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Source: Zoological Science, 27(11) : 888-894

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.27.888

ZOOLOGICAL SCIENCE **27**: 888–894 (2010)

New Carnivorous Sponges of the Genus *Abyssocladia* (Demospongiae, Poecilosclerida, Cladorhizidae) from Myojin Knoll, Izu-Ogasawara Arc, Southern Japan

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Two new species of carnivorous sponges of the genus *Abyssocladia* are described. These sponges were collected from Myojin Knoll, Izu-Ogasawara (Izu-Bonin) Arc, in southern Japan. Detailed morphological observation based on specimen both in situ and preserved revealed functional differentiation of spicule distribution. *Abyssocladia natsushimae* sp. nov. is distinct within the genus in its mop-like gross morphology, large body size, and soft tissue packed with numerous microspined microstrongyles. *Abyssocladia myojinensis* sp. nov. is characterized by possession of both typical abyssochelae and palmate abyssochelae. This is the first record of the genus from Japan.

Key words: deep sea, diversity, new species, oligotrophic, Pacific, Porifera, ROV

INTRODUCTION

A unique carnivorous feeding habit is known in poecilosclerid sponges, classified mostly in the Cladorhizidae, but also for a few species in Guitarridae and Esperiopsidae (Vacelet and Boury-Esnault, 1995; Ereskovsky and Willenz, 2007; Vacelet, 2007). These sponges display a special morphology, with a symmetrical, stipitate body, possessing lateral processes lined with hook-like spicules. Furthermore, the aquiferous system and choanocyte chambers, characters that have been regarded to be diagnostic for the phylum Porifera (Hooper and van Soest, 2002), are absent in those carnivorous sponges, with the exception of *Chondrocladia* spp. (Kübler and Barthel, 1999).

Recent investigations have shown high species diversity of carnivorous sponges in the deep ocean, especially in the Pacific; most of the species collected by manned submersibles, remotely operated vehicles (ROV), or more classical trawling, have been undescribed (Lehnert et al., 2005; Vacelet, 2006, 2008; Reiswig and Lee, 2007; Vacelet et al., 2009). However, the carnivorous sponge fauna off Japan has been poorly prospected; only three species from this region have been reported to date: *Euchelipluma arbuscula* (Topsent, 1928) and *Chondrocladia yatsui* Topsent, 1930 from Sagami Bay, and *Chondrocladia magna* Tanita, 1965 from off Kushiro and Miyako (northeast Japan). None of these sponges have been rediscovered since their original descriptions, and their feeding habits are thus unknown.

The genus *Abyssocladia*, which a few years ago contained only three species and was considered as synonymous to *Chondrocladia*, has recently increased to seven species (Vacelet, 2006). Furthermore, at least five new species from New Zealand (Vacelet and Kelly, unpublished data) and one from the Antarctic Weddell Sea (Janussen and Plotkin, 2009, unpublished data) are in the process of description. All these *Abyssocladia* are well characterized by their gross morphology, as well as their spicule complement, typically with a large variety of unusual microscleres.

In 2008, the ROV 'Hyper-Dolphin' collected pedunculate sponges attributed to the genus *Abyssocladia* in the Izu-Ogasawara (Izu-Bonin) Arc, in the northwestern Pacific. We present here the description of two new species from deep Japanese waters.

MATERIALS AND METHODS

The sponges described in the present study were collected during the NT-08-07, Leg2 cruise of the R/V 'Natsushima' of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) carried out during the period of 11–17 April 2008 (Fig. 1). This cruise was organized as part of the research program "Verification of endemicity of animal species in hydrothermal vent and seamount on the Ogasawara Arc." The sponges were collected by the ROV 'Hyper-Dolphin' (Dive#820). Underwater pictures were taken by a camera equipped on the ROV. The specimens were preserved in 90% ethanol soon after landing of the ROV on the deck, and deposited in National Museum of Nature and Science, Tokyo (NSMT).

For observation of the surface structure of the sponge, scanning electron microscopy (SEM) using a JSM 5200LV microscope, was performed on few filaments of each sponge, cut from the main body, dried, and sputter-coated with gold–palladium.

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doi:10.2108/zsj.27.888

Dry fragments of the sponge tissue were digested in hydrogen peroxide, subsequently centrifuged and resuspended in distilled water three or four times to obtain clean spicules. Cleaned spicules were placed on glass slides, and embedded in mounting medium under a cover slip before observation under a light microscope. Spicules were also dried on a small circular cover slip, coated with 400Å of gold and observed by SEM. Spicules from the filament, the main body, and the surface of the peduncle were separately prepared from the same individual using the same procedure. The main axis and its cover could not be separated for *Abyssocladia myojinensis* sp. nov. due to its small size.

Spicules were measured with a calibrated ocular micrometer directly under a microscope. Measurements were carried out along randomly chosen transects across the slide, ignoring unfocused, broken, or malformed spicules. Thirty spicules for each spicule type were selected randomly for measurement. Spicule sizes are given as mean size, range, and standard deviation.

