

An integrative taxonomic study of *Susanlimocotyle narina* n. gen. n. sp. (Monogenoidea, Dactylogyridae) from the nasal cavities of a marine catfish (Siluriformes, Ariidae) from the Atlantic Amazon Coast of Brazil and new molecular data of *Chauhanellus* spp.

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ABSTRACT

Based on a taxonomic approach, combining morphological characters with DNA sequences (*i.e.*, 18S rDNA, ITS1, 5.8S rDNA and ITS2), *Susanlimocotyle* n. gen. is proposed to accommodate *Susanlimocotyle narina* n. sp. from the nostrils of the ariid *Sciades herzbergii* (Bloch) from the coast of the state of Pará, Brazil. *Susanlimocotyle* n. gen. is characterized by species possessing: an intestinal ceca confluent posteriorly; a male copulatory organ, comprising a variable tube, articulated with the accessory piece; a sclerotized vagina, vaginal aperture dextro-ventral; an onchium; a robust ventral bar; two dorsal bars; a ventral anchor with elongated shaft and a dorsal anchor with deep root expanding into wings. In addition, new molecular data of *Chauhanellus* spp. are also provided and used for the evaluation of the phylogenetic relationships among monogenoids parasitizing siluriforms. *Susanlimocotyle* n. gen. exhibited a higher genetic divergence level for 18S rDNA (4.6 to 7.2% [83–130 bp]) with respect to *Chauhanellus* spp. despite sharing *S. herzbergii* as a host, than *Hamatopeduncularia* spp., (4.1 to 5.8% [75–110 bp]) from Oriental ariids. For the 18S rDNA, 5.8S rDNA, ITS1 and ITS2 regions, *C. boegeri* and *C. susanlimae* were observed to have the smallest interspecific distances, and *C. velum* was revealed to be the most genetically distant species to *Chauhanellus*. The proposal for *Susanlimocotyle* n. gen. is also supported by phylogenetic analysis based on the 18S rDNA gene, which supports the close relationship between the new genus and *Hamatopeduncularia* and *Chauhanellus* from ariids from the South America and Oriental regions. Moreover, the patterns towards the shared diversification between monogenoids and their ariid hosts were addressed.

1. Introduction

Marine catfishes belonging to the family Ariidae (Siluriformes) include 153 species inhabiting marine, brackish, and freshwater environments along the world's tropical and subtropical continental shelves [1,2]. Nineteen of such species can be found in Brazilian waters, while 68% of the Ariidae diversity occurs in the Atlantic Amazon Coast of Brazil [1].

In the last years, parasitological studies focused on the Ariidae have resulted in a better knowledge of the monogenoids associated with these hosts [3,4]. The diversity of monogenoids of ariid fish around the world

is composed of 72 species divided in two families (Dactylogyridae and Neocalceostomatidae) included in the order Dactylogyridea. Within the Dactylogyridae; *Chauhanellus* Bychowsky & Nagibina, 1969 includes 27 species, *Hamatopeduncularia* Yamaguti, 1953 (30), and *Neotetraonchus* Bravo-Hollis, 1968 (5), and within the Neocalceostomatidae; *Neocalceostomoides* Kritsky, Mizelle & Bilqees, 1978 includes 5 species, *Calceostomella herzbergii* Zambrano, Dezón & León, 2004 and three monotypic genera, *Neocalceostoma*, *Neocalceostoma elongatum* Tripathi, 1957, *Thysanotohaptor*, *Thysanotohaptor rex* Kritsky, Shameem, Kumari, & Krishnaveni, 2012 and *Fridericianella*, *Fridericianella ovicola* Brandes, 1894 [3–7]. However, taxonomic studies on these parasites are mainly

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based on morphological data, with limited use of molecular information [3–9].

Chauhanellus and *Hamatopeduncularia* are the most diverse monogenoid genera with species found parasitizing the gills of ariids, with 57 described species distributed worldwide [3–5]. Both genera were primarily included in Ancyrocephalinae Bychowsky, 1937, before being posteriorly transferred to Ancylo-discoidinae Gusev, 1961 [10,11]. However, the taxonomic status of Ancyrocephalinae and Ancylo-discoidinae, as well as their phylogenetic relationships among Dactylogyridae, have been under discussion for many decades [12–16]. Lim et al. [5] revised the taxonomic status all dactylogyrid genera with species infecting siluriform fishes from the Old World and raised Ancylo-discoidinae to family status. However, Mendoza-Palmero et al. [16] suggested that Ancylo-discoididae, as proposed by Lim et al. [5], has no phylogenetic support, and should be considered a junior synonym of Dactylogyridae.

In the present study, based on an integrative taxonomic approach, combining morphological characters and sequences of the 18S rDNA, ITS1, 5.8S rDNA and ITS2 genes, the monotypic *Susanlimocotyle* n. gen. is erected to accommodate the dactylogyrid *Susanlimocotyle narina* n. sp. from the nostrils of the ariid *Sciades herzbergii* (Bloch) from the coast of the state of Pará, Brazil. Using 18S rDNA sequences, the phylogenetic position of *Susanlimocotyle* n. gen. was analyzed, along with its phylogenetic relationships with *Chauhanellus* spp., *Hamatopeduncularia* spp., and other dactylogyrid parasites from Siluriformes. In addition, new molecular data of *Chauhanellus* spp. are also provided.

2. Material and methods

2.1. Sample collection, morphological study

Twenty two specimens of *Sciades herzbergii* (Bloch) were collected by trammel net; 20 from Caratateua Village, in the municipality of Bragança, in the state of Pará, Brazil (1° 59' 41.91" S, 46° 43' 21.385" W); and two from Ajuruteua Village, in the municipality of Bragança, in the state of Pará, Brazil (0° 49' 31" N; 46° 36' 29" W) on March 20, 2018, and January 10, 2019, under a License for the Collection of Zoological Material (SISBio n° 60666–2 and Sisgen n° AD28DC2).

The nasal cavities of the host specimens were examined for monogenoids. After opened with dissection scissors, the nasal cavities were washed with heated water (~65 °C) in a Petri dish, and the contents were examined under a stereomicroscope. Helminths were removed from the sediment using small probes, and were fixed in 4% formalin for morphological study, or in 95% ethanol for molecular characterization. To obtain sequences of South America species distinct from *Susanlimocotyle narina* n. gen. n. sp., the gills of *Sciades herzbergii* were removed and fixed in ethanol 95%. Specimens of *Chauhanellus boegeri* Domingues & Fehlaue, 2006, *C. susamlimae* Domingues, Soares & Watanabe, 2016, and *C. velum* Domingues, Soares & Watanabe, 2016, were collected and fixed for molecular analysis.

Some specimens were stained with Gomori's trichrome [17,18] and mounted in Damar gum to examine their internal soft structures, and others were mounted in Hoyer's medium [17,18] for the study of the sclerotized structures. Measurements, all in micrometers, were obtained according to the procedures of Mizelle and Klucka [19]. Dimensions of organs and other structures represent the highest measurements in the dorso-ventral view; lengths of curved or bent structures (bars and accessory piece) represent the straight-line distances between the extreme ends; lengths of anchors followed Soares et al. [20], and the total lengths of the male copulatory organ (MCO) was measured using ImageJ [21] on drawing tube images. Measurements are presented in micrometers as the mean followed by the range and number (n), of specimens measured, are in parentheses. Illustrations were prepared with a drawing tube a Leica DM 2500 microscope with differential interference contrast and phase contrast optics. Illustration of soft structures was carried out using pen and ink, while hard structures were scanned and redrawn on a digitizing

tablet, using Corel© [22]. Plates were also prepared in Corel© [22]. Definitions of prevalence and mean intensity followed Bush et al. [23].

Type specimens, vouchers and hologenophores (see Pleijel et al. [24] for terminology) were deposited in the Invertebrate Collection of the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará state, Brazil, under N° (MPEG 266–278). For comparative purposes, paratypes of the following species of *Chauhanellus* and *Hamatopeduncularia* were examined: *C. boegeri* Domingues & Fehlaue, 2006, (CHIOC 36821a – c), *C. hamatopeduncularioideum* Domingues, Soares & Watanabe, 2016 (CHIOC 38240b – d), *C. susamlimae* Domingues, Soares & Watanabe, 2016 (CHIOC 38251b – d), *C. velum* Domingues, Soares & Watanabe, 2016 (INPA 679) and *H. bagre* Hargis, 1955 (CHIOC 38273–38278). Scientific names of hosts follow Marceniuk et al. [1]. To comply with the regulations in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new taxa have been submitted to ZooBank.

2.2. Molecular characterization

For correct identification, each parasite specimen subjected to molecular analysis was divided using fine needles under a dissecting microscope. The anterior half of the body was placed in a 1.5 ml microtube with 96% ethanol for genomic DNA extraction. The posterior part containing the haptor complex was completely flattened under coverslip pressure and mounted in Hoyer's for species identification. Genomic DNA was extracted using Qiagen Dneasy® Blood and Tissue Kit, according to the manufacturer's protocol, with a final volume of 30 µl. Concentration of the DNA was verified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) at 260 nm.

