Two New Species and One New Genus of Glass Sponges (Hexactinellida: Euplectellidae and Euretidae), From a Transect on a Seamount in the Northwestern Pacific Ocean

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Hexactinellid sponges often form structural habitats for other organisms and thus support high biodiversity. Two representative morphotypes of hexactinellid sponges were sampled by a remotely operated vehicle along a transect (depths of 2377-2758 m) on the Ko-Hakucho Guyot in the northwestern Pacific Ocean, both new to science. One new species, Flavovirens hemiglobus gen. et sp. nov., which is pedunculate and bears the main choanosomal spicules of diactins, is clearly attributed to the euplectellid subfamily Bolosominae. Its set of microscleres present (namely, predominating oxyhexasters and discasters, and rare discohexasters and staurodiscs) characterizes it as a new genus. The other new species, Chonelasma tyloscopulatum sp. nov., which is funnel in form without dichotomous branching tubes or lateral oscula and has firm body walls supported by a three-layered dictyonal framework, is clearly attributed to the euretid genus Chonelasma (subfamily Chonelasmatinae). Its presence of surface pentactins, two types of scopules, and both oxy- and disco-tipped hexasters or hemihexasters as common microscleres, characterizes it as a new species. The placements are also supported by molecular phylogenetic evidence from nuclear 18S rDNA and 28S rDNA, and mitochondrial 16S rDNA and cytochrome c oxidase subunit I (COI) gene. More sampling efforts should be conducted to improve the understanding of the biodiversity of deep-sea seamount sponges.

Keywords: Euretidae, Euplectellidae, integrative taxonomy, Northwestern Pacific Ocean, seamount, Porifera



INTRODUCTION

Sponges are important structure-forming taxa in the deep sea; they can provide biogenic microhabitats for other invertebrates to associate with, thus increasing local habitat heterogeneity and supporting high biodiversity (Bell, 2008; Hajdu et al., 2017). Compared to other invertebrates, hexactinellid sponges often have complex and exquisite frameworks; therefore, their functional significance has been highlighted (Shen et al., 2021). As one of the two subclasses of hexactinellid sponges, Hexasterophora is characterized by the presence of microhexasters as the main microscleres; however, there are significant differences in their frameworks, thus showing diverse and distinct appearances (Reiswig and Wheeler, 2002; Tabachnick, 2002). Therein, the family Euplectellidae (Gray, 1872) belongs to the order Lyssacinosida, which features frameworks formed by loose spicules (the texture is soft); the family Euretidae (Zittel, 1877) belongs to the order Sceptrulophora with dictyonal frameworks formed by fused spicules (the texture is hard and fragile). Both are widely distributed (Reiswig and Wheeler, 2002; Tabachnick, 2002) and the main groups of habitat-forming species on deep-water seamounts (Clark et al., 2011).

Sponges in the Euplectellidae family are generally tubular, cup-like or fungus-like and sometimes pedunculate (Tabachnick, 2002). According to the method of attachment to the substratum, the body form and the type of atrialia, this family is currently divided into three subfamilies: Euplectellinae (Gray, 1872); Corbitellinae (Gray, 1872); and Bolosominae Tabachnick, 2002; with a total of 31 genera (15 of which are monotypic) and 125 valid species to date (de Voogd et al., 2022). Previously, Euplectellidae was classified into three genera: Euplectella, Corbitella, and Heterotella (Gray, 1867). Schulze (1886; 1887) subdivided it into three subfamilies based on the dermal skeleton content: Euplectellinae, Holascinae, and Taegerinae (later synonymized with Corbitellinae), with six genera. Tabachnick (2002) then divided Euplectellidae into three subfamilies with a new subfamily named Bolosominae thus to form the basic taxa consisting of 27 genera in three subfamilies. Later, Dohrmann (2016) transferred the genus Symplectella (previously belonging to the subfamily Corbitellinae) to the family Rossellidae. Recently, five new monotypic genera have been added into Euplectellidae, of which four are bolosomines (Reiswig and Kelly, 2018; Shen et al., 2019; Castello-Branco et al., 2020). The definition of Bolosominae was emended by Shen et al. (2019) to include the rhizophytous attachment together with basiphytous attachment of pedunculate euplectellids. Thus, basiphytous (or rarely rhizophytous) and pedunculate euplectellids are allocated to the subfamily Bolosominae (Tabachnick, 2002; Shen et al., 2019), currently containing 13 genera (nine of which are monotypic) and 33 valid species (de Voogd et al., 2022). It should be noted that these three subfamilies are likely not monophyletic (Dohrmann et al., 2017; Dohrmann, 2019).

