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Two new species of Cephalogonimidae Looss, 1899 (Digenea: Plagiorchioidea) from Africa (Mozambique and Guinea), including a new phylogenetic hypothesis for related plagiorchioids

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ABSTRACT

Two new species of Cephalogonimidae Looss, 1899 (from *Emoleptalea* Looss, 1900 and *Masenia* Chatterji, 1933) are described from African freshwater fishes. *Emoleptalea mozambiquensis* n. sp. infected the turquoise killifish, *Nothobranchius furzeri* Jubb, in Mozambique and differs from its nine congeners by the combination of differences in body shape and size, oral sucker shape, sucker width ratio, configuration of the digestive tract and gonads, vitelline follicle shape and vitellarium configuration. *Emoleptalea dollfusi* Srivastava, 1960 is a synonym of *Emoleptalea loossi* Srivastava, 1960, thus there are still nine accepted species. *Masenia baroensis* n. sp. infected the globe fish, *Tetraodon lineatus* L., in the Republic of Guinea and differs from its five African congeners and 15 Asian congeners by the combination of circumoral spine count, oral sucker shape, caecal extent, ovary shape, genital pore position, and configuration of the vitellarium. *Masenia dayali* (Gupta & Puri, 1984) Chandra & Saxena, 2016 and *Masenia pushpanjalii* are *nomina dubia*. We propose *Masenia ritai* (Agrawal, 1964) n. comb., with *M. ritai* Sircar & Sinha, 1970 its junior synonym. *Heterorchis* cf. *crumenifer* (identified tentatively due to egg size) is reported from the West African lungfish, *Protopterus annectens* (Owen), in Mozambique (new geographical record). *Heterorchis protopteri* Thomas, 1958 and *Heterorchis ghanensis* Thomas, 1968 are *species inquirendae*. Sequences (28S rDNA) from these parasites were included in a Bayesian phylogenetic analysis with 37 other ingroup taxa. Both new species formed a clade with *Masenia nkomatiensis* Dumbo, Dos Santos & Avenant-Oldewage, 2019 from Africa. These three species formed a sister relationship with the other available cephalogonimids: *Cephalogonimus americanus* Stafford, 1902 and *Cephalogonimus retusus* (Dujardin, 1845), both frog parasites from North America and Europe, respectively. *Heterorchis* cf. *crumenifer* represented a distinct lineage within the Plagiorchioidea but formed a polytomy with species from 10 plagiorchioid families.

1. Introduction

This study concerns specimens belonging to three species of plagiorchioid digeneans infecting fishes in Africa that were collected as part of two collection trips investigating freshwater helminth parasites; the first was to the Republic of Guinea during 2003 and the second was to South Africa and Mozambique in 2020. Specimens of two species of the Cephalogonimidae Looss, 1899 were collected. The third species belongs in *Heterorchis* Baylis, 1915, a rarely reported genus with ambiguous phylogenetic affinities. Baylis (1915) provisionally treated *Heterorchis* as related to certain genera in the Lepodermatidae Odhner, 1910. It was later determined that *Lepoderma* Looss, 1899 was a synonym of *Plagiorchis* Lühe, 1899 by a slim margin of time and the Lepodermatidae is now treated as a synonym of the Plagiorchiidae Lühe, 1901 (see Tkach, 2008). Yamaguti (1953) transferred *Heterorchis* to the Fellodistomidae Nicoll, 1909 without written explanation but more recently Prudhoe and Bray (1982) considered it to belong in the Plagiorchiidae and Tkach in Pojmańska et al. (2008) treated it as a genus *incertae sedis* in the Plagiorchioidea Lühe, 1901. Our specimens are consistent in morphology with the original description for *Heterorchis*

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crumenifer Baylis, 1915, (apart from egg size), and closely match the description of the species by Dollfus (1950). We herein tentatively identified our specimens as *H*. cf. *crumenifer* with the incongruence of egg size with the original material accounting for the decision to apply the favorable comparison designation. Partial fragments of the large subunit ribosomal DNA (lsrDNA) gene were generated for all three species and aligned with some comparable publicly available sequences from digeneans belonging in the Monorchiata Olson, Cribb, Tkach, Bray & Littlewood, 2003 and Xiphidiata Olson, Cribb, Tkach, Bray & Littlewood, 2003 within the Plagiorchiida La Rue, 1957. The alignment was subjected to Bayesian inference analysis to produce a phylogenetic tree estimating the interrelationships of the studied species within the Plagiorchioidea.

2. Materials and methods

2.1. Specimen collection

Hosts for the studied digeneans were collected from freshwater habitats in Africa. Hosts were captured by seining as well as by using baited mesh traps. During March of 2003, we discovered an undescribed digenean species belonging in the cephalogonimid genus *Masenia* Chatterji, 1933 infecting the globe fish, *Tetraodon lineatus* L., (Tetraodontiformes: Tetraodontidae) in the Niandan River (10°36′51″N, 9°41′42″W) and the Niger River (10°41′37″N, 9°38′08″W), both near Baro, Republic of Guinea. During February 2020, we discovered a second undescribed cephalogonimid species belonging in *Emoleptalea* Looss, 1900 infecting the turquoise killifish, *Nothobranchius furzeri* Jubb, (Cyprinidontiformes: Nothobranchiidae) in the Karingani Game Reserve, Mozambique (24°20′8.09″S 32°15′42.0."E). Also, during that time and from the same area as the infected killifish, we collected specimens of *H*. cf. *crumenifer* infecting the intestine of several individuals of the West African lungfish, *Protopterus annectens* (Owen), (Lepidosireniformes: Protopteridae). All hosts were dissected immediately after being euthanized by spinal severance. The digestive tracts were excised intact, sliced longitudinally to expose the lumen, and, immersed in citrated saline solution (7.0 ppt saline solution: 7 g of sodium citrate dissolved in 1 L tap water) or saline solution (0.85% sodium chloride in tap water) and examined using a Wild M5 stereo dissecting microscope. Flukes were removed from intestines using pipet, fine forceps, or artist's paintbrush, rinsed in saline solution, pipetted onto glass slides and killed with heat without pressure or pipetted into near boiling tap water. Worms were fixed in 10% neutral buffered formalin for morphological study or preserved in 95% ethanol for DNA extraction.

2.2. Morphological study

Heat-killed, formalin fixed specimens were stained in aqueous Van Cleave's plus Ehrlich's hematoxylin solutions (VCE) at [500:1] for 18 h, or in aqueous Meyer's hematoxylin solution at [1 stock solution: 5 distilled water] for 35 min. Stained worms were rinsed in tap water and gradually dehydrated using an ethanol series till reaching [70% ethanol]. Worms stained in VCE were then placed in a solution of 70% ethanol (3 ml) with four drops of cold saturated lithium carbonate in 70% ethanol solution plus two drops of butylamine (99.5%) for 10 min, and worms stained in Meyer's hematoxylin were destained using 1% hydrochloric acid in 70% ethanol solution for 10 min followed by emersion in 1% sodium hydroxide in 70% ethanol solution for 10 min. All worms were then fully dehydrated using ethanol, cleared in clove oil, and mounted on glass slides in Canada balsam using a cover slip. Measurements are reported as ranges in micrometres. Type materials and vouchers are deposited in the United States National Museum of Natural History's Invertebrate Zoology Collection (USNM, Smithsonian Institution, Washington, D.C.).

2.3. DNA extraction and preparation of nucleotide sequences

Genomic DNA was extracted from individual ethanol preserved specimens with the Qiagen DNAeasy tissue kit (Qiagen Incorporated, Valencia, California). A fragment of the lsrDNA gene was amplified from the resulting genomic DNA following protocols detailed in Truong et al. (2021). Representative nucleotide sequences are deposited in the National Institute of Health's genetic sequence database (GenBank) with accession numbers reported below and in Table 1.

2.4. Phylogenetic analysis

The three new sequences were aligned with 38 other lsrDNA nucleotide sequences from GenBank using MAFFT (Katoh and Standley, 2013). The alignment contained 40 sequences representing digeneans belonging in the Xiphidiata (34 in Plagiorchioidea, three in Microphalloidea Ward, 1901, and three in Gorgoderoidea Looss, 1901), and one sequence representing *Lissorchis kritskyi* Bamhart & Powell, 1979, belonging in the Monorchiata and functioning as the outgroup (Table 1). The lissorchid outgroup was chosen based on its previously estimated position within the Digenea Carus, 1863 from earlier phylogenetic investigations (Olson et al., 2003; Sokolov and Shchenkov, 2017; Pérez-Ponce de León and Hernández-Mena, 2019; Sokolov et al., 2019). The alignment was trimmed at both ends to match the shortest sequences (HM137608, HM137615, KF013188), resulting in an overall length of 1122 bases. JModelTest 2 version 2.1.10 was used to determine the best-fit models of nucleotide substitution for the analysis: substitution model averaging (nst-mixed) and gamma distribution to model rate-heterogeneity (Darriba et al., 2012). The alignment was subjected to Bayesian inference analysis using MrBayes software version 3.2.5 with parameters set to defaults (Ronquist and Huelsenbeck, 2003), and settings outlined in Troung et al. (2021). The resulting phylogram was generated using FigTree software version 1.4.3 (Rambaut et al., 2014). All figures were edited using Adobe Photoshop version 21.1.3 (Adobe Systems Inc., San Jose, California, USA).

3. Results

Superfamily Plagiorchioidea Lühe, 1901
Family Cephalogonimidae Looss, 1899

3.1. Genus Emoleptalea Looss, 1900

Emoleptalea mozambiquensis n. sp.

3.1.1. Description (Figs. 1–3)

[Based on three mature specimens] Body oval, 1050–1160 long, 480–520 wide at mid-body. Tegument spinous, spines minute, 5–6 long, becoming sparser near the posterior end. Oral sucker sub-terminal on ventral surface, subspherical with posterior margin truncated, 113–123 long, 115–123 wide. Prepharynx 20–25 long. Pharynx wider than long, 37–53 long, 68–75 wide. Oesophagus very short, 10 long in a single measurable specimen. Ventral sucker pre-equatorial, nearly circular, 125–138 long, 125–135 wide. Oral to ventral sucker width ratio 1:1.01–1.17. Forebody 300–325 long or 28% of body length. Intestine bifurcating in forebody at about halfway between suckers, caeca blind, terminating in vicinity of middle of post-testicular space. Postcaecal space 250–290 long or 22–27% of body length.

