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RESEARCH ARTICLE

On the taxonomic position of the genus *Parahigginsia* (Porifera: Demospongiae), with the description of a new species and the first record of the genus from the North Pacific

О таксономическом положении рода *Parahigginsia* (Porifera: Demospongiae), с описанием нового вида и первым указанием этого рода из северной части Тихого океана

V.A. Shilov, A.D. Kukhlevskiy & E.A. Nefedova

В.А. Шилов, А.Д. Кухлевский, Е.А. Нефедова

Vladimir A. Shilov, A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevskogo St., Vladivostok 690041, Russia. E-mail: shilvl@yandex.ru

Andrey D. Kukhlevskiy, A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevskogo St., Vladivostok 690041, Russia. E-mail: ad.kukhlevskiy@gmail.com

Ekaterina A. Nefedova, Zoological Institute, Russian Academy of Sciences, 1 Universitetskaya Emb., St Petersburg 199034, Russia. E-mail: ekaterina.nefedova@zin.ru

Abstract. A new species of the genus *Parahigginsia* Dendy, 1924 from the southern slope of the underwater Piip Volcano in the western Bering Sea, found at a depth of 981 m, is described. Previously, only two species of this genus were known, *P. phakelloides* Dendy, 1924 from the South Pacific and *P. strongylifera* Van Soest, Meesters et Becking, 2014 from the Caribbean. The discovery of *P. beringiensis* **sp. nov.** in the North Pacific extends the known distribution and depth ranges of *Parahigginsia*. Morphological comparison of the species from this genus has been conducted. The results of morphological examination and sequencing of the 18S and 28S rRNA genes indicate that the new species belongs to the order Suberitida and, most likely, to the family Halichondriidae.

Резюме. Новый вид рода *Parahigginsia* Dendy, 1924 описан с южного склона подводного вулкана Пийпа в западной части Берингова моря с глубины 981 м. Ранее были известны лишь два вида этого рода: *P. phakelloides* Dendy, 1924 из южной части Тихого океана и *P. strongylifera* Van Soest, Meesters et Becking, 2014 из Карибского бассейна. Находка *P. beringiensis* **sp. nov.** в северной Пацифике расширяет сведения о распространении и диапазон глубин для рода *Parahigginsia*. Проведено морфологическое сравнение видов рода. Результаты морфологического исследования и секвенирования генов 18S рРНК и 28S рРНК указывают на то, что новый вид принадлежит отряду Suberitida и, скорее всего, к семейству Halichondriidae.

Key words: Northwest Pacific, Bering Sea, Piip Volcano, Porifera, Demospongiae, Suberitida, Halichondriidae, *Parahigginsia*, new species

Ключевые слова: Северо-Западная Пацифика, Берингово море, вулкан Пийпа, Porifera, Demospongiae, Suberitida, Halichondriidae, *Parahigginsia*, новый вид

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Introduction

In recent decades, spongiologists have collected a tremendous amount of material from the North Pacific, including the Bering Sea. State-of-the-art equipment for deep-sea exploration such as human-occupied vehicles (HOV) and remotely operated vehicles (ROV) has made even the greatest depths accessible to researchers. Lehnert with co-authors (2005–2020) published a substantial number of papers on sponges from the Aleutian Islands and adjacent waters with descriptions of species living/found both in the shelf zone and at greater depths, including 30 new species. Lehnert et al. (2005) recorded a new demosponge species from a depth of 1357 m and described (Lehnert et al., 2006) nine new demosponge species from depths to 2827 m. Reiswig & Stone (2013) identified 13 new hexactinellid species from depths to 2311 m. Three demosponge species were described from the eastern Bering Sea (off the Pribilof Islands) (Lehnert et al., 2008; Lehnert & Stone, 2017). Recently, an inventory of sponges from the eastern Bering Sea has been carried out, which resulted in the identification of a new demosponge species (Stone et al., 2019). In addition, four new species and a new genus of Demospongiae have been described from the Aleutian Islands (Lehnert & Stone, 2020a, 2020b).

The 22nd research cruise aboard the research vessel (R/V) "Akademik Mstislav Keldysh" in 1990 and the 75th and 82nd cruises aboard the R/V "Akademik M.A. Lavrentyev" in 2016 and 2018 yielded rich materials from the area of the Vulkanologov Massif, including the underwater Piip Volcano, the Bering Sea. Those cruises provided ample materials for the descriptions of new taxa of Hexactinellida and Demospongiae. Tabachnick (2002) described a new genus, *Ijimaiella* Tabachnick, 2002 (Hexactinellida: Euplectellidae). Later on, additional data on *Ijimaiella* were obtained, with new findings and observations in vivo (Tabachnick et al., 2022). Menshenina & Tabachnick (2022) described the genus *Docosaccus* Menshenina et Tabachnick, 2022 (Hexactinellida: Euplectellidae). Tabachnick et al. (2023) provided information on the family Rossellidae from the Bering Sea and off the Pacific part of Bering Island and described one subspecies and three species of this family. Shilov et al. (2023) described four new species of Vulcanellidae (Demospongiae: Tetractinellida).

A total of 55 Demospongiae species belonging to 22 genera and 15 families were found in the material collected from the Piip Volcano slopes. Among them is an unknown representative of the genus *Parahigginsia* Dendy, 1924.

Parahigginsia and its type species, *P. phakelloides* Dendy, 1924, were described from New Zealand (Dendy, 1924). The species was later found off New Caledonia (Lévi & Lévi, 1983). The genus remained monotypic for a long time, and only recently, another species has been described from the Southern Caribbean, *P. strongylifera* Van Soest, Meesters et Becking, 2014. The third species, *P. beringiensis* **sp. nov.**, which we have collected on the slope of the underwater Piip Volcano in the Bering Sea, is the only known representative of the genus from the North Pacific. *Parahigginsia* is currently assigned to the family Heteroxyidae of the order Axinellida (De Voogd et al., 2023). The aim of this study is to describe the new species and to assess the taxonomic position of the genus *Parahigginsia* using morphological and molecular data.

Material and methods

Study area and sampling. The material was collected during the 82nd research cruise aboard the R/V "Akademik M.A. Lavrentyev" in 2018. That expedition was carried out in the area of the Vulkanologov Massif and the underwater Piip Volcano, Bering Sea. All samples were collected from one locality using a ROV "Comanche 18" (Sub-Atlantic, UK) on the southern slope of the underwater Piip Volcano (Figs 1, 2A). During the ROV dives, photography and video filming were conducted. The material was fixed in 96% ethanol on board. After the cruise, the collected material was deposited at the museum (MIMB) of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Vladivostok (NSCMB FEB RAS).

