ANIMAL SCIENCE

Maxiclavella and *Praeclavella* (Siphonostomatoida: Lernaeopodidae) new genera confirmed by molecular and morphological evidence

RAUL CASTRO-ROMERO, MARTIN MIGUEL MONTES & SERGIO MARTORELLI

Abstract: The new genus *Maxiclavella* is proposed to accommodate *Clavella simplex* Castro Romero and Baeza Kuroki, 1985, which differs from *Clavella* species, including long and narrow cephalothorax, small bulla, and simple armature of the antenna and antennule. *Praeclavella nasalis,* new genus and species, was found parasitizing the olfactory sacs of *Isacia conceptionis*. *Praeclavella nasalis* could be differentiated from other *Clavella* species by a biramous antenna with an exopod shorter than the endopod, the base of the cephalothorax with a large lobular and suborbicular projection on each side, a suborbicular bulla, mandibles without secondary teeth, and a suboval male body type. Males of both genera fit well in the *Clavella*-clade male type, although they differ from each other in many aspects, mainly in the position of the buccal area, which is oriented ventrally in *Maxiclavella* and distally in *Praeclavella nasalis,* and in a genital process, which is present in the latter and absent in the former. Genetic distances of mtDNA COI and 28S rDNA supported the validity of the two new genera parasitizing the same host, *I. conceptionis.* Also *Clavella*-branch (Clavellinae Wilson), a key based genera on female specimens is presented.

Key words: Antofagasta, *Clavella simplex*, integrative analysis, *Isacia*, *Maxiclavella*, *Praeclavella.*

INTRODUCTION

The most distinctive feature of the family Lernaeopodidae is the presence of modified maxillae in females for the attachment to the host's tissues. Within this family, each maxilla varies in size and in secondary characteristics, such as: condition (fused or separate); presence or absence of lateral swelling at the base (lobular or aliform); collar (distal end), small or large, and broad or narrow (among other traits); and type of bulla. According to Boxshall & Halsey (2004), the family Lernaeopodidae includes 48 genera grouped into five clades (Kabata 1979): *Salmincola* Wilson, 1915, *Lernaeopoda* von Nordmann, 1832, *Brachiella* Cuvier, 1830, *Clavella* Oken, 1815, and *Charopinus* Krøyer, 1863.

Kabata (1979) recognizes the following genera in the *Clavella*-clade: *Advena* Kabata, 1979 (subsequently renamed as *Kabatazus* by Özdikmen (2008) as a junior homonym); *Alella* Leigh-Sharp, 1925; *Anaclavella* Heegaard, 1940; *Clavellistes* Shiino, 1963; *Clavellodes* Wilson, 1915; *Clavellomimus* Kabata, 1969; *Clavellisa* Wilson, 1915; *Clavellopsis* Wilson, 1915; *Euclavellisa* Heegaard, 1940; and *Proclavellodes* Kabata, 1967. Seven genera were added later: *Clavellotis* Castro Romero and Baeza Kuroki, 1984; *Cryptova* Kabata, 1992; *Margolisius* Benz, Kabata and Bullard, 2000; *Mixtio* Kabata, 1986; *Nudiclavella* Ho, 1975 (albeit

with unknown male); *Pseudomixtio* Kabata, 1990; and *Sparidicola* Kabata and Tareen, 1987. Except for *Pseudomixtio* and *Kabatazus,* females lack posterior processes on the trunk, while they possess posterior and genital processes in *Clavellotis, Clavella*, and *Mixtio*. In this clade, the size of the maxilla varies over a wide range, from medium-sized to completely reduced. The bulla is typically short and suborbicular, but it can be suboval or elongate. The males are small, suboval to subcircular or even subtriangular in shape, and their trunk is aligned with the cephalothorax. The appendages are close to one another in the anterior region or apart from each other in ventral position. The genital process is absent or poorly developed and some genera bear caudal rami.

Molecular methods have been successfully used in taxonomic and phylogenetic studies of Crustacea. For example, Lefébure et al. (2006) found a high correlation between molecular divergence and taxonomy for shallow taxonomic levels. The mitochondrial cytochrome oxidase subunit I (mtDNA COI) gene has been proved to be useful for discriminating between and within crustacean orders (Costa et al. 2007). In Copepoda, it serves as a reliable marker for delimitation and identification of free-living (Bucklin et al. 1999, Blanco-Bercial et al. 2014, Baek et al. 2016) and parasitic species. In the latter group, the COI gene has been used for assessing richness (Muñoz et al. 2015), inferring phylogenetic relationships (Dippenaar 2009), unraveling cryptic diversity in *Nessipus* species (Dippenaar et al. 2010), and detecting polymorphism in pennellids (Castro-Romero et al. 2016). Moreover, the nuclear 28S rDNA gene is one of the most informative markers for high taxonomic levels. It has been used in phylogenetic studies addressing closely related genera of Cyclopoida (Zagoskin et al. 2014), Calanoida (Blanco-Bercial et al. 2011), and

Ergasilidae (Song et al. 2008). In addition, both 28S rDNA and COI genes were used to identify copepods parasitizing fish larvae (Muñoz et al. 2015).

The aims of this paper are to clarify the taxonomic position of *Clavella simplex* Castro-Romero and Baeza- Kuroki, 1985, to describe other copepods recently found in the olfactory sac of *Isacia conceptionis* (Cuvier, 1830), and to determine their position within the *Clavella*-branch. The results of the genetic analysis of mtDNA COI and 28S rDNA supported the morphological evidence, and therefore a new genus is proposed herein to include the newly collected specimens. In addition, the taxonomic position of *Clavella adunca* (Strøm, 1762) is discussed, as this species has been frequently reported from different hosts, localities and under various synonyms (Nunes Ruivo 1957, Kabata 1963, 1979). The present study raises the number of genera included in the *Clavella*-clade to 19. A key for all the genera within this clade is presented.

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MATERIALS AND METHODS

Specimen collection

A total of 155 fish of *Isacia conceptionis* ranging from 16.5 to 29 cm in total length were obtained from commercial artisanal fishery at Antofagasta Bay (23°39´00´´S, 70°24´00´´W) and immediately taken to the laboratory, where they were examined with the naked eye or under a stereomicroscope. Some copepods were collected and fixed in 70% alcohol, and then their appendages were dissected for morphological analysis, while others were fixed in absolute ethanol for molecular analysis. Drawings were made with the aid of a drawing tube attached to a light microscope (Olympus CH-2, Tokyo,

Japan). Measurements are in micrometers with means followed by ranges in parentheses, unless otherwise stated. The nomenclature of copepod body parts follows Huys & Boxshall (1991), except for the armature of antennule and antenna (Kabata 1979) and caudal rami (Dojiri & Ho 2013). Some fixed specimens were dehydrated through an increasing ethanol series, dried to CO_2 critical point, and sputter-coated with gold. Samples were then observed and photographed using a Philips 505 scanning electron microscope (SEM) equipped with ADDA II digital image capture software (Soft Imaging System, Lakewood, Colorado). A key was constructed based on the original descriptions of the genera included in the *Clavella*-clade (Kabata 1979) and those described later. The genera *Kabatazus*, *Clavellopsis*, and *Cryptova* included in the key are based on male type.

DNA extraction, PCR amplification and sequencing

Sequence fragments were obtained from *Clavellotis dilatata* (Krøyer, 1863) Castro-Romero and Baeza-Kuroki, 1984; *Clavella simplex* Castro-Romero and Baeza- Kuroki, 1985; *Parabrachiella auriculata* Castro Romero and Baeza Kuroki, 1987, *Parabrachiella anisotremi* Castro- Romero and Baeza-Kuroki, 1989; *Clavella applicata* Castro Romero and Baeza-Kuroki, 1985), *Clavella caudata* Castro- Romero and Baeza Kuroki, 1985 and the new species found in the olfactory sacs of *Isacia conceptionis* (Cuvier, 1830) (Supplementary Material - Table SI).

