UNIVERSITI PUTRA MALAYSIA

A SANGUINICOLID BLOOD FLUKE IN SEA BASS (*LATES CALCARIFER* BLOCH) IN COASTAL PENINSULAR MALAYSIA

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By

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LIST OF ABBREVIATIONS

ant. - anterior
c - caeca
ci - cirrus
co - anterior commissure
cp - cirrus pouch
EGC - eosinophilic granular cell
F. - female genital pore
FCR - food conversion ratio
fp - female pore
g - gland cells
M. - male genital pore
m - Mehlis' gland
MMC - melanomacrophage centre
n - nerve canal
o - ovary
oe - oesophagus
ov - oviduct
oo - ootype
PER - protein efficiency ratio
post - posterior
s - sperm
sd - sperm duct
sp - spine
sv - seminal vesicle
t - testis
u - uterus
ue - uterine egg
μ - micrometre
v - vitellaria
vd - vitelline duct
vr - vitelline reservoir

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the degree of Master of Science.

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June 1992

Chairman: Dr. Faizah Shaharom

Faculty: Fisheries and Marine Science

Cruoricola lates N. Gen., N. Sp. from the blood vessels of cultured sea bass (*Lates calcarifer* Bloch 1790) is described. It is a lanceolate sanguinicolid with a single column of submarginal, ventral spines; extensive vitellarium; and X-shaped intestine. The single, lobed testis extends laterally to the caeca. The cirrus lies dorsal to the spherical seminal vesicle. Auxiliary seminal vesicle present. The uterus is post-ovarian, partly situated between the lobes of the ovary; mid-portion is thick. *Cruoricola lates* N. Gen., N. Sp. is found in all sea bass over 15g weight in the type locality, Pulau Ketam.

The adults of *C. lates* primarily inhabit the venous circulation of *Lates calcarifer*. Eggs were found in the kidney, liver, ventricle of the heart and gills of all fish examined three months after stocking.

Cruoricola lates eggs in tissues evoke a cellular immune response consisting of encapsulation by either activated macrophages and/or endothelial cells. In the heart this is accompanied by macrophage infiltration. In the kidneys, encapsulation of eggs is followed by pigment deposition in and around the capsule. The main foci of pathological effect are the pancreatic acinar tissue, head kidney, and intertubular caudal kidney tissue. *Cruoricola lates* egg deposition in these tissues may have a negative effect on growth through reduction in food conversion ratio and depression of immunological capability.

Haematological parameters (haematocrit, serum protein, plasma protein) were so variable that no relationship between them and infection with blood flukes could be described.

Cruoricola lates was present in sea bass culture sites sampled in Penang, Johore, Pahang and Terengganu. Kelantan sites appeared not to have high incidence of infection, probably due to the freshwater influence. Intensity and prevalence of infection appear to increase with intensity of culture.

Histological and dissection techniques are complementary in giving a comprehensive picture of the location of worms and eggs in the host. As *Cruoricola lates* is readily available, and as sea bass are easily maintained under laboratory conditions, there is wide scope for further studies on this worm and its relationship with the host.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia, sebagai memenuhi sebahagian daripada keperluan untuk mendapat Ijazah Master Sains.

FLUK DARAH SANGUINICOLID DALAM IKAN SIAKAP (*LATES CALCARIFER*) DI PANTAI SEMENANJUNG MALAYSIA

oleh

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Jun 1992

Pengerusi: Dr. Faizah Shaharom

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Morfologi *Cruoricola lates* N. Gen., N. Sp. daripada saluran darah ikan siakap ternakan (*Lates calcarifer* Bloch 1790) dihuraikan. Ia adalah fluk darah sanguinicolid yang berbentuk daun dengan satu deretan spina bawah pinggiran di bahagian ventral, vitelarium yang luas dan usus berbentuk silang. Testis tunggal berlobus memanjang kebahagian sisi sekum. Sirus memanjang di bahagian dorsal vesikel sperma yang berbentuk sfera. Terdapat vesikel sperma auksiliari. Uterus terdapat di bahagian belakang ovari dan sebahagiannya terletak antara lobus ovari; bahagian tengah uterus adalah tebal. *Cruoricola lates* dijumpai dalam semua ikan siakap yang beratnya melebihi 15 grám di lokasi tertentu iaitu Pulau Ketam, Selangor. *Cruoricola*

