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Morphological and molecular characterization of *Udonella brasiliensis* n. sp. (Monogenoidea), an epibiont on *Caligus* sp. parasite of Ariidae from the southeastern coast of Brazil

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ABSTRACT

The present study describes *Udonella brasiliensis* n. sp., an epibiont found on *Caligus* sp., a parasite the ariids *Genidens barbus* (Lacepède) and *Aspistor luniscutis* (Valenciennes), caught on the coast of the state of São Paulo, Brazil. Morphological and molecular analyses (partial 18S rDNA) were carried out. The morphological data showed that *U. brasiliensis* n. sp. can be distinguished from current valid species by its morphometric attributes (*e.g.*, body, pharynx, ovary and testis), while the molecular information supports the proposal of a new species. The 18S rDNA phylogenetic analysis shows a close relationship between the new species and *Udonella australis* Carvajal & Sepulveda, in a subclade formed of species that parasitize South American fish. Finally, this study also discusses a scenario of initial irradiation for udonellids.

1. Introduction

Marine Ariidae catfish (Siluriformes) are widely distributed around the world, in both tropical and temperate zones [1]. This fish family comprises 155 species belonging to 34 genera, and 12% of its diversity occurs on the coast of Brazil [2,3]. To date, 33 ariid species around the world have been investigated for monogenoid parasites [3–11], and 75 species have been parasitizing this fish group, with representatives of the Dactylogyridea and Gyrodactylidea orders [3,12,13]. The former contains 65 species of Dactylogyridae and nine of Neocalceostomatidae, while reports from Gyrodactylidea are limited to *Udonella caligorum* Price, 1938 (Udonellidae) [3,12,13].

The udonellid group is composed of epibiont marine species that utilize either parasitic copepods or argulids as a substrate from which they feed off their fish hosts, with seven species described and recorded in a variety of geographical locations [14,15]. Udonellids have presented taxonomic challenges since their erection, related to the absence of a ciliated larvae, the lack of anchors or hooks, as well as differentiated sexual organs, which are important characteristics for the taxonomy of monogenoids [15,16]. However, recent studies based on molecular data support Udonellidae as a monophyletic group [15,17].

Based on an integrative taxonomic approach combining morphological characters, 18S rDNA sequences, and phylogenetic analyses, the present study describes a new species of the genus *Udonella* found on *Caligus* sp. from the ariids *Genidens barbus* (Lacepède) and *Aspistor luniscutis* (Valenciennes) off the southeastern coast of Brazil.

2. Material and methods

2.1. Sample collection, morphological study

Eighteen specimens of *G. barbus* and seven of *A. luniscutis* were collected using trammel net from the estuarine region of Cananéia, in the state of São Paulo, Brazil (25°02′09.2″S; 47°54′57.8″W) in April 2019, under a Collection of Zoological Material License (SISBio n° 60,666–2 and Sisgen n° AD28DC2).

Udonella-infected *Caligus* sp. were collected from the mouth and nostril of *G. barbus* and *A. luniscutis*. Udonellid specimens were removed from *Caligus* sp., and fixed in 4% formalin for morphological study, or in 95% ethanol for molecular characterization.

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Specimens were stained with Gomori's trichrome [18,19] and mounted in Damar gum to examine their internal soft structures [18,19]. Measurements were obtained in according to the procedures of Freeman and Ogawa [15]. Dimensions of organs represent the highest measurements in the dorso-ventral view. Measurements are presented as the mean followed by the range and number (n) of specimens measured in parentheses. Illustrations were prepared with a drawing tube attached to a Leica DM 2500 microscope with differential interference contrast and phase contrast optics. Illustration of soft structures was carried out using pen and ink. Plates were prepared in Corel© [20]. Definitions of prevalence and mean intensity followed Bush et al. [21].

Type specimens, paratypes, vouchers and hologenophores (see Pleijel et al. [22] for terminology) were deposited in the following collections: Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, and Invertebrate Collection of the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará state, Brazil. Scientific names of hosts follow Marceniuk et al. [1]. The taxonomic determination of the copepods parasitic follow Dojiri and Ho [23]. 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 95% ethanol for genomic DNA extraction. The posterior part containing the haptor was completely flattened under coverslip pressure and mounted in Hoyer's, which served as the voucher, that is the hologenophore *sensu* Pleijel et al. [22], of the specimen used for DNA sequencing. 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. Ten eggs of the studied udonellid specimens were also used for DNA extraction applying the same protocol.

