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A new blood fluke, *Parasanguinicola vastispina* gen. nov., sp. nov. (Trematoda: Sanguinicolidae), from sea bass *Lates calcarifer* (Centropomidae) cultured in Malaysia

Abstract A new sanguinicolid blood fluke, *Parasanguinicola vastispina*, is described from sea bass *Lates calcarifer* cultured in Malaysia. It is distinguished by its massive armature and widely spaced genital pores, the female pore being pre-ovarian. *P. vastispina* inhabits the branchial arteries, dorsal aorta, mesenteric venules and renal artery of its host. No pathological effect was observed in infected fish.

10% neutral buffered formalin. Tissues in saline were examined for sanguinicolids for up to 4 h after removal of the tissues from the fish. Additionally, histology studies were conducted to observe the parasites in situ. The heart and ventral aorta, one gill, one eye, the cardiac stomach, a pyloric caecum, the rectum and excretory bladder, part of the liver, the head and caudal kidney and the spleen were fixed in cold buffered neutral formalin, processed and sectioned using standard methods, and stained with haematoxylin and eosin. Specimens were prepared for electron microscopy using the methods described by Køie (1982).

Introduction

During studies on the blood fluke *Cruoricola lates* in cultured sea bass *Lates calcarifer* (Bloch, 1790) (Centropomidae) in Malaysia (Herbert et al. 1994), a second, previously undescribed blood fluke was found. Although sea bass have been subject to numerous parasitological investigations in this region, this new genus of blood fluke has never been recorded from sea bass. The present report describes this new blood fluke and discusses the relationship of this parasite with its host and in relation to *C. lates*.

Materials and methods

Sea bass were collected from various localities (Fig. 1) and examined for blood flukes by removal of the heart and ventral aorta, gills, dorsal aorta, kidneys, liver, and viscera to separate petri dishes filled with normal saline or modified Cortland saline (Schreck and Moyle 1990). All organs except the gills were teased apart, and the blood vessels were torn apart. The branchial arteries were removed by cutting open the gill arch and removing the arteries, which were then slit open. Sanguinicolids were removed as they emerged from blood vessels and were immediately fixed in hot
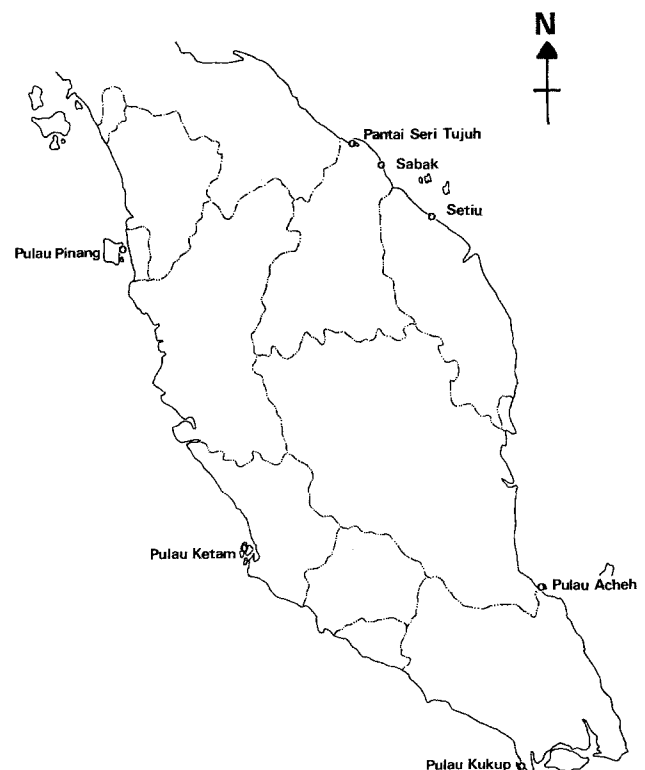
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Fig. 1 Sea-bass culture sites sampled for *Parasanguinicola vastispina* in peninsular Malaysia

Results

Morphology

Genus: *Parasanguinicola* gen. nov.
Type species: *P. vastispina*
Taxonomy: *P. vastispina* sp. nov.

