

A new species of the glass sponge genus *Walteria* (Hexactinellida: Lyssacinosida: Euplectellidae) from northwestern Pacific seamounts, providing a biogenic microhabitat in the deep sea

Chengcheng Shen^{1,2}, Hong Cheng^{1,2}, Dongsheng Zhang^{1,2,3}, Bo Lu^{1,2}, Chunsheng Wang^{1,2,3*}

¹Key Laboratory of Marine Ecosystem Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China

²Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519000, China

³School of Oceanography, Shanghai Jiao Tong University, Shanghai 200240, China

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Abstract

We report on a hexactinellid sponge new to science, *Walteria demeterae* sp. nov., which was collected from the northwestern Pacific seamounts at depths of 1 271–1 703 m. Its tubular and basiphytous body, extensive lateral processes, numerous oval lateral oscula which are irregularly situated in the body wall, the presence of microscleres with oxyoidal, discoidal and onychoidal outer ends, and the absence of anchorate discohexasters, indicate it belongs to the genus *Walteria* of family Euplectellidae, which is also supported by molecular phylogenetic evidence from 18S, 28S, 16S rRNA and cytochrome c oxidase subunit I (COI) gene sequences. The unique morphotype, which is structured by a thin and rigid framework of body wall and lateral processes consisting of diactins, characterizes it as a new species. Local aggregations of individuals of this new species coupled with their associated macrofauna in the Suda Seamount are reported, highlighting its functional significance in providing biogenic microhabitats in the deep sea.

Key words: Euplectellidae, *Walteria*, integrative taxonomy, northwestern Pacific Ocean, Porifera, sponge aggregations

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1 Introduction

Sponges are an important structure-forming component of the deep-sea benthic fauna, providing biogenic microhabitats to associated organisms and thereby increasing local habitat heterogeneity and supporting high biodiversity, especially when they occur in sponge aggregations (Bell, 2008; Hajdu et al., 2017). Due to the highly complex and exquisite frameworks, the functional significance of euplectellid sponges, well-known for the “Venus flower basket” form and its associated spongicolid shrimps, has been highlighted in enhancing biodiversity and abundance of associated organisms (Kou et al., 2018). Generally, the sponges of family Euplectellidae Gray, 1867 are tubular, cup-like or fungus-like, lophophytose, basiphytose or rhizophytose, and sometimes pedunculate (Shen et al., 2019; Tabachnick, 2002). Megascleres consist of dermal and atrial hexactins (sometimes pentactins or both), choanosomal stauractins, tauactins and diactins (sometimes as well as hexactins and pentactins), and basal anchorate spicules (rarely diactins). Microscleres vary in the outer ends including graphiocomes, floricomes, drepanocomes, and others. There are three subfamilies in this family: Euplectellinae Gray, 1867, Corbitellinae Gray, 1872, and Bolosominae Tabachnick, 2002 (but note that these taxa are likely not

monophyletic; see Dohrmann et al., 2017; Dohrmann, 2019; Shen et al., 2019). The subfamily Corbitellinae, in which the typical “Venus flower basket” form is widespread, has 11 genera (4 of them are monotypic) and 39 valid species currently recognized (de Voogd et al., 2021).

The genus *Walteria* Schulze, 1886 currently consists of two species, *W. flemmingii* Schulze, 1886 and *W. leuckarti* Ijima, 1896. They occur in the western Pacific Ocean and eastern Indian Ocean at depths of 370–4 732 m (MacIntosh et al., 2018; Reiswig and Kelly, 2018; Tabachnick, 2002; Tabachnick et al., 2019). The type species *W. flemmingii* is known from the North Central Pacific Ocean off Hawaii, through the southwest Pacific islands of New Caledonia and New Zealand, extending south to Macquarie Ridge and west to the eastern Indian Ocean of the Perth Canyon in West Australia (MacIntosh et al., 2018; Reiswig and Kelly, 2018; Tabachnick, 2002; Tabachnick et al., 2019). *Walteria leuckarti* is known from the Sagami Bay, Japan, and Marianas Basin to the southwest Pacific islands around New Zealand (Reiswig and Kelly, 2018). Another indeterminate species of *Walteria* is known from off Hawaii and New Zealand from depths of 1 446–2 210 m (Reiswig and Kelly, 2018).

By taking *W. flemmingii* as type species, Schulze (1887) and

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*Corresponding author, E-mail: wangshio@sio.org.cn

Tabachnick (2002) described the general morphology and spiculation. Schulze (1887) did not recognize the lateral processes or onychohexaster microscleres, which were supplemented by Tabachnick (2002). However, Tabachnick (2002) made some statements on the existence of prostalia lateralia, large hexactins and pentactins to support the lateral processes, and discasters as some of the disc-bearing microscleres, which were refuted by Reiswig and Kelly (2018) after further collection and examination. Moreover, the existence of primary large stauractins and their importance in generating the rectangular lattice of wall strands were ignored by earlier descriptions, but later underlined by Reiswig and Kelly (2018) in detail. In addition, it was suggested that there were only graphiocomes and floricomes as stellate microscleres (Reiswig and Kelly, 2018). The intraspecific differences between the South Australian specimens having oxyhexasters and New Zealand and Hawaiian specimens having onychohexasters were illustrated (Reiswig and Kelly, 2018). For the valid species, *W. leuckarti*, which has a unique bottle-brush-like body form, Reiswig and Kelly (2018) indicated that the floricomes are rare and difficult to find; these authors therefore assigned the subspecies *W. leuckarti longina* Tabachnick, 1988 as a synonymised name so as to avoid subspecies designations within the Hexactinellida. Furthermore, another morphotype, with tubular body, extensive side processes, pointed apex and lattice-like body wall, was suggested to be a new species (Reiswig and Kelly, 2018). This morphotype was previously recorded from off Hawaii and displayed online by the Hawaiian Undersea Research Laboratory (HURL) Archive (<http://www.soest.hawaii.edu/HURL/HURLarchive/guide.php>). One specimen of dead, eroded framework and lack of loose spiculation (*Walteria* sp. indet.), coupled with seafloor images presumably identical to it but without collections, from New Zealand was described but not named (Reiswig and Kelly, 2018).

In this study, we describe a new species, the specimens of which were collected by remotely operated vehicle (ROV) at depths of 1 271–1 703 m from the northwestern Pacific seamounts. Local aggregations of this new sponge species and its associated organisms are reported from the Suda Seamount at depths of 1 295–1 320 m. Finally, we present molecular phylogenetic results supporting the placement of the new species in the family Euplectellidae, genus *Walteria*.