lates yang matang tinggal di dalam peredaran vena *Lates calcarifer*. Telur dijumpai di dalam ginjal, hati, ventrikel jantung dan insang pada semua ikan yang diperiksa tiga bulan selepas pelepasan. Telur *C. lates* dalam tisu merangsangkan tindakan imun selular iaitu termasuk pengkapsulan sama ada oleh makrofaj teraktif dan/atau sel endotelium. Di dalam jantung tindakan ini disertai dengan penyusupan makrofaj. Di dalam ginjal pengkapsulan telur diikuti oleh endapan pigmen di dalam dan di sekeliling kapsul. Kesan patologi terutamanya terdapat di tisu asinar pankreas, kepala antara tubul ginjal dan tisu ginjal kauda. Ada kemungkinan telur *C. lates* di dalam tisu ini mempunyai kesan negatif ke atas pertumbuhan *L. calcarifer* melalui pengurangan dalam nisbah pertukaran makanan dan penurunan keupayaan keimunan. Parameter hematologi (hematokrit, protein serum, protein plasma) sangat berbeza hingga perhubungan antara parameter ini dan jangkitan fluk darah tidak dapat dihuraikan.

Cruoricola lates terdapat di dalam ternakan ikan siakap yang disampel dari Pulau Pinang, Johor, Pahang dan Terengganu. Kawasan di Kelantan tidak mempunyai insiden jangkitan yang tinggi mungkin disebabkan oleh pengaruh

air tawar. Keamatan jangkitan meningkat dengan peningkatan keamatan kultur ikan. Teknik histologi dan pembedahan adalah saling membantu dalam membuat gambaran menyeluruh tentang lokasi cacing dan telur dalam perumah. Oleh kerana *C. lates* mudah diperolehi dan ikan siakap mudah disimpan di dalam makmal maka terdapat skop yang besar bagi kajian lanjut tentang cacing ini dan perhubungannya dengan perumah.

CHAPTER I

INTRODUCTION

Foreword

Digenetic trematodes of the family Sanguinicolidae are parasites which inhabit the circulatory system of marine and freshwater fishes. They have been found in the blood vessels, lymphatic system and cardiac tissues of a variety of elasmobranchs and teleosts. Very little is known about their presence in fish from the South-East Asian region.

Sanguinicolids are the only digeneans in which adults are of economic importance in fish culture (Smith, 1972; Bauer *et al.*, 1973). Most adult digeneans live in organs which have direct connection to the outside, allowing eggs to pass out with minimal or no effect on the host. As blood flukes live and reproduce within the circulatory system, their presence may cause pathological changes in host tissues in which the parasites, or their eggs, have become lodged. Reports of blood flukes in cultured fishes are few, probably because they are difficult to find and are rarely looked for. The genus *Sanguinicola* (=*Plehniella*) has only been recorded in freshwater

fishes, while all other genera of the family have been reported in marine fishes (Short, 1954).

Sanguinicolids

Introduction

Fish blood flukes were first described from a marine fish in 1900 (Odhner, 1900) and from freshwater fish in 1905 (Plehn, 1905). *Sanguinicola* spp. were only recognised as digeneans later (Odhner, 1911), probably due to their unusual characteristic of having no suckers, which are a prominent morphological trait of most other digeneans. To date, the family Sanguinicolidae consists of eighteen genera (Overstreet and Koie, 1989). For the purposes of this discussion, "sanguinicolids" refers to members of the families Sanguinicolidae and Aporocotilidae of Yamaguti (1958, 1971), unless otherwise stated.

