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Description of a New Blood-fluke, *Cruoricola lates* n. g., n. sp. (Digenea: Sanguinicolidae), from Sea-bass *Lates calcarifer* (Bloch, 1790) (Centropomidae)

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Abstract

A new blood-fluke, *Cruoricola lates* n. g., n. sp., is described from sea-bass *Lates calcarifer* cultured in Malaysia. It is also found in Thailand and Australia. All fish examined over 15 cm in length were infected in the type-locality. This sanguinicolid is differentiated from other genera by the spherical seminal vesicle; the large, single testis extending beyond the intestinal caeca; the medial, bi-lobed ovary; and the single column of submarginal, laterally directed, evenly spaced spines. It has separate genital pores which are close together. Adults are found predominantly in the mesenteric venules of the venous circulation. Juveniles are commonest in the caudal kidney.

Introduction

The sea-bass *Lates calcarifer* (Bloch, 1790) is the most important cultured brackish water fish in Malaysia (Ong, 1983; Anon., 1990). As a result of their commercial importance in the Asian region, many investigations of pathogens, particularly parasites, have been made (e.g., Leong & Wong, 1986; Chonchouenchob et al., 1987; Glazebrook & Campbell, 1987). A previously undescribed blood-fluke was found infecting the sea-bass cultured at Pulau

Ketam, Selangor, Malaysia, during routine histopathological studies (Shaharom & Anderson, 1988). To date other reports of blood-flukes in sea-bass have been limited. Leong & Wong (1986) listed *Cardicola* sp. in a table of parasites of cultured sea-bass from southern Thailand, and Langdon (1990) reported sanguinicolid eggs from Australian fishes, including sea-bass.

The following is the description of the blood-fluke reported as *Cardicola* sp. by Leong & Wong (1986) in sea-bass from Thailand and also found in cultured sea-bass from Malaysia and Australia (Herbert et al., 1991).

Materials and methods

Sea-bass *Lates calcarifer*, collected from cage culture sites at Pulau Ketam, Selangor, were killed, and the heart, gill arteries, dorsal aorta, kidney and visceral blood vessels examined for blood-flukes. Live worms were briefly washed in saline and fixed in either hot 10% buffered formalin, AFA, or Bouin's fluid. Specimens for whole-mounts were stained with paracarmine, Mayer's carmalum, Horen's trichrome, or Mayer's haematoxylin and mounted in Canada balsam. Sectioned specimens were stained with haematoxylin and eosin or Masson's trichrome. Measurements were taken of specimens with eggs in the uterus and a clearly visible testis. Specimens for scanning electron microscopy were relaxed in saline at 7°C for up to one hour and fixed in cold 2.5% glutaraldehyde, or fixed directly in cold 2.5% glutaraldehyde. They were then washed with cacodylate buffer, post-fixed for two hours in cold cacodylate buffered osmium tetroxide and dehydrated through an acetone series. The material was then critical point dried in a Hitachi HCP-2 critical point drier, coated with gold and examined under a Hitachi scanning electron microscope.

***Cruoricola* n. g.**

Diagnosis

With characteristics of family Sanguinicolidae von Graff 1907. Body lanceolate, with single column of submarginal spines except at anterior extremity. Oral sucker absent. Oesophagus long, extending about one quarter of body length. Intestine X-shaped with short anterior caeca and long posterior caeca terminating pre-ovarially. Excretory vesicle Y-shaped with short stem and distensible antero-laterally directed arms. Testis single, pre-ovarian and posterior to caecal junction, extending laterally to posterior caeca. Sperm duct single. Seminal vesicle well developed, postovarian, contained in cirrus-sac. Cirrus present. Ovary post-testicular, in posterior quarter of body, extending laterally almost to margins of body. Uterus post-ovarian. Vitellarium follicular, extensive and pre-ovarian. Genital pores separate but close together, dorsal and post-ovarian. Parasitic in blood circulatory system of marine and brackish-water fish.

Type-species: Cruoricola lates n. sp.

Etymology: From the Latin *cruor*, blood, and *-icola* (*colere*), to dwell in. Masculine gender.

Differential Diagnosis

Body of uniform length, with lateral directed body spines in single column submarginally; margins of body not recurved ventrally. Uterus entirely postovarian; mid-portion with thick walls. Testis single, extending lateral to caeca almost to margin. Seminal vesicle spherical, within cirrus-sac. Auxiliary seminal vesicle usually indistinct. Oral sucker absent.