2 Materials and methods

2.1 Sample and video data collection

Two specimens of *Walteria demeterae* sp. nov. were collected by ROV from the northwestern Pacific seamounts. The holotype (catalogue number: SIO-POR-244) was collected on September 20, 2018 by ROV *Haima* from the Weijia Seamount in the northwestern Pacific Ocean at dive ROV12 of cruise DY51/I (12.730 9°N, 156.539 7°E, depth of 1 703 m). The paratype (catalogue number: SIO-POR-099) was collected by ROV *Hailong III* from the Suda Seamount in the northwestern Pacific Ocean on August 19, 2018 at dive ROV07 of cruise DY48/I (22.010 0°N, 159.377 2°E, depth of 1 271 m). The specimens were preserved in 95% ethanol and deposited in the Sample Repository of Second Institute of Oceanography (SRSIO), Hangzhou, China. A benthic investigation at the Suda Seamount implemented by ROV *Hailong III* on August 20, 2018 at dive ROV08 of cruise DY48/I (starting point: 22.011 0°N, 159.389 4°E, 1 295 m deep; ending point: 22.012 0°N, 159.391 1°E, 1 307 m deep) recorded a frequent occurrence of a morphotype identical to the new sponge species. Video recordings and still images collected by this investigation,

in total 3.0 h, from depths of 1 295–1 320 m, covering 550 m in distance, were examined to describe these local sponge aggregations and the presence of associated macrofauna.

2.2 Spicule and skeleton analysis

Spicules and frameworks were isolated by digesting small pieces of tissue from body wall and lateral processes, which were placed in different centrifuge tubes, in concentrated nitric acid using a metal bath maintained at about 80°C for one day until the tissues were completely dissolved. The spicules and frameworks were then rinsed twice in distilled water and later suspended in alcohol for light microscope (LM) and scanning electron microscope (SEM) observation. Slides for LM were examined with ZEISS Imager A2. Spicules for SEM (TM-1 000 Tabletop Microscope) were pipetted from the solution onto silicon wafers, and skeletons were moved onto silicon wafers, all of which were subsequently coated with platinum. Photomicrographs were obtained by SEM, and measurements were made with the software ZEN pro 2012 1.1.2.0.

2.3 Molecular work

DNA was extracted from small pieces of tissue using a DNeasy Blood&Tissue Kit (Qiagen) according to the manufacturer's instructions. The DNA quality and concentration were detected with a Nanodrop 2000 and Qubit fluorometer. Then the whole genome was sequenced via the Illumina PE150 strategy on the HiSeq™ X-Ten platform (Illumina, CA, USA) at Zhejiang Tianke High Technology Development Co., Ltd. The total of clean reads was assembled with the SPAdes genome assembler (Bankevich et al., 2012). The nuclear 18S rDNA and 28S rDNA, and mitochondrial 16S rDNA and cytochrome c oxidase subunit I (COI) genes were retrieved from the assembled contigs and used for phylogenetic analysis. The start and end of target sequences were checked by the reported universal PCR primer pairs (Dohrmann et al., 2008; Folmer et al., 1994). The 18S rDNA, 28S rDNA, 16S rDNA, and COI gene sequences are deposited in GenBank with accession numbers of MW652662, MW652660, MW652655, and MW517848, respectively.

2.4 Phylogenetic analysis

The four sequences of 18S rDNA, 28S rDNA, 16S rDNA, and COI plus reference data, were aligned using the ClustalW-algorithm with default settings (15/6.66 as gap/gap length penalties) in Geneious prime (Biomatters Ltd., New Zealand). The alignments were performed separately, and then concatenated into one file after manually trimming unalignable regions on both flanks. The phylogenetic tree was inferred with maximum likelihood (ML) analysis using IQ-TREE (Nguyen et al., 2015; Chernomor et al., 2016). The auto-selected best substitution model (-m MFP option) was used in IQ-TREE, with 1 000 bootstrap replicates (command line and input parameters: iqtree -s concatenated_alignments.phy -m MFP -p concatenated_alignments_partition.txt -bb 1 000 -alrt 1 000). The ultrafast bootstrap (UFBoot) was used to assess clade support by adding "-bb 1 000" (Hoang et al., 2018; Minh et al., 2013). An SH-aLRT test (Guindon et al., 2010) was also performed by adding "-alrt 1 000". The tree was constructed based on the concatenated alignment file of *Walteria demeterae* sp. nov. and other 31 reference species of hexactinellids by taking three species of the genus *Tretopleura* as outgroups (detailed information of sequences is shown in Table 1). The ML tree was then visualized and edited in Figtree 1.4.2 (Rambaut, 2006).

Table 1. GenBank accession numbers of marker genes of the 32 species used in the molecular phylogenetic analysis

Order: family	Species	Voucher number	Accession number				
			18S rDNA	28S rDNA	16S rDNA	COI gene	
Lyssacinosa: Euplectellidae	<i>Walteria demeterae</i> sp. nov. (holotype)	SIO-POR-244	MW652662	MW652660	MW652655	MW517848	
	<i>Walteria leuckarti</i>	SMF 10522	AM886399	AM886373	AM886337	FR848939	
	<i>Docosaccus maculatus</i>	GW5429	FM946116	FM946115	FM946105	FR848934	
	<i>Rhabdopectella tintinnus</i>	HBOI 4-X-88-2-014	AM886402	AM886371	AM886332	FR848941	
	<i>Regadrella</i> sp.	HBFH 8-VIII-09-2-001	/	FR848916	FR848917	/	
	<i>Rhizophyta yapensis</i>	SIO-POR-083	MK463603	MK463607	MK458682	MK453399	
	<i>Acoelocalyx brucei</i>	SMF 10530	AM886401	AM886370	AM886333	FR848938	
	<i>Malacosaccus coatsi</i>	SMF 10521	AM886400	AM886369	AM886334	FR848937	
	<i>Euplectella</i> sp. 1	HBOI 19-XI-86-1-001	AM886397	AM886368	AM886335	FR848935	
	<i>Euplectella</i> sp. 2	HBOI 12-XI-86-1-054	AM886398	AM886367	AM886336	/	
	<i>Saccocalyx microhexactin</i>	MBM179994	/	/	KM881702	/	
	<i>Saccocalyx pedunculatus</i>	SMF 12082	MF740862	MF684009	MF683987	/	
	<i>Hertwigia</i> sp.	USNM 1122181	FM946121	FM946120	FM946104	FR848940	
	<i>Bolosoma</i> sp.	USNM 1097546	FM946118	FM946117	FM946102	FR848942	
	<i>Bolosominae</i> n. gen. n. sp.	HURL P4-224-sp7	/	LT627534	LT627520	LT627552	
	<i>Atlantisella</i> sp.	HBOI 22-X-95-1-7	LT627547	LT627533	LT627519	/	
	<i>Holascus euonyx</i>	SMF12092	MF740859	/	MF683980	/	
	<i>Holascus taraxacum</i>	SMF12059	/	MF684005	MF683982	/	
	<i>Hyalostylus microfloricomus</i>	SMF12085	MF740860	MF684007	MF683984	/	
	<i>Hyalostylus schulzei</i>	SMF11707	/	/	MF683985	/	
	Lyssacinosa: Rossellidae	<i>Rossella nuda</i>	SMF 10531	AM886384	AM886355	AM886343	HE580217*
<i>Nodastrella asconemaoida</i>		ZMA POR18484	AM886386	AM886354	AM886344	FR848921	
<i>Caulophacus arcticus</i>		SMF 10520	AM886395	AM886360	AM886350	FR819684	
<i>Caulophacella tenuis</i>		SMF 10533	AM886392	AM886363	AM886351	FR848927	
<i>Caulophacus valdiviae</i>		SMF 10528	AM886394	AM886362	AM886348	FR848929	
<i>Caulophacus weddelli</i>		SMF 10527	AM886393	AM886361	AM886349	FR848928	
<i>Crateromorpha meyeri</i>		SMF 10525	AM886389	AM886359	AM886347	FR848923	
<i>Bathydorus spinosus</i>		SMF 10526	AM886390	AM886358	AM886341	FR848924	
Lyssacinosa: Aulocalycidae		<i>Euryplegma auriculare</i>	NIWA 43457	/	LT627535	LT627518	LT627551
Sceptrulophora: Uncinateridae		<i>Tretopleura weijica</i>	SIO-POR-090	/	/	MT176124	MT178277
	<i>Tretopleura</i> sp. 1	HURL P4-229-sp10	/	LT627542	LT627529	LT627556	
	<i>Tretopleura</i> sp. 2	HURL P5-701-sp4	/	LT627543	LT627530	LT627555	

Note: The symbol "*" refers that the COI gene of *Rossella nuda* was from the voucher SMF 11730.