Characteristics of Sanguinicolids

Few sanguinicolid life cycles have been elucidated and all, with the exception of *Aporocotyle simplex* (a marine blood fluke), are members of the genus *Sanguinicola* Plehn 1905. *Sanguinicola* spp. are exclusively freshwater (Short, 1954; Yamaguti,1958). Characters used in taxonomy of digeneans often include the characteristics of larval forms, particularly ciliated plate patterns of miracidia and excretory systems in cercariae, as these

may indicate phylogenetic relationships (Chandler and Read, 1961). Due to the lack of known life cycles, most descriptions of marine sanguinicolids are only from adult forms.

The habitat within the host is one of the diagnostic features of the sanguinicolids. As adults, they live either in the lymphatic or blood circulatory system or in the coelom of fish. Most species are found in the blood. Two species (*Deontacylix ovalis* and *Plehniella* (=*Sanguinicola*) *coelomicola* inhabit the coelom (Manter, 1947; Szidat, 1951). Koie (1982) found *Aporocotyle simplex* in the lymphatic and blood circulatory systems. *Sanguinicola inermis* may develop in the skin of their carp hosts (Sommerville and Iqbal, 1991). The only record of a sanguinicolid (*Plehniella* (=*Sanguinicola*) *dentata*) within the lumen of the intestine of a fish (Paperna, 1964 a, b) is open to question, as upon opening the intestine blood vessels will be broken, allowing sanguinicolids in them to escape.

The distinguishing morphological features of sanguinicolids, and the principal feature that gives them familial status, is the absence of a pharynx and lack of well developed suckers. All other features of the family as proposed by von Graff (1907) are absent in one or more members (Table 1). Of these, important features present

Table 1. Selected Characteristics of Marine Sanguinicolid Trematodes.

Abbreviations are as follows: ant.- anterior; post.- posterior; F- female genital pore; M- male genital pore; CP- cirrus pouch; Y- cirrus/cirrus pouch present; N- cirrus/cirrus pouch absent; V-L - ventrolateral.

Genus and Source	Uterus	Genital pores	Position of genital pore	Testes number	Testes position	Ovary position	Intestine	Armature	CP	Cirrus
Aporocotyle (Yamaguti,1958: Thulin,1980)	preovarian	common genital atrium	median, sinistral	many	intercaecal	intercaecal preuterine	H-shaped	minute, clumped spines	Y	Y
Cardicola (Short,1953; Yamaguti,1971)	postovarian	separate	M. post.&lat. to F.; sinistral; postovarian	1	intercaecal	mid-postcaecal area; level with or preuterine	H-shaped	transverse rows of small V-L spines	N[1]	Y or N
Chimaerohemecus (van der Land,1967)	postovarian	separate	M.dorsal, sinistral. F.ant. to M.	1	intercaecal	intercaecal pre-uterine	bifurcated	ventro-lateral margins	Y	Y
Deontacylix (Manter,1947; Yamaguti,1971)	extends preovarian	separate	M. post. to F. Both right of median	1	between nerve trunks	postcaecal; sinistral	H-shaped with diverticules	dorsal and lateral		N
Hyperandrotrema (Maillard and Ktari,1978)	postovarian	separate	M. post. to F. Both on left side	1	intercaecal	most intercaecal, pre-uterine, post-testicular	bifurcated	lateral	N	Y
Metaplehniella (Lebedev & Parukhin,1972)	postovarian	separate	M. post. to F. F.median, M. on left	1	mostly postcaecal	postcaecal;media n pre-uterine	H-shaped	fine; marginal; paired		N