***Cruoricola lates* n. sp.**

Type-locality: Pulau Ketam, Selangor, Malaysia.

Other localities: Jelutong, Penang; Kampung Setiu Baru, Terengganu; Pulau Kukup, Johore; Pulau Acheh, Johore.

Also found in cultured sea-bass from Hinchinbrook Channel, North Queensland, Australia, and from Songkhla, Southern Thailand.

Type-material: Holotype: from mesenteric venule of *Lates calcarifer*, Pulau Ketam, Selangor. Deposited in the National University of Singapore Zoological Reference Collection (ZRC.1992.8). Paratypes: deposited in the National University of Singapore Zoological Reference collection (ZRC.1992.9, 10), Queensland Museum (GL 17978-81), and Meguro Parasitological Museum (19567 A967-970).

Type-host: *Lates calcarifer* Bloch, 1790 (Centropomidae).

Sites in host: (figures in parentheses refer to percentage of fish from type-locality over 15 cm in length infected in that site. n = 45): all visceral blood vessels (excluding spleen and kidney) (98.97%), renal blood vessels (71.73%), pericardial blood vessels (55.5%), filamental arteries (40.2%), dorsal aorta (10.31%), rete mirabile (10%).

Prevalence of infection: 100% in type-locality in fish weighing over 10 g (n = 120). Nil in fish 7–9 g (n = 20).

Description (Figs. 1–3)

(Based on observation of live material, 55 mounted specimens and 6 sectioned worms. Measurements from mounted specimens in micrometers, ranges in parentheses).

General morphology. Body flat, lanceolate with pointed anterior end and blunt posterior end; length 1,171 (443–1,969), width 209 (99–306), 4–6 times longer than wide; maximum width in testicular region. Spines evenly spaced in single, submarginal column ventro-laterally, except anterior to mouth, where absent (Fig. 4). Length of spine about 3 long, embedded, 6–8 apart. Lateral nerve-cord conspicuous, uniting posterior to arms of excretory vesicle; anterior commissure 85 (30–132) or 5–8% of body length from anterior end, dorsal to oesophagus. Mouth small, ventral, subterminal, 6.6 (4.5–9.1) from anterior tip; grooves with pits leading from anterior margin of mouth to anterior tip of worm (Fig. 5). Oesophagus slightly sinuous, especially in distal half; anterior portion thin walled; wall gradually thickening toward intestine; granular gland-cells surround middle third of oesophagus in band of varying thickness (1–5 cells) (Fig. 3a). Intestine normally X-shaped, with 2 short caeca extending antero-laterally and 2 long caeca posteriorly. Oesophago-intestinal junction 27–30% of body length from anterior end. Anterior caecal arms usually club-shaped,

one usually slightly longer than other, 40 (10–81) long; posterior caeca fairly straight, usually slightly dilate and curved at posterior end, 492 (146–1,051) long, or 42% (33–53%) of body length, terminating anterior to ovary. Excretory vesicle Y-shaped, with short stem, distensible anteriorly directed arms.

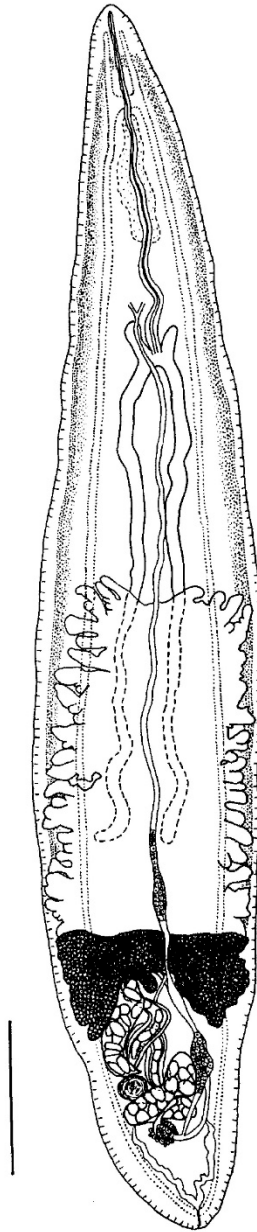


Figure 1. *Cruoricola lates* n. g., n. sp. Holotype. Dorsal view. Scale-bar: 0.2 mm.