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′) and WormB (5′ –CTTGTTACGACTTTTACTTCC– 3′) [24]. In the second round, for the nested PCRs, the primer combinations were WormA and 1270R (5′-CCGTCAATTCCTTTAAGT-3′) [24], and 930F (5′- GCATGGAATAATGGAATAGG-3′) [25] with WormB, which amplified two overlapping fragments of approximately ~1179 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 set as follows: denaturation 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. The 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. 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, state of São Paulo, Brazil), using the same primers used for 18S rDNA amplification.

2.3. Alignment, phylogenetic inference and genetic distances

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 [26] to verify the similarity of the sequences newly obtained in the present study with other sequences of monogenoids in the NCBI BioSystems database [27]. Alignments of 18S rDNA were generated using MUSCLE implemented in Geneious version 7.1.3 [28]. Five partial sequences of the 18S rDNA of the Udonellidae published in the NCBI BioSystems database [27] were aligned with three newly generated sequences of *Udonella*. Two sequences of the Gyrodactylidae were used as the outgroup (see Table 1). Ten sequences (1811–1965 bp long) were aligned; the extremes were trimmed leaving an alignment 1829 bp long. Model of evolution was selected by JModelTest 2.1.1 (University of Vigo and University of A Coruña, Spain) [29] using the Akaike information criterion. Phylogenetic analyses were performed using the Maximum likelihood (ML) and Bayesian inference (BI) methods. ML was performed in the PhyML 3.0 implemented *via* the web server (http://www.atgc montpellier.fr/phyml/) [30], with topology assessed by bootstrapping with 1000 replicates, applying the HKY85 + G model. BI was done using MrBayes v.3.0 [31] implemented *via* the computational resource CIPRES [32], under the same model, with posterior probabilities estimated from 500 thousand generations with two independent runs of four simultaneous Markov Chain Monte Carlo (MCMC) algorithms, sufficient to keep the average standard deviation below 0.001. The MCMC with 1000th tree saved, diagnostic for every 1000th generation with burn-in periods, were set to the first 25,000 generations. Trees were visualized using Figtree 1.3.1 [33] and figures prepared using Corel© [20]. Genetic divergence was determined using the p-distance model matrix in MEGA version 7 [34]. Gaps and missing data were deleted.

3. Results

Six (33%) out of the 18 host specimens of *G. barbus* and two (28%) out of the seven *A. luniscutis* examined were infected with monogenoids. Morphological and molecular analyses of the 18S rDNA gene enabled to propose a new species of the genus *Udonella*, described below.

3.1. Taxonomic summary

Class: Monogenoidea Bychowsky, 1937
Order: Gyrodactylidea Bychowsky, 1937
Family: Udonellidae Taschemberg, 1879
Genus: *Udonella* Johnson, 1835
Udonella brasiliensis n. sp. (Figs. 1 and 2)

Type-host. *Caligus* sp. on *Genidens barbus* (Lacepède) (Siluriformes: Ariidae; Fig. 2A-B, E).

Type-locality. Cananéia, state of São Paulo, Brazil (25°02′09.2"S; 47°54′57.8"W).

Site of infection. Body surfaces of *Caligus* sp., frequently on the genital segment.

Prevalence. 06 of 18 *Genidens barbus* (33%).

Mean intensity. 2.16 parasites per infected host.

Mean abundance. 0.7 parasites per host.

Specimens deposited. Holotype (CHIOC 39536a), paratypes (CHIOC 39536b–j), hologenophores (CHIOC 39536k; MPEG 000291), vouchers (MPEG 000292–000299).

Representative DNA sequence. 1811 and 1812 bp long partial sequences of the 18S rDNA gene of two parasite isolates (GenBank accession number MW763077; MW763065). 1812 bp long partial sequences of the 18S rDNA gene from eggs (GenBank accession number MW762711).

Zoobank Life Science Identifier. (LSID) for *Udonella brasiliensis* sp. n. 5F5B670F-E544-491D-B583-48951E0696A9.

Etymology. The specific name is related to the first description of

Table 1
List of monogenoids included into phylogenetic analyses, providing host species data, locality, GenBank ID, and references.

