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## A new family of poecilostomatoid copepods (Strepidae fam. nov.) associated with the sun coral, *Tubastraea coccinea* Lesson, 1829 in Taiwan

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### Abstract

Both sexes of *Strepus elongatus* **gen. et sp. nov.** are described based on specimens obtained from the coral host, *Tubastraea coccinea* Lesson, 1829, collected from shallow water reefs in Taiwan. The new species belongs to a new family of the copepod order Poecilostomatoida and displays the following morphological characteristics: (1) poecilostome type of well developed piercing mandibles, (2) absence of an oral cone, (3) highly transformed body, (4) 4-segmented antennules, (5) 2-segmented maxilliped, (6) caudal rami with only two setae, and (7) complete loss of legs 1–5 in the female, but legs 1–2 being represented by a free segment bearing two terminal setae in the male. In addition, the phylogenetic analysis based on 18S ribosomal RNA sequence data showed that *S. elongatus* clustered with a *Sabelliphilus*–*Anchimolgus* clade (*Sabelliphilidae* + *Xarifiidae* + *Rhynchomolgidae* + *Anchimolgidae*), and that the average genetic distance between *S. elongatus* **gen. et sp. nov.** and representatives of the six most closely related families (0.064) was higher than the average distances among those families (0.028). Therefore, based on both morphological and molecular data, a new family Strepidae **fam. nov.** is proposed to accommodate its only known species, *S. elongatus* **gen. et sp. nov.**

**Key words:** Copepoda, scleractinian corals, *Tubastraea coccinea*, phylogeny, Strepidae

### Introduction

Copepods are the most common and widespread crustaceans in marine ecosystems and live with other organisms in an array of symbiotic states as mutualists and parasitic forms (Humes 1985a; 1985b; 1994; Ho 2001). Based on morphological characters, the group currently comprises ten orders accommodating a total of approximately 14,712 valid species. The order Poecilostomatoida accommodates 60 valid families and contains by far the greatest number of symbiotic species on marine invertebrates (Ho 2001; Huys & Boxshall 1991; Walter & Boxshall 2014). Humes (1985a) claimed that approximately 227 and 161 copepod species were associated with hexacorals and octacorals, respectively. He also suggested that scleractinian corals provide not only food resources but also shelters, thus allowing a greater diversity of symbiotic/parasitic copepods than other invertebrate hosts. Kim's (2003, 2005, 2006, 2007, 2009, 2010) subsequent work on symbiotic/parasitic copepods resulted in the discovery of many species new to science from Madagascar, Moluccas, Eniwetok Atoll and New Caledonia. Although new copepod taxa continue to be discovered in shallow water corals, the lack of comprehensive studies (Humes 1994; Kim 2003) suggests that the number of currently known species represents only a small fraction of the species actually living with scleractinian corals and that their diversity is grossly underestimated.

Symbiotic/parasitic copepods are frequently found in association with dendrophylliid corals which are prominent components of shallow reefs in the tropical Indo-Pacific (Humes 1985a; Cheng *et al.* 2007, 2011). So far, at least nine species of copepods have been reported from corals belonging to the family Dendrophylliidae (including the genera *Turbinaria* Oken, 1815 and *Tubastraea* Lesson, 1829), *i.e.* *Cholomyzon palpiferum* Stock & Humes, 1969 and *C. brevisetigerum* Humes, 1997 from *Tubastraea micranthus* (Ehrenberg, 1834) (note that Stock & Humes (1969) reported the host under its junior synonym, *Dendrophyllia nigrescens* Dana, 1846), *C.*

*tubastraeae* Cheng, Dai & Chang, 2011 from *Tubastraea coccinea* Lesson, 1829, *Coralliomyzon tenens* Humes & Stock, 1991 and *C. latitergum* Humes, 1997 from *Turbinaria peltata* (Esper, 1794), *Xarifia hamata* Humes & Ho, 1968 from *Turbinaria* sp., *X. insolita* Cheng, Ho & Dai, 1997 from *T. coccinea*, and *X. lacerans* Humes, 1985b and *X. uncinata* Humes, 1985b from *Turbinaria frondens* (Dana, 1846).

In the present study, we present a full description of an undescribed species of poecilostomatoid copepod obtained from a dendrophylliid coral, *T. coccinea*, collected from the coastal area of Taiwan. Both morphological and molecular approaches were used to determine the taxonomic identity and phylogenetic position of this copepod.