The 18S rDNA was amplified using a two-round polymerase chain reaction (PCR). In the first round, DNA was amplified with the primer pair WormA (5'-GCGAATGGCTCATTAAATCAG – 3') [25] and WormB (5' – CTTGTTACGACTTTTACTTCC – 3') [25]. In the second round, for the nested PCRs, the primer combinations were WormA and 1270R (5' - CCGTCAATTCCTTAAAGT-3') [24], and 930F (5' - GCATGGAA-TAATGGAATAGG-3') [26] with WormB [25], which amplified two overlapping fragments of approximately ~1179 bp and ~ 1054 bp, respectively. The Internal Transcribed Spacers (ITS1 and ITS2) and the 5.8S rDNA regions were amplified also using a two-round polymerase chain reaction (PCR). In the first round, DNA was amplified with the primer pair 1200F (5'- CAGGTCTGTGATGCC – 3') [25] and D2 (5' – TGGTCCGTGTTTCAAGAC – 3') [27]. In the second round, for the nested PCR, the primer combinations were 1200F and 28SR1 (5' - GCTTCGATGTTGGGCTARTCTC-3') [28], which amplified one fragment of approximately ~1189 bp.

PCRs were performed in a Matercyler® nexus (Eppendorf, Hamburg, Germany) with a final volume of 25 µl and with the DreamTaq Green PCR Master Mix (2×) (Thermo Scientific Wilmington, USA), following the manufacturer's recommendations. A quantity of 0.1 mM of each primer and 3 µl of the extracted DNA was used in the reactions. For 18S rDNA, the PCR profile was set as follows: at 94 °C was performed for 3 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 90 s, and a final elongation at 72 °C for 10 min. For ITS1, 5.8S rDNA and ITS2, the PCR profile was set as follows: at 94 °C was performed for 5 min, followed by 35 cycles of 94 °C for 45 s, 50 °C for 30 s, 72 °C for 90 s, and a final elongation at 72 °C for 7 min. The nested PCRs were conducted with 1 µl of the product of the PCRs, diluted 1:1 in ultrapure water, with the same PCRs profile for each gene. Amplicons were electrophoresed in 2% agarose gel in a TAE buffer (Tris 40 mM, Acetic Acid 20 mM, EDTA 1 mM) stained with SYBRsafe® (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) alongside a 1 kb Plus DNA Ladder (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) at 100 V for 30 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen, USA) and sequencing was carried out with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) in a 3500

DNA sequencing analyzer (Applied Biosystems, California, USA) at Helixxa Company (Paulínia, São Paulo state, Brazil), using the same primers used for 18S rDNA amplification, and for the ITS1, 5.8 rRNA and ITS2 fragments, the 1200F and 28SR1.

2.3. Alignment, genetic distances and phylogenetic inference

Contigs were edited using Sequencher 4.1.4 (Gene Codes, Ann Arbor, MI) and deposited in GenBank under the accession numbers listed in Table 1. Standard nucleotide BLAST searches were then conducted [29] to verify the similarity of the sequences newly obtained in the present study with other sequences of monogenoids in the NCBI BioSystems database [30]. Alignments of 18S rDNA, ITS1, 5.8S rDNA and ITS2 were generated using MUSCLE implemented in Geneious version 7.1.3 [31]. To determine the position of *Susanlimocotyle narina* n. gen. n. sp., *Chauhanellus boegeri*, *C. susamlimae*, and *C. velum* among other representatives of the dactylogyrid genera, phylogenetic analyses were based on sequences of the 18S rDNA gene only. The choice of this molecular marker is related with the larger number of sequences of monogenoid parasites of arid fish available for comparison at the NCBI [30]. A total of 49 partial sequences of the 18S rDNA of species belonging to the order Dactylogyridae published in the NCBI BioSystems database [30] along with two of the Monocotylidae and two of the Capsalidae (used as the outgroup), were retrieved from GenBank (see Table 1) and aligned with the newly generated sequences of *Susanlimocotyle narina* n. gen. n. sp., *Chauhanellus boegeri*, *C. susamlimae*, and *C. velum*. Fifty-seven sequences (1619–2200 bp long) were aligned; the extremes were trimmed leaving an alignment 1787 bp long. Phylogenetic analyses were performed using the maximum likelihood (ML) and Bayesian inference (BI) methods. ML was done in the PhyML 3.0 implemented via the web server (<http://www.atgc.montpellier.fr/phyml/>) [32], with topology assessed by bootstrapping with 1000 replicates, using the GTR + I + G model of evolution selected by JModelTest 2.1.1 (University of Vigo and University of A Coruña, Spain) [33], using the Akaike information criterion. BI was done using MrBayes v.3.0 [34] implemented via the computational resource CIPRES [35], under the same model, with posterior probabilities estimated from 1 million generations with two independent runs of four simultaneous Markov Chain Monte Carlo (MCMC) algorithms, with every 1000th tree saved and an MCMC diagnostic for every 1000th generation. Burn-in periods were set to the first 25,000 generations. Trees were visualized using Figtree 1.3.1 [36] and figures prepared using Corel© [22]. Genetic divergence was determined using the p-distance model matrix in MEGA version 7 [37] separately for each genetic marker. Gaps and missing data were deleted.

3. Results

The results of the present study support the proposed creation of a new genus of Monogenoidea to harbor a new species of Dactylogyridae from the nasal cavity of *S. herzbergii* caught off the coast of the state of Pará. The morphological and 18S rDNA, ITS1, 5.8S rDNA and ITS2 data that endorse the creation of the new taxon are presented below. New molecular data of *Chauhanellus* spp. are also provided: the 18S rDNA sequence of *C. boegeri* was 1761 bp long. The combined ITS1, 5.8S rDNA and ITS2 sequence was 810 bp long, of which 354 bp corresponded to the ITS1, 149 bp to the 5.8S rDNA and 307 bp to the ITS2 region. In *C. velum* the 18S rDNA sequence was 1749 bp long. The combined ITS1, 5.8S rDNA and ITS2 sequence was 817 bp long, of which 354 bp corresponded to the ITS1, 149 bp to the 5.8S rDNA and 314 bp to the ITS2 region. For *C. susamlimae* the 18S rDNA sequence was 1751 bp long. The combined ITS1, 5.8S rDNA and ITS2 sequence was 796 bp long, of which 354 bp corresponded to the ITS1, 149 bp to the 5.8S rDNA and 293 bp to the ITS2 region.

3.1. Taxonomic summary

Class: Monogenoidea Bychowsky, 1937.

Subclass: Polyonchoinea Bychowsky, 1937.

Order: Dactylogyridae Bychowsky, 1937.

Family: Dactylogyridae Bychowsky, 1933.

Susanlimocotyle n. gen.

Type-species: *Susanlimocotyle narina* n. sp.

Type host: *Sciades herzbergii* (Bloch) (Siluriformes, Ariidae).

Site: Nasal cavities.

Type-locality: Caratateua Village, municipality of Bragança, Pará state, Brazil (1° 59' 41.91" S, 46° 43' 21.385" W) on March 20, 2018, and January 10, 2019.

Other localities: Ajuruteua Village, municipality of Bragança, Pará state, Brazil (0° 49' 31" N; 46° 36' 29" W).

Etymology. The genus name is in honor of the late Dr. Lee Hong Susan Lim, the University of Malaya, in recognition of his valuable work on the Monogenoidea. Acknowledge the fact that Dr. Lim was greatly responsible for most of our knowledge of the diversity of Monogenoidea from Asian Siluriformes.

Zoobank Life Science Identifier. (LSID) for *Susanlimocotyle* n. gen. FE2CC83C-554D-417D-9429-BA2EB12DC5E8.

3.1.1. Description

Diagnosis. Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Cephalic region with terminal cephalic lobe poorly developed. Bilateral pairs of head organs opening subterminal to tip of cephalic lobes; cephalic glands lateral or postero-lateral to pharynx. Eyes present (two pairs); accessory chromatic granules absent. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; two intestinal ceca, confluent posteriorly to gonads, lacking diverticula. Genital pore opening midventral, anterior to copulatory complex; genital atrium of soft tissue. Gonads tandem. One testis, dorsal to germarium; vas deferens looping left intestinal cecum; seminal vesicle sigmoid. One prostatic reservoir. Copulatory complex comprising articulated MCO, accessory piece; MCO sclerotized, comprising a variable tube; accessory piece sclerotized, comprising robust rod. Vagina single, sclerotized, vaginal aperture dextro-ventral. Uterus delicate. Seminal receptacle not observed; Vitellaria well developed, coextensive with ceca. Haptor armed with, 14 hooks (8 marginal, 2 central, 4 dorsal); ventral onchium; 2 pairs of anchors (1 ventral, 1 dorsal); 3 haptor bars (1 ventral, 2 dorsal). Onchium with 2 units and connected with extrinsic haptor muscles. Ventral anchor with elongate shaft. Dorsal anchor with deep root expanding into wings. Robust ventral bar.

Remarks. *Susanlimocotyle* n. gen. monotypic is characterized by a combination of the following features: (1) an intestinal ceca confluent posteriorly; (2) an MCO, comprising a variable tube, articulated with the accessory piece; (3) a sclerotized vagina, vaginal aperture dextro-ventral; (4) an onchium; (5) a robust ventral bar; (6) two dorsal bars; (7) a ventral anchor with long shaft and (8) a dorsal anchor with deep root expanding into wings. According to Kritsky et al. [6], the term 'onchium' has applied to apparently non-homologous accessory structures, usually plate- or shield-like, found in the haptors of species of: *Paradactylogyrus* Thapar, 1948, *Bychowskyella* Achmerow, 1952, *Neotetraonchus*, *Bagrobdella* Paperna, 1969, *Protoancylodiscoides* Paperna, 1969 and *Malayanodiscoides* Lim & Furtado, 1986 [5,6]. *Susanlimocotyle* n. gen., *Neotetraonchus* spp., *Bychowskyella* spp., *Bagrobdella* spp. and *Protoancylodiscoides* spp., all occurring on siluriform fishes, where *Susanlimocotyle* n. gen. and *Neotetraonchus* spp. seem to be restricted to ariids hosts. Despite knowledge about the position and different forms of the onchium in the haptors of each of these monogenoids groups (see [5,6]), its function is still unknown. In addition to the fact that members of *Susanlimocotyle* and *Neotetraonchus* are found parasitizing ariids, they also share the presence of an onchium. However, both genera can be

Table 1

List of monogenoids included into phylogenetic analyses, providing host species data, locality, GenBank ID, and references.

