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Morphological identification and DNA barcoding of a new species of *Parabrachiella* (Siphonostomatoida: Lernaeopodidae) with aspects of their intraspecific variation

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ABSTRACT

We present a detailed morphological description and a DNA barcoding of *Parabrachiella platensis* n. sp. collected from *Mugil liza* Valenciennes in Samborombon Bay (Buenos Aires, Argentina). This new species was compared with two *Parabrachiella* species parasitic on mugilids: *Parabrachiella exilis* (Shiino, 1956) and *Parabrachiella mugilis* (Kabata, Raibaut et Ben Hassine, 1971). *Parabrachiella platensis* n. sp. differs from those species in the shape of posterior processes, the anal slit with two pairs of bipartite papillae, the size of cephalothorax, the trunk, the maxilla, the microhabitat on the host, and the lack of caudal rami. On the host, the new species was in the nostrils (a new site for a species of the genus *Parabrachiella*) and in the fins base. Some minor morphological differences were observed in relation to the locations on the host. The molecular analysis conducted based on mtDNA-COI between specimens of the new species on the fins and nostrils showed a genetic similarity of 99.8%. This percentage supports that the specimens found in nostrils and fins base could represent a single species. New studies on *P. platensis* n. sp., including infection of the same fish with the two forms, could bring some new information. Anyway according to the genetic information provided and the minimal morphological differences spotted we conclude that the two forms are a single specie. The differences observed are possibly influenced by the place of the host where the two forms of copepods were found, nostrils and fins. The new species was also molecularly compared to other five species of *Parabrachiella* including *P. exilis* (parasitizing mugilid from Chile), *Parabrachiella anisotremis*, *Parabrachiella auriculata, Parabrachiella merluccii*, and *P. hugu* (the last two sequences were taken from the GenBank). The genetic distance of 9% among *P. platensis* n. sp. and *P. exilis*, which is the close morphological related species, allow to states that these two copepods on mugilids belong to different species and then validating the morphological differences found between them.

1. Introduction

The Lernaeopodidae is one of the most numerous families of copepods and its representatives are extensively adapted to parasitism. Most of the lernaeopodid species represents narrow host specificity and parasitize specific anatomical locations on their fish hosts (Piasecki et al., 2010). The genus *Parabrachiella* Wilson, 1915 is one of the most numerous genera in this species-rich family. According to Piasecki et al. (2010), the genus *Parabrachiella* contains 67 species. In Argentina, Etchegoin et al. (2006) redescribed *Parabrachiella spinicephala* Ringuelet, 1945, a parasite of the Brazilian sandperch, *Pinguipes brasilianus* Cuvier. In the same country, Sardella et al. (1995) and Alarcos and Etchegoin (2010) reported *Parabrachiella chevreuxii* (Van Beneden, 1891), parasitizing the whitemouth drummer, *Micropogonias furnieri* (Desmarest). Another congener—*Parabrachiella insidiosa* (Heller, 1865), was found by Sardella and Timi (1995) on *Merluccius hubbsi* Marini. Cantatore et al. (2012) provided a list of copepods parasites of fishes from the Argentine Sea and found *Parabrachiella amphipacifica* (Ho, 1982) infecting *Cottunculus granulosus* Karrer.

Mugilids (mullets) have been reported as hosts for many lernaeopodid copepods. In Chile, *Parabrachiella exilis* (Shiino, 1956), was reported by Castro Romero and Baeza Kuroki (1986) on flathead grey mullet, *Mugil cephalus* Linnaeus. Knoff et al. (1994) reported *P. exilis* hosted by lebranche mullet, *Mugil liza* Valenciennes, from Brazil. *Parabrachiella mugilis* (Kabata, Raibaut et Ben Hassine, 1971) was reported parasitizing golden grey mullet *Liza aurata* (Risso), in the North Atlantic, the

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Mediterranean Sea, the Lake of Tunis (lagoon), and the Gulf of Oman at Muscat (Kabata et al., 1971).

During the nostrils and fins examinations in juvenile *M. liza* from Samborombón Bay, copepods of *Parabrachiella* sp. were found.

The aim of the study was to determine the taxonomic status of the specimens parasitic on *M. liza* and its relationships with *P. exilis,* which is also parasitic on mugilid, with other three fishes species collected in Antofagasta (Chile) waters, and with another two species from GenBank.

2. Materials and methods

2.1. Specimens and taxonomy

Fish samples were collected in the Ajó River (36°20′12″S, 56°54′17″W), Samborombón Bay, Buenos Aires province, Argentina, from 15 March through 21 September 2009. Fish were captured with a modified Garlito/Bituron stationary net (Colautti 1998) plus a trawl net 10 m long with a 5 mm stretched mesh in the wings and a 2.5 mm stretched mesh in the codend. Captured fish were fixed with 10% (v/v) aqueous formalin, weighed, and measured. Some fish were carried to the laboratory alive and the parasites found were fixed for DNA extraction as we mention below. The fish specimens ranged from 3.64 to 23.4 cm in standard length and from approximately 1 to 400 g in weight. The nasal cavities were dissected under a stereomicroscope, and parasites detected were removed and stored in 10% buffered formalin. Parasite appendages were dissected, cleared in lactic acid, and examined under light microscopy. Drawings were made with the aid of a drawing tube. Measurements for females and males are given in mm as mean values followed by ranges in parentheses. Terminology follows Huys and Boxshall (1991), but detailed terminology related to body parts is based on Kabata (1979). Specimens fixed in formalin, were dehydrated in a series of increasing concentrations of ethanol, dried to the critical point in an EMITECH model K850, and sputter-coated with gold. Samples were then observed and photographed in a Philips SEM 505 microscope equipped with digital-imaging program (Soft Imaging System ADDA II [SIS]).

2.2. Molecular data

Copepod specimens, collected from the nostrils and the fins were preserved separately in 96% ethanol and kept at −20 °C until DNA extraction. Specimens of four other *Parabrachiella* species from fishes of Chile were included in the analysis: *Parabrachiella anisotremi* (Castro Romero et Baeza Kuroki, 1989); *Parabrachiella auriculata* (Castro Romero et Baeza Kuroki, 1987), *P. exilis*, and *Parabrachiella kabatai* (Luque et Farfan, 1991). Sequences of *Parabrachiella merluccii* (Bassett-Smith, 1896) and *Parabrachiella hugu* (Yamaguti, 1939), deposited in Bold Systems Public Data Portal and in GenBank, respectively, were also included in the mtDNA-*COI* analysis (Table 1). The Chilean *Parabrachiella* specimens for DNA extraction came from the private collection of one of the authors (RCR). A COI sequence of *Ergasilus* von Nordmann, 1832 from the IBOL project: TREAR, was also included as outgroup.

For DNA extraction a sample of 2–3 mm^3 of ethanol-preserved tissue, 5 ml of insect Lysis Buffer, and 0.5 ml of Proteinase K, 20 mg/ml were placed in each well of 96-well Eppendorf plate for DNA extraction. Genomic DNA was extracted according to the glass fibre (GF) protocol for DNA extraction using 96-well plates by Ivanova et al. (2006a,b). Amplification of the 5′ barcode region of COI was made using the HCO2198_t1 (Folmer et al. (1994) tailed) (CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA), and LCO1490_t1 (Folmer et al. (1994) tailed) (TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG) primers. PCR reactions were performed in 96-well plates. The reaction master mix consisted of 625 µl of 10% trehalose, 200 µl water, 125 µl of buffer, 62.5 µl $MgCl_2$ (50 mM), 6.25 µl dNTP (10 mM), 12.5 µl of each primer (10 µM), and 6 µl Taq DNA polymerase (5 U/ml). Each well contained 10.5 ml mixture and 2 ml genomic DNA. The PCR reaction profile was comprised of an initial step of 2 min at 95 °C and 5 cycles of 94 °C for 30 s, annealing at 45 °C for 40 s, and extension at 72 °C for 1 min, 35 cycles of 94 °C for 30 s, annealing at 51 °C for 40 s, and extension at 72 °C for 1 min, final extension at 72 °C for 10 min. Amplicons were visualized on 2% agarose E-GelH 96-well system (Invitrogen). The PCR amplification products where placed in 96-wells plate containing 6.25 µl of 10% trehalose and posted to the University of Guelph for DNA sequencing. The COI barcode sequence was obtained according with the protocol of Ivanova and Grainger (2006). The sequencing of Chilean fish specimens was carried out in Macrogen Inc. (Korea).