3 Results

3.1 Systematics of the new sponge species

Phylum Porifera Grant, 1836

Class Hexactinellida Schmidt, 1870

Subclass Hexasterophora Schulze, 1886

Order Lyssacinosa Zittel, 1877

Family Euplectellidae Gray, 1867

Subfamily Corbitellinae Gray, 1872

Genus *Walteria* Schulze, 1886

Type species. *Walteria flemmingii* Schulze, 1886

Walteria demeterae sp. nov.

(Figs 1–5, Table 2)

Material examined. Holotype (Fig. 1a): SIO-POR-244, SRSIO, *Haima* ROV DY51/I, dive ROV12, collected by Bo Lu, September 20, 2018, at Weijia Seamount in the northwestern Pacific Ocean (12.7309°N, 156.5397°E), depth of 1703 m, stored in 95% ethanol. Paratype (Fig. 1b): SIO-POR-099, SRSIO, *Hailong III* ROV DY48/I, dive ROV07, collected by Dongsheng Zhang, August 19, 2018, at Suda Seamount in the northwestern Pacific Ocean (22.0100°N, 159.3772°E), depth of 1271 m, stored in 95% ethanol.

Other locations (images only; Fig. 2). Suda Seamount in the northwestern Pacific Ocean: *Hailong III* ROV DY48/I, dive

ROV08, 22.0110°–22.0120°N, 159.3894°–159.3911°E, depths of 1295–1320 m, August 20, 2018.

Description. The new species is presented by a tubular body with lateral oscula and processes (Fig. 1). The holotype has a tubular body with extensive side processes, pointed apex, lattice-like body wall and basiphytous attachment (Fig. 1a). Wall is thin and rigid with numerous oval lateral oscula in irregular arrangement. Distal lateral processes are simple or dichotomously branching outgrowths of the lateral wall. Color of the specimen in ethanol is transparent white.

Five segments are totally 919.8 mm in length and 46.1 mm in maximum diameter (Fig. 1a2). The pointed apex tapers to a very small terminal osculum but is damaged at the top (Fig. 1a3). A large number of lateral processes project about 85 mm from the nodes of the lateral outer surface, forming a crudely hairy aspect (Fig. 1a4). The numerous oval lateral oscula are 15 mm in maximum diameter. Spacing between lateral oscula is nearly adjacent or with maximum edge-to-edge distance of 25 mm. The basal end forms an attachment disc with a smaller diameter than the tubular body attached to hard substrate and supported by lateral struts (Fig. 1a5). The cylindrical body is slightly oblique, the external wall is relatively smooth while the internal wall shows numerous bulging circular strands. The lateral oscula are generally

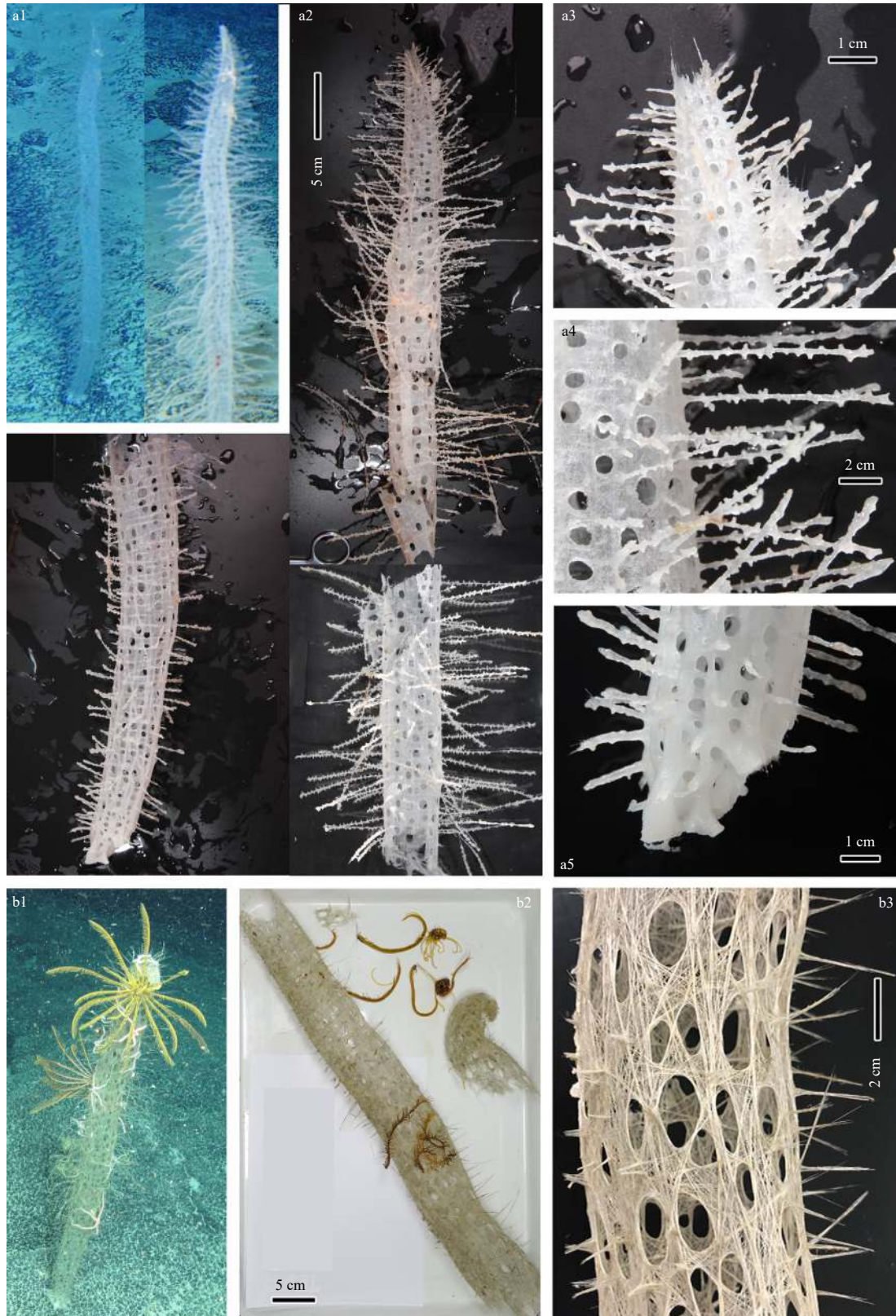


Fig. 1. *Walteria demeterae* sp. nov. specimens. Holotype: *in situ* images (a1), whole collected specimen including five segments (a2), close-up images of a slightly damaged apex (a3), middle body (a4), and basal part (a5); paratype: *in situ* image (b1), whole collected specimen including two segments (b2), and close-up image of middle body showing fused skeleton (b3).

separated by these strands.

