Blood flukes (Digenea: Aporocotylidae) of walking catfishes (Siluriformes: Clariidae): new genus and species from the Mekong River (Vietnam) with comments on related catfish aporocotylids

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Abstract: *Nomasanguinicola canthoensis* gen. et sp. n. infects the branchial vessels of bighead catfish, *Clarias macrocephalus* Günther (Siluriformes: Clariidae), in the Mekong River near Can Tho, southern Vietnam. *Nomasanguinicola* differs from all other genera of fish blood flukes (Digenea: Aporocotylidae) by the combination of lacking body spines and by having an anterior sucker with two flanking columns of large denticles, an intestine comprising several short papilla-like caeca, an inverse U-shaped uterus, and an ootype located near the separate genital pores. The new species has an ootype that is posterior to the level of the female genital pore. That feature most easily differentiates it from the only other putative aporocotylid species having an anterior sucker with two flanking columns of large denticles, *Plehniella dentata* Paperna, 1964 and *Sanguinicola clarias* Imam, Marzouk, Hassan et Itman, 1984, which have an ootype that is lateral (*P. dentata*) or anterior (*S. clarias*) to the level of the female genital pore. These two species apparently lack extant type materials, infect North African catfish, *Clarias gariepinus* (Burchell), and herein are considered *incertae sedis*, but likely comprise species of *Nomasanguinicola*. An updated list of hosts, sites of infection and geographic localities for the six species and three genera of blood flukes that mature in catfishes is provided. The new species is the first fish blood fluke recorded from Vietnam and only the third reported from a walking catfish (Clariidae).

Keywords: taxonomy, *Nomasanguinicola canthoensis*, *Sanguinicola clarias*, *Plehniella dentata*, *Clarias macrocephalus*, South East Asia

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The nominal 'blood flukes' (Digenea: Aporocotylidae) that mature in the blood vascular system of freshwater fishes total 24 species assigned to *Sanguinicola* Plehn, 1907 plus monotypic *Acipensericola* Bullard, Snyder, Jensen et Overstreet, 2008 and *Plehniella* Szidat, 1951 (see also Lebedev and Parukhin 1972), which comprise species that likely infect the body cavity of catfishes only (Szidat 1951; unpublished data) (Table 1). To date, collectively, the freshwater fish aporocotylids have been documented to infect members of Cypriniformes (28 fish host species), Siluriformes (7), Esociformes (*Esox lucius* Linnaeus), Perciformes (5 + 2 marine), Salmoniformes (4 species of *Oncorhynchus* Suckley), Characiformes [*Prochilodus platensis* (Valenciennes)], and Acipenseriformes [*Polyodon spathula* (Walbaum) and *Acipenser oxyrinchus* Mitchill].

The diversity of species presently assigned to *Sanguinicola* sensu lato that infect freshwater fishes is focused principally on cypriniforms, with nearly all parasite-host records deriving from members of the Cyprinidae (see Smith 1997a,b). Many fewer host records of non-cyprini-

forms exist and a blaring exception is that of *Sanguinicola maritimus* Nolan et Cribb, 2005, which infects marine wrasses (Labridae) of south Australia (Nolan and Cribb 2004).

Compared to the blood flukes of carps, all of which are currently assigned to *Sanguinicola*, those of catfishes (Siluriformes), including putative species of *Sanguinicola* and *Plehniella*, are few. These five aporocotylids infect the blood (Woodland 1923, 1924, Odhner 1924, Paperna 1964, 1996) and body cavity (Szidat 1951, Guidelli et al. 2002) of seven catfishes assigned to three families and six genera (Table 1). Some of these hosts are valued aquaculture species, e.g. walking catfishes (species of *Clarias* Scopoli), and because blood flukes are known pathogens of cultured fishes (Bullard and Overstreet 2002), knowledge on their taxonomic diversity, hosts and geographic distributions is important to disease diagnostics and prevention in warm-water culture systems.

Herein, we report the first record of a fish blood fluke from Vietnam and describe specimens collected from the

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Table 1. Blood flukes (Digenea: Aporocotylidae) of catfishes (Siluriformes).

	Host	Site	Locality	Reference(s)
Sanguinicola chalmersi Odhner, 1924	*Auchenoglanis occidentalis* (Valenciennes) (Claroteidae)	blood, heart	Sudan, Africa	Woodland 1923, Odhner 1924, Khalil 1969, 1971, Paperna 1996
	Synodontis schall (Block and Schneider) (Mochokidae)	mesenteric and branchial blood vessels	Cairo and Giza fish markets, Egypt	Woodland 1923, Imam et al. 1984
Sanguinicola clarias Imam, Marzouk, Hassan et Itman, 1984 *incertae sedis*	*Clarias gariepinus* (Burchell) (Clariidae) (as *C. lazera*)	'mesenteric and other blood vessels'	Cairo and Giza fish markets, Egypt	Imam et al. 1984
		not specified	Beni-Suef fish market, Egypt	Imam and El-Askalany 1990
Plehniella coelomicola Szidat, 1951	*Iheringichthys labrosus* (Lütkin) (Pimelodidae)	body cavity	La Plata River, Argentina	Szidat 1951
	Pimelodus maculatus Lacépède (Pimelodidae) (as *P. clarias*)	body cavity	La Plata River, Argentina	Szidat 1951, Lunaschi 1985
	Pimelodus albicans (Valenciennes) (Pimelodidae)	body cavity	La Plata River, Argentina	Lunaschi 1985
Plehniella dentata Paperna, 1964 *incertae sedis*	*Clarias gariepinus* (Burchell) (Clariidae) (as *C. lazera*)	'intestine' (probably mesenteric vessels)	Lake Tiberia and Hule Nature Reserve, Israel	Paperna 1964
Plehniella platyrhynchi (Guidelli, Isaac et Pavanelli, 2002) comb. n. (originally *Sanguinicola*)	*Hemisorubim platyrhnchos* (Valenciennes) (Pimelodidae)	body cavity	Paraná River, Brazil	Guidelli et al. 2002
Nomasanguinicola canthoensis gen. et sp. n.	*Clarias macrocephalus* Günther (Clariidae)	branchial vessels	Can Tho fish market, Vietnam (Mekong River)	present study