## Material and methods

**Specimen collection and morphological studies.** A total of 160 fragments of *Tubastraea coccinea* (about 3–5 cm in length and 3–5 cm in width) were collected by SCUBA diving at 1–5 m depth in four locations of coastal Taiwan, *i.e.* Yaliu (25°12.307'N, 121°41.380'E), Doufu Cape (24°58.384'N, 121°87.150'E), Wanlitong (21°59.770'N, 120°42.290'E), and Tiaoshi (21°57.555'N, 120°45.989'E), between November 2010 and August 2011. The coral fragments were placed in a zip bag during the collecting process, and transported to the laboratory for further examination. Coral fragments were placed in small (150–200 ml) containers of seawater in which 1 ml of 70% ethanol was added every 10–20 min to gradually increase the concentration to 5% ethanol. The fragments remained immersed in this solution for 6–8 hours at room temperature allowing sufficient time for the copepods to be expelled from the corals. The water was subsequently filtered through a fine net (mesh size approximately 100 µm). The copepods were picked from the sediment retained in the filtrate using a dissecting microscope and either preserved in 70% ethanol for morphological examination or in 95% ethanol for DNA extraction.

Morphological studies followed Humes & Gooding (1964) for the standard procedures of examining symbiotic copepods. Length and width of the copepods were measured from five individuals under a dissecting microscope. Prior to dissection, the copepods were cleared in 85% lactic acid for at least 1–2 h, and then dissected on a wooden slide under a dissecting microscope. The dissected body parts and appendages were examined under a compound microscope. All drawings were made under a compound microscope with the aid of a drawing tube.

**Molecular phylogenetic analysis.** Genomic DNA was extracted from a whole adult female using commercial DNA extraction kits (Genomics BioSci & Tech, Taiwan). DNA extracts were diluted 10X in TE buffer and stored at -20°C until amplification by polymerase chain reaction (PCR). The PCR conditions and thermal cycling profile for 18S ribosomal DNA amplification were based on the protocol published by Huys *et al.* (2012). Purification of the PCR products and sequencing reactions were done by Genomics BioSci & Tech (Taiwan). Nucleotide sequences of both forward and reverse strands were determined using an ABI 3730XL automated sequencer (Applied Biosystems, Carlsbad CA, U.S.A.). Sequences were assembled and edited using the SEQUENCHER version 4.2 software (Gene Code, Ann Arbor MI, U.S.A.). The sequence obtained is deposited in GenBank under accession no. KP733689. Seventy-four 18S ribosomal DNA sequences were downloaded from Genbank representing six orders, including Calanoida (two species), Poecilostomatoida (31 species), Siphonostomatoida (33 species), Misophrioida (one species), Harpacticoida (two species), Cyclopoida (two species) and three outgroups. Sequences were aligned using MEGA 5 (Tamura *et al.* 2011). Maximum likelihood tree searching was conducted in RAXML-HPC BlackBox under the CIPRES Science Portal v3.1 (Miller *et al.* 2010). We also calculated the inter-family K2P genetic distance among species closely related to *S. elongatus* to determine the genetic threshold between families based on ssrDNA sequences by using MEGA 5 (Tamura *et al.* 2011).

## Taxonomy

### Order Poecilostomatoida Thorell, 1859

#### Strepidae fam. nov.

**Diagnosis.** The diagnosis of the family coincides with that of its type and only genus *Strepus*, given below.

## *Strepus* gen. nov.

**Diagnosis.** Body elongate and cylindrical, external segmentation weakly defined. Urosome 3-segmented. Paired genital apertures located dorso-laterally. Caudal ramus with two terminal setae. Antennule 4-segmented, with armature formula: 0, 3, 2, and 5 setae in female; 0, 3+1 aesthetasc, 2, and 5 setae in male. Antenna uniramous, 3-segmented, with three small denticles on distal segment. Mandible a small blade with teeth on distal side. Maxillule represented by simple lobe tipped with two setae. Maxilla 2-segmented; syncoxa unarmed; basis with a distal lash. Maxilliped 2-segmented; first segment with two setae; second segment with two long setae and one denticle. Legs 1–5 absent in female; legs 1–2 with free segment bearing two terminal setae in male.

**Type species.** *Strepus elongatus* sp. nov.

**Etymology.** The generic name “*Strepus*” is derived from streptococcus and alludes to the body shape of the male. Gender masculine.