Etymology

Parasanguinicola: from *Para* (like, similar) and *Sanguinicola* (a genus of freshwater blood fluke), meaning similar to *Sanguinicola; vastispina:* from *vasti* (large) and *spina* (spine), due to the large marginal spines.

Description

The description is based on the holotype, three paratypes, and sections of four specimens (Figs. 2, 3). Measurements were obtained from 16 mounted specimens; ranges are given in micrometers.

The body is flat and spatulate. The anterior end may have an oral cone; the posterior end is indented and spined laterally. The length is 520 (319–714) μm and the width, 95 (55–149) μm, is nearly uniform along most of the body. Spines are single, ventro-laterally directed, equally sized and deeply embedded in the integument. The distal portion of the spine protrudes from the integument margin. The spines are 11–12 μm long and are separated by a distance of 3–5 μm with 85–90 spines occurring along each side of the body. Nerve cords are conspicuous and broad; the anterior commissure is located 86 (48–153) μm from the anterior tip. The mouth is slightly sub-terminal, appearing in an eversible oral cone measuring 5–7 in length and in width at 2–3 μm from the anterior end. The oesophagus is slightly sinuous, is 164 (113–218) μm long and is dilated about 47 (16–62) μm from the mouth, the dilated portion being anterior to the nerve commissure and surrounded by a conspicuous band of gland cells; it runs ventrally to the anterior nerve commissure. The oesophageal bulb lies immediately anterior to the intestine, measures 10 (9–13) μm in diameter and appears to be thick-walled. The intestine is usually X-shaped (often irregular, with five lobes occurring in some specimens); all caeca are short and obscured by the vitellarium.

The testis is elongate, post-intestinal and equatorial (or nearly so), is 106 (47–134) μm long and 28 (13–35) μm wide and lies between the nerve cords. It is about 4 times longer than it is wide; the lateral margins are fairly straight, the anterior margin is rounded and the posterior margin tapers into the sperm duct. The sperm duct extends posteriorly and dextrally and is usually grossly distended with sperm. A thin-walled cirrus pouch surrounds the terminal part of the sperm duct, is 28 (18–29) μm long and may be surrounded by a 2- to 5-cell-thick band
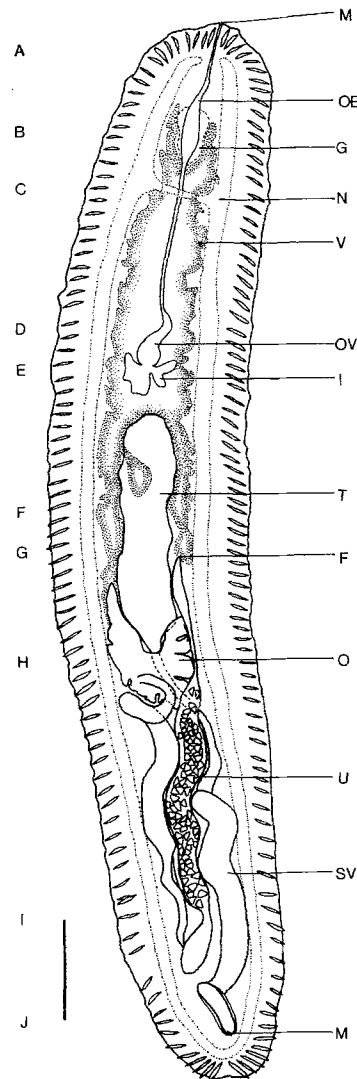

Fig. 2 *P. vastispina* gen. nov., sp. nov. holotype, dorsal view (*M* Mouth, *OE* oesophagus, *G* gland cells, *N* nerve canal, *V* vittellarium, *OV* oesophageal vesicle, *I* intestine, *T* testis, *F* female pore, *O* ovary, *U* uterus, *SV* seminal vesicle, *M* male pore). Bar= 50 μm

of prostatic cells. The male genital pore is located dorsally, medially, at a distance of 23 (16–27) μm from the posterior end. A cirrus is absent.