Parasites family/species	Hosts	Host family	Locality	GenBank ID	Reference
Udonellidae					
Udonella australis	*Caligus rogercresseyi* ex *Eleginops maclovinus*	Eleginopsidae	Chile	FJ946832	[15]
***Udonella brasiliensis* n. sp.**	***Caligus* sp. *ex Aspistor luniscutis***	**Ariidae**	**Brazil**	**MW763065**	**Present study**
***Udonella brasiliensis* n. sp.**	***Caligus* sp. *ex Genidens barbus***	**Ariidae**	**Brazil**	**MW763077**	**Present study**
***Udonella brasiliensis* n. sp.**[a]	***Caligus* sp. *ex Genidens barbus***	**Ariidae**	**Brazil**	**MW762711**	**Present study**
Udonella caligorum	*Caligus* sp. ex *Gadus morhua*	Gadidae	UK	AJ228796	[16]
Udonella caligorum	*Lepeophtheirus salmonis* ex *Salmo salar*	Salmonidae	UK	FJ946831	[15]
Udonella fugu	*Pseudocaligus fugu* ex *Takifugu niphobles*	Tetraodontidae	Japan	FJ946830	[15]
Udonella myliobati	Caligid copepod ex *Myliobatis australis*	Myliobatidae	Australia	FJ946833	[15]
Gyrodactylidae					
Gyrodactylus salaris[b]	*Salmo salar*	Salmonidae	Norway	Z26942	[40]
Gyrodactylus salmonis[b]	*Oncorhynchus mykiss*	Salmonidae	Mexico	JN230350	[41]

[a] Sequence obtained from eggs of the *Udonella brasiliensis* n. sp.
[b] Used as outgroups. Sequences obtained in the present study are in bold.

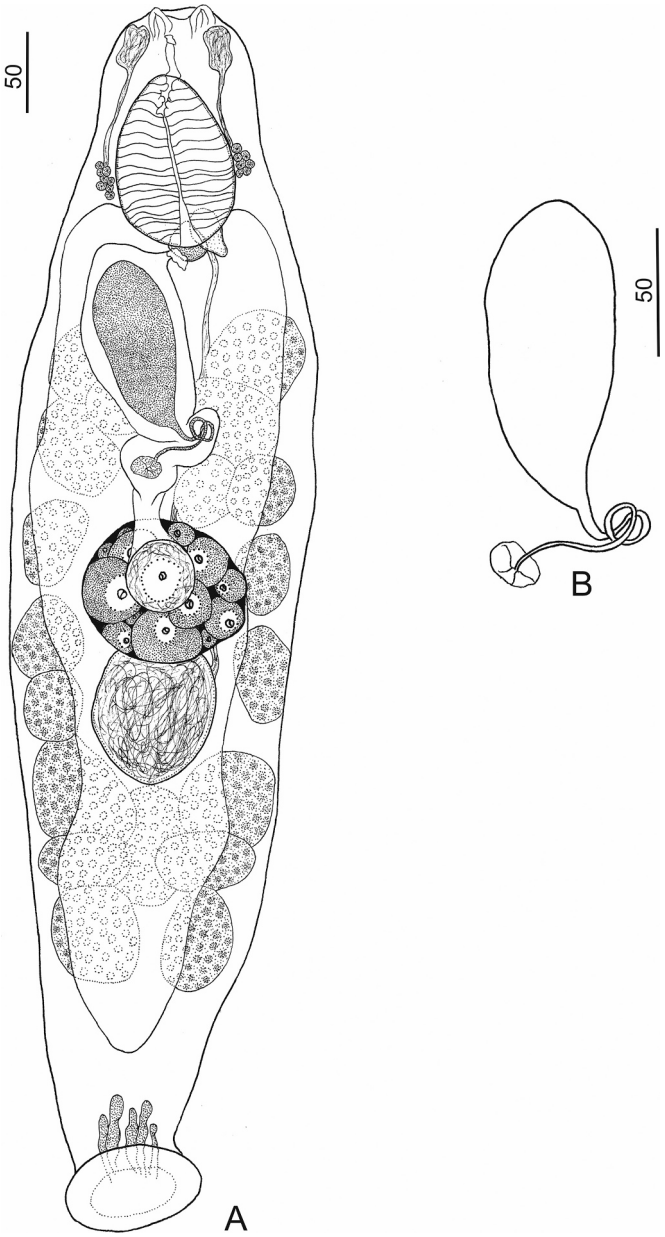

Fig. 1. *Udonella brasiliensis* n. sp. A. Holotype whole-mount, ventral; B. Egg. Scale bars Fig. 1A and B (50 μm).

Udonella from Brazilian waters.

Other records. *Caligus* sp. on *Aspistor luniscutis* (Valenciennes) (Siluriformes: Ariidae), Cananéia, state of São Paulo, Brazil (25°02′09.2"S; 47°54′57.8"W). Prevalence: 2 of 7 hosts (28%); Mean intensity: 4; Mean abundance: 1.1.

Comparative measurements. Table 2, Supplementary Table S1.

