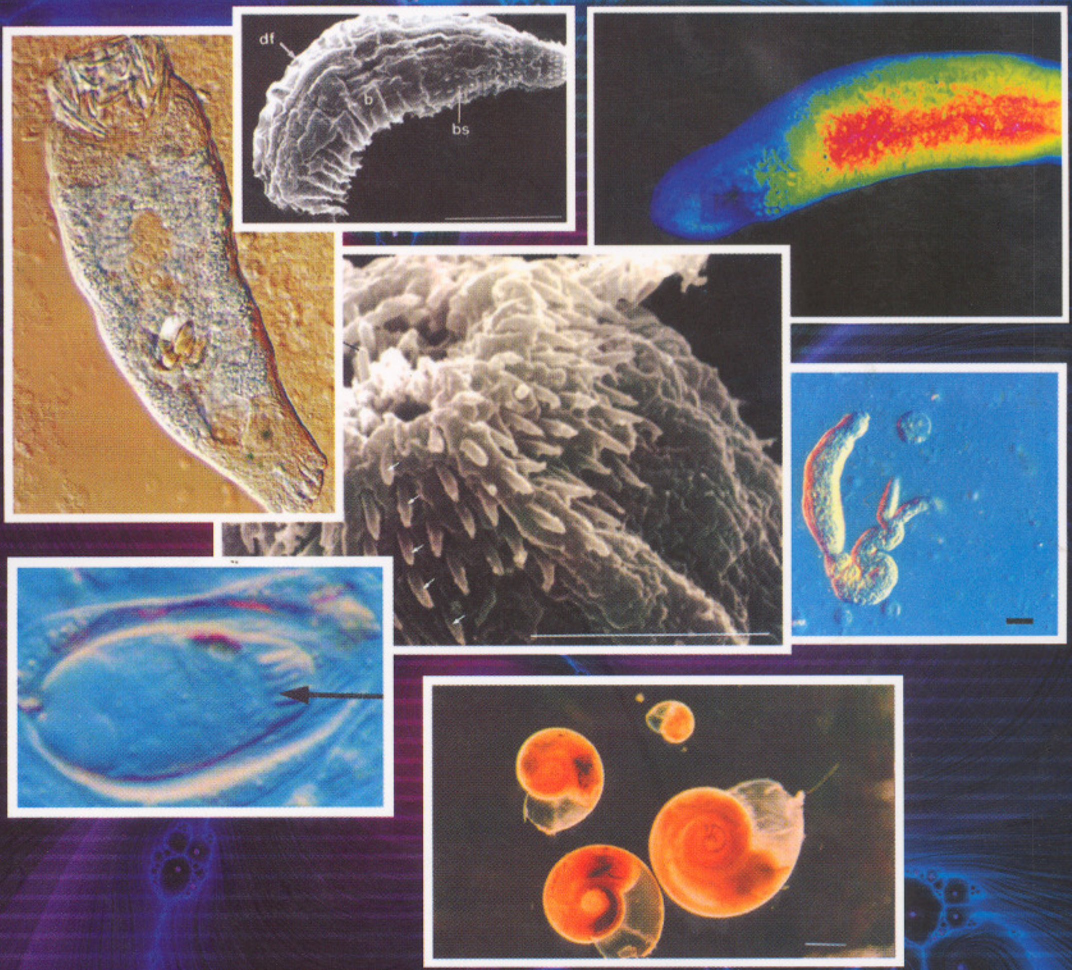
Syarahan Inaugural

The Fascinating World of Flukes



Oleh

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Some of the photographs of the marine monogenea have been taken from Ecology of marine parasites by Klaus Rohde, University of Queensland Press, 1982.