Sequence fragments ranging from 597 to 708 base pairs (bp) corresponding to mtDNA COI gene and from 696 to 866 bp corresponding to 28S rDNA gene were obtained. Only one specimen from each fish host was analyzed to achieve the highest genetic variability. *Ergasilus* sp. for COI and *Ergasilus briani* Markevich, 1933 for 28S rDNA were used as outgroup. DNA extraction

was performed using releasing reagent GeneReleaser® DNA Full Size (BioVentures, Inc.), with some modifications (Schizas et al. 1997). Each assay was performed with replicates. *Drosophila melanogaster* Meigen, 1830 larvae were used as positive control.

The mtDNA COI and 28S rDNA gene regions were amplified by Polymerase Chain Reaction (PCR) (Saiki et al. 1988). The primers used for mtDNA COI were LCO1490 fwd (5´-GGT CAA CAA ATC ATA AAG ATA TTG G-3´) and HCO2198 reverse (5´-TAA ACT TCA GGG TGA CCA AAA AAT CA-3´) (Folmer et al. 1994). The primers used for the 28S rDNA marker were 28SF (5' – ACA ACT GTG ATG CCC TTA G – 3') and 28SR (5' – TGG TCC GTG TTT CAA GAC G – 3'), following the protocol proposed by Song et al. (2008).

The reactions were prepared using Green GoTaq 5X Buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 0.2 mM of NEB Nucleotide Mix, and Flexi GoTaq polymerase enzyme (Promega). This procedure was carried out using a PTC-100 thermocycler Peltier. The PCR protocol was followed as in Burgos et al. (2003). The PCR products were analyzed by electrophoresis in 1% agarose gel using TAE 1X buffer supplemented with 2 µl of ethidium bromide in the presence of UV light.

Sequencing was carried out in a specialized laboratory Macrogen (Korea). Sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (http:// www.ncbi.nlm.nih.gov) and are available under the accession numbers indicated in Table SI.

Alignment and phylogenetic analysis

The sequences of the mtDNA COI and 28S rDNA genes were edited by eye using Geneious 6.1.8 software (Biomatters, Auckland, New Zealand) (Drummond et al. 2010). Alignments of both gene fragments were assembled using the

online version of MAFFT v.7 (Katoh & Standley 2013). For mtDNA COI sequences, the nucleotide alignment and the presence of pseudogenes in Geneious were assessed using the translated amino acid sequences based on the invertebrate genetic code. For 28S rDNA sequences, the online program Gblocks v0.91 (Castresana 2000, Talavera & Castresana 2007) was used with relaxed parameters to discard poorly aligned regions.

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, 2014). The mtDNA COI 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 (Posada 2003), TRN+G for the second (Tamura & Nei 1993), and HKY+G for the third codon position (Hasegawa et al. 1985)). For the 28S rDNA, the appropriate nucleotide substitution model implemented for the matrix resulting after the Gblock program was K80+G (Kimura 1980).

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 million generations each, to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations. Once the average standard deviation of split frequencies was determined, and it was less than 0.01, as suggested by MrBayes 3.2. The robustness of the clades was assessed using Bayesian PP, where PP > 0.95 was considered strongly supported. A majority consensus tree with clade lengths

was reconstructed after discarding the first 25% sampled trees in both analyses.

Additionally, the proportion (p) of absolute nucleotide sites (p-distance) (Nei & Kumar 2000) was obtained to compare the genetic distance among and between lineages. The p-value matrix was obtained using MEGA v.6.0 (Tamura et al. 2013), with variance estimation, with the bootstrap method (1000 replicates) and with a nucleotide substitution (transition + transversions) uniform rate.

RESULTS

Description

Maxiclavella n. gen.

ZooBank registration: urn:lsid:zoobank.org:act:60E91D2C-6782-4A64-8F3F-5BE49DCFBC5F

Female diagnosis: Long cylindrical and narrow cephalothorax, of more than two times the length of of the trunk. Small subcircular swelling on each side of the cephalothorax base. Small head. Unsegmented antennule, with simple armature having short spiniform process, 4 digitiform processes, and 6 setae. Biramous antenna, with long sympod–endopod axis and short exopod. Maxilla of medium size, with small bulla. Maxillules with 2 setae on inner lobe and 1 ventral seta on outer lobe. Subrectangular trunk, without genital process. Male of *Clavella*-clade type, pyriform in outline, without caudal rami.

Male diagnosis: Pyriform in lateral view, cephalothorax fused with trunk (sac-like distally, with rounded distal margin), without genital process or caudal rami. Uniramous antennule, shorter than the antenna, with whip in basal segment; distal segment longer than basal segment, with 4 elements. Biramous antenna; exopod shorter than the endopod, with 1 segment, endopod with 2 segments, distal segment with 3 elements. Subterminal buccal tube, as long as the basal segment of

maxilla. Mandible with strongly reduced teeth and indiscernible dental formula. Uniramous maxillule; well developed maxilla, subchelate, with strong basal segment, regularly round or suboval; subchela, tipped with claw, sharply curved distally . Maxilliped shorter than maxilla; basal segment subquadrangular, with robust and strongly curved claw closing distally against short process of distal margin in basal segment. Margin of basal segment receiving claw, with 3 short processes. Claw bifid distally, with inner ventral surface bearing at least 3 small teeth-like processes.

Taxonomic summary

Type species: Maxiclavella simplex (Castro-Romero and Baeza- Kuroki, 1985) n. comb.

Type host: Isacia conceptionis (Cuvier) (Teleostei: Haemulidae)

Site of infection: Fins, surface of caudal peduncle, and preopercular space.

Type locality: Antofagasta, Chile.

Prevalence and intensity: Prevalence of 9% (14/155 infected vs sampled host) and intensity of 1.36 (1–2).

Specimens deposited: Museo Nacional de Historia Natural, Chile (see Castro-Romero & Baeza-Kuroki 1985, MNHN Santiago No. 15051). The new material described herein were deposited in Raul Castro's private collection (University of Antofagasta) and in the Colección de Zoología de Invertebrados de Museo de la Plata, Argentina, MLP No. 27156.

Etymology: The genus name, *Maxiclavella*, is a combination of the suffix "maxi" derived from the Latin adjective "maximum", referring to the elongated cephalothorax, and the existing genus name "*Clavella*".

Maxiclavella simplex (Castro- Romero et Baeza-Kuroki, 1985) n. comb.

(Supplementary Material - Figures S1-S4)

Redescription of female (based on 8 specimens): Cephalothorax with small subcircular swelling on each side of the base. Trunk with anal tubercle on distal margin. Unsegmented antennule with simple distal armature comprising elements 1, 4, and 6, and whip (for armature details see Fig. 2 in Castro Romero & Baeza Kuroki (1985). Biramous antenna, with long sympod–exopod axis; endopod perpendicular to sympod, 2 segmented, with 2 small setae on distal margin (2, 5), 1 on mid-anterior margin (1), and row of spinules on posterior surface (4); exopod simple, bearing short spiniform process at mid length and sparsely spinulated on outer surface. Mandible lacking secondary teeth, (see Fig. 4 in Castro Romero & Baeza Kuroki (1985), dental formula P4, P1, B4, blade narrower at distal end. Maxillule distinctly slim; inner lobe with two setae (inner seta shorter and narrower than outer); ventral outer lobe with one seta. Maxilla (cf. Figs. 1, 7 in Castro Romero & Baeza Kuroki (1985)) with minute cup-shaped bulla (swelling). Maxilliped, myxal area with patch of spinules on ventral surface and single spine.