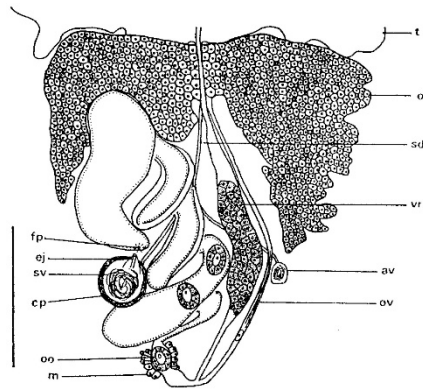


Figure 2. Terminal genitalia of *Cruoricola lates* n. g., n. sp. slightly diagrammatic to show relationships of ducts and auxiliary seminal vesicle. *Abbreviations:* av, auxiliary seminal vesicle; ej, ejaculatory duct; cp, cirrus-sac; fp, female pore; m, Mehlis' gland; o, ovary; oo, ootype; ov, oviduct; sd, sperm duct; sv, seminal vesicle; t, testis; vr, vitelline reservoir. *Scale-bar:* 0.1 mm.

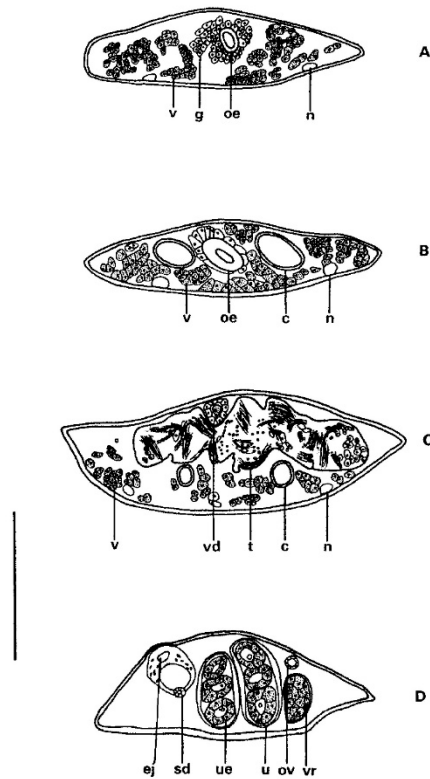


Figure 3. Transverse sections of *Cruoricola lates* n. g., n. sp.: A. Through mid-part of oesophagus; B. Through anterior caeca and terminal part of oesophagus; C. Through testis near terminal ends of posterior caeca; D. Through uterus and vitelline reservoir at seminal vesicle level. *Abbreviations:* c, caeca; ej, ejaculatory duct; g, gland cells; n, nerve canal; oe, oesophagus; ov, oviduct; sd, sperm duct; t, testis; u, uterus; ue, uterine egg; v, vitelline follicle; vd, vitelline duct; vr, vitelline reservoir. *Scale-bar:* 0.1 mm.

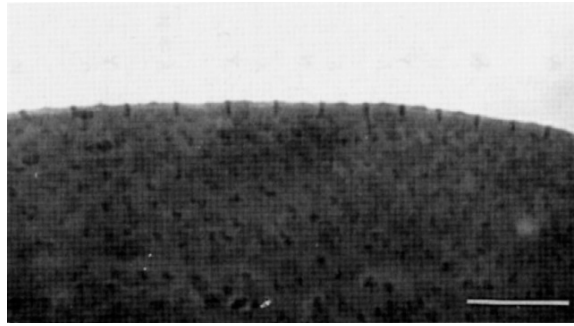


Figure 4. Spines of *Cruoricola lates* n. g., n. sp. Whole-mount. Scale-bar: 20 μ m.

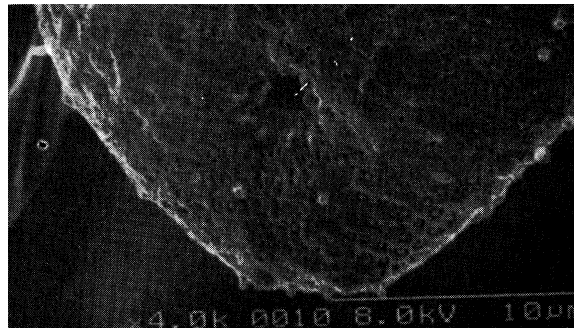


Figure 5. Mouth of *Cruoricola lates* n. g., n. sp. showing shallow grooves with pits. Papillae are present on the anterior margin. Scale-bar: 10 μ m.

