

Research Paper

**Cite this article:** Cruces C, Chero J, Ñacari LA, De Celis VR, Simões R and Luque JL (2024). Two new *Neotetraonchus* species (Dactylogyridea, Dactylogyridae) parasitising the Peruvian sea catfish *Galeichthys peruvianus* (Siluriformes, Ariidae), including molecular data. *Journal of Helminthology*, **98**, e35, 1–8  
<https://doi.org/10.1017/S0022149X24000208>.

Received: 18 December 2023

Revised: 12 March 2024

Accepted: 13 March 2024

**Keywords:**

Monopisthocotylea; fish parasites; ariids; gill parasites; 28S rDNA; taxonomy

**Corresponding author:**

J.L. Luque;

Email: [luqueufrj@gmail.com](mailto:luqueufrj@gmail.com)

# Two new *Neotetraonchus* species (Dactylogyridea, Dactylogyridae) parasitising the Peruvian sea catfish *Galeichthys peruvianus* (Siluriformes, Ariidae), including molecular data

C. Cruces<sup>1,2</sup>, J. Chero<sup>2</sup>, L.A. Ñacari<sup>3</sup>, V.R. De Celis<sup>4</sup>, R. Simões<sup>5</sup> and J.L. Luque<sup>5</sup> 

<sup>1</sup>Programa de Pós-Graduação em Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil;

<sup>2</sup>Laboratorio de Zoología de Invertebrados, Departamento Académico de Zoología, Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos (UNMSM), Av. Universitaria cruce con Av. Venezuela cuadra 34, Lima, Peru;

<sup>3</sup>Laboratorio de Ecología y Evolución de Parásitos, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, 601 Angamos, Antofagasta, Chile; <sup>4</sup>Laboratorio de Genómica y Biología Evolutiva, Instituto de Ciencias y Tecnología, Universidad Ricardo Palma (URP), Av. Alfredo Benavides 5440 Santiago de Surco, Lima, Peru and

<sup>5</sup>Departamento de Parasitología Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil

## Abstract

As part of a parasitological survey, several specimens of two new monopisthocotylean species, *Neotetraonchus celsomanueli* sp. nov. and *N. peruvianus* sp. nov. (Dactylogyridea, Dactylogyridae), were collected from the gill filaments of the Peruvian sea catfish *Galeichthys peruvianus* (Siluriformes, Ariidae) off Puerto Pizarro, Tumbes region, Peru. *Neotetraonchus celsomanueli* sp. nov. is characterised by an MCO with a T-shaped distal end and an accessory piece that is ribbed and expanded proximally with a worm-shaped termination. *Neotetraonchus peruvianus* sp. nov. is typified by its MCO, which has a sledgehammer-shaped distal end and an accessory piece with a claw-shaped distal end. Additionally, *N. peruvianus* sp. nov. is characterised by its jellyfish-shaped onchium. A partial 28S rDNA sequence was obtained from *N. celsomanueli* sp. nov., and a phylogenetic analysis was conducted. This analysis revealed the phylogenetic position of *Neotetraonchus celsomanueli* sp. nov. within a clade comprising monopisthocotylean parasites of diadromous and marine ariid catfishes, including *Hamatopeduncularia* spp., *Chauhanellus* spp., *Thysanotohaptor* Kritsky, Shameem, Kumari & Krishnaveni, 2012, and *Neocalceostomoides spinivaginalis* Lim, 1995. This finding brings the number of known *Neotetraonchus* species to seven and represents the first described *Neotetraonchus* species infecting marine catfishes from Peru.

## Introduction

The order Siluriformes constitutes an exceptionally diverse group of fishes and stands as one of the oldest in the world (Kappas *et al.* 2016). This order encompasses the family Ariidae, comprising 42 genera and 157 species. Members of this family inhabit marine and aquatic environments across the globe (Eschmeyer 2023; Marceniuk *et al.* 2024).

To date, 78 species of monopisthocotyleans have been found to parasitise Ariidae worldwide (Soares *et al.* 2023). Among these species, 69 belong to Dactylogyridae, including representatives from genera such as *Chauhanellus* Bychowsky & Nagibina, 1969, *Hamatopeduncularia* Yamaguti, 1953, *Neotetraonchus* Bravo-Hollis, 1968, and *Susanlimocotyle* Soares, Domingues & Adriano, 2021. Additionally, there are seven species classified under the Neocalceostomatidae, encompassing genera such as *Fridericianella* Brandes, 1894, *Neocalceostoma* Tripathi, 1959, *Neocalceostomoides* Kritsky, Mizelle & Bilqeas, 1978, and *Thysanotohaptor* Kritsky, Shameem, Kumari, & Krishnaveni, 2012.

The Peruvian sea catfish, *Galeichthys peruvianus* Lütken, 1874 (Siluriformes: Ariidae), is a demersal fish endemic to Peru (Kulongowski 2010; Froese and Pauly 2023; Fricke *et al.* 2023) that inhabits coastal waters (Kailola and Bussing 1995; Froese and Pauly 2023). Hitherto, surveys on monogeneans from *G. peruvianus* had been restricted to one record: the dactylogyrid *Hamatopeduncularia* sp. (Iannacone and Luque 1993; Luque *et al.* 2016).

During a study on gill ectoparasites on marine fishes from Peru, two new species of *Neotetraonchus* Bravo-Hollis, 1968, *N. celsomanueli* sp. nov. and *N. peruvianus* sp. nov., were found infecting *G. peruvianus*. Herein, the new species are figured and described.