The ovary is dendritic, being 35 (18–57) μm long and 26 (18–37) μm wide, and lies adjacent to the posterior part of the testis, ventrally to the sperm duct, in the third quarter of the body. The oviduct exits the ovary dorsally at the mid-level and is sinistral; it turns down to the median posterior border of the ovary, joins with the vitelline duct immediately anterior to the oötype and is usually packed full with spermatozoa, serving as a seminal receptacle. The oötype is relatively large, being 11 μm wide and 18 (16–27) μm long; it appears thick-walled, often accommodating 10–20 developing ova. Mehlis' gland is inconspicuous. The uterus is 195 (154–284) μm long, occupying about one-third of the body length. It extends anterio-medially in a straight path; immediately

Fig. 3A–J Sections of *P. vasti-spina* gen. nov., sp. nov. Relate levels to Fig. 1. **A** Oesophagus near the anterior end. **B** Dilated and thickened part of the oesophagus surrounded by gland cells. **C** Oesophagus and vitelline duct posterior to the anterior nerve commissure. **D** Oesophageal bulb. **E** Gut caeca. **F** Testis. **G** Testis and female pore. **H** Ovary and sperm duct. **I** Sperm duct, oviduct, vitelline duct and uterus. **J** Cirrus pouch and ejaculatory duct. Bar=20 μm
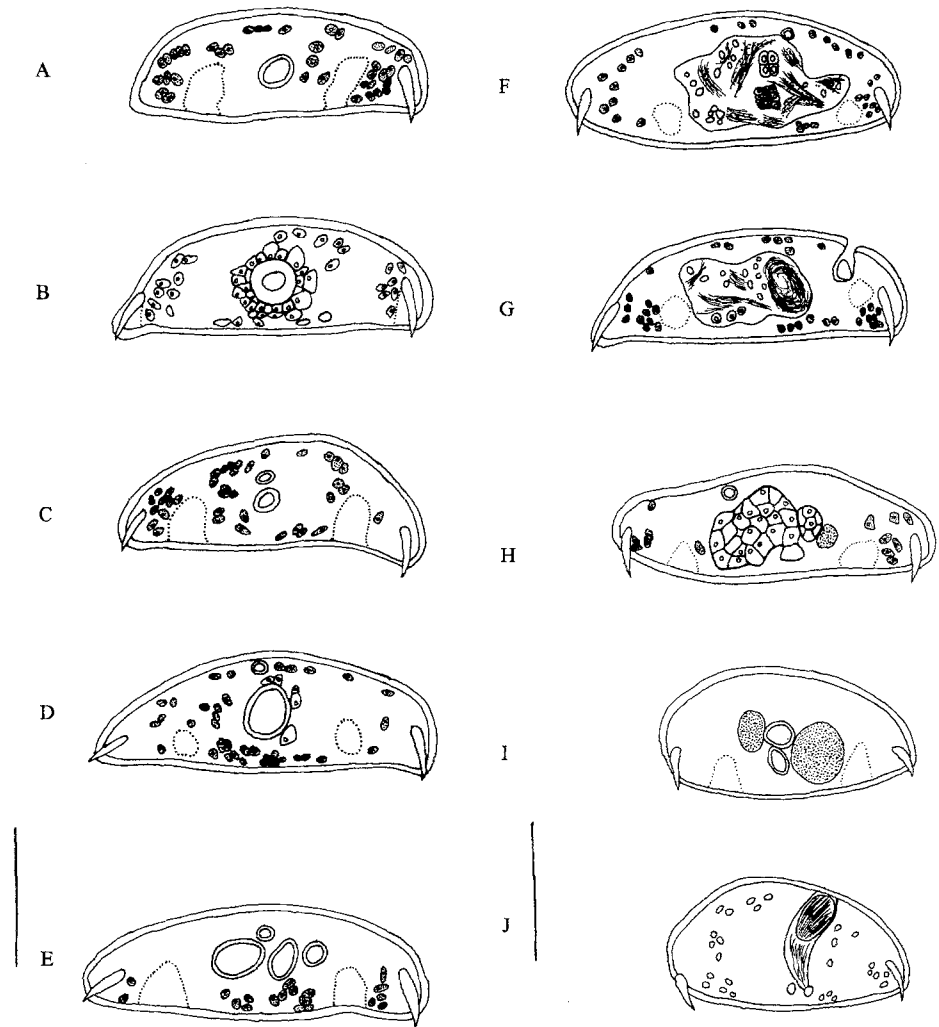
posterior to the ovary it turns to the right and extends adjacent to the ovary and testis. The female genital pore is located dorsally at 27 (18–40) μm from the dextral margin of the worm and is equatorial or slightly post-equatorial. The eggs are small, triangular in profile, measure 11–12 μm along the sides and are thin-shelled, non-quinone-tanned and anoperculate. The vitellarium is extensive and non-follicular, lies between the nerve cords, extends anteriorly to the anterior commissure and posteriorly to the ovary and is dorsal and ventral to the organs in between. The vitelline duct is single and lies ventral to the uterus and oviduct. The excretory vesicle is Y-shaped, small and sub-terminal and has inconspicuous arms; the stem is 5–7 μm long and 3–4 μm wide, and the pore is located 1–2 μm from the end of the body.