3.1.1. Morphological data

Description. (Based on eleven specimens, one mounted in Hoyer's medium and ten stained with Gomori's trichrome). Body elongate, fusiform, total length including haptor 0.673 mm (0.553–0.785; n = 10), total width at level of germarium 0.163 mm (0.120–0.205; n = 10) (Figs. 1A and 2D). Anterior head organs 2, each fed by single group of cephalic gland-cells, posterolateral to the pharynx. Sensory papillae 2, in anterior median position in relation to openings of head organs. Mouth subterminal, median. Pharynx ovate, eversible, with papillate anterior margin covered with microvilli (observed only in paratypes), 93 μm (70–113; n = 10) long, 75 μm (65–81; n = 10) wide. No esophagus. Intestine a wide tubular sac, running posteriorly in dorsal median field, extending to end of vitellaria. Testis single, subspherical, 90 μm (77–98; n = 10) long, 90 μm (79–99; n = 10) wide, posterior to ovary. Vas deferens, running on left side of ovary and uterus. Ovary single, subspherical, located in middle of body, 70 μm (46–84; n = 10) long, 76 μm (50–96; n = 10) wide. Fertilization chamber distinct, contains single large oocyte. Uterus distended. Uterus opening directly into the genital atrium. Egg pyriform, 146 μm (107–204; n = 7) long, 71 μm (42–102; n = 7) wide, with long slender unipolar elastic filament 109 μm (57–154; n = 7) long; attachment disk at free end of filament (Figs. 1B and 2A-C). Prostatic reservoir subspherical, near ejaculatory bulb. Bulb attached dorsally to genital atrium. Cirrus not differentiated. Genital aperture mid-ventral, posterior to pharynx, 16 μm (13–17; n = 5) in diameter. Vitellarium comprising 2 rows of large irregular follicles, extending in each lateral field from level of genital pore to near posterior end of intestine. Seminal vesicle, not observed. Haptor, disc-shaped, 64 μm (46–81; n = 9) in diameter, with microvilli on adhesive surface. Five to six haptoral glands with ducts opening on adhesive surface (Fig. 1A).

3.1.2. Molecular data

Three sequences of the partial 18S rDNA gene were newly generated for *U. brasiliensis* n. sp. found on *Caligus* sp. parasite of the ariids *G. barbus* and *A. luniscutis* from the southeastern coast of Brazil. Two sequences from parasite isolates are 1811 and 1812 bp long, while one sequence obtained from the monogenoid eggs is 1812 bp long. The pairwise genetic analysis revealed divergences within the *Udonella* genus ranging from 1% to 5.6% (17–107 bp) (Table 3). The smallest interspecific distances were observed between *U. brasiliensis* n. sp. and *U. australis* Carvajal & Sepulveda, 2002, 1% (17 bp), while *U. myliobati* Aken'Ova & Lester, 1996, was found to be the most genetically distant