Male reproductive system. Testis lobed, single, posterior to intestinal junction, extending lateral to caeca almost to edges of worm, bounded posteriorly by ovary; numerous sinuous masses swollen with fully formed spermatozoa present; length 370 (88–786) is 32% (18–40%) of body length; width 176 (62–285); usually 2–4 times longer than wide. Testis not visible in some specimens. Sperm duct passing ventral to uterus. Cirrus-sac sinistral, spherical, 31 (17–43) in diameter, thin-walled, containing seminal vesicle, pars prostatica and ejaculatory duct. Prostatic cells small, flattened, occupy space between cirrus-sac and seminal vesicle. Seminal vesicle spherical, diameter 23 (12–35). Ejaculatory duct short. Cirrus small, inconspicuous, dorsal to seminal vesicle, directed antero-medially toward female pore. Male genital pore dorsal, submedian, and sinistral, close to female pore. Auxiliary seminal vesicle when visible usually rounded, size apparently dependent on volume of contents, dextral, postovarian, and lateral to vitelline reservoir, about 14 (7–29) in diameter (n = 20).

Female reproductive system. Ovary bi-lobed, with fairly straight anterior margin bounded by testis and irregular lateral edges; length 43 (10–81) and 247 (90–364) (at shortest and longest points, respectively); width (at widest point) 163 (71–275); 17–19% of body length from posterior end of worm. Vitellarium extensive, reaching anteriorly past nerve commissure and posteriorly to level of the ovary, ventral to testis. Vitelline duct single, median, lying dorsal to testis, anteriorly forked in vicinity of the caecal junction; slightly dilate preovarially, widening post-ovarially into prominent vitelline reservoir dorsal to oviduct, uniting

with oviduct close to oötype. Oviduct arises from posterior border of ovary medially, extends dextral to uterus before turning across to oötype; distal portion of oviduct often enlarged. Oötype posterior to uterus, medial or nearly so, surrounded by Mehlis' gland cells; Laurer's canal absent. Uterus post-ovarian, loops anteriorly between lobes of ovary and turns back posteriorly down to female pore; terminal and proximal portions not conspicuously muscular; middle portion of uterus thick-walled laterally but not so dorsally or ventrally (Fig. 3d); uterine seminal receptacle elongate. Eggs ovoid, thin shelled, not operculate; in utero 20 (15–26) long by 11.7 (7–18) (n = 112) in fixed worms, in live slightly pressed worms 28 × 20 (n = 20).

Discussion

Cruoricola lates n. sp. is widely distributed throughout the blood-system of its host, but the majority of worms are found in the venules around the stomach, pyloric caeca, intestine and excretory bladder. It primarily inhabits the venous circulation, although it may occur in the filamental arteries or dorsal aorta. It was never found in the heart but did inhabit the pericardial vessels. Most adult worms are found in the visceral venules and veins, which is probably why this species has been previously overlooked. Juvenile worms were primarily found in the caudal kidney. Most other species of blood-flukes have been found in the heart or gill arteries, as most trematode parasitologists examine only the heart and gills, and rarely anywhere else in the circulatory system (Thulin, 1980).

In life, *C. lates* n. sp. has a thin membrane around the edges, which it appears to use as a seal to aid in attachment to smooth surfaces. However, this was not apparent in sectioned specimens. Using this, it can resist a strong jet of saline used when trying to dislodge it from the bottom of a petri dish, suggesting that the marginal spines may be an aid in maintaining position while moving within the host but play little role in attachment while stationary. Large specimens of *Sanguinicola inermis* Plehn, 1905, a species which does not possess spines, are also difficult to remove from smooth surfaces (Sommerville & Iqbal, 1991).

In whole-mounts, refractive dorso-ventral ductlike structures are present in most of the body but are especially conspicuous through the testis and ovary. They are not apparent around the oral region, uterus, or vitelline reservoir. Their presence or absence has been noted in few other species (e.g., Manter, 1947, 1954; Overstreet & Køie, 1989). In some mounted specimens of *C. lates* n. sp. they are not particularly obvious. In sections, they appear to be dorso-ventral, membrane-bound divisions in the tissue attached to the subtegumental muscles (Fig. 6). Pearson (personal communication) suggests that they are dorso-ventral muscle fibers which serve to maintain the fiat shape of the worm. Dorso-ventral muscle fibers have been observed in sections of *Aporocotyle simplex* Odhner, 1900 by Thulin (1980). The structures in *C. lates* n. sp. remain translucent in whole-mounts stained with nuclear stains. As their development apparently precedes that of the gonads and vitellarium, these organs would necessarily grow around them, thus making them conspicuous in stained whole-mounts.