## Materials and methods

### *Specimen collection and morphological analyses*

Specimens of the Peruvian sea catfish were captured by the artisanal fishery during January 2018 and February 2019 off the coastal zone of Puerto Pizarro, Tumbes region (3°29'S, 80°24'W),

northern Peru. Fish were immediately dissected upon capture, and the gill arches were removed and placed in vials containing heated sea water (60°C). Each vial underwent vigorous shaking, after which formalin was added to obtain a 4% solution. In the laboratory, the contents of each vial were examined under a dissecting microscope, and monopisthocotyleans were removed from the gill filaments or sediment using small probes. Some monopisthocotyleans were directly fixed in 70% ethanol and subsequently preserved in 90% ethanol until further processing. The anterior and posterior parts of these specimens were cut and used for morphological identification, whereas the middle sections were used for molecular procedures. Some specimens were stained with Gomori's trichrome (Humason 1979), clarified in clove oil, and mounted in Canada balsam for observing internal anatomy. Other specimens were mounted in Hoyer's medium (Humason 1979) for studying sclerotised structures. Examination and photography of specimens were conducted using a compound Nikon™ Eclipse SI photomicroscope (Tokio, Japon) equipped with phase contrast light microscopy, and drawings were made with the aid of a drawing tube. Measurements are presented in micrometers (µm), representing straight-line distances between extreme points of the structures measured, and are expressed as the range followed by the mean and number (n) of structures measured in parentheses. Dimensions of the haptor and copulatory sclerites follow the proposal by Kritsky *et al.* (2009). Body length represents the length of the body proper with the haptor. Numbering of haptor-hook pairs followed the system of Mizelle (1936) and Mizelle and Price (1963). Anatomical terms for *Neotetraonchus* follow Kritsky *et al.* (2009). The direction of the coil (clockwise vs. counterclockwise) of the male copulatory organ (MCO) follows Kritsky *et al.* (1985). Fishes were identified according to the keys of Peruvian marine fishes by Chirichigno and Vélez (1998) and Chirichigno and Cornejo (2001). The type-material of the species studied in the present paper was deposited in the Helminthological Collection of the Museum of Natural History at the San Marcos University (MUSM), Peru.

### Molecular characterisation and phylogenetic analysis

The DNA samples were isolated from a single specimen of a new monopisthocotylean species using the Qiagen QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. This DNA underwent polymerase chain reaction (PCR) amplification using primers C1 (5' -ACCCG CTGAA TTTAA GCAT- 3') and D2 (5' -TGGTC CGTGT TTCAA GAC- 3') (Littlewood *et al.* 2000; Lockyer *et al.* 2003; Olson *et al.* 2003) to amplify and sequence the partial 28S rDNA gene. The thermocycling profile applied was per Mendoza-Palmero *et al.* (2015). Samples were sequenced utilising an ABI 3730 DNA analyser from the RPT01A subunit for DNA sequencing, available at the Technological Platforms Network of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Sequences were edited and contigs were assembled using ProSeq 2.9 beta (Filatov 2002). The National Center for Biotechnology Information sequence database (henceforth 'GenBank') was searched for similar sequences using Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* 1990). Sequences generated in this study were aligned with selected sequences obtained from GenBank, using Clustal W software (Larkin *et al.* 2007) (Supplementary Table S1). *Pseudomurraytrematoides pricei* (DQ157672), *Pseudorhabdosynochus lantauensis* (AY553624), and *Pseudorhabdosynochus epinepheli* (AY553622)

(Diplectanidae) were used as the outgroup for the 28S rDNA. The aligned dataset was analysed with JModelTest2 software (Santorum *et al.* 2014), which compares different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the data for each gene. The best model found was TVM + I + G, selected with Bayesian information criterion (BIC). The model parameters were as follows: assumed nucleotide frequencies A = 0.1967, C = 0.1870, G = 0.2689, and T = 0.3474; substitution rate matrix with A-C substitution = 1.2088, A-G = 5.2010, A-T = 2.6301, C-G = 0.6526, C-T = 5.2010, G-T = 1.000; p-inv distribution and gamma distribution with shape parameters = 0.1800 and 1.0240, respectively. Next, the best model was implemented in MrBayes 3.2.7a (Huelsenbeck and Ronquist 2001) for Bayesian inference analysis (BI). For the BI analysis, unique random starting trees were used in Metropolis-coupled Markov chain Monte Carlo (MCMC) (Ronquist, *et al.* 2011). The analysis was performed for a total of 5,000,000 generations. Visual inspection of log-likelihood scores against generation time indicated that the log-likelihood values reached a stable equilibrium before 100,000 generations. Thus, a burn-in of 1000 samples was conducted; every 100th tree was sampled from the MCMC analysis, obtaining a total of 100,000 trees, and tree topology represented the 50% majority rule consensus trees. Support for nodes in the BI tree topology was obtained by posterior probability. The phylogenetic analyses were conducted using the Cyberinfrastructure for Phylogenetic Research Science Gateway V. 3.3 platform (<http://www.phylo.org/>) (Miller *et al.* 2011). Phylogenetic trees were visualised and edited in Figtree 1.4.4 (Rambaut 2009). Pairwise genetic distances between the sequences of 28S rDNA were calculated in MEGA X (Kumar *et al.* 2018) using the Kimura 2-Parameter model (Kimura 1980).

## Results

### Taxonomic account

*Class.* Monopisthocotylea Odhner, 1912

*Order.* Dactylogyridea Bychowsky, 1937; Dactylogyridae Bychowsky, 1933; *Neotetraonchus* Bravo-Hollis, 1968

*Neotetraonchus celsomanueli* sp. nov. Cruces, Chero, Ñacari & Luque

<https://zoobank.org/act:985C5E83-D43D-4263-9563-5F7E104DF719>

Figures 1A–N

*Type host.* *Galeichthys peruvianus* Lütken, 1874 (Siluriformes, Ariidae), Peruvian sea catfish.

*Site in host.* Gill filaments.

