Phylogeny of two new pheronematid sponges from the Caroline Seamount and South China Sea

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Abstract

Two new species of genus *Pheronemoides* are described in this study. The Specimens were collected from the South China sea and the Caroline seamount in the northwestern Pacific Ocean. *Pheronemoides crustiformis* sp. nov. differs from its congers in exhibiting large microamphidiscs, whip-like and slightly bent microuncinates and small spiny microdiactins. *Pheronemoides curvipentactin* sp. nov. possesses special pentactine atrialia with round terminal or tapering terminal curved pinular rays and crooked pentactins, making it easily distinguishable from its congers. Partial sequences of the 28S rDNA and 16S rDNA genes were also amplified to confirm the family assignment of the two new species and to explore the systematic status of *Pheronemoides*. 
Keywords

Caroline seamount – *Pheronemoides crustiformis* sp. nov – *Pheronemoides curvipentactin* sp. nov – phylogeny – South China Sea

Introduction

Seven genera (*Schulzeviella* Tabachnick, 1990; *Semperella* Gray, 1868; *Pheronema* Leidy, 1868; *Platylistrum* Schulze, 1904; *Polio pogon* Thomson, 1877; *Sericolophus* Ijima, 1901; *Pheronemoides* Gong & Li, 2017) are included in the family Pheronematidae. *Pheronemoides* Gong & Li, 2017 was established based on a single specimen collected on a seamount near Yap Trench. The atrial areas and dermal areas of *Pheronemoides* are on opposite sides of the body. Its basalia are not positioned exactly at the centre of the body, but rather in a basal crescent and amesially on the dermal surface. Viewed from above, the sponge is hemispherical or spherical. Observed laterally on one side, the sponge is arched and hollow inside (Gong & Li, 2017). Considering the body shape of the sponge together with the location of the basalia and the marginalia, it may be a transitional genus between *Pheronema* and *Sericolophus*.

In July 2016, a sponge specimen was collected at the Taitung County using an Agassiz trawl 121°32.621′ to 121°33.7489′E longitude and 21°12.4106′ to 21°13.2041′N latitude. This is the first report of the presence of *Pheronemoides* species in the South China Sea. In August 2017, a seamount biodiversity survey on the Caroline seamount was conducted using the R/V *Ke Xue*. Another specimen of *Pheronemoides* was collected by the remotely operated vehicle (ROV) *Fa Xian* at a depth of 1429.2 m. After further morphological examination and molecular analysis, the specimens were confirmed to be new species. These two new species are described and illustrated herein. There are now three species in *Pheronemoides*, all of which were collected in the northwestern Pacific Ocean, two of them were found on seamounts.

Materials and methods

Sample collection

One sponge sample was collected by the research ship *Ocean Researcher I* in the South China Sea using an Agassiz trawl. The sample was deposited in the National Museum of Natural Science, Taiwan. Another sponge sample was collected by the submersible remotely operated vehicle *Fa Xian* during a cruise of the research ship *Ke Xue* in the western Pacific Ocean. The sample was deposited in the Marine Biological Museum of Chinese Academy of Sciences (MBMCAS), Qingdao, China.

Spicule analysis

A small piece of sponge tissue was used to prepare the spicules by digesting them with concentrated nitric acid, and the spicules were then observed with scanning electron microscopy (SEM) and light microscopy (LM). For SEM, the spicules were first concentrated on a cover glass (diameter: 8 mm), which was then attached to a SEM stub. After coating with gold, the spicules were observed using a Hitachi S-3400N. For spicule measurements, we used an Olympus DSX500 Optodigital microscope with the manufacturer’s image analysis software.

DNA extraction and PCR amplification

Total genomic DNA was extracted using a Tissue DNA Kit (OMEGA Qingdao, China). Polymerase chain reaction (PCR) amplification
was carried out in a 25 μL total reaction volume containing 12.5 μL of Premix Taq™ (Takara, Otsu, Shiga, Japan), 1 μL of each primer, 2 μL of template DNA, and 8.5 μL of DNase-free ddH2O. The 16S rDNA and 28S rDNA sequences were amplified with the primers 16S1fw/16SH_mod (Dohrmann et al., 2008) and 28SliF1(5’–GGCGAAAGACTAATCGAACCA–3’)/28SliR1(5’–TTGGAGACCTGATGCGGTGA–3’) respectively. Amplification was performed using the following procedure: 5 min at 94°C for initial denaturing, followed by 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 48°C, extension for 60 s at 72°C, and a final extension for 5 min at 72°C.