Parasites species	Host	Host family	Locality	GenBank ID	Reference
Dactylogyridae					
Ancyrocephalinae					
<i>Anacanthorus penilabiatus</i>	<i>Piaractus mesopotamicus</i>	Serrasalmidae	Brazil	KU941837	[44]
<i>Bravohollisia tecta</i>	<i>Pomadasys maculatus</i>	Haemulidae	China	KJ571020	[45]
<i>Bravohollisia maculatus</i>	<i>Pomadasys maculatus</i>	Haemulidae	China	KJ571018	[45]
<i>Euryhaliotrema johnii</i>	<i>Lutjanus johnii</i>	Lutjanidae	China	EU836214	[45]
<i>Euryhaliotrematoides annulocirrus</i> ^a	<i>Chaetodon vagabundus</i>	Chaetodontidae	Australia	AY820602	[15]
<i>Euryhaliotrematoides berenguelae</i> ^a	<i>Chaetodon citrinellus</i>	Chaetodontidae	French Polynesia	AY820604	[15]
<i>Euryhaliotrematoides triangulovagina</i> ^a	<i>Chaetodon kleinii</i>	Chaetodontidae	Palau	AY820608	[15]
<i>Euryhaliotrematoides pirulum</i> ^a	<i>Chaetodon lunula</i>	Chaetodontidae	French Polynesia	AY820607	[15]
<i>Haliotrema pratensis</i>	–	–	–	EU836230	Sun et al. (unpublished)
<i>Haliotrema macracantha</i>	–	–	–	EU836229	Sun et al. (unpublished)
<i>Haliotrema aurigae</i>	<i>Chaetodon auriga</i>	Chaetodontidae	Australia	AY820610	[15]
<i>Haliotrema leporinus</i>	–	–	–	EU836227	Sun et al. (unpublished)
<i>Haliotrema scyphovagina</i>	<i>Forcipiger flavissimus</i>	Chaetodontidae	French Polynesia	AY820611	[15]
<i>Haliotrema eukurodai</i> ^b	–	–	–	EU836223	Sun et al. (unpublished)
<i>Lethrinotrema zhanjiangense</i>	<i>Lethrinus nebulosus</i>	Lethrinidae	China	KJ571021	[45]
<i>Lethrinotrema grossecurvitubum</i>	<i>Lethrinus nebulosus</i>	Lethrinidae	China	EU836225	[45]
<i>Mymarothecium viatorum</i>	<i>Piaractus mesopotamicus</i>	Serrasalmidae	Brazil	KU941838	[44]
<i>Protogyrodactylus amacleithrium</i>	–	–	Egypt	FM251947	Riva, C. (unpublished)
<i>Protogyrodactylus johnstoniettsi</i>	–	–	Egypt	FM251946	Riva, C. (unpublished)
<i>Pseudohaliotrema sphincterporus</i>	<i>Siganus doliatus</i>	Siganidae	Australia	AJ287568	[25]
<i>Tetrancistrum nebulosi</i>	–	–	–	HM545910	Wang et al. (unpublished)
<i>Tetrancistrum nebulosi</i>	–	–	–	HM545910	Wang et al. (unpublished)
Ancylodiscoidinae					
<i>Bychowskyella fossilisi</i>	<i>Heteropneustes fossilisi</i>	Heteropneustidae	India	KT852454	[46]
<i>Bychowskyella tchangii</i>	<i>Clarias batrachus</i>	Clariidae	India	KT852455	[46]
<i>Chauhanellus boegeri</i>	<i>Sciades herzbergii</i>	Ariidae	Brazil	MW132134; MW179607^d	Present study
<i>Chauhanellus susamlimae</i>	<i>Sciades herzbergii</i>	Ariidae	Brazil	MW144439; MW179608^d	Present study
<i>Chauhanellus velum</i>	<i>Sciades herzbergii</i>	Ariidae	Brazil	MW144823; MW179609^d	Present study
<i>Hamatopeduncularia arii</i>	<i>Arius jella</i>	Ariidae	India	KT252895	[4]
<i>Hamatopeduncularia bifida</i>	<i>Arius jella</i>	Ariidae	India	MK084781	[4]
<i>Hamatopeduncularia elongata</i>	<i>Arius jella</i>	Ariidae	India	MK084780	[4]
<i>Hamatopeduncularia madhaviae</i>	<i>Plicofollis dussumieri</i>	Ariidae	India	KT252898	[4]
<i>Hamatopeduncularia thalassini</i>	<i>Arius jella</i>	Ariidae	India	KT252900	[4]
<i>Hamatopeduncularia</i> sp.	–	–	–	KT252899	Ummej et al. (unpublished)
<i>Mizelleus longicirrus</i>	<i>Wallago attu</i>	Siluridae	India	KR296801	[47]
<i>Susanlimocotyle narina</i> n. gen. n. sp.	<i>Sciades herzbergii</i>	Ariidae	Brazil	MW144824; MW179606^d	Present study
<i>Thaparocleidus gangus</i>	<i>Wallago attu</i>	Siluridae	India	KX364087	[46]
<i>Thaparocleidus gangus</i>	<i>Wallago attu</i>	Siluridae	India	KX364088	[46]
<i>Thaparocleidus wallagonius</i>	<i>Wallago attu</i>	Siluridae	India	KX364085	[46]
<i>Thaparocleidus wallagonius</i>	<i>Wallago attu</i>	Siluridae	India	KX364086	[46]
Dactylogyrinae					
<i>Dactylogyrus falciformis</i>	<i>Cyprinus carpio</i>	Cyprinidae	Egypt	FN391583	Aquaro et al. (unpublished)
Pseudodactylogyrinae					
<i>Pseudodactylogyroides apogonis</i>	<i>Apogon semilineatus</i>	Apogonidae	Japan	AB065115	Iwashita et al. (unpublished)
<i>Pseudodactylogyrus anguillae</i>	–	–	–	AB060591	Iwashita and Ogawa (unpublished)
<i>Pseudodactylogyrus bini</i>	<i>Anguilla japonica</i>	Anguillidae	Japan	AB065113	Iwashita et al. (unpublished)
<i>Pseudodactylogyrus haze</i>	<i>Acanthogobius flavimanus</i>	Gobiidae	Japan	AB065114	Iwashita et al. (unpublished)
Diplectanidae					
<i>Lamellodiscus donatellae</i>	–	–	–	FN296209	Aquaro, G. (unpublished)
<i>Lamellodiscus donatellae</i>	–	–	–	FN296214	Aquaro, G. (unpublished)
<i>Lamellodiscus japonicus</i>	<i>Acanthopagrus s. schlegelii</i>	Sparidae	China	EU836236	[45]
<i>Lamellodiscus pagrosomi</i>	<i>Pagrus major</i>	Sparidae	China	EU836235	[45]
<i>Pseudorhabdosynochus grouperi</i>	<i>Epinephelus coioides</i>	Serranidae	Indonesia	FJ655782	[48]
<i>Pseudorhabdosynochus lantauensis</i>	–	–	–	GQ495271	Dang et al. (unpublished)
Pseudomurraytrematidae					
<i>Pseudomurraytrema ardens</i>	<i>Catostomus ardens</i>	Catostomidae	United States	AJ228793	[49]
Anoplodiscidae					
<i>Anoplodiscus cirruspiralis</i>	<i>Sparus auratus</i>	Sparidae	Australia	AJ287475	[25]
Sundanonchidae					
<i>Sundanonchus micropeltis</i>	<i>Channa micropeltis</i>	Channidae	Malaysia	AJ287579	[25]
Monocotylidae					
<i>Calicotyle affinis</i> ^c	<i>Chimaera monstrosa</i>	Chimaeridae	Norway	AJ228777	[50]
<i>Dictyocotyle coeliaca</i> ^c	<i>Amblyraja radiata</i>	Rajidae	United Kingdom	AJ228778	[49]
Capsalidae					
<i>Capsala martinieri</i> ^c	<i>Mola mola</i>	Molidae	United Kingdom	AJ276423	[25]
<i>Encotyllabe chironemi</i> ^c	<i>Chironemus marmoratus</i>	Chironemidae	Australia	AJ228780	[50]

Species sequenced in this study are in bold.

^a *Euryhaliotrematoides* was placed in subjective synonymy with *Euryhaliotrema* [51].^b *Haliotrema eukurodai* = *Euryhaliotrema eukurudai* [51].^c Species used as outgroups.^d Sequences used for the nucleotide divergence (p-distance) analyses using ITS1, 5.8S rDNA and ITS2 (Supplementary Table S1).

distinguished from each other by the position and shape of the onchium. In *Susanlimocotyle* n. gen. this structure is ventral in the haptor and is formed by 2 sclerotized plates, both connected by extrinsic haptoral muscles and associated with the ends of the ventral bar by the posterior portion of the plate. In *Neotetraonchus*, the onchium is ventral and is formed by a single sclerotized plate associated with the pair of hooks 1 (see [6]). In addition to onchium, *Susanlimocotyle* n. gen. differs from *Neotetraonchus* due to the presence of the intestinal ceca confluent posteriorly (intestinal ceca non-confluent in *Neotetraonchus*), by possessing a MCO articulated with the accessory piece (MCO non-articulated with the accessory piece in *Neotetraonchus*), by the presence of two dorsal bars (one dorsal bar in *Neotetraonchus*) and by having a ventral anchor with elongate shaft (ventral anchor with short shaft in *Neotetraonchus*).