The paratype was collected as a long dead, eroded framework with broken lateral processes and lack of loose spiculation

(Fig. 1b). Two segments of tubular body and pointed apex are totally 674.4 mm in length and 71.2 mm in maximum diameter (Fig. 1b2). The numerous oval lateral oscula are 20 mm in max-

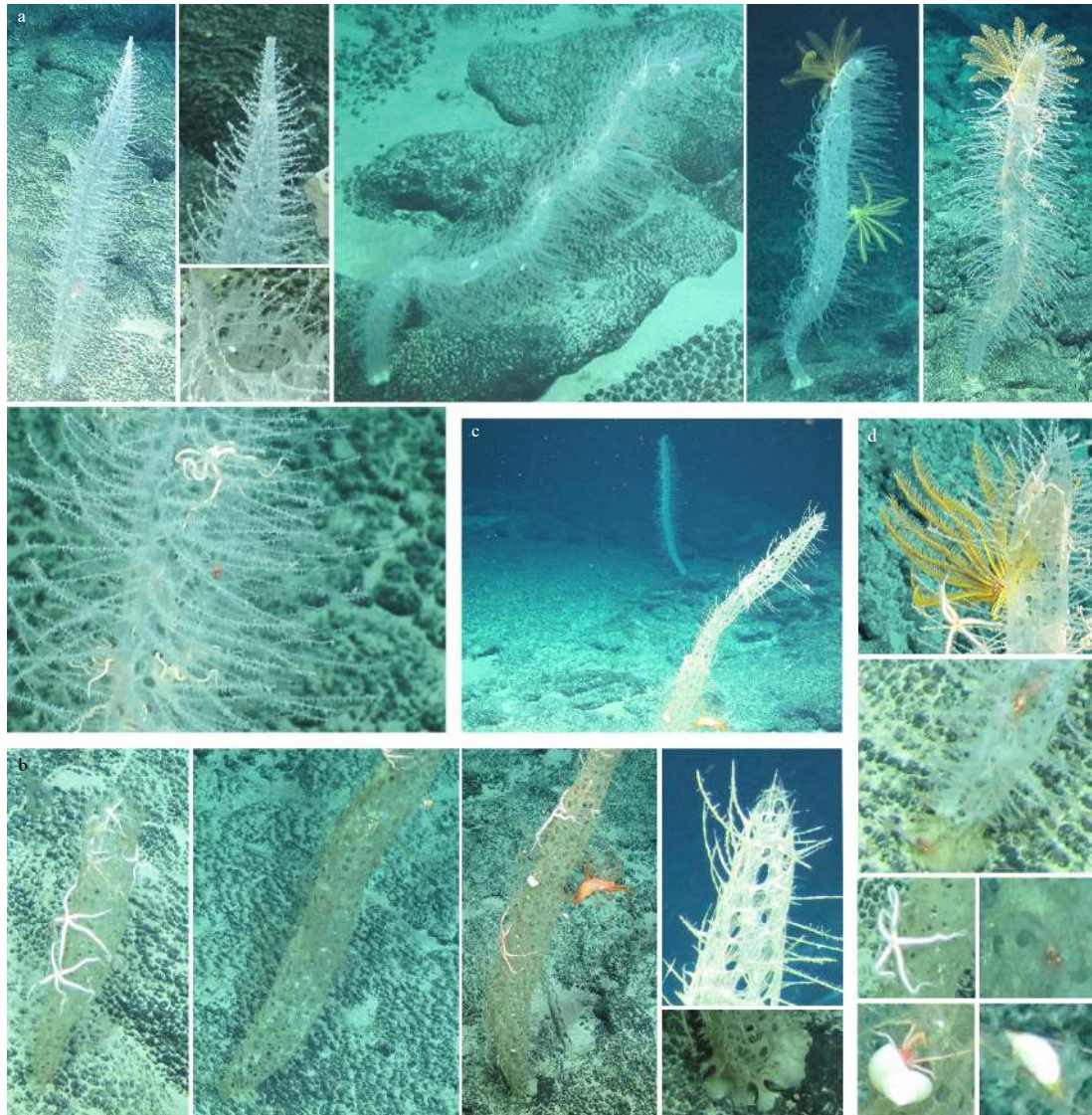


Fig. 2. *Walteria demeterae* sp. nov., seafloor images of the same morphotype observed at dive ROV08 of cruise DY48/I from the Suda Seamount in the northwestern Pacific Ocean. a. Living individuals showing different shapes of tubular body with close-up images of small terminal osculum in the pointed apex, circular strands in the inner wall, and lateral processes projecting from the body wall; b. dead individuals showing different degrees of erosion with close-up images of pointed apex and basal part; c. concurrence of both living and dead individuals in one image; d. examples of invertebrates associated with living or dead individuals, including Comatulida, Spongicolidae, *Ophioplithaca defensor*, Paguridae, Cirripedia and others (from top to bottom, then from left to right).

imum diameter. Spacing between lateral oscula is nearly adjacent or with maximum edge-to-edge distance of 25 mm. The lateral processes were broken to project at most 48 mm. The pointed apex bends and tapers to a very small terminal osculum but is damaged at the top. Longitudinal, circular and oblique strands can be observed within the framework (Fig. 1b3). Color in ethanol is light brown.

Relevant seafloor images clearly present the unique morphotype characterized by a lattice-work, tubular body with numerous lateral processes, forming a crudely hairy aspect for living individuals or a hispid body wall in different densities and lengths for dead individuals (Fig. 2). The tubular body varies in degree of bending and the pointed apex tapers sharply or tenderly.

Skeleton. The principle skeleton mainly consists of fused diactins with sparsely attached small stauractins, pentactins and sometimes hexactins (Fig. 3). The main skeletal framework of the body wall is a dominant network of crossing longitudinal, trans-

verse and oblique bundles of thick and smooth diactins closely fused by synaptacula and therein an irregular framework formed by ray-to-ray fusion of relatively thin diactins at different angles in a plane (Figs 3a and b). Longitudinal bundles are always dermal (Fig. 3a3); transverse (circular) bundles are always the most atrial (Fig. 3b3); oblique bundles and the irregular framework are situated between the longitudinal and transverse bundles. Transverse bundles are strongest and apparent to the naked eye in the collected specimen; longitudinal and oblique bundles are also strong, and the former are much thicker near the base. The framework of lateral processes consists of several parallel diactins fused by synaptacula (Fig. 3c). The principle skeleton of the body wall, especially close to the spicule bundles, is sparsely covered by attached small and smooth stauractins, pentactins and occasionally hexactins (Fig. 3d).