Phylogenetic analysis
Analysis based on 16S rDNA and 28S rDNA gene sequences were undertaken to show the systematic status of the two new species as well as the genus Pheronemoides within the family Pheronematidae. The sequence of eight additional species from six genera including all species in Pheronematidae and Hyalonema (Onconema) obtusum as the outgroup were downloaded from GenBank to construct the phylogenetic tree (table 1).

After trimming, the concatenated dataset consisted of 1535 bp (16S/28S = 417/1118 bp), alignment gaps were represented as ‘-’ and missing data were represented as '?'. The homologous sequences, including ten sequences of 16S rDNA and eight sequences of 28S rDNA genes, were aligned using MUSCLE 3.8 (Edgar, 2004) with the default parameters. The best-fitting nucleotide substitution model (16S rDNA: GTR+G; 28S rDNA: GTR+G) for each partitioned dataset was assessed with ModelTest 3.7 (Posada & Crandall, 1998). Maximum likelihood (ML) analysis was carried out using RAxMLGUI v1.5 (Silvestro & Michalak, 2012) under the GTRGAMMA substitution model for all partitions in the concatenated dataset. Bayesian inference (BI) analysis was conducted using Mrbayes 3.2 (Huelsenbeck & Ronquist, 2001), Markov Chains were run for 10 million generations, with sampling every 1000 generations. The first 25% of trees were discarded as burn-in, and the remaining trees were summarized in 50% majority rule consensus tree to estimate the posterior probabilities. The effective sample size values were examined with Tracer v1.7 (Rambaut et al., 2018) to ensure that convergence was reached.

Results

Taxonomy
Class Hexactinellida Schmidt, 1870
Subclass Amphidiscophora Schulze, 1886
Order Amphidiscosida Schrammen, 1924
Family Pheronematidae Gray, 1870
Genus Pheronemoides Gong & Li, 2017

Pheronemoides crustiformis sp. nov.
(figs. 1–2, table 2)

Material examined. Holotype: MBM286618, Caroline seamount (10°31′29.881″N, 140°11′00.083″E), 24 August 2017, 1429.2 m depth, hard bottom.

Description. The sponge is irregularly trap-

ezoidal when observed from above (fig. 1B), the diameter of the body is 530 mm, and the height of the body is 354 mm. It is fan-shaped and has a large hollow between the dermal surface and basalia when viewed from one side (fig. 1A). When viewed from another side, it is spherical. Atrial areas cover the upper sur-
face of the sponge and dermal areas are on the opposite. Marginalia present on the boundary between the atrial and dermal areas, protrud-

ing out 20 mm from the body surface. Basalia are located on the edge of dermal surface as a basal crescent (fig. 1C), thus leaving a large hollow between the basalia and dermal areas. Basalia, more than 280 mm in length, consist of many small spicule tufts. Meshes on atrial areas (of 0.1–1.5 mm diameter, fig. 1E) are
Table 1: Species, specimen museum vouchers, GenBank accession numbers, and references using in this study