Microscopy. Spicule and skeleton mounts for optical microscopy (ZEISS Primostar 3) were made according to standard methods (Hooper, 2003; Van Soest & Hooper, 2005). For scanning electron microscopy (SEM, ZEISS Sigma

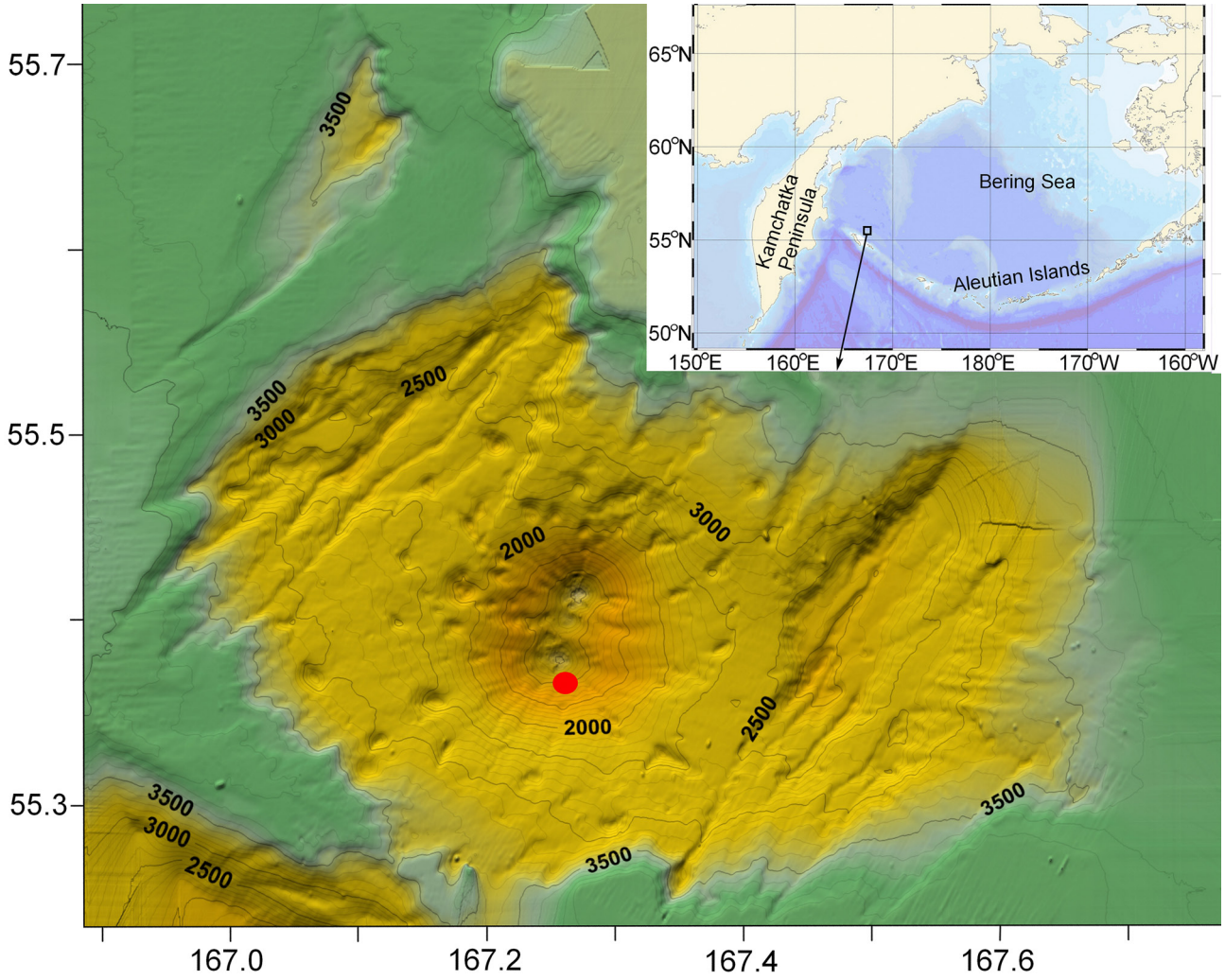

Fig. 1. Map of the study area, with the sampling locality of *Parahigginsia beringiensis* **sp. nov.** indicated by a red circle.

300 VP), a suspension of spicules and the sponge fragment were applied onto a microscope stub, and, after drying, were carbon sputter-coated. The examination was conducted at a voltage of 10 kV.

The material was processed and images were taken using a ZEISS Primostar 3 optical microscope and a ZEISS Sigma 300 VP scanning electron microscope at NSCMB FEB RAS. The sponge specimens were photographed in the laboratory.

Micro-computed tomography. Without denying the value of the imaging techniques used in this study of sponges such as embedding in epoxy resin or paraffin, we here demonstrate that micro-computed tomography (micro-CT) provides a greater array of data. It allows the most informative "virtual" slices of the object under study to be selected and located in any desired plane. Moreover, preparation of samples for micro-CT is simple and convenient, especially in order to examine relative positions of spicules in the skeleton. The samples kept in 96% ethanol were air-dried at 50–70% air humidity for about 4–5 h without pretreatment. The drying time was selected empirically. As our experience showed, this factor is important for providing high quality of the final image. Scanning was performed using a Neoscan N80 system with the following parameters: source voltage of 40 kV, source current of 120 mA, image pixel size of 2–3.5 μm, no filter, and rotation step of 0.2°. All samples were rotated 360°. The scanning included 800 to 1,000 sections, from which X-ray images were recalculated into density sections

displaying the structure of the scanned specimens using the SkyScan NRecon and DataViewer software packages. The study was carried out using the equipment of the Core Facilities Centre "Taxon" at the Zoological Institute, Russian Academy of Sciences (ZIN RAS, St Petersburg, Russia).

Molecular analyses. DNA was isolated from fragments of specimen MIMB47653 fixed in 96% ethanol using a PureLink™ Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. The primers used for the amplification of 18S and 28S rRNA and ITS1–5.8S–ITS2 gene fragments are listed in *Electronic supplementary material 1* (see Addenda).