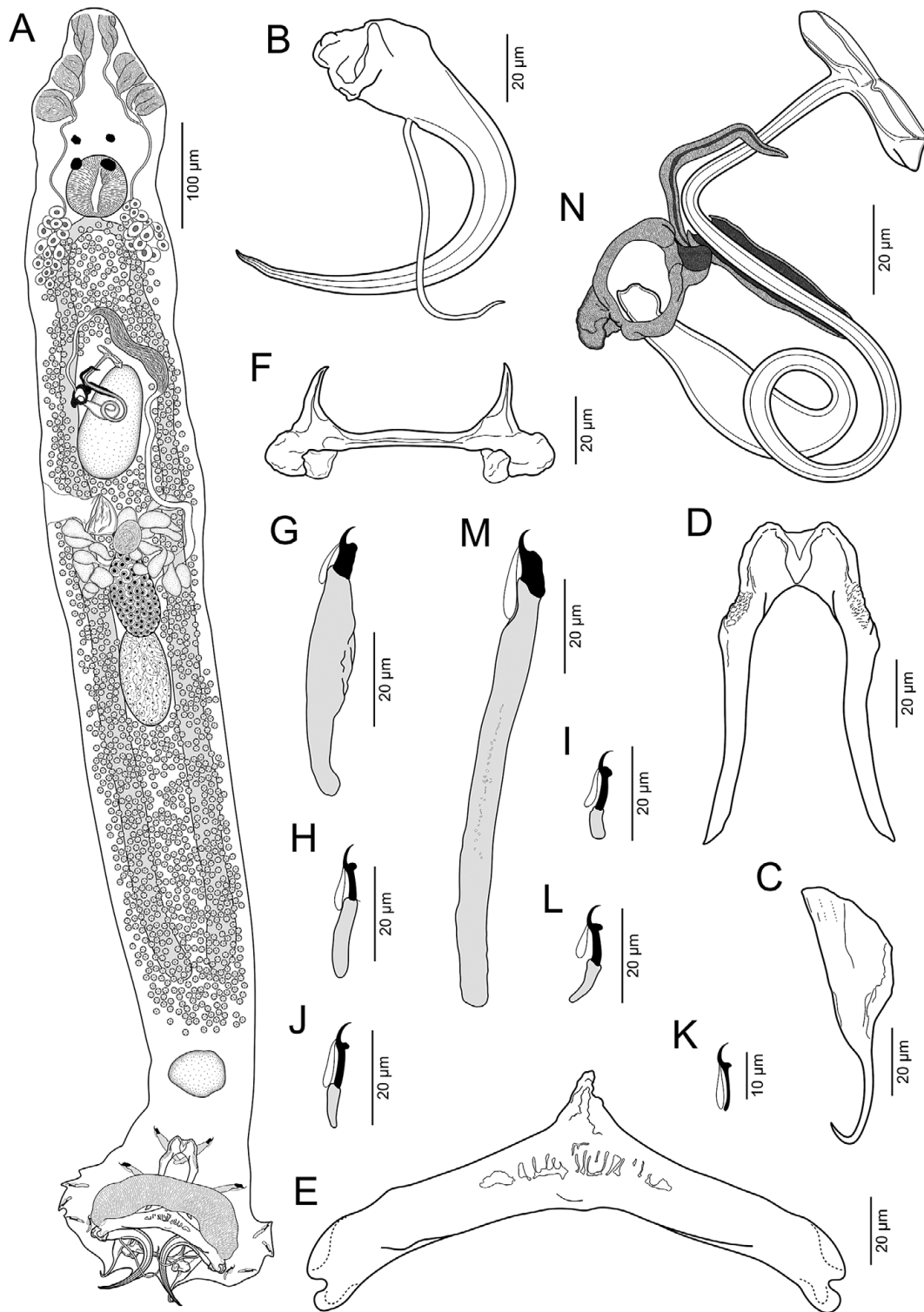
*Type locality.* Puerto Pizarro (3°29'S, 80°24'W), Tumbes, Peru, South America.

*Deposited material.* Holotype: MUSM 5402; paratypes: MUSM 5403a–j.

*Molecular sequence data.* The 28S rDNA (823 bp), obtained from one specimen, was deposited in GenBank (accession numbers OR886655).

*Etymology.* This species is named in honor of Celso Manuel Cruces Torres, father of the first author.

*Description.* Based on 5 specimens mounted in Hoyer medium and 6 specimens stained with Gomori's trichrome: Body elongate, fusiform, slender, 985–1308 (1198; n = 6) long; greatest width 112–158 (134; n = 6), usually at level of prostatic reservoir. Tegument thin, surface smooth. Cephalic region moderately broad; lateral



**Figure 1.** *Neotetraonchus celsomanueli* sp. nov. from the Peruvian sea catfish *Galeichthys peruvianus*. (A) Whole specimens (ventral view); (B) Ventral anchor; (C) Dorsal anchor; (D) Onchium; (E) Ventral bar; (F) Dorsal bar; (G) Hook pair 1; (H) Hook pair 2; (I) Hook pair 3; (J) Hook pair 4; (K) Hook pair 5; (L) Hook pair 6; (M) Hook pair 7; (N) Male copulatory organ.

lobes poorly developed; 3 bilateral pairs of conspicuous head organs; bilateral pair of cephalic glands at postpharyngeal level. Four eye-spots, equidistant, members of anterior pair smaller than those of posterior pair; chromatic granules absent. Pharynx spherical, muscle-glandular, 51–71 (62;  $n = 6$ ) in diameter; esophagus short; intestinal bifurcation postpharyngeal; intestinal ceca not

confluent posteriorly to gonads, lacking diverticula. Peduncle broad, elongate. Haptor differentiated from body proper, almost subhexagonal, with bilateral lobes, 126–187 (156;  $n = 6$ ) long; 170–219 (192;  $n = 6$ ) wide; well-developed secretory gland lying on peduncle, plate-shaped. Onchium ventral in haptor, 77–109 (93;  $n = 11$ ) long, forked-shaped, with long posteriorly directed bilateral