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ABSTRACT

This inaugural lecture is aimed at showing the interesting and fascinating aspects of studies on gill flukes and blood flukes. Flukes are the common term for parasitic worms belonging to the Phylum Platyhelminthes and class Trematoda. The author begins by giving a brief classification on flukes and quoting the lyrics of a song on Platyhelminthes. The morphological features of the gill fluke is then introduced with pictures of the skin fluke *Gyrodactylus* sp. The host specificity of Monogenea is highlighted with emphasis on a variety of mechanisms or parameters which can influence host-specificity. The site-specificity of Monogenea is also discussed with special emphasis on the site-specificity exhibited by the monogenea *Cichlidogyrus sclerosus* on the gills of tilapia *Sarotherodon mossambicus.*

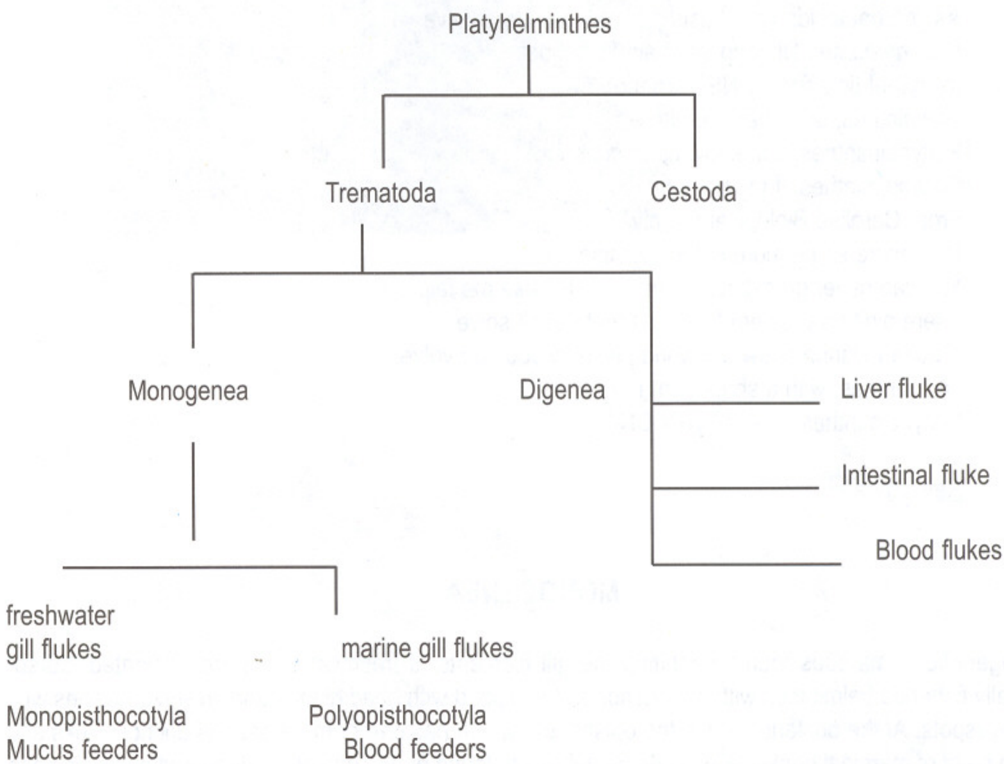
The life-cycle of monogenea is highlighted, a comparison being made between temperate and tropical egg laying periods. Studies on attachment inducing capacities of fish skin towards the larval oncomiracidia of Monogenea are also discussed.The economic importance of Monogenea are emphasized with examples from mass mortalities of the spiny sturgeon *Acipenser nudiventris* in the Aral sea in the 1930s due to the monogenea *Nitzschia sturionis* to the mass mortalities of sea bass *Lates calacarifer* in cage cultures in Malaysia due to the monogenean *Pseudorhabdosynochus latesi.* The possible methods of management are briefly discussed.

The subject on digenetic blood trematodes is next introduced beginning with the morphological description of a common blood fluke and also touching briefly on ecological aspects of infection. The life–cycle of blood flukes is next highlighted with special mention of *Sanguinicola armata* and the discovery of its intermediate host the freshwater snail *Gyraulus convexiusculus*. Laboratory experimental infections were also carried out to determine the life-cycle of *S. armata* with the grass carp *Ctenopharyngodon idella* and this was the project of one masters student. Another masters student looked at the cercarial penetration through the fish skin using histological techniques. The marine blood fluke *Cruoricola latesi* which was first discovered in 1985 is next introduced. Attempts to look for the intermediate host was carried out since 1985 but only in 1996 did we manage to secure a grant to carry out intensive study. Eventually in 1998 the intermediate host *Anodontia edentula* was found.

