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Morphology and molecular phylogeny of *Chauhanellus* Bychowsky & Nagibina, 1969 (Monogenoidea) parasitizing marine catfish (Ariidae) from the Atlantic coast of South America: a new species, supplementary taxonomic information and new insights

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Abstract

In this study, a new dactylogyrid species is described from the gills of two ariid fish species of the Brazilian coast, *Genidens barbus* and *Genidens genidens*, by combining morphological characters with partial *18S rDNA* sequences. The new species can be distinguished from its congeners by the following morphological characteristics: a male copulatory organ (MCO), a coiled tubular shaft of around one counterclockwise ring, base with a sclerotized cap; MCO with tapered distal region; a T-shaped accessory piece; a vagina, a cup-shaped vaginal vestibule, slightly sclerotized; and a pharynx comprising a muscular, glandular bulb. Supplementary taxonomic data for *Chauhanellus velum* from *Sciades couma* (type-host) are also presented and new *18S rDNA* sequences of *Chauhanellus* spp. from other ariid fish from South America are provided. Phylogenetic analyses based on partial *18S rDNA* gene sequences placed *Chauhanellus riograndinensis* n. sp. and *Chauhanellus velum* as two early divergent lineages within *Chauhanellus* from South America. Finally, a way to test the monophyly of *Chauhanellus* and *Hamatopeduncularia* is also discussed, which may be useful for future studies.

Introduction

Ariidae (Siluriformes) species, also known as marine catfish, are widespread in the tropical and subtropical continental shelves of the Atlantic, Indian and Pacific Oceans (Marceniuk *et al.*, 2012). Currently, a total of 156 Ariidae species belonging to 34 genera are known and ~20 species have been found to occur on the Atlantic coast of South America (Marceniuk *et al.*, 2012; Fricke *et al.*, 2022).

There are 77 species of Monogenoidea currently parasitizing Ariidae around the world; 68 Dactylogyridae (*Chauhanellus* Bychowsky & Nagibina, 1969, *Hamatopeduncularia* Yamaguti, 1953; *Neotetraonchus* Bravo-Hollis, 1968 and *Susanlimocotyle* Soares, Domingues & Adriano, 2021), seven Neocalceostomatidae (*Fridericianella* Brandes, 1894, *Neocalceostoma* Tripathi, 1959, *Neocalceostomoides* Kritsky, Mizelle & Bilqees, 1978 and *Thysanotohaptor* Kritsky, Shameem, Kumari, & Krishnaveni, 2012), and two Udonellidae (*Udonella* Johnson, 1835) (Lim *et al.*, 2001; Domingues *et al.*, 2016; Illa *et al.*, 2019; Soares *et al.*, 2021a, b; Soo & Tan, 2021).

Chauhanellus, as amended by Lim (1994), is the second most species-rich genus, with 30 valid species, behind only *Hamatopeduncularia*, which comprises 32 species (Lim *et al.*, 2001; Soares *et al.*, 2021a; Soo & Tan, 2021). *Chauhanellus* species possess a haptor with or usually without a digit-like extension; a dorsal anchor with or without spines at proximal base surface and slightly expanded outer roots; a ventral anchors with outer roots usually expanded and base of inner roots thickened; and a vaginal dextral opening with a sclerotized vaginal tube. Of the *Chauhanellus* species known to date, 28 have been described as infecting ariid marine

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Table 1. *Chauhanellus* species from ariid hosts.

Chauhanellus species	Type host	Type locality	References
C. alatus (Chauhan, 1945)	*Arius arius*[a]	India	Lim *et al.* (2001)
C. aspinosus Lim, 1994	*Arius venosus*	Malaysia	Lim *et al.* (2001)
C. auriculatum Lim, 1994	*Arius maculatus*	Malaysia	Lim *et al.* (2001)
C. australis (Young, 1967)	*Neoarius australis*	Australia	Lim *et al.* (2001)
C. boegeri Domingues & Fehlauer, 2006	*Genidens barbus*	Brazil	Domingues & Fehlauer (2006)
C. caelatus Lim, 1994	*Nemapteryx caelata*[b]	Malaysia	Lim *et al.* (2001)
C. chauhani Venkatanarasaiah & Kulkarni, 1990	*Plicofollis dussumieri*[c]	India	Lim *et al.* (2001)
C. digitalis Lim, 1994	*Hexanematichthys sagor*[d]	Malaysia	Lim *et al.* (2001)
C. duriensis Lim, 1994	*Netuma thalassina*[e]	Malaysia	Lim *et al.* (2001)
C. flexiosus Bychowsky & Nagibina, 1969	*A. arius*[a]	China	Lim *et al.* (2001)
C. forcipi Lim, 1994	*Hexanematichthys sagor*[d]	Malaysia	Lim *et al.* (2001)
C. hamatopeduncularoideum Domingues, Soares & Watanabe, 2016	*Amphiarius rugispinis*	Brazil	Domingues *et al.* (2016)
C. hypenocleithrum Domingues, Soares & Watanabe, 2016	*Sciades proops*	Brazil	Domingues *et al.* (2016)
C. indicus Rastogi, Kumar & Singh, 2004	*Wallago attu*[f]	India	Rastogi et *al.* (2004)
C. intermedius Lim, 1994	*Hexanematichthys sagor*[d]	Malaysia	Lim *et al.* (2001)
C. malayanus Lim, 1994	*H. sagor*[d]	Malaysia	Lim *et al.* (2001)
C. nagibinae Paperna, 1977	*Plicofollis argyropleuron*[g]	Kenya	Lim *et al.* (2001)
C. nengi (Tripathi, 1959)	*Nemapteryx nenga*[h]	India	Lim *et al.* (2001)
C. neotropicalis Domingues & Fehlauer, 2006	*Aspistor luniscutis*	Brazil	Domingues & Fehlauer (2006)
C. oculatus Bychowsky & Nagibina, 1969	*A. maculatus*	China	Lim *et al.* (2001)
C. osteogeneiosi Lim, 1994	*Osteogeneiosus militaris*	Malaysia	Lim *et al.* (2001)
C. pedunculatus Paperna, 1977	*Arius* sp.	India	Lim *et al.* (2001)
C. poculus Lim, 1994	*A. maculatus*	Malaysia	Lim *et al.* (2001)
C. pulutanus Lim, 1994	*A. maculatus*	Malaysia	Lim *et al.* (2001)
C. riograndinensis **n. sp.**	***Genidens barbus***	**Brazil**	**Present study**
C. seenghali (Kumar, 2013)	*Sperata seenghala*[i]	India	Domingues *et al.* (2016)
C. susamlimae Domingues, Soares & Watanabe, 2016	*Sciades herzbergii*	Brazil	Domingues *et al.* (2016)
C. trifidus Lim, 1994	*Hexanematichthys sagor*[d]	Malaysia	Lim *et al.* (2001)
C. tuberhamatus Zhang & Ding, 1997	*Tachysurus sinensis*[i,j]	China	Lim *et al.* (2001)
C. velum Domingues, Soares & Watanabe, 2016	*Sciades couma*	Brazil	Domingues *et al.* (2016)
C. youngi Kearn & Whittington, 1994	*Neoarius graeffei*[k]	Australia	Lim *et al.* (2001)

The new species described in this study is in boldface type.
[a] Formerly *Arius falcarius*
[b] Formerly *Arius caelatus*
[c] Formerly *Tachysurus dussumieri*
[d] Formerly *Arius sagor*
[e] Formerly *Arius thalassinus*
[f] Siluridae
[g] Formerly *Arius macrocephalus*
[h] Formerly *Arius nenga*
[i] Bagridae
[j] Formerly *Arius sinensis*
[k] Formerly *Arius graeffei*

catfish worldwide, while only two species have also been reported in non-ariid hosts (see table 1) (Lim *et al.*, 2001; Domingues & Fehlauer, 2006; Domingues *et al.*, 2016).

In South America, only six species of *Chauhanellus* have been described in Atlantic coast ariids (table 1) (Domingues & Fehlauer, 2006; Domingues *et al.*, 2016; Soares *et al.*, 2021a). However, recent studies indicate that the actual number of species in the region is probably much higher (Soares *et al.*, 2022).

During a study of monogenoids of ariid species captured from the Brazilian coast, a new species of *Chauhanellus* was found in the gills of *Genidens barbus* (Lacepède) and *Genidens genidens* (Cuvier) and is described based on morphological characters

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and partial *18S rDNA* sequences. Supplementary taxonomic data from *Chauhanellus velum* Domingues, Soares & Watanabe, 2016, a parasite of *Sciades couma* (Valenciennes) (type-host) and new partial *18S rDNA* sequences of *Chauhanellus* spp. from South American ariids, are also presented. Finally, new insights into *Chauhanellus* are provided based on morphology and molecular phylogenetic evidence.