arms. Anchors dissimilar, lacking well-defined roots. Ventral anchor with fine conspicuous inner filament, 82–88 (85; n = 4) long; evenly curved shaft; doubly recurved point. Dorsal anchor 81–83 (82; n = 4) long, with large rootless base; delicate shaft and point. Ventral extrinsic adductor muscle well developed, broadly inverted U-shaped. Ventral bar 154–177 (163; n = 4) long, tiara-shaped, with medium anteromedial projection, bilobed lateral ends. Dorsal bar 85–104 (92; n = 4) long, rod-shaped, elongated, with two developed anterolateral protuberances, expanded lateral ends. Fourteen hooks, dissimilar in size and shape; hook pairs 1–4 and 6–7 having shanks comprised of 2 variably expanded subunits; pair 1 with a grossly expanded proximal subunit of shank, near onchium; proximal subunit of pairs 2–4 and 6 variable in length and shape; hook pair 5 with uniform shank, comprised of single subunit, lying along shaft and point of ventral anchor; filamentous hook loop extends beyond level of junction of both subunits in pairs 1, 2, 4, and 7, extend at level of junction of both subunits in pairs 3 and 6, and about shank length in pair 5; hook pair 1, 59–63 (61; n = 4) long; hook pair 2, 32–33 (33; n = 4) long; hook pair 3, 23–25 (24; n = 4) long; hook pair 4, 22–25 (23; n = 4) long; hook pair 5, 15–16 (16; n = 4) long; hook pair 6, 26–31 (28; n = 4) long; hook pair 7, 101–109 (103; n = 4) long. MCO 250–316 (282; n = 11) long, tubular, with ring-shaped base and proximally expanded shaft; shaft with T-shaped tip and counterclockwise ring at distal end of proximal expansion; accessory piece ribbed and expanded proximally, with worm-shaped termination. Testis ovate, intercecal, dorsal to ovary, 90–123 (101; n = 6) long, 31–40 (36; n = 6) wide; vas deferens looping left intestinal cecum, dilating to form sinuous seminal vesicle in left side of trunk, anterolateral to MCO; single elongate ovate prostatic reservoir, dorsal to MCO. Ovary 90–119 (102; n = 6) long, 29–48 (40; n = 6) wide; ovate, overlapping anterior portion of testis. Vaginal aperture dextralateral, prominent; vaginal vestibule large, well developed, sclerotised and bowl-shaped proximally; vaginal duct narrow, short, running posteriorly to join large subspherical seminal receptacle lying near body mid-line and overlying anterior end of ovary. Oviduct, oötype, and uterus not observed. Vitelline follicles dense throughout trunk, lateral fields of follicles confluent anterior to MCO, anterior to ovary and posterior to testis. Egg not observed.

### Remarks

*Neotetraonchus celsomanueli* sp. nov. differs from all congeners mainly by having a tubular MCO with a ring-shaped base and a hammer-shaped distal end, and by having an accessory piece ribbed and expanded proximally with worm-shaped termination. Members of the new species slightly resemble *N. bychowskyi* Bravo-Hollis, 1968 from the Gulf of Mexico in the morphology of their anchors and ventral bars. However, the new species differs from *N. bychowskyi* in the morphology of the accessory piece, which is ribbed and attached to the medial region of the shaft of the MCO, as opposed to a non-ribbed rod accessory piece in *N. bychowskyi* (see Figure 2 of Kritsky et al. 2009). In addition, both species can be differentiated by the morphology of the MCO (T-shaped tip in *Neotetraonchus celsomanueli* sp. nov. vs. flared tip in *N. bychowskyi*) and by the morphology of the onchium (plate subtrapezoidal with two anterolateral knob and large anteromedial projection in the new species vs. plate subquadrangular with short anteromedial projection in *N. bychowskyi*). *Neotetraonchus celsomanueli* sp. nov. also resemble *N. bravohollisae* Paperna, 1977 from the north Atlantic Ocean in the morphology of the MCO (expanded distally). However, *N. celsomanueli* sp. nov. differ from *N.*

*bravohollisae* in the morphology of the accessory piece of the MCO copulatory complex (ribbed and expanded proximally with worm-shaped termination in *N. celsomanueli* sp. nov. vs. slender rod with sickle-shaped termination in *N. bravohollisae*), onchium (subtrapezoidal plate in the new species vs. with subquadrangular plate in *N. bravohollisae*), and morphology of the ventral bar (tiara-shaped with medium anteromedial projection in *N. celsomanueli* sp. nov. vs. an inverted U-shape with a short anteromedial projection in *N. bravohollisae*).

***Neotetraonchus peruvianus* sp. nov. Cruces, Chero, Ñacari & Luque**

<https://zoobank.org/act:4BA46D7C-17F4-49FA-94E6-F1B15A9361AA>

Figures 2A–N

*Type host.* *Galeichthys peruvianus* Lütken, 1874 (Siluriformes, Ariidae), Peruvian sea catfish.

*Site in host.* Gill filaments.

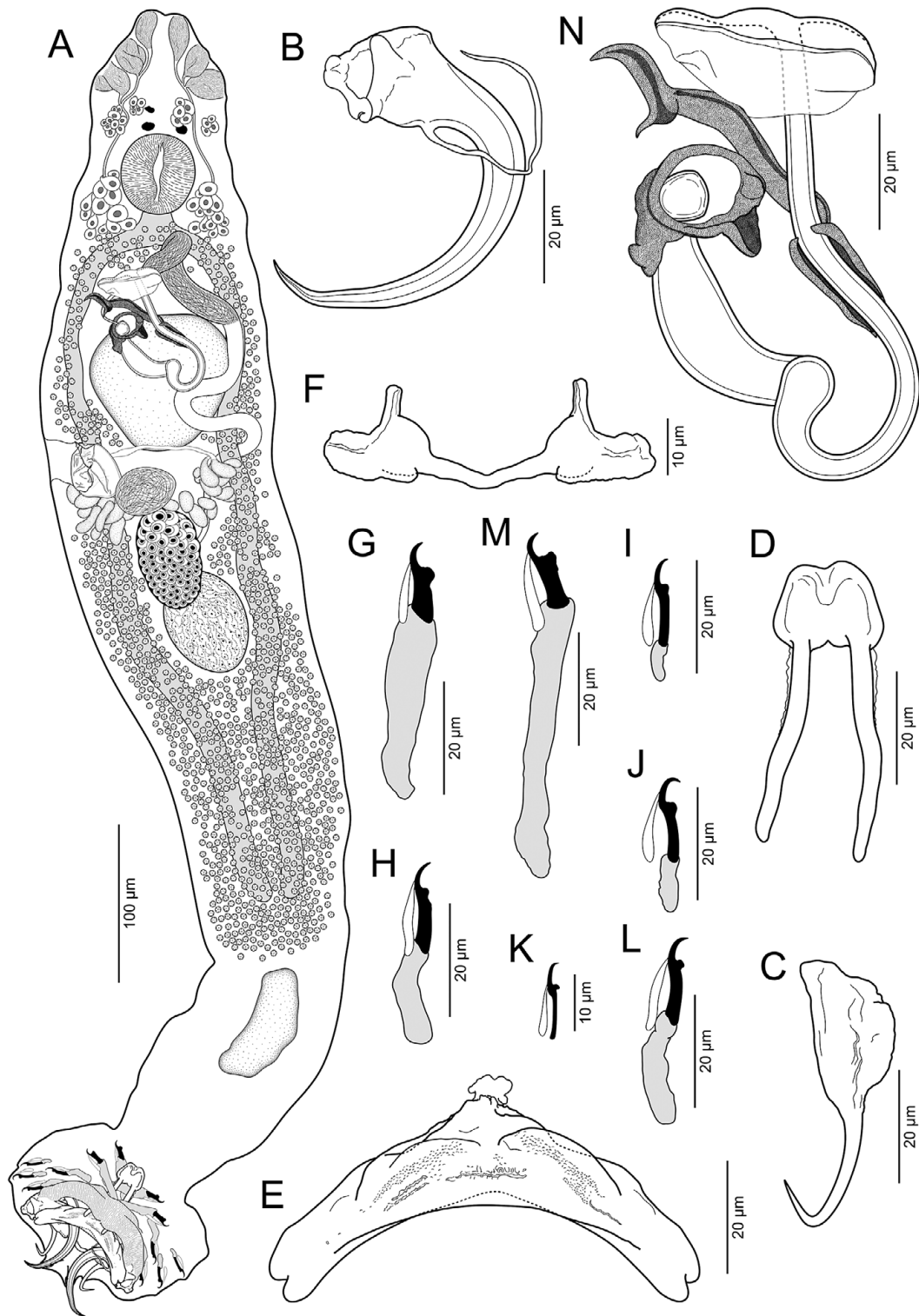
*Type locality.* Puerto Pizarro (3°29'S, 80°24'W), Tumbes, Peru, South America.