This is a world first as it is the first time that a marine bivalve was found to harbour the sporocysts of a digenean blood fluke. This research won the group the Top Ten Award for Innovative Research and Invention. 2000. Universiti Putra Malaysia (UPM).

INTRODUCTION

Flukes are the common term for parasitic worms belonging to the Phylum Platyhelminthes (see poem) and the class Trematoda. These flatworms (Platy - flat; helminthes – worms) are flattened dorso-ventrally and can be classified as follows:



Platyhelminthes

(to the tune of the Guess Who's *American Woman*)

Lyrics by *Off Yer Rockers*
World Debut: July 3, 1999
Platyhelminthes, I wanna study you.
Platyhelminthes, check out my tatoo.
I caught you squirming around my drain
Now you're just driving me insane
You know I'm gonna study you until I drop
You'll even see my picture in the donut shoppe
Oh 'minthes, when will we meet? Platyhelminthes, boring through my feet.
Platyhelminthes, to NSF I'll go
Platyhelminthes, to get some dough.
I'll get an RA and I'll grab some bucks
Then I'll head south and with a little luck
I'll make you happy as a worm can be
In the *Journal of Parasitology*.

'Helminthes, I'm gonna write about you.
Platyhelminthes, no other worm will do.
[Instrumental Break]
Platyhelminthes, I'm gonna hunt you down
Platyhelminthes, the coolest worm in town.
When I see you do your magic I will come alive
As you get inside your hosts and eat them to survive
I'll find you and I'll bring you back to school.
It's about time for my NSF grant renewal.
'Helminthes, don't be so cruel.
Platyhelminthes, you know that I'm no fool.
Platyhelminthes, if my lab runs dry
From Carolina Biological Supply
I'll procure some more in the morning mail.
You can never go extinct, no way you'll make me fail.
There ain't no problem facing us that I can't solve
'Cause I wanna show the world just how you co-evolve.
Oh 'minthes, with a shark and a ray
Platyhelminthes, I've got your DNA.

<u>Revalidate</u>

MONOGENEA

Monogenetic trematodes found inhabiting the gill filaments of freshwater fish are elongated, dorso-ventrally flattened helminthes with an anterior end equipped with head glands and in some species with four eyespots. At the posterior end is the opishaptor which has one or more pairs of anchor hooks and seven pairs of marginal hooks. (Fig. 1) Some flukes are found on the dorsal, pectoral and pelvic fins and these parasites are skin flukes namely *Gyrodactylus sp.* which lack eyespots and have eight pairs of marginal hooks. Identification of monogenean parasites are based on the structure and measurement of the opisthaptoral hooks and the chitinous copulatory appendage. Freshwater monogenetic trematodes are gill and mucus feeders belonging to the Monopisthocotyla. The presence of eyespots and chitinous copulatory appendages and the absence of a 4-tiered embryo distinguishes the dactylogyrids from the gyrodactylids. (Fig. 2)

Marine monogenea are large gill flukes with an elongated opisthaptor with numerous clamps or opisthaptoral hooks (Fig. 3). The Polyopisthocotylean are blood feeders and this is proven by the presence of haematin in the intestinal region.