Measurements (in µm, based on 8 specimens, of which 2 carried egg sacs) Cephalothorax 3,785 (3,070-4,487) length, 1,756 (1,359-2,051) width; Trunk 1,756 (1,359-2,051) length, 744 (513-769) width; Anal tubercle 173 (128-205) length, 208 (154-282) width; Maxilla 605 (513-667) length, 256 (205-308) width. Egg sac 3,000 (2,743-3,256) length, 308-(308-308) width.

Diagnosis of male (based on a single specimen): Pyriform in lateral view, small (215 long and 139 wide). Cephalothorax fused with trunk (sac-like distally, with rounded distal margin), without genital process or caudal rami. Uniramous antennule, shorter than antenna, with whip in basal segment; longest distal segment, with 4 elements: short setae (1), short processes (2, 3), digitiform process (4), and fine seta (5); shortest basal segment. Biramous
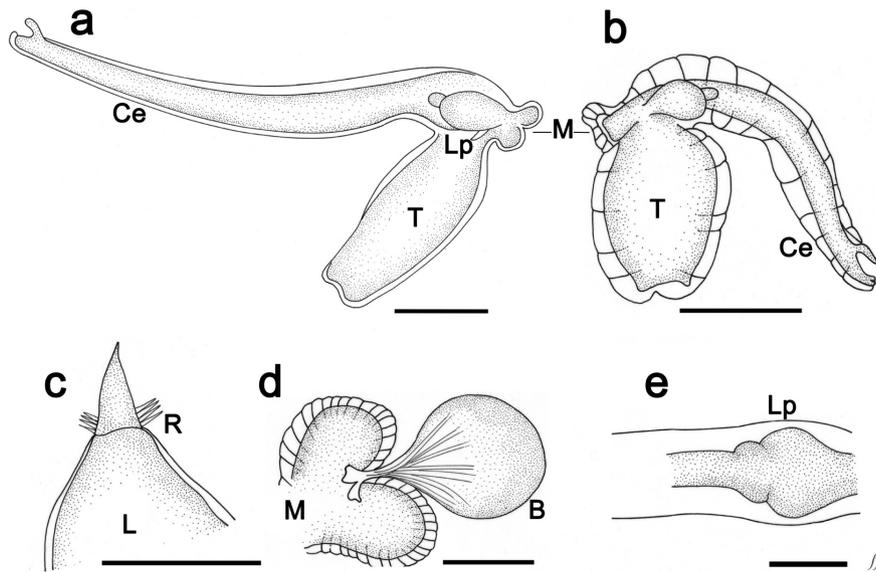
Figure 1. Female *Praeclavella nasalis* n. gen. et sp., **(a)** Lateral view, completely expanded cephalothorax; **(b)** lateral view, contracted cephalothorax (different specimen); **(c)** labrum and rostrum, dorsal view; **(d)** maxilla and bulla; **(e)** base of cephalothorax, dorsal view. Abbreviations: B = bulla, Ce = cephalothorax, L = labrum, Lp = lobular projection, M = maxilla, R = rostrum, and T = trunk. Scale bars: Fig. a and b= 500 µm, Figs. c, d and e = 25 µm.

antenna; exopod shorter than endopod, with 1 segment, with 1 small seta on outer margin and medio-distal process; endopod with 2 segments, distal segment with 3 elements: seta (4), short processes (2) and spiniform process (5). Subterminal buccal tube, as long as the basal segment of maxilla. Mandible with strongly reduced teeth and indiscernible dental formula. Uniramous maxillule; inner lobe armed with two setae of equal length. Well-developed, subchelate maxilla, with strong basal segment, regularly round or suboval; subchela, tipped with claw, sharply curved distally. Maxilliped shorter than maxilla; basal segment subquadrangular, with robust and strongly curved claw closing distally against short process of distal margin in basal segment. Margin of basal segment receiving claw, with 3 short processes. Claw bifid distally, with inner ventral surface bearing at least 3 small teeth-like processes.

Remarks

Maxiclavella n. gen. differs from genera by bearing genital processes (*Clavellotis*, *Mixtio*, and some *Clavella* spp.), posterior processes on the trunk, (*Clavellopsis*, *Margolisius*, and *Advena)*; and short or reduced maxilla (*Alella*,

Clavellisa, *Clavellodes*, *Clavellopsis*, *Clavellotis*, *Euclavellisa*, and *Proclavellodes*).

The new genus, which has a medium-sized maxilla, can be distinguished from *Anaclavella*, *Clavellistes*, *Clavellomimus*, *Margolisius*, *Mixtio*, *Nudiclavella*, and *Pseudomixtio*, mainly by the antennule, antenna armature, mandible (dental formula without secondary teeth), armature of the maxillule lobes, maxilliped (myxal area, claw with barb, spinules on the base of the claw), relative cephalothorax length compared to trunk length, shape of cephalothorax base, features of the maxilla, type of bulla and the anal tubercle (Table SII).

Maxiclavella differs from all species of genus *Clavella* by the long and narrow cephalothorax and conspicuous anal tubercle. It is worthy to note that *Clavella bathyalis* Kazatchenko and Avdeev, 1977 shares some similarity with *M. simplex* n. gen., n. comb. in the cephalothorax length, trunk shape, maxilla size, antennule with a simple armature, absence of annexed lobules (or swelling at base) in the cephalothorax and a genital process. On the other hand, *C. bathyalis* has small lobes on the trunk edge, which are absent in *M. simplex* n. gen., n. comb. In addition, *C. bathyalis* possesses an elongated
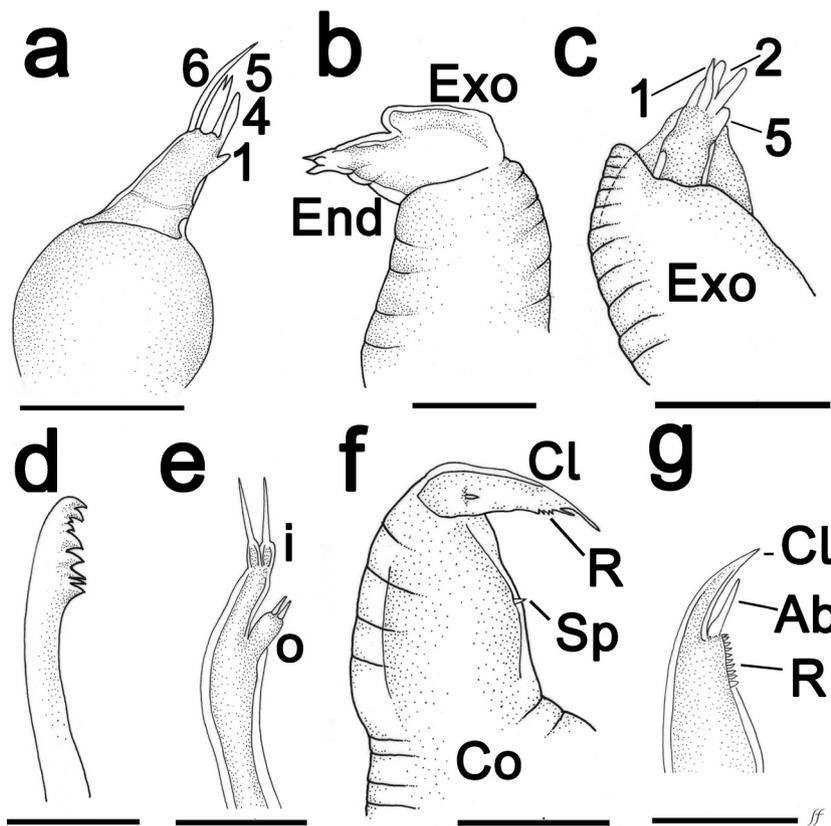
Figure 2. Female *Praeclavella nasalis* n. gen. et sp., (a) antennule; (b) antenna, lateral view; (c) antenna, dorsolateral view (different specimen); (d) mandible; (e) maxillule; (f) maxilliped; (g) claw of maxilliped, detail. Abbreviations: 1, 2, 4, 5, 6 = distal armature, Ab = accessory barb, Cl = claw, Co = corpus, End = endopod, Exo = exopod, i = inner lobe, o = outer lobe, R = row of denticles, and Sp = spine on myxal area. Scale bars: Fig. a-e and g= 25 µm, Fig. f= 50 µm.

bulla and lacks an anal tubercle, while the new genus has a tiny and subspherical bulla and an anal tubercle. The similar morphology of the body and some appendages suggest that they are closely related species, but the lack of a male description for *C. bathyalis* precludes any further comparison of our results. This species was recorded parasitizing a fish caught at 1,000 m deep (Kazatchenko & Avdeev 1977), while the fish host of *M. simplex* n. gen., n. comb. was found in shallow waters.