The amplification reaction was carried out in 10 μL of a mixture of DreamTaq polymerase (ThermoFisher) (0.1 U/μL) and 1× Taq Turbo buffer (Evrogen), forward and reverse primers at a concentration of 0.25 μM each and 0.2 mM dNTPs each in the Verity thermocycler (Applied Biosystems). Thermocycling conditions were as follows: an initial denaturation step of three minutes at 95 °C, followed by 35 cycles of denaturation at 95 °C for 20 seconds, annealing at the temperature presented in *Electronic supplementary material 1* for 30 seconds, and extension of 72 °C for one minute 30 seconds. This was followed by a final extension step of 72 °C for seven minutes and holding at 4 °C. The quality and quantity of PCR products were checked by electrophoresis in 1.5% agarose gel. Amplified DNA fragments were purified with ExoSap-IT (ThermoFisher) under the conditions recommended by the manufacturer. Amplicons were used as a template for the sequencing reaction using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). For the sequencing reaction, the same primers were used as for the amplification. Sequence reaction products were purified from unreacted terminators on Sephadex G-50 columns (GE Healthcare). Dried samples were dissolved in formamide and subjected to electrophoresis in a GA-3500 Genetic Analyser (Applied Biosystems). Sequences were assembled using the Geneious R11 program (https://www.geneious.com/) and deposited in the NCBI/GenBank database under the accession numbers PP266643 and PP250158 (for the 18S and 28S rRNA genes, respectively).

Sequences from NCBI/GenBank for the phylogenetic analysis were accessed using the Unipro UGENE v.51.0 program (https://ugene.net/). For 18S rRNA sequences, two types of inquiries were made: (1) "Heteroscleromorpha" AND "18S"; (2) "Heteroscleromorpha" AND "small" AND "subunit" AND "RNA". For 28S rRNA sequences, the inquiries were, respectively, as follows: (1) "Heteroscleromorpha" AND "28S"; (2) "Heteroscleromorpha" AND "large" AND "subunit" AND "RNA". Duplicate sequences were removed from the analysis.

To identify the region to which the NCBI/GenBank-accessed 28S rRNA sequences of sponges from the subclass Heteroscleromorpha belonged, we assembled them using our sequence (PP250158) as a reference. For this, we used the Geneious R11 program ("Geneious" algorithm).

Our sequences and those accessed from GenBank were aligned in the Geneious program using the integrated MAFFT tool (Katoh & Standley, 2013). The nucleotide substitution model was calculated in the MEGA 7 program (Kumar et al., 2016). For both fragments (18S rRNA and 28S rRNA), the TrN+I+G model was used. Phylogenetic trees were constructed by the neighbour-joining (NJ), maximum likelihood (PhyML) and Bayesian Inference Analysis (BA) methods using Geneious R11 program.

To construct maximum likelihood (ML) trees with large numbers of sequences, IQTREE Web Server was used (http://iqtree.cibiv.univie.ac.at/).

The taxonomy used in the present study is in accordance with the World Porifera Database (De Voogd et al., 2024). The morphological terminology follows Boury-Esnault & Rützler (1997).

Results

Phylum **Porifera** Grant, 1836

Class **Demospongiae** Sollas, 1885

Subclass **Heteroscleromorpha**
Cárdenas, Pérez et Boury-Esnault, 2012

Order **Suberitida** Chombard et
Boury-Esnault, 1999

Family **Halichondriidae** Gray, 1867

Diagnosis (modified from Erpenbeck & Van Soest, 2002). Suberitida with oxeotes and stylotes, i.e. spicules taking variously shapes of oxeas,

Table 1. Comparative micrometric, habit, distribution and depth data for the species of the genus *Parahigginsia*.

Species	Oxeas	Acanthomicroxeas	Habit; distribution; depth
P. beringiensis **sp. nov.** (holotype, MIMB47653)	332.0–(515.8)–650.7 × 9.2–(14.2)–17.1	63.1–(82.1)–113.1 × 3.7–(4.4)–5.3	Encrusting; Bering Sea, Piip Volcano; 981 m
P. beringiensis **sp. nov.** (paratype MIMB47654)	259.0–(441.7)–610.9 × 9.2–(13.1)-16.6	66.1–(80.5)–105.8 × 3.2–(4.1)–5.8	Encrusting; Bering Sea, Piip Volcano; 981 m
P. beringiensis **sp. nov.** (paratype MIMB47655)	265.6–(497.7)–624.2 × 9.5–(13.4)–15.8	73.6–(92.8)–131.5 × 3.9–(4.8)–5.5	Encrusting; Bering Sea, Piip Volcano; 981 m
P. phakelloides Dendy, 1924 (original description)	340 × 22	88 × 2	Lamellate; New Zealand; 129.5 m
P. phakelloides Dendy, 1924 sensu Levi & Levi (1983)	350–(400)–420 × 18–(20)–25	100–120 × 2–3	Lamellate; Nouvelle-Calédonie; depth not given
P. phakelloides Dendy, 1924 sensu Hooper (2002)	280–355 × 18–23	78–95 × 1–2	Lamellate; Nouvelle-Calédonie; depth not given
P. strongylifera Van Soest, Meesters et Becking, 2014 (original description)	Strongyles: 290–(341)–370 × 10–(15)–18	75–(93)–120 × 1–(1.8)–2.5	Encrusting; The Caribbean Netherlands, Bonaire; 238 m, on volcanic outcrops
P. cf. *strongylifera* Van Soest, Meesters et Becking, 2014 sensu Van Soest (2017)	171–(196)–204 × 9–(11.1)–14	74–(83.3)–102 × 0.5–(1.1)–2	Crust; Suriname; 104–130 m

Note. Measurements are expressed in μm, as minimum–(mean)–maximum length × width values, whenever available.

strongyloxeas, oxeas with blunt ends, and true styles, occasionally with slightly expanded tyle near one of endings or more centrally. Spicules invariably smooth (with rare exceptions), often elongate, fusiform, but also equidiametrical over much of their length, with pointed apices normally elongated and gradually narrowing to sharp point, usually slightly curved, occasionally straight or rarely crooked or doubly angulated. Spicules typically of wide size range, sometimes without distinct size categories having no clear overlap; in this case, smaller sizes often concentrated at surface. Distinct ectosomal skeletal differentiation with either tangential crust of intercrossing spicules, often easily detached due to underlying subdermal spaces, or confusedly paratangential or palisade-like arrangement, in which case surface skeleton not easily detached and coming off in "flakes". Choanosomal skeleton confused, with many of megascleres seemingly randomly strewn and often with remnants of main spicule tracts and vague interconnecting spicules or tracts. Binding spongin rare. Spicule density sometimes quite high, and in combination with large sizes of megascleres, consistency often becoming hard and brittle.