Material and methods

Sample collection, morphological study and deposit of the helminths

Specimens of distinct ariid species were collected by local fishermen with trammel nets and hooks from four locations on the Brazilian coast between December 2017 to December 2019 under a License for the Collection of Zoological Material (SISBio No. 60666-2 and Sisgen No. AD28DC2) (table 2). The gill arches were removed and placed in vials containing heated water (~65°C), following which each vial was vigorously shaken. The contents of each vial were examined in the laboratory under a dissecting microscope and helminths were removed from the gills or sediment using small probes. Helminths were fixed in either 4% formalin for morphological study or 96% ethanol for molecular characterization. Some specimens were stained with Gomori's trichrome and mounted in Damar gum to examine their internal soft structures, while others were mounted in Hoyer's medium (Humason, 1979; Boeger & Vianna, 2006) for the study of the sclerotized structures. Measurements, all in micrometres, were taken following the procedures of Mizelle & Klucka (1953). 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; anchor length measurements followed Soares *et al.* (2019); and the total length of the male copulatory organ (MCO) was measured using ImageJ (Rasband, 2022). Measurements are presented in micrometres as the mean followed by the range and the number (*n*) of specimens measured is shown in parentheses. Illustrations were prepared with a drawing tube attached to a Leica DM 2500 microscope with differential interference contrast and phase contrast optics. The soft structures were illustrated using pen and ink, while the hard structures were scanned and redrawn on a digitizing tablet using CorelDraw (2014). Plates were also prepared in CorelDraw (2014). Definitions of prevalence, mean intensity and mean abundance followed Bush *et al.* (1997). The Bray–Curtis similarity test (Bray & Curtis, 1957) was used to evaluate possible morphometric similarities between the specimens of *Chauhanellus* from *S. couma* (present study) and *C. velum* from other previously described hosts. Type specimens, vouchers and hologenophores (Pleijel *et al.*, 2008) were deposited in the Invertebrate Collection of the Museu Paraense Emílio Goeldi (MPEG. PLA), Belém, Pará, Brazil, and the collection of Platyhelminthes of the Adão José Cardoso Museum of Zoology of the State University of Campinas (ZUEC PLA), São Paulo, Brazil, respectively, under No. (MPEG.PLA 000359–000384; ZUEC PLA 186-187). The vouchers of *C. velum* CHIOC 38262 a-b, 38263 were examined for comparative purposes. Nomenclature for hosts followed Marceniuk *et al.* (2012). Details of the new taxa have been submitted to ZooBank following the International Code of Zoological Nomenclature (article 8.5 of the amended version) (International Commission on Zoological Nomenclature, 2012).

Molecular characterization of parasites

Each monogenoid specimen subjected to molecular analysis was divided using fine needles under a dissecting microscope. The anterior half of the body (without the MCO) was placed in a 1.5 ml microtube with 96% ethanol for genomic DNA extraction. The posterior part containing the haptoral complex and the MCO were flattened under coverslip pressure and mounted in Hoyer's solution for species identification. These fragments also served as vouchers (hologenophores). Genomic DNA was extracted using a Qiagen Dneasy® Blood and Tissue Kit, according to the manufacturer's protocol, with a final volume of 30 µl. DNA concentration was verified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) at 260 nm.

The partial *18S rDNA* was amplified using a two-round polymerase chain reaction (PCR). In the first round, DNA was amplified with the primer pair WormA and WormB (Littlewood & Olson, 2001). In the second round, for the semi-nested PCRs,

Table 2. Host species, locality (geographical coordinates), and monogenoid species detected in the present effort on each fish species.

Host	*N*	Locality	Parasite
Amphiarius rugispinis	10	Ajuruteua (0°49′31″ N;46°36′29″ W), Bragança, PA, Br	*Chauhanellus hamatopeduncularoideum*[a] *Chauhanellus neotropicalis*
Aspistor quadriscutis	6	Ajuruteua (0°49′31″ N; 46°36′29″ W), Bragança, PA, Br	*C. neotropicalis*[a]
	8	Caratateua (1° 59′ 41.91″ S; 46° 43′ 21.385″ W), Bragança, PA, Br	*C. neotropicalis*
Genidens barbus	18	Cananéia (25°02′09.2″ S; 47°54′57.8″ W), SP, Br	*Chauhanellus boegeri*
	31	Estuary of Patos Lagoon (32° 08′ 05.7″ S; 52° 06′ 11.2″ W), RS, Br	*C. boegeri* *Chauhanellus riograndinensis* n. sp[a]
Genidens genidens	17	Estuary of Patos Lagoon (32° 08′ 05.7″ S; 52° 06′ 11.2″ W), RS, Br	*C. boegeri* *Chauhanellus riograndinensis* n. sp
Sciades couma	6	Caratateua (1° 59′ 41.91″ S; 46° 43′ 21.385″ W), Bragança, PA, Br	*Chauhanellus hamatopeduncularoideum* *Chauhanellus velum*[a] *C. boegeri*
Sciades proops	9	Ajuruteua (0°49′31″ N;46°36′29″ W), Bragança, PA, Br	*Chauhanellus hypenocleithrum*[a]

N, number of host; PA, Pará; SP, São Paulo; RS, Rio Grande do Sul; BR, Brazil.
[a]Specimen subjected to molecular analysis, for which sequences of *18S rDNA* were used for the phylogenetic analysis.

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the primer combinations were WormA and 1270R (Littlewood & Olson, 2001) and 930F (Littlewood *et al.*, 2008) with WormB, which amplified two overlapping fragments of approximately 1179 base pairs (bp) and 1054 bp, respectively. PCRs were performed in a Matercycler® nexus (Eppendorff, Hamburg, Germany) with a final volume of 25 µl: 12.5 µl of DreamTaq Green PCR Master Mix (2×) (Thermo Scientific Wilmington, USA), following the manufacturer's recommendations, 0.5 mM of each primer, and 3 µl of the extracted DNA.

The PCR profile was performed using the cycling described in Soares *et al.* (2021a). The semi-nested PCRs were conducted with 1 µl of the product of the PCRs, diluted 1:1 in ultrapure water, applying the same cycling conditions. 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. The PCR products were purified using a 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 analyser (Applied Biosystems, California, USA) at Helixxa Company (Paulínia, São Paulo, Brazil) or at the Human Genome Research Center, of the University of São Paulo (São Paulo, Brazil), using the same primers as used for rDNA amplification.

Alignment 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 3. Standard nucleotide Basic Local Alignment Search Tool searches were then conducted (Altschul *et al.*, 1997) to verify the similarity of the newly obtained sequences from the present study with other sequences of monogenoids in the United States National Center for Biotechnology Information (NCBI) BioSystems database (Geer *et al.*, 2009). Alignment of the *18S rDNA* was generated using MUSCLE implemented in Geneious version 7.1.3 (Kearse *et al.*, 2012). A total of 34 partial sequences of the *18S rDNA* of species belonging to the Dactylogyridea order published in the NCBI BioSystems database (Geer *et al.*, 2009), along with two of the Monocotylidea order (used as the outgroup) were retrieved from GenBank (see table 3) and aligned with five newly generated sequences of *Chauhanellus* spp. from ariids from the Brazilian coast (*Chauhanellus riograndinensis* n. sp., *Chauhanellus neotropicalis* Domingues & Fehlauer, 2006, *Chauhanellus hamatopeduncularoideum* Domingues, Soares & Watanabe, 2016, *Chauhanellus hypenocleithrum* Domingues, Soares & Watanabe, 2016, and *C. velum*). Despite the availability of several partial *18S rDNA* sequences, we restricted our analysis to sequences of monogenoids >1600 bp to attain the highest number of variable and phylogenetically informative sites. Forty-one sequences (1647–2200 bp long) were aligned, and the extremes were trimmed, leaving an alignment of 1777 bp in length. The model of evolution was selected by JModelTest 2.1.1 (Darriba *et al.*, 2012) using the Akaike information criterion. Phylogenetic analyses were performed using the maximum likelihood (ML) and Bayesian inference (BI) methods. ML was performed in PhyML 3.0, implemented via a web server (http://www.atgc-montpellier.fr/phyml/) (Guindon *et al.*, 2010), with topology assessed by bootstrapping with 1000 replicates, applying the GTR + I + G model. BI was performed using MrBayes v.3.2.6 (Ronquist *et al.*, 2012), under the same model, with posterior probabilities estimated from 5×10^5 generations with two independent runs of four simultaneous Markov chain Monte Carlo (MCMC) algorithms, sufficient to keep the average standard deviation below 0.001 and the effective sample size (>200) on Tracer v1.7 (Rambaut *et al.*, 2018). The MCMC algorithms with the 1000th tree saved and diagnostics for every 1000th generation with burn-in periods, were set to the first 25,000 generations. Trees were visualized using FigTree 1.3.1 (Rambaut, 2022) and figures prepared using CorelDraw (2014). Genetic divergence was determined using the *P*-distance model matrix in MEGA version 7 (Kumar *et al.*, 2016). Gaps and missing data were deleted.