Sponges in the Euretidae family are predominately tubular, cup-funnel formed, or blade formed, with or without dichotomously branching secondary lateral tubes (Reiswig and Wheeler, 2002). According to the body form, this family is presently divided into two subfamilies: Euretinae (Zittel, 1877); and Chonelasmatinae (Schrammen, 1912); with a total of 15 genera (four of which are monotypic) and 58 valid species to date (de Voogd et al., 2022). Those with bodies without dichotomous branching are allocated to the subfamily Chonelasmatinae, including seven genera (two of which are monotypic) and 26 valid species to date (Reiswig and Wheeler, 2002; de Voogd et al., 2022). Therein, the genus Chonelasma Schulze, 1886 has the funnel-like body form without lateral oscula and currently accounts for 42% of the total species of Chonelasmatinae. The concept of family Euretidae and inclusion of taxa have varied widely; subdivision of the family into two subfamilies was proposed by Reid (1958) and modified slightly by Reiswig and Wheeler (2002) thus to form the basic classification. Previously, 16 genera were allocated into the family Euretidae by Reiswig and Wheeler (2002); later, Homoieurete Reiswig and Kelly, 2011 and Pinulasma Reiswig and Stone, 2013 were erected and added into the subfamily Chonelasmatinae, while the former was then removed based on the molecular evidence (Dohrmann et al., 2017); meanwhile, the two sceptrule- and uncinate-lacking genera, Heterorete (previously belonging to the subfamily Euretinae) and Myliusia (previously belonging to the subfamily Chonelasmatinae), were moved into Hexasterophora incertae sedis by Dohrmann et al. (2017) based on their phylogenetic placement. It should be noted that this family and its two subfamilies are not monophyletic groups; there currently seem to be two unrelated groups of euretid genera according to phylogenetic analyses and one of these two groups is closely related to the family Farreidae (Reiswig and Dohrmann, 2014; Dohrmann et al., 2017; Dohrmann, 2019).

In this study, two specimens of representative glass sponges have been sampled from a transect at depths ranging from 2377 m to 2758 m located in the northern Ko-Hakucho Guyot of the northwestern Pacific Ocean by a remotely operated vehicle (ROV); these two specimens are both described new to science, with one species attributed to a new genus of the family Euplectellidae and the other species to the genus *Chonelasma* of the family Euretidae, after both morphological and molecular assessments.

MATERIALS AND METHODS

Sample Collection

Two new specimens were collected on Sep. 16, 2020 by ROV Hailong III from the Ko-Hakucho Guyot at one transect of dive ROV01a of cruise DY61/I onboard R/V Da Yang Yi Hao. The transect is located on the upper slope of the northern Ko-Hakucho Guyot at depths from 2377 m to 2758 m; this seamount is located on the northwestern Pacific Ocean adjacent to the Marcus-Wake Seamount chain to the east and the Magellan Seamount chain to the southeast. The holotype (catalog number: SIO-POR-292) of *Flavovirens hemiglobus* gen. et sp. nov. was collected by the ROV manipulator arms at the station at 23.5152°N, 148.5779°E, at a depth of 2716 m; the holotype (catalog number: SIO-POR-294) of *Chonelasma tyloscopulatum* sp. nov. was collected by the ROV suction samplers at the station at 23.5037°N, 148.5735°E, at a depth of 2425 m. The specimens were preserved in 95% ethanol and deposited in the Sample Repository of Second Institute of Oceanography (SRSIO), Hangzhou, China.

Spicule and Skeleton Examinations

Spicules were isolated by digesting small pieces of tissue from several specific parts of the body, 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 were then rinsed twice in distilled water and later suspended in alcohol for Light Microscope (LM) and Scanning Electron Microscope (SEM) observation. Frozen sections, 1.0-2.0 mm thick, were prepared by handing slicing vertically or horizontally to examine the dictyonal framework of the specimen belonging to euretids. The sections were placed on different grooved slides and digested in concentrated nitric acid by using a heating plate. The digested sections were then rinsed twice by pipetting distilled water and dried in the air for observation. Slides for LM were examined by ZEISS Imager A2. Spicules for SEM (TM-1000 Tabletop Microscope) were pipetted from the solution onto silicon wafers, and skeletons were moved from the grooved slides 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.

Molecular Work

DNA was extracted from pieces of sponge tissue by using a DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. The DNA quality and concentration were detected by Nanodrop 2000 and Qubit fluorometer. Then the genomic DNA was sequenced via Illumina PE150 strategy. One pair-end library was constructed with the insert size of approximately 300-400 bp and sequenced on HiSeqTM X-Ten platform (Illumina, CA, USA) at Zhejiang Tianke High Technology Development Co., Ltd. The total of fastq reads were qualified, trimmed and assembled by MEGAHIT assembler (Li et al., 2015; Li et al., 2016). The nuclear 18S ribosomal RNA gene (18S rDNA) and 28S ribosomal RNA gene (28S rDNA), and mitochondrial 16S ribosomal RNA gene (16S rDNA) and cytochrome c oxidase subunit I (COI) gene were recovered from the assembly contigs. The obtained four marker genes were used for phylogenetic analyses. The markers were checked by mapping the reported universal primer pairs (Folmer et al., 1994; Dohrmann et al., 2008) to the target sequences.

Phylogenetic Analyses

Detailed information of species and their marker sequences used for phylogenetic analyses in this study is shown in **Supplementary Table 1**. The reference alignments of four marker genes of 18S rDNA, 28S rDNA, 16S rDNA and COI sequences were provided by Dohrmann (2019) with secondary structure information of 18S and 28S rDNA in alignments (available online: https://doi.org/10.6084/ m9.figshare.5951965). The newly added four marker-gene sequences of the two new species and *Tretopleura weijica* were manually aligned with those alignment files and edited in the Geneious prime software (Biomatters Ltd., New Zealand). The single-gene alignments were aligned separately, whose unalignable regions were trimmed manually, and then concatenated into one supermatrix file with partition information for exporting (available online: https://doi.org/10.6084/m9. figshare.19447883). *Semperella schultzei* QM G318562 belonging to the family Pheronematidae was selected as the outgroup. The phylogenetic tree was reconstructed with partitioned maximum likelihood (ML) analysis using RAxML GUI v.1.5 (Silvestro and Michalak, 2012; Stamatakis, 2014), with rapid bootstrapping (1000 replicates) and the GTR+G model (Lanave et al., 1984; Yang, 1994). The ML tree was visualized in Figtree (http://tree.bio.ed.ac.uk/ software/figtree/).