The ectosomal skeleton of the dermal surface is composed of a layer of mainly sword-shaped hexactins as dermalia with the

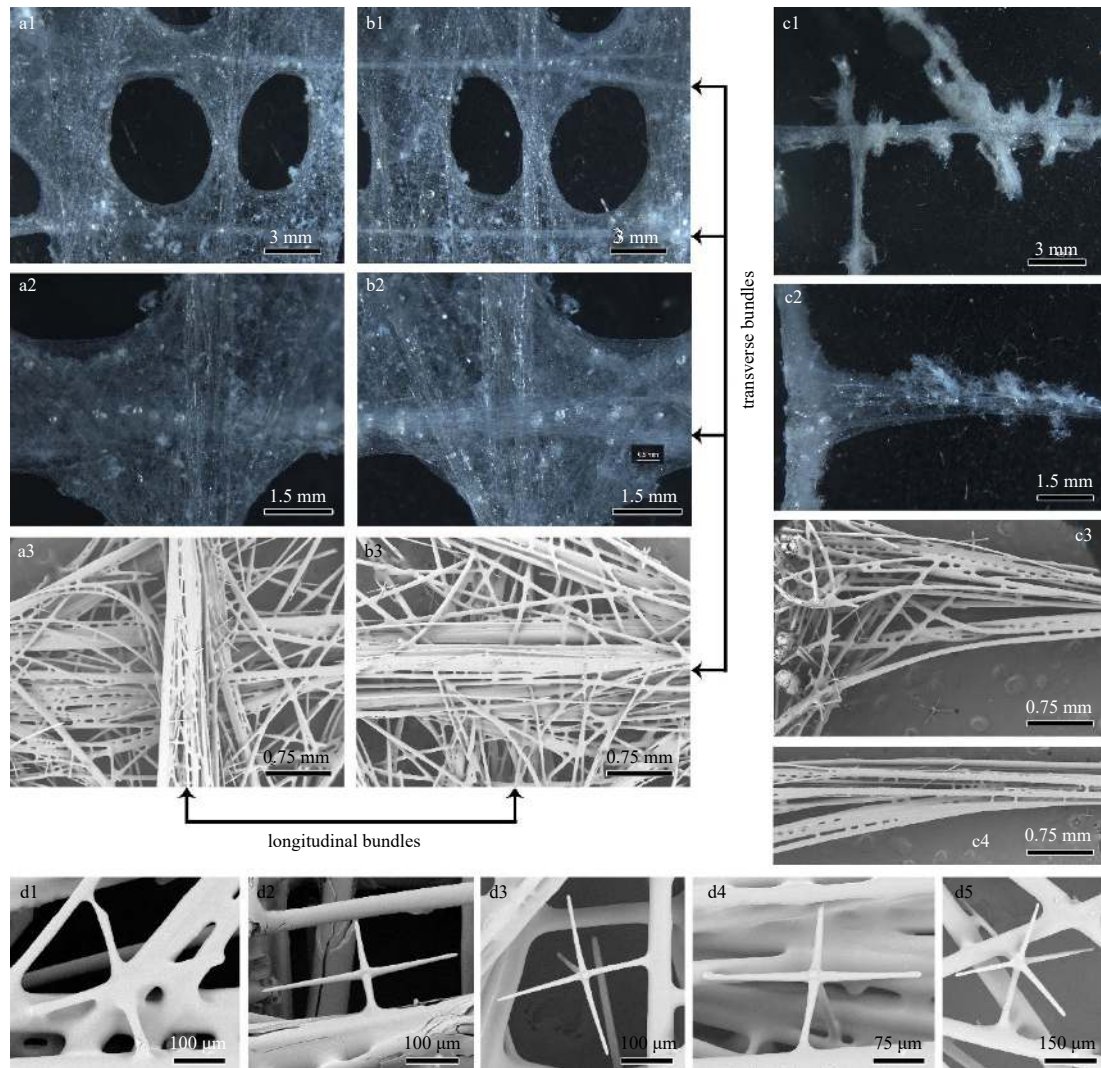


Fig. 3. *Waleria demeterae* sp. nov., surfaces of holotype. Dermal surface of body wall with lateral oscula (a1), close-up image of crossing longitudinal and transverse bundles (a2), and SEM of longitudinal bundles (a3); atrial surface of body wall with lateral oscula (b1), close-up image of crossing longitudinal and transverse bundles (b2), and SEM of transverse bundles (b3); lateral processes of dichotomously branching (c1) or simple (c2) outgrowths, the SEM of framework at junction of body wall and lateral process (c3) and its fused diactins (c4); attached stauractins (d1, d2), pentactins (d3, d4) and occasionally hexactins (d5).

short distal ray pointing outwards, covering the outer surface of the main body wall and all surfaces of the lateral processes. The inner surface of the body wall is covered by a layer of pentactins as atrialia, with the long proximal rays extending into the wall. Microscleres are dispersed among the proximal rays of dermalia and atrialia.

Spicules. Megascleres of the body are choanosomal diactins and stauractins, dermal hexactins, and atrial pentactins (Figs 4a–d; Table 2).

Choanosomal diactins (Fig. 4a) vary widely in size with 451.0–(1 071.4)–4 300.0 μm in length (some can be longer) and 8.0–(14.0)–27.5 μm in diameter (Table 2). They are straight or gently curved, subterminally rough or rarely smooth, with a generally inconspicuous or evident central swelling, rounded or conically pointed terminal ends, sometimes slightly inflated near the tips.

Choanosomal stauractins (Fig. 4b) are rarely observed and have lateral rays with rounded or blunt-pointed terminal ends which are rough and slightly inflated near the tips. The lateral rays of stauractins are generally unequal with the long ray 168.5–

(265.9)–738.9 μm in length and the short ray 107.5–(157.6)–211.3 μm in length; the ray width is 8.8–(14.2)–18.5 μm (Table 2). There is a nearly undetectable swelling or sometimes a thin and very short fifth ray in the axial cross.

Dermalia (Fig. 4c) of the body wall and lateral processes are similar sword hexactins with proclined spines covering the terminal end of each ray. The distal ray is inflated lanceolate, sometimes oval or occasionally uninflated (or slightly inflated), 88.9–(172.0)–275.0 μm long in the lateral processes, longer than that in the body wall with 49.2–(136.2)–215.0 μm in length; tangential rays have rounded and often slightly inflated terminal ends, with a mean length of about 122 μm both in the body wall and lateral processes but varying more widely in size in the body wall than in the lateral processes; the proximal ray is 1.5–(4.2)–9.4 times longer than the tangential rays with conically pointed tips, which is 243.3–(506.6)–882.0 μm long and 11.3–(14.5)–17.3 μm thick in the lateral processes, larger than that in the body wall with 114.0–(431.9)–686.7 μm in length and 6.4–(13.6)–18.7 μm in width (Table 2). Dermal sword hexactins are more common in