branchial vessels of bighead catfish, *Clarias macrocephalus* Günther (Siluriformes: Clariidae) as a new species. We also propose a new genus to accommodate this new species and use its diagnostic characters to focus attention to the only other nominal clariid blood flukes, both of which reportedly infect North African catfish, *Clarias gariepinus* (Burchell): *Plehniella dentata* Paperna, 1964 and *Sanguinicola clarias* Imam, Marzouk, Hassan et Itman, 1984) (see Paperna 1964, Imam et al. 1984).

MATERIALS AND METHODS

The infected catfish was captured locally from the Mekong River before being purchased from a fish market in Can Tho (10°01'35"N; 105°47'12"E), Vietnam, kept alive in a bucket of ambient water, euthanized by spinal severance and necropsied on 5 January 2011. The heart, gill arches and associated branchial vessels were removed, isolated in separate sample bags and exposed to freshwater heated to 60 °C immediately before filling the sample bag with and being stored in 10% neutral buffered formalin (n.b.f.).

In the laboratory at Auburn University, the fixed contents of the sample bag were examined for the presence of aporocotylids with aid of a stereo-dissecting microscope. Resulting aporocotylid specimens intended as stained, whole mounted specimens were removed from n.b.f., rinsed thoroughly with distilled water and cleaned with fine brushes to remove any debris, stained overnight in Van Cleave's hematoxylin with several additional drops of Ehrlich's hematoxylin, made basic at 70% ethanol with lithium carbonate and butylamine, dehydrated, cleared in clove oil, and permanently mounted on glass slides using Canada bal-

sam. Illustrations of stained, whole-mounted specimens were made with the aid of a Leica DM-2500 equipped with differential interference contrast optical components and a drawing tube. Measurements were obtained by using a calibrated ocular micrometer and are herein reported in micrometres (μm) as a range followed by, in parentheses, the mean and number of measurements taken. Scientific names including taxonomic authorities and dates for fishes follow Eschmeyer (2012). Common names are taken from FishBase (Froese and Pauly 2012). Higher level fish classification and nomenclature follows Nelson (2006). Nomenclature for the Aporocotylidae follows Bullard et al. (2009). Brown (1956) was used to help construct the genus name and specific epithet.

RESULTS

Nomasanguinicola gen. n.   Figs. 1–9

Diagnosis. Body of adult approximately $< 8\times$ longer than wide, dorsoventrally flattened, lacking posterolateral protuberance, more rounded posteriorly than anteriorly, ventrally concave, lacking tegumental body spines ('Marginalstacheln') and rods and bristles ('Stäbchen-Börstchen'). Rosethorn-shaped spines in posterior body end absent. Ventrolateral nerve cords and dorsolateral nerve cords present; ventrolateral nerve cord extending nearly entire body length, appearing slightly subterminal, with commissures anteriorly and posteriorly.

Anterior sucker present, demarcated from body by posterior constriction of tegument, an obvious proboscis

accommodating mouth, armed with denticles; denticles peg-like, having a broadly rounded proximal base and narrowing distally to form a slightly recurved tip, directing posteroventrally, forming two columns of four denticles each and flanking the mouth. Mouth a minute pore, medioventral, subterminal. Pharynx present, a minute muscular region surrounding terminus of oesophagus at level of anterior sucker. Oesophagus medial, without loops or extensive convolutions, < 1/4 of body length, including anterior and posterior swellings, lacking obvious oesophageal gland that stains differentially and has refractive acini (cf. *Cardicola* spp.), connecting with intestinal ramification anterodorsally. Intestine comprising several short papilla-like caeca approximately < 1/10 of oesophagus length. Caeca lacking diverticula or secondary rami but having uneven outline.

Testis single, extending from a region posterior to caeca to ovary, < 1/3 of body length, an extensively convoluted mass of basophilic testicular tissue, sperm, and vasa efferentia presenting as field with laterally directing lobes, not extending lateral to ventrolateral nerve cords; vasa efferentia coalescing at posteroventral margin of testis. Auxiliary external seminal vesicle absent. Stylet associated with cirrus absent. Cirrus-sac present, surrounding enlarged seminal vesicle. Male genital pore dorsal, sinistral, post-gonadal, slightly lateral to midline.

Ovary single, medial, margins expanding laterad and resembling 'butterfly wings', as wide or wider than testicular field, occupying posterior 1/4 of body immediately posterior to testicular field. Vitellarium follicular, coextensive with alimentary tract, gonads and reproductive tracts from level of pharynx to ovary; common vitelline duct prominent, medial, forming a wide duct at level of ventrolateral nerve commissure, extending posteriad along midline, ventral to gonads and male reproductive tract. Oviduct emanating from posteromedial ventral surface of ovary, expanding in proximal portion to form an oviducal seminal receptacle, connecting with distal portion of vitelline reservoir before joining with ootype; ootype longer than wide, located approximately at level of genital pores. Laurer's canal not evident.

Uterus inverse U-shaped, lacking coils or extensive convolutions along its length, having approximately equal-length ascending and descending segments; uterine seminal receptacle not evident; uterine eggs spheroid, lacking appendages or filaments, lacking triangular habitus. Metraterm indistinct. Female genital pore near level of male genital pore. In blood vascular system of 'walking catfishes' (Siluriformes: Clariidae).