Results

A total of 100% of the 10 host specimens of *Amphiarius rugispinis* (Valenciennes), 100% of the fourteen *Aspistor quadriscutis* (Valenciennes), five (16%) of the 31 host specimens of *G. barbus*, seven (42%) of the seventeen *G. genidens*, three (50%) of the six *Sciades couma* and 100% of the nine *S. proops* (Valenciennes) examined were infected with monogenoids (table 2).

The morphological, morphometric and partial *18S rDNA* data endorsing the proposition of the new taxon, the supplementary taxonomic of *C. velum* and insights into *Chauhanellus* are presented below.

Taxonomic acts

Taxonomic summary

Class Monogenoidea Bychowsky, 1937

Subclass Polyonchoinea Bychowsky, 1937

Order Dactylogyridea Bychowsky, 1937

Dactylogyridae Bychowsky, 1933

Chauhanellus Bychowsky & Nagibina, 1969

Chauhanellus riograndinensis n. sp. (fig. 1)

Synonym. Chauhanellus sp. (Soares *et al.*, 2022)

Type-host. Genidens barbus (Lacepède), (Siluriformes, Ariidae).

Site of infection. Gills.

Type locality. Estuary of Patos Lagoon, Municipality of Rio Grande, Rio Grande do Sul State, Brazil (32° 08′ 05.7″ S; 52° 06′ 11.2″ W).

Other records. Genidens genidens (Cuvier), Ariidae (prevalence: 42% of 17 hosts; mean intensity: 47; mean abundance: 1.1), Estuary of Patos Lagoon, Municipality of Rio Grande, Rio Grande do Sul State, Brazil (32° 08′ 05.7″ S; 52° 06′ 11.2″ W).

Prevalence. 16% of 31 hosts examined.

Mean intensity. 32 parasites per infected host.

Mean abundance. two parasites per host.

Specimens deposited. Holotype, MPEG.PLA 000359; paratypes, MPEG.PLA 000360–000372; vouchers, MPEG.PLA 000373–000379; hologenophore, ZUEC PLA 187.

Representative DNA sequence. 1689 bp long partial sequence of the *18S rDNA* gene of one parasite isolates (GenBank accession number, OQ517175).

Etymology: The specific name refers to the municipality of Rio Grande, Rio Grande do Sul State, Brazil, where the type host was collected.

Number of ZooBank. 7E70DF71-791A-4333-B6B7-7989BB6F4EA1

Comparative measurements. table 4

Description. (Based on fourteen specimens, eight mounted in Hoyer's medium and six stained with Gomori's trichrome). Body fusiform, total length excluding haptor 863 (743–972;

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Table 3. List of monogenoids included in the phylogenetic analyses, providing host species data, locality, GenBank ID and references. Data for the present study are highlighted with boldface type.

Parasites species	Host	Host family	Locality	GenBank ID	Reference
Dactylogyridae					
Ancyrocephalinae					
Anacanthorus penilabiatus	*Piaractus mesopotamicus*	Serrasalmidae	Brazil	KU941837	Muller *et al.* (2016)
Bravohollisia tecta	*Pomadasys maculatus*	Haemulidae	China	KJ571020	Sun *et al.* (2014)
Bravohollisia maculatus	*P. maculatus*	Haemulidae	China	KJ571018	Sun *et al.* (2014)
Euryhaliotrema johnii	*Lutjanus johnii*	Lutjanidae	China	EU836214	Sun *et al.* (2014)
Euryhaliotrematoides annulocirrus[a]	*Chaetodon vagabundus*	Chaetodontidae	Australia	AY820602	Plaisance *et al.* (2005)
Euryhaliotrematoides berenguelae[a]	*Chaetodon citrinellus*	Chaetodontidae	French Polynesia	AY820604	Plaisance *et al.* (2005)
Euryhaliotrematoides triangulovagina[a]	*Chaetodon kleinii*	Chaetodontidae	Palau	AY820608	Plaisance *et al.* (2005)
Euryhaliotrematoides pirulum[a]	*Chaetodon lunula*	Chaetodontidae	French Polynesia	AY820607	Plaisance *et al.* (2005)
Haliotrema aurigae	*Chaetodon auriga*	Chaetodontidae	Australia	AY820610	Plaisance *et al.* (2005)
Haliotrema scyphovagina	*Forcipiger flavissimus*	Chaetodontidae	French Polynesia	AY820611	Plaisance *et al.* (2005)
Lethrinitrema zhanjiangense	*Lethrinus nebulosus*	Lethrinidae	China	KJ571021	Sun *et al.* (2014)
Lethrinitrema grossecurvitubu	*L. nebulosus*	Lethrinidae	China	EU836225	Sun *et al.* (2014)
Mymarothecium viatorum	*Piaractus mesopotamicus*	Serrasalmidae	Brazil	KU941838	Muller *et al.* (2016)
Pseudohaliotrema sphincteroporus	*Siganus doliatus*	Siganidae	Australia	AJ287568	Littlewood & Olson (2001)
Ancylodiscoidinae					
Bychowskyella fossilisi	*Heteropneustes fossilisi*	Heteropneustidae	India	KT852454	Verma *et al.* (2017a)
Bychowskyella tchangi	*Clarias batrachus*	Clariidae	India	KT852455	Verma *et al.* (2017a)
Chauhanellus boegeri	*Sciades herzbergii*	Ariidae	Brazil	MW132134	Soares *et al.* (2021a)
Chauhanellus hamatopeduncularoideum	***Amphiarius rugispinis***	**Ariidae**	**Brazil**	**OQ511493**	**Present study**
Chauhanellus hypenocleithrum	***Sciades proops***	**Ariidae**	**Brazil**	**OQ511507**	**Present study**
Chauhanellus neotropicalis	***Aspistor quadriscutis***	**Ariidae**	**Brazil**	**OQ511509**	**Present study**
Chauhanellus riograndinensis **n. sp**	***Genidens barbus***	**Ariidae**	**Brazil**	**OQ517175**	**Present study**
Chauhanellus susamlimae	*Sciades herzbergii*	Ariidae	Brazil	MW144439	Soares *et al.* (2021a)
Chauhanellus velum	*S. herzbergii*	Ariidae	Brazil	MW144823	Soares *et al.* (2021a)
C. velum	***Sciades couma***	**Ariidae**	**Brazil**	**OQ511558**	**Present study**
Hamatopeduncularia arii	*Arius jella*	Ariidae	India	KT252895	Illa *et al.* (2019)
Hamatopeduncularia bifida	*A. jella*	Ariidae	India	MK084781	Illa *et al.* (2019)
Hamatopeduncularia elongata	*A. jella*	Ariidae	India	MK084780	Illa *et al.* (2019)
Hamatopeduncularia madhaviae	*Plicofollis dussumieri*	Ariidae	India	KT252898	Illa *et al.* (2019)
Hamatopeduncularia thalassini	*A. jella*	Ariidae	India	KT252900	Illa *et al.* (2019)
Mizelleus longicirrus	*Wallago attu*	Siluridae	india	KR296801	Verma *et al.* (2017b)
Susanlimocotyle narina	*S. herzbergii*	Ariidae	Brazil	MW144824	Soares *et al.* (2021a)
Thaparocleidus gangus	*W. attu*	Siluridae	India	KX364088	Verma *et al.* (2017a)

(*Continued*)

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Table 3. (*Continued.*)