Sequences were edited by eye using the platform GENEIOUS 5.1.7. Barcode fragment alignments were assembled using MAFFT v.7 (Katoh and Standley, 2013). We checked the nucleotide alignment for the presence of pseudogenes in Geneiuos Pro, using the translated amino acid sequences based on the invertebrate mitochondrial genetic code. The best partitioning scheme and substitution model for each DNA partition was chosen under the Bayesian Information Criterion (BIC; Schwarz 1978) using the "greedy" search strategy in Partition Finder v. 1.1.1 (Lanfear et al., 2012). The barcode fragment dataset was partitioned into first-, second-, and third-codon positions with the appropriate nucleotide substitution model implemented for each codon position (TIM + G for the first and second (Posada 2003); and HKY + G for the third codon position (Hasegawa et al., 1985)). *Ergasilus* sp. was used as the outgroup for the COI data set.

The phylogenetic reconstruction was carried out using Bayesian Inference (BI) through MrBayes v. 3.2.1 (Ronquist et al., 2012). The phylogenetic trees were reconstructed using two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMC) for 20×10^6 generations each to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations. The first 25% of the sampled trees were discarded as 'burn in'.

All *P. platensis* n. sp. sequences, trace files (electrophenogram), primer sequences used, and the specimen data were deposited in the public project "Parasites of fish and Invertebrates from Argentina (Code = TREAR) in the Barcode of Life Database (BOLD) (www.barcodinglife.org). All obtained sequences were also deposited in GenBank (Table 1). The holotype, the allotype, and the paratypes of the new species were deposited in the invertebrate collection of the Museo de La Plata, Argentina.

Family Lernaeopodidae Milne Edwards, 1840

Genus *Parabrachiella* Wilson C.B., 1915

Parabrachiella platensis n. sp.

Type-host: *Mugil liza* (Mugiliformes: Mugilidae); local name 'lisa', English name "Lebranche mullet".

Type locality: Ajó River, south of Samborombón Bay, Argentina (36°20′S, 56°54′W).

Attachment site: Nostrils (primary) and fins.

Prevalence: 49.30% (nostrils) and 17.61% (fins).

Mean intensity: 2.16 (nostrils) and 1.7 (fins).

Type material: Deposited in the invertebrate collection of the Museo de La Plata, Argentina. Holotype adult female: MLP-Cr 26948 and allotype adult male: MLP-Cr 26949. Four paratypes adult females with the male: MLP-Cr 26945-47 and MLP-Cr 26950

Etymology: The species name "*platensis*" refers to the name of the estuary of La Plata River where the parasite was found.

Description (Figs. 1–11)

Adult Female [Based on 20 ovigerous specimens.] Measurements in Table 2. Body typically lernaeopodid. Cephalothorax (Fig. 1A and B) subcylindrical, dorsal shield widening terminally, reinforced by more sclerotized cuticle, (Fig. 1C). Antennule (Figs. 1D and 3C) four-segmented with swollen basal segment, and short solus at boundary between third and fourth segment (Fig. 3C). Distal segment armature (Figs. 1D and 3C) with short process 3, simple seta 6, bifid seta 5, and

Table 1
Details of copepods parasites of marine fishes Chile used in this study.

Copepod parasites species [Code	Host (Family)			A	C	G	T
Poecilostomatoida							
Ergasilidae							
Ergasilus sp. [Erg]	*Mugil liza* Valenciennes, 1836 (Mugilidae)	KU557411	678	172	118	141	247
Siphonostomatoida							
Lernaeopodidae							
Parabrachiella hugu (Yamaguti, 1939)	*"Spheroides rubripes" = Takifugu rubripes*	KT030285	558	163	68	100	227
Parabrachiella merlucci (Bassett-Smith, 1896)	*Merluccius merluccius* (Linnaeus, 1758)	KT208689	667	205	88	105	269
Parabrachiella anisotremi (Castro Romero & Baeza Kuroki, 1989)	*Anisotremus scapularis* Tschudi,1846 (Pomadasidae)	KX815887	597	186	78	90	243
		KX815888	615	193	77	96	249
		KX815889	639	202	80	95	262
		KX815890	651	204	83	97	267
Parabrachiella auriculata (Castro Romero & Baeza Kuroki, 1987)	*Cilus gilberti* Abbott, 1899 (Sciaenidae)	KX815906	603	191	79	95	238
		KX815907	630	197	84	102	247
		KX815908	654	209	86	102	257
Parabrachiella exilis (Shiino, 1956)	*Mugil cephalus* Linnaeus, 1758	KY026072	609	194	78	96	241
sensu Castro-Romero & Baeza-Kuroki 1986		KY026073	681	218	90	109	264
		KY026074	699	220	93	110	276
sensu Knoff et al. (1986)	*Mugil liza* Valenciennes, 1836 (Mugilidae)						
sensu Shiino (1956)	"*Kyphosus lembus*" = *Kyphosus vaigiensis* (Quoy et Gaimard, 1825)						
Parabrachiella kabatai (Luque & Farfan, 1991)	Isacia conceptionis (Cuvier, 1831)	KY026075	666	200	74	289	103
		KY026076	666	199	75	104	288
		KY026077	666	199	75	104	288
		KY026078	627	193	67	94	273
Parabrachiella platensis n. sp. (nostrils)	*Mugil liza* Valenciennes, 1836 (Mugilidae)	KY026080	666	211	78	100	277
		KY026081	666	210	79	101	276
		KY026082	666	210	78	101	277
		KY026083	660	207	77	101	275
Parabrachiella platensis n. sp. (fins)	*Mugil liza* Valenciennes, 1836 (Mugilidae)	KY026084	666	210	78	101	277
		KY026085	666	210	78	101	277

seta 4 plus short process 1 (not forming gibber). Antenna (Figs. 2A and B, 3D) biramous, sympod-exopod long axis, exopod globose with short distal seta and another distolaterally margin (Fig. 3D). Endopod two-segmented. Apical armature (Figs. 2B, 3D) with robust curved hook 1, slender seta 2, and seta 5, process 4 on lateral side, and ventrally to the latter a pad of scale-like denticles on ventral margin. Labrum and labium forming buccal cone (Fig. 3E). Labrum bearing rostral seta ventrally (Fig. 2C), and fine setules. Labium margin with rows of dense setae, without sensilla on disto-ventral surface (Fig. 3E) or with two sensilla (one on each side) near distal margin (Fig. 3F) (only observed in the SEM). Mandible (Fig. 2D) with coxa globose, short; gnathobase blade with 3 secondary teeth. Dental formula: P1, S1, P1, S1, P1, S1, B4. Last secondary tooth smallest. Maxilla (Fig. 1A and B) medium size, arms separated, not fused (partially fused, in some specimens), with short nipple-like structure near base (Figs. 1A and B, 3A). Bulla short (Fig. 2G) with manubrium and expanded anchor (Fig. 2H). Maxillule (Figs. 2E, 3E and F) bilobate, inner lobe with two long setae of unequal length (differences in length shown in Fig. 3E and F), outer lobe with one (in copepods from fins) or two short setae of unequal length (in Fig. 3F only one seta visible). Maxilliped (Fig. 2F) subchelate. Corpus robust, no armature on myxal area. Claw (Figs. 2I, 4B and C) slightly curved, barb stout, shaft with denticulate disto-ventral margin. A row with at least 5 denticles (commonly 5) on surface of claw near its base (Fig. 4B), at each side (not observed with optic microscopy) and two denticles on lateral surface close to the base in in copepods located on fins (Fig. 4B).