Testes subglobular, oblique, slightly overlapping with each other or separated by slight space, largely intercaecal but anterior testis overlapping caeca on one specimen; anterior testis 175–215 long, 168–205 wide, posterior testis 198–205 long, 198–220 wide. Post-testicular space 325–390 long or 31–34% of body length. Cirrus sac curving in a reverse C-shape, extending to near anterior margin of ventral sucker and partially overlapping sucker, 335–414 long, 60–70 wide. Cirrus sac containing bipartite seminal vesicle, pars prostatica with well-defined

Table 1
Partial large subunit ribosomal DNA sequences used for phylogenetic analysis.

Species	Family	GenBank accession no.[a]	Reference
Suborder Monorchiata			
Monorchioidea			
Lissorchis kritskyi	Lissorchiidae	EF032689	Curran et al. (2006)
Suborder Xiphidiata			
Gorgoderoidea			
Encyclometra colubrimorum	Encyclometridae	AF184254	Tkach et al. (2000b)
Gorgodera cygnoides	Gorgoderidae	AF151938	Tkach et al. (2000b)
Xystretrum solidum	Gorgoderidae	KF013188	Cutmore et al. (2013)
Microphalloidea			
Maritrema poulini	Microphallidae	KJ144177	Presswell et al. (2014)
Maritrema prosthometra	Microphallidae	AY220631	Tkach et al. (2003)
Prosthogonimus ovatus	Prosthogonimidae	MN726975	Schwelm et al. (2020)
Plagiorchioidea			
Auridistomum chelydrae	Auridistomidae	AY116872	Olson et al. (2003)
Brachycoelium salamandrae	Brachycoeliidae	AF151935	Tkach et al. (2000a)
Cephalogonimus americanus	Cephalogonoimidae	HM137615	Razo-Mendivil and Pérez-Ponce de León (2011)
Cephalogonimus retusus	Cephalogonimidae	AY222276	Olson et al. (2003)
Choanocotyle hobbsi	Choanocotylidae	EU196356	Tkach and Snyder (2007)
Choanocotyle nematoides	Choanocotylidae	EU196358	Tkach and Snyder (2007)
Dasymetra nicolli	Reniferidae	AF433672	Tkach et al. (2001b)
Emoleptalea mozambiquensis n. sp.	Cephalogonimidae	**MW586927**	Present study
Glypthelmins pennsylvaniensis	Glypthelminthidae	HM137608	Razo-Mendivil and Pérez-Ponce de León (2011)
Glypthelmins quieta	Glypthelminthidae	AY222278	Olson et al. (2003)
Haematoloechus varigatus	Haematoloechidae	AF151916	Tkach et al. (1999)
Heterorchis cf. *protopteri*	*Incertae sedis*	**MW586924**	Present study
Leptophallus nigrovenosus	Leptophallidae	AF151914	Tkach et al. (1999)
Macrodera longicollis	Leptophallidae	AF151913	Tkach et al. (1999)
Macroderoides texanus	Macroderoididae	EU850398	Tkach et al. (2008)
Macroderoides typicus	Macroderoididae	HQ680849	Tkach and Kinsella (2011)
Masenia baroensis n. sp.	Cephalogonimidae	**MW586925**	Present study
Masenia nkomatiensis	Cephalogonimidae	MH142268	Dumbo et al. (2019a)
Mesocoelium sp.	Mesocoeliidae	AY222277	Olson et al. (2003)
Mesocoelium sp.	Mesocoeliidae	AF433677	Tkach et al. (2001b)
Neoglyphe locellus	Plagiorchiidae	AF300330	Tkach et al. (2001a)
Neoglyphe sobolevi	Plagiorchiidae	AF300329	Tkach et al. (2001a)
Omphalometra fexuosa	Omphalometridae	AF300333	Tkach et al. (2001a)
Opisthioglyphe ranae	Telorchiidae	AF151929	Tkach et al. (2001a)
Orientocreadium batrachoides	Orientocreadiidae	MK496882	Dumbo et al. (2019b)
Orientocreadium pseudobagri	Orientocreadiidae	MF611697	Sokolov and Shchenkov (2017)
	Leptophallidae	AF151910	Tkach et al. (1999)
Paralepoderma cloacicola	Plagiorchiidae	AF184250	Tkach et al. (2000b)
Plagiorchis muelleri	Plagiorchiidae	AF151931	Tkach et al. (2000a)
Plagiorchis vespertilionis	Reniferidae	HQ665459	Santoro et al. (2011)
Renifer aniarum	Reniferidae	AF433671	Tkach et al. (2001b)
Renifer kansense	Omphalometridae	AF300331	Tkach et al. (2001a)
Rubenstrema exasperatum	Haematoloechidae	AY222279	Olson et al. (2003)
Skrjabinoeces similis	Telorchiidae	AF151915	Tkach et al. (1999)
Telorchis assula			

[a] Bold GenBank accession numbers produced in the present study.

prostatic bulb, and elongated cirrus (apparently unarmed). Proximal portion of bipartite seminal vesicle larger than distal portion; proximal portion 85–139 long, 53–63 wide; distal portion 75 long, 58–65 wide. Prostatic bulb pear-shaped, 43–53 long, 25–33 wide. Cirrus 165–188 long. Cirrus sac communicating with genital atrium dorsal to ventral sucker; genital atrium 26–38 long, 13–15 wide. Genital pore opening dorso-median, immediately posterior to anterior margin of oral sucker.

Ovary subglobular, submedian, amphitypic, situated on same side as posterior testis, 160–173 long, 140–148 wide. Seminal receptacle a transversely elongated to nearly subspherical sac containing sperm, dorso-median to and overlapping ovary, always smaller than ovary, 98–125 long, 75–80 wide. Oviduct leaving anterior ovary, extending toward median line of body, forming ootype surrounded by Mehlis' gland between ovary and anterior testis (not clearly observed, partially observable in one dorsal specimen). Laurer's canal communicating with oviduct near junction with seminal receptacle, leading toward dorsal surface, opening at ovarian level on dorsal surface. Vitellarium comprised of two lateral bands of large irregularly shaped follicles; follicles surrounding caeca, extending from level at posterior margin of pharynx to approximately posterior third of testicular zone. Vitelline reservoir roughly triangular, median, or slightly submedian, overlapping posterior third of ventral sucker, situated ventral relative to ovary but with collection ducts running dorsal to ovary and anterior testis. Proximal uterus descending from ovarian complex between testes in dorsal hindbody, coiling extensively and occupying most of hindbody. Distal uterus ascending ventrally on ab-ovarian side. Metraterm thick-walled, adjacent to dorsally, and following path of cirrus sac, 190–275 long, 25–33 wide (width measured near proximal end). Eggs filling uterus, oval, operculated; distal eggs 23–25 long, 13–18 wide.

Excretory bladder Y-shaped, main stem (visible only in largest specimen), 310 long or 27% of body length, branching 50 posteriorly from posterior margin of posterior testis. Excretory system opening through excretory pore at terminal end with glandular cells surrounding stem near pore.

Type host: turquoise killifish, *Nothobranchius furzeri* Jubb, 1971, (Cyprinidontiformes: Nothobranchiidae).

Site in host: intestine.

Type locality: Karingani Game Reserve, Mozambique.

Prevalence: 3 worms infected one of five fish examined.

Specimens deposited: Holotype USNM 1642499; 2 paratypes USNM 1642450-1.

Sequence deposited: GenBank No. MW586927.

ZooBank Life Science Identifier: urn:lsid:zoobank.org:pub:00977A33-A27A-4150-9385-7AF29064523C.

Etymology: The species is named for the country from which it was originally collected.

3.1.2. Remarks

Emoleptalea mozambiquensis n. sp. conforms to the diagnosis for the

Cephalogonimidae in having a small oval body, spinous tegument, genital pore at anterior extremity, and being parasitic in the digestive tract of a freshwater fish (Jones and Bray, 2008). We placed the new species in *Emoleptalea* because the excretory vesicle lacks lateral diverticula, testes are oblique, the vitelline follicles are in lateral groups that span the region of the ventral sucker, and anterior tegumental spines are not enlarged and do not form a circumoral circlet. The new species has an amphitypic ovary (Fischthal and Kuntz, 1963). Two of the three specimens had the ovary on the right side of midline and the third had the ovary on the left side of midline. The cirrus sac approached the genital atrium from the left side of the body, the anterior testis was on the left side, and the seminal receptacle on the right in the two similar specimens (Fig. 1). In contrast, the positions of these same gonadal organs were reversed in the single specimen with the ovary on the right side (Figs. 2 and 3).

There were nine accepted species belonging in *Emoleptalea*, all of which infect freshwater catfishes as adults. The discovery of *E. mozambiquensis* in a non-catfish host is remarkable but considering that only three specimens were collected from a single infected fish we cannot rule out the possibility that *N. furzeri* represents an accidental host and additional survey of a broad variety of fishes from the type-locality should be conducted. Four of the accepted species were described and are known from Africa: *Emoleptalea exilis* (Looss, 1899) Looss, 1900, *Emoleptalea synodontidos* Dollfus, 1950, *Emoleptalea rifaati* (Ramadan, Saoud & Taha, 1987) Jones & Bray, 2008, and *Emoleptalea nwanedi* King, Smit, Baker & Luus-Powell, 2018. Five species were described and are known from India: *Emoleptalea horai* (Gupta, 1955) Jones & Bray, 2008, *Emoleptalea dollfusi* Srivastava, 1960, *Emoleptalea loossi* Srivastava (1960), *Emoleptalea hardayali* (Kumar & Agrawal, 1980) Jones and Bray (2008), and *Emoleptalea kanungoi* (Agrawal and Agrawal, 1985) Jones & Bray, 2008. *Emoleptalea mozambiquensis* is herein differentiated from eight congeners using combinations of features, including body size and shape, characteristics of suckers, gonads, digestive system, and position of the genital pore. We refrain from making comparisons with *E. dollfusi* and discuss this below.

Emoleptalea mozambiquensis differs from the type-species, *E. exilis*, which infects the bayad, *Bagrus bajad* (Forsskål) in the Nile River, Egypt, by having a more oval, less elongated body, oral sucker slightly smaller rather than much larger than the ventral sucker, shorter forebody representing ~ 28% rather than ~ 35% of body length, and more elongated bands of irregular vitelline follicles extending well into the testicular zone rather than condensed bands of elongated follicles confined posteriorly to the ovarian zone (Looss, 1899).

