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Oceanitis abyssalis sp. nov., a new deep-sea fungus from sunken wood collected at the depth of 5707 m in the Northwest Pacific Ocean

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ABSTRACT

A new deep-sea fungus, *Oceanitis abyssalis* sp. nov. was described based on analyses of the LSU rDNA sequences and morphological characteristics. The new species was found on a branch of *Prunus* sp. collected at 5707 m depth of the abyssal plain in the Northwest Pacific Ocean, east off the Japan Islands. To the best of our knowledge, our discovery is the deepest record of the genus *Oceanitis* and also for the obligate deep-sea fungi. Morphologically, *Oceanitis abyssalis* is closely similar to *O. scuticella* Kohlmeyer and both were collected from deep-sea water. *Oceanitis abyssalis* can be distinguished from *O. scuticella* by having unicellular ascospores, smaller deciduous polar appendages and occasionally tree-like appendages, semi-persistent asci, smaller ascomata that are drop-shaped, and cream-colored. We also reviewed the taxonomic status of *O. scuticella*. The phylogenetic position of the authentic *O. scuticella* remains unclear due to the lack of genetic information and the inaccessibility of the type specimen. However, our investigation of the molecular and morphological characteristics of specimens previously identified as *O. scuticella* suggests that they include several cryptic, undescribed species. Notably, the Kuril-Kamchatka Trench material (M0229768), previously identified as *O. scuticella*, is suggested to be a representative of *Oceanitis abyssalis*.

Key words: Ascomycota, Halosphaeriaceae, marine fungi, *Prunus* sp.

INTRODUCTION

The deep sea is recognized as an extreme environment, characterized by the absence of sunlight irradiation, predominantly low temperatures (occasionally extremely high, >400 °C near hydrothermal vents), high hydrostatic pressure (up to 110 MPa) and reduced oxygen and nutrient sources (Nagano & Nagahama 2012). Despite this harsh environment, it is also presumed that fungi play an important role in the decomposition of sunken wood in deep-sea environments, just as in terrestrial environments. To date, there have been six species described as obligate deep-sea fungi found in water depths between 500–5315 m (Raghukumar 2017). *Alisea longicolla* J.Dupont & E.B.G. Jones, *Allescheriella bathygena* Kohlm., *Bathyascus vermispurus* Kohlm., *Oceanitis scuticella* Kohlm. and *Periconia abyssa* Kohlm. have been discovered from the sunken wood collected in deep sea and *Abyssomyces hydrozoicus* Kohlm., has been found from the deep-sea hydrozoa (Dupont *et al.* 2009; Dupont & Schwabe 2016; Kohlmeyer 1969, 1977; Nagano *et al.* 2019). Limited data is available regarding fungi associated with sunken wood in deep-sea environments, mainly due to its difficulty of access and rareness of discovery in the vast deep ocean floor (Nagano *et al.* 2019).

Oceanitis was established by Kohlmeyer (1977) to accommodate *Oceanitis scuticella* that was originally described from the sunken wood collected from a depth of 3975 m in the Gulf of Angola, Atlantic Ocean (Kohlmeyer 1977). Unfortunately, the holotype material (J.K. 2971a) was not found in the New York Botanical Garden (Wijayawardene