In the present study, we describe the male of *Maxiclavella* n. gen. for the first time, and we propose to include it in the *Clavella*-clade type, based on the fusion of the cephalothorax with the trunk and a short pyriform body. In this male, appendages (maxilla and maxilliped) are located just in the middle of the ventral surface. Among the genera discussed here, the males of *Margolisius*, *Nudiclavella*, and *Mixtio* have not

been reported yet. *Anaclavella* males are typical of the *Clavella*-clade in that their appendages are placed close to each other in the anterior part of the body. *Pseudomixtio* also bears anteriorly located appendages, but its body is subtriangular in shape. The males of *Clavellistes* have an elongated body, which is unique among males of *Clavella*-clade genera. The female and male characteristics described above allow the separation of the presently described specimen parasitizing *I. conceptionis* from the other genera included in the *Clavella*-clade

Castro-Romero & Baeza-Kuroki (1985) considered that the main differences of *C. simplex* compared with other *Clavella* spp. are the length of cephalothorax, the small bulla, and the simplification of the armature of the antenna and antennule. These differences, together with the presence of a well-developed anal tubercle, would justify the transfer of this
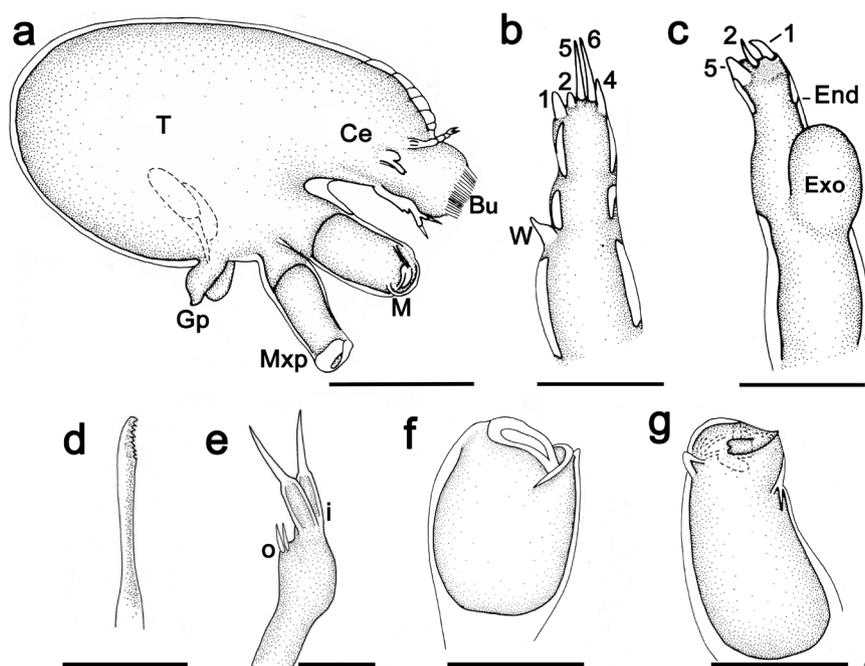
Figure 3. Male *Praeclavella nasalis* n. gen. et sp., (a) Whole male, lateral view; (b) antennule; (c) antenna; (d) mandible; (e) maxillule; (f) maxilla; (g) maxilliped. Abbreviations: 1, 2, 4, 5, 6 = armature, Bu = buccal tube, Ce = cephalothorax, End = endopod, Exo = exopod, Gp = Genital process, i = inner lobe, M = maxilla, Mxp = maxilliped, o = outer lobe, T = trunk, and w = whip. Scale bars: Fig. a = 100 μm, Figs. b and c = 15 μm, Figs. d and e = 25 μm, Figs. f and g = 50 μm.

species to another genus, but the male was not available at that time (Castro-Romero & Baeza-Kuroki 1985). The present paper provides a description of the male and the re-examination of some female appendages supporting the placement of this species in a new genus within the *Clavella*-clade.

Praeclavella n. gen.

ZooBank registration: urn:lsid:zoobank. org:act:CF6E61CA-4846-4C5F-8673-0F2E4E984D0A

Diagnosis: *Clavella*-clade, female as *Clavella*: Cephalothorax cylindrical, about equal or shorter than trunk, base of cephalothorax with or without swelling. Trunk variable in shape (oval, elongated, subcircular). Maxilla, short or reduced. Trunk with or without genital process, caudal rami only in *Praeclavella caudata* (Castro-Romero & Baeza-Kuroki 1985) n. comb. Antennule with variable segmentation, and distal armature. Biramous antenna (exopod short or reduced). Mandible with secondary dentition.

Male *Clavella* type, small, suboval or, subtriangular, cephalothorax fused with trunk, no body segmentation.

Taxonomic summary

Type species: Praeclavella stichaei (Kroyer, 1863) n. comb.

Etymology: The genus name, *Praeclavella*, is a combination of the Latin suffix "prae" meaning "before", which refers to the fact that a biramous antenna is a plesiomorphy, whereas a uniramous antenna is a derived condition, and the existing genus name "*Clavella*".

Praeclavella nasalis n. sp.

ZooBank registration: urn:lsid:zoobank. org:act:C0AE1E0B-966F-4165-9D7C-6587F3BCB3DC (Figs. 1-3, Figure S5)