Parasites species	Host	Host family	Locality	GenBank ID	Reference
Thaparocleidus wallagonius	*W. attu*	Siluridae	India	KX364085	Verma *et al.* (2017a)
Diplectanidae					
Lamellodiscus japonicus	*Acanthopagrus s. schlegelii*	Sparidae	China	EU836236	Sun *et al.* (2014)
Lamellodiscus pagrosomi	*Pagrus major*	Sparidae	China	EU836235	Sun *et al.* (2014)
Pseudorhabdosynochus grouperi	*Epinephelus coioides*	Serranidae	Indonesia	FJ655782	Wu *et al.* (2005)
Pseudomurraytrematidae					
Pseudomurraytrema ardens	*Catostomus ardens*	Catostomidae	United States	AJ228793	Littlewood *et al.* (1998)
Anoplodiscidae					
Anoplodiscus cirrusspiralis	*Sparus auratus*	Sparidae	Australia	AJ287475	Littlewood & Olson (2001)
Sundanonchidae					
Sundanonchus micropeltis	*Channa micropeltis*	Channidae	Malaysia	AJ287579	Littlewood & Olson (2001)
Monocotylidae					
Calicotyle affinis[b]	*Chimaera monstrosa*	Chimaeridae	Norway	AJ228777	Littlewood *et al.* (1999)
Dictyocotyle coeliaca[b]	*Amblyraja radiata*	Rajidae	United Kingdom	AJ228778	Littlewood *et al.* (1998)

[a] *Euryhaliotrematoides* was placed in subjective synonymy with *Euryhaliotrema* (Kritsky, 2012).
[b] Species used as outgroups.

n = 6), total width at the level of germarium 141 (126–168; *n* = 6) (fig. 1a). Cephalic margin tapered; poorly developed terminal lobes; four bilateral pairs of head organs with rod-shaped secretion; cephalic glands unicellular, lateral to pharynx. Eyes four, equidistant; accessory chromatic granules absent. Mouth subterminal, midventral, prepharyngeal; pharynx comprising muscular, glandular bulb, spherical, 72 (63–85; *n* = 6) long, 71 (56–88; *n* = 6) wide. Oesophagus elongate; two intestinal caeca, non-confluent posteriorly, lacking diverticula. Common genital pore midventral near level of intestinal bifurcation; genital atrium muscular, unarmed. Gonads intercaecal, testis post-germarial, dorsal to germarium. Testis bacilliform, 120 (109-134; *n* = 6) long, 52 (39-65; *n* = 6) wide. Vas deferens looping left of intestinal caecum; seminal vesicle a dilatation of vas deferens, sigmoid. Single saculiform prostatic reservoir, lying posterior to the base of MCO. Copulatory complex comprising MCO, accessory piece; MCO, sclerotized, coiled tubular shaft of around one counterclockwise ring, base with a sclerotized cap, 85 (73–102; *n* = 8) long, tapered distal region. Accessory piece sclerotized, T-shaped, proximally spatulate, guarding termination of MCO; non-articulated with MCO (fig. 1c). Germarium piriform, 108 (79-164; *n* = 6) long, 69 (48-92; *n* = 6) wide. Uterus delicate. Vagina comprises a vaginal aperture with dextroventral opening, submarginal; vaginal vestibule cup-shaped, slightly sclerotized; narrow vaginal canal, a loop at the distal portion before entering the seminal receptacle (fig. 1d). Seminal receptacle ovoid. Mehlis' glands, bilateral to the uterus. Eggs, ootype not observed. Vitellaria coextensive with intestinal caeca; transverse vitelline duct anterior to seminal receptacle, dorsal to intestinal caeca. Haptor digitiform, 242 (213-273; *n* = 4) long, 222 (180-242; *n* = 4) wide, with four haptoral glands (two ventral and two mid-dorsal). Anchors dissimilar. Ventral anchor, outer 48 (44–55; *n* = 8) long, inner 31 (27–35; *n* = 8) long; base 34 (29–38; *n* = 8) long; with divergent roots; truncate superficial root; expanded deep root, evenly curved elongate shaft, slightly straight short point extending at the level of tip of the superficial root (fig. 1g). Dorsal anchor, outer 46 (41–57; *n* = 8) long, inner 42 (38–51; *n* = 8) long; base 25 (23–27; *n* = 8) long; robust, with inconspicuous roots, slightly curved short shaft, elongate point extending well past the level of the tip of the inner base; anchor spine blunt (fig. 1h). Ventral bar, 114 (93-144; *n* = 3) long, 27 (25-29; *n* = 3) wide, broadly open U-shaped rod with bifid ends for articulation with ventral anchor (fig. 1e). Dorsal bar, 105 (86–141; *n* = 5) long, 21 (18–24; *n* = 6) wide, V-shaped, with bifurcation on both ends, acute anterior protuberance, rounded posterior protuberance; elongated posteromedial process (fig. 1f). Hooks similar in shape, 17 (15–19; *n* = 16) long, shank elongated, without inflation, erect thumb, lightly curved long shaft and delicate point (fig. 1b). Filamentous hook loop comprising 80% of the length of the shank.

Remarks

The new species seems to be closely related to *Chauhanellus susamlimae* Domingues, Soares & Watanabe, 2016, by having a digitate haptor; a ventral anchor with divergent roots, a truncate superficial root and an expanded deep root; evenly curved shaft; a dorsal anchor, robust, with inconspicuous roots; slightly curved shaft; elongated point; and anchor spine blunt. However, *C. riograndinensis* n. sp. can be easily distinguished from this species because the new species possesses MCO, a coiled tubular shaft of around one counterclockwise ring, base with a sclerotized cap, MCO with tapered distal region and accessory piece,

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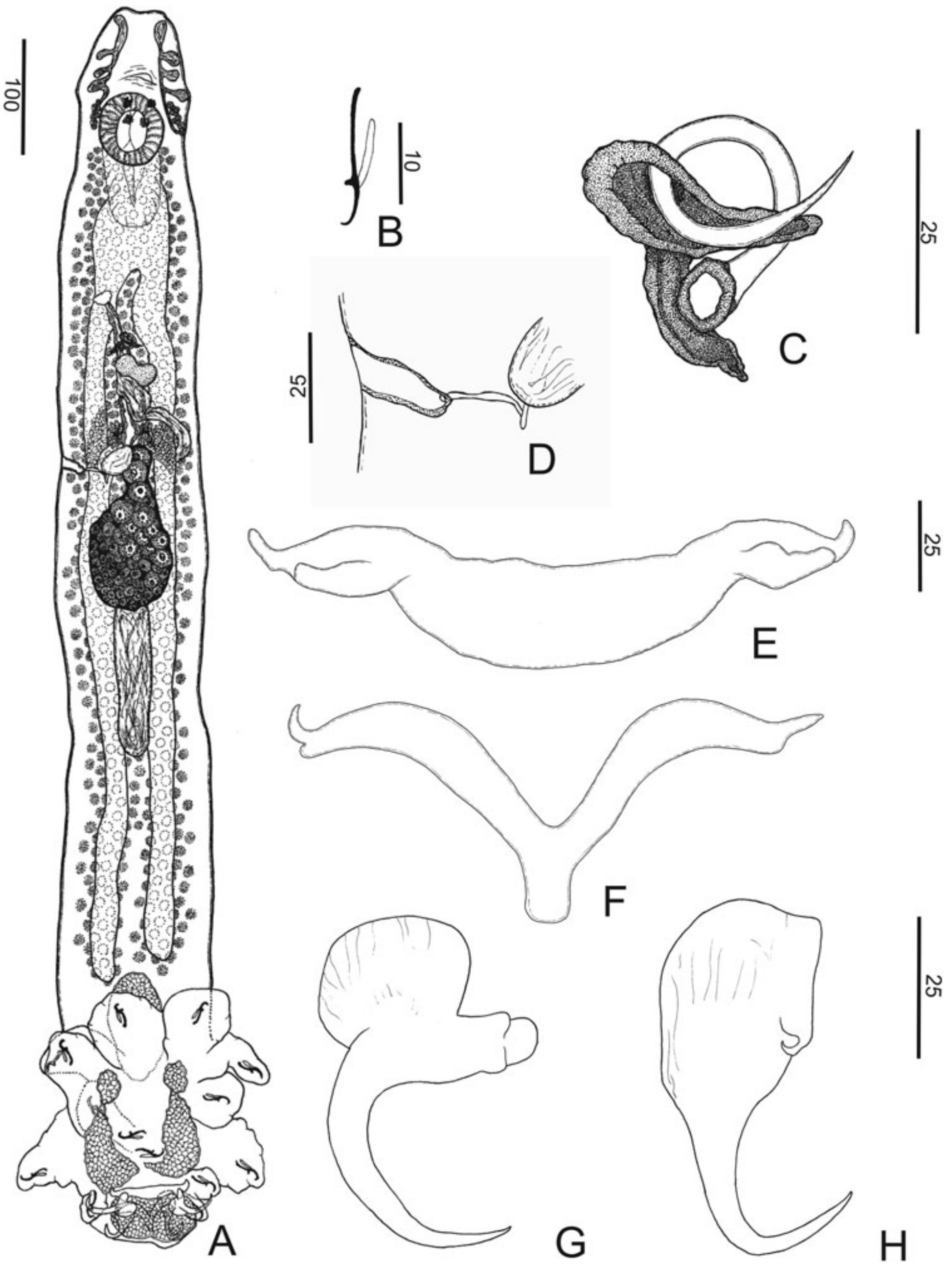

Fig. 1. *Chauhanellus riograndinensis* n. sp. (a) Holotype whole-mount, ventral; (b) hook; (c) copulatory complex; (d) vagina; (e) ventral bar; (f) dorsal bar; (g) ventral anchor; (h) dorsal anchor. Scale bars: (a) 100 μm; (b) 10 μm; (c–h) 25 μm.

