Phylogeny of two new phoronematid 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 congeners 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 congeners. 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; *Poliopogon* 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′-GGCGAAAGACTAATC-GAACCA-3′)/28SliR1(5′-TTGGAGACCTGAT-GCGGTGA-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 out-group 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 surface of the sponge and dermal areas are on the opposite. Marginalia present on the boundary between the atrial and dermal areas, protruding 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
		16S	28S	
<i>Pheronemoides crustiformis</i> sp. nov. (holotype)	MBM286618		MN165729	This study
<i>Pheronemoides curvipentactin</i> sp. nov. (holotype)	NMNS-8130-001	MN165705	MN165730	This study
<i>Pheronemoides fungosus</i> Gong & Li, 2017 (holotype)	YM30037	KU175224	MN165731	Gong et al., 2017; this study
<i>Pheronema</i> sp.		AM886323	AM886381	Dohrmann et al., 2008
<i>Semperella schulzei</i> (Semper, 1868)		AM886324	AM886372	Dohrmann et al., 2008
<i>Semperella jiaolongae</i> Gong & Li, 2015 (holotype)	MBM179993	KM881703		Gong et al., 2015
<i>Sericolophus hawaiiicus</i> Tabachnick & Lévi, 2000		AM886325	AM886380	Dohrmann et al., 2008
<i>Schulzeviella</i> sp.	P4-224 sp5	LT627531	LT627545	Dohrmann et al., 2017
<i>Poliopogon microuncinata</i> Kersken, Janussen & Martínez Arbizu, 2018	SMF 11698	MF683973		Kersken et al., 2018
<i>Poliopogon distortus</i> Gong & Li, 2018 (holotype)	MBM286037	MF098799		Gong et al., 2018
<i>Hyalonema</i> (<i>Onconema</i>) <i>obtusum</i> Lendenfeld, 1915	SMF 12072	MF683971	MF684003	Kersken et al., 2018

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

FIGURE 1 Holotype MBM286618 of *Pheronemoides crustiformis* sp. nov. A, photograph showing the specimen in its natural habitat; B, the external morphology of atrial areas; C, the external morphology of dermal areas and basalia; D, mesh structure of dermal areas; E, mesh structure of atrial areas.

(fig. 2 L–N). 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

FIGURE 2 Spicules of holotype MBM286618 of *Pheronemoides crustiformis* sp. nov. A, choanosomal pentactin; B, the anchor of basalia; C, atrial pinular pentactin; D, dermal pinular pentactin; E–G, micramphidiscs; H, microdiactin I; I, microdiactin II; J, shaft of macrouncinate; K, shaft of mesouncinate; L–N, Sceptre; O–P, the anchor of basalia.

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*).

TABLE 2 Measurements of the spicules of holotype MBM2866i8 of *Pheronemoides crustiformis* 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, 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
Choanosomalia, 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
Mesouncinate				
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, 517m depth.

Description. Specimen is curved (fig. 3A). Dermal surface and atrial surface are on opposite sides. Sponge only has small amount

FIGURE 3 Holotype NMNS-8130-001 of *Pheronemoides curvipentactin* sp. nov. A, the external morphology of dermal areas (scale bar = 5 cm); B, the external morphology of atrial areas.

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

FIGURE 4 Spicules of holotype NMNS-8130-001 of *Pheronemoides curvipentactin* sp. nov. A, choanosomal pentactin; B–C, dermal pinular pentactins; D–H, atrialia; D–E, pinular pentactins; F, crooked pentactin; G–H, special pentactins; I, shaft of macrouncinate; J, microuncinate; K, shaft of microuncinate; L, terminal of microuncinate; M, micramphidisc; N, microdiactin; O–Q, the anchor of basalia; R–S, marginalia.

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)

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
Macrourcinate				
length	3	4182	4018–4355	168
width	3	16	15–17	1
Microurcinate				
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

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 basalialia are not positioned exactly at the centre of the body but amesially on the dermal surface.

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).

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 schultzei* 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

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 *Semperella*, *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 atrialia, which makes it easily distinguished from the other 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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References

- Dohrmann, M. (2018) Progress in glass sponge phylogenetics: a comment on Kersken et al (2018). *Hydrobiologia*. doi:10.1007/s10750-018-3708-7.
- Dohrmann, M., Janussen, D., Reitner, J., Collins, A.G. & Wörheide, G. (2008) Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). *Syst. Biol.*, 57(3), 388–405. doi:10.1080/10635150802161088.
- Dohrmann, M., Kelley, C., Kelly, M., Pisera, A., Hooper, J.N. & Reiswig, H.M. (2017) An integrative systematic framework helps to reconstruct skeletal evolution of glass sponges (Porifera, Hexactinellida). *Front. Zool.*, 14, 18. doi:10.1186/s12983-017-0191-3.
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32, 1792–1797. doi:10.1093/nar/gkh340.
- Gong, L. & Li, X. (2017) A new genus and species of Pheronematidae (Porifera: Hexactinellida: Amphidiscosida) from the western Pacific Ocean. *Zootaxa*, 4337(1), 132–140. doi:10.11646/zootaxa.4337.1.7.
- Gong, L., Li, X. & Qiu, J.W. (2015) Two new species of Hexactinellida (Porifera) from the South China Sea. *Zootaxa*, 4034(1), 182–192. doi:10.11646/zootaxa.4034.1.9.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755. doi:10.1093/bioinformatics/17.8.754.
- Kersken, D., Kocot, K., Janussen, D., Schell, T., Pfenniger, M. & Arbizu, P.M. (2018) First insights into the phylogeny of deep-sea glass sponges (Hexactinellida) from polymetallic nodule fields in the Clarion-Clipperton Fracture Zone (CCFZ), northeastern Pacific. *Hydrobiologia*, 811(1), 283–293. doi:10.1007/s10750-017-3498-3.
- Posada, D. & Crandall, K.A. (1998) ModelTest: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818. doi:10.1093/bioinformatics/14.9.817.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in bayesian phylogenetics using tracer 1.7. *Syst. Biol.*, 67(5), 901–904. doi:10.1093/sysbio/syy032.
- Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.*, 12, 335–337. doi:10.1007/s13127-011-0056-0.
- Tabachnick, K.R. & Menshenina, L.L. (1999) An approach to the phylogenetic reconstruction of Amphidiscophora (Porifera: Hexactinellida). *Mem. Queensl. Mus.*, 44, 607–615.
- Tabachnick, K.R. & Menshenina, L.L. (2002) Family Pheronematidae Gray, 1870. In: J.N.A. Hooper & R.W.M. Van Soest (Eds) *Systema Porifera. A guide to the Classification of Sponges*, pp. 1267–1280. Kluwer Academic/Plenum Publishers, New York. doi:10.1007/978-1-4615-0747-5_129.
- Van Soest, R.W.M., Boury-Esnault, N., Hooper, J.N.A., Rützler, K., de Voogd, N.J., Alvarez, B., Hajdu, E., Pisera, A.B., Manconi, R., Schönberg, C., Klautau, M., Picton, B., Kelly, M., Vacelet, J., Dohrmann, M., Díaz, M.C., Cárdenas, P., Carballo, J.L., Ríos, P. & Downey, R. (2019) World Porifera Database. Pheronematidae Gray, 1870. Accessed online on 9 July 2019: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=131685>

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