Emoleptalea mozambiquensis resembles *E. synodontidos*, which infects the onespot squeaker, *Synodontis notatus* Vaillant, in the Democratic

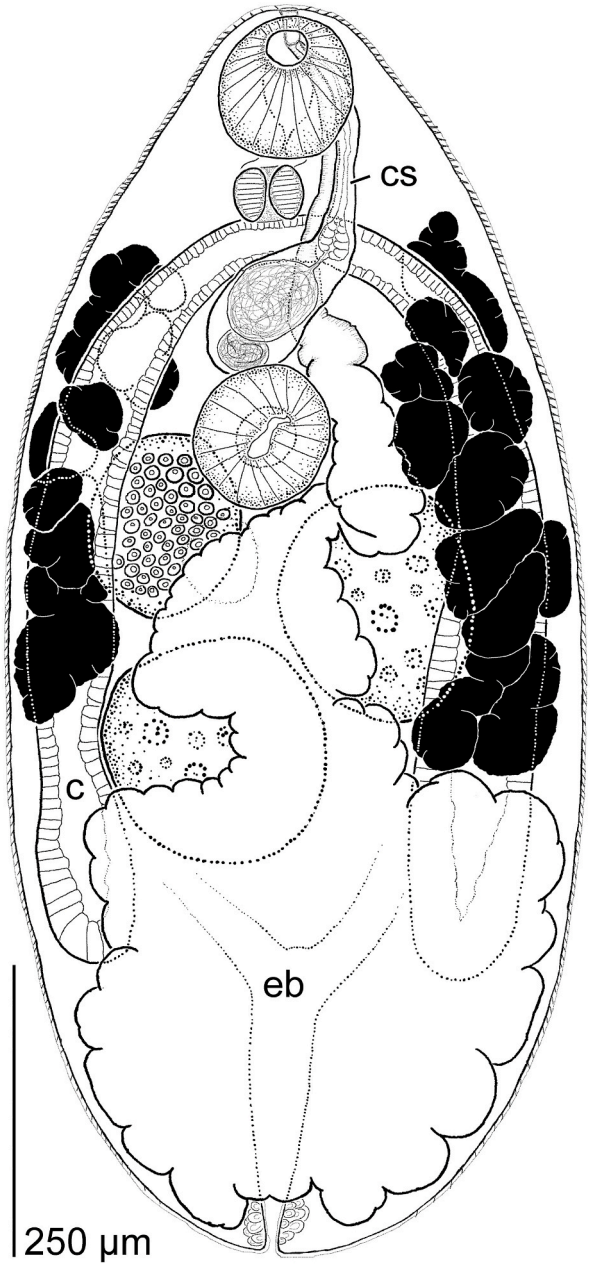

Fig. 1. *Emoleptalea mozambiquensis* n. sp. from intestine of turquoise killifish, *Nothobranchius furzeri* Jubb. Ventral view of holotype. Eggs are omitted from uterus and outline of uterus does not show extensive coiling. Abbreviations: caecum, c; cirrus sac, cs; excretory bladder, eb.

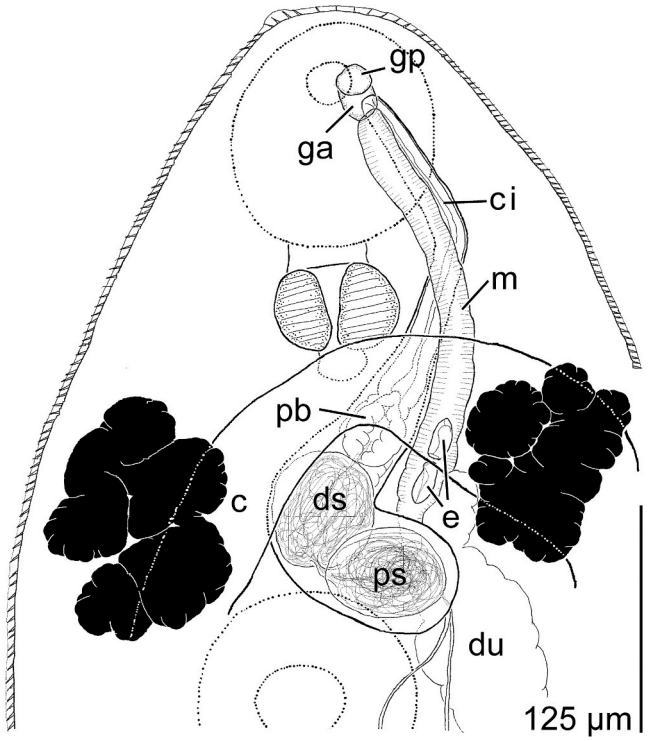

Fig. 2. *Emoleptalea mozambiquensis* n. sp. from intestine of turquoise killifish, *Nothobranchius furzeri* Jubb. Dorsal view of anterior end. Note the caeca are surrounded by the vitelline follicles and the terminal genitalia run ventral relative to caeca. This specimen has the ovary on the left side of body. Abbreviations: caecum, c; cirrus, ci; distal portion of seminal vesicle, ds; distal uterus, du; egg, e; genital atrium, ga; genital pore, gp; prostatic bulb, pb; proximal portion of seminal vesicle, ps.

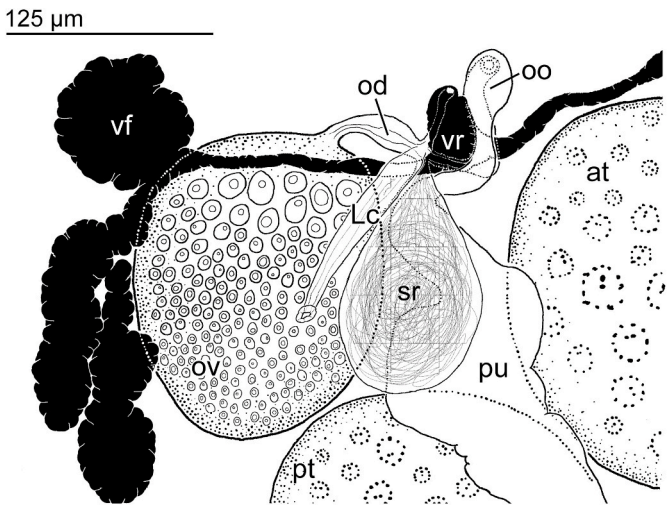

Fig. 3. *Emoleptalea mozambiquensis* n. sp. from intestine of turquoise killifish, *Nothobranchius furzeri* Jubb. Dorsal view of ovarian complex. Mehlis' gland cells and eggs are omitted. Abbreviations: anterior testis, at; Laurer's canal, Lc; ovary, ov; oviduct, od; ootype, oo; posterior testis, pt; proximal uterus, pu; seminal receptacle, sr; vitelline follicle, vf; vitelline reservoir, vr.

Republic of the Congo, but may be differentiated by having a smaller body (1050–1160 µm compared with 1770 µm long), much shorter oesophagus (10 µm compared with 48 µm long), and most significantly, the genital pore opens dorso-median near the anterior end of the body instead of submedian on the ventral surface at a level even with the posterior margin of the ventral sucker (Dollfus, 1950).

Emoleptalea mozambiquensis differs from *E. horai*, which infects the stinging catfish, *Heteropneustes fossilis* (Bloch), in India, by having a subspherical rather than a funnel-shaped oral sucker, caeca extending into the post-testicular space rather than limited to the posterior margin of the ovary, and by having the genital pore opening dorso-median near the anterior end of the body instead of ventro-lateral on the left side at the level of the pre-pharynx (Gupta, 1955).

Emoleptalea mozambiquensis resembles *E. loossi*, which was described on the basis of specimens from *H. fossilis* in India, but may be differentiated by the near absence of a prepharynx and oesophagus (20–25 µm long and 0–10 µm long, respectively) compared with each being about the pharynx length, a shorter forebody (~28% compared with ~34% of body length), genital pore not submedian at the anterodorsal margin of oral sucker, and having more extensive vitelline bands that span the ventral sucker region and enter the forebody compared with being confined anteriorly at mid-ventral sucker level (Srivastava, 1960).

Emoleptalea mozambiquensis differs from *E. hardayali*, which infects the striped dwarf catfish, *Mystus vittatus* (Bloch), in India, by having oblique rather than tandem testes and the genital pore opening dorso-median near the anterior end of the body instead of ventrally in the pharyngeal zone (Agrawal and Agrawal, 1985).

Emoleptalea mozambiquensis differs from *E. kanungoi*, which infects the freshwater bagrid catfish *Rita rita* (Hamilton), in India, by having a subspherical rather than a funnel-shaped oral sucker, a short oesophagus, caeca extending into the post-testicular space rather than limited to the posterior margin of the testes, and a genital pore opening dorso-median near the anterior end of the body instead of laterally on the right side at the mid-level of the oral sucker (Agrawal and Agrawal, 1985).

Emoleptalea mozambiquensis resembles *E. rifaati*, which infects two mochokid catfishes (*Synodontis schall* [Bloch & Schneider] and *Synodontis serratus* Rüppell), in the Nile River Delta, Egypt, but may be differentiated by having a subspherical rather than funnel-shaped oral sucker, having the oral sucker slightly smaller than ventral sucker rather than the reverse condition (oral to ventral sucker width ratio 1:1.01–1.17 compared with 1.07–1.16:1 in *E. rifaati*), testes more anterior in the hindbody (post-testicular space 31–34% of body length) compared with ~19% in *E. rifaati*, and having vitelline bands extending well into the forebody compared with limited to the anterior margin of the ventral sucker in *E. rifaati* (Ramadan et al., 1987).

Emoleptalea mozambiquensis differs from *E. nwanedi*, which infects the silver catfish, *Schilbe intermedius* Rüppell, in Limpopo Province, South Africa, by having a larger body (1050–1160 µm compared with 582–722 µm long), a subspherical rather than elongated oral sucker, seminal receptacle smaller rather than larger than ovary, and the genital pore opens dorso-median near the anterior end of the body instead of submedian at the lateral edge of the ventral sucker as in *E. nwanedi* (King et al., 2018).

3.2. Genus *Masenia* Chatterji, 1933

Masenia baroensis n. sp.

