pseudamphistominae

Microtrema Kobayashi, 1915
Type species: *Microtrema truncatum* Kobayashi, 1915
Type host: Pig (*Sus scrofa*) (Mammalia: Artiodactyla: Suidae).
Type locality: not designated, Japan

Pseudamphistomum Lühe, 1908
Type species: *Pseudamphistomum truncatum* (Rudolphi, 1819) (syn. *Distomum truncatum* Rudolphi, 1819)
Type host: *Phoca vitulina* (Mammalia: Canivora: Phocidae)
Type locality: Europe

Ratziinae

Ratzia Poche, 1926
(Synonym *Brachymetra* Stossich, 1904)
Type species: *Ratzia parva* (Stossich, 1904) (syn. *Brachymetra parva* Stossich, 1904)
Type host: *Rana esculenta* Linnaeus (Amphibia: Anura: Ranidae) (progenetic metacercariae)
Type locality: Italy (Europe)

Tubangorchiinae

Tubangorchis Skrjabin, 1944
Type species: *Tubangorchis caintaensis* (Tubangui, 1928) (syn. *Metorchis caintanensis* Tubangui, 1928)
Type host: *Hypotaenidia philippensis* Pelzeln, 1873 (syn. *Rallus owstoni* (Rothschild, 1895)) (Aves: Gruiformes: Rallidae)
Type locality: Cainta, Rizal Province, Luzon, the Philippines

DEFINITIVE HOSTS

It is the definitive host of members of this family that provides the site for development of opisthorchiid metacercariae into the adult forms, and thus the means to reproduce. Adult opisthorchiid worms exploit all major vertebrate groups as definitive hosts, with birds being the most common exclusive host for adult flukes. However, like humans and other mammals, this is probably due to the fact that fish are a major dietary component (genera in **bold** are specific to the vertebrate class):

Fish

***Agrawalotrema* Sahay & Sahay, 1988**
***Allogomtiotrema* Yamaguti, 1958**
Cladocystis Poche, 1926
***Gomtia* Thapar, 1930**
Opisthorchis Blanchard, 1895
Pseudamphistomum Lühe, 1908
***Pseudogomtiotrema* Gupta & Jain, 1991**
***Satyapalia* Lakshminarayana & Hafeezullah, 1974**
***Thaparotrema* Gupta, 1955**
***Witenbergia* Vaz, 1932**

Amphibians

Ratzia Poche, 1926

Reptiles

Amphimerus Barker, 1911
***Cyclorchis* Lühe, 1908**
***Evranorchis* Skrjabin, 1944**
***Oesophagicola* Yamaguti, 1933**
Ratzia Poche, 1926
***Trionychotrema* Chin & Zhang, 1981**

Birds

Amphimerus Barker, 1911
Cladocystis Poche, 1926
Clonorchis Looss, 1907
***Diasiella* Travassos, 1949**
***Erschoviorchis* Skrjabin, 1945**
***Euamphimerus* Yamaguti, 1941**
***Hepatiarius* Fedzullaev, 1961**
***Holometra* Looss, 1899**
Metametorchis Morozov, 1939
Metorchis Looss, 1899
***Nigerina* Baugh, 1958**
Opisthorchis Blanchard, 1895
Pachytrema Looss, 1907
***Plotnikovia* Skrjabin, 1945**
***Pseudamphimerus* Gower, 1940**
***Tubangorchis* Skrjabin, 1944**

Mammals

Amphimerus Barker, 1911
Clonorchis Looss, 1907
***Delphinicola* Yamaguti, 1933**
Metametorchis Morozov, 1939
Metorchis Looss, 1899
***Microtrema* Kobayashi, 1915**
Opisthorchis Blanchard, 1895
Pachytrema Looss, 1907
***Parametorchis* Skrjabin, 1913**
***Paropisthorchis* Barker, 1912**
Pseudamphistomum Luhe, 1908

LIFE CYCLES

The life cycles of Opisthorchiidae flukes are complex in that they have various larval stages, three different hosts (two intermediate and one definitive), and both asexual and sexual stages of reproduction. Although opisthorchiid flukes can have several generations within different larval stages, there are five main larval stages before the sixth and final adult form emerges. Each stage of the fluke's life cycle is morphologically distinct from the others, making each stage uniquely suited to its environment and role in the development of the adult form.