Genus ***Parahigginsia*** Dendy, 1924

Type species: *Parahigginsia phakelloides* Dendy, 1924.

Diagnosis (modified from Hooper, 2002). Erect, lamellate, lobate growth or crust forms. Surface smooth, not hispid. Ectosome glabrous, containing only spongin and scattered acanthoxeas lying tangentially to surface. Extra-axial skeleton not clearly delineated from axis but consisting of irregularly anastomosing and ascending primary and secondary tracts of choanosomal oxeas not protruding through surface but becoming more ordered and compact near periphery, forming dense crust below ectosome. Choanosomal skeleton with condensed axial reticulation of oxeas forming irregular isotropic tracts, without distinct fibers or obvious collagenous spongin. Megascleres as smooth oxeas of single category; microscleres as curved acanthose microxeas.

***Parahigginsia beringiensis* sp. nov.**
(Figs 2–4)

Holotype. MIMB47653, R/V "Akademik M.A. Lavrentyev" cruise 82, Station LV82–7, sample 2–19, **Russia**, *Bering Sea*, southern slope of Piip Volcano, 55.3688°N 167.2662°E, 981 m, on skeletons of hexactinellid sponges, 17 June 2018, coll. V. Shilov (MIMB).

Paratypes. Two specimens: MIMB47654 (sample 2–12) and MIMB47655 (sample 2–17), with all other data same as for the holotype (MIMB).

Description. General appearance as in Fig. 2B–D.

Sponge form as thin crust covering skeleton of hexactinellid sponges, 2.5 × 2.5 cm in size and 0.5–1.5 mm thick. Colour light grey (in ethanol). Surface smooth, wavy, at thinnest parts repeating structure of substrate surface. Surface layer easily detached from base. Oscula invisible. Consistency soft.

Skeleton. Ectosomal skeleton (Fig. 3A, B) consisting of thin, non-dense layer of acanthomicroxeas supported by horizontally oriented choanosomal oxeas. Pores of 70–100 μm in diameter, visible in dermal membrane (Fig. 3C). Choanosomal skeleton halichondrioid (Fig. 3A, D–F), formed by loose bundles of oxeas and single oxeas. In some places, sponge overgrowing hexactinellid skeleton on both sides (Fig. 3E, F), but megascleres reaching into porous substrate to no more than 0.5–0.7 mm. Acanthomicroxeas, being also abundant in choanosome (Fig. 3E), present in deeper layers of porous substrate, but not everywhere. Sponge presumably growing flat over substrate surface and in its upper layers.

Spicules (Fig. 4A–C). Megascleres. Oxeas (Fig. 4A, B) straight or slightly curved, 259–(441.7)–610 × 9.2–(13.1)–16.6 μm. Acanthomicroxeas (Fig. 4C) slightly curved, 66–(80.5)–106 × 3.2–(4.1)–5.8 μm.

Morphological comparison. The three studied specimens have similar ectosomal skeleton structures. The spicule sizes of the specimens differ insignificantly (Table 1). They have been assigned to the genus *Parahigginsia* based on the presence of oxea megascleres and acanthomicroxea microscleres, organised as a dermal skeleton composed of acanthomicroxeas, and a choanosomal skeleton with tangled bundles of oxeas, as well as dispersed acanthomicroxeas.

In the body structure, the new species is closest to *P. strongylifera* sensu Van Soest, 2017.

There are similarities in the structure of the ectosomal skeleton of *P. beringiensis* **sp. nov.** with the other two species of *Parahigginsia*, *P. phakelloides* and *P. strongylifera*. In all three species, the skeleton is a thin crust of acanthomicroxeas lying on a layer of tangled choanosomal fibers and individual oxeas. The presence of acanthomicroxeas in the choanosome is also a common trait.

There are, nevertheless, differences in the structure of the choanosomal skeleton. In *P. phakelloides*, an isotropic reticulation of the choanosomal skeleton is present (Hooper, 2002: "...choanosomal skeleton a dense petrosiid-like isotropic reticulation of robust oxeas forming thick irregular tracts..."). In *P. strongylifera*, the isotropic reticulation is less pronounced (Van Soest et al., 2014: "...confused choanosomal reticulation (Fig. 15d)"). In *P. beringiensis* **sp. nov.**, the choanosomal skeleton is rather halichondrioid, formed by indistinct bundles of oxeas and single oxeas, and lacks any isotropic reticulation. In *Parahigginsia* spp., a relationship is observed between the sponge body shape (lamellate, lobate, or crustose) and the skeletal structure supporting this shape.

Acanthomicroxeas reach a greater thickness in *P. beringiensis* **sp. nov.**, whereas oxeas vary in length to a greater extent than those in other species of the genus (Table 1).

Molecular analysis. For specimen MIMB47653 (the holotype of the new species), we obtained the following nuclear DNA fragments: (1) a fragment, including an almost complete 18S rRNA nucleotide sequence (without beginning), the complete ITS1, 5.8S rRNA, and ITS2 sequences, the beginning of the 28S rRNA gene (about 70 nucleotides) (GenBank accession # PP266643); (2) a fragment of the 28S rRNA gene (approximately from nucleotide 800 to nucleotide 2150) (GenBank accession # PP250158). We could not amplify the sequence of the beginning of the 28S rRNA gene from this specimen. With an increase in the temperature of primer annealing, the amplification of the DNA fragment did not proceed; with a decrease, a fragment was obtained that, however, belonged to other organisms: fungi (*Malassezia restricta* Guého, Guillot et Midgley, 1996 and *Exophiala oligosperma* Calandron ex de