Diagnosis of female (based on 8 specimens): Cylindrical cephalothorax (Fig. 1a, b) 2,320 (1,590–2,671) in length and 278 (205–385) in width; more than twice the length of the trunk when not extended, and almost three times the trunk when completely extended. Base of cephalothorax with large lobular and suborbicular projection on each side (Fig. 1a, b, e); without dorsal shield. Buccal area with subtriangular labrum and short apical rostrum bearing four setules on each side (Fig. 1c). Subrectangular trunk, 1,194
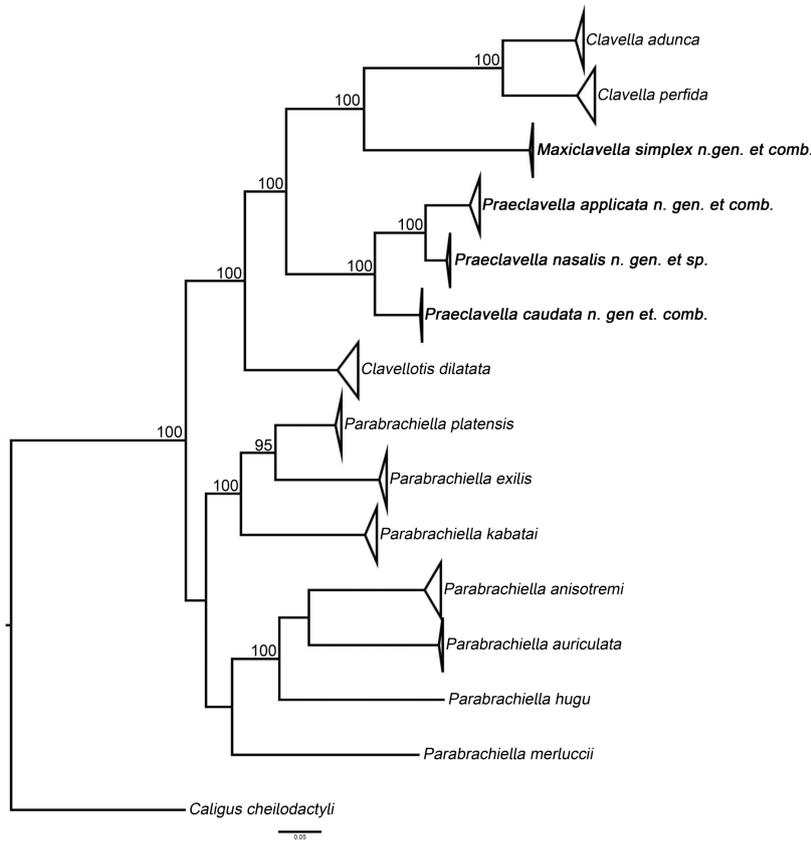
Figure 4. Phylogenetic tree of all the available *Clavella*-branch of the Lernaeopodidae family sequences constructed with Bayesian inference analysis of mtDNA COI sequences.

(897–1,333) in length and 751 (590–872) in width, with pronounced disto-lateral margin. Absent genital and posterior processes. Inconspicuous anal area. Genital orifices located disto-laterally. Multiseriate egg sacs (not drawn). Antennule (Fig. 2a) with 3 segments and armature of medium complexity with four elements: spiniform process (1), digitiform processes (4), bifid seta (5) and seta (6); absent solus and whip. Antenna (Fig. 2b, c) with long sympod–exopod axis, biramous with separated rami; lobular exopod, shorter than endopod and armed laterally with spiniform process; bisegmented endopod with strong, bifid processes (2), fine seta (1) and short process (5) (Fig. 2c). Mandibles (Fig. 2d) with 2 adjacent secondary teeth (dental formula P2, S2, P2, B3). Maxillules (Fig. 2e); inner lobe with 2 papillae bearing 1 seta each; outer lobe, ventrally, with papillae bearing 2 setae of equal size. Reduced maxilla (Fig. 1a, b), 167

(103–231) in length and 242 (205–282) in width. Suborbicular Bulla, longer than maxilla, with slender manubrium; Subspherical anchor (Fig. 1d). Maxillipeds (Fig. 2f) with strong corpus bearing 1 spine in myxal area. Claw base with barb; subchela with spiniform process on third basal and row of distal denticles (Fig. 2g).

Diagnosis of male (based on a single specimen): Males (Fig. 3a) typically suboval in general outline; narrow anterior margin; appendages occupying about one-third of body length. Trunk wider than long, blunt, with prominent genital process (modified) just posterior to the maxilliped base. Antennule (Fig. 3b) with 3 segments, distally with 5 elements: process (1), short process (2), digitiform seta (4), seta (5), and seta (6); basal whip. Antenna (Fig. 3c); lobular exopod, without armature, shorter than endopod; endopod with two setae (1 and 2) and spiniform processes (5). Mandible (Fig.
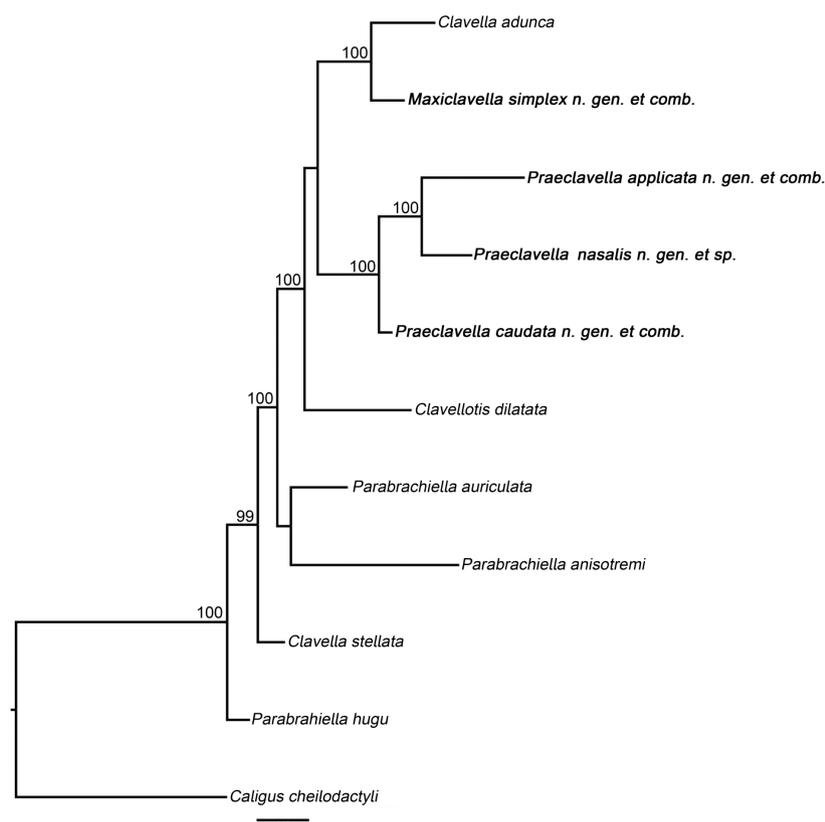
Figure 5. Phylogenetic tree of all the available Lernaeopodidae family sequences constructed with Bayesian inference analysis of 28S rDNA sequences.

3d) without secondary teeth (with at least eight teeth of equal size). Maxillule (Fig. 3e); inner lobe with two papillae bearing one seta each; short outer lobe, laterally located, with two short equal setae. Strong, suboval, subchelate maxilla (Fig. 3f), with a gently curved claw inserting tip in pronounced distal margin. Maxilliped narrower than maxilla (Fig. 3g); strongly curved claw.

Taxonomic summary

Type host: Isacia conceptionis (Cuvier, 1830).
    Type locality: Antofagasta, Chile.
    Site of infection: Olfactory sacs.
    Prevalence and intensity: Prevalence of 4.52% (7/155 infected vs sampled host) and intensity of 1.14 (1–2).
    Specimens deposited*:* Holotype C-NMHN-1157, Museo Nacional de Historia Natural de Santiago de Chile.

Etymology: The specific name "*nasalis*" refers to the site of infection.

Remarks

The described specimens were compared with species of *Clavella* and *Praeclavella* n. gen. lacking genital process and having biramous antenna (*Praeclavella alata* (Brian, 1909) n. comb.; *Praeclavella applicata* (Castro-Romero et Baeza-Kuroki, 1985) n. comb.; *Praeclavella bathyalis* (Kazatchenko et Avdeev, 1977) n. comb.). As proposed above, this species would be included into the new genus as n. comb by having a biramous antenna, until validation is accomplished through either molecular approaches or description of the male; *Praeclavella bowmani* (Kabata, 1963) n. comb.; *Praeclavella canaliculata* (Wilson, 1915) n. comb.; *Praeclavella caudata* (Castro-Romero et Baeza-Kuroki, 1985) n. comb.; *Praeclavella chiloensis*

(Castro-Romero, 1994) n. comb.; *Praeclavella convergentis* (Castro-Romero, 1994) n. comb.; *Praeclavella embiotocae* (Dojiri, 1981) n. comb.; *Praeclavella fortis* (Castro et Gonzalez, 2009) n. comb.; *Praeclavella gracilis* (Hansen, 1923) n. comb.; *Praeclavella hardingi* (Nunes-Ruivo, 1964) n. comb.; *Praeclavella irina* (Wilson, 1915) n. comb.; *Praeclavella levis* (Wilson, 1915) n. comb.; *Praeclavella longicauda* (Ho, 1993) n. comb.; *Praeclavella ovata* (Yamaguti, 1939) n. comb.; *Praeclavella parva* (Wilson, 1915) n. comb.; *Praeclavella porogadi* (Nunes-Ruivo, 1964) n. comb.; *Praeclavella scombrois* (Yamaguti, 1939) n. comb.; *Praeclavella singularis* (Castro-Romero et Gonzalez, 2009) n. comb.; *Praeclavella stichaei* (Krøyer, 1863) n. comb.; *Praeclavella tumida* (Wilson, 1915) n. comb.