*Deposited material.* Holotype: MUSM 5404; paratypes: MUSM 5405a–g.

*Molecular sequence data.* It was not possible to obtain the molecular sequences of this species.

*Etymology.* The specific epithet of the new species refers to the country where it was discovered.

*Description.* Based on 4 specimens mounted in Hoyer medium and 4 specimens stained with Gomori's trichrome: Body elongate, fusiform, slender to robust, 847–1094 (976; n = 4) long; greatest width 137–164 (152; n = 4), usually at level of prostatic reservoir. Tegument thin, surface smooth. Cephalic region moderately broad; lateral lobes poorly developed; 3 bilateral pairs of conspicuous head organs; bilateral pair of cephalic glands at pre and postpharyngeal level. Four, equidistant, members of anterior pair smaller than those of posterior pair; chromatic granules absent. Pharynx spherical, eye spots muscle-glandular, 53–69 (60; n = 4) in diameter; esophagus short; intestinal bifurcation postpharyngeal; intestinal caeca not confluent posteriorly to gonads, lacking diverticula. Peduncle broad, elongate. Haptor differentiated from body proper, almost subrectangular, with bilateral lobes, 95–124 (109; n = 4) long; 136–164 (149; n = 4) wide; well-developed secretory gland lying on peduncle, elongate. Onchium ventral in haptor, 44–58 (53; n = 8) long, jellyfish-shaped, with long posteriorly directed bilateral arms. Anchors dissimilar, lacking well-defined roots. Ventral anchor with fine conspicuous inner filament, 49–55 (52; n = 4) long; evenly curved shaft; doubly recurved point. Dorsal anchor 46–51 (49; n = 4) long, with large rootless base; delicate shaft and point. Ventral extrinsic adductor muscle well developed, broadly inverted U-shaped. Ventral bar 106–113 (111; n = 4) long, broadly inverted V-shaped, with broad anteromedial projection, bilobulated lateral ends. Dorsal bar 73–76 (74; n = 4) long, bandeau-shaped, elongated, with two developed anterolateral projections, expanded lateral ends. Fourteen hooks, dissimilar; hook pairs 1–4 and 6–7 having shanks comprised of 2 variably expanded subunits; pair 1 with grossly expanded proximal subunit of shank, near onchium; proximal subunit of pairs 2–4 and 6 variable in length and shape; hook pair 5 with undilated shank, comprised of single subunit, lying along shaft and point of ventral anchor; filamentous hook loop extends beyond level of junction of both subunits in pairs 1, 2, 4, and 7, extend at level of junction of both subunits in pairs 3 and 6, and about shank length in pair 5; hook pair 1, 40–45 (42; n = 4) long; hook pair 2, 26–31 (29; n = 4) long; hook pair 3, 23–26 (25; n = 4) long; hook pair 4, 23–27 (25; n = 4) long; hook pair 5, 16–17 (16; n =



**Figure 2.** *Neotetraonchus peruvianus* sp. nov. from the Peruvian sea catfish *Galeichthys peruvianus*. (A) Whole specimens (ventral view); (B) Ventral anchor; (C) Dorsal anchor; (D) Onchium; (E) Ventral bar; (F) Dorsal bar; (G) Hook pair 1; (H) Hook pair 2; (I) Hook pair 3; (J) Hook pair 4; (K) Hook pair 5; (L) Hook pair 6; (M) Hook pair 7; (N) Male copulatory organ.