RESULTS

Systematics of New Species of Euplectellidae

Phylum Porifera (Grant, 1836) Class Hexactinellida (Schmidt, 1870) Subclass Hexasterophora (Schulze, 1886) Order Lyssacinosida (Zittel, 1877) Family Euplectellidae (Gray, 1867) Subfamily Bolosominae (Tabachnick, 2002) Genus *Flavovirens* gen. nov. Type species. *Flavovirens hemiglobus* gen. et sp. nov. (described below).

Diagnosis

Body is hemiglobular, pedunculate, basiphytous with a wide, upright opening. No apparent extended margin or marginalia. Dermal and atrial surfaces are cratered, without apparent lattice covers. Choanosomal spicules are mainly diactins, plus uncommon hexactins, pentactins, stauractins, tetractins and triactins. Dermalia and atrialia are pinular hexactins. Microscleres predominate as oxyhexasters and discasters and rare discohexasters and staurodiscs.

Etymology

The new genus *Flavovirens* refers to the *in situ* color of the peduncle of holotype which is yellowish green (described below; **Figure 1A**). The new name is formed from *flavus* (Latin word for yellow) and *virens* (Latin word for green); the gender is neuter.

Remarks

Due to the main choanosomal spicules of diactins and the presence of a long peduncle, in addition to the presence of discasters and oxyhexasters as the main microscleres, the new specimen is clearly attributed to the subfamily Bolosominae of the family Euplectellidae. It is distinguished from other bolosomines by the set of microscleres present, namely, predominating oxyhexasters and discasters and rare discohexasters and staurodiscs.

The absence of graphiocomes prevents its assignment to *Trachycaulus*, *Vityaziella*, *Trychella*, *Neocaledoniella* or *Advhena*. In addition, *Trachycaulus* lacks disco-tipped microscleres which are present in the new specimen; *Vityaziella* and *Trychella* present amphidiscs which are absent in the new specimen; *Neocaledoniella* and *Advhena* have discohexasters in the form of calycocomes, while



FIGURE 1 | *Flavovirens hemiglobus* gen. et sp. nov., specimen of holotype.
(A) *In situ* image. (B) Whole collected specimen, lateral view of body head
(B1), top view of opening (B2), and bottom view of body head (B3).
(C) Close-up view of dermal surface. (D) Close-up view of atrial surface.

the new specimen has discohexasters mainly in the form of discasters but not calycocomes. The absence of plumicomes prevents its assignment to Caulocalyx (bearing only plumicomes and discohexasters as microscleres) or Saccocalyx (various microscleres including other codonhexasters, drepanocomes, and spirodiscohexasters). The absence of floricomes prevents its assignment to Amphoreus, Hyalostylus or Rhabdopectella. In addition, Amphoreus features an amphora shape, while the new specimen has a hemiglobular body with a stalk; Hyalostylus and Rhabdopectella both have sigmatocomes. The absence of amphidiscs prevents its assignment to Amphidiscella. The presence of abundant oxy-tipped microscleres prevents its assignment to Rhizophyta or Bolosoma since they have only disco-tipped microscleres. Therefore, the set of microscleres renders the new specimen unique among bolosomines and thus justifies the proposal of a new genus.

Flavovirens hemiglobus gen. et sp. nov. (Figures 1, 2; Table 1).

Material Examined

Holotype (**Figure 1**): SIO-POR-292 (original number: DY61-ROV01a-B06), SRSIO, Hailong III ROV DY61/I, dive ROV01a, collected by Chengcheng Shen, Sep. 16, 2020, on the Ko-Hakucho Guyot in the northwestern Pacific Ocean (23.5152°N, 148.5779°E), depth of 2716 m, stored in 95% ethanol.

Description

The new species presents a large, soft, hemispherical body borne on a moderately long, robust, slightly curved peduncle with a disc-like base of attachment (**Figure 1A**). The atrium is approximately 14.4 cm in depth, and the osculum is upright and wide, approximately 33.5 cm in diameter, without an extended margin or marginalia (**Figure 1B**). The inhalant and exhalant systems are composed of wide and open compound apertures without lattice covers, cratering the dermal and atrial surfaces (**Figures 1C, D**). The inhalant and exhalant apertures are 5.1 cm×3.6 cm in maximum size. *In situ* color is white for the body and yellowish green for the below half of the peduncle (**Figure 1A**); on the desk and preserved, the body is light beige (**Figure 1B**).

Megascleres

Surface megascleres of the body are dermal, atrial and marginal pinular hexactins; choanosomal megascleres are common diactins (two types, together >56.0%), a number of hexactins (two types, together <22.4%) and pentactins (<11.2%), and rare stauractins (<3.7%), tetractins (<1.9%) and triactins (<4.7%; **Figures 2A–I**; **Table 1**).