Differential diagnosis. Ventrolateral tegumental body spines absent. Anterior sucker present, with denticles; denticles peg-like, directing posteroventrally, forming a column per each side of mouth. Pharynx present, minute, at terminus of oesophagus. Oesophagus < 1/4 of body length, including anterior and posterior swellings. Intestine comprising several short papilla-like caeca. Auxiliary external seminal vesicle and stylet associated with cirrus absent. Cirrus-sac present. Ovary butterfly wing-shaped. Common vitelline duct medial, forming a wide duct at level of ventrolateral nerve commissure. Oviduct expanding proximally to form oviducal seminal receptacle; ootype located approximately at level of genital pores. Laurer's canal absent. Uterus inverse U-shaped, lacking coils, having approximately equal-length ascending and descending segments. Genital pores separate.

Type species: *Nomasanguinicola canthoensis* sp. n.

Etymology: The Greek 'nomas' for nomad or wanderer refers to the terrestrial affinity of *Clarias* spp. (so-called 'walking catfishes') as well as alludes to the natural history of species assigned to *Nomasanguinicola* as probably divergent from those of *Sanguinicola*.

Remarks. The new genus differs from marine aporocotylid genera that include species infecting bony fishes (Euteleostei) by the combination of lacking ventrolateral transverse rows of tegumental body spines and by having an intestine comprising several short caeca rather than elongate, paired anterior and posterior caeca. It differs from the blood fluke genera including species that infect chondrichthyans by lacking robust, C-shaped lateral tegumental body spines and a Laurer's canal. *Nomasanguinicola* is easily differentiated from the other aporocotylid genera including species that infect freshwater fishes, i.e. *Sanguinicola*, *Plehniella* and *Acipensericola*, by characteristics of the anterior sucker, body spination, oesophagus, intestine, and genitalia. It most closely resembles *Sanguinicola* sensu lato by having an anterior oesophageal swelling, a prominent common vitelline duct that is medial and dorsal to the testis and genital ducts (Ejsmont 1926), a lobed testis that is post-caecal, and a 'double-winged' or 'butterfly-shaped' ovary that is primarily posttesticular.

Nomasanguinicola differs from *Sanguinicola* mostly notably by the spination of the anterior sucker. We regard the presence of two columns of denticles and flanking the mouth as the marquee feature that differentiates *Nomasanguinicola* from all other blood fluke genera. Other aporocotylids have minute, straight spines typically arranged in concentric rows distributing anterior to the mouth (Meade and Pratt 1965, Schell 1974, Guidelli et al. 2002, Bullard 2012); however, those spines should not be confused with the denticles described herein and diagnostic for *Nomasanguinicola*. Moreover, we doubt that the denticles of *Nomasanguinicola* and the concentric, minute spines of aporocotylids are homologous. Abundant morphological evidence suggests that the latter spines are paedomorphic and homologous to the spines of the specialized penetration organ of aporocoytylid cercariae (Cribb et al. 2011). Likewise, the tri-radiate 'tooth-like mouth apparatus' that Ejsmont (1926) observed in *S. armata* and that Rašín

(1929) observed in *S. volgensis* (as *Janickia volgensis*) should not be confused with the denticles described herein for the type species of *Nomasanguinicola*.

Plehniella, the only other aporocotylid genus that includes species infecting catfishes, differs from *Sanguinicola* and *Nomasanguinicola* by lacking lateral tegumental body spines or denticles associated with the anterior sucker and by having an ovoid body (rather than an elongate), a gut comprising six short caeca (rather than four or five), testicular lobes angled at 45° from the midline and that each form a V-shape, a massive seminal vesicle enveloped by a glandular region, and a compact ovary that is entirely post-testicular and deeply-lobed. An ecologically unique attribute of species of *Plehniella* is that they mature in the body cavity of catfishes (Table 1); whereas the other freshwater aporocotylid genera include species infecting the blood. All of the blood flukes that have been reported to infect the body cavity of freshwater fishes infect pimelodid catfishes and all are assigned to *Plehniella*. No blood fluke infecting the body cavity of a species of Clariidae has been reported to date. The taxonomic status of *Plehniella* and its species will be treated in greater detail elsewhere (in preparation, SAB).

Acipensericola, which accommodates the blood flukes of basal actinopterygians (Acipenseriformes) has a bowl-shaped anterior sucker, robust peg-like lateral tegumental spines, a column of testes, and a Laurer's canal, among other diagnostic features (Bullard et al. 2008).

Nomasanguinicola canthoensis sp. n.　　　Figs. 1–9

Diagnosis of adult based on light microscopy of four whole-mounted specimens: With characters of *Nomasanguinicola* (see above). Body elongate oval in shape, 840–1060 (943; 4) long, 230–250 (240; 4) wide or 3.5–4.6 (3.9; 4) × longer than wide; body-margin straightened, not constricted ventrally in any specimen studied, having regularly-spaced aggregates of tegumental papillae; papillae aggregates having approximately 3–6 papillae each approximately 1 long and 1 wide, near limits of light microscopy (Figs. 1, 2).

Ventrolateral nerve cords 5–10 (8; 4) wide near midbody at widest level, difficult to trace for most of length in most specimens, 30–38 (35; 3) or 12–17% (15%; 4) of body width from body margin, contiguous at body ends, becoming confluent with paired cord 18–20 (19; 4) or 2% of body length from posterior body end; secondary branches and dorsolateral nerve cords not evident; commissure of ventrolateral nerve cord 105–123 (114; 4) or 12–13% (12%; 4) of body length from anterior body end, 50–65 (58; 3) across width of worm or 22–26% (24%; 4) of body width, 8 (4) in diameter, perpendicular to long axis of body, coursing dorsal to oesophagus (Fig. 1).