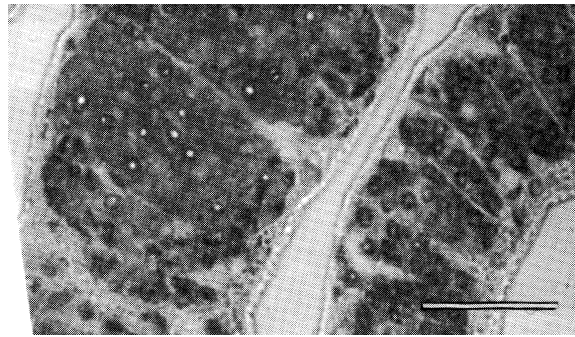


Figure 6. *Cruoricola lates* n. g., n. sp. Dorso-ventral structures, presumed to be muscle fibers, running through ovary and vitellarium. Scale-bar: 50 μ m.

Of 100 ovigerous adults, 45 had no discernible testis, which suggests that the testis involutes or degenerates when spent. A similar phenomenon observed by Thulin (1980) in *A. simplex*, and was also noted in *Spirorchis haematobium* (Stunkard, 1922) Price, 1934, a spirorchiid blood-fluke of turtles (Stunkard, 1923). All mature worms without a visible testis, and juvenile worms with a partly developed testis, when observed alive, had active sperm in both the seminal vesicle and the uterus.

The auxiliary seminal vesicle is not apparent in all specimens, and its size and shape depends on its fullness. In sanguinicolid, only *Pearsonellum corventum* Overstreet & K oie, 1989 has an auxiliary seminal vesicle. As in *P. corventum*, sperm in the auxiliary seminal vesicle sometimes appeared to be degenerate. The auxiliary seminal vesicle in *C. lates* differs from that in *P. corventum* in its close proximity to the ovary and vitelline reservoir. Its location and often empty state make it indistinct in many specimens.

Scanning electron microscopy (SEM) allows the genital pores of *C. lates* n. sp. to be seen clearly (Fig. 7).

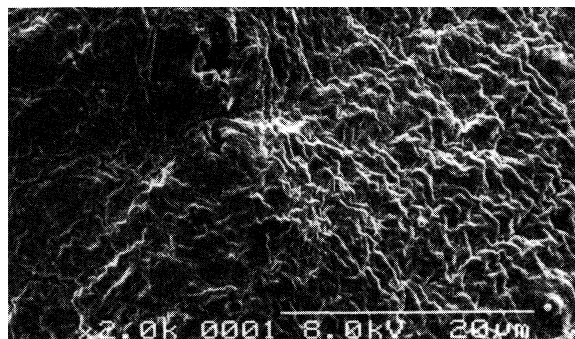


Figure 7. Genital pores of *Cruoricola lates* n. g., n. sp. Female pore closest to top. Scale-bar: 20 μ m.

The relative importance of some of the characters used in taxonomic studies of blood-fluke was reported by Short (1954) and van der Land (1967). The following discussion will center on both these morphological characters and several others of significance (refer to Table 1).