Susanlimocotyle narina n. sp. (Fig. 1).

Type-host. *Sciades herzbergii* (Bloch), (Siluriformes, Ariidae).

Type-locality. Caratateua Village, municipality of Bragança, Pará

state, Brazil (1° 59' 41.91" S, 46° 43' 21.385" W) on March 20, 2018 and January 10, 2019.

Other records. *Sciades herzbergii* (Prevalence: 50% of 2 hosts; Mean intensity: 1; Mean abundance: 0.5), Ajuruteua Village, municipality of Bragança, Pará state, Brazil (0° 49' 31" N; 46° 36' 29" W).

Site. Nasal cavities.

Specimens deposited. Holotype (MPEG 266), paratypes (MPEG 267–274), hologenophore (MPEG 275), voucher (MPEG 276).

Representative DNA sequence. GenBank accession number MW144824, MW179606.

Zoobank Life Science Identifier. (LSID) for *Susanlimocotyle narina* sp. n. 652748AF-9770-4C58-B5DB-3D2186D29AFC.

Etymology. The specific name is derived from the site of infection *narina*.

Prevalence. 60% of 20 hosts examined.

Mean intensity. 1.83 parasites per infected host.

Mean abundance. 1.1 parasites per host.

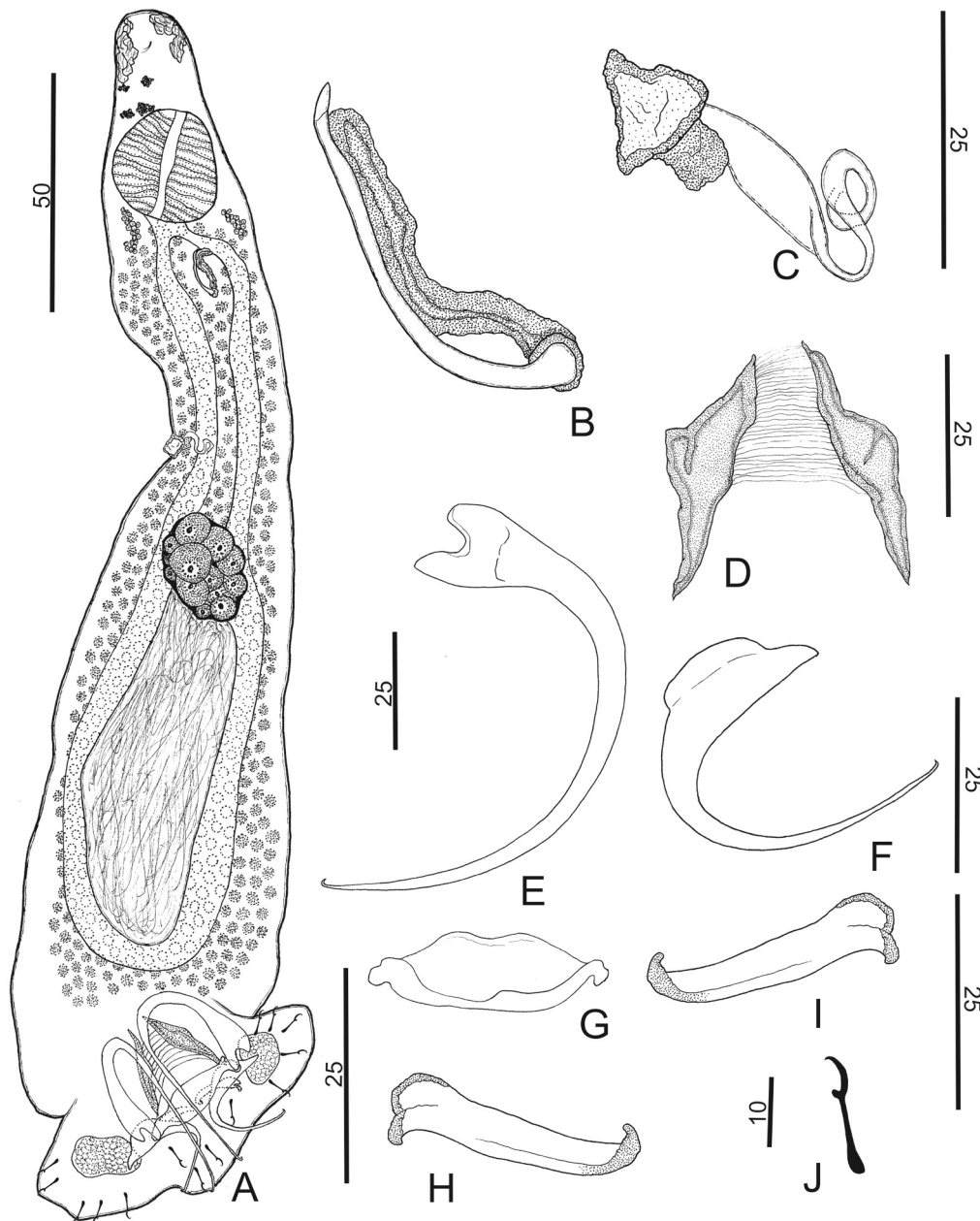


Fig. 1. *Susanlimocotyle narina* n. gen. n. sp. A. Holotype whole-mount, ventral; B. Copulatory complex; C. Vagina; D. Onchium; E. Ventral anchor; F. Dorsal anchor; G. Ventral bar; H, I. Dorsal bars; J. Hook. Scale bars Fig. 1A (50 µm), Figs. 1B-1C, 1D-1H (25 µm); Fig. 1J (10 µm).

Table 2

Comparative measurements (in μm) of specimens of *Susanlimocotyle narina* n. sp. parasite of the nasal cavity of *Sciades herzbergii* from two localities in the state of Pará, Brazil.

	Caratateua village	N	Ajuruteua village	N
MCO length	51 (40–60)	6	53	1
Ventral Bar				
Length	38 (28–43)	5	35	1
Width	12 (9–13)	4	10	1
Dorsal Bar				
Length	38 (30–45)	5	37	1
Width	6 (6–7)	6	6	1
Ventral Anchor				
Outer	99 (90–104)	5	100	1
Inner	81 (72–86)	6	85	1
Base	20 (18–21)	6	20	1
Dorsal Anchor				
Outer	34 (32–38)	8	32	1
Inner	26 (23–46)	9	23	1
Base	13 (12–16)	9	12	1
Hook				1
Length	16 (15–18)	9	15	1

Comparative measurements. Table 2.

Description. (Based on nine specimens, four mounted in Hoyer, five mounted in Gomori's trichrome): Body fusiform, total length excluding haptor 318 (227–446; $n = 4$); total width at level of germarium 64 (40–92; $n = 4$) (Fig. 1A). Cephalic margin tapered; moderately developed terminal lobes; five to six bilateral pairs of head organs with rod-shaped secretion; cephalic glands unicellular, posterolateral to the pharynx. Eyes 4, posterior pair larger than anterior pair; accessory chromatic granules absent. Pharynx ovate 34 (25–46; $n = 3$) long, 33 (25–48; $n = 3$) wide. Testis saculiform 74 (70–78; $n = 2$) long, 30 (25–35; $n = 2$) wide. Prostatic reservoir subspherical, near to MCO (observed only in paratypes). MCO, 51 (40–60; $n = 6$) long, elongated tube, frequently appearing J-shaped, with tapered distal portion; base of MCO with sclerotized margin (Fig. 1B). Accessory piece comprising elongated rod, convoluted. Germarium ovate, 24 (23–25; $n = 2$) long, 14 (14–15; $n = 2$) wide. Eggs, Mehlis' glands, Seminal receptacle, ootype not observed. Vagina heavily sclerotized, vaginal pore dextral, marginal, vaginal vestibule cup-shaped, long vaginal canal sclerotized, with expanded proximal region and distal compressed and sigmoid (Fig. 1C). Uterus delicate. (observed only in paratypes). Vitelline follicles dense. Haptor subhexagonal, 61 (47–70; $n = 3$) long, 43 (33–50; $n = 3$) wide, with 3 haptor glands (2 ventral, 1 dorsal). Onchium (Figs. 1D, 2A), 2

sclerotized plates, with tapered ends and median expansion, connected by extrinsic haptor muscles, associated with the ends of the ventral bar by posterior portion of plate. Anchors dissimilar. Ventral anchor, outer 99 (90–104; $n = 5$) long, inner 81 (72–86; $n = 6$) long; base 20 (18–21; $n = 6$) wide; with poorly developed superficial and deep roots of similar length, subtriangular; shaft long, evenly curved, point with fish-hook-like termination (Figs. 1E, 2B). Dorsal anchor, outer 34 (32–38; $n = 8$) long, inner 26 (23–46; $n = 9$) long; base 13 (12–16; $n = 9$) wide; with inconspicuous roots; superficial root triangular, developed; expanded deep root; shaft recurved near mid-length; point with fish-hook-like termination (Figs. 1F, 2B). Ventral bar, 38 (28–43; $n = 5$) long, 12 (9–13; $n = 4$) wide, trapezoidal-shape with short groove at each end for articulation with ventral anchor (Fig. 1G). Pair dorsal bars, no connection between them, with anterior end with strongly sclerotized protuberance, bifid posterior end for articulation with dorsal anchor, each dorsal bar with 38 (30–45; $n = 5$) long, 6 (6–7; $n = 6$) wide (Figs. 1H–I, 2B). Hooks similar in shape, 16 (15–18; $n = 9$) long, shank without inflation, depressed thumb, lightly curved short shaft, delicate point, shank with pin-head-like distal portion; filamentous hook loop not observed (Fig. 1J).