Trunk (Fig. 1A and B), with two pairs of short, blunt posterior (lateral) processes, dorsal anal region encircled by single pair of short papillae on each side (not visible with optic microscopy) with short ventral lobes bearing oviduct-orifice (Fig. 3A and B). Very short ventral genital process (Fig. 3B). Anal area with three short tubercles in specimens attached to fins (Fig. 4C). Egg sacs with 30–60 eggs (Fig. 1A).

Adult Male [Based on 20 specimens.] Measurements in Table 2. Body male type A (Fig. 5A) according to Kabata (1979). Cephalothorax about 40% trunk length. Antennule three segmented (Figs. 5B, 6A–C). Basal segment longest and second and third segments approximately same length. Distal segment armed with elements 1, 2, 3, 4, 5, and 6 (Fig. 6C). Solus present (Fig. 6B) between second and third segment (not detected with optic microscopy). Whip not detected. Antenna (Figs. 5C, 6A and D) biramous, elongate, and prehensile. Sympod

Table 2
Measurements of *Parabrachiella platensis* n. sp. from the nostrils and fins.

	Measurements	*P. nasalis* n. sp. from nostrils	*P. nasalis* n. sp. from fins
FEMALES	Body long	3.19 (2.59–3.97)	2.18 (1.24–3.32)
	Cephalothorax long by wide	1.98 (1.65–2.51) by 0.33 (0.28–0.36)	1.25 (0.73–1.97) by 0.37 0.30–0.46)
	Maxila long by wide	0.72 (0.57–0.84) by 0.28 (0.22–0.38)	0.49 (0.46–0.62) by 0.24 (0.18–0.33)
	Bulla long	0.28 (0.22–0.38)	
	Trunk long by wide	1.21 (0.94–1.46) by 0.92 (0.78–1.27)	0.93 (0.51–1.35) by 0.70 (0.26–1.08)
	Trunk lateral processes	0.23 (0.19–0.34) by 0.13 (0.11–0.2)	0.13 (0.11–0.15) by 0.09 (0.07–0.13)
	Trunk Ventral lobes bearing oviduct, long by wide	0.11 (0.09–0.14) by 0.07 (0.04–0.08)	
	Egg Sac long by diameter	1.27 (1.13–1.46) by 0.41 (0.32–0.54)	1.22 (0.72–1.76) by 0.44 (0.40–0.51)
MALES	Body long	0.61 (0.54–0.68)	0.67 (0.59–0.77)
	Cephalothorax long by wide	0.26 (0.24–0.28) by 0.27 (0.23–0.32)	0.27 (0.26–0.30) by 0.18 (0.14–0.21)
	Trunk long by wide	0.35 (0.31–0.40) by 0.28 (0.18–0.38)	0.39 (0.34–0.47) by 0.16 (0.15–0.77)

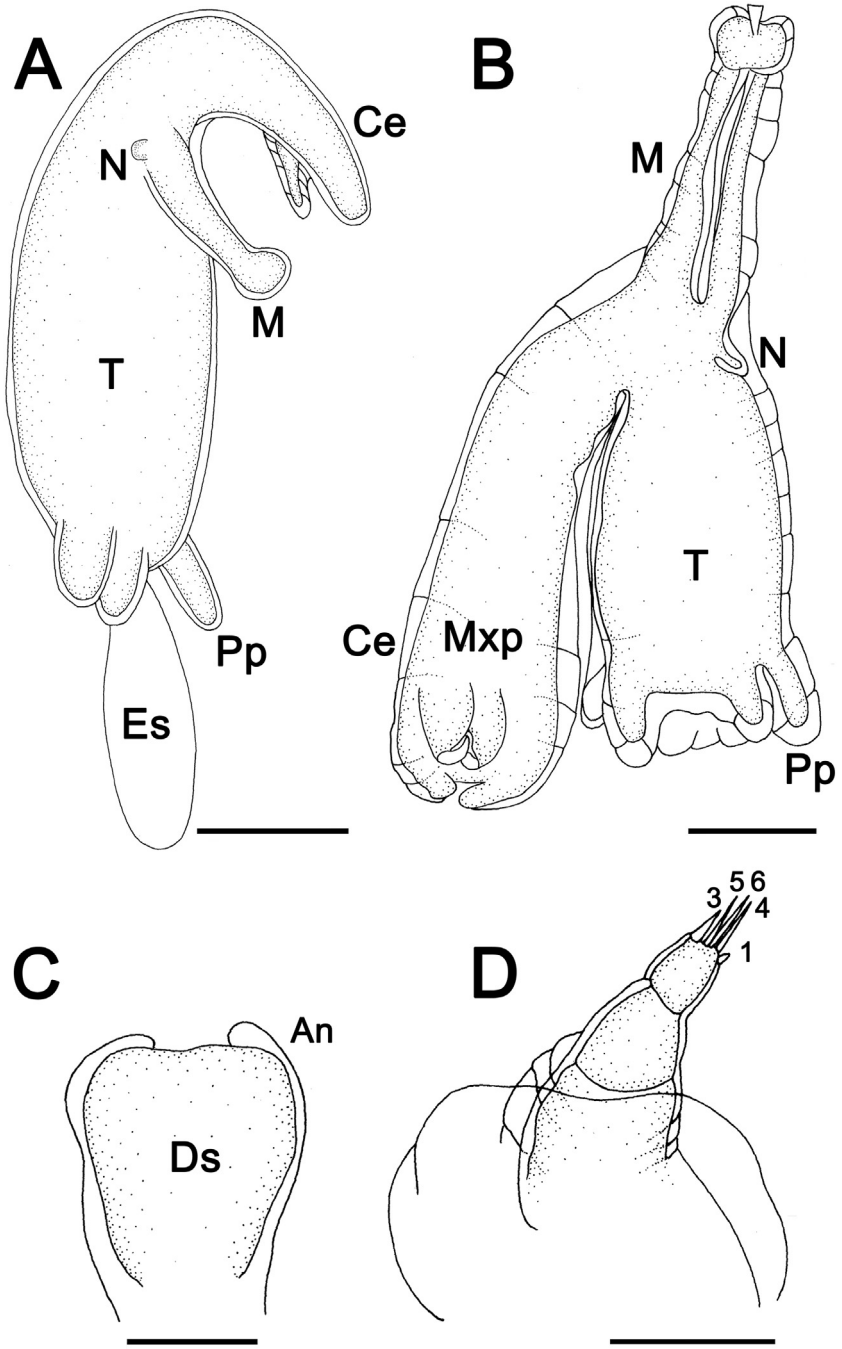

Fig. 1. *Parabrachiella platensis* n. sp. from nostrils. Female. A. Lateral view. *Parabrachiella platensis* n. sp. from fins Female. B. Ventral view. C. Dorsal shield of cephalothorax. D. Antennule. *Abbreviations*: 1, 3, 4, 5, 6, armature; An, antenna; Ce, Cephalothorax; Ds, Dorsal shield; Es, Egg sac; M, Maxilla; Mxp, Maxilliped; N, Nipple like structure; Pp, Posterior process; T, Trunk. *Scale bars*: A, 500 μm; B, 250 μm; C, 150 μm; D, 5 μm.

cylindrical, unarmed. Exopod bulbous, one-segmented, with two short spines on dorsal surface (in nostrils specimens). Endopod, two segmented, longer than exopod. Terminal segment with hook 1, seta 2, process 4 on outer surface, and seta 5 on ventral margin; ventral surface of distal segment with pad of denticles. Buccal cone (Fig. 6A and D) formed by labrum and labium. Labrum armed distally with setiform process, labium without sensilla. Mandible (Fig. 5F) blade with at least three teeth;. Maxillule (Fig. 6A and D) bilobate; inner lobe with 2 large, unequal subcylindrical setae and outer lobe with minute seta in ventral position (Fig. 6D). Maxilla (Figs. 5D, 6A) with basal segment of length slightly greater than width, subchela strongly curved distally. Maxilliped (Figs. 5E, 6E) with robust corpus without armature; subchela with robust base, nearly cylindrical, tapering at apex. Claw strongly curved.