Pinular dermal hexactins (Figure 2A) are spined in the ray ends but smooth in the cross center and most of the tangential and proximal rays. The pinular ray is oval or clavate, 28-(98)-148 μ m long, 1.3–(2.6)–5.2 times larger than its maximum width; the tangential rays have bluntly pointed, clavate or spindle-like ray tips, usually inflated near the tips, 153-(203)-285 µm long; the proximal ray is usually long and tapers to a bluntly conical ray rip, up to three times longer than the tangential rays, while sometimes short with a clavate ray tip, less than half of the tangential rays; the proximal ray is $6-(9)-15 \mu m$ thick, slightly thinner than the tangential rays. In addition, there may be more than one pinular ray in a single pinular hexactin or one pinular ray and one rough clavate ray. Pinular atrial hexactins (Figure 2B) have the same form and ray ornamentation as the dermal hexactins but are slightly larger in dimension. Furthermore, the pinular hexactins in the margin of the dermal and atrial surfaces are quite different from the dermal and atrial ones. Pinular marginal hexactins (Figure 2C) are microspined over the whole body and may be sparsely spined or smooth in the middle of the proximal ray; the pinular ray is club-like with very short thorns; they are larger in dimension with more robust tangential and proximal rays, approximately 1.5 times thicker than the dermal and atrial rays.

Choanosomal diactins (Figure 2D), slightly curved or straight, have rough terminal ends and a smooth center. They occur as two types in different forms and sizes. The larger one (diactin I; Figure 2D1), 1750-(3770)-7070 µm long and 14-(25)-45 µm thick, has conically pointed terminal ends and inconspicuous central swellings. The smaller one (diactin II; Figure 2D2), onethird as long as and half the width of the larger one, has conically pointed, rounded or seldomly clavated terminal ends, and the shapes of their centers vary with inconspicuous or four swellings. Choanosomal hexactins (Figure 2E) are smooth in the cross center but slightly rough in the ray tips. Rays of choanosomal hexactins usually taper to sharply pointed tips, forming a regular form of hexactins (hexactin I; Figure 2E1), 250-(346)-436 µm in ray length and 11-(18)-26 µm in ray width. However, one ray therein may occur with a clavate tip (hexactin II; Figure 2E2), which is 64-(114)-235 µm in ray length, approximately one-third



FIGURE 2 | Flavovirens hemiglobus gen. et sp. nov., spicules of holotype (SEM). (A) Pinular dermal hexactin, whole and enlarged ray ends. (B) Pinular atrial hexactin, whole and enlarged ray ends. (C) Pinular marginal hexactin, whole and enlarged ray ends. (C) Pinular marginal hexactins, whole and enlarged ray ends. (C) Two types of choanosomal diactins, whole and enlarged tips and center. (E) Two types of choanosomal hexactins, whole and enlarged ray ends. (F) Choanosomal pentacin, whole and enlarged tip and cross center. (G) Choanosomal tetractin, whole and enlarged tip and cross center. (H) Choanosomal stauractin, whole and enlarged tip and cross center. (J) Choanosomal stauractin, whole and enlarged tip and cross center. (J) Two examples of oxyhexasters, whole and enlarged secondary ray tips. (K) Two types of discohexasters, whole and enlarged secondary ray tip and terminal disc. (L) Staurodisc, whole and enlarged ray tip.

as long as the other rays with sharply pointed tips. Choanosomal pentactins (**Figure 2F**) have the same ray shape and ornamentation and similar dimensions as regular choanosomal hexactins. They are smooth or rarely microspined and flat in the cross center without swelling. Choanosomal tetractins (**Figure 2G**) have hemispherical swellings in the smooth cross center and sparsely spined ray ends tapering to sharply pointed tips. Their dimensions are similar to those of choanosomal stauractins. Choanosomal stauractins (**Figure 2H**) are smooth in the cross center, sometimes with hemispherical swelling, while microspined

in ray ends with sharply pointed tips. Each ray in a single stauractin is unequally long, with an average of 406–(498)–620 μm in length and 13–(19)–22 μm in width. Choanosomal triactins (**Figure 2I**) have the same form and ornamentation as tetractins, but sometimes the proximal ray may be short and end in a rounded tip. Their rays are approximately 15 μm in average width, slightly thinner than tetractins, and almost the same length as tetractins.

Microscleres

Microscleres are common oxyhexasters (>51.9%) and discohexasters (two types, together >44.2%) and rare staurodiscs (<3.9%; **Figures 2J–L**; **Table 1**).

Oxyhexasters (**Figure 2J**) are entirely smooth and vary greatly in size, of which the whole diameter ranges from 52 to 193 μ m with an average of 116 μ m. The short primary rays support 8–12 secondary rays that diverge and are straight or slightly curved. Secondary rays vary greatly in length, ranging from 18 to 74 μ m with an average of 43 μ m. The longer secondary rays taper gently and usually end in bluntly pointed tips or rarely spherical knobs (**Figure 2J1**), while the shorter secondary rays quickly taper to sharply pointed tips (**Figure 2J2**). The secondary rays are thin, 1–(2)–3 μ m in width.

Discohexasters (Figure 2K) are spherical with very short, smooth primary rays and sparsely spined or smooth, straight secondary rays; they occur in two types. The more common type (discohexaster I; Figure 2K1), 160-(195)-223 µm in diameter, is in the general high-density form of discasters; the primary rays are smooth and bear approximately 25 secondary rays per primary ray; the secondary rays are sparsely microspined and 3–(3)–4 μm in width; the terminal discs are 15-(18)-20 µm in diameter, with 11-17 well-developed marginal teeth. The uncommon type (discohexaster II; Figure 2K2) features thinner secondary rays and smaller terminal discs; the primary rays bear approximately 20 secondary rays on each primary ray; the secondary rays below the discs are usually swollen, while most of the secondary rays are thin, approximately 2 µm in width; the terminal discs are very small, 3-(4)–5 μ m in diameter, with 12–13 undeveloped marginal teeth. The two types of discohexasters are similar in their diameters, which are larger than those of oxyhexasters.