Ventral tegumental sensory papillae present, distributing across ventral surface of body from level of anterior sucker to posterior end, approximately 3 in diameter, apparently lacking cilia but having a central pore-like structure that is less basophilic than surrounding tegument. Dorsal tegumental sensory papillae not evident with light microscopy.

Anterior sucker base width 38–40 (39; 4) or 16–17% (16%; 4) of body width, 18–23 (22; 4) long or 1.7–2.1 (1.8; 4)× wider than long; terminal papillae on anterior margin of anterior sucker present, paired, each approximately 1 long and 1 wide; denticles of anterior sucker approximately 2 (4) wide, with each flanking column 12–18 (16; 4) long or 52–100% (74%; 4) of anterior sucker length (Figs. 1, 3, 5–7). Mouth 2 (4) in diameter, medioventral, 4–6 (5; 4) or 17–33% (22%; 4) of anterior sucker length from anterior end; pharynx minute, approximately 7 long and 4 wide, comprising an oblong enveloping region surrounding distal end of oesophagus at level of anterior sucker, not extending posteriad along oesophagus beyond posterior margin of anterior sucker, indistinct, in our experience, at magnifications < 2 000×.

Oesophagus 230–305 (265; 4) long or 27–30% (29%; 4) of body length, beginning as a narrow tube extending posteriad 40–60 (51; 4) or 4–6% (5%; 4) of body length before connecting with anterior oesophageal swelling; anterior oesophageal swelling 29–35 (33; 4) long or 14–24% (19%; 4) of oesophagus total length, 10–18 (14; 4) wide or 4–7% (6%; 4) of maximum body width, at level midway between ventrolateral nerve commissure and anterior sucker.

Oesophagus narrowing to 3 posterior to anterior oesophageal swelling and extending posteriad; posterior oesophageal swelling immediately anterior to caeca 23 (4) long or 8–10% (9; 4) of oesophagus length, 20–23 (21; 4) wide or 1.3–2.3 (1.8; 4)× maximum oesophagus width.

Oesophagus wall approximately 2 thick at level of commissure of ventrolateral nerve cord, approximately 5 thick at level of posterior oesophageal swelling; connecting with intestine dorsomedially. Oesophageal gland not clearly differentiated from surrounding parenchyma in any specimen examined.

Intestine star-shaped, having five clearly-differentiated caeca in all specimens examined; caeca length measuring (clockwise from oesophagus-intestine connection) 20–30 (25; 3), 20–30 (25; 3), 25–30 (27; 3), 20–33 (26; 3), and 17–25 (21; 3) or approximately 2–3% of body length and 6–9% of oesophagus length, caeca base width measuring (clockwise from oesophagus-intestine connection) 13–16 (14; 3), 13–14 (13; 3), 12–18 (15; 3), 10–18 (13; 3), and 10–25 (16; 3) or approximately 5–8% of maximum body width; postcaecal distance 240–320 (276; 4) or 28–32% (29%; 4) of body length (Figs. 1, 4).