Trunk with long axis of genital trunk slightly curved ventrally (Fig. 5A). Two distal genital lobes (Fig. 5G).

Parabrachiella exilis Shiino,1956

Type-host: *Mugil cephalus* (Mugiliformes: Mugilidae); local name "lisa", English name "grey mullet".

Type locality: Antofagasta, Chile.

Attachment site: fins.

Prevalence: 17.61% (fins).

Mean intensity: 1.

Adult Female [Based on 5 ovigerous specimens. Only a small number of details have been added concerning trunk posterior margin processes, which have been revealed *via* SEM.]

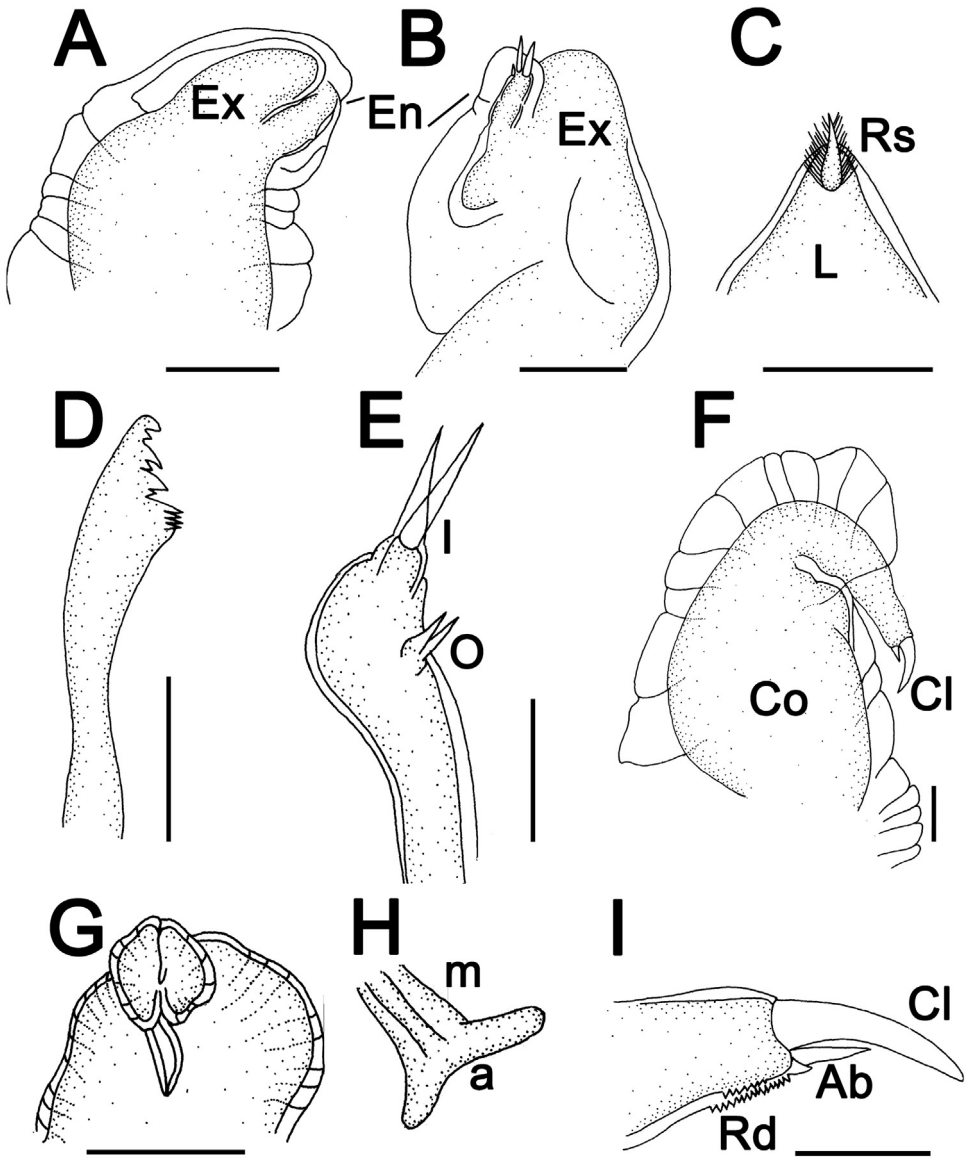

Fig. 2. *Parabrachiella platensis* n. sp. A. Antenna. B. Apical detail of antenna armature. C. Labrum. D. Mandible. E. Maxillule. F. Maxilliped. G. Maxilla showing the distal end of bulla. H. Bulla. I. Claw of maxilliped. *Abbreviations*: a, anchor; Ab, Anexed barb; Cl, Claw; Co, Corpus; En, Endopod; Ex, Exopod; I, Inner lobe; L, Labium; O, outer lobe; Rd, Row denticles; Rs, Rostral setules. *Scale bars*: A and B, 5 μm; C, 10 μm; D, 25 μm; E, 10 μm; F, 50 μm; G, 250 μm; H, 100 μm: I, 25 μm.

Female trunk subrectangular with two pairs of posterior, blunt, processes and a short, dorsal, caudal rami (Fig. 7A and B).

2.3. Molecular results

The content of adenine, guanine, cytosine, and thymine for *Parabrachiella* species is listed in Table 1. The six specimens of *P. platensis* n. sp. analysed (four from the nostrils and two from the fins) showed a close distance with a similarity of 99.8%, with sequences differing only by 1 or 2 bp.

The genetic distance was only 0.2–0.4% among the specimens collected in fins and nostrils Table 4. This result confirmed that both sets parasitizing different habitats on the host would belong to the unique species showing minimal morphological differences.

The interspecific genetic distance (Table 3) among *P. exilis* and *P. platensis* n. sp. is 9%, both forming a clade more apomorphic than the others species studied (Fig. 8). *Parabrachiella platensis* showed a 16% of genetic distance from *P. auriculata*, 12% from both *P. anisotremi* and *P. kabatai,* and 14% from also both *P. merluccii* and *P. hugu.*

It is notorious the position of *P. hugu*, which is located more basal han all other species treated (Fig. 8), presenting a genetic distance of 14–18% from the other species used in this study (Table 3).

3. Discussion

Most of the 138 copepods specimens acquired from the host were dissected from the nostrils and only few specimens were collected from the fins. For this reason, we assumed that the preferable location of the new species is the nostrils. *Parabrachiella* specimens collected from fins showed several minor morphological differences with respect to those located in the nostrils. *Parabrachiella platensis* n. sp. residing within the nostrils has a pair of small papillae on each side of the anal slit; while the specimens from the fins have three tubercles in that region. Also, near the distal margin of the labium, the fins specimens have a pair of seta that are absent in *P. platensis* n. sp. located on the nostrils. Detailed examination of the maxilliped with SEM reveals that *P. platensis* n. sp. from nostrils has an external armature with two groups of at least 5 denticles each at the base of the claw, whereas the specimens from fins have only two denticles. The maxillule also provides other differences; *P. platensis* n. sp. copepods from nostrils bears two setae on the outer