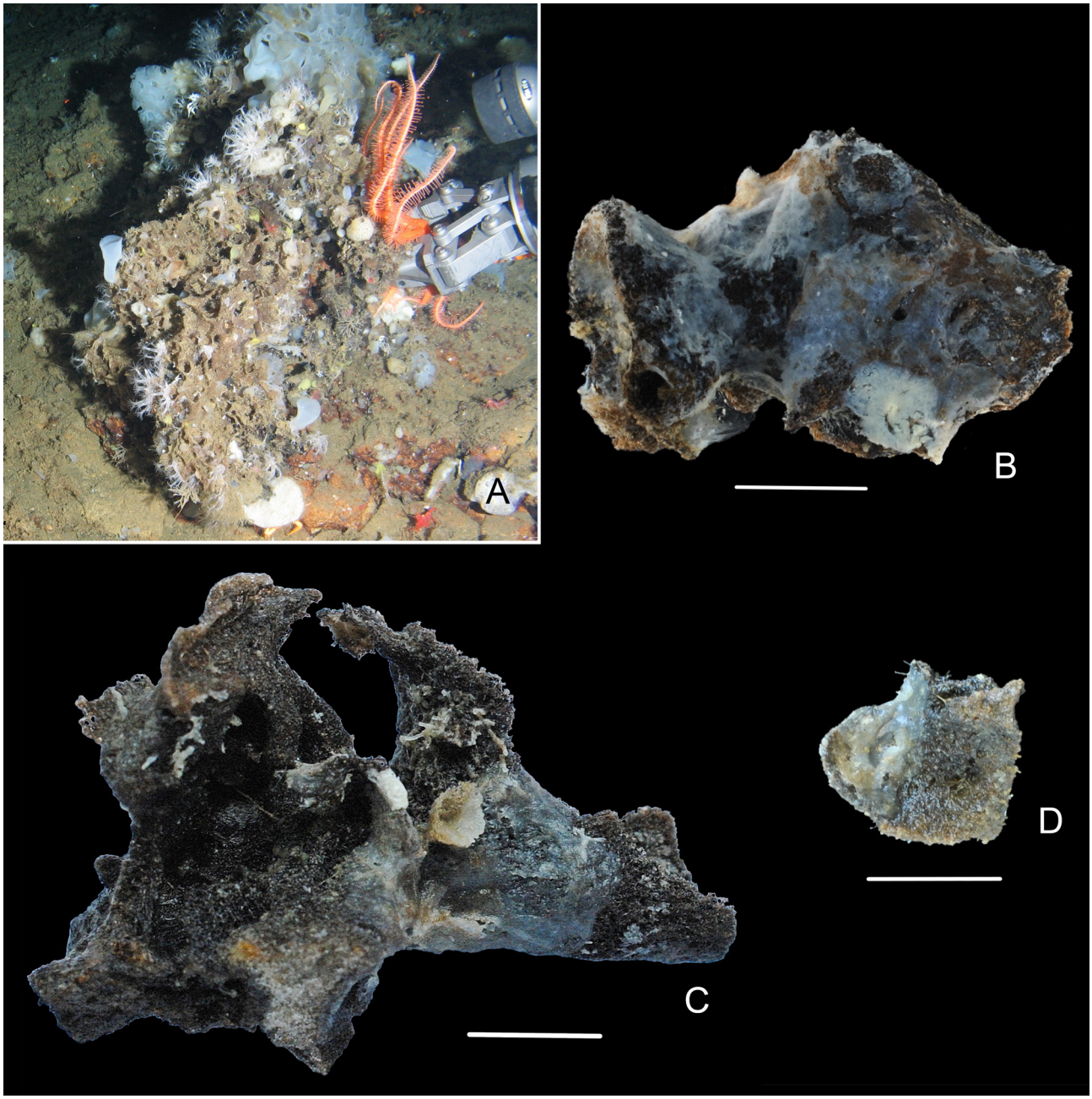

Fig. 2. *Parahigginsia beringiensis* **sp. nov.**, general view of the type specimens. **A**, aggregation of skeletons of hexactinellid sponges *Farrea* sp., on which specimens of *P. beringiensis* **sp. nov.** were found; **B**, holotype; **C**, paratype MIMB47654; **D**, paratype MIMB47655. Scale bars (B–D): 1 cm.

Hoog et Tintelnot, 2003), free-living flatworms (Prolecithophora), and gorgonian corals (whip coral, *Junceella fragilis* Ridley, 1884).

We attempted to find the closest matches for our specimen using the NCBI BLASTn algorithm. The closest matches for the 18S rRNA sequence of our specimen were *Homaxinella subdola* (Bowerbank, 1866) (KC901944) (Redmond et al., 2013) and *Halichondria* (*Halichondria*) *genitrix* (Schmidt, 1870) (AY348885) (Borchiellini et al., 2004), with a 98% similarity. These species belong to the order Suberitida. The 18S rRNA sequence of our specimen had a 97% homology with other representatives of the subclass Heteroscleromorpha (orders Clionaida, Merliida, Trachycladida, Bubarida, and Tethyida).