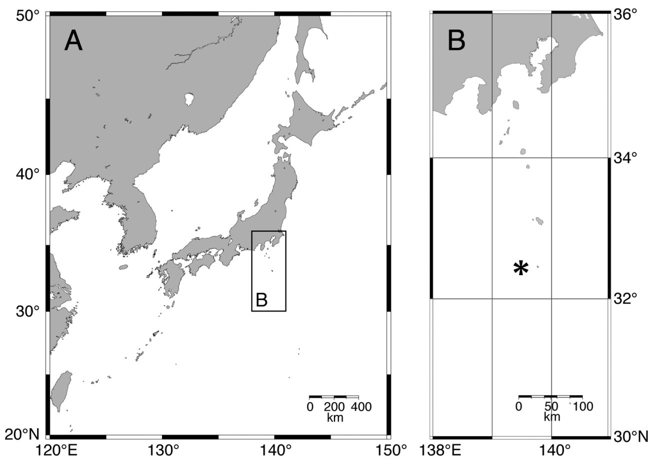

Fig. 1. Location of sampling site. Asterisk indicates the location of Myojin Knoll.

TAXONOMIC ACCOUNT

Order Poecilosclerida Topsent, 1928
Family Cladorhizidae Dendy, 1922
Genus *Abyssocladia* Lévi, 1964

Type species: *Abyssocladia bruuni* Lévi, 1964, fixed by monotypy.

Diagnosis: Cladorhizidae with abyssochelae and sigmancistras, most often pedunculate and disciform with a radial skeleton (Vacelet, 2006).

Abyssocladia natsushimae sp. nov.
(Figs. 2–5)

Material examined

Holotype: NSMT-Po-1892. Inside of caldera of Myojin Knoll, Izu-Ogasawara Arc, southern Japan (Fig. 1), 13 April 2008, 32°4.539′N, 139°51.095′E, 862 m, collected during NATSUSHIMA cruise, dive#820 of the ROV 'Hyper-Dolphin', attached on brown stone.

Etymology

The specific epithet refers to the R/V 'Natsushima' that operated the present cruise.

Distribution

This species is presently known only from type locality.

Description of holotype

Body. Mop-like, elongated, and flattened, with long, thin peduncle attached to solid substratum by enlarged base (Fig. 2A, B, D). Total length 88/82 mm including/excluding apical