Genitalia (Figs. 1, 8, 9) restricted to posterior quarter of body, primarily occupying space between nerve cords. Testicular field comprising a mass of testicular tissue, sperm and vasa efferentia, approximately rectan-
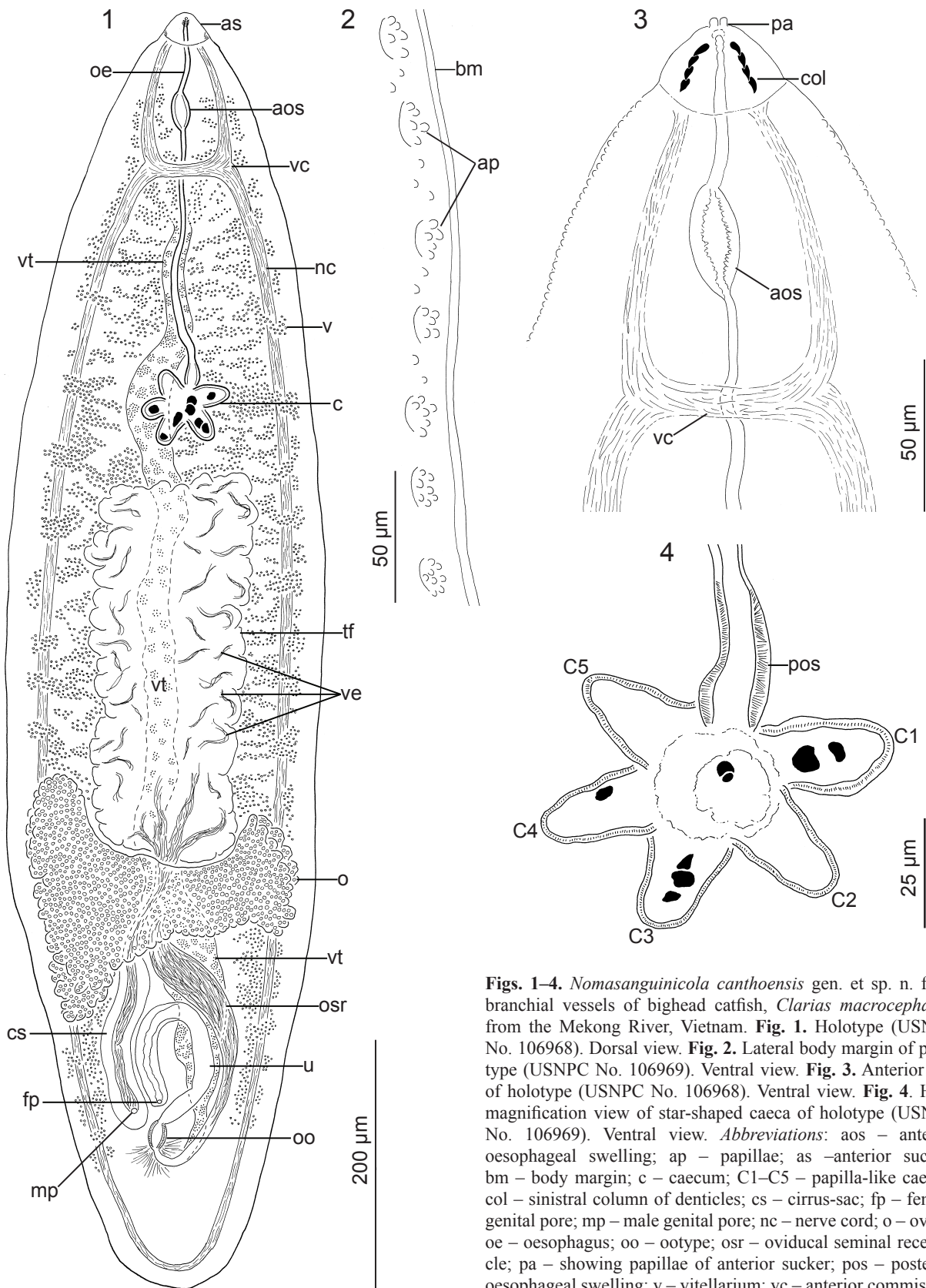
Figs. 1–4. *Nomasanguinicola canthoensis* gen. et sp. n. from branchial vessels of bighead catfish, *Clarias macrocephalus*, from the Mekong River, Vietnam. **Fig. 1.** Holotype (USNPC No. 106968). Dorsal view. **Fig. 2.** Lateral body margin of paratype (USNPC No. 106969). Ventral view. **Fig. 3.** Anterior end of holotype (USNPC No. 106968). Ventral view. **Fig. 4.** High magnification view of star-shaped caeca of holotype (USNPC No. 106969). Ventral view. *Abbreviations*: aos – anterior oesophageal swelling; ap – papillae; as –anterior sucker; bm – body margin; c – caecum; C1–C5 – papilla-like caecae; col – sinistral column of denticles; cs – cirrus-sac; fp – female genital pore; mp – male genital pore; nc – nerve cord; o – ovary; oe – oesophagus; oo – ootype; osr – oviducal seminal receptacle; pa – showing papillae of anterior sucker; pos – posterior oesophageal swelling; v – vitellarium; vc – anterior commissure of ventrolateral nerve cord; ve – vasa efferentia; vt – primary vitelline duct; tf – testicular field; u – uterus.
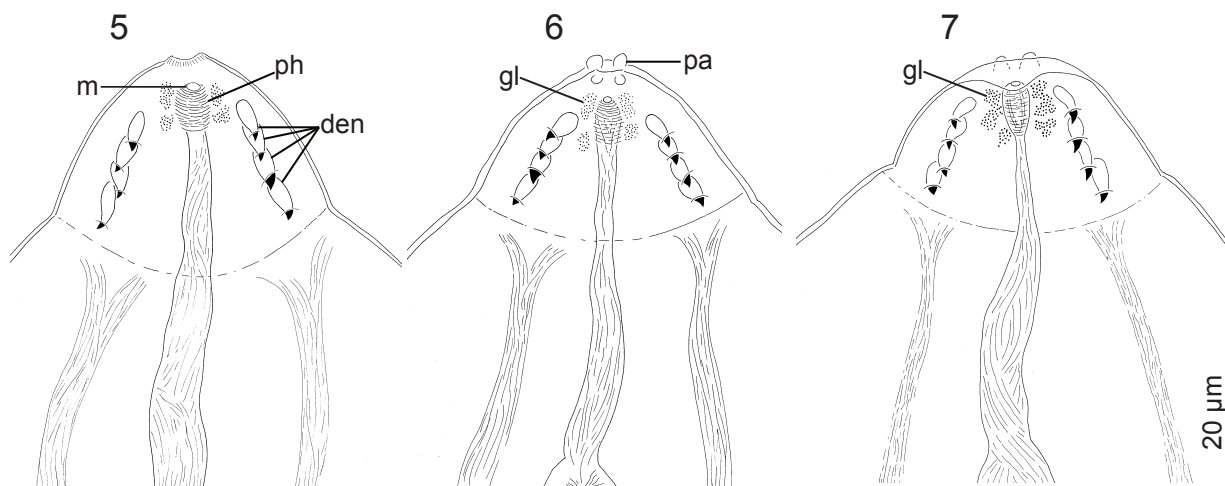
Figs. 5–7. Anterior sucker of *Nomasanguinicola canthoensis* gen. et sp. n. from branchial vessels of bighead catfish, *Clarias macrocephalus* from the Mekong River, Vietnam. Ventral view. **Fig. 5.** Paratype (USNPC No.106969). **Fig. 6**. Holotype (USNPC No. 106968). **Fig. 7.** Paratype (USNPC No. 106970). *Abbreviations*: den – denticles; gl – glandular area surrounding pharynx; m – mouth; pa – papillae associated with apex of anterior sucker; ph – pharynx.

gular in shape, entirely postcaecal, with slightly irregular lateral margins, 238–480 (332; 4) long or 28–45% (35%; 4) of body length, 120–130 (124; 4) in maximum width or 48–54% (52%; 4) of body width at level of mid-body, 2.0–3.8 (2.7; 4) × wider than long; post-testicular space 325–405 (358; 4) long or 33–43% (38%; 4) of body length (Figs. 1, 8, 9).