et al. 2018). Dupont *et al.* (2009) reported *O. scuticella*-like fungi from the small wood fragments and the sugar cane debris collected from the tropical Southwest Pacific, the Vanuatu archipelago at depths between 551 m and 1273 m and from the sunken wood collected in the deep sea of the South China Sea at depths between 1205 m and 1397 m. The molecular phylogenetics of these specimens based on small subunit (SSU) and large subunit (LSU) rDNA placed *O. scuticella* in a monophyletic clade with *Ascosalsum cincinnatulum*, *A. unicaudatum* and *A. viscidulum*. The latter three species were transferred to *Oceanitis*, an earlier generic name. The genus *Oceanitis* currently contains four species of which *O. scuticella* was described from the deep-sea sample. *Oceanitis unicaudatum* is recorded from littoral and subsurface water at a depth of 130–430 m (Jones & Le Campion-Alsumard 1970a, 1970b). Dupont & Schwabe (2016) also reported *O. scuticella*-like fungus from the 1 m long sunken birch wood collected from the Kurile Kamchatka Abyssal Plain, the Northwest Pacific at depths between 5229 m and 5217 m. Dupont *et al.* (2009) noted that their fungal collection from the Vanuatu archipelago has aggregated stromatic ascomata. Furthermore, Dupont & Schwabe (2016) reported that their fungal collection from the abyssal depth in the Northwest Pacific Ocean (specimen M0229768) has unicellular ascospores and drop-shaped ascomata. Both collections by Dupont *et al.* (2009) and Dupont & Schwabe (2016) showed morphological differences from the original description by Kohlmeyer, which was characterized by one-septate ascospores and globose to ellipsoidal ascomata (Kohlmeyer 1977). The analyses of the internal transcribed spacer region of ribosomal DNA (ITS rDNA) sequence between the Kuril-Kamchatka Trench material M0229768 (Dupont & Schwabe 2016) and the Vanuatu materials (Dupont *et al.* 2009) showed 2.5–3.0% difference (Dupont & Schwabe 2016). While Dupont *et al.* (2009) and Dupont & Schwabe (2016) attributed their collections to inter-specific variations within *O. scuticella*, Dupont & Schwabe (2016) also suggested the possibility of a distinct species and emphasized that discovering new specimens is important. However, the phylogenetic position of *O. scuticella sensu stricto* has not been elucidated due to the lack of genetic information and the type specimen is no longer available, making future acquisition difficult. Therefore, even though these incongruences may indicate the existence of cryptic species within the *O. scuticella* species complex, this possibility has never been investigated so far.

In this study, we report an unknown *Oceanitis* species on sunken wood collected during our survey on the abyssal plain seafloor in the Northwest Pacific Ocean, which has similar characters to the Kurile Kamchatka Abyssal Plain collection (M0229768), namely drop-shaped ascomata with unicellular ascospore. The morphology of the newly discovered fungus differs from that of the other four *Oceanitis* species, including the deep-sea taxon *O. scuticella*. Thus, it is described here as a new species. The morphological and phylogenetic analyses on the previously described as *Oceanitis scuticella* isolates are also presented and discussed here.

MATERIALS AND METHODS

Wood sampling

During our expedition YK19-11 on the abyssal plain seafloor at St. 10 (35°55.61'N, 144°57.87'E) in the Northwest Pacific Ocean under the Kuroshio Extension (Nakajima *et al.* 2021), an over 50 cm sized relatively fresh brown branch with bark was found and collected by using the manipulator of the human occupied vehicle *Shinkai* 6500 (Dive no. 6K#1555 conducted on 2 September 2019) at a depth of 5707 m (Fig. 1A). Dozens of creamy-white ascomata was found on the branch (Fig. 1B), which was identified as a *Prunus* sp. by PCR targeting the *rbcL* gene. The surface of the wood log with ascomata was cut into small pieces and placed in sterile bottles with sterile seawater or 10% glycerol on board and stored at 4 °C and -80 °C, respectively, until the observation.

Morphology

Morphological characteristics were observed using Olympus SZ61 dissecting microscope (Olympus) and Olympus BX51 compound microscope (Olympus) equipped with TouP Tek XCAM1080PHA (TouP Tek) digital imaging system. Ascomata were squashed and mounted in seawater to prepare the specimens for photography and all measurements. Vertical sections of ascomata were prepared using a Leica CM1100 cryostat (Leica Biosystems). Upon identifying a new species, it was registered in an online database, MycoBank (<http://www.MycoBank.org>). Single spore isolation was done following the method described by Overly *et al.* (2019), utilizing several fungal media, such as glucose yeast agar (GYA), potato dextrose agar (PDA), marine agar (MA), Czapek-Dox agar (CDA), and malt yeast peptone agar (MYPA) (6g malt extract, 2g yeast extract, 2g Polypeptone, 15g agar, 1L artificial seawater). Additionally, liquid media with sterile balsa wood in culturing flasks were also used. All media were prepared using artificial seawater and contained antibiotics. All media were incubated at 4°C, 11°C, and 20°C for several months to years. However, the