T-shaped, proximally spatulate, guarding termination of MCO (MCO, a sclerotized tube, sigmoid, and accessory piece comprising an elongated sheath in *C. susamlimae*) and vagina sclerotized (a muscular vagina [unsclerotized] in *C. susamlimae*).

Chauhanellus velum Domingues, Soares & Watanabe, 2016 (fig. 2)

Type-host. *Sciades couma* (Valenciennes) (Siluriformes, Ariidae).

Site of infection. Internal borders of the primary lamellae of the gills.

Type-locality. Fish market, Municipality of Bragança, State of Pará, Brazil.

Other records. *Sciades couma*, Caratateua, Municipality of Bragança, State of Pará, Brazil (1° 59′ 41.91″ S, 46° 43′ 21.385″ W) (present study); *Sciades herzbergii*, Caratateua, Municipality of Bragança, State of Pará, Brazil (Soares *et al.*, 2021a); *Sciades herzbergii,* Furo da Ostra, municipality of Curuça, State of Pará, Brazil; *Sciades passany*, Fish market, Municipality of Bragança, State of Pará, Brazil (Domingues *et al.*, 2016).

Prevalence. 50% of six hosts examined.

Specimens deposited. Vouchers five (MPEG.PLA 000380–000384).

Specimens studied. Vouchers of *C. velum* CHIOC 38262 a-b, 38263.

Representative DNA sequence. 1654 bp long partial sequence of the *18S rDNA* gene of one parasite isolates (GenBank accession number, OQ511558).

Comparative measurements. table 5

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Table 4. Comparative measurements (in µm) of specimens of *Chauhanellus riograndinensis* n. sp. from *Genidens barbus* and *Genidens genidens* from Rio Grande, Rio Grande do Sul, Brazil.

	Genidens barbus	*N*	*Genidens genidens*	*N*
Male copulatory organ				
Length	85(73–102)	8	79(75–92)	6
Ring	17(16–18)	5	18(17–19)	3
Ventral bar				
Length	114(93–144)	3	109(105–114)	2
Width	27(25–29)	3	15(13–18)	3
Dorsal bar				
Length	105(86–141)	5	77	1
Width	21(18–24)	6	19(16–22)	3
Ventral anchor				
Outer	48(44–55)	8	45(38–52)	7
Inner	31(27–35)	8	27(22–33)	7
Base	34(29–38)	8	31(26–36)	7
Dorsal anchor				
Outer	46(41–57)	8	43(33–53)	8
Inner	42(38–51)	8	38(30–47)	8
Base	25(23–27)	8	20(15–25)	8
Hook length	17(15–19)	16	17(16–19)	16

N, number of parasite.

Redescription. (Based on five specimens, two mounted in Hoyer's medium and three stained with Gomori's trichrome). Body fusiform, total length excluding haptor 898 (766–1143; *n* = 3), total width at the level of germarium 178 (148–232; *n* = 3) (fig. 2a). Cephalic margin tapered; poorly developed terminal lobes; four bilateral pairs of head organs with rod-shaped secretion; cephalic glands not observed. Eyes four, equidistant; accessory chromatic granules absent. Mouth subterminal, midventral; pharynx subspherical, 82 (70–100; *n* = 3) long, 69 (57–79; *n* = 3) wide. Oesophagus short; two intestinal caeca, non-confluent posteriorly, lacking diverticula. Common genital pore midventral, anterior to the copulatory complex near the pharynx; genital atrium muscular, unarmed. Gonads intercaecal, testis post-germarial, dorsal to germarium. Testis subspherical, 83 (72–93; *n* = 2) long, 72 (70–75; *n* = 2) wide. Vas deferens looping left of intestinal caecum; seminal vesicle a dilatation of vas deferens, sigmoid. Single spherical prostatic reservoir, lying posterior to the base of MCO. Copulatory complex comprising MCO, accessory piece; MCO sclerotized, coiled tubular shaft of around one counterclockwise ring, base with an expanded sclerotized cap, 103 (85–113; *n* = 4) long, MCO acute distal region. Accessory piece sclerotized, comprising two regions, proximal region with three small projections (one projection serrated on the inner margin) and duct throughout the MCO pass, distal region an elongated sheath; non-articulated with MCO (fig. 2b). Germarium piriform, 121 (115–130; *n* = 3) long, 64 (50–81; *n* = 3) wide. Uterus delicate. Vagina comprises vaginal aperture with dextroventral opening, submarginal; vaginal vestibule with soft tissue; vaginal canal, short, sclerotized, knob shape, before entering the seminal receptacle. Seminal receptacle ovoid. Mehlis' glands, bilateral to the uterus. Eggs, ootype not observed. Vitellaria coextensive with intestinal caeca. Haptor subcircular, velum-like, 314 (232–383; *n* = 3) long, 273 (199–331; *n* = 3) wide, with four haptoral glands. Anchors dissimilar. Ventral anchor, outer 47 (39–54; *n* = 4) long, inner 44 (38–39; *n* = 4) long; base 39 (37–43; *n* = 4) long; with divergent roots; truncate superficial root; expanded deep root, mildly curved elongate shaft, slightly curved short point extending at the level of the tip of the superficial root (fig. 2f). Dorsal anchor, outer 44 (36–49; *n* = 3) long, inner 32 (31–33; *n* = 3) long; base 33 (28–37; *n* = 3) long; robust, with inconspicuous roots, expanded margin, slightly curved short shaft, elongate point extending well past the level of the tip of the inner base; anchor spine blunt (fig. 2g). Ventral bar 53 (42–67; *n* = 5) long, 12 (10–13; *n* = 4) wide, curved in the posterior direction, with bifid ends for articulation with ventral anchor (fig. 2d). Dorsal bar 53 (38–76; *n* = 5) long, 10 (7–14; *n* = 5) wide, slightly straight shaped, with rounded ends; mid-posteromedial process (fig. 2e). Hooks similar in shape, 18 (17–19; *n* = 5) long, shank elongated, without inflation, erect thumb, evenly curved shaft point (fig. 2c). Filamentous hook loop comprising 80% of the length of the shank.

Remarks

A comparative analysis of the vouchers of *C. velum* (CHIOC 38262 a-b, 38263), provided by Domingues *et al.* (2016), and specimens of *Chauhanellus* from *S. couma*, Caratateua, Municipality of Bragança, in Pará, Brazil, indicated that they are conspecific, mainly because they both share the same morphology of the copulatory complex, haptor velum-like, bars and anchors (fig. 2) (see also molecular data results). Moreover, the Bray–Curtis morphometric analysis of the morphological structures of *C. velum* from *S. couma* (present study) and *S. herzbergii*, *S. couma* and *S. passany* (table 5) identified a similarity of 88–95% across the specimens from each host (fig. 3; Online supplementary table S1). In addition, we provide supplementary morphological data and new illustrations of *C. velum* (fig. 2).

The ventral anchor observed in the specimens in the present study possesses a truncate superficial root and an expanded deep root (fig. 2f). This characteristic was also observed in the vouchers examined (CHIOC 38262 a-b, 38263); however, not as clearly as in the specimens of the present study, as it appears to be compressed by the coverslip sheet, making its definition difficult. It is true that the species has a truncate superficial root, although it was not clear in the drawing from the original description (Domingues *et al.*, 2016, p. 312, fig. 27). The compression of this structure by the coverslip sheet may have caused a misinterpretation by Domingues *et al.* (2016). In addition, with respect to the intestinal caeca, vas deferens, genital pore, genital atrium, seminal vesicle, seminal receptacle, uterus and Mehlis' gland, none of which were observed in the original description of the species, the present study provides a better definition of these structures (see above).