Genus and Source	Uterus	Genital pores	Position of genital pore	Testes number	Testes position	Ovary position	Intestine	Armature	CP	Cirrus
Neoparacardicola (Yamaguti, 1970; 1971)	extends preovarian	separate	M. post. to F. at marginal notch	2	ant. intercaecal post. postcaecal	postcaecal; between ant. margin of post. testis and right side of body	X-shaped with diverticules	two V-L rows	Y	Y
Orchispirium (Madhavi and Rao, 1970)	extends preovarian	common	submedian postovarian	1	intercaecal transversely coiled tube	postcaecal; post-testicular; right of median; mostly pre-uterine	bifurcated	? possibly lost	Y	Y
Paracardicola (Martin, 1960)	postovarian	separate but close	near mid seminal vesicle level	2	postcaecal; preovarian and postovarian	postcaecal preuterine	H-shaped	laterally spined		N
Paracardicoloides (Martin, 1974)	preovarian	common	preovarian, on right side	2	postcaecal; in hindbody	postuterine; postcaecal, between testes	bifurcated	V-L band sheathed in tegument	Y	Y
Paradeontacylix (McIntosh, 1934; Ogawa & Egusa, 1986)	postovarian	separate	F. median; M. sinistral and post. to F.	many	2 median intercaecal rows	inter- or postcaecal; preuterine	H-shaped	V-L rows of spines; rose thorn hooks posterior	Y[2]	Y or N
Pearsonellum (Overstreet & Koie, 1989)	extends preovarian	separate	M. dorsal sinistral; post. to F.; F. anteromedial.	1	mostly intercaecal	postcaecal; post-testicular; median	H-shaped	V-L transverse rows	Y	Y
Plethorchis (Martin, 1975)	preovarian	common	near posterior on left	>100	postcaecal	post uterine postcaecal	bifurcated	lateral clusters of 3-4	Y	Y
Psettarium (Goto & Ozaki, 1929; 1930)	postovarian	separate	F. median; M. post. to and sinistral to F.	1 (?) diffuse	not clear, extends postovarian	postcaecal, laterally bound by testis; dextral	H-shaped	ventro-lateral transverse rows	Y	Y

Table 1 (Continued)

Genus and Source	Uterus	Genital pores	Position of genital pore	Testes number	Testes position	Ovary position	Intestine	Armature	CP	Cirrus
Psettaroides (Lebedev & Parukhin, 1972)	postovarian	separate	M. lateral on right; F. median & ant.	1	preovarian	postcaecal; posttesticular; median	H-shaped with diverticules	V-L transverse rows	?	Y
Psuedocardicola (Parukhin, 1985)	Extends preovarian	separate	F. sub-median & ant. to marginal M.	5	intercaecal preovarian	postcaecal, sinistral	X-shaped	?	Y	Y
Selachohemecus (Short, 1954)	postovarian	common	median	1	preovarian; postcaecal	pre-uterine; posttesticular; median	four very short caeca (=X)	V-L margin single spines		N
Cruoricola	postovarian	separate	sinistral, M. sinistral & lateral. to F.	1	preovarian postcaecal	postcaecal posttesticular width of body	H-shaped	V-L single row	Y	Y

Table 1 (Continued)

1. Except *C. congruenta* Lebedev and Mamaev, 1968 appears to have a cirrus pouch.
2. If cirrate.

in most genera are the X- or H-shaped intestine with short anterior limbs, median ovary or ovaries, lack of Laurer's canal, and Y-shaped excretory vesicle. Hooks and/or spines, although not mentioned by von Graff, have been found in most sanguinicolids described to date.

Life Cycle

All blood flukes have a simple, two host life cycle (Koie, 1982). The life cycle of aquatic sanguinicolids usually involves a mollusc, the intermediate host where cercariae develop, and a fish, which is the definitive host. Most of the sanguinicolid life cycles elucidated to date belong to the genus *Sanguinicola*. The life cycle of only one marine blood fluke, *Aporocotyle simplex* Odhner 1900, has been determined (Koie, 1982).

All sanguinicolids produce eggs, which most often hatch within the gill filaments. The eggs may or may not be operculate. In *Sanguinicola* spp. the miracidia break through the gill epithelium to the outside (Bauer *et al.*, 1973; Evans 1974a; Anderson and Shaharom-Harrison, 1986). Presumably, most sanguinicolid eggs develop in the host and the miracidia actively escape, although in some cases the eggs escape from damaged gill filaments before hatching (Grabda, 1991). One sanguinicolid, *Chimaerohemecus trondheimensis*, is unique in that 9-20 miracidia develop inside one egg (Thulin, 1982).

If the miracidium locates a suitable host it bores in, and after two generations of sporocysts, cercariae are produced. No