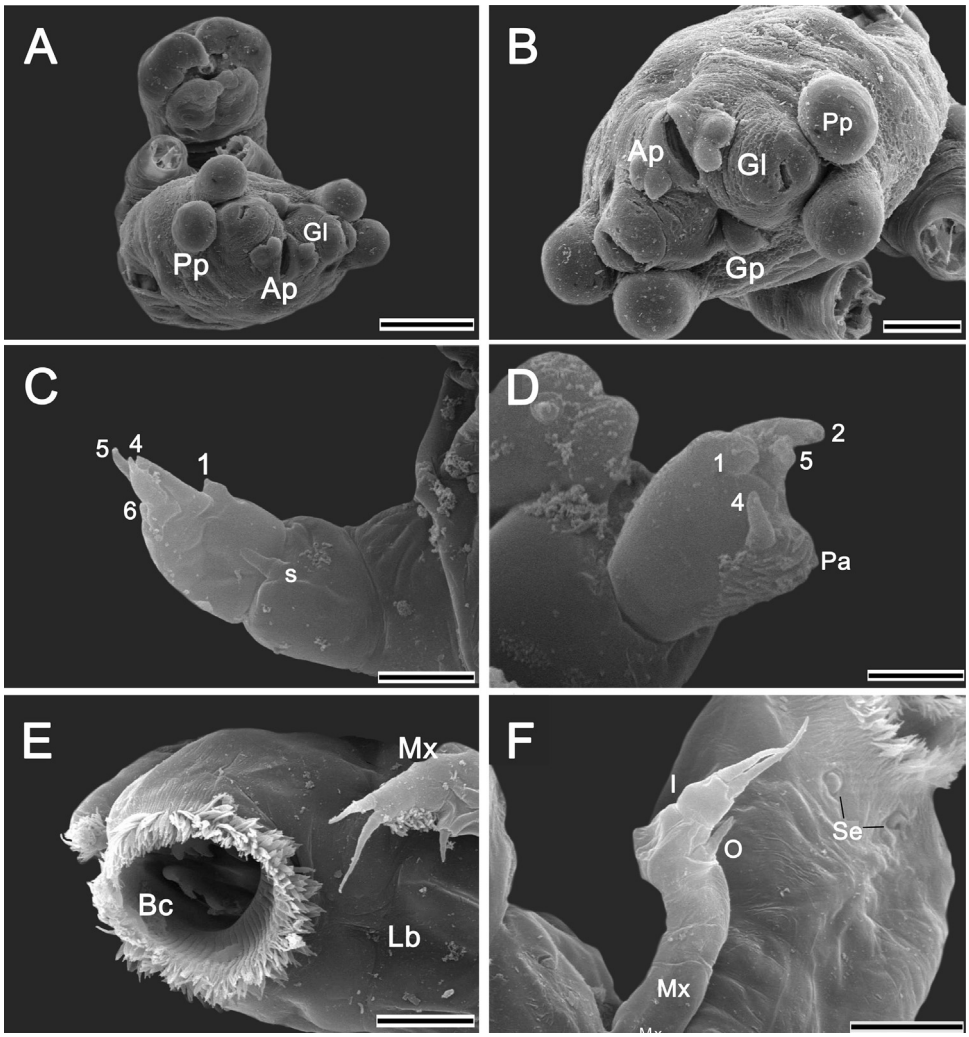

Fig. 3. SEM *Parabrachiella platensis* n. sp. from nostrils. Female. A. Ventral view showing the trunk and processes. B. Detail of processes and papillae. C. Lateral view of the antennule, showing the solus and armature. D. Armature of endopod of the antenna. E. Buccal cone and maxillule. F. Maxillule and labium of *Parabrachiella platensis* n. sp. from fins. *Abbreviations*: 1, 2, 4, 5, 6, armature; Ap, Anal papilae; Bc, Buccal cone; Gl, genital lobe bearing oviduct opening; Gp, Genital process; I, Inner lobe; Lb, labium; Mx, maxillule; O, outer lobe; pa, pad; Pp, posterior processes; S, solus; Se, sensilia. *Scale bar:* A, 200 μm; B, 100 μm;C, 10 μm; D, 5 μm; E, 20 μm, F, 20 μm.

lobe while fin specimens present only one seta. Finally, the antennules of *P. platensis* n. sp. from nostrils have a well-developed solus which is not observed in the specimens from the fins.

Together, the low intraspecific genetic distance between the specimens collected from the fin and nostrils (0.2–0.4%) and the close morphological similarity between the two morphotypes, do not support the description as two separate species. This, differences agree with those presented by Costa et al. (2007) and Hill et al. (2001) who reported a distance of 0.5–0.8% among three geographic separate populations of crustaceans *Calanus helgolandicus* Claus, 1863. Despite of that, Burns et al. (2007) using COI sequences noticed that some valid butterfly species differ “only by one to three nucleotides”. New studies on *P. platensis* n. sp., including infection of the same fish with the two forms, could bring some new information. Anyway according to the genetic information provided and the minimal morphological differences spotted we conclude that the two forms are a single specie. The differences observed are possibly influenced by the place of the host where the two forms of copepods were found, nostrils and fins.

Parabrachiella includes at least 67 species (Piasecki et al., 2010) of which 35 hold two pairs of posterior processes on the trunk (Castro Romero and Baeza Kuroki, 1987; Piasecki et al., 2010). The parasitic specimens examined from the host *M. liza* in Argentina during the present study were compared with this last group of species. Close examination led to the conclusion that the now described specimens are not conspecific with any of the above species. The difference between the new species and those described for mugilids are the size and shape of the posterior processes, the length of maxilla, the shape of the maxillary arm, the shape and relative size of the trunk and finally, it’s appendages.

A comparison was made between the newly described specimens parasitizing *M. liza*, with two species of copepods infecting Mugilidae fishes: *P. exilis* and *P. mugilis*

The females of *P. platensis* n. sp. are smaller than the specimens of *P. exilis* found by Knoff et al. (1994), but similar in size to those examined by Shiino (1956) and Castro-Romero and Baez-Kuroki (1986). Other differences between *P. platensis* n. sp. and the *P. exilis* specimen lie in the sizes of the cephalothorax (1.98 vs. 2.48 long), the second maxilla (0.72 vs. 1.28 long), the egg sac (1.27 vs. 2.14 long), and the trunk (1.21 vs. 1.62 long). Furthermore, the specimens described by Shiino (1956) had a longer trunk (1.69 long). *Parabrachiella platensis* n. sp. differs from *P. exilis* in the shape of the posterior processes—which are short and obtuse in *P. platensis* n. sp. vs. subconical in *P. exilis* (sensu Shiino, 1956). In addition, the small digitiform processes, (i.e., caudal rami), dorsally located occurring in *P. exilis*, (Fig. 7, and Fig. 19A and C of Shiino, 1956) are not present in the new species. *Parabrachiella platensis* n. sp. compared with *P. exilis* has short oviduct-orifice processes (similar in size to the posterior processes), two pairs of small bipartite papillae laterally flanking the anal slit (in nostrils specimens),