The body of *P. vastispina* is covered with very fine cilium-like structures. These are less than 1 μm long and are distributed all over the body. The mouth of *P. vastispina* is small, surrounded by a sphincter-like band of muscle and marginally sub-terminal when the oral cone is everted. The anterior part of the oesophagus measures 1–1.5 μm in diameter. Sea bass erythrocytes are 5–6 μm in diameter.

Generic diagnosis

The characters are those of the family Sanguinicolidae von Graff, 1907. The body is spatulate and spined marginally with partly embedded spines. A pharynx and an oral sucker are absent; the oesophagus is relatively long and swollen in the anterior part. The intestine has an irregular shape, all caeca being short; the oesophageal vesicle is located immediately anterior to the intestine. The testis is single and post-caecal, being situated between the intestine and the ovary; a cirrus sac is present but a cirrus is absent; the male genital pore is located close to the posterior extremity. The ovary is post-testicular, single and dendritic, lying posterior to the female pore; the vitellarium extends from the anterior nerve commissure to the level of the ovary. The excretory vesicle is Y-shaped and small. This organism is parasitic in the vascular system of teleosts.

Differential diagnosis

The spines are well developed and partly embedded, protruding ventro-marginally. The intestinal shape is irregu-

lar and all lobes are short. An oesophageal vesicle is present. The testis is single and post-intestinal, a cirrus is absent and the male genital pore is located in the posterior part of the body. The ovary is post-testicular; the uterus extends pre-ovarially and the female genital pore is pre-ovarian.

Material deposited: Holotype (Fig. 2) from the branchial artery of *Lates calcarifer* Bloch 1790, Pulau Ketam, B. Herbert, National University of Singapore Zoological Reference Collection ZRC.1993.6875
Paratypes: ZRC.1993.6876-7
Queensland Museum G211242-4
Meguro Parasitological Museum 19594, 19595

Localities

Type locality: Pulau Ketam, Selangor, West Malaysia
Other Localities: Pantai Seri Tujuh, Kelantan; Setiu, Terengganu; Pulau Kukup, Johore; Jelutong, Penang
Hosts: Type host, *L. calcarifer* (Bloch 1790)
Sites in host: branchial arteries, dorsal aorta, mesenteric venules