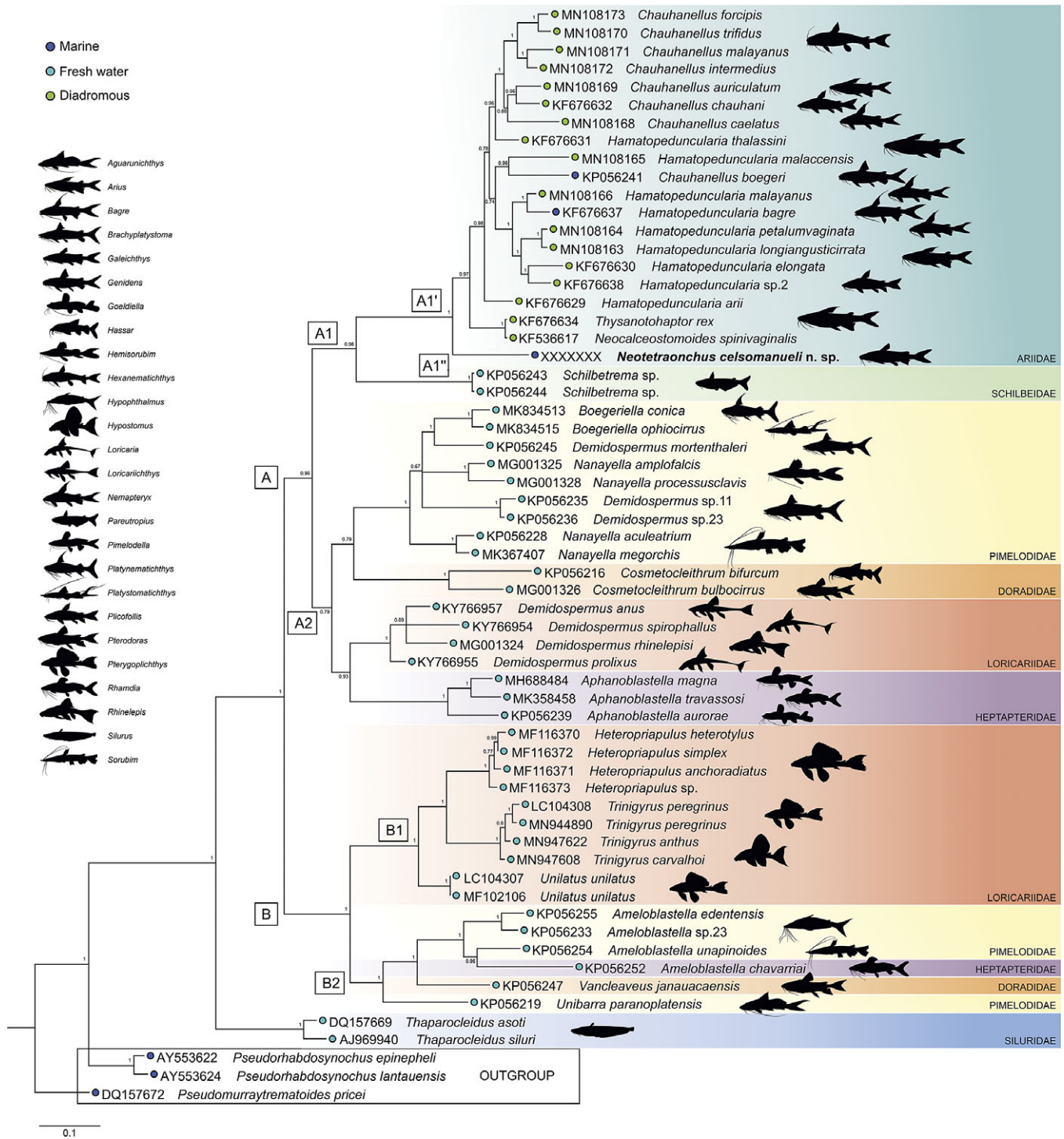
3) long; hook pair 6, 31–36 (33; n = 4) long; hook pair 7, 62–66 (65; n = 4) long. MCO 172–226 (194; n = 8) long, tubular, with ring-shaped base and proximally expanded shaft; shaft with sledgehammer-shaped distal end, with counterclockwise ring at distal end of proximal expansion; accessory piece ribbed and expanded proximally, with claw-shaped distal end. Testis ovate,

intercecal, dorsal to ovary, 57–79 (70; n = 4) long, 38–53 (46; n = 4) wide; vas deferens looping left intestinal cecum, dilating to form sinuous seminal vesicle in left side of trunk, anterolateral to MCO; single well-developed, ovate prostatic reservoir, dorsal to MCO. Ovary 72–86 (79; n = 4) long, 41–48 (45; n = 4) wide; ovate, overlapping anterior portion of testis. Vaginal aperture

dextralateral, prominent; vaginal vestibule large, well developed, sclerotised, and funnel-shaped proximally; vaginal duct narrow, short, running posteriorly to join large subspherical seminal receptacle lying near body mid-line and overlying anterior end of ovary. Oviduct, oötype, and uterus not observed. Vitelline follicles dense throughout trunk, lateral fields of follicles confluent anterior to MCO and posterior to testis. Egg not observed.

**Remarks**

*Neotetraonchus peruvianus* sp. nov. is unique among its congeners by its MCO, which has a sledgehammer-shaped distal end and an accessory piece with claw-shaped distal end. In addition, *N. peruvianus* sp. nov. is typified by having a jellyfish-shaped onchium.



**Figure 3.** Phylogenetic tree based on 28S region for *Neotetraonchus celsomanueli* sp. nov. (Dactylogyridae) inferred by Bayesian inference (BI) to show their relationships with other species of Dactylogyridae infecting arid hosts. Numbers along branches indicate the bootstrap values obtained from the posterior probability of BI. GenBank accession numbers are shown, and the scale bar indicates the number of nucleotide mutations per site.



### Phylogenetic relationships

In this study, a partial 28S rDNA sequence of 823 base pairs (bp) was obtained from *Neotetraonchus celsomaneli* sp. nov. (OR886655). BLAST analyses revealed no significant match with any other available Monopisthocotylea sequences in GenBank. The closest similarities were observed with *Hamatopeduncularia thalassini* (KF676631), *Thysanotopator rex* (KF676634), and *Chauhanellus auriculatum* (MN108169) of ariid catfishes, showing a range of similarity between 82.61–83.11%. (See [supplementary table S1](#)). The genetic divergences between *Neotetraonchus celsomaneli* sp. nov. and *Thysanotopator rex*, as well as *Neocalceostomoides spinivaginalis*, varied by 16% (84 bp). Between *Hamatopeduncularia* spp. and *Chauhanellus* spp., the divergence ranged from 17 to 23% (83–112 bp). (See [supplementary Table S2](#)).

The phylogenetic analysis was performed based on the partial 28S rDNA gene of dactylogyrid species exclusively from Siluriformes, including our newly identified species. The analyses revealed two distinct clades with robust support in Bayesian inference (BI) analyses ([Figure 3](#)). Clade A exhibited strong support and further divided into two well-supported clades: clade A1, which subdivided into A1' and A1''. Clade A1' comprised monopisthocotylean parasites of diadromous and marine catfishes (Ariidae) such as *Hamatopeduncularia* spp., *Chauhanellus* spp., *Thysanotopator rex*, *Neocalceostomoides spinivaginalis*, and *Neotetraonchus celsomaneli* sp. nov. Clade A1'' included *Schilbetrema* sp. from freshwater catfishes (Schilbeidae), closely related to dactylogyrid species of Ariid fishes. Clade A2 encompassed species parasitising freshwater catfishes (Pimelodidae, Doradidae, Loricariidae, and Heptapteridae) ([Figure 3](#)).