3.2.1. Description (Figs. 4 and 5)

[Based on 10 mature specimens] Body, oval, small, 660–854 long, 335–450 wide near midbody. Tegument covered by robust slightly recurved spines except near posterior end; body spines 8–11.5 long. Oral sucker funnel-shaped, terminal with subterminal mouth opening, 108–140 long, 105–153 wide, with opening surrounded by two rows of alternating elongated conical spines; rows containing 35–36 spines each (usually a total of 72 but spines may have dislodged in some specimens), interrupted dorso-terminally. Circumoral spines generally slightly larger than body spines; oral row 8–14 long; aboral row 8–11.5 long. Prepharynx directing dorsally and diagonally from base of oral sucker, very short, 5–20 long in two measurable specimens. Pharynx nearly spherical, 40–53 long, 40–55 wide. Oesophagus directing dorsally, very short, 10 long in a single measurable specimen. Ventral sucker nearly circular, 108–208 long, 128–208 wide. Oral to ventral sucker width ratio 1:1.1–1.3 in 9 specimens without pressure (1:1.6 in single compressed specimen). Forebody 150–250 long or 22–30% of body length (nine unflattened specimens). Intestine bifurcating in forebody immediately anterior to ventral sucker, caeca blind, extending to vicinity of posterior margin of posterior testis.

Testes subglobular, oblique, contiguous or overlapping, intercaecal; anterior testis submedian either on left or right side of body 88–165 long, 93–180 wide, posterior testis 98–160 long, 113–160 wide. Post-testicular space 170–220 long (nine measured) or 23–29% of body length. Cirrus sac club-shaped, sigmoidal, extending to posterior margin of ventral sucker, 300–430 long (eight measured), 75–165 wide (eight measured at widest portion across proximal seminal vesicle). Cirrus sac containing bipartite seminal vesicle, pars prostatica, and unarmed cirrus. Proximal portion of seminal vesicle 13–75 long, 25–55 wide (five measured), distal portion always larger, 75–135 long, 56–70 wide (six measured). Pars prostatica lacking diverticulum or well-defined prostatic bulb, 50–100 long, 33–70 wide (six measured). Cirrus elongate, 100–188 long (six measured). Cirrus sac communicating with small, elongated genital atrium; genital atrium dorsal to oral sucker, median, 20–30 long, 10–13 wide (six measured). Genital pore opening dorso-median, immediately posterior to terminal interruption of oral spines.

Ovary distinctly 2–4 lobed, submedian, amphitypic on the same side as posterior testis, at level of anterior testis but extending slightly anterior relative to it, sometimes overlapping anterior testis ventrally, 93–150 long, 63–120 wide. Ootype surrounded by Mehlis' gland immediately adjacent and median relative to ovary (exact configuration obscured by eggs in uterus in all specimens). Seminal receptacle dorso-median to and immediately adjacent to ovary, ventral relative to and overlapping Mehlis' gland, 50–128 long, 38–68 wide (three measured). Laurer's canal not observed. Vitellarium comprised of two lateral groups of 7–12 large subglobular to irregular-shaped follicles; follicles surrounding caeca, groups confined to region of ventral sucker. Vitelline reservoir transversely oval shaped, 38–50 long, 28–38 wide (two

measured), immediately antero-ventral to seminal receptacle, submedian on same side as ovary. Proximal uterus dorso-median, descending from ovarian complex, coiling, and occupying most of hindbody. Distal uterus ascending ventrally on abovarian side, following curvature of cirrus sac, and communicating with indistinct metraterm; metraterm dorsal and parallel with cirrus sac, thin-walled, connecting with posterior end of genital atrium. Eggs filling uterus, oval, operculated, 23–28 long, 15–19 wide (20 measured from distal region of uterus).

Excretory bladder Y-shaped, main stem (visible in one specimen), 205 long or 27% of body length, terminating 18 posteriorly from posterior margin of posterior testis. Excretory system opening through excretory pore at terminal end.

Type host: globe fish, *Tetraodon lineatus* L., (Tetraodontiformes: Tetraodontidae).

Site in host: intestine.

Type locality: Niandan River, Republic of Guinea.

Other locality: Niger River, Republic of Guinea.

Prevalence: Six worms from one of two fish examined from the Niger River; four worms from one of three fish examined from Niandan River.

Specimens deposited: Holotype USNM 1642452; 9 paratypes USNM 1642453-1642461.

Sequence deposited: GenBank No. MW586925.

ZooBank Life Science Identifier: urn:lsid:zoobank.org:pub:9C6-BAB41-1974-41C3-ACCA-554730276E8A.

Etymology: The species is named for the town of Baro, Republic of Guinea, which is surrounded by the drainage from which the specimens were collected.

3.2.2. Remarks

Masenia baroensis n. sp. conforms to the diagnosis for the Cephalogonimidae in having a small, oval body, spinous tegument, genital pore at the anterior extremity, and being parasitic in the digestive tract of a freshwater fish (Jones and Bray, 2008). We placed the new species in *Masenia* because it has two circumoral rows of approximately 35–36 spines each (72 total), encircling the oral sucker. Just as in the previously described species, we observed amphitypic orientation of the ovary and gonadal systems in *M. baroensis*. Specimens having the ovary on the left side have the anterior testis on the right side and the cirrus sac and metraterm approach the genital atrium from the right side of the body (Fig. 4). In contrast, specimens having the ovary on the right side have the anterior testis on the left side and the cirrus sac and metraterm approach the genital atrium from the left side of the body (Fig. 5).

Prior to this study, *Masenia* contained 24 accepted species, with five confined to infecting freshwater catfishes in Africa, and approximately 19 species confined to infecting Asian fishes (12 limited to freshwater catfishes, two infecting a snakehead species [Channidae] in India, and four infecting marine fishes in Indian coastal waters) (Chandra and Saxena, 2016; Madhavi and Bray, 2018; Scholz et al., 2018; Dumbo et al., 2019a). The accepted African species are: *Masenia proteropora* (Thomas, 1958) Kudlai, Scholz & Smit, 2018, which infects a clariid catfish, *Clarias anguillaris* (L.), in Ghana; *Masenia bangweulensis* (Beverly-Burton, 1962) Kudlai, Scholz & Smit, 2018, which infects the clariid *Clarias ngamenis* Castelnau, in Zambia; *Masenia ghanensis* (Fischthal & Thomas, 1968) Kudlai, Scholz & Smit, 2018, which infects a clariid, *Heterobranchus longifilis* Valenciennes, in Ghana; *Masenia synodontis* (Khalil & Thurston, 1973) Kudlai, Scholz & Smit, 2018, which infects a mochokid, *Synodontis victoriae* Boulenger, in Lake Victoria; and *Masenia nkomatiensis* Dumbo, Dos Santos & Avenant-Oldewage, 2019, which infects a clariid, *Clarias gariepinus* (Burchell), in Mozambique.

Masenia baroensis is unique among its African congeners in having a non-catfish host, a distinctly 3–4 lobed ovary, and has an exceptionally high number of circumoral spines (72). The ovary is entire in *M. bangweulensis*, *M. ghanensis*, *M. proteropora*, and *M. nkomatiensis* but may be slightly lobed in *M. synodontis* (see Khalil and Thurston, 1973). *Masenia proteropora* has 50–58 circumoral spines (Dumbo et al., 2019a). *Masenia bangweulensis* has 48 circumoral spines (Beverley-Burton, 1962). *Masenia ghanensis* has 56 circumoral spines (Fischthal and Thomas, 1968). *Masenia synodontis* has 36–40 circumoral spines (Khalil and Thurston, 1973). *Masenia nkomatiensis* has 50 circumoral spines (Dumbo et al., 2019a). *Masenia baroensis* differs further from *M. proteropora* and *M. synodontis* in having a funnel-shaped rather than subspherical oral sucker (see Thomas, 1958a; Khalil and Thurston, 1973), and uniquely among the African congeners, *M. synodontis* has a ventral sucker smaller than the oral sucker (Khalil and Thurston, 1973).

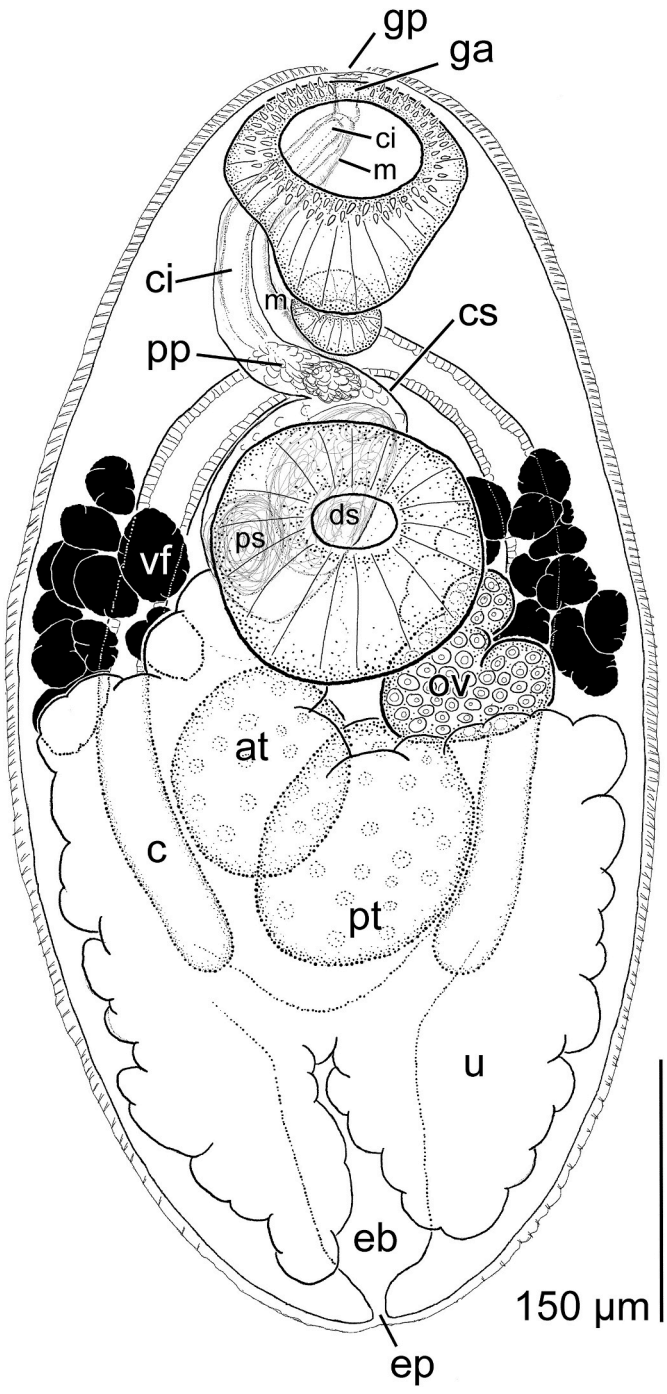

Fig. 4. *Masenia baroensis* n. sp. from the intestine of the globe fish, *Tetraodon lineatus* L. Ventral view of holotype. Abbreviations: anterior testis, at; caeca, c; cirrus, ci; cirrus sac, cs; excretory bladder, eb; distal portion of seminal vesicle, ds; excretory pore, ep; genital atrium, ga; genital pore, gp; metraterm, m; ovary, ov; pars prostatica, pp; proximal portion of seminal vesicle, ps; posterior testis, pt; uterus (drawn with eggs omitted), u; vitelline follicle, vf.