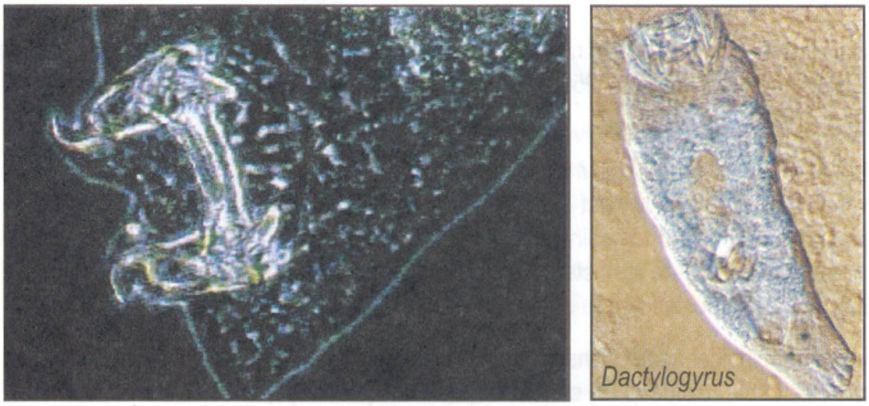


Fig. 1: Opisthaptoral hooks of *Dactylogyrus* sp

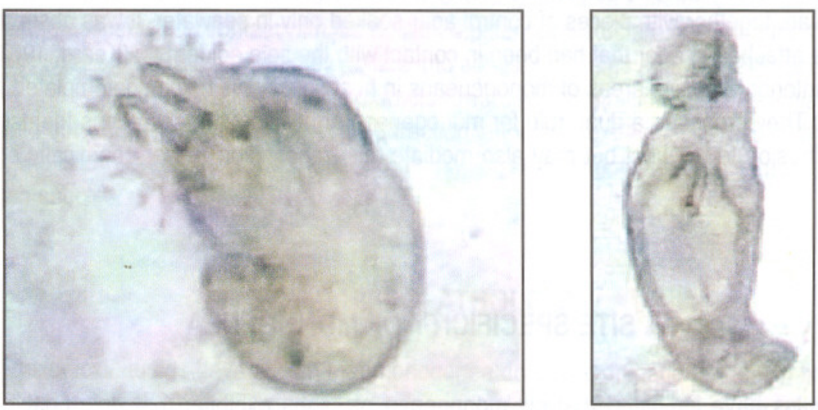


Fig. 2: The monogenea *Gyrodactylus* species

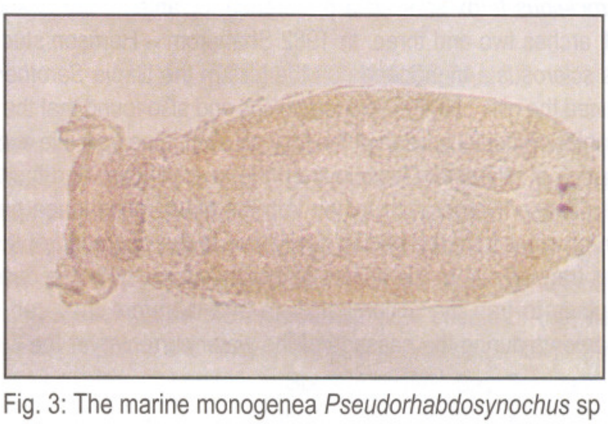


Fig. 3: The marine monogenea *Pseudorhabdosynochus* sp

HOST SPECIFICITY OF MONOGENEA

Monogeneans have a high degree of host specificity and there has been numerous reports regarding this (Whittington et al 2000, Llewellyn, 1956, Bychowsky, 1961; Rohde, 1979; Kohn & Cohen, 1998; Fletcher & Whittington, 1998).

Of more than 900 specimens of gill monogeneans collected from 2104 fish representing 17 species of fish all except two were strictly host specific. Bychowsky (1961) reported that 711 of 957 species of monogeneans were confined to a single host while Rohde (1979) found that 340 species out of 435 species of monogeneans were restricted of a single host species, 388 species to one genus, 420 species to one family and 429 species to one order.

Specialised morphological adaptations of the monogenea to the site of attachment is one of the factors which play a role in the maintenance of host – specificity. (Llewellyn, 1956; Williams, 1960, 1961, 1970).

A variety of mechanisms or parameters may influence host-specificity of monogenea. In a series of elegant experiments Kearn (1967) has shown that 90% of the larvae of the marine monogenea *Entobdella solea* attached to the scales of the host fish *Solea solea* compared to other fish scales. In a second series of experiments agar jelly that had been in contact with live sole skin was offered to freshly hatched *E. solea* larvae. together with pieces of control agar soaked only in seawater. It was observed that 92% of the larvae attached to agar that had been in contact with the sole epidermis. (Kearn, 1967). Currently the role of anterior adhesive areas of monogeneans in host specifity is being contemplated (Whittington et al, 2000). They proposed a dual role for monogenean adhesives: the secretions themselves clearly generate adhesion to the host but may also mediate this "recognition" either chemically or via nearby receptors.