ascospores did not germinate. Herbarium specimen was deposited in National Museum of Nature and Science (TNS), Tsukuba, Japan.

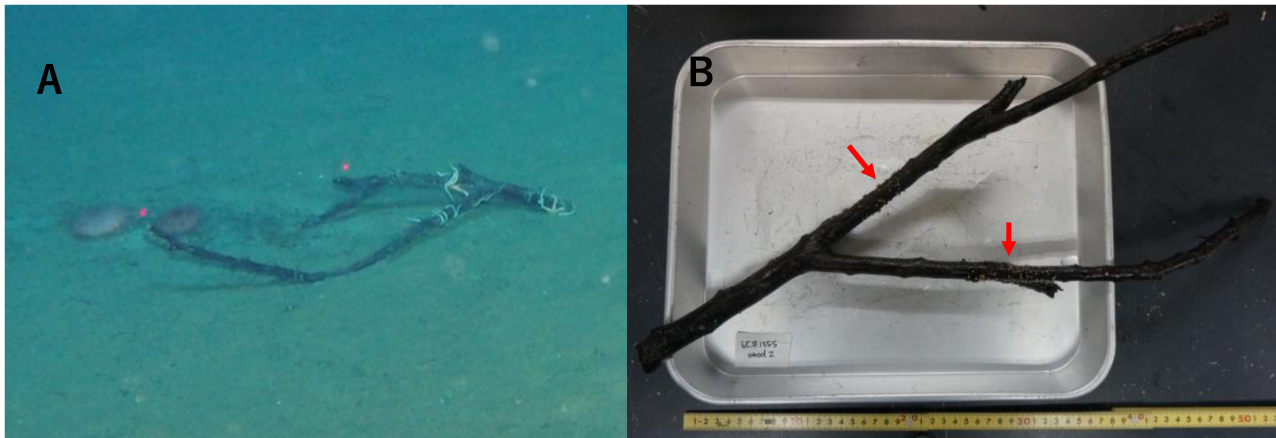


FIGURE 1. The photos of the sunken wood, *Oceanitis abyssalis* 6K1555_SW2 (= TNS-F-70722) was found. A: In deep sea on-site photo of the collected sunken wood. B: Ascomata of *O. abyssalis* spread on the sunken wood. Red arrows indicate the ascomata locations.

DNA extraction, amplification, sequencing and sequence alignment

Genomic DNA was extracted from fungal ascomata using the DNeasy UltraClean Microbial Kit (QIAGEN) following the manufacturer's instructions. Extracted DNA was stored at $-30\text{ }^{\circ}\text{C}$, prior to PCR amplification. Fungal DNA was amplified with three different primer sets, targeting the 18S-ITS1-5.8S-ITS2-26S rDNA regions. SSU rDNA was amplified by employing NS1 (5'-GTAGTCATATGCTTGCTC-3') (White *et al.* 1990)/nu-SSU-1196-3 (5'-TCTGGACCTGGTGAGTTTCC-3') (Borneman & Hartin 2000). ITS rDNA was amplified by employing ITS1F (5'-CTGGTATTAGAGGAAGTAA-3') (Gardes & Bruns 1993)/ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). LSU rDNA was amplified by employing LR0R (5'-ACCCGCTGAACTTAAGC-3')/LR7 (5'-TACTACCACCAAGATCT-3') (Vilgalys & Hester 1990). PCR reaction was carried out using TaKaRa Ex Taq (TaKaRa) with $0.2\text{ }\mu\text{M}$ (each) of a pair of primers and $1\text{ }\mu\text{L}$ of DNA template under the following PCR thermal cycling conditions in a Veriti 96-well Thermal Cycler 9902 (Applied Biosystems): $94\text{ }^{\circ}\text{C}$ for 5 min followed by 30 cycles of $94\text{ }^{\circ}\text{C}$ for 30 s, $55\text{ }^{\circ}\text{C}$ for 30 s, $72\text{ }^{\circ}\text{C}$ for 1 min, followed by a final extension at $72\text{ }^{\circ}\text{C}$ for 7 min. PCR products were purified by *ExoSAP-IT® Express* (Thermo Fisher Scientific) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3730xl DNA Analyzer (Applied Biosystems). Obtained confirmed sequences were submitted to GenBank (SSU: LC789976, ITS: LC777827, LSU: LC789975) and also compared with sequences stored in the GenBank database using the BLAST alignment software (<http://www.blast.genome.ad.jp/>).