| Species                          | Vouchers     | GenBank accession numbers | References                           |
|----------------------------------|--------------|---------------------------|--------------------------------------|
| Pheronemoides crustiformis sp. nov. (holotype) | MBM286618  | MN165729                  | This study                           |
| Pheronemoides curvipentactin sp. nov. (holotype) | NMNS-8130-001 | MN165705                  | MN165730                            | This study                           |
| Pheronemoides fungosus           | YM30037      | KU175224                  | MN165731 | Gong et al., 2017; this study |
| Gong & Li, 2017 (holotype)       |              |                           |                                      |                                      |
| Pheronema sp.                    |              | AM886323                  | AM886381 | Dohrmann et al., 2008 |
| Semperella schulzei             |              | AM886324                  | AM886372 | Dohrmann et al., 2008 |
| (Semper, 1868)                   |              |                           |                                      |                                      |
| Semperella jiaolongae           | MBM179993    | KM881703                  | Gog et al., 2015                     |
| Gong & Li, 2015 (holotype)       |              |                           |                                      |                                      |
| Sericolophus hawaiicus          | P4-224 sp5   | LT627531                  | LT627545 | Dohrmann et al., 2017 |
| Schulzeviella sp.               |              |                           |                                      |                                      |
| Poliopogon microuncinata        | SMF 11698    | MF683973                  | Dohrmann et al., 2008               |
| Kersken, Janussen & Martinez Arbizu, 2018 |              |                           |                                      |                                      |
| Poliopogon distortus            | MBM286037    | MF098799                  | Gong et al., 2018                   |
| Gong & Li, 2018 (holotype)       |              |                           |                                      |                                      |
| Hyalonema (Onconema) obtusum     | SMF 12072    | MF683971                  | MF684003 | Kersken et al., 2018 |
| Lendenfeld, 1915                |              |                           |                                      |                                      |

Wider than dermal areas (of 0.1–1 mm diameter, fig. 1D).

Spicules. Pentactins (fig. 2A) with smooth rays make up the choanosomal skeleton, and tangential rays are 969–5499 μm long. Dermal pinular pentactins (fig. 2D) and atrial pentactins (fig. 2C) are similar in shape, and the tangential rays, which are slightly spiny, are 75–144 μm and 66–186 μm in length, respectively. Pinular rays, with conical or sharply pointed apex, are 189–307 μm and 75–144 μm in length, respectively. Basalia are two-toothed anchors and monaxones (probably diactins). Anchors have spiny shaft in proximal part (fig. 2O) and smooth shaft in distal part (fig. 2P), with diameter of 402–506 μm and length can be more than ten centimeters. Monaxones are easily broken and none of a complete one was observed, we speculate that they are diactins. Marginalia are sceptres (length: 3091–8120 μm; width: 16–34 μm) with spiny proximal part and smooth distal part.
TWO NEW SPECIES OF PHERONEMOIDES

Uncinates consist of two types. Macrouncinates (fig. 2J) and mesouncinates (fig. 2K) are similar in shape, and are both covered with numerous short spines. Macrouncinates and mesouncinates are 5182–5873 μm and 511–2376 μm in length, respectively.

Microscleres consist of microamphidiscs and microdiactins. Microamphidiscs (fig. 2E–G), with palmate head 33 % of the total length, have shafts covered by numerous spines. Total length of microamphidiscs are 23.2–37.8 μm, umbel length are 18–28 μm, and umbel width are 13–27 μm. Microdiactins have two types. Microdiactins I (fig. 2H) are whip-like in shape and covered with short spines, 236–367 μm in length. Microdiactins II (fig. 2I) have stronger teeth than microdiactins I, and their total length is 96–177 μm.

Etymology. Crustiformis is Latin for shell-shaped, in reference to the body shape similar to a thin shell viewed from the lateral side of the new specimen.

Remarks. Observed from the lateral side, the new species is arched and hollow inside, and it obviously belongs to Pheronemoides. P. crustiformis sp. nov. differs from the P. fungosus Gong & Li, 2017 by having bigger microamphidiscs (54–86 μm versus 23–38 μm in P. fungosus), a whip-like and slightly bent microuncinate (vs. straight microuncinate)
in *P. fungosus*), microdiactins covered by small spiny teeth (vs. microdiactins covered by long irregular teeth in *P. fungosus*) and a hemispherical body shape when observed from above (vs. a spherical body shape in *P. fungosus*).
TWO NEW SPECIES OF *PHERONEMOIDES* | 10.1163/18759866-20191422