Staurodiscs (**Figure 2L**) are large with a maximum diameter of 160 μ m or small with a minimum diameter of 45 μ m; their terminal discs are in the same form as those of common discohexasters with well-developed marginal teeth.

Ecology

Flavovirens hemiglobus gen. et sp. nov. is distributed on the Ko-Hakucho Guyot in the northwestern Pacific seamounts (the holotype was collected at a depth of 2716 m). The bottom disc of the long peduncle was attached to hard substrates.

Etymology

The new species name *hemiglobus* refers to the hemiglobular body.

Remarks

The new specimen described here has a hemiglobular body with a wide, upright opening and a moderately long peduncle

TABLE 1 | Spicule dimensions of Flavovirens hemiglobus gen. et sp. nov., holotype (in µm).

Spicule/µm	mean	s.d.	min	max	Ν
Dermal pinular hexactin, pinular ray length	98	29.3	28	148	30
tangential ray length	203	32.1	153	285	29
proximal ray length	329	177.8	45	530	18
pinular ray basal width	16	4.5	9	26	30
pinular ray max. width	38	11.4	15	65	30
tangential ray width	10	2.1	6	15	30
proximal ray width	9	2.1	6	15	30
Atrial pinular hexactin, pinular ray length	122	29.6	62	161	30
tangential ray length	199	37.6	134	282	29
, , , , , , , , , , , , , , , , , , ,				690	22
proximal ray length	408	180.9	42		
pinular ray basal width	20	4.9	11	31	30
pinular ray max. width	50	12.5	19	72	30
tangential ray width	11	1.8	8	14	30
proximal ray width	11	2.0	6	14	30
Marginal pinular hexactin, pinular ray length	99	23.5	50	158	30
tangential ray length	266	52.9	178	374	30
proximal ray length	477	139.6	133	722	28
pinular ray basal width	32	8.1	20	57	30
pinular ray max. width	56	11.1	36	81	30
tangential ray width	16	2.8	11	22	30
proximal ray width	15	3.1	9	21	30
Choanosomal diactin I, length	3770	1888.9	1750	7070	15
width	25	8.9	14	45	30
Choanosomal diactin II, length	1180	260.8	670	1670	30
width	13	3.3	5	20	30
Choanosomal hexactin I, ray length	346	59.3	250	436	9
ray width	18	4.2	11	26	9
Choanosomal hexactin II, distal ray length	114	47.0	64	235	15
tangential ray length	302	62.5	218	419	15
proximal ray length	321	137.5	165	544	11
distal ray width	15	4.5	10	26	15
distal ray max. width	18	4.5	12	26	15
tangential ray width	17	4.4	10	25	15
proximal ray width	16	3.0	10	22	15
Choanosomal pentactin, tangential ray length	397	111.9	147	520	12
proximal ray length	307	81.8	205	388	4
tangential ray width	20	5.2	5	27	12
proximal ray width	17	4.8	5	25	12
Choanosomal tetractin, tangential ray length	483	24.0	466	500	2
proximal ray length	365	45.0	333	397	2
tangential ray width	21	2.9	19	23	2
proximal ray width	18	1.3	18	19	2
Choanosomal stauractin, ray length	498	94.2	406	620	4
ray width	19	4.3	13	22	4
Choanosomal triactin, tangential ray length	493	111.4	376	667	5
proximal ray length	457	283.6	217	770	3
tangential ray width	15	4.0	11	21	5
proximal ray width	15	4.4	11	22	5
Oxyhexaster, diameter	116	33.4	52	193	40
primary ray length	9	1.9	5	13	37
secondary ray length	43	15.5	18	74	40
primary ray width	7	1.1	5	9	37
secondary ray width	2	0.3	1	3	40
Discohexaster I (discaster), diameter	195	13.9	160	223	40
diameter of fused primary rays	23	4.0	17	31	30
secondary ray length	81	6.5	68	95	30
secondary ray width	3	0.3	3	4	30
diameter of disc	18	1.3	15	20	30
Discohexaster II, diameter	190	15.1	172	205	4
primary ray length	10	1.0	9	11	3
secondary ray length	76	4.6	70	80	4

(Continued)

Spicule/µm	mean	s.d.	min	max	Ν
primary ray width	11	1.7	10	13	3
secondary ray width	2	0.1	2	2	4
diameter of disc	4	0.9	3	5	4
Staurodisc, diameter	86	63.9	45	160	3
ray length	40	28.6	23	73	3
ray width	3	0.6	2	4	3
diameter of disc	12	3.7	9	16	3

(approximately three times longer than the body head) with a plate-like base attached to hard substrates. It bears diactins as the main choanosomal spicules, plus uncommon hexactins, pentactins, stauractins, tetractins and triactins. Therefore, it is clearly attributed to the subfamily Bolosominae of the family Euplectellidae. However, the new specimen cannot be accommodated in any of the currently accepted genera in this subfamily. It is distinguished from other bolosomines by the set of microscleres present, namely, predominating oxyhexasters and discasters, rare discohexasters and staurodiscs (described above). Thus, we propose a new monospecific genus within Bolosominae to include *Flavovirens hemiglobus* gen. et sp. nov.