The new species can be easily distinguished from *P. caudata* n. comb. by the absence of caudal rami, in addition to other differences related to the antenna armature, the shape and size of the swelling at the base of the cephalothorax, the size of the maxilla, and the shape of the bulla.

Praeclavella nasalis n. gen., n. sp. can be separated from the other species without genital process by a large oval swelling at the base of the cephalothorax and a smaller one situated posteriorly, the reduced maxilla with a wider apical end, the subcircular bulla, the antennule segmentation and armature and, finally, by the antenna armature (Table SIII).

Maxiclavella simplex n. gen. et comb. male versus Praeclavella nasalis n. gen. et sp. male

The males of these species are compared because the females were found parasitizing the same host species and the male of *Maxiclavella* n. gen. is herein described for the first time. The male of *M. simplex* n. gen. n comb. has a pyriform body and is smaller than the male of *P. nasalis* n. gen. n. sp., which is more rounded. The maxilla and maxillipeds are located ventrally in

M. simplex n. gen. n comb. but anteriorly in *P. nasalis* n. gen. n. sp. The male of *Maxiclavella* n. gen. lacks caudal rami, while they are present in *P. nasalis* n. gen. n. sp. The antennule is shorter than the endopod of the antenna in *Maxiclavella* n. gen., but their length is almost the same in *Praeclavella* n. gen. The antennule armature consists of four elements in *Maxiclavella* n. gen., but five in *P. nasalis* n. gen. n. sp. In *Maxiclavella* n. gen. the maxillule is only composed of an inner lobe, and the exopod of the antenna is cylindrical, which reaches the base of the last segment of the endopod and has a spine and a distal tubercle. In *P. nasalis* n. gen. n. sp., the maxillule is biramous and the exopod of the antenna is globular, which reaches the middle of the distal segment of the endopod and bears no armature. Moreover, in both taxa the endopod of the antenna bears 4 elements, but two of them are well-developed in *Maxiclavella* n. gen., while they are equally long in *P. nasalis* n. gen., n. sp. In brief, the male types (i.e., general body shape) of these two new genera are very different from each other and from other presently known males of the *Clavella*-clade genera.

Clavella Oken, 1816

Diagnosis: Adopted from Kabata (1979) and Wilson (1915). Cylindrical cephalothorax, variable in length, with a distinguishable dorsal shield. With or without genital process, caudal rami present only in *Clavella deminuta* Kabata, 1992. Antennule obscurely segmented or unsegmented, well-developed apical armature. Uniramous antenna, with long sympod–endopod axis. Mandible with variable dental formula, and sometimes primary and secondary teeth are not clearly distinguishable; diastema present or absent. Maxillule with ventral exopod. Short, small maxilla, fused or clearly separated. Subchelate maxilliped. Male usually suboval, small, no division between cephalothorax and trunk, unsegmented body.

The present revision confirms the validity of the following currently recognized species: *C. adunca*; *Clavella diversia* Ho, 1993; *Clavella gadomi* Ho, 1993; *Clavella insolita* Wilson, 1915; *Clavella okamurai* Ho, 1993; *Clavella pinguis* Wilson, 1915; *Clavella sokodara* Ho, 1993; *Clavella squamigera* Wilson, 1915; *Clavella tumidula* Kabata, 1992; and *Clavella zini* Kabata, 1979. The description of the remaining species within *Clavella* is incomplete, at least regarding the condition of the antenna, and further revision is required to allow their proper classification into genera.

Molecular phylogenetic analysis

A total of 22 COI and 7 28S rDNA barcoding section sequences were obtained from individuals assigned to *C. dilatata,* (n = 4 and n = 1, respectively), *M. simplex* n. gen. n. comb. (n = 3 and n = 1, respectively), *Parabrachiella* (*P. auriculata* n = 3 and n = 1, *P. anisotremi* n = 4 and n = 1, respectively), and *Praeclavella* n. gen. (*P. applicata* n. comb. n = 3 and n = 1, *P. caudata* n. comb. n = 3 and n = 1, *P. nasalis* n. sp. n = 2 and n = 1, respectively). The number of sequences of COI and 28S rDNA markers retrieved from GenBank were as follows: *C. adunca* n = 7 and 1, respectively; *Clavella perfida* n = 0 and n = 7, respectively; *Clavella stellata* (Krøyer, 1863) n = 1 and n = 0, respectively; and *Caligus cheilodactyli* n = 1 and n = 1, respectively (Table SI).

The COI genetic distance (Table I) between *M. simplex* n. gen. n. comb. and *Clavella adunca* was 21%, and between *P. nasalis* n. gen. n. sp. and *P. applicata* n. comb. was 7%. The genetic distance between species of *Praeclavella* n. gen. and *C. adunca* was 22%, and that between *C. adunca* and *C. perfida* was 16%. The intragroup

Table I. Genetic distances matrix (uncorrected p-distance) for copepods used in this manuscript. The percentage values are derived from the COI mt-DNA.

	OG	1	2	3	4	5	6	7	8	9	10	11	12	13	Intra Group Distance
outgroup															
1.- *Parabrachiella anisotremi*	20														0,76
2.- *Parabrachiella auriculata*	20	14													0,14
3.- *Parabrachiella exilis*	19	14	17												0,87
4.- *Parabrachiella platensis*	16	12	16	9											0,35
5.- *Parabrachiella kabatai*	18	16	17	12	12										0,72
6.- *Parabrachiella merlucci*	21	15	16	15	14	16									n/c
7.- *Parabrachiella hugu*	19	15	18	16	14	15	18								n/c
8.- *Clavellotis dilatata*	22	18	19	17	16	16	18	20							2,06
9.- *Praeclavella applicata*	22	16	19	16	15	16	18	17	16						0,14
10.- *Praeclavella nasalis*	22	16	19	16	16	16	18	17	17	7					0,43
11.- *Praeclavella caudata*	23	16	21	17	16	16	18	19	17	10	9				0,00
12.- *Clavella adunca*	27	23	25	23	23	23	24	25	20	22	22	22			0,87
13.- *Clavella perfida*	22	18	21	19	18	19	21	22	20	19	20	18	16		1,55
14.- *Maxiclavella simplex*	25	25	26	25	24	25	26	24	22	22	22	22	21	22	0,29

genetic distances were very low, the highest being 2.06% for *C. dilatata*, followed by 1.55% for *C. perfida*, and below 1% for the remaining species.

The 28S rDNA genetic distances are provided in Table II. The genetic distance between *P. nasalis* n. gen., n. sp. and *P. caudata* n. comb. was 4%, and between *P. nasalis* n. gen. n. sp. and *P. applicata* n. comb. was 8%.

Bayesian phylogenetic relationships inferred from the COI and 28S rDNA sequences are shown in Figures 4 and 5, respectively.