Clade B also displayed strong support and was divided into two subclades (B1 and B2), including species parasitising freshwater catfish. Clade B1 comprised dactylogyrid species exclusively parasitising loricariids, such as *Heteropriapulus* spp., *Trinigyryus* spp., and *Unilatus unilatus*. Clade B2 encompassed species like *Ameloblastella* spp. from Pimelodidae and Heptapteridae, *Vancleaveus janauacaensis* from Doradidae, and *Unibarra paranoplatensis* from Pimelodidae ([Figure 3](#)).

### Discussion

The present survey revealed the presence of two new species of *Neotetraonchus*, *N. celsomaneli* sp. nov. and *N. peruvianus* sp. nov., found on marine catfish *Galeichthys peruvianus*, a fish common to local markets in central and northern Peru but little studied in relation to its helminth parasites.

*Neotetraonchus* was proposed and included in the family Neotetraonchidae by Bravo-Hollis (1968) due to specific characteristics such as possessing 16 larval hooks (eight pairs), a sacciform (non-bifurcating) gut, and a sclerotised tube-like copulatory complex. However, a subsequent revision by Kritsky *et al.* (2009) reevaluated the genus, suggesting that the *Neotetraonchus* species actually features 14 hooks (seven pairs) and a gut composed of two caeca, aligning more with the typical features of the Dactylogyridae. To date, four valid *Neotetraonchus* species have been reported infesting marine ariid catfishes (Kritsky *et al.* 2009, Soares *et al.* 2023).

The BI phylogenetic analysis ([Figure 3](#)), employing partial 28S rDNA sequences, shows the phylogenetic position of the genus *Neotetraonchus* within Clade A1', grouping with members of the Neocalceostomidae (*Thysanotopator* and *Neocalceostomoides*) and Dactylogyridae (*Chauhanellus* and *Hamatopeduncularia*). However, this suggests a possible confusion in the taxonomic

position of the neocalceostomids, which is likely closely related to the Dactylogyridae. The Neocalceostomatidae has not been definitively established or universally accepted, yet the family is tentatively recognised to include species with an adult disc-shaped haptor armed with a pair of ventral anchors and 14 pairs of hooks (10 marginal and 4 ventral) (Boeger and Kritsky 2001; Kritsky *et al.* 2012).

Traditionally, the Dactylogyridae comprised the subfamilies Ancylo-discoidinae Gusev, 1961 and Ancyrocephalinae Bychowsky, 1937 (Boeger and Kritsky 1993). The BI phylogenetic analysis supports the possibility that the families Neotetraonchidae Bravo-Hollis, 1968 (now Dactylogyridae) and Neocalceostomidae Lim, 1995 could potentially be reclassified as subfamilies within the Dactylogyridae, as Neotetraonchinae and Neocalceostominae, respectively. However, determining the precise subfamily within the Dactylogyridae, from a molecular perspective, requires further sequence data from closely related taxa.

*Neotetraonchus celsomaneli* sp. nov. and *N. peruvianus* sp. nov. are the first species of the genus found infecting a *Galeichthys* species inhabiting Peruvian waters. Based on the present study and other recent reports on the monopisthocotyleans infecting fishes from northern Peru, we assume that the actual diversity of monopisthocotyleans in Peru and in the Southeast Pacific is still poorly known, and description of many new taxa, especially on poorly studied hosts, can be anticipated.

**Supplementary material.** The supplementary material for this article can be found at <http://doi.org/10.1017/S0022149X24000208>.

**Acknowledgements.** The authors are grateful to the following people who helped with collection of fishes in Peru: Ivette Cuellar, Eva G. Huancachoque, Alexander Reyes, Milagros Carrillo, and Cynthia E. Rodríguez, all from the National University Federico Villarreal. We also want to thank Arnaldo Maldonado Júnior for his kindness in lending us his laboratory facilities.

**Financial support.** C.L. Cruces was supported by a student fellowship from the Coordenação de Aperfeiçoamento de Pessoal do Ensino Superior, Brazil - Finance Code 001. J.L. Luque was supported by a researcher fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

**Competing interest.** The authors declare that they have no conflicts of interest.

**Ethical standard.** All applicable institutional, national, and international guidelines for the care and use of animals were followed.

**Data availability.** All data that support the findings of this study are available in the main text and supplementary material.

### References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Boeger WA, Kritsky DC (1993). Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* **26**, 1–32. <https://doi.org/10.1007/BF00009644>.
- Boeger WA, Kritsky DC (2001). Phylogenetic relationships of the Monogenoidea. In Littlewood DTJ, Bray RA (eds). *Interrelationships of the Platyhelminthes*. London: Taylor & Francis, 92–102.
- Bravo-Hollis M (1968). Helmintos de peces del Pacífico Mexicano. XXVII. Descripción de *Neotetraonchus bychowskyi* gen. nov., sp. nov. (Neotetraonchidae fam. nov.) de las branquias de *Galeichthys seemani* (Günther). *Anales del Instituto de Biología* **39**, 13–28.