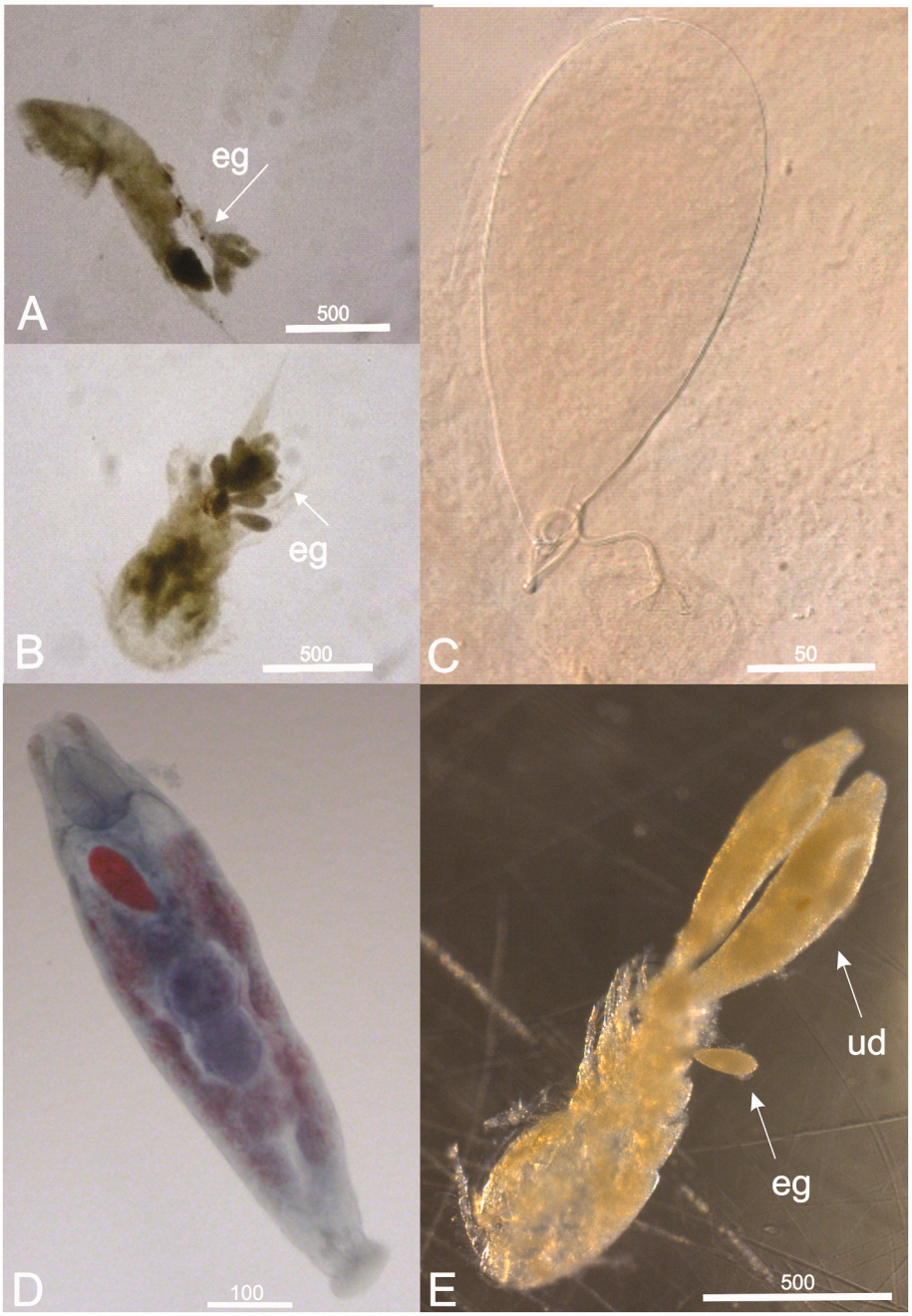

Fig. 2. *Udonella* b*rasiliensis* n. sp. epibiont on *Caligus* sp. from *Genidens barbus* from Cananéia, São Paulo, Brazil. A. *Caligus* sp., side view; B. *Caligus* sp., dorsal view; C. Egg of the *Udonella brasiliensis* n. sp. D. *Udonella brasiliensis* n. sp., ventral view. E. *Caligus* sp. carrying two specimens of *Udonella brasiliensis* n. sp. and one eggs (eg). Scale Fig. 2A, B, E (500 μm); Fig. 2C (50 μm); Fig. 2D (100 μm).

species to *Udonella* 5.6% (107 bp). The genetic divergence among the specimens of *Udonella* from *G. barbus* and *A. luniscutis* was 0.1% (only 2 bp). The 18S rDNA sequences obtained from the eggs and worms of udonellid from *G. barbus* were 100% identical.

ML and BI analyses yielded similar tree topology (Fig. 3). With strong statistical support, *U. brasiliensis* n. sp. appeared as the derived species in a sister position to *U. australis* found on *Eleginops maclovinus* (Cuvier) (Perciformes: Eleginopsidae) from Chile. Also, with strong support, this clade grouped with that composed of *U. caligorum* from UK. *Udonella fugu* Freeman & Ogawa, 2010 from Japan, and *U. myliobati* from Australia arose as early divergent udonellids.

Remarks. Due to the absence of haptoral sclerites and a differentiated copulatory organ, the morphological features with which *Udonella* spp. can be distinguished are limited (*e.g.,* body size, haptor diameter, size of ovary relative to testis and position of genital pore). Nevertheless, *U. brasiliensis* n. sp. can be distinguished from the other species by at least two such characters (Table 2 and Supplementary Table S1). Morphologically, the new species resembles *U. murmanica* Kornakova & Timofeeva, 1981, *U. australis* and *U. fugu*, by having the genital pore in a mid-ventral position. Regarding this character, *U. brasiliensis* n. sp. differs from *U. caligorum sensu* Price 1938, *U. papillifera* Van der Land, 1967, and *U. ophiodontis* Ching & Leighton, 1993, in which the genital pore is sinistral to the median line, or positioned sub-marginally. Furthermore, *U. brasiliensis* n. sp. can be distinguished from all these species by possessing a smaller body, haptor diameter, pharynx, ovary, and testis.

Table 2
Morphological measurements for *Udonella brasiliensis* n. sp. compared with other members of the genus.