Table 1. Selected characteristics of marine sanguinicolid trematodes

Genus (Source)	Uterus	Genital pores	Position of genital pore	Testes number	Testes position	Ovary position	Intestine	Armature	Cirrus-sac	Cirrus
<i>Aporocotyle</i> (Yamaguti, 1958; Thulin, 1980a)	Pre-ovarian	Common genital atrium	Median, sinistral	Many	Intercaecal	Intercaecal; pre-uterine	H-shaped	Minute, clumped spines	Y	Y
<i>Cardicola</i> (Short, 1953; Yamaguti, 1971)	Post-ovarian	Separate	M. post. & lat. to F.; sinistral; post-ovarian	1	Intercaecal	Mid-post-caecal area; level with or pre-uterine	H-shaped	Transverse rows of small V-L spines	N ¹	Y or N
<i>Chimaerohemecus</i> (van der Land, 1967)	Post-ovarian	Separate	M. dorsal, sinistral. F. ant. to M.	1	Intercaecal	Intercaecal pre-uterine	Bifurcate	Ventrolateral margins	Y	Y
<i>Deontacylix</i> (Manter, 1947; Yamaguti, 1971)	Extends pre-ovarially	Separate	M. post. to F. Both right of median	1	Between nerve trunks	Postcaecal; sinistral	H-shaped with diverticules	Dorsal and lateral		N
<i>Hyperandrotrema</i> (Maillard & Ktari, 1978)	Post-ovarian	Separate	M. post. to F. Both on left side	1	Intercaecal	Most intercaecal; pre-uterine; post-testicular	Bifurcate	Lateral	N	Y
<i>Metaplehnella</i> (Lebedev & Parukhin, 1972)	Post-ovarian	Separate	M. post. to F. F. median, M. on left	1	Mostly postcaecal	Post-caecal; median; preuterine	H-shaped	Fine; marginal; paired		N
<i>Neoparacardicola</i> (Yamaguti, 1970, 1971)	Extends pre-ovarially	Separate	M. post. to F. at marginal notch	2	Ant. intercaecal Post. post-caecal	Post-caecal; between ant. margin of post. testis and right side of body	X-shaped with diverticules	2 V-L rows	Y	Y
<i>Orchispirium</i> (Madhavi & Rao, 1970)	Extends pre-ovarially	Common	Submedian; postovarian	1	Intercaecal transversely coiled tube	Post-caecal; post-testicular; right of median; mostly pre-uterine	Bifurcate	? possibly lost	Y	Y
<i>Paracardicola</i> (Martin, 1960)	Post-ovarian	Separate but close	Near mid-seminal vesicle level	2	Post-caecal; pre-ovarian and post-ovarian	Post-caecal; pre-uterine	H-shaped	Laterally spined		N
<i>Paracardicoloides</i> (Martin, 1974)	Pre-ovarian	Common	Pre-ovarian, on right side	2	Post-caecal; in hindbody	Post-uterine; post-caecal, between testes	Bifurcate	V-L band sheathed in tegument	Y	Y

Table 1. *Continued*

Genus (Source)	Uterus	Genital pores	Position of genital pore	Testes number	Testes position	Ovary position	Intestine	Armature	Cirrus-sac	Cirrus
<i>Paradeontacylix</i> (McIntosh, 1934; Ogawa & Egusa, 1986)	Post-ovarian	Separate	F. median; M. sinistral and post. to F.	Many	2 median intercaecal rows	Inter- or post-caecal; pre-uterine	H-shaped	V-L rows of spines; rose thorn hooks posterior	Y ²	Y or N
<i>Pearsonellum</i> (Overstreet & Køie, 1989)	Extends pre-ovarially	Separate	M. dorsal sinistral; post. to F.; F. antero-medial	1	Mostly intercaecal	Post-caecal; post-testicular; median	H-shaped	V-L transverse rows	Y	Y
<i>Plethorchis</i> (Martin, 1975)	Pre-ovarian	Common	Near posterior on left	> 100	Post-caecal	Post-uterine; post-caecal	Bifurcate	Lateral clusters of 3-4	Y	Y
<i>Psettarium</i> (Goto & Ozaki, 1929; 1930)	Post-ovarian	Separate	F. median; M. post. to and sinistral to F.	1 (?) diffuse	Not clear, extends post-ovarian	Post-caecal, laterally bound by testis; dextral	H-shaped	Transverse rows	Y	Y
<i>Psettarioides</i> (Lebedev & Parukhin, 1972)	Post-ovarian	Separate	M. lateral on right; F. median & ant.	1	Pre-ovarian	Post-caecal; post-testicular; median	H-shaped with diverticules	V-L transverse rows	?	Y
<i>Pseudocardicola</i> (Parukhin, 1985)	Extends pre-ovarially	Separate	F. sub-median & ant. to marginal M.	5	Intercaecal; pre-ovarian	Post-caecal, sinistral	X-shaped	?	Y	Y
<i>Selachohemecus</i> (Short, 1954)	Post-ovarian	Common	Median	1	Pre-ovarian; post-caecal	Pre-uterine; post-testicular; median	4 very short caeca (= X)	V-L margin single spines		N
<i>Cruoricola</i>	Post-ovarian	Separate	Sinistral, M. sinistral & lateral to F.	1	Pre-ovarian post-caecal	Post-caecal, post-testicular width of body	H-shaped	V-L single row	Y	Y

1. Except *C. congruenta* Lebedev & Mamaev, 1968, which appears to have a cirrus sac.

2. If cirrate.

Abbreviations: ant., anterior; post., posterior; F, female genital pore; M, male genital pore; Y, cirrus/cirrus-sac present; N, cirrus/cirrus-sac absent; V-L, ventro-lateral.