Systematics of New Species of Euretidae

Phylum Porifera (Grant, 1836) Class Hexactinellida (Schmidt, 1870) Subclass Hexasterophora (Schulze, 1886) Order Sceptrulophora (Mehl, 1992) Family Euretidae (Zittel, 1877) Subfamily Chonelasmatinae (Schrammen, 1912) Genus Chonelasma (Schulze, 1886) Type species. Chonelasma lamella (Schulze, 1886) Chonelasma tyloscopulatum sp. nov. (Figures 3, 4; Table 2).

Material Examined

Holotype (**Figure 3**): SIO-POR-294 (original number: DY61-ROV01a-B09), SRSIO, Hailong III ROV DY61/I, dive ROV01a, collected by Chengcheng Shen, Sep. 16, 2020, on the Ko-Hakucho Guyot in the northwestern Pacific Ocean (23.5037°N, 148.5735°E), depth of 2425 m, stored in 95% ethanol.

Description

The specimen was collected as four slightly undulating, plate-like fragments from a funnel-like body form (**Figures 3A, B**). The funnel-shaped body protrudes from a cylindrical peduncle (**Figure 3A**). The preserved fragments are 4.4 cm×2.8 cm in maximum size and 2.0–3.8 mm in thickness. The texture is hard and fragile. Dermal and atrial surfaces are smooth (**Figures 3C, D**). Channels (epirhyses and aporhyses) are observable on both surfaces. Epirhyses, approximately 1.9 mm×1.1 mm in maximum size, are covered by a porous tissue membrane (**Figures 3C2, C3**). Aporhyses, approximately 2.6 mm×1.4 mm in maximum size, are arranged longitudinally, most of which lack lattice-like covers (**Figures 3D2, D3**). The color *in situ* is white (**Figure 3A**); on the desk and preserved, it is light beige (**Figure 3B**).



FIGURE 3 | Chonelasma tyloscopulatum sp. nov., specimen, surfaces and skeletons of holotype. (A) *In situ* images of holotype (A1) and two other same morphotypes (A2, A3). (B) Whole collected specimen of holotype including four segments (dermal surface upward). (C) Dermal surface, close-up images in different scales with epirhyses in surface view (C1–C3), symbiotic anthozoan indicated by an arrowhead (C1) and SEM of cleaned skeleton (C4), growth margin upward. (D) Atrial surface, close-up images in different scales with aporhyses in surface view (D1–D3), symbiotic anthozoan indicated by an arrowhead (D1) and SEM of cleaned skeleton (D4), growth margin upward.
(E) Vertical-longitudinal section of the dictyonal framework, consisting of dermal cortex, primary middle layer and atrial cortex, dermal side upward.
(F) Close-up view of dermal cortex with digitate spurs (SEM).

Skeleton

The choanosomal skeleton is a three-layered dictyonal framework consisting of a primary middle layer with elongated rectangular meshes and two irregularly meshed cortical layers on both the dermal and atrial sides (**Figures 3C4, D4, E**). Dictyonal skeletons are often sparsely spined and attached by rough, digitate spurs (**Figure 3F**). The ectosomal skeleton is composed of a lattice of loose pentactins overlying all choanosomal framework surfaces and entrances to epirhyses but not most of the openings of aporhyses (**Figures 3C3, D3**).

Megascleres

Megascleres of the body are surface pentactins, scopules (two types) and uncinates (Figures 4A–C; Table 2).

The surface pentactins (**Figure 4A**) are in regular form with slight or rare hemispherical swelling in the cross center. They are uniformly microspined over the whole body and similar on both dermal and atrial surfaces. The tangential rays taper to bluntly conical or clavate ray tips, $166-(272)-404 \mu m$ in length, $6-(12)-21 \mu m$ in width; the proximal ray is slightly shorter and thinner than the tangential rays, $93-(228)-350 \mu m$ in length and $6-(11)-18 \mu m$ in width, with a tapering and bluntly conical tip.

Scopules (**Figure 4B**) occur as two types in different forms and sizes. The smaller one (scopule I; **Figure 4B1**), 352-(422)-545 µm in total length and 3-(5)-6 µm in shaft width, is in the form of strongyloscopules; its narrow or wide neck carries four or rarely three straight or slightly curved tines ending in triangular or rounded tips; the shaft is sparsely spined ending in a bluntly pointed tip. The larger one (scopule II; **Figure 4B2**) is in the form of tyloscopules, 469-(550)-644 µm in total length, and almost twice as wide as the smaller one in shaft width. The narrow neck bears four or rarely five straight or slightly curved tines ending in spherical knobs; the tines are profusely covered with fine



FIGURE 4 | Chonelasma tyloscopulatum sp. nov., spicules of holotype (SEM). (A) Surface pentactin, whole and enlarged ray ends and cross center. (B) Two types of scopules, whole and enlarged heads and tips. (C) Uncinate, whole and enlarged tips and center. (D) Hemioxyhexaster (D1) and oxyhexaster (D2), whole and enlarged ray end. (E) Oxyhexactin, whole and enlarged cross center. (F) Discohexaster (F1) and hemidiscohexaster (F2), whole and enlarged secondary ray end. (G) Onycho-tipped hexaster, whole and enlarged secondary ray end.

recurved spines, but the distal surfaces of the knobs are bare; most of the shaft is sparsely spined ending in an abrupt sharp tip.

Uncinates (**Figure 4C**) are generally straight and sometimes slightly curved, $610-(1000)-1250 \mu m$ long and $3-(6)-10 \mu m$ thick, with barbs curving out from the spicule surface. One end is full of well-developed barbs, which are oriented toward the other end; the barbs on the other end are undeveloped and similar to forward-curved spines. Both tips are sharply pointed.