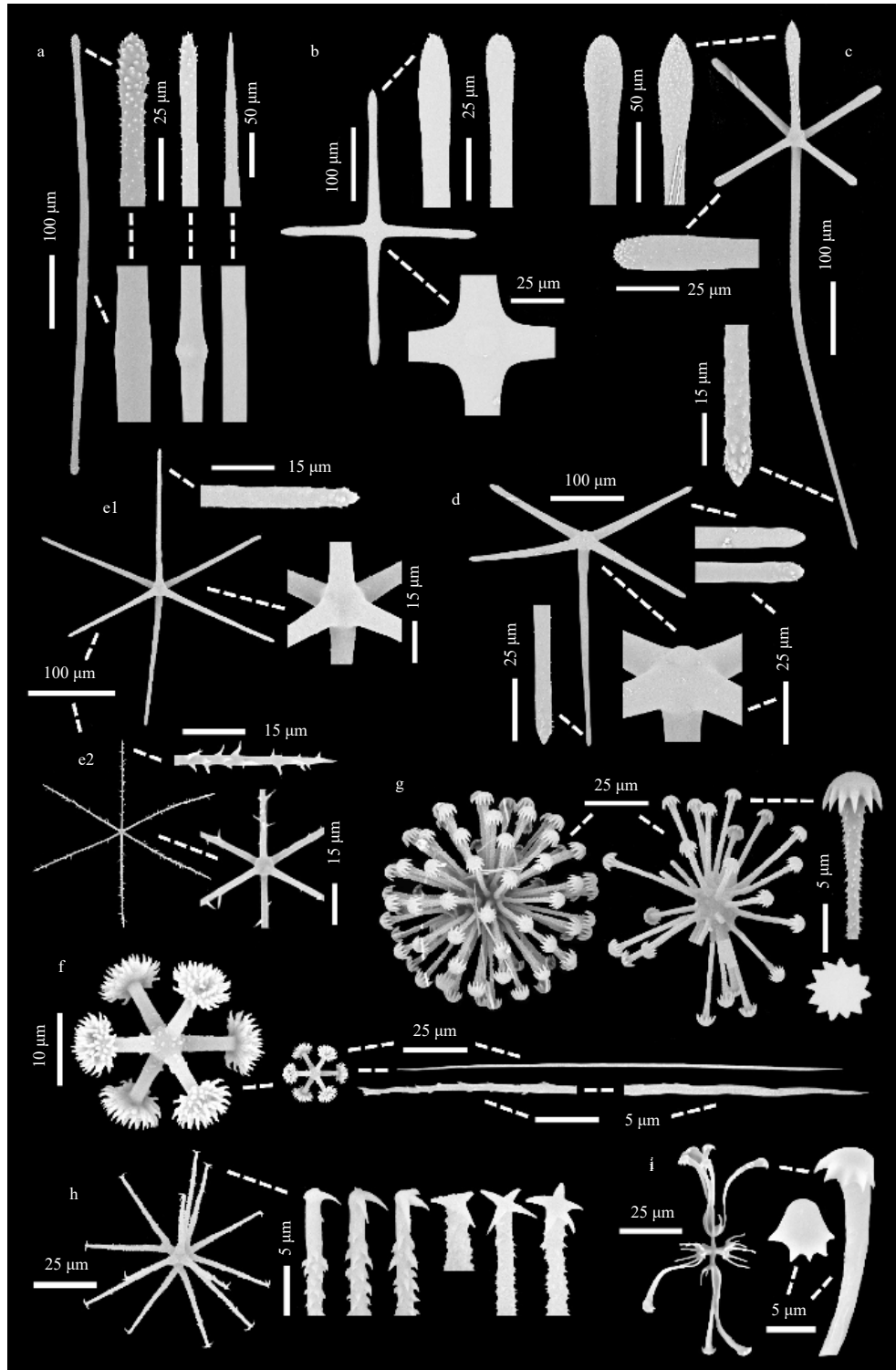


Fig. 4. *Walteria demeterae* sp. nov., spicules. a. Choanosomal diactin, whole and enlargements of three types of rays and middle segments; b. choanosomal stauractin, whole and enlargements of axial centre and two types of rays; c. dermal sword hexactin, whole and enlargements of tangential, proximal and two types of distal rays; d. atrial pentactin, whole and enlargements of axial centre, proximal ray, and two types of tangential rays with a smooth or slightly rough tip; e. oxyhexactins, which are sparsely covered by fine spines (located all over the body, e1) or entirely covered by macrospines (restricted to the basal part, e2), whole and enlargements of centres and rays; f. graphiocombe, primary centre and terminal ray at same scale, enlargements of primary centre and both the basal part and tip of terminal ray; g. discohexasters in common dense form or rare sparse form (corresponding to “discohexaster 1” and “discohexaster 2” described for *W. flemmingii* and *W. leuckarti* by [Reiswig and Kelly, 2018](#)), whole and enlargements of terminal ray and disc; h. onychohexaster, whole and enlargements of six types of onychoid tips with 1–5 claws; i. floricome, whole (not intact) and enlargements of terminal ray and tip.