Vasa efferentia difficult to trace, 4–8 (7; 4) wide, an interconnecting meshwork of fine ducts entwining throughout testicular tissue, containing spermatozoa, extending primarily along ventral surface of testis, uniting in posterior region of testis to form vas deferens. Vas deferens an extremely thin-walled duct extending 80–85 (83; 3) or 8–10% (9%; 4) of body length posteriad, 8–12 (10; 3) wide at level of posterior margin of testicular field, narrowing posteriorly to 2–7 (4; 3) before connecting with cirrus sac and internal seminal vesicle, ventral to ovary, curving sinistrad, containing sperm in all specimens.

Cirrus-sac 22–28 (26; 3) in maximum width or 10–11% (10%; 3) of body width, with wall 5–8 (7; 3) thick; internal seminal vesicle robust, oblong, 126–160 (138; 3) long, 8–13 (10; 3) wide or 12–16 (13; 3) × longer than wide, orienting diagonally posteriad. Gland surrounding seminal vesicle indistinct. Male genital pore approximately 8 wide, 90–150 (123; 3) or 11–14% (13%; 3) of body length from posterior body end, immediately posterolateral to female genital pore.

Ovary dorsal to vas deferens, having superficial lobes extending laterad in lateral regions of ovary near body margin, extending laterad beyond level of ventrolateral nerve cord, having dextral and sinistral halves of ovary measuring 84–158 (114; 3) and 90–200 (139; 3) long or approximately 10–17% (13; 3) of body length, 175–210 (197; 3) in maximum width or 76–85% (82%; 3) of body width,

0.4–1.0)× wider than long; post-ovarian space 123–150 (133; 3) long or 12–18% (14%; 3) of body length (Figs. 1, 8, 9).

Oviduct arching in dextral half of body posterior to ovary and medial to cirrus sac, 175–263 (223; 3) long or 21–25% (24%; 3) of body length, slightly shorter than combined length of vas deferens and cirrus sac, including a short proximal duct, a dilated portion (= oviducal seminal receptacle), and a narrow distal portion; short proximal duct emanating from posteroventral surface of ovary and extending posteriad 10–15 (12; 3) before dilating, 2–5 (3) wide; oviducal seminal receptacle filled with sperm in all specimens, 100–140 (120; 3) long or 38–69% (56%; 3) of total oviduct length, 13–25 (20; 3) wide or 5.6–7.8 (6.3; 3)× longer than wide, occupying space between cirrus-sac and dextral nerve cord, post-ovarian. Distal portion of oviduct 7–8 (8; 3) wide or 32–62% (42; 3) of oviducal seminal vesicle width, continuing posteriad approximately in parallel with dextral body margin before connecting with ootype.

Vitellarium primarily indistinct; secondary collecting ducts indistinct; primary vitelline duct originating in anterior region of body at level between ventrolateral nerve commissure, 610–770 (710; 3) long, 15–28 (22; 4) wide at level of caeca, 29–58 (46; 3) wide at level of seminal vesicle; pre-vitelline duct distance 155–175 (162; 3) or 15–17% (17%; 3) of body length. Ootype 23–28 (25; 3) long, 10–13 (12; 3) wide, 1.9–2.3 (2.1; 3)× longer than wide, sinistral, immediately posterior to male genital pore, connecting with vitelline duct and oviduct on its posterior surface, orienting parallel with long axis of body (not transverse); post-ootype distance 103–125 (112; 3) or 10–13% (12%; 3) of body length. Mehlis' gland 40–60 (50; 3) wide, surrounding ootype and occupying region

Figs. 8, 9. Genitalia of *Nomasanguinicola canthoensis* gen. et sp. n. from branchial vessels of bighead catfish, *Clarias macrocephalus* from the Mekong River, Vietnam. **Fig. 8.** Holotype (USNPC No. 106968). Dorsal view. **Fig. 9.** Paratype (USNPC No. 106969). Ventral view. *Abbreviations*: au – ascending segment of uterus; cs – cirrus-sac; du – descending segment of uterus; fp – female genital pore; isv – internal seminal vesicle; junc – junction of vitelline duct and oviduct; mp – male genital pore; mg – Mehlis' gland; oo – ootype; o – ovary; osr – oviducal seminal receptacle; vit – primary vitelline duct; t – testis; ve – vasa efferentia; vd – vas deferens.

mostly posterior to connection of ootype with vitelline duct and oviduct.

Uterus inverse U-shaped, post-caecal, post-gonadal, occupying space between cirrus sac and oviducal seminal receptacle; ascending uterus 75–138 (102; 3) long or 9–13% (10; 3) of body length, 17–40 (26; 3) in maximum width; descending uterus 65–118 (87; 3) long or 84–87% (85%; 3) ascending uterus length, 12–53 (27; 3) in maximum width. Uterine eggs lacking thick shell, oblong, 16 (4) long, 10 wide (4) or 1.6× longer than wide. Female genital pore anteromedial to male genital pore, sinistral, postovarian, 133–158 (144; 3) or 13–17% (15%; 3) of body length from posterior body end. Excretory system indistinct.

T y p e a n d o n l y h o s t : Bighead catfish, *Clarias macro-cephalus* Günther (Siluriformes: Clariidae).

T y p e l o c a l i t y : Fish market at Can Tho (10°01'35"N; 105°47'12"E), Mekong River, southern Vietnam.

S i t e i n h o s t : Adults in branchial vessels.

P r e v a l e n c e a n d i n t e n s i t y o f i n f e c t i o n : Four specimens infected one bighead catfish.

S p e c i m e n s d e p o s i t e d : Holotype and one paratype at the United States National Parasite Collection (USNPC Nos. 106968–106970); one paratype at the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice (IPCAS D-693).

E t y m o l o g y : The specific epithet '*canthoensis*' is for the type locality, Can Tho, Vietnam.