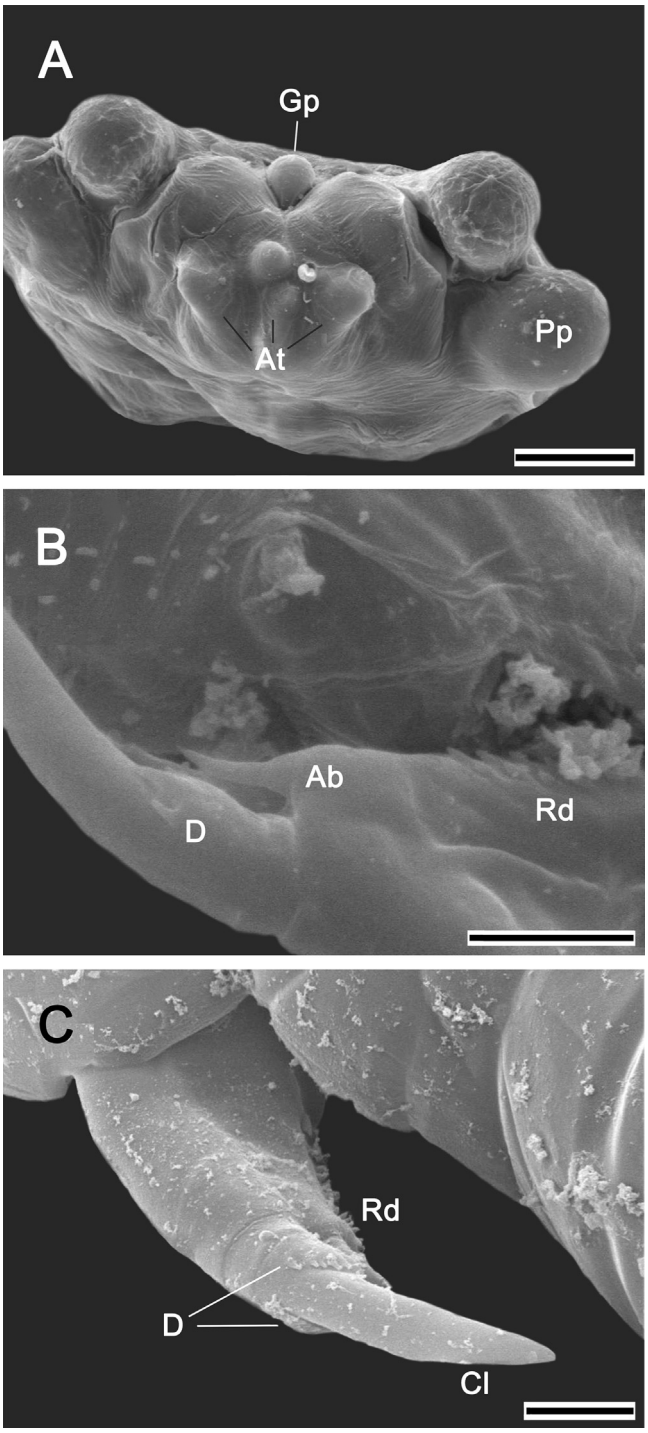

Fig. 4. *Parabrachiella platensis* n. sp. A. Posterior view of the *Parabrachiella platensis* n. sp. from fins. B. Armature claw maxilliped. A. Claw of maxilliped of the *Parabrachiella platensis* n. sp. from fins. *Abbreviations*: At, anal tubercles; Ab, Anexed barb; Cl, Claw; D, denticles; Gp, Genital process; Pp, Posterior process; Rd, Row of denticles. *Scale bars*: A, 100 μm; B, 20 μm;C, 10 μm.

and lack of caudal rami. The pairs of bipartite papillae flanking the anal slit has not been previously reported in other *Parabrachiella* species.

The comparison of *P. mugilis* parasitic on *L. aurata* and leaping mullet, *Liza saliens* (Risso), from Tunis (Kabata et al., 1971) with the described specimen likewise indicates that the two copepods are different species. *Parabrachiella mugilis* has a single pair of posterior processes (caudal rami) at the end of the trunk along with a short genital process, while *P. platensis* n. sp. has two posterior processes on the trunk, in addition to small papillae around the anal slit lacking caudal rami. Furthermore, the species occupy different microhabitats on the host: *P. mugilis* lives attached to the fins, while *P. platensis* n. sp. were found either on inside the nasal cavity (a new site for a species of the genus *Parabrachiella*), or attached to the fins base. In this case, the females compared have a longer trunk (1.31 vs. 1.21 long), a smaller cephalothorax (1.56 vs. 1.98 long), and bigger maxilla (1.5 vs. 0.72 long). The male of *P. mugilis* is smaller than the male of *P. platensis* n. sp. (0.33 vs. 0.61 long).

The morphology per se indicates that *Parabrachiella* parasitizing mugilids (*P. exilis*, *P. mugilis* and *P. platensis* n. sp.) are different species.

According to the description presented in this paper, it is clear that the *Parabrachiella* species that parasitize South American mugilids represent a close related species, at least in the case of species that parasitize *M. liza* from Argentina and *M. cephalus* from Chile. Similarities between these copepod species make difficult to distinguish between them, but a high-resolution analysis of the posterior margin of the trunk has revealed significant differences in the anal region.

The genital area, which includes processes and lobes associated with the oviducts orifices, exhibit different shapes and degrees of development, which depend on the age or sexual maturity of the female specimen, for this, we must work only with adult gravid females. The anal area can also show morphological variation concerning associated projections, which are often species-specific. These anal tubercles are typically not considered caudal rami. *Parabrachiella exilis* bears the caudal rami located dorsally in that region (see present Fig. 7A and B) corresponding to the “a much shorter dorsal pair, whose members are closely adjoining each other on midline” sensu Shiino (1956; Fig. 19A and C).

When the posterior region of the trunk is examined using light microscopy, several processes are often seen in the same plane, obscuring their identity. When viewed using SEM, however, features of the distal margin or surface of the trunk become clearer, and anal processes or papillae-like structures become evident. The description of this area in new species must be approached with care, especially concerning the presence of caudal rami.

The presence of sensilla on the labium surface is observed only in the specimens of *P. platensis* n. sp. from fins base and not in those from nostrils. This feature seems to be relevant for the feeding activity of these copepods in their microhabitat. No previous reports have ever been made of this kind of setae at that position.

DNA barcoding (Hebert et al., 2003) analysis has demonstrated to be useful in free-livings copepods (Blanco-Bercial et al., 2011, 2014; Buckling et al., 1995). Also in species-level identifications of different taxa including crustaceans (Costa et al., 2007; Dippenaar et al., 2010; Morales-Serna et al., 2014) and used recently by González et al. (2016), for identification and description of a new species for the caligid *Lepeophtheirus confusum* González, Castro, Muñoz et López, 2016.

The morphological and the mtDNA-COI evidence allow to separate *P. platensis* n. sp. from P. *exilis* (with a genetic distance of 9%). They are forming a clade, (both parasitizing mugilids), with a genetic distance of 12–16% from other *Parabrachiella* species here compared.

Avise (2000) and Waugh (2007) gave values of 1–2% for the intraspecific distance of mitochondrial genes and less than 10% of interspecific variation. In some cases, a lower genetic distance can differentiate species using the COI gene.

For caligids the level of genetic distance observed is bigger compared with other free living and parasitic copepods, González et al. (2016), report the level of genetic distance for *Lepeophtheirus confusum* range from 17 to 25% with other *Lepeophtheirus* species (based on COI). Morales-Serna et al. (2014) working with 11 *Caligus* species states the genetic distances among species ranging from 8.42 to 20.87%. Oines and Heuch (2005) found distances of 18–20% among *Caligus* species.

For free living crustaceans, Costa et al. (2007) report genetic distances inside the same species of 4.92% in one crab genus to 31–39% in the amphipods.