Observations

Parasites were found in the branchial arteries, dorsal aorta, mesenteric venules and renal artery of *L. calcarifer*. In the type locality the prevalence was 50% and the intensity, 2.5 (*n*=110). In other localities, infection rates were as follows: Jelutong, Penang, 10/11; Setiu, Terengganu, 2/11; Pulau Kukup, Johore, 2/10; and Pantai Seri Tujuh, Kelantan, 1/7. Most infections involved one or two worms; only a couple of fish had more than ten worms. Most worms were found in the branchial arteries (71.2%) or dorsal aorta (49.1%), although some were found in the mesenteric venules (22%) or renal artery (3.4%). Pairs of worms were most frequently found in the branchial arteries or dorsal aorta; in the mesenteric venules, single individuals were most commonly encountered. The maximum number of worms encountered in a single fish was 14, with 10 being found in the branchial arteries and 4 in the dorsal aorta. Worms were not found in fish from Pulau Acheh, Pahang (*n*=17) or Hinchinbrook Channel, Australia (*n*=6).

Discussion

Parasanguinicola vastispina was not abundant in any of the sites from which sea bass were collected, two or four specimens being found in most fish. It was found only in fish over 20 cm long and 100 g in weight. *P. vastispina* was distributed throughout sites in Malaysia, but small numbers were found at Kelantan, Terengganu and Johore. They were relatively abundant at Selangor and Penang at the times fish were sampled. As this parasite was uncommon in intensively cultured fish, it is probable that

it is very rare in wild fish. *P. vastispina* is very small and, thus, easily overlooked. It appears that once it escapes the blood vessels it is susceptible to rapid degeneration, as many individuals had large internal vacuolations in the anterior half of the body. These vacuolations, which were present in most specimens, appeared to displace the vitelline cells and obscured other structures.

The presumed dorso-ventral muscle fibres previously observed in *Cruoricola lates* (Herbert et al. 1994), *Pearsonellum corventum* (Overstreet and Køie 1989), and other sanguinicolids were observed in the testis and ovary of *P. vastispina* but were not as prominent as they are in other species. As this worm is small and narrow, perhaps these structures do not need to be as large if they serve to maintain its shape. *P. vastispina* swims with vigorous dorsal and ventral contractions of the whole body, which in saline does not result in any noticeable forward movement. The worm moves along flat surfaces by peristaltic movements.

In the blood vessels the spines of *P. vastispina* push into the endothelial cell walls but apparently do not penetrate them. The branchial arteries and dorsal aorta are among the largest vessels in fish, and it is probable that the well-developed armature is necessary to resist the strong blood flow in these areas. No *P. vastispina* was ever found associated with the heart or ventral aorta.

Parasanguinicola eggs were rarely observed in histological sections or gill scrapes. Possibly, the small egg size and the heavy infection of renal and hepatopancreatic tissue with *C. lates* eggs precluded observation of *P. vastispina* eggs. Also, the small number of worms in fish would have limited the chances of seeing eggs in sections. As the eggs of *P. vastispina* are only slightly larger than sea bass erythrocytes, eggs should be capable of passing through all but the smallest capillaries. However, some eggs should lodge in the vascular organs, particularly the kidney and spleen. None were ever observed in any of these organs in the 35 infected fish histologically examined. In one fish, degenerated (presumed) *P. vastispina* eggs were found in the rete mirabile.

The ootype of *P. vastispina* appears capable of processing numerous eggs simultaneously as evidenced by the numbers of eggs inside it and by its large size relative to the eggs. The eggs of *Sanguinicola inermis* Plehn, 1905 and *S. armata* Plehn, 1905 are triangular in profile, similar to those of *Parasanguinicola*, and are also anoperculate. Eggs of *S. inermis* are 40 µm long in utero, and those of *S. armata* measure 44×22 µm after they have been laid (Scheuring 1920; Anderson and Shaharom-Harrison 1986). Eggs of some sanguinicolids appear to enlarge after being laid. The eggs of *Parasanguinicola* are much smaller than those of *S. inermis* and *S. armata*, which probably explains why they are not easily seen in the gills or other tissues of the host.