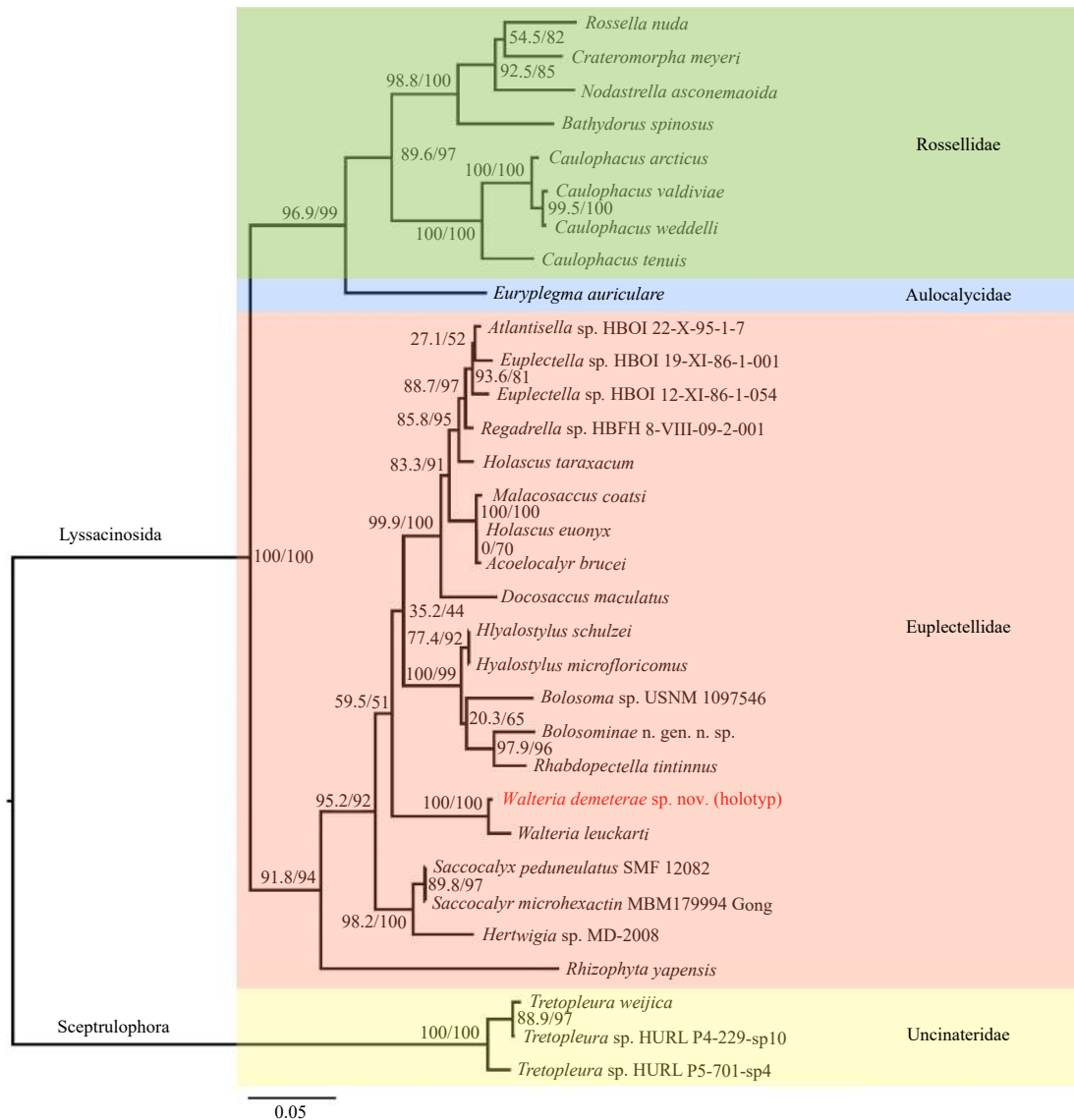


Fig. 5. Maximum-likelihood phylogenetic tree of 32 species from four families of Class Hexactinellida (by taking three species of the genus *Tretopleura* as outgroups), showing the relationships of the species *Walteria demeterae* sp. nov. and related taxa. The tree was inferred from the concatenated alignments of 18S rDNA, 28S rDNA, 16S rDNA and COI. Numbers at nodes are SH-aLRT/UFBoot support values (all based on 1 000 replicates).

the lateral processes but more variable in the body wall.

Atralia are regular smooth pentactins (Fig. 4d), mainly present within the body wall, with a small or rarely spherical swelling; tangential rays are 111.5–(158.0)–225.0 μm long with tips smooth or slightly rough, rounded or bluntly pointed, rarely inflated; the proximal ray varies in length of 83.3–(310.2)–654.0 μm with slightly rough and blunt-pointed tips (Table 2).

Microscleres are common oxyhexactins and graphiocomes, a number of discohexasters, some onychohexasters, and rare floricommes (Figs 4e–i; Table 2).

Oxyhexactins (Fig. 4e) in the whole body wall and lateral processes are sparsely covered by fine spines over the entire surface or rarely smooth, both similar in size and shape with rougher and parabolic terminal ends (smooth-rough oxyhexactins; Fig. 4e1). In addition, there is another kind of oxyhexactin entirely covered by macrospines, 3.0–(7.1)–12.0 μm in maximum length, with sharply pointed tips (macrospined oxyhexactins; Fig. 4e2; Table 2), which are restricted to the basal part. Rays of the former

are 115.0–(180.3)–265.0 μm long and 3.9–(8.0)–12.4 μm thick, larger than the macrospined ones with 71.5–(117.7)–195.3 μm in length and 2.3–(3.0)–4.0 μm in width (Table 2).

Graphiocomes (Fig. 4f) are typical for Euplectellidae with 22.7–(25.6)–29.7 μm in centre diameter; the primary rays are 6.3–(7.5)–10.0 μm long, ornamented with short smooth hemispheric knobs or rarely smooth; terminal rays (raphides) are very thin and 100.0–(158.9)–179.0 μm long, bearing sparse recurved spines (Table 2); each primary ray supports on its distal swelling about 60 bases of raphides.

Discohexasters (Fig. 4g) are spherical, 77.5–(88.9)–110.0 μm in diameter, with very short smooth primary rays 3.5–(5.8)–9.0 μm in length and rough straight terminal rays 30.0–(35.7)–46.1 μm in length (Table 2); they occur in either the more common dense form with more than 12 terminal rays per primary ray or rare sparse form with about 6 terminal rays on each primary ray; marginal teeth on terminal discs number 11–13.

Onychohexasters (Fig. 4h) are 70.0–(83.0)–100.0 μm in dia-

Table 2. Spicule dimensions of *Walteria demeterae* sp. nov., holotype

Spicule	Dimension	Mean	S.D.	Min	Max	N	
Choanosomal diactin	length/ μm	1 071.4	881.9	451.0	4 300.0	30	
	width/ μm	14.0	3.4	8.0	27.5	30	
Choanosomal stauractin	long ray length/ μm	265.9	164.7	168.5	738.9	11	
	short ray length/ μm	157.6	32.7	107.5	211.3	11	
	ray width/ μm	14.2	2.9	8.8	18.5	11	
Dermal sword hexactin (lateral processes)	distal ray length/ μm	172.0	44.8	88.9	275.0	30	
	distal ray basal width/ μm	15.2	2.1	11.1	20.0	30	
	distal ray maximum width/ μm	19.4	3.8	14.0	30.0	30	
	tangential ray length/ μm	122.3	24.6	72.2	186.0	30	
	tangential ray width/ μm	13.2	1.3	9.3	15.3	30	
	proximal ray length/ μm	506.6	140.2	243.3	882.0	30	
	proximal ray width/ μm	14.5	1.4	11.3	17.3	30	
	Dermal sword hexactin (body wall)	distal ray length/ μm	136.2	53.0	49.2	215.0	14
		distal ray basal width/ μm	15.1	3.8	7.4	22.7	14
		distal ray maximum width/ μm	19.4	5.8	10.0	33.3	14
tangential ray length/ μm		121.7	46.5	64.3	215.3	14	
tangential ray width/ μm		13.1	3.4	6.8	18.0	14	
proximal ray length/ μm		431.9	181.2	114.0	686.7	14	
proximal ray width/ μm		13.6	3.0	6.4	18.7	14	
Atrial pentactin	tangential ray length/ μm	158.0	30.7	111.5	225.0	30	
	tangential ray width/ μm	13.7	2.6	8.3	18.7	30	
	proximal ray length/ μm	310.2	137.2	83.3	654.0	30	
	proximal ray width/ μm	14.8	3.2	8.3	21.7	30	
Oxyhexactin	ray length/ μm	180.3	35.7	115.0	265.0	40	
	ray width/ μm	8.0	2.1	3.9	12.4	40	
Oxyhexactin (base)	ray length/ μm	117.7	29.8	71.5	195.3	30	
	ray width/ μm	3.0	0.5	2.3	4.0	30	
	spine max. length/ μm	7.1	2.3	3.0	12.0	30	
	centre diameter/ μm	25.6	1.8	22.7	29.7	30	
Graphiocomes	primary ray length/ μm	7.5	0.8	6.3	10.0	30	
	primary ray width/ μm	2.1	0.2	1.8	2.5	30	
	raphide length/ μm	158.9	13.2	100.0	179.0	50	
	diameter/ μm	88.9	6.6	77.5	110.0	30	
Discohexaster	primary ray length/ μm	5.8	1.2	3.5	9.0	30	
	secondary ray length/ μm	35.7	2.8	30.0	46.1	30	
	diameter/ μm	83.0	8.8	70.0	100.0	25	
Onychohexaster	primary ray length/ μm	4.1	0.9	2.5	6.7	25	
	secondary ray length/ μm	37.0	3.9	30.0	45.2	25	
	diameter/ μm	122.1	54.0	84.7	184.0	3	
Floricomae	primary ray length/ μm	5.8	2.3	3.8	8.3	3	
	secondary ray length/ μm	53.5	26.4	33.3	83.3	3	

meter, a little smaller than discohexasters; the primary rays are 2.5–(4.1)–6.7 μm long, with a bulge extending beyond the origins of the terminal rays; the terminal rays are 30.0–(37.0)–45.2 μm in length, covered by either fine or large recurved spines (Table 2); onychoid tips in individual spicules are often distinct with 1–5 claws.