Egg

The egg or, more specifically, the encapsulated embryo of opisthorchiid worms is the result of the only sexual stage of reproduction, as well as being the first stage of asexual reproduction in the fluke's life cycle. Adult worms of *Clonorchis sinensis* are capable of producing up to 4000 eggs per day (Roberts and Janovy, 1996) and these are passed from the definitive host in the faeces. Based on the original descriptions, eggs of species of the family Opisthorchiidae have measurements in the range 21-100 μm in length and 10-120 μm in breadth, they are usually operculate and yellow/brown in colour. Opisthorchiid eggs closely resemble those of heterophyid flukes and this causes problems in identification, which is important in cases of human infection (Ditrich et al. 1990). Indeed, Ditrich et al. (1992) also showed that not only were opisthorchiid and heterophyid eggs similar, but that great variability was present among eggs from the same species.

Miracidia

The miracidia are already contained within the eggs and are well developed, with their internal organization being somewhat asymmetrical (Roberts and Janovy, 1996). The eggs are passed from the definitive host in the faeces and the miracidia will only hatch upon the eggs being ingested by the first intermediate host. This host is usually a gastropod (Prosobranchia) mollusc of the families Bithyniidae, Hydrobiidae, or Thiaridae; for example, the most common species ingesting *Clonorchis sinensis* eggs are: *Parafossarulus manchouricus*, *Bulimus fuchsianus* (= *Bithynia fuchsiana*), and *Alocima longicornis* (= *B. longicornis*) (Smyth, 1994). As the diet of aquatic snails such as these consists mainly of vertebrate faeces, the eggs are passively transferred to the first intermediate host where they hatch and begin subsequent stages of development (La Rue, 1951). Following hatching from the egg, the miracidia migrate along the digestive tract to the snail's gut where the next stage of development can begin.

Sporocyst

The second developmental stage within the first intermediate host is that of the sporocyst, which develops directly from the miracidium. Sporocysts have no mouth, but absorb nutrients directly from the host tissue, in this case the snail's gut wall. In the case of *Clonorchis sinensis*, sporocysts develop from the miracidia within 4 hours of the eggs being ingested, and then to develop into redia within 17 days (Roberts and Janovy, 1996).

Redia

The penultimate stage of intra-molluscan development is that of the redia, which develop from sporocysts. As reported by La Rue (1951), daughter generations of rediae are morphologically indistinct from one another and so it is difficult to accurately determine how many different generations will have developed within the molluscan host. Adam et al. (1995), however, have gone some way to further define the larval stages of *Opisthorchis viverrini* with their study on the ultrastructure of redia and pre-emergent cercaria of this species.

Cercaria

The cercariae of opisthorchiid flukes are the larval forms that enter the second intermediate host. Although Yamaguti (1975) reported that the cercaria of opisthorchiid trematodes was of a variety of types, the classification of Brooks et al. (1985) is much more succinct and states, among other things, that opisthorchiid cercariae are oculate with dorsoventral fin folds present on the tail (pleurolophocercous). From other literature it seems that this is an accepted general description of opisthorchiid cercariae (Smyth, 1994; Roberts and Janovy, 1996). The morphology of opisthorchiid cercaria from Laos has also been examined by Ditrich et al. (1992). Upon leaving the snail host, the cercaria must actively find their second intermediate (fish) host and this is achieved by hanging upside down in the water and slowly sinking to the bottom. When any object comes into contact with the cercaria, such as a swimming fish, or even the currents they create, they rapidly swim upwards, before beginning to sink again. This adaptation favours contact with the fish host and on touching the epithelium of the fish (usually a species of the Cyprinidae), the cercaria attaches by its oral sucker, loses its tail and forms a cyst underneath the scales or in the muscle.

Metacercaria

The final larval stage, and that which takes place in the second intermediate host, is the development of the metacercaria. Although the metacercarial stage is not unique to the Opisthorchiidae, it is not found in all trematodes either; for example, the cercaria of other species can directly penetrate the skin of, or be eaten by, the definitive host (Smyth, 1973). Metacercaria of *Clonorchis sinensis* can also be found in certain crustaceans; namely, *Caridina*, *Macrobrachium*, and *Palaemonetes*, and were found to be experimental infective to guinea pigs (Roberts and Jonovy, 1996). Encysted in the muscle of the fish host, the metacercariae excyst upon consumption of the infected tissue by the definitive host and the young flukes migrate to the liver where they mature to adults. La Rue (1951) reported that the juvenile flukes can either migrate to the liver through chemotaxis, or by following the actual physical track of the bile duct. Behaviour of the metacercaria is also covered by Cable (1972), along with a review of the behaviour of the other larval stages of digenetic trematodes.