Cruoricola is the more abundant of the two blood flukes found in sea bass cultured at Pulau Ketam. It is found in 100% of sea bass over 90 in length mm (10 g), and the average intensity is 17, although this varies with

age and, possibly, season. *Parasanguinicola* is much less common, being found in 50% of fish in the same size range, with an average intensity per fish of 2.5. The site preferences of these two worm genera differ but are not mutually exclusive. About half of gill infections were concurrent. In the dorsal aorta, which was less commonly infected by *Cruoricola,* about 40% of infections were concurrent. *Parasanguinicola* was much less frequently found in the mesenteric venules.

Different modes of attachment are probably the reason for the different site preferences. *Cruoricola* appears to have a weaker attachment mechanism than *Parasanguinicola. Cruoricola* possesses small, fine spines, and *Parasanguinicola* has massive spines. Presumably this would give greater purchase in the branchial arteries and the dorsal aorta. Vessel size may be a factor, as the few *Parasanguinicola* found in the mesenteric venules were always from the larger vessels, whereas *Cruoricola* invariably emerged from small vessels.

Holmes (1971) observed spatial separation of *Psettarium sebastodorum* Holmes 1971 and *Aporocotyle macfarlani* Holmes, 1971 in rockfish *Sebastes* spp. He attributed the restriction of *Aporocotyle* to the heart and that of *Psettarium* to the ventral aorta and afferent branchial arteries to the different modes of attachment. *Aporocotyle* was more suited to the heart, where it could thread itself through the intertrabecular spaces. *Psettarium* tended to drift to the more distal gill arteries, where its lateral spines could wedge into the arterial walls. Both *Aporocotyle* and *Psettarium* inhabited sites different from those occupied by *Cruoricola* and *Parasanguinicola.* Blood flukes were never found in the heart or ventral aorta of sea bass in Malaysia, although *Cruoricola* was found in the pericardial vessels. Possibly ventrolateral rows of spines, or clusters of spines, are necessary for attachment in those areas.

Presumably *P. vastispina* feeds on serum, as its mouth and oesophagus appear to be too small to ingest sea bass erythrocytes. Several other sanguinicolids live either in the coelom or in the lymph for at least part of their life (Linton 1915; Koie 1982; Sommerville and Iqbal 1991). This suggests that erythrocytes are not an essential part of the diet in all sanguinicolids. The abbreviated gut suggests that some of the nutritional requirements of *P. vastispina* may be met by absorption across the tegument. Possibly, the cilium-like structures that are abundant on the surface of *P. vastispina* serve to enlarge the surface area and facilitate absorption of food.

Only two sanguinicolid members, namely, *Selachohemecus olsenii* Short, 1954 and *Sanguinicola spp.,* have an abbreviated intestine composed of short caeca similar to those in *Parasanguinicola.* The intestine of *P. vastispina* is obscured by the vitellarium but appears to be X-shaped and composed of short lobes, which may be quite broad. *Selachohemecus* has four short, thick-walled caeca, two of which are directed antero-laterally and two, posteriorly. The caeca of *P. vastispina* are different in that they are not always regular in shape and are not as thick-walled. *Selachohemecus* also has a widening of the

oesophagus, but this is not surrounded by gland cells and is located posterior to the anterior commissure. *Sanguinicola* spp. normally have an intestine with four short caeca, although *S. armata* Plehn, 1905 has five intestinal caeca. Similar to *S. armata, P. vastispina* has a widening of the oesophagus anterior to the anterior nerve commissure. *Parasanguinicola* differs from *S. armata* in having a continuous distribution of spines around the body, a prominent oesophageal vesicle and a different reproductive-system morphology. An oesophageal vesicle is found in only one other sanguinicolid, *Orchispirium heterovitellatum* Mahdavi and Rao, 1970, which differs from *Parasanguinicola* in having a bifurcated gut, a transversely coiled testis and a common genital pore.