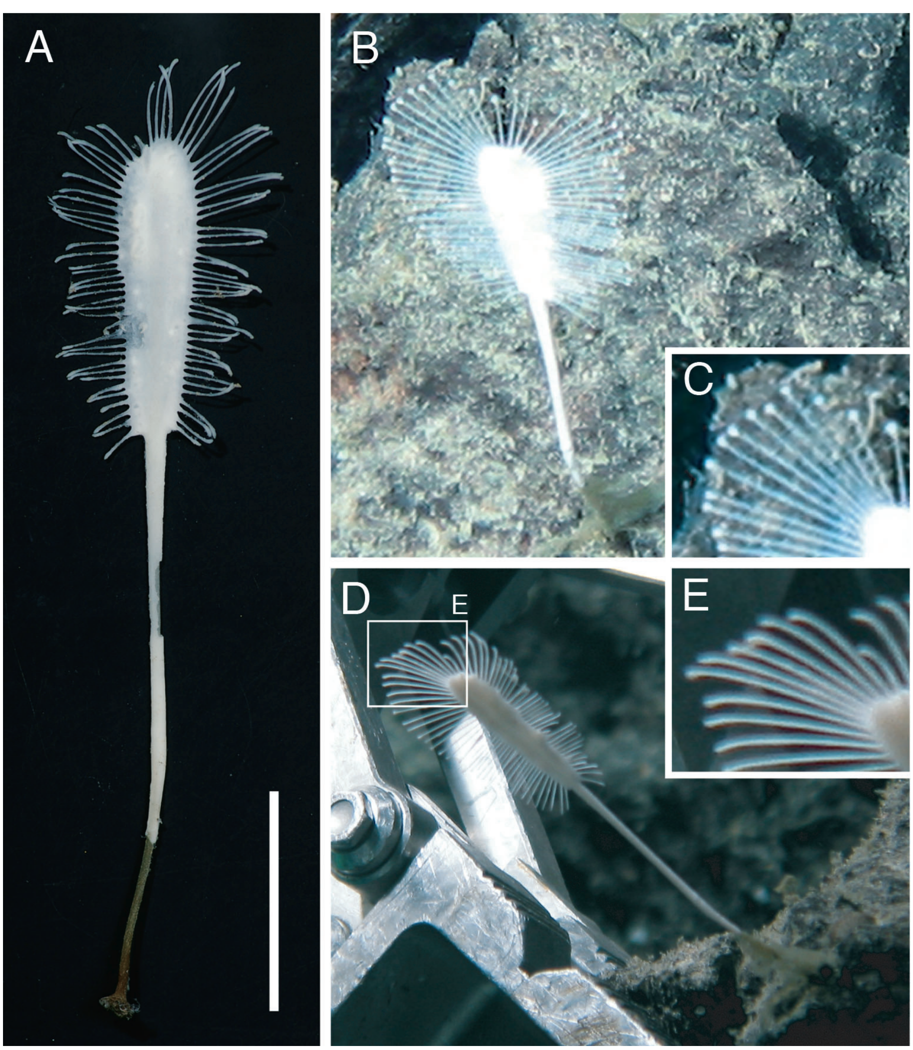

Fig. 2. *Abyssocladia natsushimae* sp. nov. **(A)** Preserved specimen of the holotype (NSMT-Po-1892). **(B)** Field image of the holotype. **(C)** Magnified view of tip of the filament before sampling. **(D)** Sampling of the holotype by ROV 'Hyper-Dolphin'. **(E)** Magnified view of distal end of the filament after sampling. Note distal ends of filaments are shrunk compared to Fig. 2C. Scale bar: 2 cm.

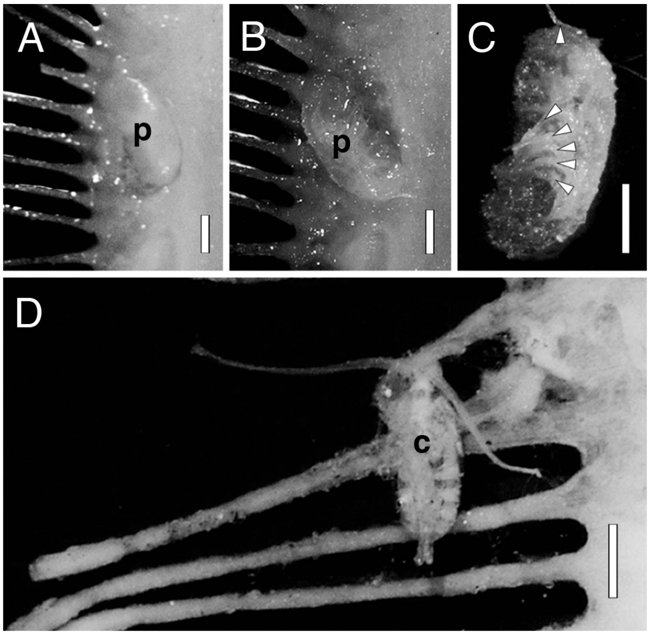

Fig. 3. Prey trapped by *Abyssocladia natsushimae* sp. nov. (Holotype, NSMT-Po-1892). **(A, B)** Partly detached prey from body of the sponge. **(C)** Detached prey. Multiple appendages are shown in arrowheads. **(D)** Copepod trapped by the filament of the sponge. p, prey. c, copepod. Scale bars: 1 mm.

filament, respectively. Peduncle 53.7 mm long, 1.7–2.2 mm thick, covered with soft tissue. Main body 28 mm long, 7.5 mm wide, and less than 1 mm thick; bilaterally symmetrical with radially arranged lateral and apical filaments; body surface with several unidentified small masses (most likely representing engulfed prey animals, including small crustaceans), covered by soft tissue (Fig. 3A–C). Single, nearly intact copepod found trapped by filament in fixed and preserved holotype (Fig. 3D). Filaments 77 in number, 2.8–9.7 mm in length (average 5.6 mm), 150–200 μm in width, covered by thin soft tissue. Tip of each filament inflated into small spherical swellings in situ (Fig. 2B, C), but shrank during capture of specimen with remote-controlled arm of ROV (Fig. 2D, E). No visible aquiferous system. Tissue pure white; lower part of peduncle without soft tissue brown. Base enlarged, 3.4 mm in maximum diameter.

Skeleton. Axis of peduncle consisting of tightly packed long styles longitudinally and spirally arranged, elongating to the center of the body. Upper part of peduncle covered by soft tissue packed with numerous microstrongyles and few abyssochelae. Basal enlarged part of peduncle composed of substrongyle and short microstrongyle. Main body covered by numerous microstrongyles, sigmancistras and abyssochelae; these abyssochelae being larger than those found in filaments. Axis of filaments consisting of bundles of styles in various lengths, covered by soft tissue heavily packed with microstrongyles and sigmancistras (Fig. 4A–E). Abyssochelae embedded in soft tissue, especially concentrated in distal (Fig. 4B, C) and proximal (Fig. 4E) portions of filaments, but only barely, or completely not, found in middle portion (Fig. 4D); tooth and alae of abyssochelae directed outward (Fig. 4C, E).