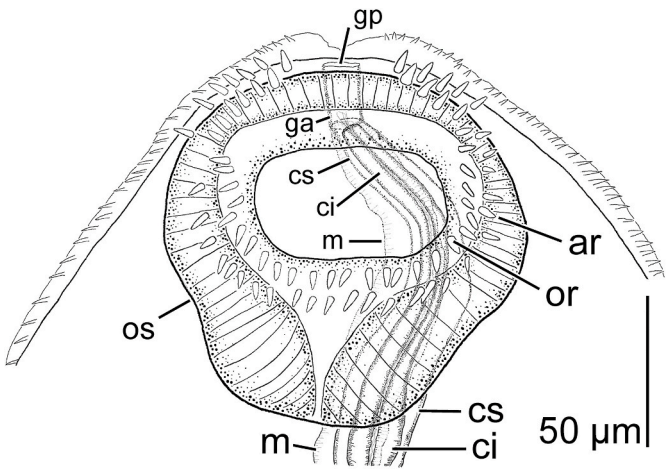

Fig. 5. *Masenia baroensis* n. sp. from the intestine of the globe fish, *Tetraodon lineatus* L. Anterior portion from a specimen having the ovary on the right side of the body. Abbreviations: aboral row of circumoral spines, ar; cirrus, ci; cirrus sac, cs; genital atrium, ga; genital pore, gp; metraterm, m; oral row of circumoral spines, or; oral sucker, os.

There is considerable confusion related to the taxonomy of Asian species in *Masenia*. Jones and Bray (2008) and Dumbo et al. (2019a) accepted 18 Asian species of *Masenia* and the latter study listed the hosts and countries from which they were described. Chandra and Saxena (2016) accepted the additional species *Masenia dayali* (Gupta & Puri, 1984) Chandra & Saxena, 2016, and we address that species in the discussion. Herein we accept 17 Asian species belonging in *Masenia* and compare *M. baroensis* with 15 of those. This decision is explained in the discussion. We differentiated *M. baroensis* from the 15 Asian congeners using circumoral spine count, sucker width ratio, configuration of the vitellarium, body shape, features of the male terminal genitalia, and extent of the caeca in the hindbody.

In having ~72 circumoral spines, *M. baroensis* is easily differentiated from the following eight Asian congeners: *Masenia collata* Chatterji, 1933 (~53 circumoral spines), *Masenia moradabadensis* (Srivastava, 1951) Dumbo, Dos Santos & Avenant-Oldewage, 2019 (52 circumoral spines), *Masenia dayali* Gupta, 1955 (~55 circumoral spines), *Masenia fossilisi* Gupta, 1955 (~52 circumoral spines), *Masenia vittatusia* Agrawal, 1963 (52 circumoral spines), *Masenia fukienensis* (Tang & Lin, 1973) Dumbo, Dos Santos & Avenant-Oldewage, 2019 (50–64 circumoral spines), *Masenia quiloni* (Gupta & Tandon, 1984) Madhavi, 2011 (58 circumoral spines), *Masenia gwaliorensis* (Bhadauria & Dandotia, 1986) Dumbo, Dos Santos & Avenant-Oldewage, 2019 (56 circumoral spines) (see Chatterji, 1933; Srivastava, 1951; Gupta, 1955; Agrawal, 1963; Tang and Lin, 1973; Bhadauria and Dandotia, 1986; Madhavi and Bray, 2018). In having the oral sucker smaller than the ventral sucker, *M. baroensis* is differentiated from two more Asian species (both marine) that have an oral sucker larger than ventral sucker: *Masenia orissai* Gupta & Tandon, 1985, *Masenia upeneusi* Gupta & Puri, 1984 (see Gupta and Tandon, 1985; Gupta and Puri, 1984). The distribution of the vitelline follicles serves to differentiate *M. baroensis* from two additional Asian congeners. *Masenia chauhani* Agarwal & Singh, 1989, and *Masenia jaunpurensis* Maurya & Singh, 2004 both have vitelline follicles extending anteriorly to the oesophageal level (see Agrawal, 1963; Dumbo et al., 2019a), whereas the vitelline follicles are confined to clusters on either side of the ventral sucker in *M. baroensis*. *Masenia baroensis* differs from both *Masenia gomtia* Agrawal, 1963 and *Masenia ritai* Sircar & Sinha, 1970 (name discussed below) by having a broader, less elongated body (ratio of body length to width ~1: 0.5 in *M. baroensis* compared with ~1: 0.25 in *M. gomtia* and ~1: 0.038–0.39 in *M. ritai*). Additionally, the proximal part of the bipartite seminal vesicle is smaller than the distal part in *M. baroensis*; whereas the opposite is true for both *M. gomtia* and *M. ritai* (see Agrawal, 1963; 1964; Sircar and Sinha, 1970). *Masenia baroensis* superficially resembles the fifteenth and final Asian congener compared, the marine species *Masenia carangai* Gupta & Tandon, 1985, which was described and remains known based on a single specimen that infected jack (*Carangoides armatus* [Ruppell]) in the Bay of Bengal on the northeastern coast of India. Both species have approximately 72 circumoral spines, similar oral to ventral sucker width ratios, similar anatomy of the male terminal genitalia (proximal portion of bipartite seminal vesicle is smaller than distal), and similar configurations of the vitellarium. Never-the-less, *M. baroensis* differs from *M. carangai* by having a relatively wider, less elongated body (ratio of body length to width ~1: 0.5 compared with ~1: 0.26 in *M. carangai*), and the caeca terminate further in the hindbody in *M. baroensis* (to the post-testicular zone rather than middle of the testicular zone in *M. carangai*) (see Gupta and Tandon, 1985).

3.3. Genus Heterorchis Baylis, 1915

Heterorchis cf. *crumenifer* Baylis, 1915

3.3.1. Description (Figs. 6 and 7)

[Based on 12 mature specimens] Body oval, 1600–2900 long, 580–1125 wide near midbody. Tegument and suckers entirely covered by robust scale-like spines. Oral sucker subglobular, subterminal, 125–250 long, 173–300 wide. Prepharynx, very short, 18–50 long (seven measured), surrounded by muscular, lip-like rim emerging from anterior margin of pharynx. Pharynx dolioform, 78–135 long, 88–130 wide, surrounded by gland cells. Oesophagus present, longer than prepharynx, 18–85 long. Ventral sucker nearly circular, 290–470 long, 268–490 wide. Oral to ventral sucker width ratio 1:1.5–1.8 (10 specimens). Forebody 320–720 long, or 20–26% of body length. Intestine bifurcating at level about midway between suckers, caeca blind, extending to near posterior region of hindbody. Postcaecal space 125–260 long or 6–11% of body length.

Testes elongate, opposite, intercaecal in middle of hindbody; right testis 420–710 long, 130–210 wide, left testis 320–700 long, 120–190 wide; right testis longer than left testis (n = 7), wider than left testis (n = 9), usually extending more posteriorly than left testis (90%, n = 39). Post-testicular space 400–850 or 21–33% of body length. Cirrus sac with anterior end curving sharply toward left side, extending posterior past ventral sucker, 500–1000 long, 110–140 wide, containing bipartite seminal vesicle, pars prostatica, and elongated bending or coiling cirrus. Proximal portion of seminal vesicle always larger than distal portion; proximal portion 188–245 long, 68–105 wide; distal portion 78–135 long, 55–80 wide. Prostatic bulb longer than wide, 65–133 long, 28–125 wide. Cirrus 280–360 long, 23–45 wide, extruded in two specimens; extruded cirrus with irregular surface, possibly caused by minute spines. Cirrus sac emptying into elongated tubular genital atrium on left side; genital atrium submarginal, 50–133 long, 28–63 wide. Genital pore slightly submarginal, opening on left side at level of intestinal bifurcation.

Ovary, distinctly 4–7 lobed, elongate, submedian on right side, immediately anterior to and usually contiguous with right testis, 235–480 long, 125–250 wide. Seminal receptacle usually overlapping or immediately adjacent to left edge of ovary, with posterior margin extending slightly more posterior than ovary, subspherical, 93–155 long, 90–133 wide (n = 5), or transverse elongated, 88–135 long, 58–83 wide (n = 3). Ootype and Mehlis' gland immediately adjacent and median to anterior half of ovary. Laurer's canal not observed. Vitellarium comprised of two lateral groups of irregularly shaped follicles; follicles extra-caecal and surrounding caeca, extending from mid-level of ventral sucker to a level approximately 2/3 of body length from the anterior end. Vitelline reservoir median, triangular to digitiform, 38–88 long, 15–50 wide (seven measured), communicating with oviduct. Uterus occupying most of ventral hindbody. Proximal uterus descends from ootype in dorsal hindbody toward the posterior end, with coils

expanding in posterior hindbody prior to ascending in ventral hindbody and communicating with prominent, highly muscular metraterm. Metraterm surrounded by gland cells, situated immediately adjacent to cirrus sac and following contour of cirrus on left side; proximal portion 175–400 long, 25–50 wide, surrounded by thin layer of gland cells (10 measured, width measurement not including gland cells); distal portion 200–400 long, 30–90 wide (nine measured, width measurement not including gland cells); entire metraterm 375–725 long (nine measured). Eggs filling uterus, operculated, 20–30 long, 10–15 wide (28 measured from distal region of uterus near junction of metraterm).

Excretory bladder Y-shaped, main stem an enormous tear-shaped swollen bladder occupying much of dorsal hindbody, extending anteriorly into ovarian level. Main collecting ducts immediately swelling near junction with main stem, forming oval to elongate bladders, 125–415 long, 38–75 wide (seven measured); bladders reverting to narrow main collection ducts 15–25 in diameter, extending anteriorly and laterally in forebody to level of oesophagus then descending posteriorly in lateral forebody. Excretory pore, dorsal, comprising an enormous dorsal circular opening, 210–510 long, 210–620 wide. Posterior margin of dorsal opening at 220–575 from posterior end or 13–19% of body length.