Molecular data

The sequencing of the partial *18S rDNA* of *C. riograndinensis* n. sp. was 1689 bp in length. Beyond this, four new partial *18S rDNA* sequences were obtained for four other species of *Chauhanellus* (*C. neotropicalis* from *A. quadriscutis* – 1699 bp long, *C. hamatopeduncularoideum* from *A. rugispinis* [type-host] – 1611 bp long, *C. hypenocleithrum* from *S. proops* [type-host] – 1653 bp long and *C. velum* from *S. couma* [type-host] –1654 bp long) (table 2).

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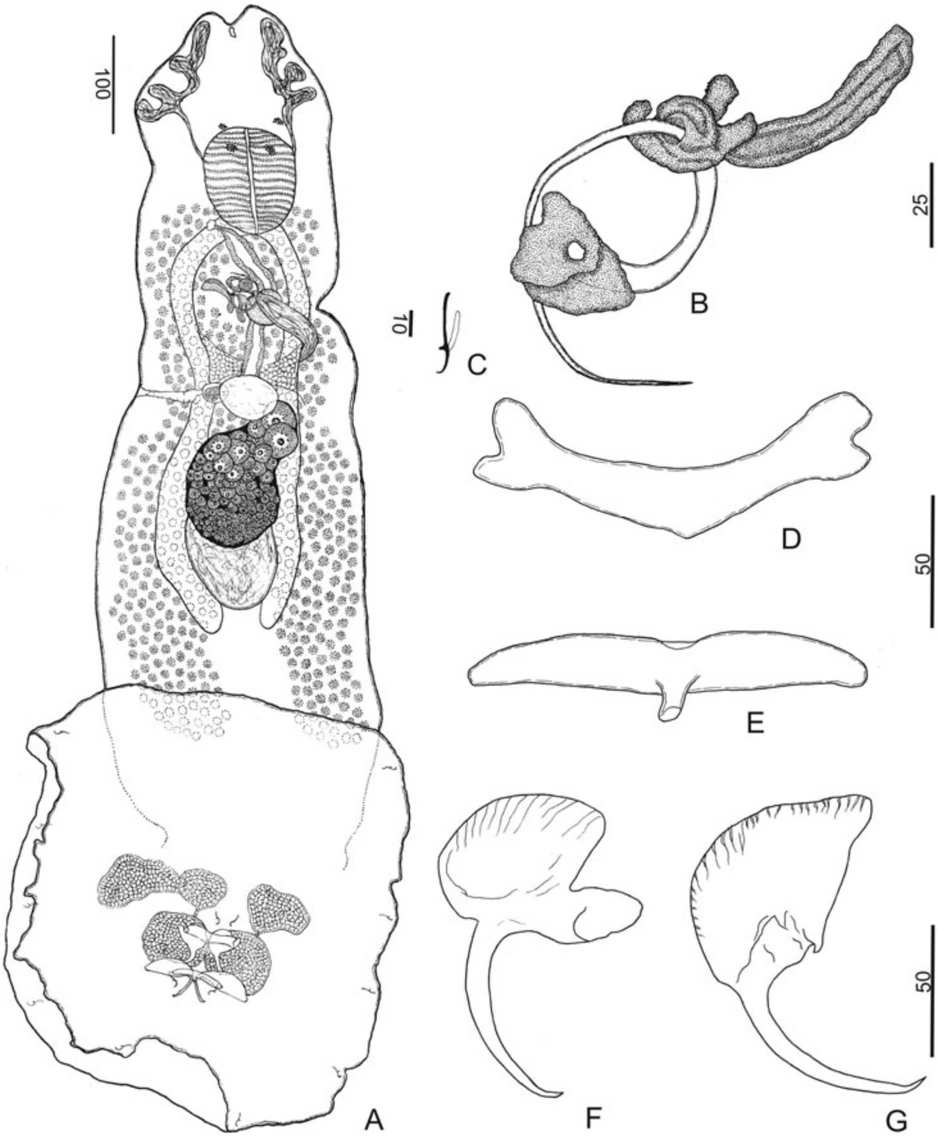

Fig. 2. *Chauhanellus velum* Domingues, Soares & Watanabe, 2016. (a) Voucher specimen whole-mount, ventral; (b) copulatory complex; (c) hook; (d) ventral bar; (e) dorsal bar; (f) ventral anchor; (g) dorsal anchor. Scale bars: (a) 100 µm; (c) 10 µm; (b) 25 µm; (d–g) 50 µm.

The genetic divergence between *Chauhanellus* species and monogenoid species from Siluriformes was compared, varying from 3.2 to 11.8% (table 6). Interspecific divergence within *Chauhanellus* ranged from 0.3 to 4.6% (7–189 bp). The genetic divergence among *C. riograndinensis* n. sp. and other *Chauhanellus* species was between 2.3 and 4.3% (39–77 pb). The divergence between *C. riograndinensis* n. sp. and the most similar morphological species, *C. susamlimae*, was 2.2% (39 pb). The smallest interspecific distance was observed between *C. boegeri* Domingues & Fehlauer, 2006 and *C. neotropicalis* at only 0.3% (7 bp), while *C. velum* was the most genetically distant species of *Chauhanellus* (4.6%). There was no intraspecific divergence between sequences of *C. velum* from the distinct hosts *S. herzbergii* and *S. couma*.

Phylogenetic evidence

The ML and BI phylogenetic analyses based on the *18S rDNA* gene converged with similar topologies, and only the BI tree was presented, with the statistical support of both methods (fig. 4). Monogenoid species from Siluriformes fish arose in clade S, with highly supportive nodes in both ML and BI analyses, and were further divided into three subclades, S1, S2 and S3 (fig. 4). S1 exclusively comprises parasites of freshwater catfish from the Oriental region: *Mizellus longicirrus* (Tripathi, 1959) from Siluridae, *Bychowskyella* spp. from Heteropneustidae and Clariidae and *Thaparocleidus* spp. from Siluridae. S2 clustered exclusively parasites of marine catfish (Ariidae) from South America and the Oriental region: *Susanlimocotyle narina* from *S. herzbergii* arises forming a strongly supported lineage closely related to *Hamatopeduncularia* spp. (*Hamatopeduncularia arii* Yamaguti, 1953, *Hamatopeduncularia bifida* Illa, Shameem, Serra, Melai, Mangam, Basuri, Petroni & Modeo, 2019, *Hamatopeduncularia elongata* Lim,1996, *Hamatopeduncularia thalassini* Bychowsky & Nagibina, 1968 [all from *Arius jella* Day] and *Hamatopeduncularia madhaviae* Illa, Shameem, Serra, Melai, Mangam, Basuri, Petroni & Modeo, 2019, from *Plicofollis dussumieri* [Valenciennes]) from Oriental ariids (fig. 4).

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Table 5. Comparative measurements (in μm) of specimens of *Chauhanellus velum* Domingues, Soares & Watanabe 2016 from *Sciades couma*, *Sciades herzbergii* and *Sciades passany* from different ariids' hosts.

	S. couma[a]	*N*	*S. couma*[b]	*N*	*S. herbergii*[b]	*N*	*S. passany*[b]	*N*
Male copulatory organ length	103(85–113)	4	110	1	104(97–112)	2	85	1
Ventral bar								
Length	53(42–67)	5	46	1	49(46–52)	2	55(52–57)	2
Width	12(10–13)	4	11	1	10(9–11)	2	14(12–15)	2
Dorsal bar								
Length	53(38–76)	5	52	1	49 (41–57)	2	67 (64–69)	2
Width	10(7–14)	5	12	1	14 (12–15)	2	15	2
Ventral anchor								
Outer	47(39–54)	4	62	1	43 (41–44)	2	37	1
Inner	44(38–39)	4	–	–	28 (25–31)	2	37	1
Base	39(37–43)	4	40	1	29 (27–31)	2	45	1
Dorsal anchor								
Outer	44(36–49)	3	–	–	123	1	165	1
Inner	32(31–33)	3	–	–	115	1	150	1
Base	33(28–37)	3	–	–	75	1	63	1
Hooks length	18(17–19)	5	16	2	16 (15–17)	2	16	2

N, number of parasite.
[a]Present study.
[b]Domingues *et al.* (2016).