Spicules. Styles (mycalostyles) long and straight (Fig. 5A), almost uniform in width, except at both ends. Larger end blunt, thinner than shaft width (Fig. 5B). Tips acerate or blunt (Fig. 5C). In main body, 1657 ± 158 (1350–1940) μm in length and 24 ± 2.5 (19–26.5) μm in width; in filament very variable, 1016 ± 395.1 (395–1790) μm in length and 16 ± 4.1 (10–24) μm in width.

Substrongyle (Fig. 5D), fusiform,

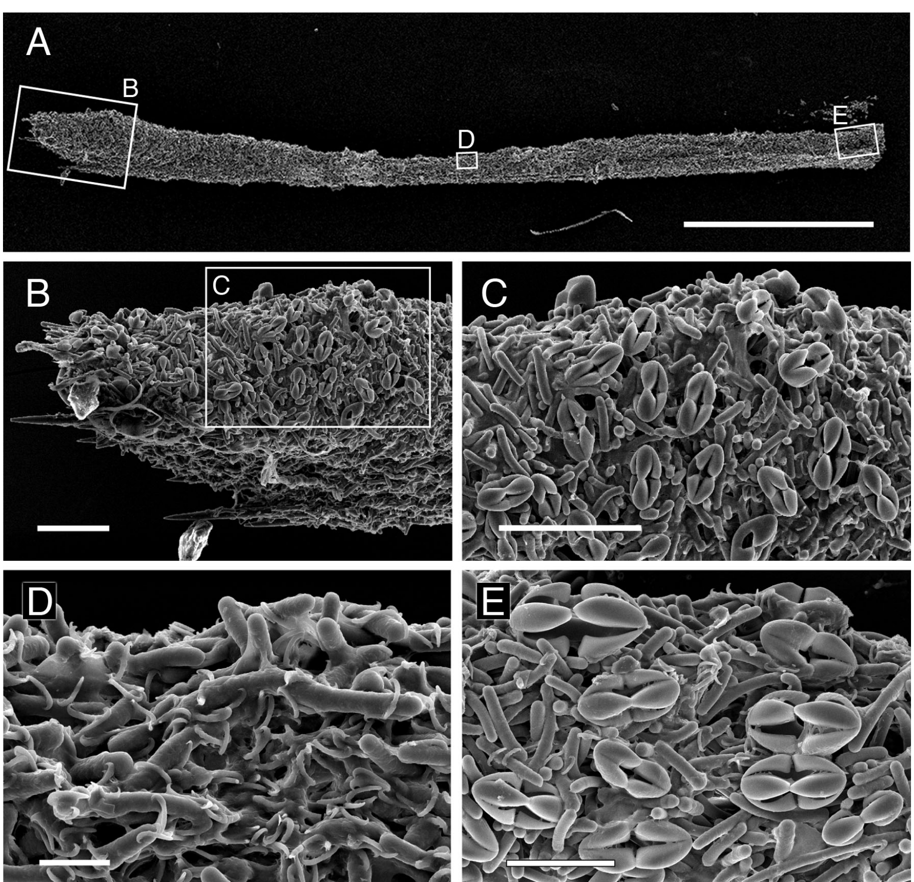

Fig. 4. SEM images of filament of *Abyssocladia natsushimae* sp. nov. (Holotype, NSMT-Po-1892). **(A)** View of single filament. **(B, C)** Magnified view of distal end of the filament. Abyssochelae arranged with the alae directed outward. **(D)** Magnified view of the shaft of the filament. Note there is no abyssochelae. **(E)** Magnified view of proximal end of the filament. Larger abyssochelae are arranged with their shaft embedded in soft tissue and the alae directed outward. Scale bars: 1 mm (A), 100 μm (B, C), 20 μm (D), 50 μm (E).