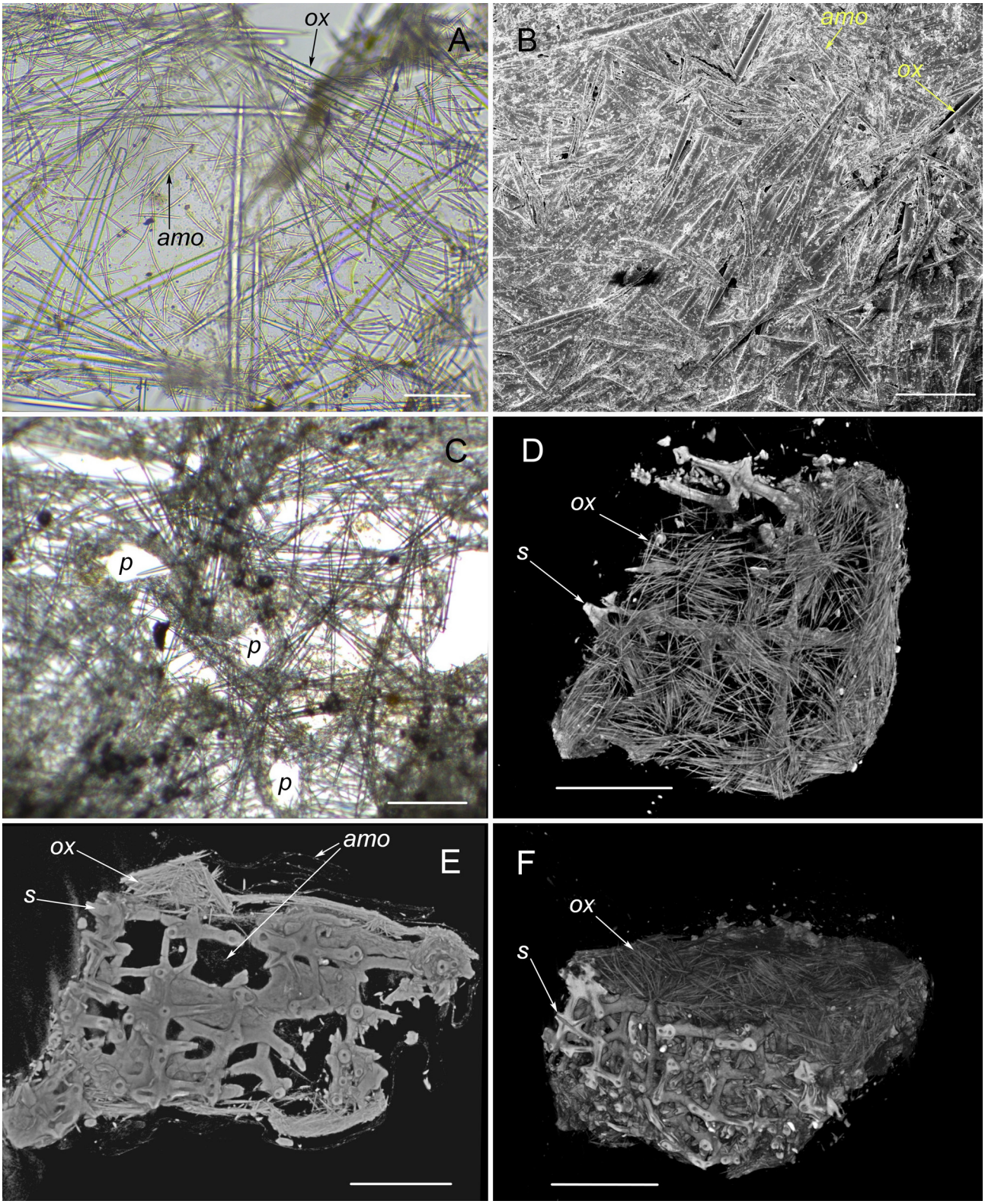

Fig. 3. *Parahigginsia beringiensis* **sp. nov.**, holotype (A, C – light microscopy images; B – SEM image; D–F – micro-CT images). **A**, ectosomal acanthomicrooxeas and supporting oxeas; **B**, surface; **C**, pores on surface; **D**, surface; **E**, skeleton, a section perpendicular to the surface; **F**, fragment of hexactinellid skeleton, overgrown by *P. beringiensis* **sp. nov.** on the surface of hexactinellid skeleton. Abbreviations: *amo* – acanthomicrooxeas; *ox* – oxeas; *s* – hexactinellid skeleton; *p* – pores. Scale bars: 100 μm (A, B), 200 μm (C), 1 mm (D, E, F).

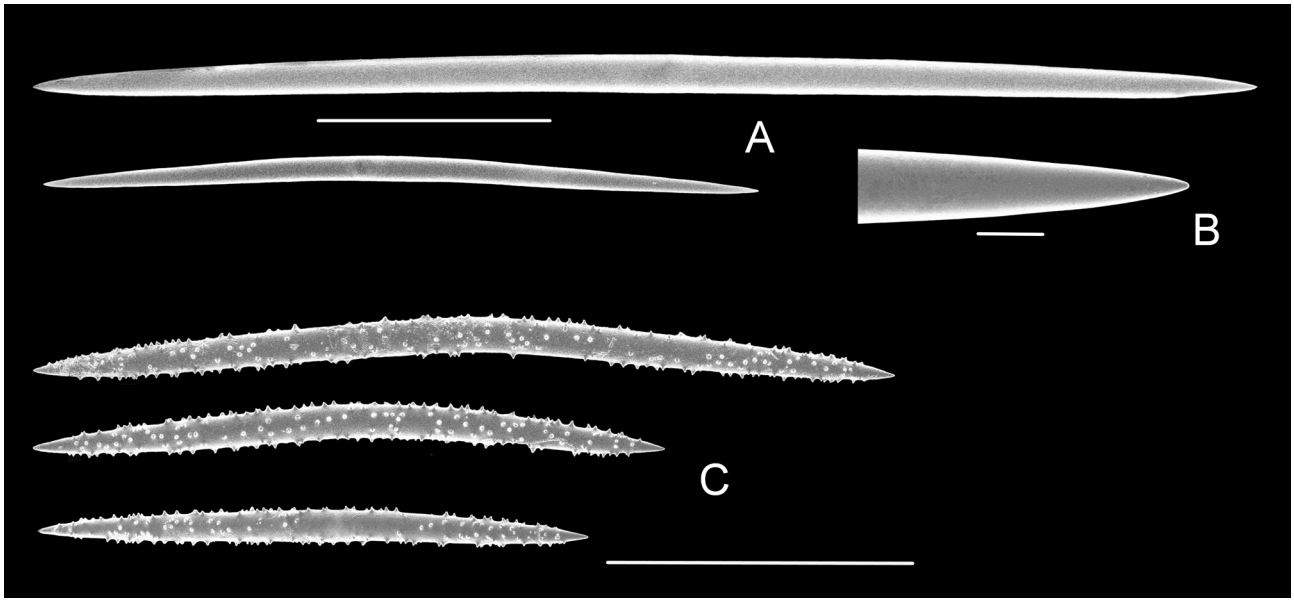

Fig. 4. *Parahigginsia beringiensis* **sp. nov.**, SEM images of spicules. **A**, oxeas; **B**, detail of the extremity of oxea; **C**, acanthomicroxeas. Scale bars: 100 μm (A), 10 μm (B), 50 μm (C).

An analysis of the 28S rRNA sequence revealed a 97% homology with the representatives of the order Suberitida: *Terpios aploos* de Laubenfels, 1954 (KC869465) (Thacker et al., 2013), *Hymeniacidon heliophila* (Wilson, 1911) (KC869620) (Thacker et al., 2013), *Pseudosuberites* sp. (AY561917) (Nichols, 2005), *Suberites domuncula* (Olivi, 1792) (AJ620113) (Perovic-Ottstadt et al., 2004), and *S. latus* Lambe, 1893 (OR129860–OR129863) (Turner et al., 2024). A slightly lower homology (96%) was revealed with representatives of other orders of the subclass Heteroscleromorpha.