For host plant identification, genomic DNA was extracted from the diced small piece of the plant by employing DNeasy Plant Pro and Plant Kit (QIAGEN) following the manufacturer's instructions. For PCR, the primer set, gPlantF (5'-AGTCTTGATCGTTACAAAGG-3')/gPlantR (5'-GTAAAATCAAGTCCACCRCG-3') (developed by Bioengineering Lab. Co., Ltd., unpublished) was used with the following PCR conditions: $94\text{ }^{\circ}\text{C}$ for 2 min, 30–35 cycles of $94\text{ }^{\circ}\text{C}$ for 30 s, $50\text{ }^{\circ}\text{C}$ for 30 s, and $72\text{ }^{\circ}\text{C}$ for 1 min, followed by $72\text{ }^{\circ}\text{C}$ for 5 min.

Phylogenetic analyses

The LSU rDNA region was aligned with other *Oceanitis* sequences and additional appropriate sequences, as referred to in Sakayaroj *et al.* (2011), and retrieved from GenBank using the CLUSTALX program. Alignment positions that were hyper variable or high in insertions/deletions, were removed from all sequences in the alignment manually. The alignment was deposited in TreeBASE (<http://www.treebase.org>) under the accession numbers S31009. A maximum-likelihood (ML) tree was constructed using GTR+G+I model with 1000 bootstrap replicates. The Maximum-parsimony (MP) tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). All these phylogenetic analyses were conducted in MEGA11 (Tamura *et al.* 2021).

RESULTS AND DISCUSSION

Phylogenetic study

The LSU rDNA sequence dataset consisted of 9 *Oceanitis* strains, along with 4 other Halosphaeriales and 3 outgroup taxa. The maximum parsimony dataset comprised 994 characters, with 799 constant characters, 121 parsimony-informative characters, and 74 parsimony-uninformative characters. The parsimony analysis resulted in eight equally parsimonious trees with a tree length of 296 steps (CI = 0.736, RI = 0.787, RC = 0.579, HI = 0.264). The ML analysis yielded the best scoring tree, with a final ML optimization likelihood value of -2930.257 (ln), as presented in Fig. 4, which includes ML/MP bootstrap support values at the nodes. The LSU rDNA phylogeny indicated that our new taxon, *Oceanitis abyssalis*, is distinct from the previously described *Oceanitis* species, and clustered together with the Kuril-Kamchatka Trench material (M0229768).

Taxonomy

Oceanitis abyssalis Y. Nagano & Abdel-Wahab *sp. nov.* Figs. 2–3

Mycobank no.: MB 851445.

Diagnosis: This species differs from the other four species of *Oceanitis*, namely *O. scuticella*, *O. cincinnatum*, *O. unicaudatum* and *O. viscidulum* by having unicellular ascospores that are fusiform, curved or sigmoid in shape, two type of ascospores appendages: one polar uncoiling appendage and tree-like appendages that develops throughout the ascospores' length; semi-persistent asci and cream-colored, drop-shaped ascomata (Dupont *et al.* 2009; Kohlmeyer 1977; Jones & Le Campion-Alsumard 1970a, 1970b).