## *Strepus elongatus* gen. et sp. nov.

(Figs. 1–2)

**Type locality.** Taiwan, north coast; off Yaliu (25°12.307'N, 121°41.380'E), northwest of Keelung; washings of *Tubastraea coccinea* Lesson, 1829.

**Type material.** A total of 31 copepods (9 ♀♀, 24 ♂♂) were obtained from four localities in Taiwanese waters. Holotype ♀ (from type locality; NTUIO-CONF 0001), allotype ♂ (from type locality; NTUIO-CONF 0002) and remaining paratypes (NTUIO-CONF 0003) were deposited in the Institute of Oceanography, National Taiwan University, Taipei, Taiwan.

**Prevalence.** 15% at Yaliu; 17.5% at Doufu–Cape; 5% at Tiaoshi; 0% at Wanlitong.

**Mean intensity.** 1.5 at Yaliu; 3.1 at Doufu–Cape; 1.0 at Tiaoshi; 0 at Wanlitong.

**Mean abundance.** 0.23 at Yaliu; 0.55 at Doufu–Cape; 0.05 at Tiaoshi; 0 at Wanlitong.

**Description of female.** Body (Fig. 1A–B) elongate and cylindrical, measuring 2.74 (2.66–2.90) mm in total length and 0.80 (0.77–0.82) mm in greatest width, based on five specimens. Ratio of body length to greatest width 3.43:1. Segmentation of body distinct, but incomplete. Head (Fig. 1A–B) very small, distinctly set off from trunk. Urosome (Fig. 1C) 3-segmented, much shorter than prosome; genital and postgenital segments recurved upward in some specimens. Areas of attachment of egg sacs located dorso-laterally. Egg sac not observed. Caudal ramus (Fig. 1D) elongate, covered with denticles; bearing two terminal setae. Surface of body unornamented (Fig. 1A–B).

Rostral area (Fig. 1E, G) distinctly protruded and covered with denticles. Antennule (Fig. 1E, G) 4-segmented; armature: 0, 3, 2, and 5 setae; all setae naked. Antenna (Fig. 1H) stronger and roughly, 3-segmented; basal and second segment unarmed and broad; distal segment with three small denticles.

Labrum (Fig. 1E) elongate and swollen. Mandible (Fig. 1E–F, I) slender, with pointed blade fringed with teeth on distal side. Maxillule (Fig. 1E–F, J) a small lobe tipped with two long setae. Maxilla (Figs. 1E–F, 2A) 2-segmented; proximal segment large and unarmed; distal segment small, with a distal process fringed with spinules on outer side. Maxilliped (Fig. 2B) 2-segmented; first segment largest, with two spiniform inner setae; second segment tipped with two long setae and one denticle. Legs 1–5 absent.