Type host: marbled lungfish, *Protopterus aethiopicus* Heckel, 1851, (Lepidosireniformes: Protopteridae).

Other hosts: West African lungfish, *Protopterus annectens* (Owen, 1839) (Dollfus, 1929, 1950; present study).

Site in host: intestine.

Prevalence: Eighty-four worms were collected from four of six fish examined; mean intensity = 21 +/− 19 (standard deviation).

Type locality: Lake Victoria, Uganda.

Other localities: Maroua, Cameroon (Dollfus, 1929); Ogooué River, Democratic Republic of the Congo (Dollfus, 1950); Karingani Game Reserve, Mozambique (present study).

Specimens deposited: 10 voucher specimens (USNM 1642462-1642471).

Sequence deposited: GenBank No. MW586924.

3.3.2. Remarks

The unique condition of the excretory system of these worms allowed us to easily identify them as belonging in the enigmatic genus *Heterorchis*. Thus far, four species, all from Africa, have been described in the genus. *Heterorchis crumenifer* (type species) was originally described infecting the intestine of a specimen of *P. aethiopicus* collected from Ugandan waters of Lake Victoria (Baylis, 1915). Dollfus (1950) reported *H. crumenifer* infecting the intestine of *P. annectens* from Democratic Republic of the Congo and provided a description for two specimens in his collection. Dollfus (1929) discovered juvenile forms of a worm that he described as *Distoma protopteri* Dollfus, 1929 infecting the intestine of *P. annectens* collected at Maroua, Cameroon. Dollfus (1950) later considered these forms to represent a synonym of *H. crumenifer*, but it should be noted that this identification (based on immature traits) must be considered tentative. Our material and adult material studied by Baylis (1915) and Dollfus (1950) compare favorably except that Baylis (1915) reported egg size of 40 μm by 20 μm, which is substantially larger than eggs we observed (20–30 by 10–15 μm) and sizes reported by Dollfus (1950), (28–31 by 15–16 μm). Baylis (1915) deposited the original type material for *H. crumenifer* in the Natural History Museum, London, UK, but we were not able to access the museum during preparation of the manuscript in 2019–20 to confirm the egg size in specimens. The present identity of our material should therefore remain tentative as (*Heterorchis* cf. *crumenifer*), until a thorough comparison with the type material can be made and egg sizes from the type material can be confirmed. Three other species have been described in *Heterorchis* since its erection.

Heterorchis protopteri Thomas, 1958 was described from the intestine of *P. annectens* collected from the Volta River, Ghana. Thomas (1958b) fixed his specimens using “slight coverslip pressure” and differentiated his specimens from *H. crumenifer* by them having a fully marginal genital pore, relatively larger ventral sucker (oral to ventral sucker width ratio of 1:1.8) compared with 1:1.5 (Baylis, 1915), 1:1.4–1.6 (Dollfus, 1950) and 1.1.5–1.8 (present study), posterior margin of testes being pointed rather than more rounded, slightly more posterior seminal receptacle, and egg size. Except for egg size, we think the differences of specimens described by Thomas (1958b) can all be produced by adding pressure. We could not identify any useful features for differentiating *H. protopteri* from *H. crumenifer*. Consequently, we consider *H. protopteri* a *species inquirenda*.

Heterorchis ghanensis Fischthal & Thomas, 1968 was described based on a single specimen infecting the intestine of a frog (*Hyperolius nitidulus* Peters) collected from Accra, Ghana. Fischthal and Thomas (1968) believed that this specimen differed from *H. crumenifer* and *H. protopteri* because they observed a connection between the caeca and excretory bladder. This remarkable feature needs confirmation. Otherwise, we find the description overlaps that of *H. crumenifer*. We consider *H. ghanensis* a *species inquirenda*.

Heterorchis senegalensis Vassiliadès & Richard, 1970 was described infecting the intestine of *P. annectens* from Dakar, Senegal. Vassiliadès and Richard (1970) described this species based on well-fixed specimens lacking pressure and considered it to differ from the three other named species based on testis shape and testis size and placed emphasis on the left testis being the more posterior one vs it being the more anterior one in the three previously named species. We observed the left testis extending more posteriorly than the right one in 10% of the specimens of *H.* cf. *crumenifer* we collected. While this trait is therefore not effective for differentiating species of *Heterorchis*, we do consider *H. senegalensis* distinguishable from *H. crumenifer* on the basis that the caecal extent is further posterior in *H. crumenifer* (to near the posterior end of the body or 6–11% of body length) compared with slightly beyond the posterior margin of posterior testis or ~21% of body length in *H. senegalensis* (Baylis, 1915; Dollfus, 1950; Vassiliadès and Richard, 1970).

3.4. Molecular results

Single sequences of the lsrDNA gene were generated for *E. mozambiquensis* n. sp. (1586 bp), and *M. baroensis* n. sp. (1252). Two sequences of the lsrDNA gene were generated for *H.* cf. *crumenifer* (identical over 1577 bases). The new lsrDNA sequence generated from *M. baroensis* differed from that of *M. nkomatiensis* (GenBank No. MH142268) at 51 positions (including gaps) (4%) when aligned across 1251 bases plus gaps.

3.5. Phylogenetic results

The phylogram inferred by the Bayesian analysis of fragments of the lsrDNA consists of three strongly supported clades, each represented by the superfamilies Gorgoderoidea, Microphalloidea, and Plagiorchioidea, respectively (Fig. 8). Herein, the Cephalogonimidae is represented by three previously available sequence fragments (two species in *Cephalogonimus* Poirier, 1886, and *M. nkomatiensis*), plus both new species (*E. mozambiquensis* and *M. baroensis*). These five species formed a strongly supported clade within the Plagiorchioidea. Both new taxa are closely related and formed a polytomy with a third African species (*M. nkomatiensis*) from a catfish. The other two species, *Cephalogonimus retusus* Walton, 1938 from Bulgaria and *Cephalogonimus americanus* Stafford, 1902 from Mexico, both from frogs, formed a closely related pair on their own. *Heterorchis* cf. *crumenifer* represents a distinct branch among a large polytomous group of plagiorchioid families (Auridistomidae Lühe, 1901, Cephalogonimidae, Choanocotylidae Jue Sue & Platt, 1998, Glypthelminthidae Cheng, 1959, Haematoloechidae Freitas & Lent, 1939, Leptophallidae Dayal, 1938, Macroderoididae McMullen, 1937, Orientocreadiidae Yamaguti, 1958, Reniferidae Platt, 1902, and Telorchiidae Looss, 1899). The topology confirms that *H.* cf. *crumenifer* is a plagiorchioid but its affinity for any one family remains ambiguous.

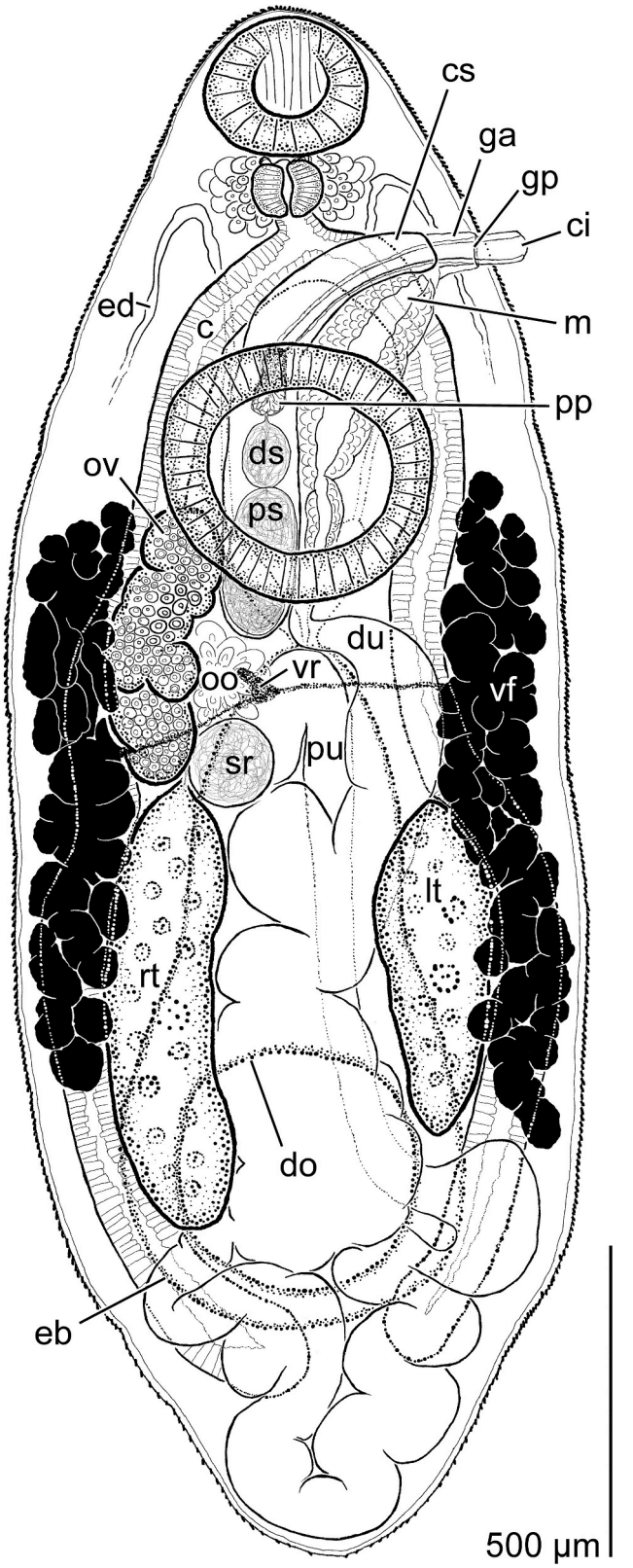

Fig. 6. Ventral view of *Heterorchis* cf. *crumenifer* from the intestine of the West African lungfish, *Protopterus annectens* (Owen). Abbreviations: caecum, c; cirrus, ci; cirrus sac, cs; dorsal opening to excretory bladder, do; distal portion of seminal vesicle, ds; distal uterus, du; excretory bladder, eb; excretory duct, ed; genital atrium, ga; genital pore, gp; left testis, lt; metraterm, m; ootype, oo; ovary, ov; pars prostatica, pp; proximal portion of seminal vesicle, ps; proximal uterus, pu; right testis, rt; seminal receptacle, sr; vitelline follicle, vf; vitelline reservoir, vr.