**Table 2** Measurements of the spicules of holotype MBM286618 of *Pheronemoides crustiformis* sp. nov. (in μm) (n, number of spicules measured; s.d., standard deviation; range, range from the minimum to the maximum)

| Spicule Type                      | n  | mean | range | s.d. |
|-----------------------------------|----|------|-------|------|
| **Dermalia, pinule**              |    |      |       |      |
| pinular ray length                | 20 | 238  | 189–307 | 30   |
| pinular ray width                 | 20 | 14   | 12–17  | 2    |
| tangential ray length             | 20 | 110  | 75–144 | 19   |
| tangential ray width              | 20 | 10   | 9–16   | 2    |
| **Atrialia, pinule**              |    |      |       |      |
| pinular ray length                | 20 | 214  | 149–309 | 43   |
| pinular ray width                 | 20 | 12   | 9–17   | 2    |
| tangential ray length             | 20 | 112  | 66–186 | 29   |
| tangential ray width              | 20 | 9    | 7–13   | 1    |
| **Choanosomalıa, pentactin**      |    |      |       |      |
| tangential ray length             | 14 | 2643 | 969–5499 | 1181 |
| tangential ray width              | 14 | 38   | 25–53  | 9    |
| **Macrouncinate**                 |    |      |       |      |
| length                            | 3  | 5464 | 5182–5873 | 362  |
| width                             | 3  | 20   | 18–23  | 3    |
| **Mesouninate**                   |    |      |       |      |
| length                            | 4  | 1881 | 1511–2376 | 361  |
| width                             | 4  | 10   | 8–12   | 1    |
| **Anchor**                        |    |      |       |      |
| diameter                          | 7  | 458  | 402–506 | 35   |
| **Sceptre**                       |    |      |       |      |
| length                            | 6  | 5656 | 3091–8120 | 2020 |
| width                             | 20 | 24   | 16–34  | 5    |
| **Microdiactin I**                |    |      |       |      |
| length                            | 17 | 284  | 236–367 | 39   |
| **Microdiactin II**               |    |      |       |      |
| length                            | 20 | 139  | 96–177 | 24   |
| **Micramphidisc**                 |    |      |       |      |
| length                            | 20 | 67   | 54–86  | 8    |
| umbel length                      | 20 | 22   | 18–28  | 3    |
| umbel diameter                    | 20 | 18   | 13–27  | 3    |

*Pheronemoides curvipentactin* sp. nov.

(figs. 3–4, table 3)

*Material examined.* Holotype: NMNS-8130-001, Lanyu Township, Taitung County, Taiwan, Pacific Ocean (21°13.2041′N, 121°33.7489′E), 29 May 2013, Agassiz trawl, 57m depth.

*Description.* Specimen is curved (fig. 3A). Dermal surface and atrial surface are on opposite sides. Sponge only has small amount.
of basalia left when collected. In addition, there is no available natural living picture of the sponge. From the remaining body shape, we inferred that the sponge has a typical body form of *Pheronemoides*: when viewed from above, it is fan-shaped (fig. 3B), when viewed from one side, there may be a big hollow between the dermal surface and basalia. The diameter of the sponge is 240 mm. Marginalia are present at the boundary between the atrial and dermal areas, protruding several centimeters from the body surface. Meshes of atrial areas and dermal areas are inconspicuous. Basalia may be located on the dermal surface, and only few basal spicules can be observed.

**Spicules.** The choanosomal pentactins (fig. 4A) have smooth proximal rays and tangential rays (length: 404–4826 μm). Dermalia are pinular pentactins (fig. 4B–C) with tangential rays (length: 89–297 μm) covered with spines and pinular rays (length: 43–77 μm) that are spindle-like and bushy with spines. Atrialia include normal and special pentactins. The normal forms (fig. 4D–E) with four spiny tangential rays (length: 177–251 μm) and one spindle-like pinular ray (length: 36–63 μm), are slenderer than the dermalia. Atrialia of special form are pentactins which have a sparsely spined pinular ray with round or tapering terminal curved ray (length: 140–261 μm), and four smooth tangential rays (length: 40–124 μm) (fig. 4G–H). Crooked pentactins (fig. 4F) with a smooth primary ray, two smooth tangential rays, a curved pinular tangential ray with a large hooked terminus, and a smooth tangential ray with an expanded terminus bearing thin teeth were also observed. This kind of curved pentactin has not been observed in other pheronematid species. Basalia are two-toothed anchors (diameter 364–609 μm) and monaxones (probably diactins). The shaft of anchors are ordered as smooth, spiny, smooth and tapering apex from the proximal part to distal part (fig. 4O–Q). According to marginal spicule of other pheronematida species, though we only observed the shaft and apex of marginalia (fig. 4R–S), we infer that they are probably scepters and/or diactins. The width
of marginalia are 16–37 μm. Uncinates are of two types: macrouncinates (fig. 4I) and microuncinates (fig. 4J). Macrouncinates (length: 4018–4355 μm) are covered by many tiny barbs on the shaft. Microuncinates (length: 175–366 μm) are thin with tiny spines on the shaft (fig. 4K) and a sharp terminal (fig. 4L).