IMPORTANCE TO HUMAN HEALTH

Far and away the most important members of family Opisthorchiidae, with regards to human health, are *Clonorchis sinensis*, *Opisthorchis viverrini* and *O. felineus*. These species are responsible for the diseases of clonorchiasis and opisthorchiosis, respectively, and collectively it is thought that these species are responsible for infecting over 30 million people worldwide (Roberts and Janovy, 1996) with a further 290 million people at risk (Rim et al., 1994). It should be noted, however, that different levels of infection are consistently quoted in literature, so these figures can be in no way absolute. In addition to the health problems (outlined further, below) associated with theses helminth species, comes a huge economic burden for countries where infection with these species is endemic; for example, infections with *O. viverrini* alone, can cost Northeast Thailand $120 million per year (Murrell et al., 1991).

Clinically, clonorchiasis and opisthorchiasis are indistinguishable from one another as they both produce symptoms of chills, diarrhoea, fever, lower abdominal pain, jaundice, swelling of the liver, and malnutrition. Morphologically the species are very similar, evident by the fact that some authors refer to the first species as *Opisthorchis sinensis* (see Kassai, 1999), and are only distinguishable by the branched (dendritic) testes of *Clonorchis* in comparison to the lobed testes of *Opisthorchis*. For the purpose of convenience and ease of explanation, therefore, all human infections caused by any of these species shall be referred to as clonorchiasis, unless otherwise stated.

The flukes causing clonorchiasis inhabit the liver of their host, which may make them somewhat less immediately life threatening than the flukes that cause schistosomiasis, for example. Nevertheless, this disease can have devastating effects on entire communities in addition to the aforementioned symptoms, in cases of prolonged and heavy infections where the worm load is in excess of 200, cirrhosis, pancreatitis, and cholangiocarcinoma can also occur. Indeed, cholangiocarcinoma, rare in other areas of the world, has a high prevalence in countries where clonorchiasis is endemic (Haswell-Elkins et al., 1992; Lee et al., 1994).

Clonorchiasis caused by *Clonorchis sinensis* and *Opisthorchis viverrini* is endemic throughout Southeast Asia in countries such as China, Korea, Japan, Taiwan, Vietnam, Laos, Thailand, and Cambodia (Tinga et al., 1999). Infection with *Opisthorchis felineus* is prevalent in Russia, particularly Siberia, and also in Kazakhstan and the Ukraine (Mas-Coma and Bargues, 1997). The disease can also be found in Europe and North America, although to a much lesser extent, and probably only due to the increasing import of fish from countries where the disease is widespread. Perhaps the most prominent reason for such a distribution of the disease lies in the traditional eating habits of the inhabitants of the aforesaid Asian and Russian countries. Many traditional Asian dishes such as yue-shan chuk (a Cantonese delicacy) consist of raw fish with rice, vegetables, and soup. Since the fish in these dishes is eaten raw or only slightly cooked, the metacercarial cysts can survive to excyst on consumption and mature to adults in their definitive host, in this case humans. Species of cyprinid fish are cultivated for human consumption and the increased breeding of the grass carp *Ctenopharyngodon idellus* for this purpose creates a wealth of second intermediate hosts for opisthorchiid cercariae, which can increase the prevalence rates of clonorchiasis (Bisseru and Chong, 1969). In Russia, frozen, raw, pickled or smoked fish are eaten and a study by Fan (1998) showed that metacercariae from *Clonorchis sinensis* can actually remain viable, even after the fish has been frozen or salted for several days. Other piscivorous mammals such as cats, dogs, and farm livestock can also act as definitive hosts such a wide range of definitive hosts ensures that large numbers of eggs are washed into water reservoirs with faecal matter and so the life cycle can begin again.

The living conditions to which people are exposed also contribute to a high incidence of this disease in Asian countries. As the main occupation in many areas is fishing or agriculture, many villages are built on the edges of lakes or reservoirs. Such villages rarely have adequate plumbing and consistently lack sanitation facilities, thus people usually defecate either close to or actually in the water, which allows the opisthorchid eggs to begin the aquatic stages of their life cycle (Ditrich et al., 1990).