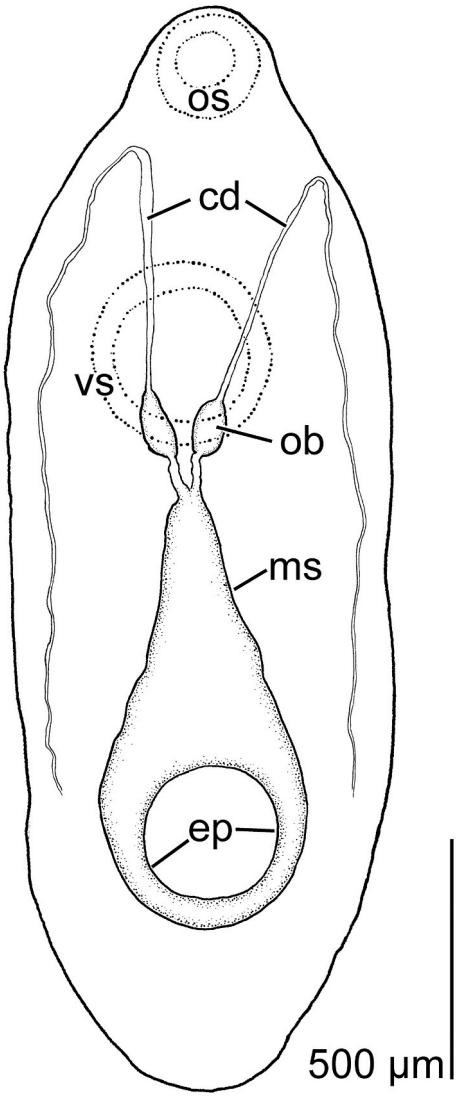

Fig. 7. Dorsal view of excretory system of *Heterorchis* cf. *crumenifer* from intestine of the West African lungfish, *Protopterus annectens* (Owen). Abbreviations: cd; collection ducts, ep; excretory pore, ms; main stem of excretory bladder, ob; oval or elongated bladders, os; oral sucker, vs; ventral sucker.

4. Discussion

4.1. Taxonomy related to the new cephalogonimid species

Emoleptalea has species ranging in Africa and South Asia. *Masenia* spp. range in Africa, South Asia, and eastern Asia. Species in both genera have thus far been reported to have limited biogeographical distributions in either Africa or Asia with no crossover. Nevertheless, it is desirable to compare new taxa with all congeners whenever possible in naming new taxa. In the present study this was possible for all African taxa but the task of making differential comparisons between the new species and all Asian congeners was complicated. We encountered major taxonomic problems with one Asian species of *Emoleptalea*, (*Emoleptalea dollfusi* Srivastava, 1960), and several Asian species of *Masenia* that thwarted a complete differential comparison. Furthermore, type materials from species in *Emoleptalea* and *Masenia* from Asia are not deposited in any lending museum. Remarkably, only five species belonging in either *Emoleptalea* or *Masenia* are represented by vouchered specimens: Gupta (1955) deposited type specimens for *E. horai*, *M. dayali*, and *M. fossilisi* in G.S. Thapar's personal Helminthology Collection, Lucknow University, India; Sircar and Sinha (1970) deposited specimens of *M.*

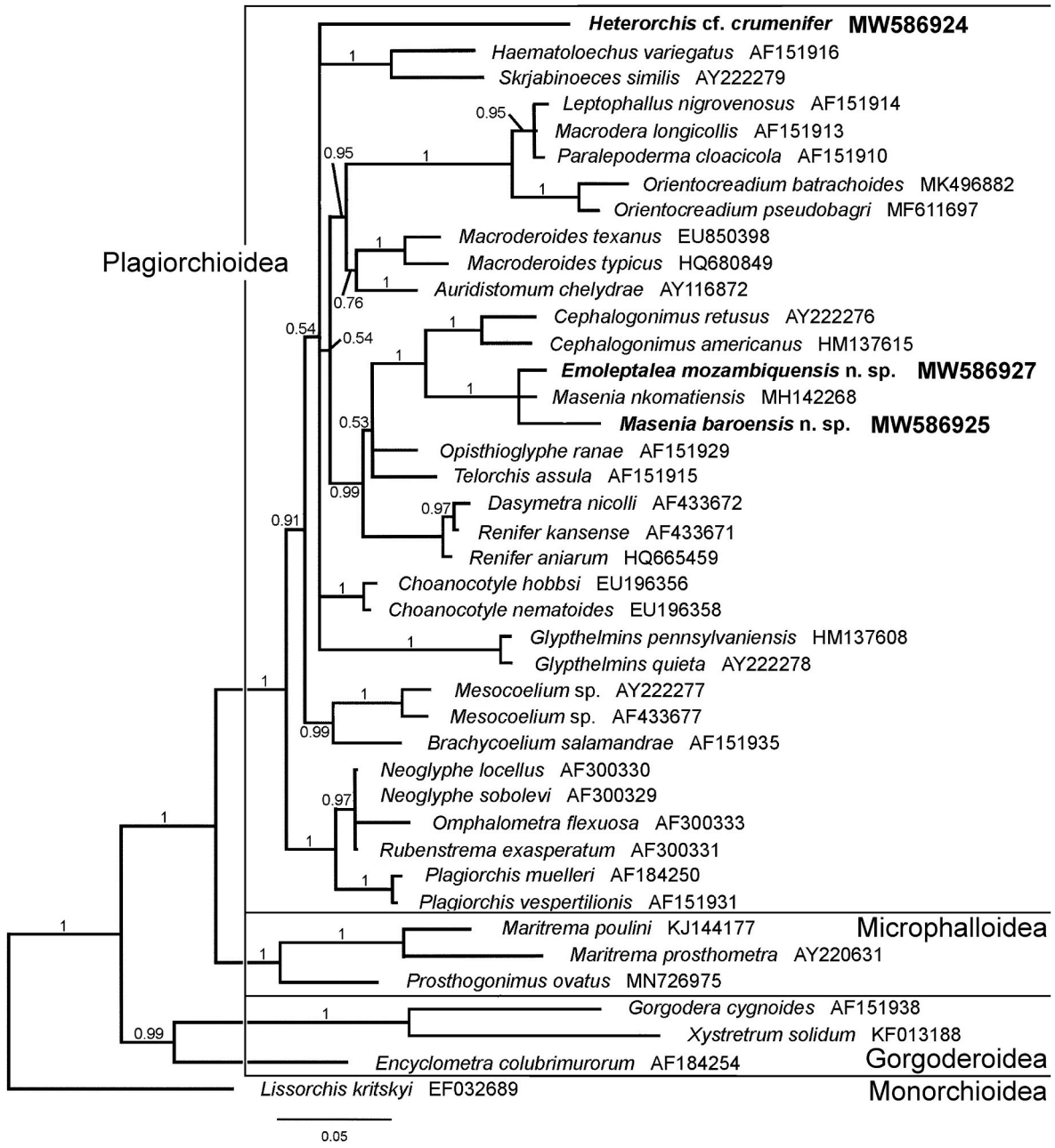

Fig. 8. Estimated phylogeny inferred from Bayesian analysis of aligned fragments of the lsrDNA sequences from 40 species of digeneans comprising the ingroup plus one outgroup taxon (*Lissorchis kritskyi*). Species names are followed by the GenBank accession number for the sequence. New sequences represented by the three studied species are in bold. Posterior probabilities are reported on branches. Scale bar indicates a 5% nucleotide difference.

ritai in the helminthology collection of the Department of Zoology, Science College, Patna University; and Gupta and Puri (1984) deposited types for *M. upeneusi* in G.S. Thapar's personal collection. Neither collection is a lending museum, which is contrary to recommendations 16C and 72F of the International Code on Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 1999). Problems associated with *E. dollfusi* and five species of *Masenia* not compared with *M. baroensis* in this study are subsequently explained.

Emoleptalea dollfusi is currently considered an accepted species (see King et al., 2018; WoRMS Editorial Board, 2020) but we elected not to compare it with *E. mozambiquensis* because we suspect fixation artifact was instrumental in naming it. Srivastava (1960) described both *E. loossi* and *E. dollfusi* (in that order) from a single individual catfish (*H. fossilis*) from Raipur, India. The species allegedly differ by the arrangement of the testes (opposite in *E. loossi* and diagonal in *E. dollfusi*). We consider specimens described as *E. dollfusi* to be slightly smaller, flattened specimens of *E. loossi*, with the excess pressure causing the distortion of the testes into the diagonal arrangement. *Emoleptalea dollfusi*, described after *E. loossi* in the paper, should be considered a junior synonym of *E. loossi*. Consequently, with the addition of *E. mozambiquensis*, we now accept five species of *Emoleptalea* from Africa and four species from South Asia, with the total number of accepted species staying at nine.

We were either unable or unwilling to compare *M. baroensis* with four of the 19 accepted species belong in *Masenia* from Asia. We were unable to obtain descriptions or accounts related to two of the 19 species: *Masenia agarwali* Hasnain & Sahay, 1994, which is one of three congeners that infects the stinging catfish, *Heteropneustes fossilis* (Bloch) in India, and *Masenia kwangtungensis* (Pan, 1984) Jones & Bray, 2008, which infects a catfish (*Clarias* sp.) in China (Jones and Bray, 2008). Two other species were described inadequately (i.e., *Masenia pushpanjalii*

Singh, Shankar, Singh & Gupta, 2006 and *Masenia dayali* [Gupta & Puri, 1984] Chandra & Saxena, 2016, and we chose not to compare *M. baroensis* with them. Gupta and Puri (1984) described *Eumasenia dayali* Gupta & Puri, 1984 based on a single specimen infecting a marine carangid (*Alepes djedaba* [Forsskål]) from the Bay of Bengal. Jones and Bray (2008) considered *Eumasenia* Srivastava, 1951 a junior synonym to *Masenia*, but did not formally create a new combination for *E. dayali*. Consequently, Chandra and Saxena (2016) proposed the new combination for the marine worm despite the name *M. dayali* being preoccupied by *Masenia dayali* Gupta, 1955, an accepted species from the freshwater catfish (*C. batrachus*) from Saharanpur in northern India and obviously different than the marine worm. Furthermore, Chandra and Saxena (2016) did this based on observations they made on two specimens they identified as *M. dayali* infecting a freshwater catfish (*M. vittatus*) in Lucknow (central India) that clearly represent a different species than *M. dayali sensu* Gupta, 1955. We consider the form they studied to certainly differ from the marine form previously known as *E. dayali* Gupta & Puri, 1984. Consequently, we consider the name *M. dayali* (Gupta & Puri, 1984) Chandra & Saxena, 2016 inapplicable. Ideally, a new species name should be proposed in *Masenia* for *E. dayali* Gupta & Puri, 1984 but we refrain from doing so here for fear that *E. dayali* represents one of the already named marine species in *Masenia*. The material studied by Chandra and Saxena (2016) needs re-evaluation, but those authors did not deposit any specimen. Similarly, *M. pushpanjalii* is an accepted species that was inadequately described from a snakehead (*Channa gachua* [Hamilton]), from the Gomati River (tributary of the Ganges River) in Jaunpur, India. Singh et al. (2006) placed this species in *Masenia* without describing or illustrating circumoral spines surrounding the oral sucker, which is the fundamental generic feature for the genus. Furthermore, they failed to deposit a specimen in any museum (violating Article 16, Recommendation 16C, and 72F of International Commission on Zoological Nomenclature, 1999), and they named the species after the first author's (female?) first name (despite it having the masculine ending *ii*) and without specifically specifying gender (disregarding Article 30.2, including Recommendation 30A, and failing to meet criteria for Article 31.1.2 of International Commission on Zoological Nomenclature, 1999). Based solely on the description, there is not enough information to diagnose the species to a genus within the Cephalogonimidae. Considering these issues and the lack of adherence to the International Commission on Zoological Nomenclature (1999), we propose that both *Masenia dayali* (Gupta & Puri, 1984) Chandra & Saxena, 2016 and *Masenia pushpanjalii* Singh, Shankar, Singh & Gupta, 2006 be considered *nomina dubia*.