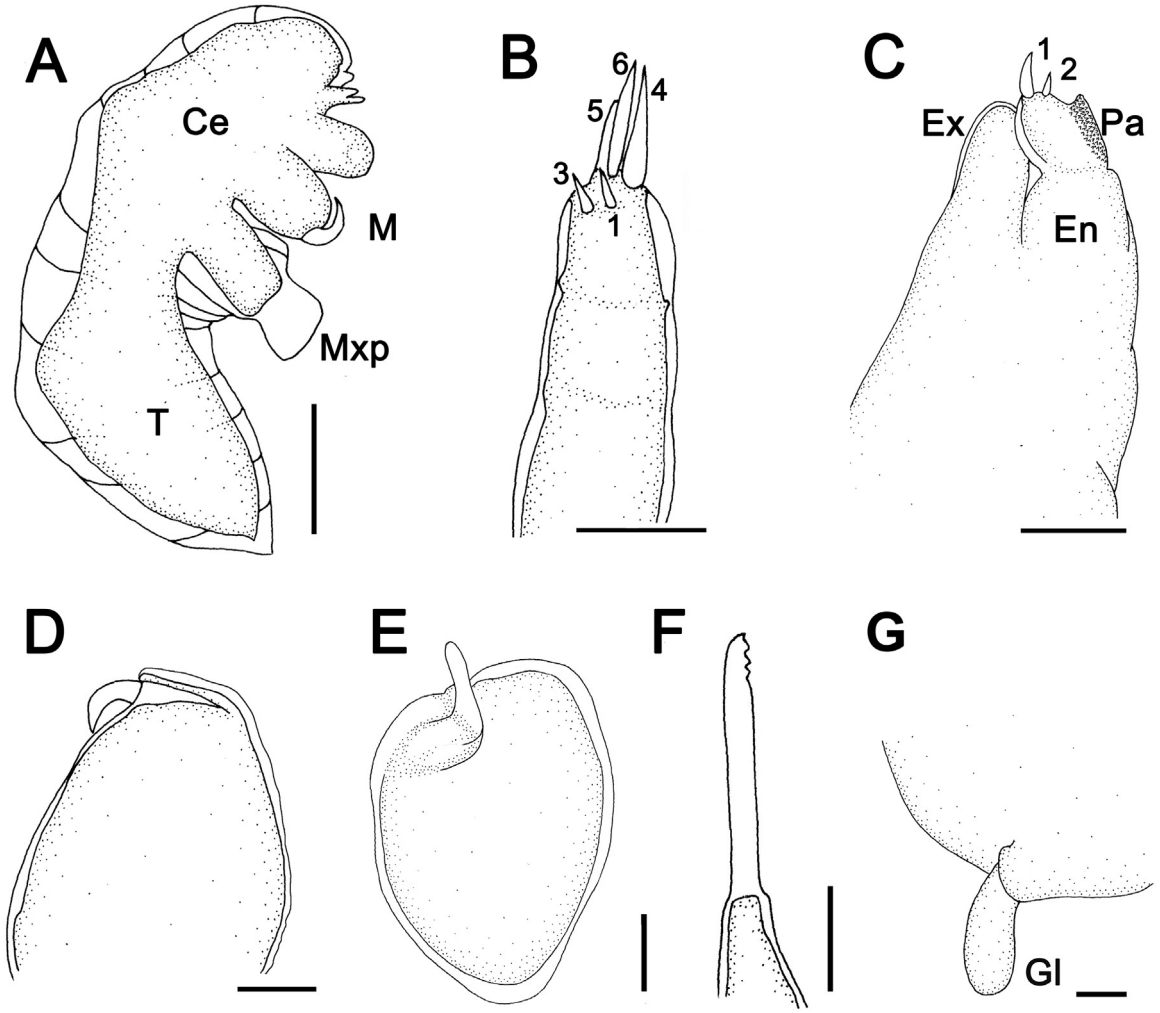

Fig. 5. *Parabrachiella platensis* n. sp. from fins. Male. A. Lateral view. B. Antennule. C. Antenna. D. Maxilla. E. Maxilliped. F. Mandible. G. Caudal rami. *Abbreviations*: 1, 2, 3, 4, 5, 6 armature; Ce, Cephalothorax; En, Endopodo; Ex, exopodo; Gl, Genital lobe; M, maxilla; Mxp, Maxilliped; T, Trunk. *Scale bars*: A, 125 μm. B, 17 μm. C, 12 μm. D, 25 μm. E, 25 μm. F, 13 μm. G, 13 μm.

Costa et al. (2014) found genetic distance of 6–16% distinguishing three lineages in *Acartia tonsa*, previously known only by its morphology.

Castro-Romero et al. (2016) reported genetic distance among Pennellidae: *Peniculus* cf. *fistula* Nordmann, 1832 specimens from different host differ by 0.95%, *Metapeniculus antofagastiensis* Castro-Romero et Baeza-Kuroki, 1985 specimens 0.44%, and for *Trifur* cf. *tortuosus* Wilson, 1917 2.25%. By the other hand the genetic distance among *P.* cf. *fistula* and *M. antofagastiensis* is 17.86%, and *P.* cf. *fistula* differing from *T.* cf. *tortuosus* by 18.16%, these tree species of pennellidae genera well recognized and differentiated by its morphology.

Dippenaar et al. (2010) reported the use of COI in crustaceans to reveal possible cryptic species of symbiotic copepods *Nessipus orientalis* on elasmobranch. These authors found an average distance within the two clades 17.44% clearly a level expected for interspecific relationships. The genetic distance among different parasitic copepods can present a wide range of variation, in accord to their reproductive history, and evolutionary speed. Hebert et al. (2003), states that low genetic distance is due to short histories of reproductive isolations which need to be studied in each order, family, genus, and its species in order to have a wide picture of this aspect for the parasitic copepods on fishes.

At the moment *Parabrachiella* species that parasitize South American mugilids include *P. exilis* (on fins) in Chile and Peru, and *P. platensis* n. sp. (in nostrils and fins), in Argentina. The exact identity of *P. exilis* for those specimens reported from Brazil by Knoff et al. (1994) must be tested using both morphology and molecular studies, they could belong to *P. platensis* n. sp. based on the host species and morphology, but can not be affirmed without a morphological and molecular study of that copepod. This study disagree with Lebepe and Dippenaar (2016) who suggest only one species parasitizing Mugilids.

It is important to note that in species of medium to short size, like those reported here, the armature of the antennules or the antenna are sometimes hard to define, even at high magnification using optical microscopy. Also, it is necessary to use SEM to detect their real armature.

Summarizing, the new species is characterized by having two pairs of posterior processes on the posterior margin of the trunk, a papillae around the anal cavity, a lack of caudal rami, and near its base, and different to the secondary teeth reported for some species of the genus, the presence of at least 5 denticles in a row on the lateral surface of the maxilliped claw, near its base different to the secondary teeth reported for some species of *Parabrachiella*. In addition to describing a new species of *Parabrachiella*, the results obtained allow to report some features of its interspecific variation and also to know the DNA barcode for other two *Parabrachiella* (*P. exilis* and *P. kabatai*) for which this genetic information was unknown until now. The presence on the same host of *P. platensis* n. sp. on the fins and in the nasal cavities demonstrates a case of radiation into specialized microhabitats.