C. lates n. sp. has a post-ovarian uterus, in common with many other genera (see Table 1). Only members of the genus *Paradeontacylix* McIntosh, 1934 have similarities in the length position and path of the uterus. Nevertheless, in *C. fates* n. sp. the uterus extends anteriorly between the lobes of the ovary, and the ovary extends almost to the margins. In this species the uterine position and shape determines the shape of the ovary. The uterus is fully developed before the ovary, and its anterior coils prevent posterior expansion of the ovary medially.

Few sanguinicolids have the single, broad testis of *C. lates* n. sp. (see Table 1). *P. corventum* and members of *Psettarooides* Lebedev & Parukhin, 1972 are the only sanguinicolids in which the single testis extends beyond the intercaecal field. In both, the testis extends anterior to the caecal junction, although this is not a generic character. Most sanguinicolids have a cirrus and cirrus-sac (see Table 1). *C. lates* n. sp. is unique in having a spherical seminal vesicle and poorly developed cirrus. Most members of *Cardicola* Short, 1953 are acirrate, except three (*C. whitteni* Manter, 1954; *C. congruenta* Lebedev & Mamaev, 1968; and *C. grandis* Lebedev & Mamaev, 1968) which are cirrate but lack a seminal vesicle. *P. corventum* has a large, well-developed cirrus and cirrus-sac, and *Metaplethniella lethrini* Lebedev & Parukhin, 1972 is acirrate.

In sanguinicolids, the ovarian position is fairly constant in relation to body length, being located in the posterior third of the body (see Table 1). Its position is normally considered relative to the uterus, as discussed above. The shape of the ovary may be quite variable within a genus and may vary in *C. lates* n. sp., where, although it normally has a fairly straight anterior margin, it is bi-lobed and extends almost to the margins. All other sanguinicolids have a comparatively narrower and/or smaller ovary than *C. lates* n. sp.

Two other morphological characters, not listed by Short (1954), are the spination and the presence or absence of a cirrus. Spines are universally present in marine sanguinicolids, except for *Orchispirum heterovitellatum* Mahdavi & Rao, 1970, which may have lost its spines during processing (Madhavi & Rao, 1970). In *C. lates* n. sp. spines cannot be seen in many mounted specimens, although they are always seen in life. Shape, size, and arrangement of spines are useful taxonomic characters. No other sanguinicolids possess fine, single, laterally directed, evenly spaced, ventrolateral spines similar to those of *C. lates* n. sp. *Pseudocardicola emmelichthy* Parukhin, 1985 has spines closely spaced on the margins, whereas in *C. lates* n. sp. they are more spread apart.

Although there are many monotypic sanguinicolid genera, the characteristics of *C. lates* n. sp. are distinct and clearly warrant its separation from all other genera. However, *C. lates* n. sp. bears many similarities to *Cardicola*; *Metaplethniella* Lebedev & Parukhin, 1972; and *Pearsonellum* Overstreet & Køie, 1989, and is closely allied to them. The generic features of *Cardicola* (see Short, 1953; Yamaguti, 1958) include a ventrally recurved margin and short, transverse rows of small spines, both of which are absent in *C. lates* n. sp. Additionally, members of *Cardicola* have widely spaced genital pores, whereas in *C. lates* n. sp. they are very close. *C. lates* n. sp. differs from *Metaplethniella lethrini* in its armature, having single spines. The widely spaced genital pores, ramified ovary, post-caecal testis, and rudimentary oral sucker of *M. lethrini* separate it from *C. lates* n. sp. *P. corventum* has a muscular metraterm, well-developed cirrus, oral sucker, and transverse rows of spines, all of which

are absent in *C. lates* n. sp. The distinguishing features of *C. lates* are the testis, armature, and almost spherical seminal vesicle.

The blood-flukes of marine fishes are a poorly studied group, with descriptions of several genera relying on very limited material. It is probable that there are many more marine sanguinicolids yet to be described. With a greater number of species for comparison the present unclear status of relationships between blood-fluke genera can be clarified. Re-examination of type-material may be required to eliminate discrepancies. In particular, the relative importance of the surface armature, intestinal shape, and reproductive system morphology warrant further investigation.

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