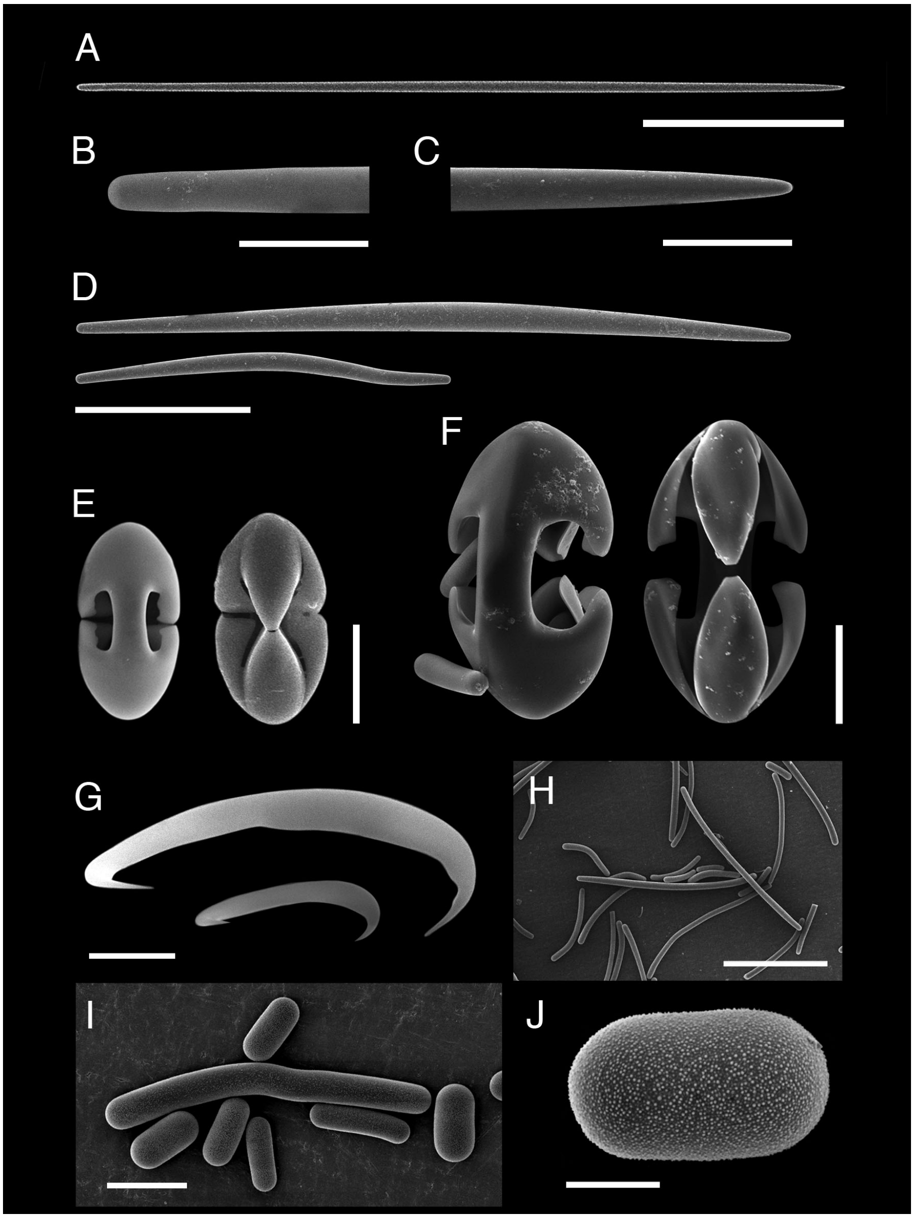

Fig. 5. SEM images of spicules of *Abyssocladia natsushimae* sp. nov. (Holotype, NSMT-Po-1892). **(A)** Style (mycalostyle). **(B)** Larger end of style. **(C)** Tip of style. **(D)** Substrongyles from enlarged attachment base. **(E)** Smaller abyssochelae. **(F)** Larger abyssochelae and microstrongyle. **(G)** Sigmancistra I (above) and sigmancistra II (below). **(H)** Microstrongyles in various sizes from filament. **(I)** Microstrongyles from cover of peduncle. **(J)** Short microstrongyle and view of the surface. Scale bars: 300 µm (A), 50 µm (B, C), 200 µm (D), 20 µm (E, F), 5 µm (G), 100 µm (H), 20 µm (I), 5 µm (J).

slightly bent at middle part, only distributed in enlarged base; 684 ± 172.1 (395–980) µm in length, 36 ± 6.2 (22–45) µm in width.

Abyssochelae with curved shaft (Fig. 5E, F). In smaller abyssochelae (Fig. 5E), frontal tooth, and lateral alae well developed, in contact with opposite tooth and alae, strongly laterally folded inside. In larger abyssochelae (Fig. 5F), tooth and alae not in contact with the opposite ones. Abyssochelae of intermediate size and shape also observed. All being oval in dorsal view; 54 ± 10.3 (38–75) µm in length, 9 ± 2.1 (6–12) µm in shaft width.

Sigmancistra I (Fig. 5G above), few; shaft slightly contorted, the larger end with a notch; 22 ± 1.4 (20–23) µm in length.

Sigmancistra II (Fig. 5G below), very abundant; shaft contorted; 10.7 ± 0.7 (9–12) µm in length.

Microstrongyles (Fig. 5H–J), very abundant, microspined (Fig. 5J), straight or slightly bent on various parts of shaft, very variable in length (Fig. 5H). Larger ones more appropriately referred to as strongyles; 64 ± 66.6 (14–250) µm in length, 6 ± 0.8 (4–10) µm in width. Microstrongyles in covering tissue of peduncle very short compared to those of other parts of sponge body (Fig. 5I).