Remarks. *Susanlimocotyle narina* n. sp. is characterized by: (1) onchium composed of 2 sclerotized plates, with tapered ends and median expansion, connected by extrinsic haptor muscles, associated with the ends of the ventral bar by the posterior portion of the plate; (2) a pair of unconnected dorsal bars, with an anterior end with a strongly sclerotized protuberance, a bifid posterior end for articulation with the dorsal anchor; (3) a heavily sclerotized vagina, vaginal pore dextral, marginal, vaginal vestibule cup-shaped, long sclerotized vaginal canal, with expanded proximal region, distal, compressed and sigmoid and (4) MCO, elongated tube, frequently appearing J-shaped, with tapered distal portion. *Susanlimocotyle narina* n. sp., represents the first occurrence of monogenoids in the nostrils of ariids in the world.

Molecular data. The sequence of 18S rDNA of *S. narina* n. gen. n. sp. was 1619 bp long. The combined ITS1, 5.8S rDNA and ITS2 sequence was 869 bp long, of which 354 bp corresponded to the ITS1, 150 bp to the 5.8S rDNA and 365 bp to the ITS2 region.

3.2. Phylogenetic position of *Susanlimocotyle narina* n. gen. n. sp. and *Chauhanellus* spp. within *Dactylogyridae*

Phylogenetic analyses built on ML and BI criteria, based on the 18S rDNA gene, yielded similar topologies. We therefore chose to present only the BI tree, with the statistical support of both methods (Fig. 3).

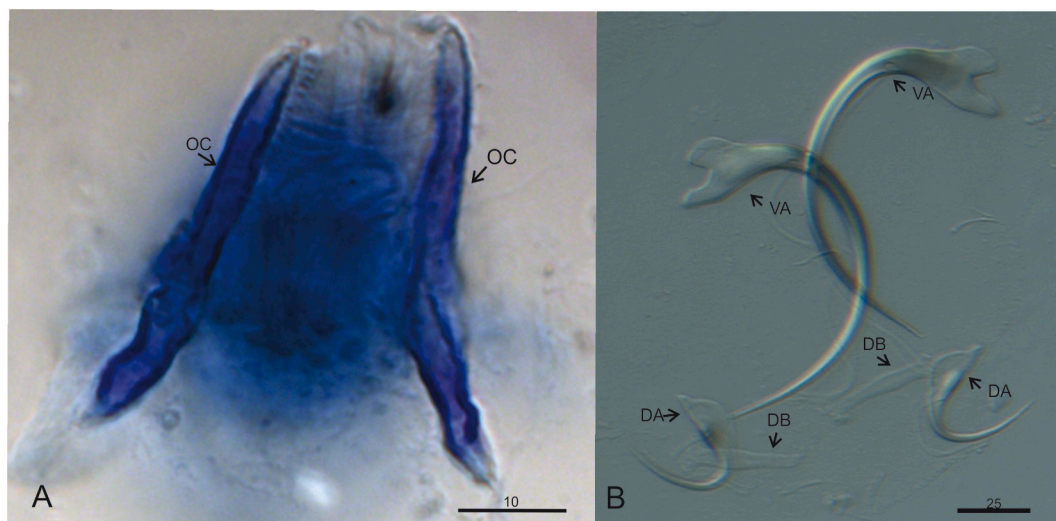


Fig. 2. *Susanlimocotyle narina* n. gen. n. sp. parasite of nasal cavity of *Sciades herzbergii*. Haptor complex. (OC) onchium; (VA) ventral anchor; (DA) dorsal anchor; (DB) dorsal bar. Scale Fig. 2A (10 μm), Fig. 2B (25 μm).

In the present study, we analyzed the clade composed only of dactylogyrid species from Siluriformes, which appear in a single clade (Fig. 3, clade A) with highly supportive nodes in both ML and BI analyses, divided into two subclades (Fig. 3, clade A1 and A2).

Clade A1 comprises species exclusively parasitizing freshwater catfish from the Oriental region (Fig. 3, clade marked in red): *Mizellus longicirrus* (Tripathi, 1959) from Siluridae, *Bychowskyella* spp. (*B. fossilisi* Majumdar & Agarwal, 1989 from Heteropneustidae and *B. tchangi* Gusev, 1976 from Clariidae) and *Thaparocleidus* spp. (*T. gangus* Verma, Chaudhary & Singh, 2016 and *T. wallagonius* Jain, 1952 [all from Siluridae]).

Clade A2 comprises species exclusively parasitizing marine catfish (Ariidae) from South America and the Oriental region (Fig. 3, clade marked in light blue): *Chauhanellus* spp. (*C. boegeri*, *C. susanlimae* and *C. velum*) and *Susanlimocotyle narina* n. gen. n. sp. all from *Sciades herzbergii* and *Hamatopeduncularia* spp. (*Hamatopeduncularia* sp., *H. arii* Yamaguti, 1953, *H. bifida* Illa, Shameem, Serra, Melai, Mangam, Basuri, Petroni & Modeo, 2019, *H. elongata* Lim, 1996, *H. thalassini* Bychowsky & Nagibina, 1968 [all from *Arius jella* Day] and *H. madhaviae* Illa, Shameem, Serra, Melai, Mangam, Basuri, Petroni & Modeo, 2019, from *Plicofollis dussumieri* [Valenciennes]). *Chauhanellus* spp. appeared as a sister group to the clade including *Susanlimocotyle narina* n. sp. and *Hamatopeduncularia* spp. *Susanlimocotyle narina* n. sp. forms a strongly supported lineage closely related to *Hamatopeduncularia* spp. from Oriental ariids. The morphological difference between *Susanlimocotyle narina* n. sp., *Chauhanellus* spp. and *Hamatopeduncularia* spp. along with the results yielded by both phylogenetic analyses, indicate that *S. narina* n. sp. in fact represents a lineage genetically and morphologically different to *Chauhanellus* spp. and *Hamatopeduncularia* spp.

3.3. Genetic divergence of *Susanlimocotyle narina* n. gen. n. sp. and *Chauhanellus* spp.

The genetic divergences with the 18S rDNA gene were compared using the sequences of dactylogyrid species from Siluriformes (Fig. 3, Table 3). The difference within the *Chauhanellus* genus ranged between 1.4 and 4.7% (28–88 bp). Among *Chauhanellus* and the most closely related genera, *Susanlimocotyle* n. gen., that sharing *S. herzbergii* as a host, showed higher divergence levels (4.6 to 7.2% [83–130 bp]), while *Hamatopeduncularia* spp. which parasite Oriental ariids fish spanned from 4.1 to 5.8% (75–110 bp).

Considering the ITS1, 5.8S rDNA and ITS2 genes, only the species sequenced herein were tested (Supplementary Table S1), as there are no sequences of the other species available in NCBI database. Genetic divergence among *S. narina* n. gen. n. sp. and *Chauhanellus* spp. from *S. herzbergii* ranged between 30.1 and 33.7% (120–161 bp) for ITS1, 7.8 and 9.6% (3–11 bp) for 5.8S rDNA and 41.5 and 47.5% (155–165 bp) for ITS2, while the interspecific distances between *Chauhanellus* spp. ranged between 15.6 and 25.2% (89–123 bp) for ITS1, 1.7 and 2.6% (2–6 bp) for 5.8S rDNA and 24.4 to 50.7% (62–157 bp) for ITS2, thereby demonstrating that the ITS1 and ITS2 genes are highly variable for these organisms. The smallest interspecific distances were observed between *C. boegeri* and *C. susanlimae* for each marker, while *C. velum* was revealed as the most genetically distant species to *Chauhanellus*, 4.7% (88 bp) for 18S rDNA, 25.2% (123 bp) for ITS1, 2.6% (6 bp) for 5.8S rDNA and 50.7% (157 bp) for ITS2.