Microscleres consist of microamphidiscs and microdiactins. Microamphidiscs (fig. 4M) have smooth shafts, with total length of 27–42 μm, umbel length of 8–10 μm and umbel width of 7–10 μm. The number of microamphidiscs is small, and their palmate head represents 28% of the total length. Microdiactins (fig. 4N)
have long teeth and their total length is 118–157 μm.

**Etymology.** Curvipentactin from *curv* (meaning curved), *pent* (meaning five), and *aktis* (meaning rays), refers to this species containing curved pentactin spicules.

**Remarks.** Although the new specimen is incomplete, and a hollow between the atrial and dermal surfaces is not observed, the body is arched, and we can infer that its basalia are not positioned exactly at the centre of the body but amesially on the dermal surface.

**Table 3** Measurements of the spicules of holotype NMNS-8130-001 of *Pheronemoides curvipentactin* sp. nov. (in μm) (n, number of spicules measured; s.d., standard deviation; range, range from the minimum to the maximum)

|                        | n   | mean | range  | s.d. |
|------------------------|-----|------|--------|------|
| **Dermalia, pentactin**|     |      |        |      |
| pinular ray length    | 20  | 122  | 89–297 | 43   |
| pinular ray width     | 20  | 19   | 12–27  | 4    |
| tangential ray length | 20  | 59   | 43–77  | 11   |
| tangential ray width  | 20  | 12   | 7–15   | 2    |
| **Atrialia, normal pentactin** |  |      |        |      |
| pinular ray length    | 20  | 216  | 177–251| 20   |
| pinular ray width     | 20  | 12   | 10–15  | 1    |
| tangential ray length | 20  | 48   | 36–63  | 8    |
| tangential ray width  | 20  | 8    | 6–11   | 1    |
| **Atrialia, special pentactin** |  |      |        |      |
| primary ray length    | 15  | 214  | 140–261| 30   |
| primary ray width     | 15  | 8    | 6–10   | 1    |
| tangential ray length | 15  | 81   | 40–124 | 26   |
| tangential ray width  | 15  | 6    | 4–7    | 0.7  |
| **Choanosomalia, pentactin** |  |      |        |      |
| tangential ray length | 20  | 1550 | 404–4826| 1041 |
| tangential ray width  | 20  | 24   | 14–38  | 7    |
| **Macrouncinate**     |     |      |        |      |
| length                | 3   | 4182 | 4018–4355| 168  |
| width                 | 3   | 16   | 15–17  | 1    |
| **Microuncinate**     |     |      |        |      |
| length                | 18  | 219  | 175–366| 53   |
| **Marginalia**        |     |      |        |      |
| width                 | 20  | 29   | 16–37  | 5    |
| **Anchor**            |     |      |        |      |
| length                | 6   | 505  | 364–609| 85   |
| **Microdiactin**      |     |      |        |      |
| length                | 20  | 138  | 118–157| 15   |
| **Micramphidisc**    |     |      |        |      |
| length                | 4   | 32   | 27–42  | 7    |
| umbel length         | 4   | 9    | 8–10   | 1    |
| umbel diameter       | 4   | 9    | 7–10   | 1    |
Therefore, we infer that the new species belongs to Pheronemoides. The new species can be easily distinguished from its congeners by possessing special pentactine atrialia with round terminal or tapering terminal curved pinular rays and crooked pentactins. Additionally, the new species contains macrouncinates and microuncinates while P. fungosus contains three types of uncinates and P. crustiformis contains macrouncinates and mesouncinates.