Species[a,b]	*U. brasiliensis* n. sp.[c]	*Udonella caligorum*[d]	*Udonella papillifera*[e]	*Udonella murmanica*[f]	*Udonella ophiodontis*[g]	*Udonella myliobati*[h]	*Udonella Australis*[i]	*Udonella fugu*[j]
Body length (mm)	0.673 (0.553–0.785; n = 10)	(1.1–1.4)	Up to 2	5.7(4.5–7.1)	2–2.8	0.62(0.43–0.83)	1.5(1.12–2.6)	2.42(1.9–3.1)
Body width (mm)	0.163 (0.120–0.205; n = 10)	0.255	0.45	0.850 (0.69–1.04)	0.303–0.743	0.238 (0.160–0.396)	0.259 (0.125–0.480)	0.66(0.5–0.8)
Haptor diameter (μm)	64(46–81; n = 9)	187–210	–	510(410–640)	245–327	137(96–185)	209.6(130−310)	296 (210–380)
Pharynx length (μm)	93(70–113; n = 10)	150–152	200	350(320–460)	164–205	94(64–112)	166(112.5–200)	173 (140–200)
Pharynx width (μm)	75(65–81; n = 10)	85–95	–	390(300–470)	131–164	73(58–88)	116.5(70–165)	216 (150–280)
Ovary length (μm)	70(46–84; n = 10)	–	–	440(230–670)	–	67(32−100)	126.9(77.5–250)	314 (260–430)
Ovary width (μm)	76(50–96; n = 10)	133	100–160	360(210–510)	135–270	97(56–152)	125.1(75–210)	326 (270–430)
Testis length (μm)	90(77–98; n = 10)	–	–	600(420–890)	–	93(56–128)	296(175–600)	265 (140–370)
Testis width (μm)	90(79–99; n = 10)	76–95	200	3800 (280–520)	189–286	99(54–144)	177.3(100–340)	325 (200–400)
Egg length (μm)	146(107–204; n = 7)	133	200–250	240(210–260)	147–155	164(136–188)	181.7 (135–247.5)	211 (150–290)
Egg width (μm)	71(42–102; n = 7)	42	100	120(100–160)	74	70(68–76)	65(57.5–77.5)	116(90–190)
Egg filament length (μm)	109(57–154; n = 7)	600–800	–	1000–1400	–	69(68–72)	418.3(220–600)	238 (160–310)
Genital pore	Mid-ventral, posterior to pharynx	Sinistral, submarginal, slightly anterior to level of posterior of the end of pharynx	Ventral to the posterior part of the pharynx, to the left of the median line	Mid-ventral, posterior to pharynx	Submarginal	Mid-ventral, posterior to pharynx	Mid-ventral posterior to pharynx	Mid-ventral, posterior to pharynx

[a] Freeman and Ogawa [15].
[b] Carvajal and Sepulveda [42].
[c] On *Genidens barbus* (present study).
[d] Price (1938).
[e] Van der Land (1967).
[f] Kornakova & Timofeeva (1981).
[g] Ching & Leighton (1993).
[h] Aken'Ova & Lester (1996).
[i] Carvajal & Sepulveda (2002).
[j] Freeman & Ogawa (2010).

Table 3
Pairwise genetic identities of 18S rDNA selected sequences of species belonging to the order Gyrodactylidea adjusted for missing data. The upper triangular matrix shows the number of nucleotide differences while lower triangular matrix shows differences in terms of nucleotide percentage. Sequences obtained in the present study are in bold.

	1	2	3	4	5	6	7	8	9	10
1.*Gyrodactylus salaris* Z26942	–	33	339	341	351	351	349	328	328	330
2.*Gyrodactylus salmonis* JN230350	12	–	331	336	345	345	341	319	319	321
3.*Udonella myliobati* FJ946833	15	14.9	–	81	102	102	110	105	105	107
4.*Udonella fugu* FJ946830	15.4	15.1	4.1	–	64	64	80	76	76	78
5.*Udonella caligorum* FJ946831	15.7	15.4	5	2.9	–	0	54	56	56	58
6.*Udonella caligorum* AJ228796	15.7	15.4	5	2.9	0	–	54	56	56	58
7.*Udonella australis* FJ946832	15.6	15.2	5.4	3.7	2.5	2.5	–	17	17	19
8.*Udonella brasiliensis* n. sp.[a]	15.6	15.2	5.5	3.8	2.7	2.7	1	–	0	2
9.*Udonella brasiliensis* n. sp.[b]	15.6	15.2	5.5	3.8	2.7	2.7	1	0	–	2
10.*Udonella brasiliensis* n. sp.[c]	15.7	15.3	5.6	3.9	2.8	2.8	1	0.1	0.1	–

[a] On *Genidens barbus*.
[b] Eggs on *G. barbus*.
[c] On *Aspistor luniscutis*.