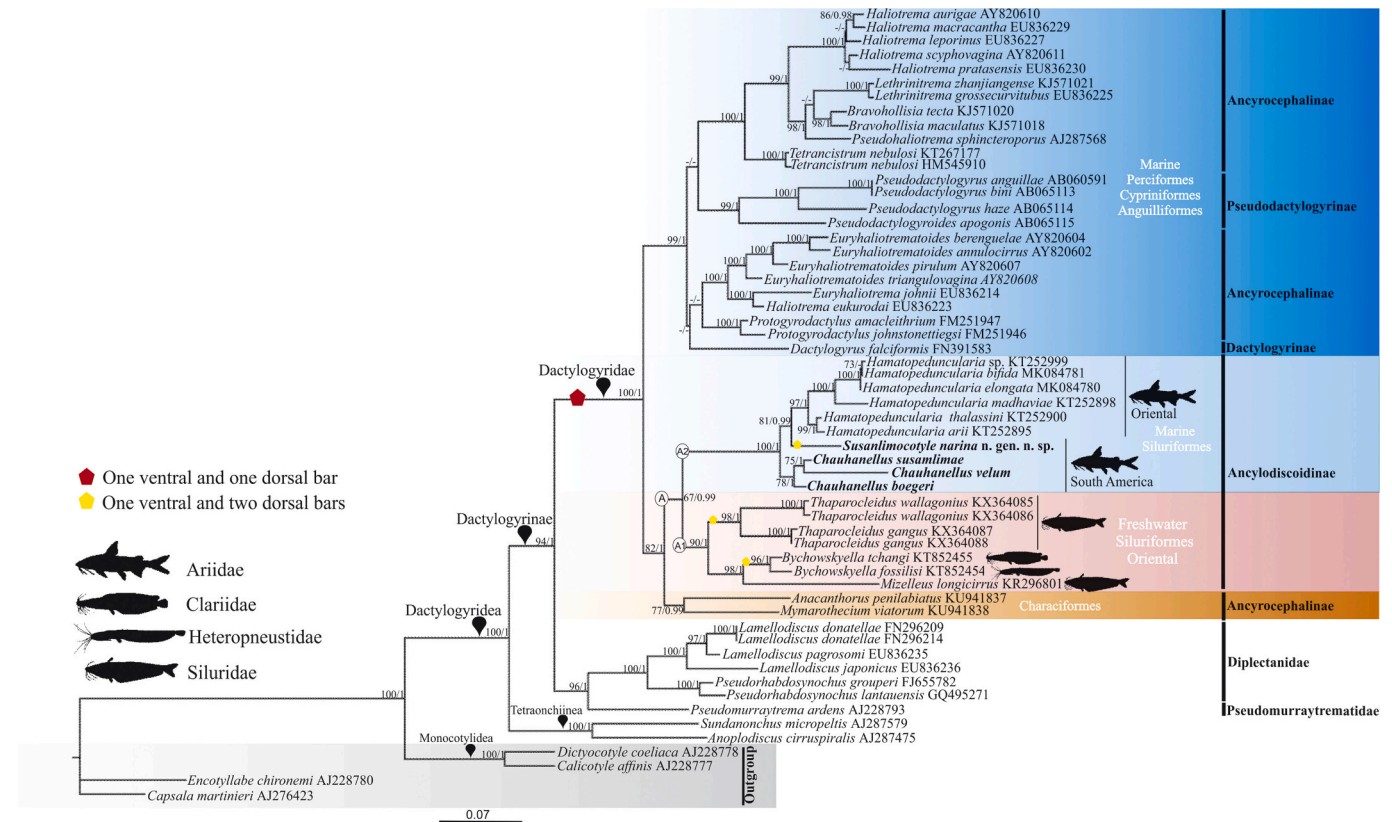


Fig. 3. Molecular phylogeny of the Dactylogyridea estimated by Bayesian inference using partial sequences of the 18S rDNA gene (1787 bp long). Species newly sequenced for the present study are in bold. Species name precedes the GenBank sequence ID. ML bootstrap support values and posterior probabilities are given above the branches (bootstrap values <60 and posterior probabilities <0.90 not reported).

Table 3

Pairwise genetic identities of 18S rDNA sequences selected from Dactylogyridae species from Siluriformes adjusted for missing data. The upper triangular matrix shows the number of differences of nucleotides and the lower triangular matrix shows the differences in terms of percentage of nucleotides. Sequences obtained in the present work are in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. <i>Mizelleus longicirrus</i> KR296801	–	234	235	206	207	333	342	328	332	319	318	164	198	216	224	240	225
2. <i>Chauhanellus velum</i>	12.5	–	130	88	83	119	130	121	120	103	104	197	206	180	186	193	192
3. <i>Susanlimocotyle narina</i> n. gen. n. sp.	12	7.2	–	83	85	110	105	94	95	75	76	198	194	167	173	181	178
4. <i>Chauhanellus boegeri</i>	10.8	4.7	4.6	–	28	89	93	85	84	60	62	169	183	144	150	164	161
5. <i>Chauhanellus susamlimae</i>	11.1	4.3	4.7	1.4	–	86	90	82	81	61	60	168	182	147	153	164	161
6. <i>Hamatopeduncularia madhaviae</i> KT252898	11.7	6.3	5.8	4.7	4.5	–	75	62	61	68	68	199	220	177	185	215	198
7. <i>Hamatopeduncularia</i> sp. KT252899	12.1	7	5.7	5.3	5	3.8	–	12	14	65	62	198	217	177	185	215	199
8. <i>Hamatopeduncularia bifida</i> MK084781	11.7	6.5	5.2	4.7	4.4	3.3	0.5	–	1	54	51	189	211	169	177	205	189
9. <i>Hamatopeduncularia elongatum</i> MK084780	11.7	6.4	5.2	4.7	4.4	3.2	0.6	0.1	–	55	52	188	210	170	178	208	192
10. <i>Hamatopeduncularia arii</i> KT252895	11	5.6	4.1	3.4	3.3	3.6	3.5	2.9	3	–	15	182	200	155	163	193	177
11. <i>Hamatopeduncularia thalassini</i> KT252900	10.9	5.6	4.1	3.4	3.3	3.5	3.2	2.7	2.8	0.7	–	181	199	152	160	190	174
12. <i>Bychowskyella tchangi</i> KT852455	7.7	10.1	9.8	8.7	8.6	9.8	9.9	9.4	9.4	8.9	8.9	–	52	150	156	152	159
13. <i>Bychowskyella fossilisi</i> KT852454	8.2	10.9	10.5	9.5	9.5	10.6	10.6	10.3	10.2	9.7	9.7	1.9	–	166	174	185	179
14. <i>Thaparocleidus gangus</i> KX364088	10.6	10	8.9	7.9	8.2	9.4	9.4	8.9	9	8.3	8.1	7.1	7.9	–	6	118	114
15. <i>Thaparocleidus gangus</i> KX364087	11	10.3	9.3	8.3	8.6	9.7	9.7	9.3	9.4	8.6	8.5	7.4	8.2	0.3	–	124	120
16. <i>Thaparocleidus wallagonius</i> KX364086	10.9	10.5	9.5	8.9	8.9	10.2	10.3	9.8	9.9	9.2	9.	7.3	8.3	5.9	6.2	–	11
17. <i>Thaparocleidus wallagonius</i> KX364085	10.9	10.4	9.4	8.7	8.7	9.9	10.1	9.7	9.7	9.	8.8	7.7	8.6	5.7	6.1	0.6	–

4. Discussion

The ML and BI phylogenetics analysis (Fig. 3) using partial 18S rDNA sequences corroborate the phylogenetic relation of the Dactylogyridae with the Pseudomurraytremitidae and Diplectanidae [12,13,15]. Nevertheless, this is the first time that the phylogenetic relationships of monogenoids parasites of ariids based on the partial 18S rDNA sequences have been examined. Moreover, the combination of morphological and molecular data supports *Susanlimocotyle* as a new genus of Dactylogyridae.

To date, 72 valid species belonging to nine monogenoids genera (*Chauhanellus* [27 species], *Calceostomella* [1 species], *Fridericianella* [1 species], *Hamatopeduncularia* [30 species], *Neotetraonchus* [5 species], *Neocalceostomoides* [5 species], *Neocalceostoma* [1 species] and *Thysanotophaptor* [1 species]) have been reported parasitizing marine catfish from the Ariidae family around the world. Except for *Fridericianella* from the eggs of the *Genidens barbatus*, all the species have been reported from the gills [3–7]. The new genus described herein represents the first monogenoid reported parasitizing the nostrils of ariids.

According to Boeger and Kritsky [12], the presence of one ventral and one dorsal bar in the haptor seems to be a synapomorphy for the clade Dactylogyridae + Tetraonchinae, while sharing one ventral and two dorsal bars represented a synapomorphy for two independent clades in both suborders: Sundanonchidae + Tetraonchidae, and Diplectanidae + Pseudomurraytremitidae. Domingues et al. [3] proposed that occurrences of one ventral and two dorsal bars in the haptor of some dactylogyrid species (i.e., *Curvianchoratus* Hanek, Molnar & Fernando, 1974, *Trinibaculo cauda* Tripathi, 1959, *Trinibaculum* Kritsky, Thatcher & Kayton, 1980, *Thaparocleidus tengra* [Tripathi, 1959] and *Hamatopeduncularia bagre* Hargis, 1955) is apparently derived within the family and represents autapomorphies for those taxa. Our phylogenetic reconstruction based on the 18S rDNA sequences suggests the presence of one ventral and two dorsal bars in some dactylogyrid taxa (i.e., *Susanlimocotyle* n. gen., *Thaparocleidus* and *Bychowskyella*) (Fig. 3, clade A) appear independently within the family as suggested by Domingues et al. [3]. The presence of two dorsal bars in the haptor of *S. narina* n. sp. and *H. bagre* may indicate that those species are closely related. However, the lack of 18S rDNA sequences of *H. bagre* does not allow us to infer the phylogenetic relationships among *S. narina* n. sp. and *H. bagre*. Nevertheless, *S. narina* n. sp. can be easily distinguish from *H. bagre* by possessing intestinal ceca confluent posteriorly (intestinal ceca non-confluent in *H. bagre*), MCO articulated with the accessory piece (MCO

non-articulated with the accessory piece in *H. bagre*), onchium (this structure is absent in *H. bagre*) and the lack of haptor digitations (haptor digitations present in *H. bagre*). In addition, *S. narina* n. sp. has a different site of infection and host to *H. bagre*, evidencing it as a new genus of Monogenoidea.