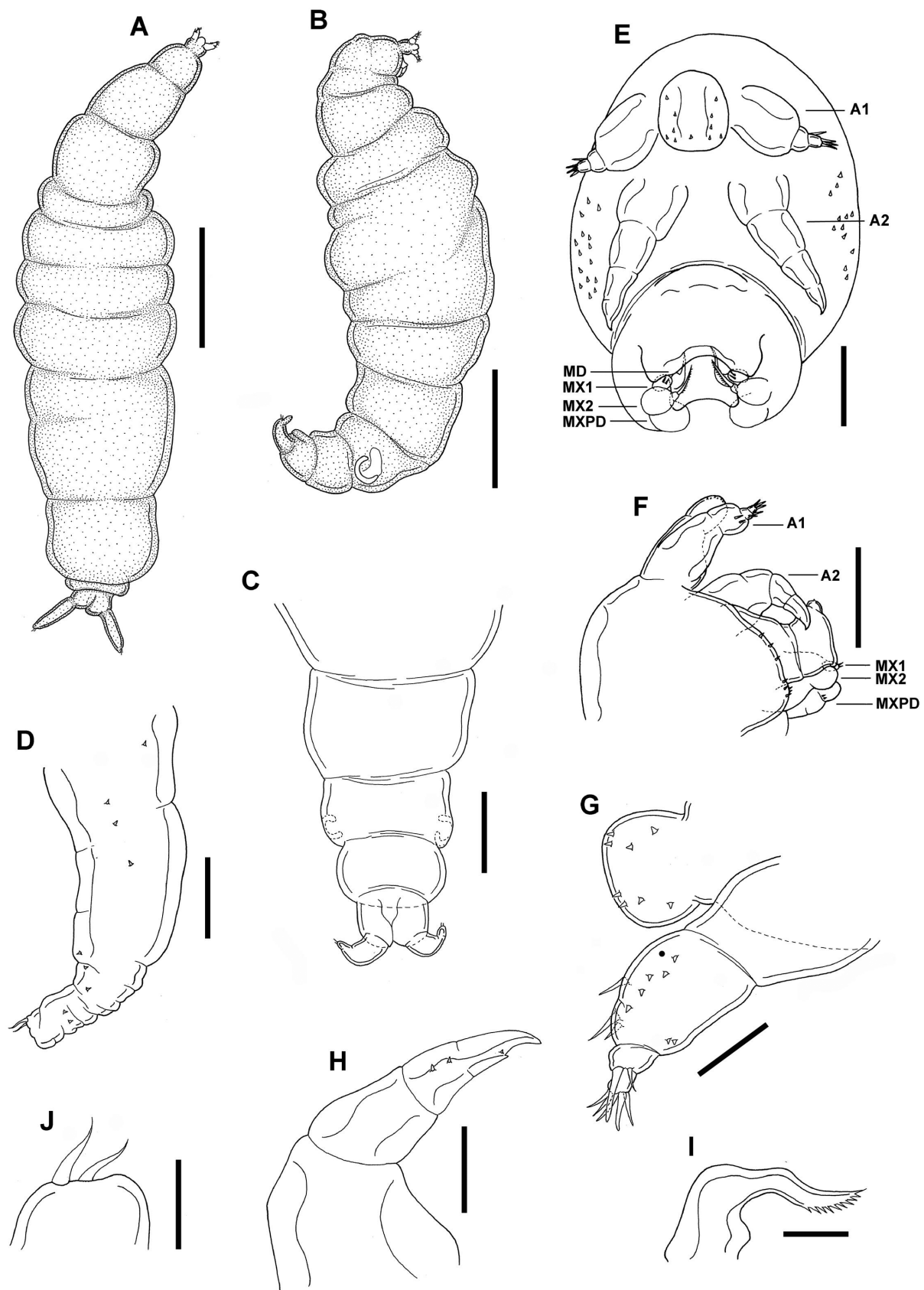
**Description of the male.** Body (Fig. 2C) generally as in female, but much more slender, with abdominal region arched upward. Length 2.20 (2.10–2.48) mm and greatest width 0.39 (0.37–0.41) mm, based on five specimens. Ratio of body length to greatest width 5.64:1. Caudal ramus elongate, shaped and armed as in female.

Antennule, antenna, mandible, maxillule, maxilla, and maxilliped like those of female, but one additional aesthetasc present on second segment of antennule (at point indicated by a dot in Fig. 1G).