More than half of the 19 described genera of sanguinicolids have a single testis. In most of these genera it is centrally placed, as it is in *P. vastispina.* Only three of these are acirrate (*Deontacylix, Metaplehniella* and *Selachohemecus*). *Cardicola* also has some members that are acirrate. *Deontacylix* and *Metaplehniella* have a testis that ramifies or is dendritic, respectively, differing from the entire testis of *P. vastispina.* The male system of *Selachohemecus* is similar to that of *Parasanguinicola* in that the sperm duct is enlarged to form a seminal vesicle and the testis tapers into the sperm duct. However, it differs in the absence of a cirrus pouch and of separate genital pores.

The ovary of *Parasanguinicola* is dendritic, although this is not apparent in all specimens. The ovary is always medial and immediately posterior to the testis in common with that of most sanguinicolids. The uterus of *Parasanguinicola* extends anteriorly to the ovary, and the female genital pore opens anteriorly to the ovary. *Parasanguinicola* is unique among sanguinicolids in having a pre-ovarian female pore and a post-ovarian male pore. *Sanguinicola (Plehniella) dentata* (Paperna, 1964) also has widely separated genital pores. Yamaguti (1971) synonomised *Plehniella* Szidat, 1951 with *Sanguinicola* and believed that Paperna had misinterpreted the position of the female genital pore of *S. dentata.* Whatever the interpretation, the female genital pore of *S. dentata* is post-ovarian.

Parasanguinicola appears to be related to *Selachohemecus* as it shows similarities in the seminal vesicle/sperm duct and intestine. However, *Parasanguinicola* exhibits differences in the armature, position of the ootype, shape and length of the oviduct, presence of an oesophageal vesicle and position of the genital pores. *Selachohemecus* is parasitic in sharks, whereas *Parasanguinicola* parasitises a teleost.

Parasanguinicola bears some similarities to *S. armata* and *S. rutili* Simon-Martin, Rojo-Vazquez and Simon-Vicente, 1987, in its large spines, oesophageal dilation, irregularly shaped intestine with short caeca, and morphology and in the presence of a cirrus pouch but differs in its reproductive-system morphology, particularly its single testis and widely separated genital pores. *Parasanguinicola* has spines distributed continuously around the margins, whereas *S. inermis* and *S. rutili* do not have

spines at the anterior and posterior extremities. Also, *Sanguinicola* spp. are parasites only of freshwater fishes, whereas *Lates calcarifer* is a euryhaline species, being cultured in Malaysia in estuaries. The type locality and several other localities had a fairly constant salinity of 31–33 ppt, although *P. vastispina* was present in localities with low salinity, tending to fresh water in the wet season. *P. vastispina* was far more common in areas where the entire life cycle would have to be completed in seawater.

The sole apomorph of *P. vastispina* is the widely separated genital pores and pre-ovarian female genital pore, which clearly distinguish it from all other genera. Some investigators believe that there are probably too many monotypic genera in the Sanguinicolidae (e.g. Overstreet and Køie 1989, at present there are 14 monotypic genera in the 19-genus family). This is probably a reflection more of the paucity of described species than of over-enthusiasm in raising new genera. That two new sanguinicolids were found in an intensively studied fish (sea bass) suggests that many more sanguinicolids await discovery. The nature of most parasitological examinations, which generally overlook much of the blood circulatory system, is probably the reason why they have previously been overlooked. Examinations directed specifically at sanguinicolids require up to 4 h for the flukes to escape from the blood vessels they inhabit. Many parasitologists discard the gut and mesenteries after scraping and/or agitation; hence, the majority of sanguinicolids are also discarded. In addition, smaller sanguinicolids, such as *P. vastispina*, can be easily overlooked, especially if they are moribund or dead.

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