Remarks. We think that the nominal aporocotylids that infect clariids probably are congeners. The present study revealed some uncanny similarities between *N. canthoensis* and the published descriptions of two putatively distinct aporocotylids that infect a congeneric clariid (*Clarius gariepinus*) in Israel and Egypt, *Plehniella dentata* Paperna, 1964 and *Sanguinicola clarias* Imam, El-Askalany, Hassan et Itman, 1984. Most notably, *N. canthoensis*, *P. dentata* and *S. clarias* each have two columns of denticles (sensu Paperna [1964]; = 'rose thorne spines' of Imam et al. [1984]) embedded in the anterior sucker (= 'small sucker' of Paperna [1964]; = 'well defined snout' of Imam et al. [1984]) and flanking the mouth. Aside from perhaps *Sanguinicola chalmersi* Odhner, 1924 (see Discussion; Imam et al. 1984), these are the only nominal aporocotylids that reportedly have two columns of denticles flanking the mouth.

Also noteworthy is that the published descriptions of these aporocotylids list the following characters, which are diagnostic for *Nomasanguinicola*: anterior sucker present, pharynx minute and located at terminus of oesophagus (fig. 2.3 of Imam et al. 1984), oesophagus < 1/4 of body length, intestine comprising several short papilla-like caeca, auxiliary external seminal vesicle or cirrus stylet lacking, cirrus-sac present, ovary having butterfly wing shape, ootype at approximate level of genital pores, Laurer's canal absent, and separate genital

pores. Specific detail of the oviduct needs confirmation in *P. dentata* and *S. clarias*, it likely expands proximally to form an oviducal seminal receptacle (fig. 1.1 of Imam et al. 1984; fig. 33 of Paperna 1964).

Moreover, the new species (see Description) and *S. clarias*, and probably *P. dentata* (see Discussion), differ from the type species of *Sanguinicola* (*S. armata*) and several other putative species of *Sanguinicola* by having an inverse U-shaped uterus that has both ascending and descending segments. Descriptions of putative species assigned to *Sanguinicola* reportedly have a uterus that is abbreviated, comprising a short ascending segment only between the ootype and female genital pore. This has been repeatedly described and confirmed in *Sanguinicola armata* Plehn, 1905 (type species) (see Plehn 1905, Woodland 1923, 1924, Odhner 1924, Ejsmont 1926, Ong and Shaharom 1995), as well as in the closely related *Sanguinicola inermis* Plehn, 1905 (see fig. 4 of Kirk and Lewis 1993).

Supporting reassignment of *P. dentata* also is that it has several key generic features that are lacking in *Plehniella coelomicola* Szidat, 1951 (type species): denticles, a testis without V-shaped lobes, a seminal vesicle that reportedly lacks a glandular enveloping region, and an ovary that reportedly lacks deep lobes. These comprise features that make its assignment to *Plehniella* dubious, while not excluding it from *Nomasanguicola*. The new species is most easily differentiated from *P. dentata* and *S. clarias* by having an ootype that is posterior to the female genital pore, whereas *P. dentata* and *S. clarias* have an ootype that is lateral (Paperna 1964) or slightly anterior (Imam et al. 1984) to the female genital pore, respectively.

DISCUSSION

At the time of the writing of this manuscript, no extant type materials could be located for *Plehniella dentata* and *Sanguinicola clarias*. The only reported type material for *P. dentata* was the holotype (No. 2044), and it may have been destroyed during a 1983 fire in Ilan Paperna's laboratory in Eilat, Israel. Regarding *S. clarias*, Imam et al. (1984) did not report deposition of type materials and we cannot locate type materials for *S. clarias*. Hence, new specimens sourced from the type host and type localities of each need re-collection and re-description. Pending those results, we regard *P. dentata* and *S. clarias* as *incertae sedis*, likely representing a single species or two closely related species of *Nomasanguincola*. Below, however, we intend to offer a few helpful comments to those attempting to identify specimens of these putative species. Although clearly differentiated from the new species, some features reported by Paperna (1964) and Imam et al. (1984) need confirmation because they seem strikingly uncharacteristic for fish blood flukes related to members of *Sanguinicola*, *Nomasanguinicola*, or *Plehniella*.

Paperna's (1964) diagnostic description of *P. dentata* was based on two stained, whole-mounted specimens

collected from the 'intestine' (presumably the mesenteric blood vessels) of two North African catfish, *Clarias gariepinus* (Burchell, 1822), (as *C. lazera*) (Siluriformes: Clariidae) sampled at Lake Tibeiras and at Hule Nature Reserve (Israel). Regarding the uterus, Paperna (1964) may have misinterpreted its course because he illustrated it as distally swollen with sperm, extending anteriad, and ending immediately posterior to the ovary. No nominal species of the Aporocotylidae has a uterus including a thin-walled, distal portion serving as a uterine seminal receptacle and no nominal species of *Sanguinicola* or *Plehniella* reportedly has a female genital pore that opens dorsomedially at level of the ovary.

Paperna's interpretation of the uterus may have derived from previous descriptions of *S. armata* (see Ejsmont 1926), wherein the uterus is abbreviated, extending anteriad only a short distance from the ootype before opening dorsally at level of the male genital pore. It seems likely, however, given the anatomy of other aporocotylids, that the swollen part of the duct illustrated and labeled by Paperna (1964) as the uterus was actually the proximal portion of the oviduct functioning as an oviducal seminal receptacle, which many aporocotylids have. We deduce this also because the feature labeled as the uterus by Paperna is in the precise location as the oviducal seminal receptacle in Imam et al.'s (1984) description of the blood fluke from this host species as well as in our description of *N. canthoensis*. Hence, we suspect that Fig. 33 of Paperna (1964) mislabels the oviduct as the uterus. In addition, we think that Paperna's Fig. 33 shows that the female genital pore in *P. dentata* opens lateral to the ootype and the male genital pore is anteromedial to the ootype.