Problems associated with the taxonomy of an additional accepted Indian cephalogonomid, *Masenia ritai* Sircar & Sinha, 1970, that we compared with *M. baroensis*, deserves further attention. Agrawal (1964) described *Eumasenia ritai* Agrawal, 1964 from a bagriid catfish (*Rita rita* [Hamilton]), collected from the Gomti River in Lucknow, India, and although Jones and Bray (2008) considered *Eumasenia* a junior synonym of *Masenia*, they did not name a new combination for this species. Presently it is not an accepted species in *Masenia*. Sircar and Sinha (1970) described *Masenia ritai* Sircar & Sinha, 1970, also infecting *R. rita*, but lower in the same drainage system in Patna, India. We consider both forms to be identical based on comparison of the descriptions (Agrawal, 1964; Sircar and Sinha, 1970). Consequently, we propose the first named form *Masenia ritai* (Agrawal, 1964) n. comb. represents the valid form with *M. ritai* Sircar & Sinha, 1970 being a junior synonym of the former. Thus, with the addition of *M. baroensis*, we consider there to be six African species in *Masenia* and we tentatively accept 17 Asian species: *M. agrawali*, *M. carangai*, *M. chauhani*, *M. collata*, *M. dayali*, *M. fossilisi*, *M. fukienensis*, *M. gomtia*, *M. gwaliorensis*, *M. jaunpurensis*, *M. kwangtungensis*, *M. moradabadensis*, *M. orissai*, *M. quiloni*, *M. ritai*, *M. upeneusi*, and *M. vittatusia*. Many of the Asian species of *Masenia* share common hosts and morphology as pointed out by Jones and Bray (2008). *Masenia collata*, *M. dayali*, and *M. gwaliorensis* are all described from *C. batrachus* and differ only slightly based on morphological features that could be influenced by differences in pressure during the fixation process or interpretations (presence or absence of a dorsal space interrupting the circumoral spines, seminal receptacle shape, caecal extent, size of proximal vs distal component of the bipartite seminal vesicle, and whether or not eggs are reported as operculated or not) (Chatterji, 1933; Gupta, 1955; Bhadauria and Dandotia, 1986). Similarly, *M. fossilisi*, *M. moradabadensis*, and *M. agarwali* are all described from *H. fossilis*, and although we are unable to obtain the description for *M. agarwali*, we note that *M. fossilisi* and *M. moradabadensis* differ only based on whether there is a dorsal interruption of spines and if eggs are reported as operculated or not (Srivastava, 1951; Gupta, 1955). Likewise, *M. rita* and *M. chauhani* are both described from *R. rita*, and the latter fluke species is reported (based on a single specimen) to differ from the former based on the anterior extent of the vitellarium, a feature possibly prone to variation (Agrawal, 1964; Sircar and Sinha, 1970; Maurya et al., 1989). Regardless, the description of *M. chauhani* is so poorly detailed as to be of little use in identifying the animal at the species level (Maurya et al., 1989). These examples serve to emphasize the need for a review of Asian species of *Masenia* and clarification of species using nucleotide data may go a long way to increasing the understanding of diversity in *Masenia* from Asia.

4.2. Systematics of Heterorchis

No information is available related to the cercariae for species from *Heterorchis*, and the excretory system exhibited in adult worms is so unusual that the position of *Heterorchis* among the flukes has been debated since the genus was erected. Baylis (1915) tentatively classified the genus in the Plagiorchiidae based on a general collection of features that conform to species in the plagiorchioid group: a scaled tegument, configuration of the alimentary tract, Y-shaped excretory bladder, small operculated tanned eggs, and a marginal anterior genital pore on the left side. Subsequent workers have either tentatively agreed with Baylis (Dollfus, 1950; Prudhoe and Bray, 1982; Bray, 1988) or classified the genus in the Fellodistomatidae (Yamaguti, 1953, 1958, 1971; Thomas, 1958b; Fischthal and Thomas, 1968; Vassiliadès and Richard, 1970; Boeger and Thatcher, 1983). The modern concept of the Fellodistomatidae entails a marine life history and the absence of a designated seminal receptacle (see Bray, 2002), both of which are violated by *Heterorchis* spp., which are entirely freshwater, and all have a prominent seminal receptacle. A fluke (*Kalipharynx piramboae* Boeger & Thatcher, 1983) discovered infecting South American lungfish, *Lepidosiren paradoxa* Fitzinger (Lepidosirenidae) from the Amazon region of Brazil may represent the closest relative to *Heterorchis* (Boeger and Thatcher, 1983). *Kalipharynx piramboae* is a monotypic species, and like *Heterorchis*, *Kalipharynx* Boeger & Thatcher, 1983 is presently considered *incertae sedis* in the Plagiorchioidea (Tkach in Pojmańska et al., 2008). While the excretory system of *K. piramboae* is incompletely described, it does have a terminal excretory pore rather than a large dorsal one, but otherwise, overall morphology is very similar between species in the two genera. Both groups share scale-like spines covering the body, robust suckers, a submarginal genital pore, extensive uterus with small operculated eggs, and the ovarian complex is nearly identical in both forms. We collected a single adult individual of *K. piramboae* from the intestine of *L. paradoxa* from Iquitos, Peru, and the excretory system of the specimen is largely obscured by the gonads and extensive coils of the uterus. However, convoluted lateral excretory collecting ducts are visible in the anterior half of the worm, suggesting the bladder may be Y-shaped. Metacercariae belonging in *Kalipharynx* were reported in two species of planorbid snails in Argentina (*Biomphalaria tenagophila* [D'Orbigny] and *Biomphalaria occidentalis* Paraense), but the excretory system was not further described in the specimens (Virginia-Fernández et al., 2013). Closer study of the museum specimens from that study may provide insight into the condition of the excretory bladder. The addition of more detailed study of the larval stages of *K. piramboae* and appropriation of nucleotide data from that species may provide great insight into the

relationship between *Kalipharynx* and *Heterorchis*, and ultimately their position and family status within the Plagiorchioidea.

4.3. Phylogenetic analysis

The overall topology of the phylogram (Fig. 8) is consistent with earlier studies that found the Plagiorchioidea and Microphalloidea to be sister groups, with the Gorgoderoidea closely related and basal to both (Tkach et al., 2001b, 2003; Razo-Mendivil et al., 2005; Sokolov and Shchenkov, 2017; Müller et al., 2018).

The present topology infers the Cephalogonimidae as a well-supported clade within the Plagiorchioidea and is consistent with previous phylogenetic studies involving the family and using lsrDNA (Olson et al., 2003; Razo-Mendivil and Pérez-Ponce de León, 2011; Dumbo et al., 2019a). The strongly supported cephalogonimid clade suggests that *Emoleptalea* and *Masenia* form a close relationship with each other and likewise that two species of *Cephalogonimus* are sister to each other. The position of *Emoleptalea*, branching close with two African species of *Masenia*, further corroborates the observations of Dumbo et al. (2019a) that the phylogenetic relationships among these cephalogonimid genera are tied to their biogeographic distributions, with *Emoleptalea* spp. + *Masenia* spp. being distributed in Africa and Asia and *Cephalogonimus* spp. being restricted in the Americas and Europe. The fact that *Emoleptalea* and *Masenia* are not resolved using lsrDNA in the present study is possibly the result of having a limited number of nucleotide sequences available for the analysis. More nucleotide sequences from species from each genus will be necessary to resolve this issue.

The topology suggests that *H.* cf. *crumenifer* forms a distinct lineage. This plus the unique morphology of the excretory system of species in *Heterorchis* might constitute enough evidence for establishment of a family within Plagiorchioidea to accommodate the genus. However, we consider understanding the relationship between *Heterorchis* and *Kalipharynx* to be of great potential for assessing the status of a family level group and advocate that both genera remain as taxa *incertae sedis* until either more life history or nucleotide data become available.

5. Conclusions

Prior to the present study nine species of *Emoleptalea* and 24 species of *Masenia* were accepted. All previously known species of *Emoleptalea* infected freshwater catfish in either Africa or South Asia. We herein added a new species, *E. mozambiquensis*, from an unusual definitive host (Cyprinidontiformes) in Africa. We consider *E. dollfusi* a junior synonym of *E. loossi*, and thus there are still nine species in the genus. *Masenia baroensis* is also herein described from an unusual definitive host (Tetraodontiformes) in the headwaters of the Niger River and represents the sixth species in the genus from Africa and the first from a non-catfish host on the continent. We consider *M. dayali* (Gupta & Puri, 1984) Chandra & Saxena, 201 and *M. pushpanjalii* both *nomina dubia*, and we consider *M. ritai* Sircar & Sinha, 1970 to represent a junior synonym to *Masenia ritai* (Agrawal, 1964) n. comb., thus the accepted number of species in the genus is reduced to 23. The phylogenetic analysis confirmed the presence of *Heterorchis* within the Plagiorchioidea but failed to provide enough evidence for placing it in an existing family or establishing a family group name for the genus.

Declaration of competing interest

The authors do not have any conflicts of interest.

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