Remarks

The possession of abyssochelae and sigmancistras clearly attributes the present species to the genus *Abyssocladia*. The present species is distinct within the known species of the genus in its mop-like gross morphology, the large size of its body, and its soft tissue packed with numerous microspined microstrongyles. Gross morphology of the currently known species of *Abyssocladia* can generally be divided into two categories. Sponges in the first category possess pedunculate, disciform bodies, including the following six species: *A. bruuni* Lévi, 1964, *A. claviformis* Koltun, 1970, *A. dominalba*, Vacelet, 2006, *A. huitzilopochtli* Vacelet, 2006, *A. inflata* Vacelet, 2006, and *A. oxeata* Koltun, 1970. The second comprises feather-like forms so far only known in *A. naudur* Vacelet, 2006, but a second representative of this category, *A. myojinensis* sp. nov. will be described in the following part of the present paper. The mop-like morphology of *A. natsushimae* can be included in the first category, as its gross morphology can be interpreted as an elongated disciform body with peduncle; however, it is clearly distinct from the congeners, which have more circular bodies.

The soft tissue packed with numerous microspined microstrongyles covering the surface of the sponge is another distinct feature in the genus. In addition to the present species (Fig. 5D), microstrongyles and/or substrongyles are reported from the basal part of the peduncle in e.g., *A. huitzilopochtlia* and *A. naudur* (Vacelet, 2006); these might serve to reinforce the attachment part of the peduncle to the substrate. However, *A. natsushimae* sp. nov. has a cover of numerous microstrongyles throughout the whole surface of the body which may contribute to its unusual large body size (88 mm in total length) compared to the other congeners (less than 45 mm). Such a cover of microspined spicules, rather difficult to categorize as either megascleres or microscleres, are known in *A. oxeata* and *A. inflata* (Vacelet, 2006), but they are double bent oxea instead of irregularly bent strongyles.

Abyssocladia myojinensis sp. nov.
(Figs. 6–7)

Material examined

Holotype: NSMT-Po-1893. Inside of caldera of Myojin Knoll, Izu-Ogasawara Arc, southern Japan (Fig. 1), 13 April 2008, 32°4.537′N, 139°51.056′E, 870 m, collected during NATSUSHIMA cruise, dive#820 of the ROV ‘Hyper-Dolphin’, attached on brown stone.

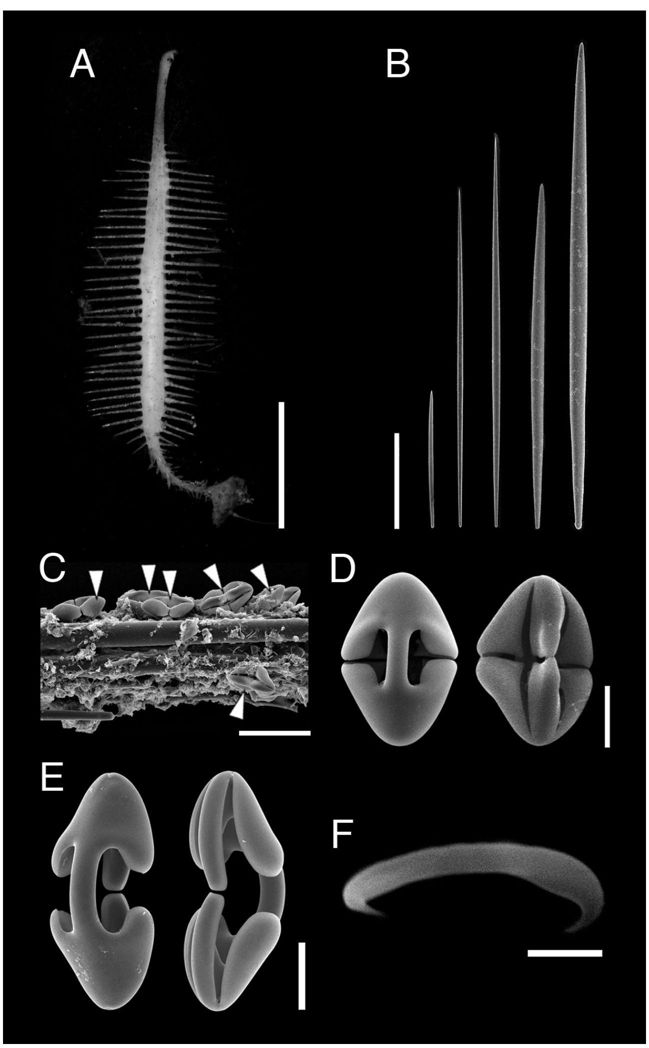

Fig. 6. *Abyssocladia myojinensis* sp. nov. (Holotype, NSMT-Po-1893). **(A)** View of the holotype. **(B)** Styles (mycalostyles). **(C)** Arrangement of abyssochelae I on the filament (arrowheads). Bundle of styles composed axis of the filament. **(D)** Abyssochelae I. **(E)** Abyssochelae II. **(F)** Sigmancistra. B–F, SEM photographs. Scale bars: 5 mm (A), 200 μm (B), 50 μm (C), 10 μm (D), 20 μm (E), 2 μm (F).

Etymology

The specie is named after the type locality, Myojin Knoll.