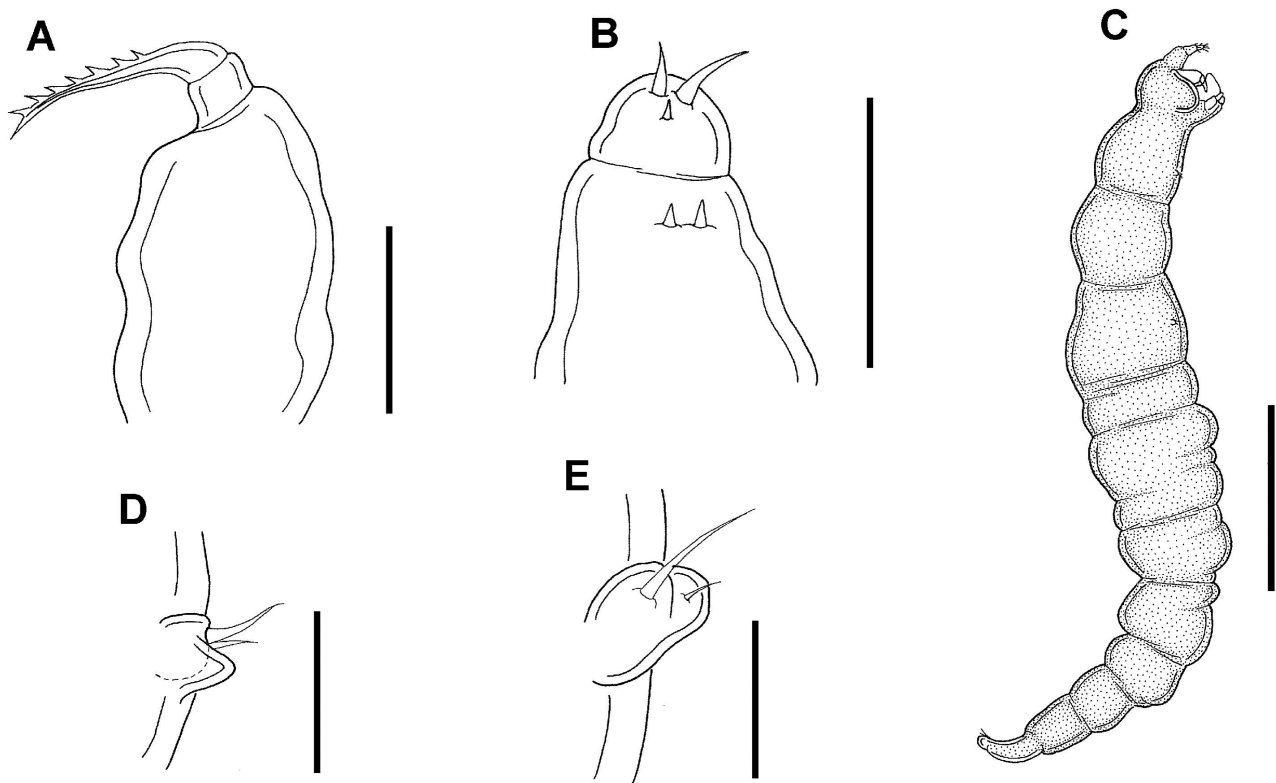
Legs 1–2 (Fig. 2D–E) a small unornamented free segment carrying two terminal setae. Legs 3–5 absent.

**Etymology.** The species name is derived from the Latin *elongatus*, meaning “elongate”, and alludes to the slender body of the new species.

**Molecular analysis.** A phylogenetic analysis using 18S rDNA sequence data was undertaken to determine the phylogenetic position of *S. elongatus* within the Copepoda, and to elucidate its phylogenetic relationships with the other families in the Poecilostomatoida (Fig. 3). After trimming several indels, the final alignment set with total length of 1,662 bp was used to generate a Maximum Likelihood tree. *Strepus elongatus* gen. et sp. nov. was



**FIGURE 1.** *Strepus elongatus* gen. et sp. nov. (female). A, habitus, dorsal; B, habitus, lateral; C, urosome; D, caudal ramus; E, cephalosome, ventral; F, cephalosome, lateral; G, rostrum and antennule (dot indicating position of aesthetasc in male); H, antenna; I, mandible; J, maxillule. A1 = antennule, A2 = antenna, MD = mandible, MX1 = maxillule, MX2 = maxilla, MXPD = maxilliped. Scale bars: A–B = 0.5 mm; C = 0.2 mm; D–E = 0.04 mm; F = 0.1 mm; G–J = 0.02 mm.