Our phylogenetic analyses showed that *Susanlimocotyle* n. gen., *Hamatopeduncularia* spp. and *Chauhanellus* spp. are closely related, appearing as sister lineages of the ancylostoidin species parasite of freshwater catfish from the Oriental region (Fig. 3, clade A). Many authors have proposed a phylogenetic proximity among *Chauhanellus* and *Hamatopeduncularia* based only on morphologically shared features [3,5,9,38,39]. Some members of *Chauhanellus* and *Hamatopeduncularia* share morphological characteristics that have been used to distinguish each other in the past (e.g., *Chauhanellus intermedius* Lim, 1994, *C. digitalis* Lim, 1994, *C. aspinous* Lim, 1994, *C. pedunculatus* Paperna, 1977, *C. hamatopeduncularoideum* and *C. susamlimae* possess features found in both *Chauhanellus* [i.e., roots expanded into wings and ventral bar with protuberances at each end] and *Hamatopeduncularia* [i.e., digitation of the haptor and absent of spines on the dorsal anchor]; *Hamatopeduncularia arii*, *H. thalassini*, *H. pulchra* Bychowsky & Nagibina, 1969, and *H. pearsoni* Kearns & Whittington, 1994, also exhibit features found in both *Hamatopeduncularia* [i.e., digitation of the haptor and absent of spines on the dorsal anchor] and *Chauhanellus* [i.e., roots expanded into wings and ventral bar with protuberances at each end] see [3,5,8,9]), suggesting that these features cannot be used as synapomorphies to differentiate both genera, and raises the question of synonymy. However, our analyses revealed phylogenetic support for the validity of both genera, as well as the validity of the *Susanlimocotyle* n. gen. within the Dactylogyridae, suggesting that the morphologic re-evaluation of *Chauhanellus* and *Hamatopeduncularia* is necessary.

Phylogenetic relationships based on partial sequences of the 18S rDNA gene of species of *Chauhanellus*, *Susanlimocotyle* n. gen., and *Hamatopeduncularia* confirms the monogenoids from Ariidae form a monophyletic group and suggests that these parasites colonized the hosts only once in the evolutive group history, followed by diversification. The Ariidae is a monophyletic group supported by morphological and molecular data [1,40]. The group is divided into three subfamilies, Galeichthyinae, Bagreinae, and Ariinae [1,41]. Betancur-R [40] suggested that the biogeographical distribution of Ariinae (ariines from the New and Old World) was driven by vicariance associated with events during the fragmentation of Gondwana ~105–41 MY ago. According to the author, the New World ariines are basal and probably originate from

South America, while the Old World taxa form a nested clade further subdivided into groups endemic to major areas (i.e., Africa, Madagascar, India-SE Asia and Australia-New Guinea).

The emergence, in our phylogenetic reconstruction of monogenoids from ariids from South America (ariines from the New World); *Chauhanellus* spp. (*C. boegeri* + *C. velum* + *C. susamlimae*) from *S. herzbergii*, *S. couma*, *S. passany*, *Genidens barbatus* and *G. genidens* (see [3]) located at a basal position of the tree, along with *Susanlimocotyle* n. gen. from *S. herzbergii*, as a sister lineage of monogenoids from Oriental ariids (ariines from the Old World); *Hamatopeduncularia* spp. from *Arius jella* and *Plicofollis dussumieri*, as a derived lineage (Fig. 3, Clade A2, Supplementary Fig. S1), points towards shared biogeographic patterns of Gondwanan vicariance for the monogenoids and their host ariines at least 105–41 MY ago *sensu* Betancur-R [40]. Similarly, Razzolini [42] suggested, based on molecular clock estimates, that *C. boegeri* from South America ariids arises clustered together as a sister group to *Hamatopeduncularia* from Oriental ariids with divergence times ~64 MY ago, which largely coincides with the timing of ariine diversification in the context of Gondwanan vicariance.

Some studies suggest that monogenoids from Ariidae can be used as a model to reconstruct the phylogenetic histories of their hosts [6,16,43]. Moreover, phylogenetic affinity among monogenoids from ariids found herein, indicates patterns towards diversification shared among ariids and their monogenoid parasites congruent with diversification scenarios in the context of Gondwanan vicariance.

5. Conclusion

The present study provides first insights into the molecular phylogeny of monogenoids parasitizing Ariidae from the New and Old World. Morphological and molecular data suggest *Susanlimocotyle* as a new genus of Dactylogyridae and indicate the closer relationship of this genus to monogenoids parasitizing Oriental ariids. The acknowledgment of phylogenetic relationships between these parasite lineages found herein contribute to a much better comprehension of the evolutionary history involving this parasite-host system.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parint.2020.102271>.

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References

- [1] A.P. Marceñiuk, N.A. Menezes, M.R. Brito, Phylogenetic analysis of the family Ariidae (Ostariophysi: Siluriformes), with a hypothesis on the monophyly and relationships of the genera, Zool. J. Linn. Soc. 165 (3) (2012) 534–669, <https://doi.org/10.1111/j.1096-3642.2012.00822.x>.
- [2] R. Fricke, W.N. Eschmeyer, R. Van der Laan (Eds.), Eschmeyer's catalog of fishes: genera, species, references, 2020. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> (Accessed 07 July 2020).
- [3] M.V. Domingues, G.B. Soares, A. Watanabe, Monogenoidea (Polyonchoinea: Dactylogyridae) parasitizing the gills of marine catfish (Siluriformes: Ariidae) inhabiting the Atlantic Amazon Coast of Brazil, Zootaxa 4127 (2) (2016) 301–326, <https://doi.org/10.11646/zootaxa.4127.2.4>.
- [4] K. Illa, U. Shameem, V. Serra, M. Melai, S. Mangam, C.K. Basuri, G. Petroni, L. Modeo, Multidisciplinary investigation on the catfish parasite *Hamatopeduncularia* Yamaguti, 1953 (Monogenoidea: Dactylogyridae): description of two new species from India, and phylogenetic considerations, Eur. Zool. J. 86 (1) (2019) 132–155, <https://doi.org/10.1080/24750263.2019.1597931>.
- [5] L.H.S. Lim, T.A. Timofeeva, D.I. Gibson, Dactylogyridae monogeneans of the siluriform fishes of the old world, Syst. Parasitol. 50 (2001) 159–197, <https://doi.org/10.1023/A:1012237801974>.
- [6] D.C. Kritsky, E.F. Mendoza-Franco, S.A. Bullard, V.M. Vidal-Martínez, Revision of the amphiamerican *Neotetraonchus* Bravo-Hollis, 1968 (Monogenoidea: Dactylogyridae), with a description of *N. vegrandis* n. sp. From the gill lamellae of the blue sea catfish *Ariopsis guatemalensis* (Siluriformes: Ariidae) off the Pacific Coast of Mexico, Syst. Parasitol. 74 (2009) 1–15, <https://doi.org/10.1007/s11230-009-9203-9>.
- [7] D.C. Kritsky, U. Shameem, C.H. Padma Kumari, I.A. Krishnaveni, New Neocalceostomatid (Monogenoidea) from the Gills of the Blackfin Sea Catfish, *Arius jella* (Siluriformes: Ariidae), In the Bay of Bengal, India, J. Parasitol. 98 (3) (2012) 479–483, <https://doi.org/10.1645/GE-3041.1>.
- [8] L.H.S. Lim, Chauhanellus Bychowsky, Nagibina, 1969 (Monogenea) from arid fishes (Siluriformes) of Peninsular Malaysia, Syst. Parasitol. 28 (1994) 99–124, <https://doi.org/10.1007/BF00009590>.
- [9] L.H.S. Lim, Eight new species of *Hamatopeduncularia* Yamaguti, 1953 (Monogenea: Ancyrocephalidae) from Ariidae of Peninsular Malaysia, Syst. Parasitol. 33 (1996) 53–71, <https://doi.org/10.1007/BF01526634>.
- [10] B.E. Bychowsky, L.F. Nagibina, Ancyrocephalinae (Dactylogyridae, Monogenoidea) from fishes of the family Ariidae, Parazitologiya. 3 (1969) 357–368.
- [11] A.V. Gusev, Freshwater Indian Monogenoidea. Principles of systematics, analysis of the world faunas and their evolution, Indian J. Helminthol. 25 & 26 (1973 & 1974) (1976) 1–241.
- [12] W.A. Boeger, D.C. Kritsky, Revision and phylogeny of the class Monogenoidea Bychowsky, 1937, Syst. Parasitol. 26 (1993) 1–32, <https://doi.org/10.1007/BF00009644>.
- [13] W.A. Boeger, D.C. Kritsky, Coevolution of Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny, Int. J. Parasitol. 27 (12) (1997) 1495–1511, [https://doi.org/10.1016/S0020-7519\(97\)00140-9](https://doi.org/10.1016/S0020-7519(97)00140-9).
- [14] W.A. Boeger, D.C. Kritsky, Interrelationships of the Monogenoidea, in: T. Littlewood, R.A. Bray (Org.) (Eds.), Interrelationships of Platyhelminthes 200, Taylor & Francis Inc, London, 2001, pp. 92–102.
- [15] L. Plaisance, D.T.J. Littlewood, P.D. Olson, S. Morand, Molecular phylogeny of gill monogeneans (Platyhelminthes, Monogenea, Dactylogyridae) and colonization of indo-West Pacific butterflyfish hosts (Perciformes, Chaetodontidae), Zool. Scr. 34 (4) (2005) 425–436, <https://doi.org/10.1111/j.1463-6409.2005.00191.x>.
- [16] C.A. Mendoza-Palmero, I. Blasco-Costa, T. Scholz, Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes), Parasit. Vectors 8 (2015) 164, <https://doi.org/10.1186/s13071-015-0767-8>.
- [17] G.L. Humason, Animal tissue techniques, W. H. Freemanco, EUA, 1979, p. 661.
- [18] W.A. Boeger, R.T. Vianna, Monogenoidea, in: V. Thatcher (Ed.), Aquatic Biodiversity in Latin America. Amazon fish parasites (Second edition), Pensoft publishers, Sofia-Moscow, 2006, pp. 42–116.
- [19] J.D. Mizelle, A.R. Klucka, Studies on monogenetic trematodes. XVI. Dactylogyridae from Wisconsin fishes, Am. Midl. Nat. 49 (1953) 720–733.
- [20] G.B. Soares, K.X. Magalhães, A.C. Silva, J.S. Carneiro, L.L. Barbosa, N.G.S. Costa, M.V. Domingues, Monogenoids (Polyonchoinea, Dactylogyridae) from *Hydrolycus armatus* (Characiformes, Cynodontidae) with the description of a new species of *Rhinoxenus* and the proposal of a new genus from the Xingu River, Pará, Brazil, Zootaxa 4700 (2) (2019) 229–245, <https://doi.org/10.11646/zootaxa.4700.2.3>.
- [21] W.S. Rasband, ImageJ. US National Institutes of Health, Bethesda, aryland, USA, Available at, <http://imagej.nih.gov/ij/> (accessed 22 October 2019).
- [22] C. Corel, Copyright ©, 2014.
- [23] A.O. Bush, K.D. Lafferty, J.M. Lotz, W. Shostak, Parasitology meets ecology on its own terms: Margolis et al. Revisited, J. Parasitol. 83 (1997) 575–583, <https://doi.org/10.2307/3284227>.
- [24] F. Pleijel, U. Jondelius, E. Norlinder, A. Nygren, B. Oxelman, C. Schander, P. Sundberg, M. Thollesson, Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies, Mol. Phylogenet. Evol. 48 (2008) 369–371, <https://doi.org/10.1016/j.ympev.2008.03.024>.
- [25] D.T.J. Littlewood, P.D. Olson, SSU rDNA and the Platyhelminthes: Signal, noise, conflict and compromise, in: D.T.J. Littlewood, R.A. Bray (Eds.), Interrelationships of the Platyhelminthes, Taylor & Francis, London, 2001, pp. 262–278.
- [26] D.T.J. Littlewood, A. Waeschenbach, P.N. Nikolov, In search of mitochondrial markers resolving the phylogeny of cyclophyllidean tapeworms (Platyhelminthes, Cestoda) - a test study with Davaineidae, Acta Parasitol. 53 (2) (2008) 133–144, <https://doi.org/10.2478/s11686-008-0029-4>.
- [27] X.Y. Wu, X.Q. Zhu, M.Q. Xie, A.X. Li, The radiation of *Haliotrema* (Monogenea, Dactylogyridae, Ancyrocephalinae): molecular evidence and explanation inferred from LSU rDNA sequences, Parasitology. 132 (2006) 659–668, <https://doi.org/10.1017/S003118200500956X>.
- [28] B.T. Dang, A. Levsen, C. Schander, G.A. Bristow, Some *Haliotrema* (Monogenea, Dactylogyridae) from Cultured Grouper (*Epinephelus* spp.) with Emphasis on the