Regarding the intestinal caeca, Paperna (1964) clearly illustrated and reported that *P. dentata* had four short, symmetrical caeca comprising the intestine. However, the caeca of species of *Sanguinicola, Plehniella*, and *Nomasanguinicola* are easily missed: the wall of the caeca is thin, delicate, refractive to staining, and unlike that of other aporocotylids that infect marine fishes, which have a thick, refractive, well-staining caecal wall that is typically obvious even if specimens are not studied alive, heat-killed, or well-stained. Moreover, we observed that in some specimens of *N. canthoensis*, the gut can be easily misinterpreted as having only four caeca, rather than five, because the anteriormost caecum can be immediately dorsal to the posterior oesophageal swelling and obscured by it.

Other features of *P. dentata* reported by Paperna (1964) included body size and shape, lateral tegumental body spines absent (likening it to species of *Plehniella* and *Nomasanguinicola*), mouth opening at terminus of small anterior sucker, four denticles flanking mouth and increasing in size anteriorly to posteriorly, oesophagus dilated anteriorly, male genital pore near ootype, and ovary bilobed. All these features correspond well with the generic diagnosis provided herein for *Nomasanguinicola*.

Imam et al.'s (1984) description of *S. clarias* was based on ten whole-mounted specimens collected from the mesenteric and other blood vessels and heart of *C. gariepinus* from Cairo and Giza, Egypt. There are several key features that need confirmation in this species upon recollection of voucher materials. First is that their narrative description of *S. clarias* seems to be reversed, i.e. their fig. 1 is likely a dorsal view. Regarding the lateral tegumental spines, Imam et al. (1984) reported that *S. clarias* has a tegument provided with regularly arranged marginal spines, but spines were not illustrated, measured, or counted.

In *N. canthoensis*, dense clusters of papillae-like tegumental protrusions along the ventrolateral body margin give the impression of spines; however, spines need to be confirmed in *S. clarias*. Again, given the uncanny morphological similarity between *P. dentata* and *S. clarias*, both from the same catfish species in the same geographic region, and given that Paperna (1964) did not report tegumental spines, the presence of such spines in *S. clarias* would certainly be diagnostic.

Regarding a pharynx, Imam et al. (1984) reported that *S. clarias* had an elongated pharynx surrounding a portion of the oesophagus. Figures 1 and 3 of Imam et al. (1984) show an expansion of the oesophagus that seems morphologically comparable to the luminal expansion (= anterior oesophageal swelling) described by Paperna (1964) for *P. dentata* and by us herein for *N. canthoensis*. That this region comprises a true pharynx, i.e. a muscular bulb surrounding the oesophagus, rather than a lateral expansion of the lumen of the oesophagus needs confirmation since the presence of a pronounced muscular pharynx is likely a feature of generic significance within Aporocotylidae, and indeed a minute pharynx is present in *N. canthoensis*. It is also possible that the region is weakly muscular and that the feature is homologous to a pharynx but presents as a pliable oesophageal wall.

Ejsmont (1926) also identified an area in the anterior portion of the oesophagus that he labeled a pharynx. Woodland (1923) illustrated an anterior oesophageal swelling/dilation (labeled as a pharynx) that was distensible in the specimens of *S. chalmersi* that he studied. Herein, we call this region an oesophageal swelling because we do not see evidence of muscle surrounding the oesophagus. Regarding the intestinal caeca, Imam et al. (1984) reported an x-shaped intestinal caecum (including photomicrographs of the gut compared with other species of *Sanguinicola*). Regarding the testis, Imam et al. (1984) described the testis as having 6–7 pairs of acini located medially. Other features of *S. clarias* reported by Imam et al. (1984) included body size and shape, anterior sucker present and bearing denticles, mouth terminal, dilation of oesophagus (= posterior oesophageal swelling) at level of caeca present, cirrus sac large, distinct, ovary double winged, medial vitelline duct, and uterus inverse

U-shaped. All of these features correspond well with the generic diagnosis provided herein for *Nomasanguinicola*.

Noteworthy also is that Imam et al. (1984) reported that one specimen of the catfish blood fluke *Sanguinicola chalmersi* Odhner, 1924 had seven denticles on either side of the mouth. No such denticles were illustrated or included in Odhner's (1924) original description, and it seems unlikely to us that this feature would vary intraspecifically, but this characteristic needs confirmation in newly collected specimens. All of the above would be beyond speculation if type materials for these taxa were extant but they apparently are not.

The above information extracted from the descriptions by Paperna (1964) and Imam et al. (1984) indicate that these flukes, both recorded from the same host species and from neighbouring geographic regions (Israel and Egypt), may be conspecific. Imam et al. (1984), apparently unaware of Paperna's (1964) description of *P. dentata* from the same host species, did not differentiate their new species from or mention *P. dentata*. New collections and studies of *P. dentata* and *S. clarias* from *C. gariepinus* (= type host) from Israel and Egypt (= type localities) could test the notion that these species are distinct as well as confirm their systematic position within *Nomasanguinicola*.

Regarding the life cycles, Paperna (1996) stated that eggs of *P. dentata* contain a fully developed miracidium when accumulated in the kidney of *C. gariepinus*. He speculated that perhaps the fluke exited the host via the urinary system. If so, this would be a significant evolutionary departure from the life cycles of other blood flukes wherein the miracidium develops in the gill before the egg hatches and the miracidium bores through the fish's gill epithelium. Perhaps this life history feature is worthy of future investigation, i.e. complementing routine histopathology with isolation and/or culture of aporocotylid eggs from kidney to confirm they contain ciliated larvae, whenever infected North African catfish are examined.

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