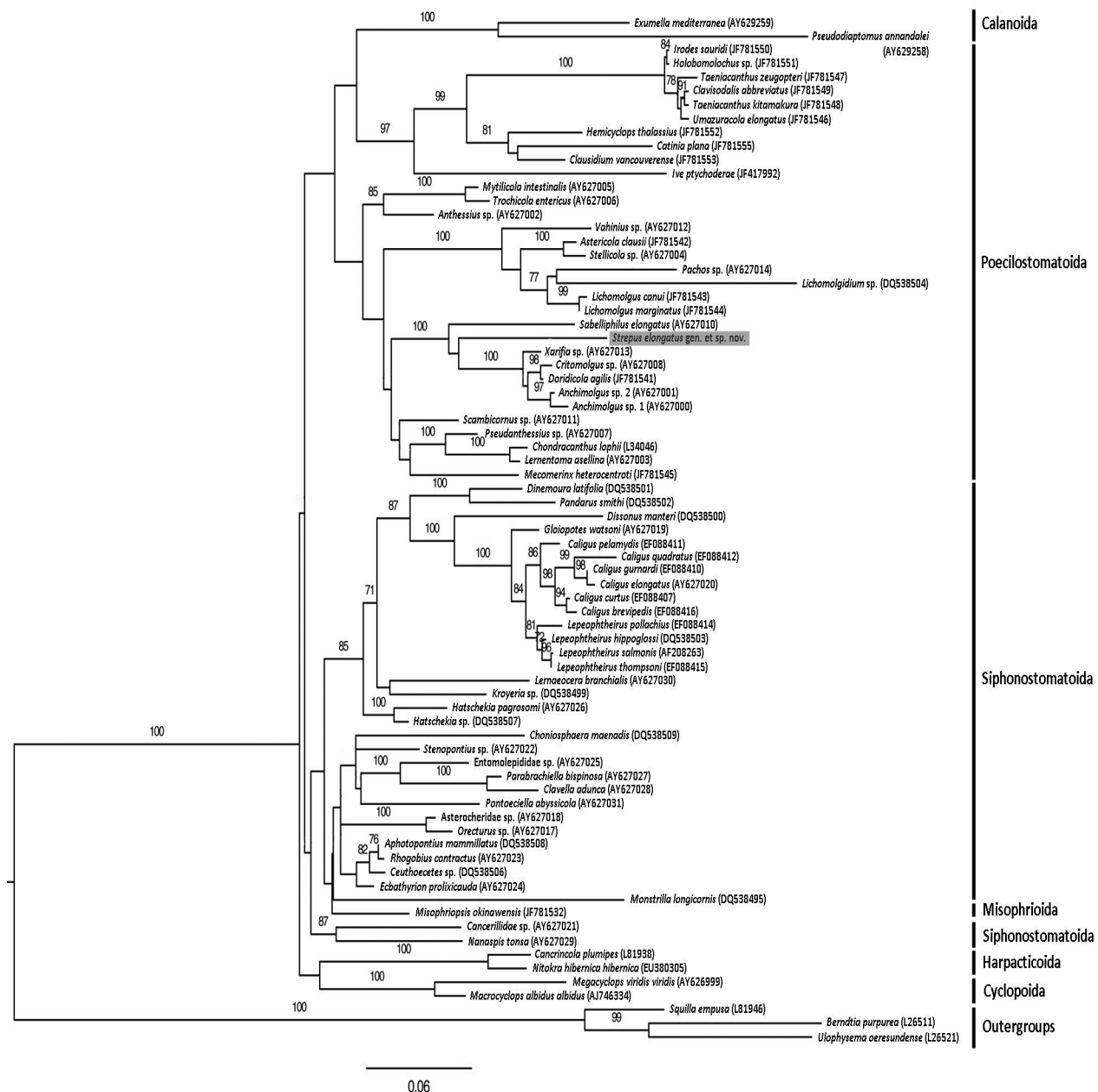


**FIGURE 2.** *Strepus elongatus* **gen. et sp. nov.** (female, A–B; male, C–E). A, maxilla; B, maxilliped; C, habitus, lateral; D, leg 1; E, leg 2. Scale bars: A–B, D–E = 0.02 mm; C = 0.5 mm.

identified as a member of the Poecilostomatoida and sister of a clade including *Xarifia* sp. (Xarifiidae), *Critomolgus* sp. and *Doridicola agilis* (Rhynchomolgidae), *Anchimolgus* sp. 1 and *Anchimolgus* sp. 2 (Anchimolgidae) in the ML tree with strong bootstrap support (100). The average distance between these species, which represent five families, is 0.028, while the distance between *S. elongatus* and those species is 0.064 (Table 1).

**TABLE 1.** K2P genetic distance between *Strepus elongatus* **gen. et sp. nov.** and *Sabelliphilus elongatus*, *Xarifia* sp., *Critomolgus* sp., *Doridicola agilis*, *Anchimolgus* sp. 1 and *Anchimolgus* sp. 2 based on a 1,662 bp fragment of the 18S rRNA gene.

	<i>Anchimolgus</i> sp. 1	<i>Anchimolgus</i> sp. 2	<i>Strepus</i> <i>elongatus</i> <b>gen. et sp. nov.</b>	<i>Critomolgus</i> sp.	<i>Doridicola</i> <i>agilis</i>	<i>Sabelliphilus</i> <i>elongatus</i>	<i>Xarifia</i> sp.
<i>Anchimolgus</i> sp. 1							
<i>Anchimolgus</i> sp. 2	0.007						
<i>Strepus elongatus</i> <b>gen. et sp. nov.</b>	0.062	0.063					
<i>Critomolgus</i> sp.	0.019	0.014	0.062				
<i>Doridicola agilis</i>	0.017	0.012	0.060	0.004			
<i>Sabelliphilus elongatus</i>	0.060	0.057	0.073	0.058	0.056		
<i>Xarifia</i> sp.	0.014	0.014	0.061	0.014	0.011	0.059	



**FIGURE 3.** Maximum likelihood tree of 75 species of Copepoda based on 18S rDNA sequences. Numbers below nodes represent support based on 1,000 bootstraps; only boot strap values higher than 70 are indicated on the nodes.