- Phylogenetic Position of *Haliotrema cromileptis*, J. Parasitol. 96 (1) (2010) 30–39, <https://doi.org/10.1645/GE-2140.1>.
- [29] S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (17) (1997) 3389–3402, <https://doi.org/10.1093/nar/25.17.3389>.
- [30] L.Y. Geer, A. Marchler-Bauer, R.C. Geer, L. Han, J. He, S. He, C. Liu, W. Shi, S. H. Bryant, The NCBI biosystems database, Nucleic Acids Res. 38 (2009) D492–D496, <https://doi.org/10.1093/nar/gkp858>.
- [31] M. Kearse, R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Simon, Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, A. Drummond, Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data, Bioinformatics 28 (2012) 1647–1649, <https://doi.org/10.1093/bioinformatics/bts199>.
- [32] S. Guindon, J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, O. Gascuel, New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0, Syst. Biol. 59 (2010) 307–321, <https://doi.org/10.1093/sysbio/syq010>.
- [33] D. Darriba, G.L. Taboada, R. Doallo, D. Posada, jModelTest 2: more models, new heuristics and parallel computing, Nat. Methods 9 (2012) 772, <https://doi.org/10.1038/nmeth.2109>.
- [34] F. Ronquist, J.P. Huelsenbeck, MrBayes 3: Bayesian phylogenetic inference under mixed models, Bioinformatics 19 (12) (2003) 11572–11574, <https://doi.org/10.1093/bioinformatics/btg180>.
- [35] M.A. Miller, W. Pfeiffer, T. Schwartz, Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in: SC10 Workshop on Gateway Computing Environments (GCE10), New Orleans, Louisiana, 2010, pp. 1–8.
- [36] A. Rambaut, FigTree v1.1.1: Tree Figure Drawing Tool, available from, 2008, <http://tree.bio.ed.ac.uk/software/figtree/>.
- [37] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0, Mol. Biol. Evol. 33 (7) (2016) 1870–1874, <https://doi.org/10.1093/molbev/msw054>.
- [38] G.C. Kearn, I.D. Whittington, Ancyrocephaline monogeneans of the genera *Chauhanellus* and *Hamatopeduncularia* from the gills of the blue catfish, *Arius graeffei*, in the Brisbane river and Moreton bay, Queensland, Australia, with descriptions of four new species, Int. J. Parasitol. 24 (4) (1994) 569–588, [https://doi.org/10.1016/0020-7519\(94\)90149-X](https://doi.org/10.1016/0020-7519(94)90149-X).
- [39] L.H.S. Lim, Diversity of monogeneans in Southeast Asia, Int. J. Parasitol. 28 (10) (1998) 1495–1515, [https://doi.org/10.1016/S0020-7519\(98\)00061-7](https://doi.org/10.1016/S0020-7519(98)00061-7).
- [40] R. Betancur-R, Molecular phylogenetics and evolutionary history of ariid catfishes revisited: a comprehensive sampling, BMC Evol. Biol. 9 (2009) 175, <https://doi.org/10.1186/1471-2148-9-175>.
- [41] P.A. Acero, R. Betancur-R, Monophyly, affinities, and subfamilial clades of the sea catfishes (Siluriformes: Ariidae), Ichthyol Explor Freshwaters. 18 (2) (2007) 133–143.
- [42] E.L. Razzolini, Dactylogyriinae: filogenia, classificação e ecologia histórica, Tese de Doutorado Universidade Federal do Paraná, UFPR, Paraná, 2019, p. 153.
- [43] E.F. Mendoza-Franco, M.D.C.R. Tun, A.J.D. Anchevida, R.E.D.R. Rodríguez, Morphological and molecular (28S rRNA) data of monogeneans (Platyhelminthes) infecting the gill lamellae of marine fishes in the Campeche Bank, southwest Gulf of Mexico, ZooKeys 783 (2018) 125–161, <https://doi.org/10.3897/zookeys.783.26218>.
- [44] M.I. Müller, P.S. Ceccarelli, M.T. Ueta, Supplementary studies on *Anacanthorus* and *Mymarothecium viatorum* (Monogenea: Dactylogyridae) from *Piaractus mesopotamicus* (Characiformes: Serrasalminae) in Brazil, Acta Parasitol. 61 (3) (2016) 508–515, <https://doi.org/10.1515/ap-2016-0067>.
- [45] Y. Sun, M. Li, T. Yang, Studies on *Lethrinotrema* Lim & Justine, 2011 (Monogenea: Dactylogyridae), with the description of two new species, a key to the genus and a phylogenetic analysis based on rDNA sequences, Syst. Parasitol. 88 (2014) 119–139, <https://doi.org/10.1007/s11230-014-9482-7>.
- [46] C. Verma, A. Chaudhary, H.S. Singh, Redescription of two species of *Thaparocleidus* (Monogenea: Dactylogyridae), with the description of *T. armillatus* sp. n. from *Wallago attu* and a phylogenetic analysis based on 18S rDNA sequences, Acta Parasitol. 62 (3) (2017) 652–665, <https://doi.org/10.1515/ap-2017-0079>.
- [47] C. Verma, A. Chaudhary, H.S. Singh, Morphology, molecular and systematic analyses of *Bychowskyella* (Monogenea: Dactylogyridae) in siluriform fish from India, J. Helminthol. 91 (2) (2017) 197–205, <https://doi.org/10.1017/S0022149X16000122>.
- [48] X.Y. Wu, A.X. Li, X.Q. Zhu, M.Q. Xie, Description of *Pseudorhabdosynochus seabasi* sp. n. (Monogenea: Diplectanidae) from *Lates calcarifer* and revision of the phylogenetic position of *Diplectanum grouperi* (Monogenea: Diplectanidae) based on rDNA sequence data, Folia Parasitol. 52 (3) (2005) 231–240, <https://doi.org/10.14411/fp.2005.031>.
- [49] D.T.J. Littlewood, K. Rohde, K.A. Clough, The phylogenetic position of *Udonella* (Platyhelminthes), Int. J. Parasitol. 28 (8) (1998) 1241–1250, [https://doi.org/10.1016/S0020-7519\(98\)00108-8](https://doi.org/10.1016/S0020-7519(98)00108-8).
- [50] D.T.J. Littlewood, K. Rohde, K.A. Clough, The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules, Biol. J. Linn. Soc. 66 (75) (1999) 114, <https://doi.org/10.1111/j.1095-8312.1999.tb01918.x>.
- [51] D.C. Kritsky, Dactylogyrids (Monogeneoidea: Polyonchoinea) parasitizing the gills of snappers (Perciformes: Lutjanidae): revision of *Euryhaliotrema* with new and previously described species from the Red Sea, Persian gulf, the eastern and indo-West Pacific Ocean, and the Gulf of Mexico, Zoologia. 29 (2012) 227–276, <https://doi.org/10.1590/S1984-46702012000300006>.