Microscleres

Microscleres are common hemioxyhexasters, rare oxyhexasters, a number of oxyhexactins, common discohexasters, rare hemidiscohexasters and onychohexasters (Figures 4D–G; Table 2).

Hemioxyhexasters and oxyhexasters (**Figure 4D**) are 59–(77)–100 μ m in diameter; the cross center is spherical and spined; the primary and secondary rays are entirely covered by short, relined hooks; hemioxyhexasters have 1–5 rays branched, and they bear two or rarely three thin, straight or out-curved secondary rays tapering to sharply pointed tips; primary rays are 9–(13)–19 μ m long, and approximately half of the secondary rays (primary to secondary ray length ratio ranging from 0.3 to 0.7). Oxyhexactins (**Figure 4E**) have the same dimensions, ray shape and ornamentation as hemioxyhexasters.

Discohexasters and hemidiscohexasters (**Figure 4F**) are stellate in form. They have the same spherical cross center and ornamentation as those of the hemioxyhexasters, but they are smaller in dimensions, 28– (39)–50 μ m in diameter, approximately half the diameter of the oxytipped one; hemidiscohexasters usually have five rays branched, and they bear 2–7 straight or slightly sigmoid secondary rays ending in small discs with about eight marginal teeth; primary rays are 4–(6)–12 μ m long, approximately half of the secondary rays (primary to secondary ray length ratio ranging from 0.3 to 0.8); primary rays are relatively robust, with 2–(2)–3 μ m in width.

In addition, only one onycho-tipped hexaster (**Figure 4G**) was observed, which has the same dimension, form and ray ornamentation as those of the discohexasters. Its terminal ray tips are onychoid or pointed, and the onychoid tips are distinct with 1-3 claws.

Ecology

Chonelasma tyloscopulatum sp. nov. is distributed on the Ko-Hakucho Guyot in the northwestern Pacific Ocean (the holotype was collected at a depth of 2425 m). The bottom disc of the short peduncle is attached to hard substrates. Small anthozoans were found both in the dermal and arial cavities, which are probably symbionts (**Figures 3C1, D1**), and this scenario has also been found in other euretid specimens (Reiswig and Kelly, 2011).

Etymology

The new species name *tyloscopulatum* refers to the large scopules (scopule II), which are in the form of tyloscopules and have not been observed in other valid species of this genus to date.

Remarks

The new specimen is plate-like in fragments with body form of a single-wall funnel; its firm body wall is supported by a three-layered dictyonal framework; dermalia and atrialia are pentactins; scopules and uncinates present; microscleres are

TABLE 2 Spicule dimensions of Chonelasma tyloscopula	<i>itum</i> sp. nov., holotype (in µm).
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Spicule/µm	mean	s.d.	min	max	N
Surface pentactin, tangential ray length	272	59.0	166	404	30
proximal ray length	228	64.8	93	350	30
tangential ray width	12	4.3	6	21	30
proximal ray width	11	3.6	6	18	30
Scopule I, length	422	40.4	352	545	30
head length	77	13.4	45	109	30
tine length	68	12.0	38	92	30
shaft width	5	0.8	3	6	30
tine width	4	3.7	2	23	30
Scopule II (tyloscopule), length	550	45.2	469	644	24
head length	91	11.5	53	106	28
tine length	77	10.8	43	89	28
shaft width	8	1.4	6	11	28
tine width	7	1.3	4	9	28
Uncinate, length	1000	251.3	610	1250	6
width	6	2.2	3	10	11
Hemioxyhexaster/oxyhexaster, diameter	77	9.8	59	100	30
primary ray length	13	2.6	9	19	30
secondary ray length	28	4.1	21	36	30
primary ray width	2	0.2	1	2	30
Oxyhexactin, diameter	73	7.8	64	88	10
ray length	37	4.1	32	44	10
ray width	2	0.1	2	2	10
Discohexaster/hemidiscohexaster, diameter	39	5.6	28	50	30
primary ray length	6	1.6	4	12	30
secondary ray length	12	1.9	8	16	30
primary ray width	2	0.3	2	3	30

predominantly oxy- and disco-tipped; therefore, this new specimen is attributed to the family Euretidae. Therein, due to its funnel-like body form without dichotomous branching tubes or lateral oscula, the new specimen belongs to the subfamily Chonelasmatinae. Furthermore, its body walls consisting of a middle primary layer with elongated rectangular meshes and two irregular-meshed cortical layers with epirhyses on dermal side or aporhyses on atrial side, its spiny pentactins on both surfaces, and its microscleres including hemioxyhexasters/oxyhexasters, discohexasters/hemidiscohexasters and rare onychohexasters support its assignment to the genus *Chonelasma*. It differs from each of the 11 previously recognized valid species by significant features of spiculation.

The pentactine surface spicules prevent its assignment to C. hamatum (Schulze, 1886); C. doederleini (Schulze, 1886); C. chathamense (Reiswig and Kelly, 2011); or C. oreia (Reiswig, 2014), each of which has hexactine surface spicules. The new specimen bears both oxy- and disco-tipped hexasters or hemihexasters as common microscleres. However, disco-tipped microscleres are absent or scarce in C. lamella (Schulze, 1886); C. choanoides (Schulze and Kirkpatrick, 1910); or C. glaciale (Reiswig and Kelly, 2011); oxy-tipped microscleres are absent or scarce in C. ijimai (Topsent, 1901); C. australe (Reiswig and Kelly, 2011); or C. biscopulatum (Reiswig and Kelly, 2011). The new specimen cannot be assigned to C. bispinula (Kersken et al., 2019), since C. bispinula has only hemihexasters as microscleres with two rudimentary primary rays lying in one axis, and this feature is more various in the new specimen; in addition, the pentactins of the new specimen are finely spined, but C. bispinula is covered by large spines; the new specimen has two types of scopules, while *C. bispinula* has only one type; the body size of *C. bispinula*, less than 6 cm in height, is much smaller than those of the other valid species as well as the new specimen.