The phylograms are constructed with the little number of sequences reported for the Lernaeopodidae family, but despite this, and based on the two analyzed genes, we showed that *Maxiclavella* n. gen. is the sister group of *Clavella* spp., and *Praeclavella* n. gen. is the sister group of both genera. In addition, *Praeclavella nasalis* n. gen. n. sp. is placed within *Praeclavella* n. gen. (with biramous antenna).

The *Parabrachiella* seems to be a natural group, distinct from *Clavella* spp. The former holds a plesiomorphic position (Figs. 4 and 5). In the Fig. 5, *P. hugu* is placed at the base of the phylogram and separated from *P. auriculata* and *P. anisotremi*. The analysis of the 28S

rDNA genetic distance shows a close similarity between *P. hugu* with *C. stellata* (8%) and *P. auriculata* (7%). The analysis of the mtDNA COI genetic distance shows a high percentage of similarity among *Parabrachiella* spp. The differences observed in both genetical analysis raise the questions wether *P. hugu* was correctly sequenced and deposited in the GenBank–or belongs to another genus.

DISCUSSION

In the phylogenetic tree of Lernaeopodidae constructed by Kabata (1979), the *Clavella*-clade included 11 genera, mainly characterized by the absence of posterior processes and the presence of a modified caudal rami ("uropod"): *Anaclavella, Alella, Clavella, Clavellodes, Clavellomimus, Clavellisa, Clavellistes, Clavellopsis, Euclavellisa, Advena* (*Kabatazus*), and *Proclavellodes*. Some members were subsequently added to this family: *Clavellotis* (for *Anchorella dilatata* Krøyer, 1863) *Mixtio* (for *Clavella inversa* Wilson, 1913); *Pseudomixtio* (for *Clavellopsis parasargi* Roubal, 1981) *Cryptova, Margolisius,* and *Sparidicola* (Kabata 1986, 1990, 1992, Kabata & Tareen 1987, Castro & Baeza 1984, Benz et al. 2000).

Table II. Genetic distances matrix (uncorrected p-distance) for copepods used in this manuscript. The percentage values are derived from the 28S rDNA.

	OG	1	2	3	4	5	6	7	8	9
outgroup										
1.- *Praeclavella applicata*	32									
2.- *Praeclavella nasalis*	29	8								
3.- *Praeclavella caudata*	28	9	4							
4.- *Maxiclavella simplex*	28	16	12	11						
5.- *Clavella adunca*	27	16	12	11	7					
6.- *Parabrachiella auriculata*	27	15	10	10	10	12				
7.- *Clavella stellata*	27	15	10	10	10	11	4			
8.- *Parabrachiella hugu*	27	16	13	11	12	13	7	8		
9.- *Clavellotis dilatata*	29	15	10	10	12	13	10	9	11	
10.- *Parabrachiella anisotremi*	31	21	15	16	17	19	12	13	15	17

The identification key provided in this study includes *Kabatazus*, *Cryptova*, and *Clavellopsis*, in disagreement with that of Kazačenko (2001). These genera are usually included within the *Clavella*-clade based on: (1) the presence of trunk projections in females (not considered to the posterior processes of other lernaeopodids (e.g., *Parabrachiella*); and (2) a closer resemblance with the *Clavella*-clade males compared to other lernaeopodid males.

Although Kabata (1979) positioned *Clavellistes* at the bottom of the *Clavella*-clade based on female characteristics, its elongated male lacks the typical *Clavella*-clade shape. Further studies are needed to elucidate the affiliation of this genus.

Kabata & Tareen (1987) included *Sparidicola* in the *Clavella*-clade, even though the male morphology is simpler than that of other males in this clade. Moreover, the male has short posterior processes, and the female has an oval genital process. These characteristics raise some doubts about the correct placement of *Sparidicola* in this clade.

Herein, we described the male of *Maxiclavella* n. gen, (with *Maxiclavella simplex* n. comb.) and the male of *Praeclavella nasalis* n. gen. n. sp. for the first time, both of which were isolated from the same host, *I. conceptionis* (caught off the Chilean coast) and fit well within *Clavella*-clade of Kabata (1979).

By generic definition, the genus *Clavella* comprises species with uniramous antenna (Wilson 1915, Kabata 1979). Notwithstanding this, Hansen (1923) assigned two species having biramous antenna in the old *Clavella* instead of establishing a new genus. Kabata (1963) reviewed the antennae of the species included in *Clavella* at that time, and decided not to make any taxonomic modification. Boxshall & Halsey (2004) emphasized the importance of the character state "uniramous antenna" to characterize *Clavella* in their key of Lernaeopodidae. According to Kabata (1963), the main axis of the antenna runs through the sympod and endopod in *Clavella* species that have a reduced exopod, while it runs through the sympod and exopod in *Clavella* species and other *Clavella*-branch genera showing a developed exopod. Subsequent reexamination led Kabata (1986, 1990) to propose the genera *Mixtio* and *Pseudomixtio* for *Clavella inversa* and *Clavellopsis parasargi*, respectively, which bear a biramous antenna.

Clavella stellata was found attached to the pelvic and pectoral fins of *Merluccius merluccius* (Linnaeus, 1758) from Scotland and North Ireland. In this study, it is placed at the base of the 28S rDNA phylogenetic tree, outside the *Clavella* and *Parabrachiella* branches possessing uniramous and biramous antennae, respectively. The use of other molecular markers and sequences from different specimens and more genera of this family will possibly contribute to clarify the phylogenetic position of *C. stellata* in the Lernaeopodidae tree. According to Kabata (1979), this species has an antenna with the sympod forming an angle with the endopod, and a short exopod, a maxilla type maxilla with both rami bearing a pair of small suborbicular swellings each, and a mandible with 3 secondary teeth, allowing its distinction from *Praeclavella* n. gen. and *Maxiclavella* n. gen. *Clavella stellata*, shows an intermediate feature between the uniramous and the biramous conditions by having a broad and flat anchor, but still requires a depth revision of its taxonomic status, which may result in the erection of a new genus.

The *Praeclavella* here proposed includes species (previously included in *Clavella*) bearing a biramous antenna, and the new *Praeclavella nasalis* n. gen. et sp. This study emphasizes the importance of a proper description of the antenna -in combination with other

characters- to differentiate between *Praeclavella* n. gen. and *Clavella*. However, it should be borne in mind that some structures and features are difficult to see under the light microscope (e.g., segmentation, apical armature, whip, solus and number of rami). Indeed, it is hard to detect the presence of a single spine placed dorsally on a small exopod, allowing the distinction among *Clavella* species. In this context, electron microscopy along with molecular approaches can provide more reliable results.

The molecular markers (mtDNA COI and 28S rDNA) used herein were useful to clarify the relationship among *Parabrachiella*, *Clavella*, *Clavellotis*, *Maxiclavella* n. gen., and *Praeclavella* n. gen. according to the information we have until now. We are conscious that, in comparison of the large number of genus inside the Lernaeopodidae family, this is a first approach that could lead to other copepodologist to sequence their samples and not only publish morphological papers. More sequences and a phylogenetic study is needed to assess the taxonomic status of *Clavellotis*, *Clavellopsis*, and *Clavellistes* within the *Clavella*-clade and their relationships with other clades of Lernaeopodidae, as mentioned above. Likewise, it is necessary to determine the position of *Parabrachiella* (with a large number of species) within the *Clavella*-clade, Ho et al. (2007) suggest it could belong to *Clavella* -clade due to the resemblance of the free-swimming stages, but the female and male differ morphologically from those of *Clavella*.