Due to the medical significance of this disease, many studies have been carried out to investigate the impact that it has on human populations. Such studies provide information on, among other things, the prevalence and spread of the disease in endemic regions, which drugs are best at combating infection, and how public health programmes can help to curb the spread of the disease (see Rim et al., 1994 for review; Yu, 1996). One such study by Sornami et al. (1973) highlighted the problems caused by the construction of the Ubolratana dam in Northeast Thailand, with regards to the health status of individuals living in the newly built villages around the banks of the man-made lake. The villagers were traditionally farmers living in the area flooded by the building of the dam; it was a natural progression, therefore, that they moved to the banks of the lake and replaced farming with fishing. The study was carried out over a period of several months during the early rainy season of the area, and was primarily concerned with the gathering of information to investigate the general health status of the villagers. The results clearly showed that the living conditions of the villagers greatly increased their chances of being the definitive host for the opisthorchiid worms as well as other helminth parasites. The traditional dishes of raw or undercooked fish, coupled with the poor sanitation facilities (only one house from the three villages had a latrine) provided a near perfect environment for the spread of infections (Sornmani et al., 1973).

Another study, carried out by Joo et al. (1997), reported on the changing patterns of clonorchiasis in Korea over a period of two years. This study also provides information on the prevalence of metacercariae in species of fresh-water fishes common to the area, and highlights the need for a continued and amplified treatment programme.

Just as there is a wealth of information on the rates of infection of clonorchiasis, there is also a multitude of studies as to what is the best form of treatment for this disease. Many studies (e.g., see Rim et al., 1981; Chen et al., 1983; Radomyos et al., 1998) have concentrated on the use of praziquantel as the most effective drug with which to treat cases of clonorchiasis, and outline extensively the best dosage and frequency of administration. However, other studies have questioned the effectiveness of this drug in the treatment of clonorchiasis; namely, Hong et al. (1998) and Tinga et al. (1999) who showed that certain dosages of praziquantel were ineffective as a means of treating clonorchiasis. However, due to the fact that praziquantel targets more than one helminth species means that it will probably remain the drug of choice for diseases such as clonorchiasis.

VETERINARY IMPORTANCE

Although Kassai (1999) states that opisthorchiid trematodes are of low veterinary significance, these helminths can still have adverse affects on domestic and wild animals. Since humans and other animals are inextricably linked in many Asian communities, where farming and fishing are the main sources of employment, such diseases as clonorchiasis can be so prevalent that they become established within entire communities. Obviously, the health risks to farm animals can be debilitating for the community as the disease weakens the animals, thus lowering the quality of the produce derived from them, or renders them too weak to be used for manual work. The situation is worsened by the fact that such animals act as reservoir hosts for the opisthorchiid worms and so their close contact with humans can exacerbate the problems of infection and control within human populations.

Similarly, wild animals can act as reservoir hosts for many helminths, and this allows the parasites to have a continual presence in the environment. As discussed by Shimalov et al. (2000), otters (*Lutra lutra* Linnaeus, 1758) in Russia are a host to many species of helminths, including three species from the family Opisthorchiidae; namely, *Opisthorchis felineus*, *Pseudamphistomum truncatum*, and *Metorchis bilis*. Introduced animal species in Russia are also hosts to a wide range of helminths. Ivanov and Semenova (2000) studied introduced animal species, including American muskrats (*Ondatra zibethicus*), American mink (*Mustela vison*), and Siberian raccoon dogs (*Nyctereutes procyonoides*), and found that they had acquired parasitic fauna of the area, which includes *M. bilis* and *P. truncatum*.

CONCLUSIONS

It is clear that even with invalid genera removed from the family, the Opisthorchiidae are still an interesting and cosmopolitan group of digeneans, and although much work has been done on the medically important species of family, relatively little is known about the other genera. This review is intended to be the first step in a critical re-examination of the

Opisthorchiidae, the subsequent steps of which should be based on the examination of specimens. It is hoped that this review has gone some way to providing a truer classification of the family as a whole, at genera-level at least, and that this information may be expanded upon in future studies to further resolve inconsistencies and invalidities within the family, particularly with regards to subfamily classification.

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