SITE SPECIFICITY OF MONOGENEA

Several studies have indicated that gill monogenea of fishes exhibited not only host but also site specificity for gill arches. (Llewellyn, 1956; Wiles, 1968; Suydam, 1971) Llewellyn (1956) found that *Diclidophora merlangi* occurred most frequently on gill arch 1 of *Gadus merlangus*. Wiles (1968) found that *Diplozoon paradoxum* occurred most often on gill arches 1 and 11 of *Abramis brama*. Hanek and Fernando found that *Urocleidus ferox* showed a preference for anterior sides and medial sections of hemibranchs and for gill arches two and three. In 1982 Shaharom – Harrison studied some aspects of biology of *Cichlidogyrus sclerosus* a monogenea on the gills of the tilapia *Sarotherodon mossambicus*. Shaharom (1985) examined the gills of 928 *S. mossambicus* and also found that the adult monogenea *C. sclerosus* preferred the anterior medial section of the first gill arch. One possible explanation for the site–specificity was the presence of microbranchiospines on the second, third and fourth gill arches but absent on the first gill arch. These microbranchispines trap the larval monogenea passing with the water current but on expulsion of water from the buccal cavity due to the operating of the gill flap the filtered material is blown from its trapped site to the mucus of the buccal cavity. In the case of the first gill arch there are no branchiospines to trap the oncomiracidium and therefore they can subsequently attach themselves to the gill filaments during the passage of the water current over the first gill arch.

LIFE CYCLE OF MONOGENEA

Most monogeneans have a direct life cycle that is no intermediate hosts are involved and most monogeneans are oviparous but a minority the gyrodactylids are viviparous. In oviparous species adult monogenea lay oval eggs equipped with a polar filament at the opercular end. Eggs hatch into oncomiracidium bearing cilia borne on epidermal plates and has gland cells opening at the anterior end and a posterior armed haptor. (Fig. 4) Many species have one or two pairs of eyespots. In most freshwater monogenea the eggs are laid singly with a short polar filament but for some marine monogenea the eggs have elongated appendages which attach the eggs together in groups or to the substrate by means of sticky droplets. In most species the eggs fall to the bottom where they may become attached to the substrate eg *Entobdella solea* and *Acanthocotyle lobianchi.*

The incubation period for monogenean eggs are controlled by temperature. In the tropics the incubation period for *Cichlidogyrus sclerosus* eggs at 25°C is 3-4 days. (Shahrom - Harrison 1984). While that of *Dactylogyrus nobilis* eggs is 3-4 days at 32°C (Shaharom-Harrison, 1986). But in the temperate region the incubation period is longer at lower temperature. Prost (1963) found that development of larvae of *Dactylogyrus anchoratus* eggs lasted 7-8 days at 15-17c. In the egg prior to hatching the embryo moved rapidly and pushed open the operculum. The oncomiracidium larva spiraled out and perform a series of ascents and descents. On contact with the host the epidermal plates are shed and the larva are attached to specific sites on the gills. Where they mature into adults Yoshinaga et al (2002) studied the attachment inducing capacities of fish skin epithelial extracts towards oncomiracidia of *Benedenia seriolae* but his results did not yield any significant specific attraction to the skin extracts. His results differed from Kearn (1968) who showed that there was a strong affinity for skin extracts of the common sole compared to other fish species.

HISTOPATHOLOGY

Histopathological studies have shown that monogenea do cause damage to the fish host (Bullard et al, 2000; Stephens et al 2001) .Gill flukes cause hyperplasia of the epithelium of the primary gill lamellae, hypertrophy of the connective tissue, atrophy of the gill capillaries and atrophy of the secondary gill lamellae. (Lutta, 1941; Oliver, 1977; Stephens et al, 2001; Roubal, 1995). Skin flukes cause erosion of dorsal and tail fins causing slow and stunted growth of fish.