Floricomae (Fig. 4i) were only observed thrice in the body wall, with a diameter of 84.7–(122.1)–184.0 μm ; the primary rays are smooth and 3.8–(5.8)–8.3 μm long; terminal rays are 33.3–(53.5)–83.3 μm long and at about nine per primary ray with five terminal claws, of which the outer surfaces are sparsely covered by fine spines (Table 2).

Etymology. The new species name *demeterae* refers to the goddess of fertility and harvest in Greek mythology named Demeter, referring to the abundant organisms often associating with the new species as well as its attractive appearance *in situ*.

Remarks. The new species described here is tubular, basiphytous but not pedunculate, bearing choanosomal diactins and stauractins, dermal hexactins, atrial pentactins and various microscleres, and is therefore clearly attributed to the subfamily Corbitellinae of family Euplectellidae (Tabachnick, 2002). Its extensive lateral processes, numerous oval lateral oscula which are irregularly situated in the body wall, the presence of microscleres with oxyoidal (microhexactins and graphiocomes), discoidal and onychoidal outer ends, and the absence of anchorate discohexasters, suggest it belongs to the genus *Walteria* (Tabachnick, 2002), which is also confirmed by our molecular phylogenetic study (see Section 3.3 below).

The new species shares the form of a hollow body with lateral oscula and processes with the two valid species and their loose spiculation is highly similar in composition and form, but the new species is significantly differentiated in the framework of the

body wall. *Walteria flemmingii* has a net-like, barrel-shaped body with a reticulate wall consisting of a sieve-like network of thin strands; the central elements of strands are primarily large stauractins and diactins generally fused by synaptacula into a rigid network. The new species has a tubular body with thin wall and pointed apex similar to that of *W. flemmingii* but is distinguished by its principle skeleton consisting mostly of diactins (without substantial contribution of stauractins). For the loose spiculation, there are choanosomal tauactins, floricones in variable abundance, and sometimes oxyhexasters occurring in *W. flemmingii*, but none of choanosomal tauactins or oxyhexasters was observed in the new species and only three floricones were observed in the holotype.

Walteria leuckarti has a bottle-brush-like body with a slender, tubular main stem composed of diactins soldered together at contact points or connected by synaptacula to form a thick stem wall with oval lateral oscula. The new species has primary spicules of the body wall framework as diactins and the oval lateral oscula similar to that of *W. leuckarti* but is distinguished by its much larger diameters of both cylindrical body and lateral oscula while much smaller thickness of the body wall. For the loose spiculation, there are choanosomal tauactins and sometimes oxyhexasters occurring in *W. leuckarti*, but none of these two types of spicules was observed in the new species.

In addition, the dead and eroded framework (*Walteria* sp. indet.) described by Reiswig and Kelly (2018) was a choanosomal skeleton consisting of a framework of fused diactins, suggesting a tubular body with oval lateral oscula but no evidence of lateral processes. Its lateral oscula are much more pronounced than in the paratype of the new species, which was also collected as an eroded framework; neither transverse or circular bundles are obvious, and nor do spicules attach to the longitudinal bundles, suggesting it is different from the new species described here. Furthermore, the related records of seafloor images from off Hawaii and New Zealand are almost identical to the appearance of the new specimens and *in situ* images from this study and can be allocated to the same morphotype rather than the eroded framework (*Walteria* sp. indet.) described by Reiswig and Kelly (2018). But whether they are the same species remains to be determined until a related living specimen from off Hawaii or New Zealand is collected.

3.2 Ecology of the new sponge species

Walteria demeterae sp. nov. is distributed in the northwestern Pacific seamounts (depths of 1 271–1 703 m; specimens and images). The bottom disc of the tubular body is attached to hard substrate. Local aggregations of *Walteria demeterae* sp. nov. were observed on the Suda Seamount in the northwestern Pacific Ocean at depths of 1 295–1 320 m (Fig. 2). At least 56 individuals characterized by the unique morphotype of the new sponge species were counted, representing 90% of the total individuals of sponges recorded in the track covering 550 m in distance. There were 33 individuals alive and others dead in different degrees of erosion.

Numerous ophiuroids (such as *Ophioplithaca defensor*, *Ophioleila elegans*), crinoids (Comatulida), crustaceans (such as Spongicolidae, Paguridae, Cirripedia) and other taxa were often observed associating with both living and dead individuals of *Walteria demeterae* sp. nov. (Fig. 2). Echinoderms, in particular crinoids and ophiuroids, were often found in high abundances attached to the sponges, with ophiuroids on or inside the canals and several crinoids often on the upper portion (Beazley et al., 2013; Na et al., 2021). Spongicolid shrimps, which often take eu-

plectellid sponges as their hosts (Kou et al., 2018; Xu et al., 2017), were observed on or inside the canals. Other crustaceans, such as hermit crabs and barnacles, were observed to prefer dead individuals of *Walteria demeterae* sp. nov. over living ones.

3.3 Molecular phylogenetic analysis of the new sponge species

The length of the 18S rDNA, 28S rDNA, 16S rDNA, and COI gene fragments was confirmed by respective primers sequence (Dohrmann et al., 2008; Folmer et al., 1994), and the PCR primers of the four target fragments could be located in the correct position of the marker genes. The data set of 18S rDNA, 28S rDNA, 16S rDNA partial sequence and COI complete sequence of *Walteria demeterae* sp. nov. (holotype; catalogue number: SIO-POR-244) contained 1 955 bp, 1 225 bp, 495 bp and 709 bp, respectively. The phylogeny confirms that the new species is closely related to *W. leuckarti*, supporting its systematic position within the family Euplectellidae, genus *Walteria* (Fig. 5).

4 Conclusions

According to both morphological and molecular assessment, the hexactinellid specimens described here clearly belong to a species new to science and are attributed to the genus *Walteria* (family Euplectellidae). This new species, *Walteria demeterae* sp. nov., shows a unique morphotype characterized by a lattice-work, tubular body with numerous lateral processes. Its structural complexity plays a key role in providing biogenic microhabitats and accordingly increasing biodiversity and abundance of associated organisms in the deep sea. Due to the rare specimens but relatively common records in seafloor images of the morphotype of this new sponge species, new materials should be collected and analyzed to examine intraspecific differences.

The method we used here for searching marker genes in genome-level datasets of marine macrobenthos has proven effective when applied to sponges. It can also provide basic information to design or edit primers when amplification of the gene fragments of relevant species is needed.

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