## Discussion

The structure of the mouthparts identifies the new species, *Strepus elongatus*, as a member of the order Poecilostomatoida. In particular, the well developed poecilostome type of piercing mandibles and the absence of an oral cone are the most significant features in support for this ordinal assignment. Some other unusual characteristics of this new species are: (1) highly transformed body (Fig. 1A–B), (2) 4-segmented antennule (Fig. 1G), (3) 2-segmented maxilliped (Fig. 2B), (4) caudal ramus with two setae (Fig. 1D); and (5) reduced legs 1–5 in the female (Fig. 1A–B). As far as we are aware, 60 valid families of copepods are currently known in the order Poecilostomatoida (Walter & Boxshall 2014), but none of them displays more than three of the aforementioned distinctive features of *Strepus*. However, Huys *et al.* (2006) suggested that some highly modified copepods could be placed as advanced members in a more inclusive clade. In the present study, the molecular evidence indicated a

close phylogenetic relationship with a clade consisting of the families Xarifiidae, Rhynchomolgidae and Anchimolgidae which is supported by high bootstrap values in the ML tree. Also, the pairwise K2P genetic distances between the most closely related families revealed the genetic uniqueness of *S. elongatus*. In addition, the morphological evidence, such as the 2-segmented maxilliped and the reduced legs 1–5 in the female, is relatively unique. Therefore, we suggest *S. elongatus* should be placed in a new family of Poecilostomatoida, Strepidae **fam. nov.** instead of being considered a highly advanced member of the Xarifiidae.

The morphology of the Strepidae is somewhat similar to that of the Mesoglicolidae. The Mesoglicolidae contains a single species, *Mesoglicola delagei* Quidor, 1906, found in the mesoglea of the corallimorpharian anthozoan, *Corynactis viridis* Allman, 1846. Conradi & López-González (1996) redescribed and revealed the ornamentation of the cephalic appendages and genital apertures of *M. delagei* with the aid of scanning electron microscopy. Their results indicated that the mandible is absent in *M. delagei* obtained from *C. viridis* in the infralittoral zone of the Strait of Gibraltar. Thus, the Strepidae, possessing typical poecilostome type of mandibles can be readily distinguished from the Mesoglicolidae.

Boxshall & Halsey (2004) recognized a group of four families of poecilostomatoids (Antheacheridae, Corallovexiidae, Lamippidae, and Mesoglicolidae), all of which utilize cnidarians as hosts. The group is characterized by (1) the transformation of the trunk, (2) the fusion of somites or loss of articulations between somites of the trunk, (3) a well developed labrum, and (4) the reduction or loss of mandibles and maxillules. Huys *et al.* (2006) employed molecular data to examine the phylogenetic relationships of copepods and identified a scleractinian-associated clade encompassing the families Anchimolgidae, Xarifiidae and Rhynchomolgidae (*AXR*). They also suggested that the common ancestor of the *AXR* clade had already established a symbiotic relationship with scleractinians. According to the aforementioned distinctive features, molecular data, and host preference, we suggested that the Strepidae should also be considered a member of this scleractinian-associated lineage (Huys *et al.* 2006). Further molecular studies including wider sampling of taxa belonging to the Antheacheridae, Corallovexiidae, Lamippidae, and Mesoglicolidae are necessary to understand the evolutionary relationships between these cnidarian-associated copepods. In addition, it is also interesting to note that most copepods of the Strepidae–Anchimolgidae clade (Strepidae + Xarifiidae + Rhynchomolgidae + Anchimolgidae) are exclusively associated with cnidarian hosts and being a sister group to the Sabelliphilidae (polychaete associates). Previous studies have demonstrated that many copepods can be obtained from corals that have associated polychaetes (Sebastian & Pillai 1974; Cheng *et al.* 2014). Whether or not some lineages of cnidarian-associated copepods originated from polychaete-associated ancestors requires further testing.

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