- Bychowsky BE, Nagibina LF (1969). Ancyrocephalinae (Dactylogyridae, Monogenoidea) from fishes of the family Ariidae. *Parazitologiya* 3, 337–368. (In Russian).
- Chirichigno N, Vélez M (1998). Clave para identificar los peces marinos del Perú. *Publicación Especial del Instituto del Mar*, 2nd edn. Callao: Instituto del Mar del Perú.
- Chirichigno N, Cornejo RM (2001). Catálogo comentado de los peces marinos del Perú. *Publicación Especial del Instituto del Mar*. Callao: Instituto del Mar del Perú.
- Filatov DA (2002). Proseq: A software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes* 2, 621–624. <https://doi.org/10.1046/j.1471-8286.2002.00313.x>.
- Fricke R, Eshmeyer WN, van der Laan R (eds) (2023). *Eshmeyer's Catalog of Fishes: Genera, Species, References*. Available at <https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>. (accessed 30 November 2023).
- Froese R, Pauly D (2023). FishBase. <http://www.fishbase.org> (accessed 3 October 2023).
- Huelsenbeck JP, Ronquist F (2001). MrBAYES: Bayesian inference for phylogeny trees. *Bioinformatics* 17, 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>.
- Humason GL (1979). *Animal Tissue Techniques*, 4th edn. San Francisco: W. H. Freeman and Co.
- Iannacone J, Luque JL (1993). Aspectos ecológicos de los parásitos branquiales del bagre, *Galeichthys percutaneous* (L.) (Pisces: Teleostei) en la costa central del Perú. *Boletín de Lima (Perú)* 88, 69–73.
- Kappas I, Vittas S, Pantartzis CN, Drosopoulou E, Scouras ZG (2016). A time-calibrated mitogenome phylogeny of catfish (Teleostei: Siluriformes). *PLoS One* 11, e0166988. <https://doi.org/10.1371/journal.pone.0166988>.
- Kailola PJ, Bussing WA (1995). Ariidae. Bagres marinos. In Fischer W, Krupp F, Schneider W, Sommer C, Carpenter KE, Niem V (eds) *Guía FAO para Identificación de Especies para lo Fines de la Pesca. Pacífico Centro-Oriental*. 3 Vols. Rome: FAO, 860–886.
- Kimura MA (1980). Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111–120. <https://doi.org/10.1007/BF01731581>.
- Kritsky DC, Boeger WA, Thatcher VE (1985). Neotropical Monogenea. 7. Parasites of the Pirarucu, *Arapaima gigas* (Cuvier), with descriptions of two new species and redescription of *Dawestrema cycloancistrum* Price and Nowlin, 1967 (Dactylogyridae: Ancyrocephalinae). *Proceedings of the Biological Society of Washington* 98, 321–331.
- Kritsky DC, Mizelle JD, Bilquees FM (1978). Studies on Monogenea of Pakistan. III. Status of the Calceostomatidae (Parona and Perugia, 1890) with a redescription of *Neocalceostoma elongatum* Tripathi, 1957 and the proposal of *Neocalceostomoides* gen. n. *Proceedings of the Helminthological Society of Washington* 45, 149–154.
- Kritsky DC, Shameem U, Kumari CP, Krishnaveni I (2012). A new Neocalceostomatid (Monogenoidea) from the gills of the blackfin sea catfish, *Arius jella* (Siluriformes: Ariidae), in the Bay of Bengal, India. *Journal of Parasitology* 98, 479–483. <https://doi.org/10.1645/GE-3041.1>.
- Kritsky DC, Mendoza-Franco EF, Bullard SA, Vidal-Martínez VM (2009). 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. *Systematic Parasitology* 74, 1–15. <https://doi.org/10.1007/s11230-009-9203-9>.
- Kulongowski C (2010). Revision of the ariid catfish genus *Galeichthys* Valenciennes (subfamily Galeichthyinae), with description of a new species from South Africa and designation of a neotype for *G. ater* Castelnau. *Smithiana Bulletin* 12, 9–23.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547–1549. <https://doi.org/10.1093/molbev/msy096>.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>.
- Littlewood DTJ, Curini-Galletti M, Herniou EA (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution* 16, 449–466. <https://doi.org/10.1006/mpev.2000.0802>.
- Lockyer AE, Olson PD, Littlewood DTJ (2003). Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): Implications and a review of the cercomer theory. *Biological Journal of the Linnean Society* 78, 155–171. <https://doi.org/10.1046/j.1095-8312.2003.00141.x>.
- Luque JL, Cruces C, Chero J, Paschoal F, Alves PV, Da Silva AC, Sánchez L, Iannacone J (2016). Checklist of metazoan parasites of fishes from Peru. *Neotropical Helminthology* 10, 301–375.
- Marceniuk AP, Oliveira C, Ferraris Jr CJ (2024). A new classification of the family Ariidae (Osteichthyes: Ostariophysi: Siluriformes) based on combined analyses of morphological and molecular data. *Zoological Journal of the Linnean Society*, 200, 426–476. <https://doi.org/10.1093/zoolinnean/zlad078>.
- Mendoza-Palmero CA, Blasco-Costa I, Scholz T (2015). Molecular phylogeny of neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). *Parasites & Vectors* 8, 164. <https://doi.org/10.1186/s13071-015-0767-8>.
- Miller MA, Pfeiffer W, Schwartz T (2011). The CIPRES science gateway: A community resource for phylogenetic analyses. In *Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery*. Article 41, 1–8. <https://doi.org/10.1145/2016741.2016785>.
- Mizelle JD (1936). New species of trematodes from the gills of Illinois fishes. *American Midland Naturalist* 17, 785–806.
- Mizelle JD, Price CE (1963). Additional haptor hooks in the genus *Dactylogyryus*. *Journal of Parasitology* 19, 785–806.
- Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* 33, 733–755. [https://doi.org/10.1016/s0020-7519\(03\)00049-3](https://doi.org/10.1016/s0020-7519(03)00049-3).
- Rambaut A (2009). FigTree v1. 3.1. <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 22 Jun 2023).
- Ronquist F, Huelsenbeck J, Teslenko M (2011). Draft MrBayes version 3.2 manual: Tutorials and model summaries. Distributed with the software from [https://bioweb.pasteur.fr/docs/modules/mrbayes/3.1.2/Manual\\_MrBayes\\_v3.2.0\\_draft.pdf](https://bioweb.pasteur.fr/docs/modules/mrbayes/3.1.2/Manual_MrBayes_v3.2.0_draft.pdf).
- Santorium JM, Darriba D, Taboada GL, Posada D (2014). jmodeltest.org: Selection of nucleotide substitution models on the cloud. *Bioinformatics* 30, 1310–1311. <https://doi.org/10.1093/bioinformatics/btu032>.
- Soares GB, Domingues MV, Adriano EA (2021). 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. *Parasitology International* 81, 102271.
- Soares G, Adriano E, Domingues M, Balbuena J (2023). Diversification processes between monogenoids (Dactylogyridae) and their marine catfish (Siluriformes: Ariidae) from the Atlantic coast of South America. *Parasitology* 150, 184–194. <https://doi.org/10.1017/S0031182022001615>.