Distribution

This species is presently known only from type locality.

Description of holotype

Body. Pinnate, slender, and flattened, bearing numerous lateral filaments bilaterally symmetrical, thin axis attached to solid substratum by enlarged base (Fig. 6A). Size, 19 mm in total length, 0.8 mm in width, less than 0.5 mm thick at main part; enlarged base 2.2 mm in maximum diameter. Filaments almost regularly arranged perpendicularly in two opposite lateral rows along the axis, with a spacing of 0.2–0.3 mm, alternating on each side, totally lacking in the apical area, being very short in the peduncle. Filaments 66 (32 and 34 respectively on each side) in number, 2.4 mm in maximum length, 50–100 μm in diameter. No visible aquiferous system. Colour white. Possible spherical embryos arranged in a row in the middle part of the axis observed through semitransparent body.

Skeleton. Main axis consisting of large styles longitudinally arranged, spirally twisted in bundle in the peduncle. Axis of the filaments consisting of bundle of styles (Fig. 6B), surface of which lined by abyssochelae I with the tooth directed outward, and the shaft lying parallel to the axis (Fig. 6C, D). Abyssochelae II (Fig. 6E) distributed only on main body and peduncle, lacking on filaments, arranged with the tooth directed outward. All parts of the tissue containing numerous sigmancistras (Fig. 6F). Enlarged attachment base composed of bundles of small styles (see below) and few abyssochelae of both types.

Spicules. 1. Styles (mycalostyles) (Fig. 6B) from the main axis, the coating of the main axis and the lateral filaments, almost straight, usually thickest at middle part, fusiform in larger spicule. Larger end blunt, slightly subspherical in large spicule. Tips usually sharply pointed, sometimes

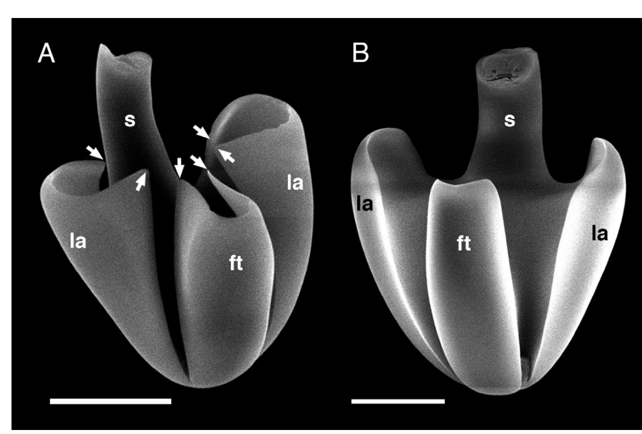

Fig. 7. Details of tooth and alae of two abyssochelae of *Abyssocladia myojinensis* sp. nov. (Holotype, NSMT-Po-1893). Both abyssochelae are broken on the shaft. **(A)** Abyssochelae I. Frontal tooth and lateral alae are laterally strongly folded inside (arrows). **(B)** Abyssochelae II. Lateral alae are nearly palmate. s, shaft. ft, frontal tooth. la, lateral alae. Scale bars: 10 μm.

acerate or blunt. Styles from the enlarged attachment base, straight, almost uniform in width, except at both ends. Larger end blunt, slightly elongated. Tips acerate. In main body and coating of the main body, very variable, 768 ± 208.7 (277.5–1052.5) μm in length and 10 ± 9.0 (7–34) μm in width; in filament, 773 ± 70.5 (605–890) μm in length and 15 ± 2.1 (11–18) μm in width; in enlarged attachment base, small and almost uniform in size compared to those of the other parts of the body, 301 ± 18.8 (272.5–350) μm in length and 5 ± 0.6 (4–6) μm in width.

2. Abyssochelae I (Fig. 6D). Shaft curved. Frontal tooth and lateral alae well developed, in contact with the opposite tooth and alae, laterally strongly folded inside (Fig. 7A); 36 ± 4.1 (28–44) μm in length, 3.5 ± 0.4 (3–4) μm in shaft width.

3. Abyssochelae II (Fig. 6E). Shaft curved. Frontal tooth long, isodiametric, nearly in contact with the opposite frontal tooth. Lateral alae nearly palmate (Fig. 7B), similar to isochelae; 68 ± 4.1 (59–78) μm in length, 7 ± 0.6 (6–8) μm in shaft width.

4. Sigmancistra (Fig. 6F), slightly contorted, without notch; 5–6 μm in length.

Remarks

The present species closely resembles *Abyssocladia naudur* collected from off Easter Island, in its gross morphology and in its abyssochelae II with their lateral alae nearly palmate (Fig. 6E). However, the present species has additional abyssochelae (Fig. 6D) not observed in *A. naudur*. Furthermore, sigmancistras of the present species are only of one type, compared to the two types in *A. naudur*. Several other carnivorous sponges display a gross morphology similar to that of *A. naudur* and of this new species, such as *Cladorhiza segonzaci* Vacelet, 2006 collected from same locality as *A. naudur* or *Euchelipluma* spp (Topsent, 1909, 1928; Lehnert et al., 2006; Vacelet and Segonzac, 2006). However the spicule composition of these species is completely different.