Subclade S3 arises in a sister position to clade S2 and comprises *Chauhanellus* species that are exclusive parasites of marine catfish (Ariidae) from South America: *C. hamatopeduncularoideum* from *A. rugispinis*, and *C. susamlimae* from *S. herzbergii*, appearing as the derived species in a sister position to *C. hypenocleithrum* from *S. proops*. In turn, with strong support, this lineage appears as sister to the one composed of *C. boegeri* from *S. herzbergii* and *C. neotropicalis* from *A. quadriscutis*. *Chauhanellus riograndinensis* n. sp., a parasite of *G. barbus* and *G. genidens*, and *C. velum* from *S. couma* and *S. herzbergii*, arose as early divergent *Chauhanellus* species.

Discussion

Several genera have been proposed for the monogenoids of ariid fishes worldwide (Soares *et al.*, 2021a, b). *Fridericianella*, *Neocalceostoma*, *Neocalceostomoides* and *Thysanotohaptor* are harboured in the Neocalceostomatidae and *Udonella* in Udonellidae (Soares *et al.*, 2021a, b). The four others, *Chauhanellus*, *Hamatopeduncularia*, *Neotetraonchus* and *Susanlimocotyle* belong to the Dactylogyridae (Soares *et al.*, 2021a).

Of the six valid *Chauhanellus* species so far reported to be infesting South American ariid catfishes (table 1), only three have available sequences (Soares *et al.*, 2021a). In the present study, we sequenced all known South American *Chauhanellus* species, as well as the new species described here.

Only two prior reports exist of *Chauhanellus* species parasitizing *G. barbus* and *G. genidens* from the Brazilian coast: *C. boegeri* on *G. barbus* and *G. genidens* from Guaratuba, Paraná, Brazil (Domingues & Fehlauer, 2006); and an undetermined species of

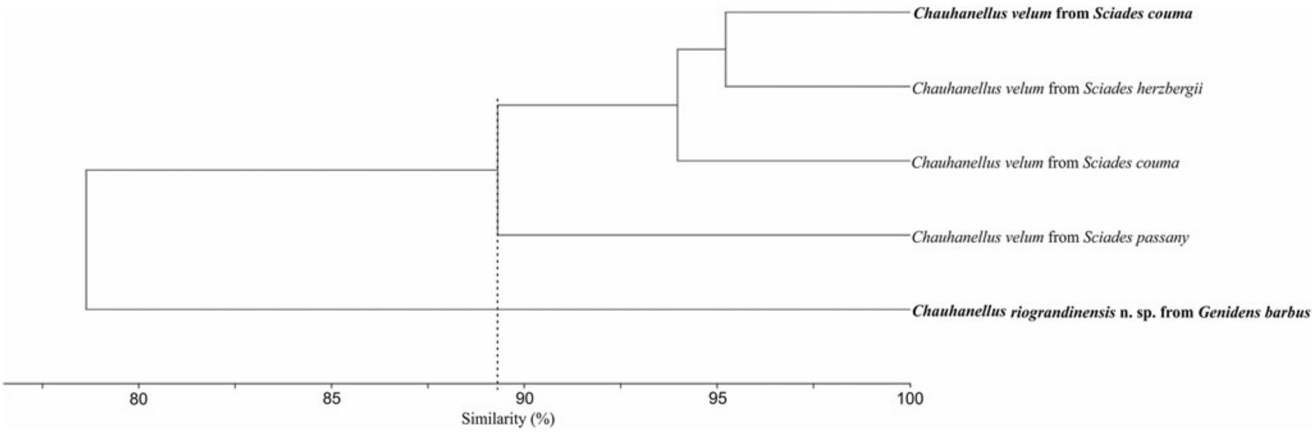

Fig. 3. Morphometric similarity dendrogram by Bray–Curtis method for *Chauhanellus velum* from different hosts and the new species described.

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Table 6. Pairwise genetic identities of *18S rDNA* sequences selected from Dactylogyridae species from Siluriformes adjusted for missing data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. *Mizelleus longicirrus* KR296801	–	189	162	214	225	454	465	208	192	430	194	399	403	184	346	237	246	222	223
2. *Bychowskyella fossilisi* KT852454	7.6	–	56	174	188	195	206	182	164	186	168	186	183	155	323	225	223	205	206
3. *Bychowskyella tchangi* KT852455	7.2	1.6	–	150	159	197	199	176	158	176	163	176	175	151	296	195	198	179	180
4. *Thaparocleidus gangus* KX364088	9.8	7.2	6.7	–	114	167	184	164	131	148	138	153	151	135	291	177	177	152	155
5. *Thaparocleidus wallagonius* KX364085	10.2	8.1	7.4	5.3	–	178	198	174	152	167	147	169	167	144	299	198	199	174	177
6. *Susanlimocotyle narina* MW144824	11.2	9.9	9.5	8.6	8.9	–	242	115	78	196	76	196	193	74	259	110	105	76	75
7. *Chauhanellus velum* MW144823[a]	11.8	10.3	9.8	9.4	9.9	6.6	–	0	77	189	84	179	184	80	279	119	131	104	103
8. *C. velum*[b]	11.8	10.3	9.8	9.4	9.9	6.6	0	–	76	78	83	78	73	80	214	107	118	95	96
9. *Chauhanellus riograndinensis* n. sp	10.5	9.1	8.5	7.6	8.6	4.4	4.3	4.3	–	45	51	43	39	39	195	77	105	55	57
10. *Chauhanellus boegeri* MW132134	10.4	9.1	8.6	7.8	8.4	4.3	4.4	4.4	2.7	–	7	104	99	32	251	89	96	62	60
11. *Chauhanellus neotropicalis*	10.6	9.3	8.8	8	8.4	4.4	4.6	4.6	3	0.3	–	36	32	36	202	82	88	59	59
12. *Chauhanellus hamatopeduncularoideum*	10.7	9.3	8.6	7.9	8.4	4.6	4.3	4.3	2.3	1.7	1.8	–	44	21	255	93	100	67	66
13. *Chauhanellus susamlimae* MW144439	10.7	9.3	8.6	8.1	8.5	4.3	4.1	4.1	2.2	1.5	1.7	0.6	–	15	251	86	93	60	61
14. *Chauhanellus hypenocleithrum*	10.6	8.9	8.3	7.9	8.3	4.3	4.4	4.4	2.2	1.8	2	1	0.8	–	194	80	86	59	60
15. *Hamatopeduncularia elongate* KT252896	15.2	13.4	12.9	13	13	11.2	12.4	12.4	10.7	10.9	11.1	10.9	10.9	11.3	–	232	220	232	232
16. *Hamatopeduncularia madhaviae* KT252898	11.0	9.7	9.2	8.6	9.1	5.1	6	6	4.3	4.3	4.4	4.4	4.1	4.6	9.5	–	75	68	68
17. *Hamatopeduncularia bifida* KT252899	11.2	9.8	9.3	8.9	9.6	4.9	6.7	6.7	4.6	4.3	5	4.9	4.6	5	9.1	3.2	–	62	65
18. *Hamatopeduncularia thalassini* KT252900	10.5	9.2	8.8	7.7	8.4	3.7	5.4	5.4	3.2	3.5	3.5	3.6	3.4	3.6	9.7	3.2	2.8	–	15
19. *Hamatopeduncularia arii* KT252895	10.5	9.3	8.9	8	8.7	3.7	5.5	5.5	3.4	3.5	3.5	3.6	3.5	3.6	9.7	3.2	3.1	0.7	–

The upper triangular matrix shows the number of differences in nucleotides and the lower triangular matrix shows the differences in terms of percentage of nucleotides.
Sequences obtained in the present work are shown in in boldface type.

[a] In *Sciades herzbergii*.

[b] In *Sciades couma*.