The ITS1–5.8S rRNA–ITS2 region of our specimen showed homology with similar sequences of sponges of the order Suberitida (although only 80% and lower, probably because of the large variability of this region within the whole genome).

In order to further clarify the taxonomic position of our specimen, we accessed the 18S rRNA nucleotide sequences of all the representatives of the subclass Heteroscleromorpha longer than 1,500 bp from the NCBI database. Some sequences were subsequently rejected because of multiple unidentified bases or long gaps. Ultimately, there were 667 remaining 18S rRNA sequences of sponges of the subclass Heteroscleromorpha, including ours. The sequences were then aligned in the MAFFT program (included in Geneious) using the "Auto" and "E-INS-I" algorithms. In both cases, the obtained alignments were not entirely satisfactory to us (sometimes the gaps were not quite good, and the nucleotide bases were not correctly aligned with each other). However, we could not make manual adjustments to such a large matrix, as the obtained data could have become distorted or inaccurate. After constructing the phylogenetic trees based on both alignments using different software and algorithms [Geneious R11 (NJ) and IQTREE Web Server (http://iqtree.cibiv.univie.ac.at/) (ML)], we obtained the trees in which the same sequences clustered with ours in the same branch (phylogenetic trees are included in *Electronic supplementary material 2*; see Addenda).

A fragment of this tree containing the sequence under study is shown in Fig. 5. As can be seen in the cladogram, our specimen appears on the branch where the representatives of the order Suberitida (Suberitidae/Halichondriidae) are clustered [with *Homaxnella subdola* (KC901944) (Redmond et al., 2013) and *Halichondria genitrix* (AY348885) (Borchiellini et al., 2004)], being basal and sister to this cluster. It should be noted that in the presented cladogram, the families Suberitidae and Halichondriidae are not grouped into separate clades but are arranged rather chaotically.

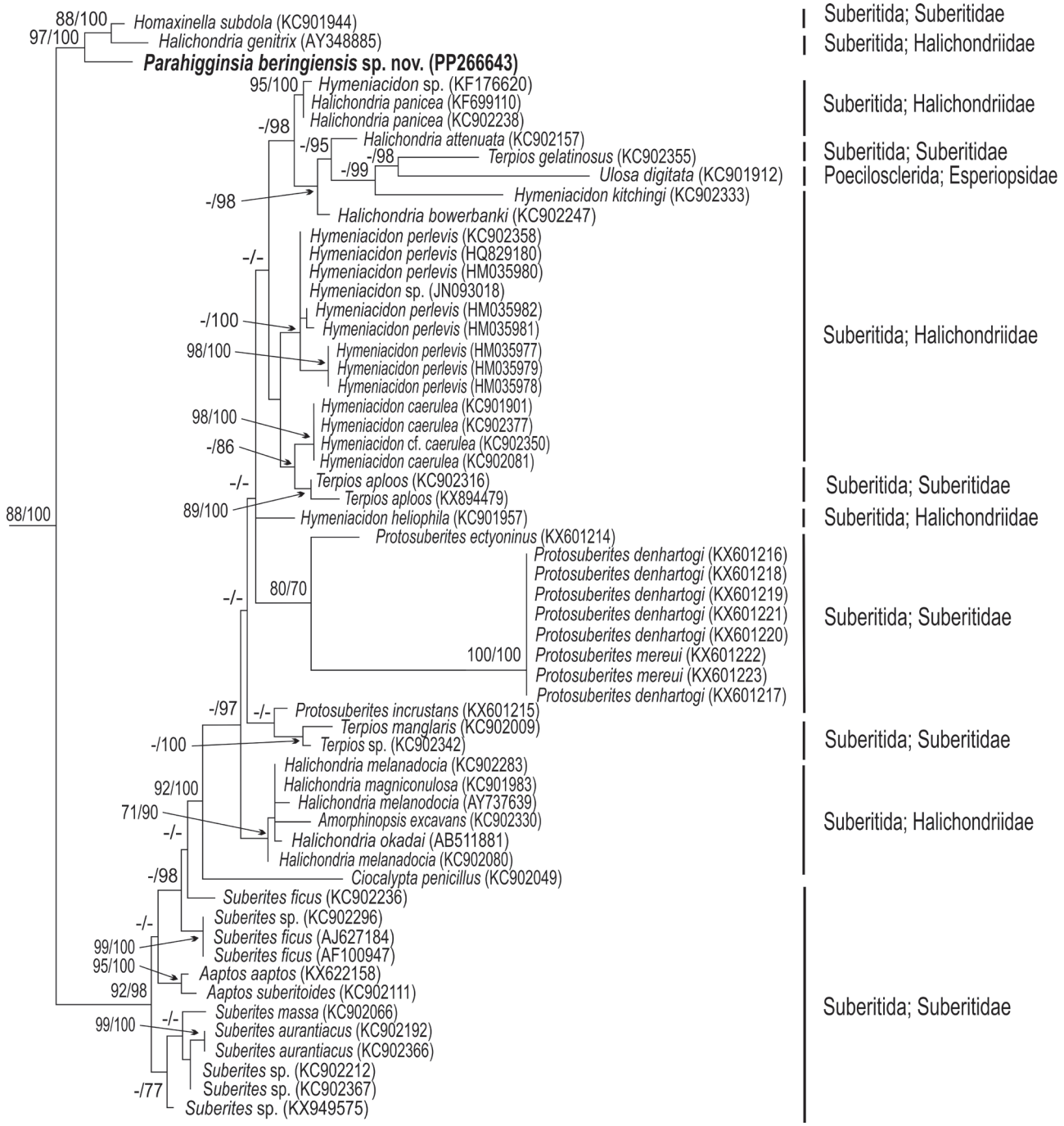

Fig. 5. A fragment of phylogenetic tree of the subclass Heteroscleromorpha (branch with our specimen) inferred from the analysis of the 18S rRNA gene using the NJ and ML methods (see Addenda: *Electronic supplementary material 2*, for the complete tree). Numbers at nodes are bootstrap supports for NJ/ML trees (values greater than 70).