ECONOMIC IMPORTANCE

Best known because of its economic importance are effects of *Nitzschia sturionis* on the spiny sturgeon *Acipenser nudiventris* in the Aral Sea Fish were dying in large numbers due to heavy infestation of this monogenea, the maximum intensity of infection was 600. According to Osmanov (1959) mortalities due to the parasite in the Aral Sea occurred in 1936 and sturgeon fisheries attained no commercial significance for the next 20 years. Pathological effects due to gill monogenea may also depend on water quality. Skinner (1982) compared the histopathological effects of the monogenean *Neodiplectanum wenningeri, Ancyrocephalus* sp and *Ancyrocephalus parvus* on the fish *Gerres cinerus, Lutjanus griseus* and *Strongylura timucu* from 2 habitats in South Biscayne Bay, Florida one heavily polluted with high amounts of ammonia, trace metals and pesticides and the other not polluted. Fish from polluted habitat were heavily infected and showed marked pathological effect such as deflection and adherence of lamellae, clubbing of filaments and obliteration of normal filament structure.

In Malaysia cultured marine fishes have been found to be heavily infected with one or more species of monogeneans (Leong and Wong, 1995). The population (=intensity) of *Pseudorhabdosynochus latesi* infecting seabass is found to be directly related to stocking densities (Balasuriya & Leong, 1994). The large monogenean population causes a lot of stress to seabass cultured in floating cages and is one of the major contributing factors to outbreaks of disease.

POSSIBLE METHODS OF MANAGEMENT

Several chemotherapeutic methods have been applied the latest being Prasiquantel (F.J Stephens et al 2001; Ki & Jae, 2000) but so far not many are effective in eradicating monogenea Stephens et al (2001) found that praziquantal baths at 2 mg li1 were the most safe and effective treatment for the monogenean *Heliotrema abaddon* on the gills of the maricultured dhufish *Glaucosoma hebraicum* in Western Australia. The use of chemicals lead to pollution problems. Hence new methods are now underway for removing monogenea. The prospects for vaccines was discussed by Buchmann (2001). Currently the use of cleaner fish to clean the host of its parasites are being studied. Another possible alternative is to develop a method of attracting the oncomiracidia to a source of attractant where they get permanently attached and the infected source can be removed.

DIGENETIC BLOOD TREMATODES

These helminthes are found inhabiting the blood vessels of the alimentary system, the afferent and efferent blood vessels and the bulbous arteriosus of the definitive host.

These flukes are translucent, lanceolate, dorso-ventrally flattened, devoid of oral and ventral suckers. (Fig. 4 & Fig. 5). Some species are armed with marginal spines on the lateral side of the body and these spines enable the fluke to move and attach to selected habitats. Tegumental spines have been reported by Schell (1974), Thulin (1980), Overstreet & Koie (1989), Kirk & Lewis (1993), Shaharom et al (1983), Herbert et al (1994) and Herbert & Shaharom (1995).

According to Azimov (1972) blood flukes are classified in the family Spirorchidae, Sanguinicolidae, Ornithobilharzidae and Schistosomatidae. The spirorchids are normally found in turtles while the sanguinicolids are mainly found in fish. Smith (1997) have reviewed publications regarding spirorchids and sanguinicolids and concluded that more parasitologists are now searching fish and turtle species for blood flukes and that more species of both sanguinicolid and spirorchid blood flukes remain to be discovered. Overstreet and Koie (1989) also pointed out that most fish species have not been examined for sanguinicolid blood flukes.