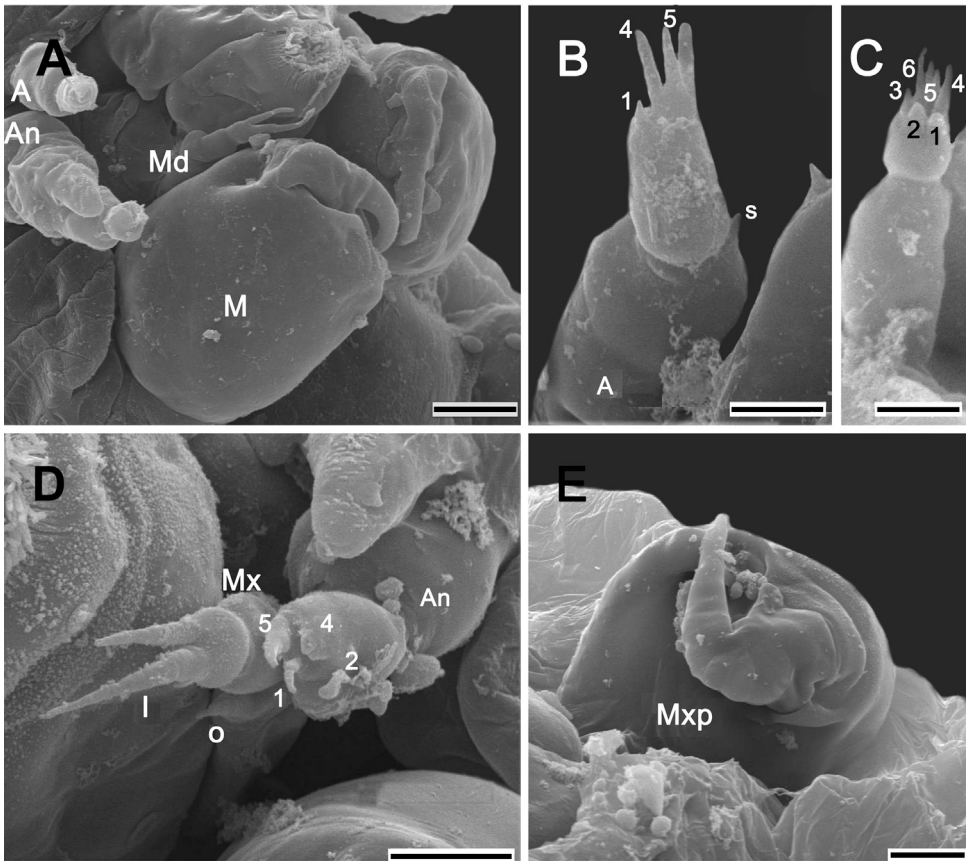

Fig. 6. SEM of *Parabrachiella platensis* n. sp. from nostrils. Male A. Lateral view. B, C Antennule. D. Antenna, buccal cone and maxillule. E. Maxilliped. *Abbreviations*: 1, 2, 3, 4, 5, 6, armature; A, antennules; An, antenna; I, inner lobe; M, maxilla; Mx, maxillule; Mxp, maxilliped; O, outer lobe; s, solus. *Scale bar:* A, C and E, 20 μm; B and D, 10 μm.

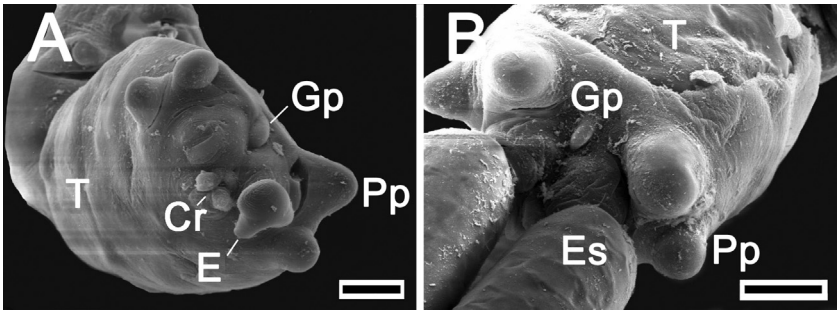

Fig. 7. SEM of *Parabrachiella exilis*, female on fins of *Mugil cephalus* from Chile. A. Posterior view without sac eggs. B. Posterior end with sac eggs. *Abbreviations*: Cr, caudal rami; E, egg; Es, Egg sac; Gp, Genital process; Pp, Posterior process; T, Trunk. *Scale bars*: A, 100 μm; B, 200 μm.

Table 3
Genetic distance (expressed in percentage) among species of copepods. *Abreviattions*: Erg., *Ergasilus*; P. ani, *Parabrachiella anisotremis*; P. aur., *Parabrachiella auriculata*; P. exi., *Parabrachiella exilis*; P. hug., *Parabrachiella hugu*; P. kab., *Parabrachiella kabatai*; P. mer., *Parabrachiella merlucci*; P. pla., *Parabrachiella platensis* n. sp.

	Erg.	P. aur.	P. ani.	P. kab.	P. pla.	P. mer.	P. exi.	P. hug
Erg.		2	2	2	2	2	2	2
P. aur.	27		1	2	2	2	2	2
P. ani.	28	13		2	1	1	2	2
P. kab.	29	16	16		1	2	1	2
P. pla.	27	16	12	12		1	1	2
P. mer.	30	16	15	16	14		2	2
P. exi.	27	17	14	12	9	15		2
P. hug.	26	18	16	15	14	18	16	

Table 4
Intraspecific genetic distances (expressed in percentage) in copepods. *Abreviattions*: Dist., Intraespecific genetic distance; Erg., *Ergasilus*; P. ani, *Parabrachiella anisotremis*; P. aur., *Parabrachiella auriculata*; P. exi., *Parabrachiella exilis*; P. hug., *Parabrachiella hugu*; P. kab., *Parabrachiella kabatai*; P. mer., *Parabrachiella merlucci*; n/c, not calculated, P. pla., *Parabrachiella platensis* n. sp.; Var., Variance.

	Dist.	Var.
Erg.	n/c	n/c
P. aur.	0.1	0.1
P. ani.	0.7	0.3
P. kab.	0.7	0.3
P. pla.	0.3	0.2
P. mer.	n/c	n/c
P. exi.	0.8	0.3
P. hug.	n/c	n/c

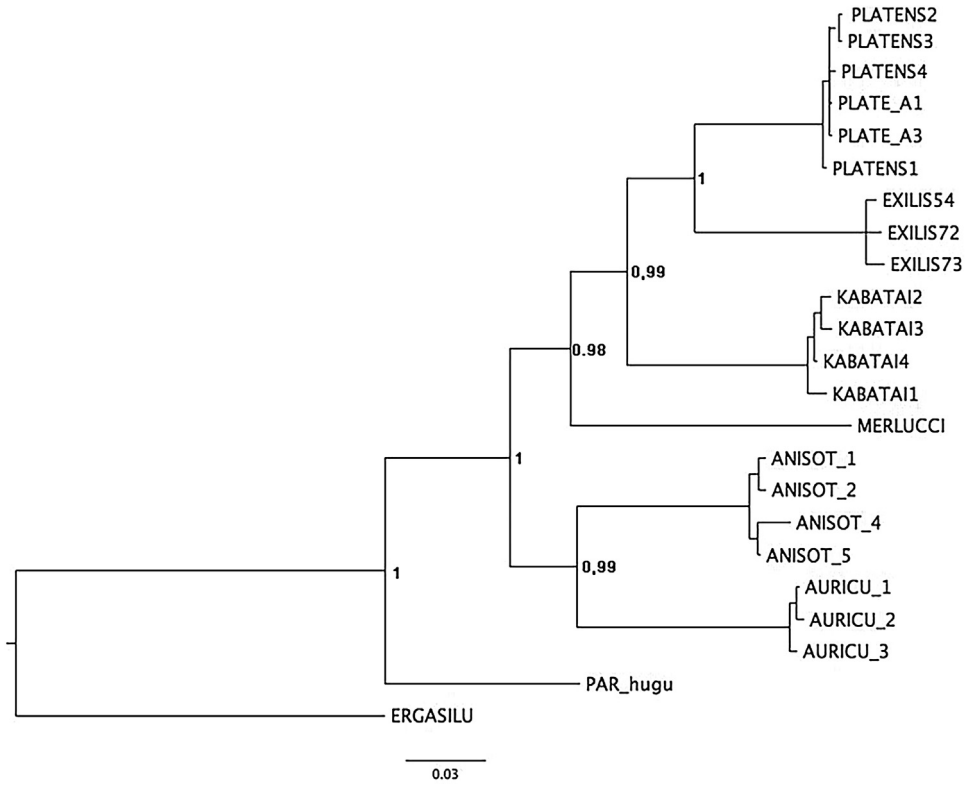

Fig. 8. Phylogenetic position of the new species of *Parabrachiella platensis* n. sp. from fins and nostrils based on the COI gene obtained with the analysis by Bayesian Inference algorithm. *Ergasilus* sp. was used as the outgroup. Abbreviations: ANISOT, *Parabrachiella anisotromi*; AURICU, *Parabrachiella auriculata*; EXILIS, *Parabrachiella exilis*; HUGU, *Parabrachiella hugu;* KABATAI, *Parabrachiella kabatai;* MERLUCCI, *Parabrachiella merlucci;* PLATENS = *Parabrachiella platensis* n. sp. from the nostrils; PLATE_A1 and PLATE_A3, *Parabrachiella platensis* n. sp. from the Fins.

New studies including more species of *Parabrachiella* could elucidate the real relationships among the genus species and especially for the case of *P. hugu* which appears as plesiomorphic to the species treated here.

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