It is difficult to establish a cut-off value of genetic distance that can be used as an indicator for species or genus assignment because of the high genetic variability of copepods and the low information available. Edmands (2001) found a remarkable intraspecific genetic distance (up to 23%) for the entire distribution range of *Tigriopus californicus* (Baker, 1912), but

considered that it was not unexpected for this species. Our results are in agreement with those of Bucklin et al. (2003), who studied Calanidae and Clausocalanidae and reported that the COI genetic distance within species ranged between 1 and 4%. Dippenaar et al. (2010) reported that the COI divergence between two clades of *Nesippus orientalis* Heller, 1868 was about 17–18% and assumed the possible existence of cryptic species, while Rocha-Olivares et al. (2001) found higher genetic distance values (21–27%) in populations of *Cletocamptus deitersi* (Richard, 1897). Bucklin et al. (2003) reported an interspecific COI variation of 9–25% for Calanidae and Clausocalanidae, while we obtained lower COI genetic distances among species of *Praeclavella* n. gen. and *Parabrachiella* (7–10% and 9-18%, respectively). The analysis of a large number of *Parabrachiella* species (with particular focus on *P. hugu*) using morphological and molecular markers will help to resolve interspecific relationships. The genetic distance found between the harpacticoid copepod *Macrosetella gracilis* (Dana, 1847) and *Miracia efferata* Dana, 1849 was 23–25% (Eberl et al. 2007), while that between the genera *Peniculus* von Nordmann, 1832, *Metapeniculus* Castro Romero and Baeza Kuroki, 1985 and *Trifur* Wilson, 1917 (morphologically well differentiated) was 18–24% (Castro-Romero et al. 2016). In the present work, the distances among the genera (about 17–25%) fell within the range reported by Eberl et al. (2007). Larsen et al. (2014) noted that small genetic (COI) divergence was independent of the occurrence of morphological differences among tanaidacean species, and this might also apply to some copepod taxa.

The genetic analysis using COI mtDNA and 28S rDNA supports the morphological information provided here. Undoubtedly, all the species presently placed in *Clavella* need to be revised combining morphological analysis

by SEM with molecular approaches. Future phylogenetic studies of the Lernaeopododidae based on novel techniques and descriptions that are more complete will reveal new species or genus and could validate the relationships among genera initially presented here.

Clavella-branch (Clavellinae Wilson) genera key, based on female specimens

1.- Reduced maxilla ....Group I
2. Maxilla of variable size, shorter than cephalothorax..Group II
3. Maxilla at least as long as the cephalothorax, broad; developed genital process; antennule with only 2 elements........*Clavellistes* Shiino, 1963.

Group I.
1.- Subrectangular trunk; no genital process; elongated, narrow bulla................ *Proclavellodes* Kabata, 1967.
- Trunk of different shape..2
2. Subquadrangular trunk 5
- Suboval trunk...3
3.- Maxilla base with a large collar-like projection; elongated bulla; antennal exopod as long as endopod, mandible with 3 secondary teth.............
....*Alella* Leigh-Sharpe, 1925.
- Short maxilla, without collar; antennule with a long seta; mandible without secondary teeth; exopod wider and shorter than endopod; bulla short and funnel-shaped.......................................
Anaclavella Heegaard, 1940.
4. Trunk with (5) posterior processes 6
- Trunk with genital processes only 5
5. Trunk with long, narrow projections with genital process; mandible without secondary dentition........................*Kabatazus* (Kabata, 1979) Özdikmen, 2008.
- Trunk without posterior projections, cephalothorax longer than trunk, with re-entrant margins; tiny genital process; antennal exopod

longer than endopod, mandible with secondary dentition............................*Clavellodes* Wilson, 1915.
6. Maxilla base with swelling; not connected posterior processes; mandible with 3 secondary teeth....................................*Clavellopsis* Wilson, 1915.
- Maxilla base without swelling; posterior processes connected by skirt-like cuticle; mandible with 3 secondary teeth............................
Cryptova Kabata, 1992.

Group II.
1.- Suborbicular trunk...2
- Pyriform trunk ....*Nudiclavella* Ho, 1975.
- Subrectangular trunk 5
2.- Cephalothorax, in normal position -anterior part of trunk -...3
- Cephalothorax on mid-surface of trunk, dorsally 4
3.- With aliform process; well-developed antennal exopod and endopod..8
- Without aliform process...9
4.- Antennule with four segments; not articulated antennal exopod; not segmented male abdomen ....*Clavellisa* Wilson, 1915.
-Antennule with two segments; antennae with two-segmented exopod; segmented male abdomen........................ *Euclavellisa* Heegard, 1940.
5.- Trunk of uniform width along entire length 6
- Trunk wider at posterior end; well-developed cephalothorax, narrower and longer than trunk; trunk with anal tubercle; medium-sized maxilla; antenna with subequal rami,. small suborbicular swelling at the base of the cephalothorax.............................*Maxiclavella* n. gen.
6.- Two pairs of lateral papillae on the anterior part of the trunk.....*Sparidicola* Kabata and Tareen, 1987.
- Without lateral papillae on the anterior part of the trunk 7
7.- Maxilla about 1/2 length of the cephalothorax; unsegmented antennules, with 3 elements,

one/1 bifid; antennal exopod shorter than endopod;, endopod with bilobed apex ………………… …………………………………………………… *Margolisius* Benz, Kabata et Bullard, 2000.

- Maxilla about 1/3 length of the cephalothorax; bulla elongated and distally rounded; tiny genital process; antennules with 3 indistinct segments; antenna with well-separated rami; exopod globose and longer than endopod………………………………………*Clavellomimus* Kabata, 1969.

8.- Suborbicular trunk with genital process of variable size; the base of the cephalothorax has aliform processes and other short processes; maxillule inner lobe with 2 long setae, mandible with 3 secondary teeth ……………………………………… …………………………*Clavellotis* Castro and Baeza, 1984.

- Suborbicular trunk, posterior margin protruding; base of the cephalothorax with aliform processes; maxillule inner lobe with 2 setae and 1 short dorsal seta at base; mandible with 2 secondary teeth…………*Mixtio* Kabata, 1986.

9.- Suboval trunk; medium-sized maxilla; present genital process; well-developed antennal exopod and endopod; mandible with 2 secondary teeth………*Pseudomixtio* Kabata, 1990.

- Suborbicular or variable trunk; genital process present or absent………………………………………10.

10.- Uniramous antenna, not developed exopod…………………………………*Clavella* Oken, 1815.

- Biramous antenna, short, lobular (unisegmented) exopod or reduced to a short spine……………………………………*Preclavella* n. gen.

Acknowledgments

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SUPPLEMENTARY MATERIAL

Figures S1, S2, S3, S4, S5
Tables SI, SII, SIII

RAUL CASTRO-ROMERO[1]
https://orcid.org/0000-0001-5358-4554

MARTIN MIGUEL MONTES[2]
https://orcid.org/0000-0002-7177-333X

SERGIO MARTORELLI[2]
https://orcid.org/0000-0002-5375-9155

[1]Universidad de Antofagasta, Facultad de Ciencias del Mar, Depto. de Ciencias Acuáticas y Ambientales, Casilla 170, 1240000 Antofagasta, Chile

[2]Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata (CCT, CONICET-UNLP), Centro de Estudios Parasitológicos y Vectores (CEPAVE), Boulevard 120 s/n e/60 y 64, 1900 Buenos Aires, Argentina

Correspondence to: **Martin Miguel Montes**
E-mail: martinmiguelmontes@gmail.com

Author contributions

Raul Castro Romero: Fish search, parasite morphology study, principal writer of the manuscript. *Martin Montes*: Material and Methods and Result section of the molecular analysis, phyllogenetical studies and help with the photos and figures edition. *Sergio Martorelli*: analysis of the bibliography, editing and revision, source founding and manuscript supervision.