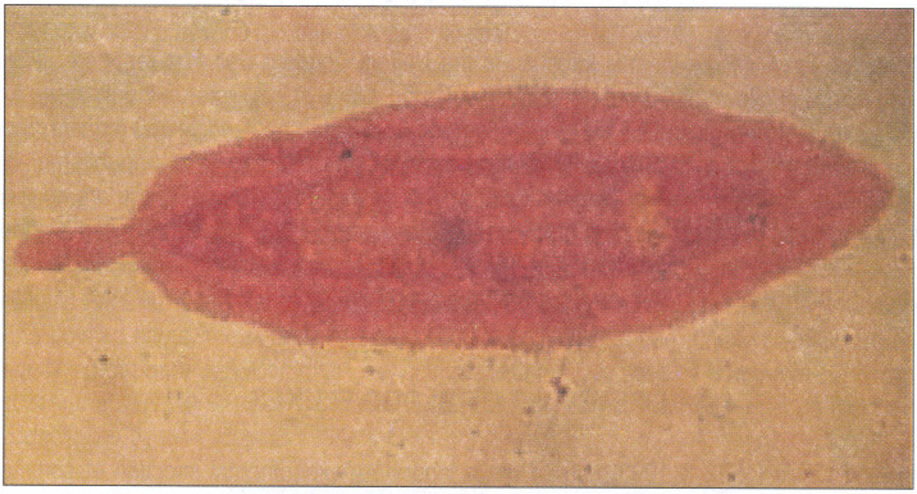


Fig. 4: A possible new species of blood fluke fom bulbous arteriosus of *Channa striatus* (toman) from Tasik Kenyir, Terengganu

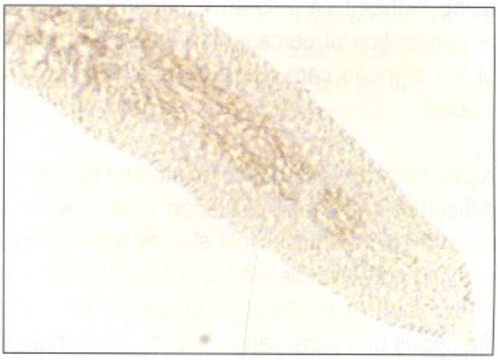


Fig. 5: The blood fluke Cruoricola lates with marginal spines and devoid of suckers

ECOLOGICAL ASPECTS OF INFECTION

An outbreak of *Sanguinicola inermis* infection in the United Kingdom was first reported by Sweeting (1978) and this was followed by further outbreaks covering a total of five water authority areas England. R.S. Kirk & Lewis (1994) described the distribution and host range of *Sanguinicola armata. S. inermis* and *S. volgensis* in British freshwater fish from information collected from unpublished records, published papers, British thesis and information from fish disease consultants. There are no records of *Sanguinicola* species in either Scotland or Wales. *S. inermis* probably entered the United Kingdom with imported carp stocks in the 1950s and 1960s. (Smith, 1997). The first record of *Sanguinicola* infection in Malaysia and South East Asia was made by Anderson and Shaharom – Harrison in 1986 (Smith, 1997).

LIFE–CYCLE OF BLOOD FLUKES

R.S. Kirk and Lewis (1992 & 1993) carried out a laboratory experimental infection of common carp *Cyprinus carpio* with *Sanguinicola inermis* using the snail *Lymnaea peregra* The life-cycle of *Sanguinicola armata* is hardly known and there was difficulty in looking for the intermediate host. This blood fluke was first found in Malaysia in 1983 under the Fish Parasite Malaysia Phase project funded by IDRC. *S. armata* was redescribed in 1995 (Ong B.L. & F. Shaharom-Harrison, 1994, 1995). Although work was carried out to find the intermediate host it was only in 1992 that the intermediate host *Gyraulus convexiusculus* was found. This was carried out by putting infected fish in an aquarium tank in the lab and exposing it to various pond snails. The snails were examined daily till eventually in 1992 we were successful. Once the intermediate host was found then Kua, a masters student carried out the laboratory infection. She followed the method of Kirk and Lewis (1992, 1993) and managed to get sporocysts and cercaria. (Kua B.C., Shaharom-Harrison F.M. & Ali J. 1999 a, 1999b).

But as her experimental infection did not have sufficient replicates hence another student Nor Azila carried out further experimental infections and penetration of miracidium into the fish using histopathological methods. But she had problem getting the host grass carp hence she eventually carried out the research using bighead carp. She managed to look at penetration of cercaria into the epidermis of the bighead carp using histopathological studies.