**Molecular data.** The phylogenies of BI and ML analyses were highly congruent. The phylogeny based on 28SrRNA and 16SrRNA (fig. 5) shows that the two new species and P. fungosus forms into a clade with the exclusion of other pheronematids, thus supporting the family assignment, with support values >50 for both the ML and BI analyses.

**Discussion**

The phylogeny of the family Pheronematidae through molecular approaches had been explored by Kersken's (2018) and Dohrmann's (2018). And they have obtained similar phylogenetic tree. In our study, we added the molecular data of Poliopogon distortus and the two new species, and a more comprehensive tree is provided. Our tree was generally consistent with the previous results except the status of Semperella schulzei which was sister to Semperella jialongae+Poliopogon+Schulzeviella while it was clustered to Pheronema+Sericolophus+Pheronemoides in the previous studies. This was probably due to the different choices of alignment and substitution models, the molecular sequences of three additional species, as well as one or two reduced molecular markers used in our analysis. Though there were some differences, our tree revealed same relationship among the different genera of Pheronematidae as the previous studies, as well as the genus Semperella was a non-monophyletic group. More species of definitive Semperella are need to be sequenced in further studies to elucidate the status of this genus.

The seven genera of Pheronematidae Gray, 1870 are often distinguished from each

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**Figure 5** Bayesian inference trees of pheronematid species based on the 16S rDNA and 28S rDNA sequence data. Numbers at each node are Bayesian posterior probabilities (left) and ML analysis bootstrap values (right).
other mainly by their external body shape, whereas the morphologies and diversity of the spicules are less important (Tabachnick & Menshenina, 2002). Approaches on the phylogeny of Pheronematidae based on morphology were explored once (Tabachnick & Menshenina, 1999; Dohrmann et al., 2017). In our tree, Pheronematidae is divided into two clades. In one clade, Pheronema is sister to Sericolophus + Pheronemoides. This is consistent with Tabachnick et al. (1999), who consider Sericolophus to have evolved independently in relation to Pheronema species, and Gong et al. (2017), who recognized Pheronemoides has a closed relationship with Pheronema and Sericolophus. The other clade included Sempereilla, Poliopogon and Schulzeviella, which differed from the results of Dohrmann, who inferred that Schulzeviella was the sister group to the remaining pheronematids (Dohrmann et al., 2017). Since the phylogeny based on molecular data was not consistent with the morphology-based hypotheses, the special morphology of different genera within Pheronematidae may not be enough to be treated as a reliable synapomorphy. And the unique morphological characteristics (i.e., the body being bilaterally symmetrical or not, the atrial cavity being open or closed, atrialia being a common surface or not and basalia in a compact tuft or a broad tuft) used to identify pheronematida species into different genera might need refinement.

Pheronemoides, which is fan-like, exhibit an atrial concave side and a dermal convex side without basalia (fig. 2). It shows a typical body shape similar to Poliopogon species. When we established the genus, there was confusion about whether the genus was effectively defined according to morphological approaches. In our tree, Pheronemoides and Poliopogon are distant, and all the species of Pheronemoides and Poliopogon were grouped together, which proves that Pheronemoides is a valid genus. P. curvipentactin sp. nov. and P. fungosus exhibit a closer relationship than P. crustiformis. Ecologically, P. curvipentactin sp. nov. and P. fungosus occur at the hard bottom on seamounts in the northwestern Pacific Ocean, while P. crustiformis sp. nov. occurs in the South China sea (substrate unknown due to the absence of a natural living image). Morphologically, only P. crustiformis sp. nov. exhibits special pentactine atralia, which makes it is easily distinguished from the others species.

Forty-eight valid species of pheronematid have been reported (Van Soest et al., 2019); here, we only include 6 valid species in our tree (without the two new species and two species that were unable to be classified at the species level). Therefore, more taxon coverage and molecular markers will be needed in future studies to explore the phylogeny of Pheronematidae.

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