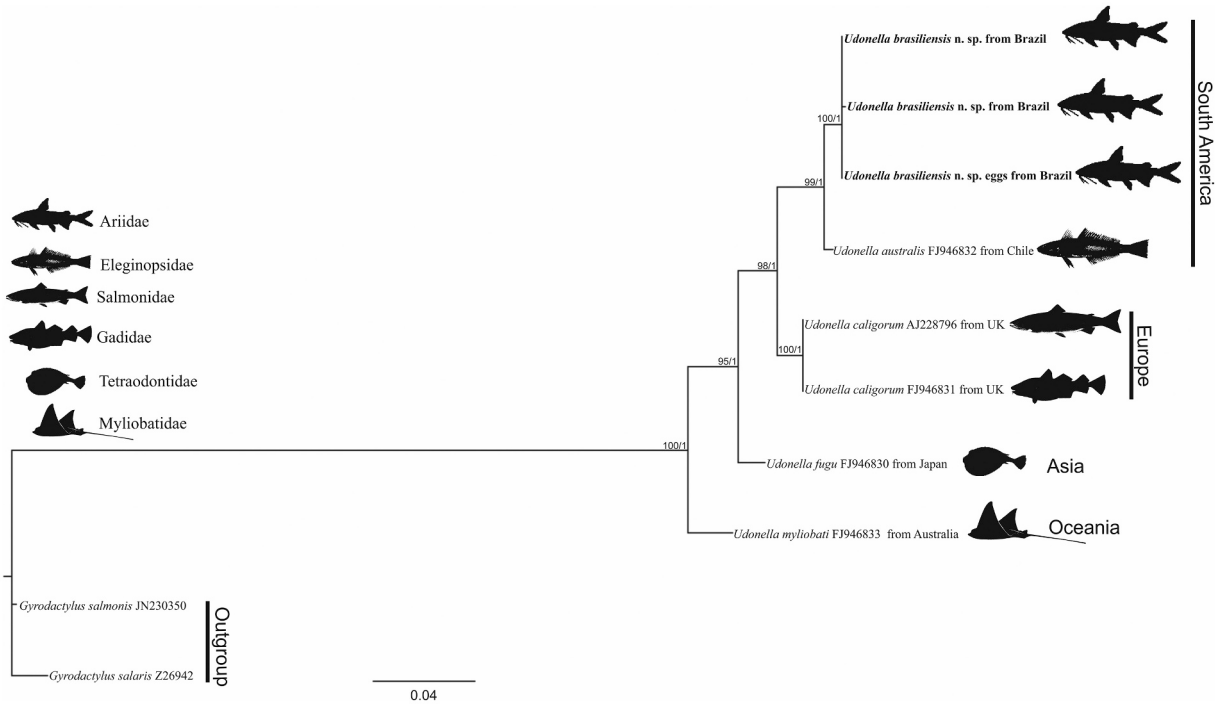

Fig. 3. Molecular phylogeny of the Udonellidae estimated by Maximum likelihood (ML) and Bayesian inference (BI) using partial sequences of the 18S rDNA gene (1829 bp long). Species newly sequenced for the present study are in bold. Species name precedes the GenBank sequence ID. ML bootstrap support values and posterior probabilities are given above the branches (bootstrap values <60 and posterior probabilities <0.90 not reported).

Udonella caligorum sensu Fuentes Zambrano et al. [12], which was also reported from the ariid *Sciades herbergii* (Bloch), differs from *U. brasiliensis* n. sp. in body length [1.44 mm (1.21–159) *vs* 0.673 mm (0.553–0.785)], ovary size [157 μm length and 135 μm width *vs* 70 μm (46–84) length and 76 μm (50–96) width] and testis size [270 μm length and 246 μm width *vs* 90 μm (77–98) length and 90 μm (79–99) width] (Supplementary Table S1).

Genetically, the closest species was *U. australis*, which diverged by only 1% (Table 3). However, morphological differences between *U. brasiliensis* n. sp. and *U. australis* can be observed regarding body length [0.673 mm (0.553–0.785) *vs* 1.5 mm (1.12–2.6)], ovary size [70 μm (46–84) length and 76 μm (50–96) width *vs* 126.9 μm (77.5–250) length and 125.1 μm (75–210) width] and testis size [90 μm (77–98) length and 90 μm (79–99) width *vs* 296 μm (175–600) length and 177.3 μm (100–340) width] (Table 2). Furthermore, *U. brasiliensis* n. sp. and *U. australis* occur in different hosts and geographic regions. Thus, the combination of morphological, molecular data, and biological traits (*e.g.,* molecular data, host species and geographic distribution, as suggested by Freeman and Ogawa [15]), support the erection of a new species. *Udonella brasiliensis* n. sp. specimens found on *Caligus* sp. infecting *G. barbus* and *A. luniscutis* are morphologically similar (Table S1), with an intraspecific genetic variation of only 0.1% (2 bp) (Table 3).