Fig. 6: The freshwater snail *Gyraulus convexiusculus*

Again her work was not complete and hence difficult to send for publication in an international journal. Life–cycle studies are difficult and challenging. Most parasitologists are interested in carrying out life-cycle work but do not go further because of the difficulties involved. But our team were determined to continue to elucidate life-cycle of blood flukes as there is little information on the life-cycles of marine sanguinicolid and marine spirorchid blood flukes (Smith, 1997).

The marine blood fluke *Cruoricola lates* was first reported in 1988 (Shaharom-Harrison & Anderson, 1988). We knew of its presence through histopathological studies but we did not know how the adult fluke look like. Hence after months of searching for the adult specimen we eventually found it one night at 3 am in the morning in the former laboratory where the present administrative building of KUSTEM now stands. From 1985 to 1998 the quest for the intermediate host was carried out. Studies on this blood fluke from sea bass was eventually carried out by a masters student from Australia Brett Herbert. He found three species of blood flukes in sea bass but only described two (Herbert et al 1994 & Herbert et al 1995). There was no apparent host response to the presence of adults of *Cruoricola lates* in the mesenteries, blood vessels, kidney and pericardial vessels of sea bass (Herbert et al 1995). Scanning electron microscopy of *C. lates* was carried out by Shaharom et al 1993 showing the lanceolate body with a subterminal mouth and pointed snout, the surface has circumferential folds of rugae amongst which are scattered microvillus-like protruberances. Confocal laser scanning microscopy used by Shaharom-Harrison in 1995 revealed that the seminal vesicle of *C. lates* is flask-shaped rather than spherical as reported in the original description by Herbert et al 1994.

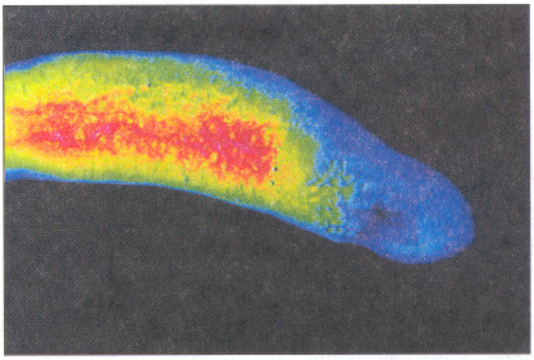


Fig. 7: The blood fluke *Cruoricola lates* under confocal laser scanning microscopy

Meanwhile the search for the intermediate host continued and over the years snails and molluscs were examined but were unsuccessful. We were facing the same predicament as Thulin (1980) who examined about 1500 specimens of mollusk (representing 12 species) and tubiculous polychatete annelids (representing seven species) in Swedish waters for the intermediate host of *Aporocotyle simplex* but was unsuccessful. Eventually in 1996 money was obtained for an intensive study and by 1998 we found the sporocysts in the marine bivalve *Anodontia edantula*. This is also a world first as it is the first time that a marine bivalve was found to harbour the sporocysts of a digenean blood fluke. (Shaharom-Harrison et al 1999). This research won us the Top Ten Award for Innovative Research & Invention from Universiti Putra Malaysia in 2000. Actually despite finding the intermediate host this work still has to undergo electrophoretic techniques for confirmation before it will be accepted by any of the prestigious journals of Parasitology.

This is not surprising as at present the only known life-cycle of a marine blood fluke is that described by Koie (1982a, b & c). She found the apharyngeat furcocercous cercariae in the terebellid polychaete annelid *Artacama proboscidea* and carried out laboratory experimental infections for confirmation. Prof Ogawa of University of Tokyo is still looking for the intermediate host of *Paradeontacyclix* sp.

Currently we have found another blood fluke in the bulbous arteriosus of the toman fish *Channa striatus* from Tasik Kenyir. This is possibly another new species. We are in the process of describing it. We will also have to look for its intermediate host which probably could be a freshwater snail. But we are going to use electrophoretic techniques to work out the life cycle instead of the traditional infection experiment method. This is in line with current requirements for publishing articles on life-cycle studies in Parasitological journals.

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