DISCUSSION

This is the first record of the genus *Abyssocladia* from Japanese waters, adding two new species to the genus. These species are well characterized by their body shape and spicule complement, although both are known from a single specimen, as is often the case for these small deep-sea sponges. They display isochelae of the abyssochela type that, as in other representatives of the genus, are difficult to assign precisely to an arcuate or palmate type (Vacelet, 2007). The presence of small crustaceans in the process being digested in *A. natsushimae* sp. nov. reconfirms that the representatives of the genus are carnivorous (Fig. 3).

The recent development of deep-sea sampling and observational techniques, such as manned submersibles and ROVs, has contributed to new discoveries of carnivorous sponges (Lehnert et al., 2005; Vacelet, 2006, 2008; Reiswig and Lee, 2007). These methods have revealed unknown and unexpected morphology in situ of these sponges as in *A. natsushimae* in the present study (Fig. 2), and enabled us to collect them intact as good unbroken samples for taxonomic study, which provide accurate ecological and morphological data. Accumulation of these data will contribute to revision of systematics of carnivorous sponges in the future. Unfortunately, the described species are usually known by a single specimen, which precludes recognition of their variability (Vacelet, 2006). However, the genus *Abyssocladia* displays high diversity of spicule type, and most of the species, including the two species here described, are well characterized by their shape, spicule complement, and the locations of each spicule type.

Carnivorous sponges exhibit clear individuality or symmetry compared to the usual irregularly massive, encrusting, or cryptic demosponges. In addition, their bodies can usually be divided into peduncle, filament, and the main body; even more regional functional specialization has been reported in some species (Reiswig and Lee, 2007; Riesgo et al., 2007). In the present study, *Abyssocladia myojinensis* sp. nov. has different types of chelae in the filament and main body, respectively, with abyssochelae in the filaments and isochelae-like abyssochelae in the main body (Fig. 6D, E). In *A. natsushimae* sp. nov., abyssochelae of the filaments are smaller than those of the main body (Fig. 5E, F). A greater number of morphological characters would provide a basis for constructing a more robust taxonomical framework. Spicule preparations separately made for each body part may contribute to a revision of a species complex such as *Chondrocladia concrescens* Schmidt, 1880 (see Vacelet, 2006).

Some deep-sea carnivorous sponges belonging to the genera *Chondrocladia* and *Cladorhiza* have a root system referred to as rhizoids composed of long spicule bundles deeply anchored into the mud bottom to support the body (Vacelet, 2007), which is considered to be an adaptation to living on the soft benthic bottom. This adaptation is absent in the seven known species of *Abyssocladia*, and in the two new species here described, which were all attached to solid substrata by an enlarged base (Lévi, 1964; Koltun, 1970; Vacelet, 2006). The relatively low species number of the genus before the use of direct observation techniques may be due to a sampling bias, as deep-sea rocky substrata are difficult to access by traditional deep-sea sampling methods, such as beam trawling. The genus is probably highly diversified in the deep oligotrophic rocky bottom, which are inaccessible to traditional sampling methods. Furthermore, the deep central Pacific is much less thoroughly investigated than the deep Atlantic. The idea that *Abyssocladia* is a Pacific-endemic genus is a possible alternate explanation for the low number of the known species of the genus.

Since the discovery of chemosynthetic ecosystems on the deep sea floor, biological researches using ROVs have concentrated on chemosynthetic communities (e.g. Desbruyères et al., 2006; Fujikura et al., 2008), however deep-sea communities incorporated in photosynthetic ecosystems are still uninvestigated, with few exceptions, such as deep-sea corals (e.g. Hovland, 2008) and planktonic aninsmals (e.g. Lindsay et al., 2001; Miyake et al., 2005). The present study demonstrates that a careful and extensive survey of the deep-sea oligortophic rocky bottom may also contribute to a better understanding of the diversity of deep-sea communities.

ACKNOWLEDGMENTS

We are grateful indebted to K. Inoue of the Ocean Research Institute, University of Tokyo, and H. Watanabe of the Japan

Agency for Marine-Earth Science and Technology (JAMSTEC) for planning the present cruise, to H. Miyake of Kitazato University for taking photographs during the dive, to S. Nemoto of Enoshima Aquarium for finding *Abyssocladia myojinensis* sp. nov., to K. Fujikura of JAMSTEC for helpful discussions. We sincerely thank the captain and crew of the R/V 'Natsushima' as well as the operations team of the ROV 'Hyper-Dolphin' for their dedicated efforts. The editor and two anonymous reviewers greatly improved the manuscript.

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(Received December 24, 2009 / Accepted May 27, 2010)