4. Discussion

The erection of the new species is supported by a combination of the differences observed in morphological, molecular and the biological traits of *Udonella* spp. To date, 75 valid species belonging to three monogenoid families (Dactylogyridae [65 species], Neocalceostomatidae [9 species] and Udonellidae [1 species]) have been reported parasitizing marine catfish of the Ariidae family around the world [3,12,13]. The new species described herein represents the first udonellid reported parasitizing ariids in the South Atlantic.

Hitherto, there are only two reports of udonellids from ariid hosts: *U. caligorum* parasitizing *Caligus* sp. found on *Arius herzbergii* (Burgess) (now *S. herzbergii*) from Margarita Island, Venezuela, and an undetermined species of *Udonella* found on *G. barbus* from the south of Brazil [12,17]. *Caligus* spp. have also been previously reported on ariids fish from Western South Atlantic coast [35]. It is possible that *Udonella* sp. reported, but not described by Boeger et al. [17] from *G. barbus* is the species described herein. However, the authors did not deposit voucher specimens in any museum collection, as they stated, and we were therefore unable to assess the true taxonomic status of these specimens.

As indicated in the remarks section, udonellid taxonomy has morphological limitations, which contributes to an under-estimation of the species-richness of the group and also explains the numerous reports of *U. caligorum* from various global locations (see Freeman and Ogawa [15] for a historical perspective). Recently, Freeman and Ogawa [15] suggested that in addition to the morphological characteristics traditionally used to distinguish *Udonella*, the host species combined with geographic distribution and molecular data of the parasite, are important for delimiting species of this genus. We herein described *U. brasiliensis* n. sp. based on such evidence, *sensu* Freeman and Ogawa [15].

Although in the past it was believed that udonellids fed on their copepod hosts, it is currently known that these organisms are obligatory fish parasites and epibiont on copepods, thus feeding on the fish [15,36–38]. Freeman and Ogawa [15], observed that *Udonella* is more host-specific to fish than to copepods, suggesting that the fish host and phylogeography are potentially important in identifying species of *Udonella*. However, our results shows that phylogenetically related host fish can share infections by the same udonellid species.

Our 18S rDNA phylogenetic analyses consistently placed the udonellids in the present study into four lineages (Fig. 3): the South American group, composed of *U. brasiliensis* on *G. barbus* and *A. luniscutis* (Ariidae) from Brazil and *U. australis* on *E. maclovinus* (Eleginopsidae) from Chile, forming a derived clade; *U. caligorum* on *Salmo salar* Linnaeus (Salmonidae) and *Gadus morhua* Linnaeus (Gadidae) from UK (Europe); *U. fugu* on *Takifugu niphobles* (Jordan & Snyder) (Tetraodontidae) from Japan (Asia); and *U. myliobati* on *Myliobatis australis* Macleay (Chondrichthyes: Myliobatidae) from Australia (Oceania), as

the early divergent species. Such results show that chondrichthyans must have served as hosts for the initial lineages of the udonellids, and additionally suggest a scenario of initial irradiation for udonellids, referring to the Pangea supercontinent ~250 MY ago *sensu* Rogers and Santosh [39]. Similarly, Boeger et al. [17] suggested, based on ultrametric analyses (using the 18S rDNA fragment) that the initial divergence of the Gyrodactylidae + Oogyrodactylidae clade with Udonellidae occurred about ~468–216 MY ago, largely coinciding with the timing of a scenario of initial irradiation of udonellids in the Pangea supercontinent. However, it is still not clear what other factors influence these groupings because *Udonella* spp. have been reported in different host groups (myliobatids, gadids, salmonids, tetraodontids, eleginopsids and ariids). Nevertheless, older geographical proximity seems to have contributed to the udonellid radiation.

5. Conclusion

The present study emphasizes the need for an integrative taxonomic approach to achieve accurate monogenoid species delimitation and classification. The proposal of *U. brasiliensis* n. sp. is a clear example of the power of this tool, especially for udonellids, for which morphological features are somewhat limited. Moreover, our results showed that *U. brasiliensis* n. sp. represents a derived lineage within udonellids. In parallel, this study represents the first description of a *Udonella* species from Brazilian waters.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2021.102371.

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