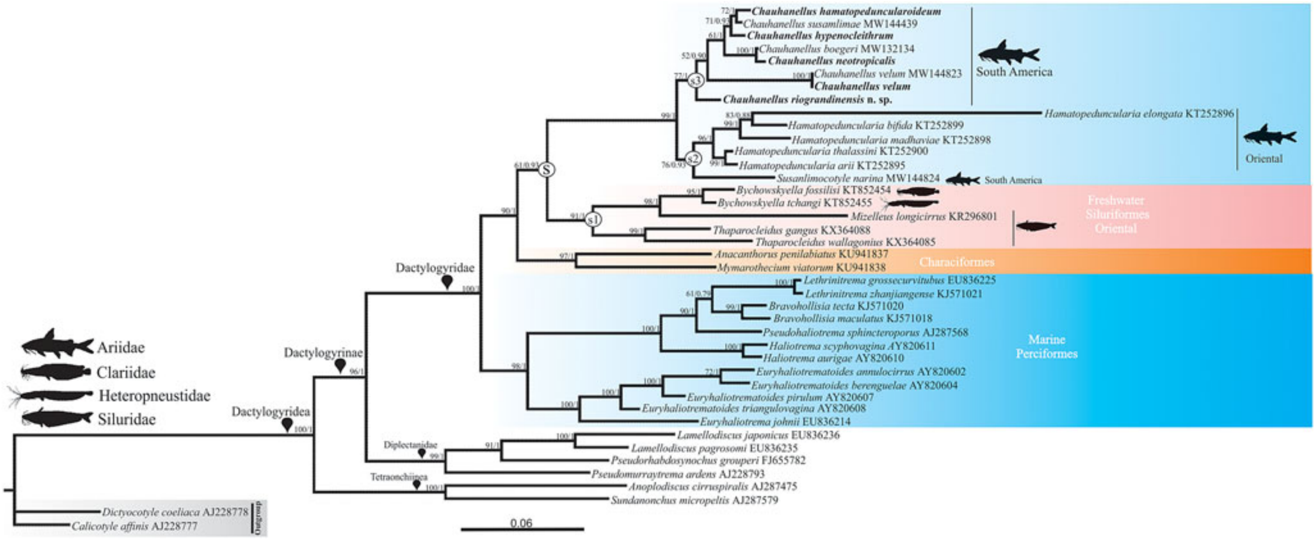

Fig. 4 Molecular phylogeny of the Dactylogyridea estimated by Bayesian inference using partial sequences of the *18S rDNA* gene (1777 base pairs long). Species newly sequenced for the present study are in boldface type. Species name precedes the GenBank sequence ID. Maximum likelihood bootstrap support values and posterior probabilities are given above the branches (bootstrap values <50 and posterior probabilities <0.70 not reported).

Chauhanellus found on *G. barbus* and *G. genidens* from the south of Brazil (Soares *et al.*, 2022). Nevertheless, a *Chauhanellus* species reported but not described by Soares *et al.*, 2022 is formally described here.

Chauhanellus was proposed by Bychowsky & Nagibina (1969) to accommodate two new species, *C. oculatus* Bychowsky & Nagibina, 1969 and *C. flexiosus* Bychowsky & Nagibina, 1969, from ariids in the south of China, as well as to transfer *Ancyrocephalus alatus* Chauhan, 1945 to *Chauhanellus* as *C. alatus* (Chauhan, 1945). The genus was proposed as closely related to *Hamatopeduncularia* (Bychowsky & Nagibina, 1969), differing from the latter by the absence of digitation on the haptor. Later, Lim (1994) proposed an amended diagnosis to the genus to accommodate some species that possess a haptor with or usually without digit-like extension and a dorsal anchor with or without spines at the proximal base surface, which are characteristics also found in *Hamatopeduncularia* (see Lim *et al.*, 2001). Here, *C. riograndinensis* n. sp. is described as possessing a combination of *Chauhanellus*-like characteristics (i.e. expanded deep roots on the ventral anchors, spine on the dorsal anchor, dumbbell-shaped ventral bar and dorsal bar with posteromedial process) and *Hamatopeduncularia*-like characteristics (i.e. haptoral digits).

Our analyses based on partial sequences of the *18S rDNA* gene revealed phylogenetic support for the validity of *C. riograndinensis* n. sp., suggesting that this species is an early divergent *Chauhanellus* species from South America (fig. 4). Our results, based on morphological, morphometric and molecular data, also suggest that the specimens of *Chauhanellus* from *S. couma* reported in the present study and *C. velum* from *S. herzbergii*, *S. couma* and *S. passany* reported by Domingues *et al.* (2016) and Soares *et al.* (2021a) are conspecific (figs 3 and 4, tables 5 and 6, Online supplementary table S1).

To date, there are ten known monogenoid species that possess a combination of the characteristics of *Chauhanellus* and *Hamatopeduncularia* (Kearn & Whittington, 1994; Lim, 1994, 1996; Lim *et al.*, 2001; Domingues *et al.*, 2016). For example, *Chauhanellus intermedius* Lim, 1994, *C. digitalis* Lim, 1994, *C. aspinous* Lim, 1994, *C. pedunculatus* Paperna,1977, *C. hamatopeduncularoideum* and *C. susamlimae* possess characteristics found in *Chauhanellus* (i.e. anchor with root expanded into wings and ventral bar with protuberances at each end) and *Hamatopeduncularia* (i.e. haptoral digits and absence of spines on the dorsal anchor); while *Hamatopeduncularia arii*, *H. thalassani*, *H. pulchra* Bychowsky & Nagibina, 1969 and *H. pearsoni* Kearn & Whittington, 1994, exhibit characteristics found in *Hamatopeduncularia* (i.e. haptoral digits and absence of spines on the dorsal anchor) and *Chauhanellus* (i.e. anchor with root expanded into wings and ventral bar with protuberances at each end). The sharing of these morphological characteristics between *Chauhanellus* and *Hamatopeduncularia* has led some authors to raise the question of synonymy (Kearn & Whittington, 1994; Lim, 1994, 1996; Lim *et al.*, 2001; Domingues *et al.*, 2016; Soares *et al.*, 2021a). However, Soares *et al.* (2021a), based on phylogenetic analyses (using the partial *18S rDNA* sequences >1700 bp) to support the validity of both genera and suggested that a morphological reevaluation of *Chauhanellus* and *Hamatopeduncularia* is required.

Recent studies based on molecular data (partial sequences of *18S rDNA* and *28S rDNA* < 900 bp) have shown that some species of *Chauhanellus* appear nested with other species of *Hamatopeduncularia* in their phylogenetic analyses, suggesting the non-monophyly of each genus (Illa *et al.*, 2019; Soo & Tan, 2021). However, the use of inappropriate sequences (e.g. short or unpublished sequences) may have contributed to these results.

The use of short sequences in phylogenetic analysis limits comparison, especially in relation to closely related taxa, as it restricts the number of variable and phylogenetically informative sites (Littlewood & Olson, 2001). In addition, small changes in alignment can have major effects on phylogenetic reconstruction (Winnepenninckx & Backeljau, 1996), and the effects of missing data can have an undesirable influence on resulting trees (Barriel, 1994; Wilkinson, 1995). We suggest that future studies that seek to test the monophyly of *Hamatopeduncularia* and *Chauhanellus* use long sequences, molecular markers from different regions and include a wider range of taxa, including the type species of each genus (*H. arii* and *C. oculatus*) and representatives of New-World and Old-World lineages *sensu* Soares *et al.*, 2021a.

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Despite the fact that *G. barbus* from Cananéia, São Paulo, Brazil (present study) and *G. barbus* and *G. genidens* from Guaratuba, Paraná state, Brazil (Domingues & Fehlauer, 2006) had been examined, the current new species was not found in these locations. On the other hand, *C. boegeri* from Guaratuba, Paraná, Brazil, was found in *G. barbus* and *G. genidens* obtained from the current study area (i.e. Cananéia, São Paulo, south-eastern Brazil and Rio Grande, Rio Grande do Sul state, southern Brazil), as well as on *S. couma* from Caratateua, Pará, northern Brazil (table 2). According to Lim *et al.* (2001), the presence of different species of *Chauhanellus* on the same host species in different biogeographical regions (e.g. *Arius maculatus* (Thunberg) from Hainan, China, and from the western coast of Peninsular Malaysia) suggests that habitat differences may affect the presence or absence of certain parasite species. However, the limitations of insufficient numbers of host samples and potentially misidentified host species should also be considered.

Conclusion

The present study provides morphological and molecular characterization (partial *18S rDNA*) of a new species of *Chauhanellus* and new *18S rDNA* sequences of *Chauhanellus* spp. from South American ariids. Our results showed that *C. riograndinensis* n. sp. and *C. velum* represent two early divergent lineages within *Chauhanellus* from South America. Moreover, the confirmation of the conspecificity of *Chauhanellus* specimens from *S. couma* (present study) and *C. velum* from *S. couma*, *S. herzbergii*, and *S. passany* reported by other authors emphasizes the need for an integrative taxonomic approach to ensure accurate delimitation of monogenoid species. Finally, we suggest one way for future studies that seek to test the monophyly of *Chauhanellus* and *Hamatopeduncularia*.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X23000135.

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Conflicts of interest. None.

Ethical standards. Permission to collect the hosts was given by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) under a License for the Collection of Zoological Material (SISBio No. 60666–2) and for access to genetic heritage (Sisgen No. AD28DC2).

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