To analyse the 28S rRNA gene of our specimen, we downloaded all the sequences of this gene for the subclass Heteroscleromorpha from NCBI/GenBank. For this, we used the Unipro UGENE v.51.0 program (https://ugene.net/). Thus, we obtained a total of 5345 sequences of the 28S rRNA gene for the subclass Heteroscleromorpha. These sequences belonged to different regions of the 28S rRNA gene and, moreover, authors frequently missed indicating the part of the gene they sequenced. Therefore, direct alignment seemed challenging. To select the sequences suitable for

the analysis, we acted as follows: in the Geneious program, we assembled all sequences of the 28S rRNA gene from GenBank using our sequence (PP250158) (the Geneious algorithm) as a reference one. A total of 1132 sequences were included in the contig and 4213 did not get in it. The sequences beyond the contig belonged either to the beginning or to the end of this gene (the sequence we obtained covered region D3–D8) or showed a homology below 85% with ours. As a result, we had two regions of maximum overlap: (1) from the beginning of our sequence (region D3–D5), approximately 650 nucleotides; (2) with a small gap from the first overlap, a region spanning about 670 nucleotides (region D6–D8).

We extracted the sequences covering region D3–D5 of the 28S rRNA gene, removed the short ones (the ends of the sequences whose major part were in another region), aligned them using the MAFFT-E-INS-I algorithm, and trimmed the protruding ends. As a result, we obtained an alignment of 520 sequences of 656 bp in length (including gaps). We then built a ML tree using the IQTREE Web Server (*Electronic supplementary material 3*; see Addenda). As it shows, our specimen is on the same branch with the representatives of the order Suberitida.

We did the same with the second overlap region (D6–D8 of the 28S rRNA gene). After all the manipulations, we obtained an alignment of 673 bp including 233 sequences and built a ML tree using the IQTREE Web Server (*Electronic supplementary material 4*; see Addenda). In it, our specimen also appears on a branch where the representatives of the order Suberitida are mainly clustered.

At last, we selected the sequences that most fully overlapped our 28S rRNA sequence. As a result, the alignment contained 82 sequences and amounted to 1282 bp (with gaps). We built BA and ML (PhyML) trees in the Geneious program. The results are presented in *Electronic supplementary material 5* (28S rRNA_D3–D8-region). As can be seen, our specimen falls in the cluster Suberitida.

Etymology. The specific epithet is an adjective referring to the region of finding, the Bering Sea.

Distribution and habitat. The new species is known only from the type locality at a depth of 981 m in the Bering Sea, at the southern slope of Piip Volcano (55.3688°N 167.2662°E). The substrate is skeletons of hexactinellid sponges. It should also be noted that the depth at which the type specimens of *P. beringiensis* **sp. nov.** were collected (981 m) was much greater than the depths of findings of *P. phakelloides* and *P. strongylifera* (122 and 238 m, respectively).

Discussion

Dendy (1924) originally placed *Parahigginsia* in the subfamily Axinellinae Lendenfeld, 1889, section Heteroxyeae Dendy, 1922. Laubenfels (1936) transferred *Parahigginsia* to the family Jaspidae Laubenfels, 1936, in the subfamily Rhaphidistinae Laubenfels, 1936 that he erected. Bergquist (1970) considered this transfer erroneous and placed *Parahigginsia* in Desmoxyidae Hallmann, 1917, which was then supported by Lévi & Lévi (1983), Hooper (2002), and Kelly et al. (2009). Van Soest & Hooper (2005) used the name Heteroxyidae Dendy, 1905 for a group of genera formerly assigned to Desmoxyidae. Morrow et al. (2012) placed *Parahigginsia* in Stelligeridae Lendenfeld, 1898, but Van Soest et al. (2014) found the evidence insufficient for this and left *Parahigginsia* in the Heteroxyidae, to which the genus still belongs (De Voogd et al., 2024).

As can be seen in the ML trees that we constructed based on 18S and 28S rRNA sequences, our specimen clusters with the representatives of the order Suberitida. Based solely on the data obtained by DNA analysis, it is not yet possible to assign the genus *Parahigginsia* to any family of the order Suberitida. However, with the data of morphological analysis (spicule composition and skeletal structure) taken into account, we propose placing the genus *Parahigginsia* in the family Halichondriidae. For complete certainty, other species of this genus should be also sequenced. In addition, sequencing of mitochondrial genes (COI) is required.

Morrow et al. (2012) noted that the data obtained using the 28S rRNA and COI genes mainly reflect the current morphological classification, but may sometimes contradict it. It is worth noting that the specimen of *Ulosa digitata* (Schmidt, 1866), belonging to a different order,

Poecilosclerida, was present and clustered on the branch of interest (Suberitida) in almost all trees: 18S rRNA – KC901912; 28S rRNA (Part1_D3-D5) – HQ379295; 28S rRNA (Part2_D6-D8) – HQ379363. All these sequences belonged to the same specimen under the voucher number BELUM:Mc4523. It is unclear whether this resulted from misidentification, contamination during the analysis, or what Morrow et al. (2012) warned about.

The same applies to the 28S rRNA sequence of the specimen from the sponge *Haplosclerida* sp. (MN153806). In all trees based on 28S rRNA, it appeared along with our specimen in the cluster, while other representatives of this order were all absent from the tree (*Electronic supplementary materials 3, 4 and 5*; see Addenda) because they had homologies less than 85% with our sequence (no higher than 75%).

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Addenda

Electronic supplementary material 1

The list of the primers used and their annealing temperature in the amplification reaction. File format: PDF.

Electronic supplementary material 2

Phylogenetic trees of the subclass Heteroscleromorpha based on the sequences of the 18S rRNA gene using the NJ (Geneious) and ML (IQTREE Web Server) methods. File formats: NEWICK, SVG, as 7Z archive.

Electronic supplementary material 3

Phylogenetic tree of the subclass Heteroscleromorpha based on the sequences of the 28S rRNA gene (D3–D5 region) using the ML method (IQTREE Web Server). File formats: NEWICK, SVG, JPEG, as 7Z archive.

Electronic supplementary material 4

Phylogenetic tree of the subclass Heteroscleromorpha based on the sequences of the 28S rRNA gene (D6–D8 region) using the ML method (IQTREE Web Server). File formats: NEWICK, SVG, JPEG, as 7Z archive.

Electronic supplementary material 5

Phylogenetic tree of the subclass Heteroscleromorpha based on the sequences of the 28S rRNA gene (D3–D8 region) using the ML (IQTREE Web Server) and BA (Geneious) methods. Numbers at nodes are bootstrap supports for ML/BA trees. File format: JPEG, as 7Z archive.

All these materials are available from: https://doi.org/10.31610/zsr/2025.34.2.298

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