It should be noted that the tyloscopules have not been observed in other valid species of the genus *Chonelasma* to date. The presence of tyloscopules combined with oxyhexasters and discohexasters has been reported in the genera *Periphragella* (belonging to the subfamily Chonelasmatinae) and *Lefroyella* (belonging to the subfamily Euretinae). Despite of their similarity in funnel-like body form, the new species clearly cannot be assigned to these two genera due to its absence of dichotomous branching tubes or lateral oscula. Also, it should be noted that the new species does not group with *C. bispinula* or *Lefroyella decora* in the phylogenetic tree (described below; **Figure 5**).

Molecular Phylogenetic Analysis of New Sponge Species

The lengths of 18S rDNA, 28S rDNA, 16S rDNA sequences and COI gene were confirmed by universal PCR primers as noted in the Methods section. The sequences of the PCR primers were located in the relevant base position of the marker genes correctly. The dataset of 18S rDNA, 28S rDNA, 16S rDNA partial sequence and COI complete sequence of *Flavovirens hemiglobus* gen. et sp. nov. (holotype) contained 1954 bp, 1233 bp, 494 bp and 1274 bp, respectively (deposited in GenBank with accession numbers OK245429, OK253063, OK245431 and OK255492, respectively); the same four marker genes of *Chonelasma tyloscopulatum* sp. nov. (holotype) contained 735 bp, 1239 bp, 540 bp and 709 bp, respectively (deposited in GenBank with accession numbers OK245428, OK253062, OK245430 and OK254902, respectively).

The ML tree was reconstructed based on the concatenated alignment file of the two new species and 41 other reference species of hexactinellid sponges (**Figure 5**).

The phylogenetic relationship was largely congruent to that of glass sponges in a previous study (Dohrmann, 2019). Flavovirens hemiglobus gen. et sp. nov. grouped well with the other Bolosominae. It should be noted that this new genus grouped with the other two specimens which were assigned to undetermined species of genus Bolosoma but no morphological evidence present. More molecular and morphological data should be collected to further study the relationship between the new genus and the genus Bolosoma. For the euretid genera, it is confirmed that there are currently two separate branches (Dohrmann, 2019); Chonelasma tyloscopulatum sp. nov. grouped with the branch consisting of Bathyxiphus and Chonelasma and mixing with Homoieurete and Sarostegia from Sceptrulophora incertae sedis. Moreover, Chonelasma tyloscopulatum sp. nov. grouped with another genus Bathyxiphus rather than the congeneric species (C. bispinula SMF 12084), which showed that this genus currently is not monophyletic. In addition to the difference in spiculation, especially

for the presence of tyloscopules in the new species, it indicated that either *Chonelasma* is not a valid genus, or the new species might have to be classified in a new genus in future revisions.

CONCLUSIONS

According to both morphological and molecular assessments, these two specimens described here are both clearly new to science, with one species attributed to a new genus of the family Euplectellidae (named *Flavovirens hemiglobus* gen. et sp. nov.) and the other species to the genus *Chonelasma* of the family Euretidae (named *Chonelasma tyloscopulatum* sp. nov.). It should be noted that the new genus *Flavovirens* grouped with two undescribed specimens (currently assigned to the genus *Bolosoma*); the relationship of the two genera should be further studied with more molecular and morphological data. Also, the new *Chonelasma* species, with the presence of tyloscopules, did not group with the congeneric species (*C. bispinula* SMF 12084), implying that *Chonelasma* may be not a valid genus. Otherwise,



FIGURE 5 | Maximum-likelihood phylogenetic tree of 43 species of hexactinellid sponges interred from the concatenated alignment of four molecular markers (18 rDNA, 28S rDNA, 16S rDNA sequences and COI gene) with RAxML. Scep. inc. sed. refers to Sceptrulophora *incertae sedis*. Bootstrap values are shown next to nodes (based on 1000 replicates).

the new species might have to be classified in a new genus in future revisions. More *Chonelasma* spp. should be sampled for molecular systematics to clarify this issue.

As important components of deep-sea ecosystems, hexactinellid sponges have still not been sufficiently collected, described, or identified. The two specimens collected in this study as the representative morphotypes of sponges were both identified new to science, which further indicated the shortage of sampling efforts in the deep sea. Limited sampling efforts should be conducted to consider both the morphological typicality of samples and their diversity, so as to assist in improving the understanding of the biodiversity of seamount sponges and to provide a reference status for the conservation and management of vulnerable marine ecosystems on seamounts.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below:

NCBI (accession: OK245429, OK253063, OK245431, OK255492, OK245428, OK253062, OK245430 and OK254902).

AUTHOR CONTRIBUTIONS

CS and CW conceptualized the study. CS, HC, and DZ (chief scientist) participated in the research cruise and collected

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specimens. CS performed the sponge identification. HC performed the molecular phylogenetic analysis. CS and CW wrote the manuscript with contributions from HC and DZ. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022. 852498/full#supplementary-material

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