Type: The Northwest Pacific Ocean, off the coast of Japan, (35°55.61'N, 144°57.87'E) under the Kuroshio Extension, on the sunken wood found on the abyssal plain at 5707 m water depths, 2 Sep 2019, Y. Nagano, holotype TNS-F-70722.

Gene sequences holotype: LC789975 (LSU), LC777827 (ITS), LC789976 (SSU).

Etymology: The epithet *abyssalis* refers to the abyssal plain where this species was collected.

Ascomata 1.2–1.6 mm high, 0.98–1.2 mm diam., superficial, drop-shaped, papillate, ostiolate, cream-colored, yellowish or brownish, subiculate, fleshy, single or gregarious. *Ostioles* 470–545 µm in length, 90–110 µm diam., ostiolar canal periphysate; periphyses 21–39 µm in length, 1–2 µm wide. *Peridium* 290–400 µm wide at the upper part of the ascomata, 120–160 µm wide at the lower part of the ascomata, 3-layered, forming a *textura angularis*; outer layer 40–50 µm wide in the upper part of the ascomata and consists of 5–7 cell layers of light-brown, thick-walled, polygonal cells, 10–20 µm wide in the lower part of the ascomata and consists of 2–4 cell layers of flattened, thick-walled cells; median layer 230–280 µm wide in the upper part of the ascomata and consists of 15–28 cell layers of hyaline, thick-walled, polygonal cells that are with large lumina and elongated to outside and small to inside, 10–20 µm wide in the lower part of the ascomata and consists of 5–9 cell layers of polygonal, thick-walled cells; inner layer 28–45 µm thick, consists of 7–11 cell layers of hyaline, flattened, parallel, thick-walled cells. *Paraphyses* absent, center of immature ascomata is filled with hyaline, thin-walled, polygonal pseudoparenchymatous cells which are eventually compressed by the asci. *Asci* 83–115 × 15–20 µm (mean = 95.7 × 17.1 µm, n=25), 8-spored, unitunicate, clavate or fusiform, thin-walled, with rounded apex, semi-persistent, short pedicellate, ripening successively on an ascogenous tissue at the bottom of the ascomatal venter, becoming detached at maturity at the base and pushed upward by young asci. *Ascospores* 58–77 × 4–5 µm, elongate fusiform, curved or sigmoid, unicellular, hyaline, guttulate, with one apical appendage; appendages 6–16 µm long, 0.5–2 µm diam, filamentous, uncoiling, tapering toward the apex, wavy, adhering with its base to the apex of the ascospore, deciduous; occasionally ascospores develop tree-like appendages throughout its length while within the ascus or after its release (Fig. 3).

Taxonomic discussion of *Oceanitis abyssalis*

Oceanitis abyssalis is morphologically similar to *O. scuticella* and both fungi were collected from deep-sea water. However, *O. abyssalis* differs from *O. scuticella* by having unicellular ascospores that are elongated fusiform, curved or sigmoid with much smaller deciduous polar appendage and occasionally with several tree-like appendages throughout the ascospores length, semi-persistent asci, smaller ascomata (1.2–1.6 mm high, 0.98–1.2 mm diam.), that are drop-shaped, and cream-colored. *Oceanitis scuticella* has one-septate ascospores, with a whip-like polar appendage that is 32–50 µm long, 2–3 µm wide; early deliquescing asci and globose to ellipsoidal ascomata (1.4–2.03 mm high, 1.4–1.78 mm diam.) that are brown to dull orange-coloured and seated on hypostroma. Hypostroma were not observed in

O. abyssalis. We believe that these significant morphological differences cannot be representative of variation within a species. Therefore, we concluded *O. abyssalis* should be described as a new species.

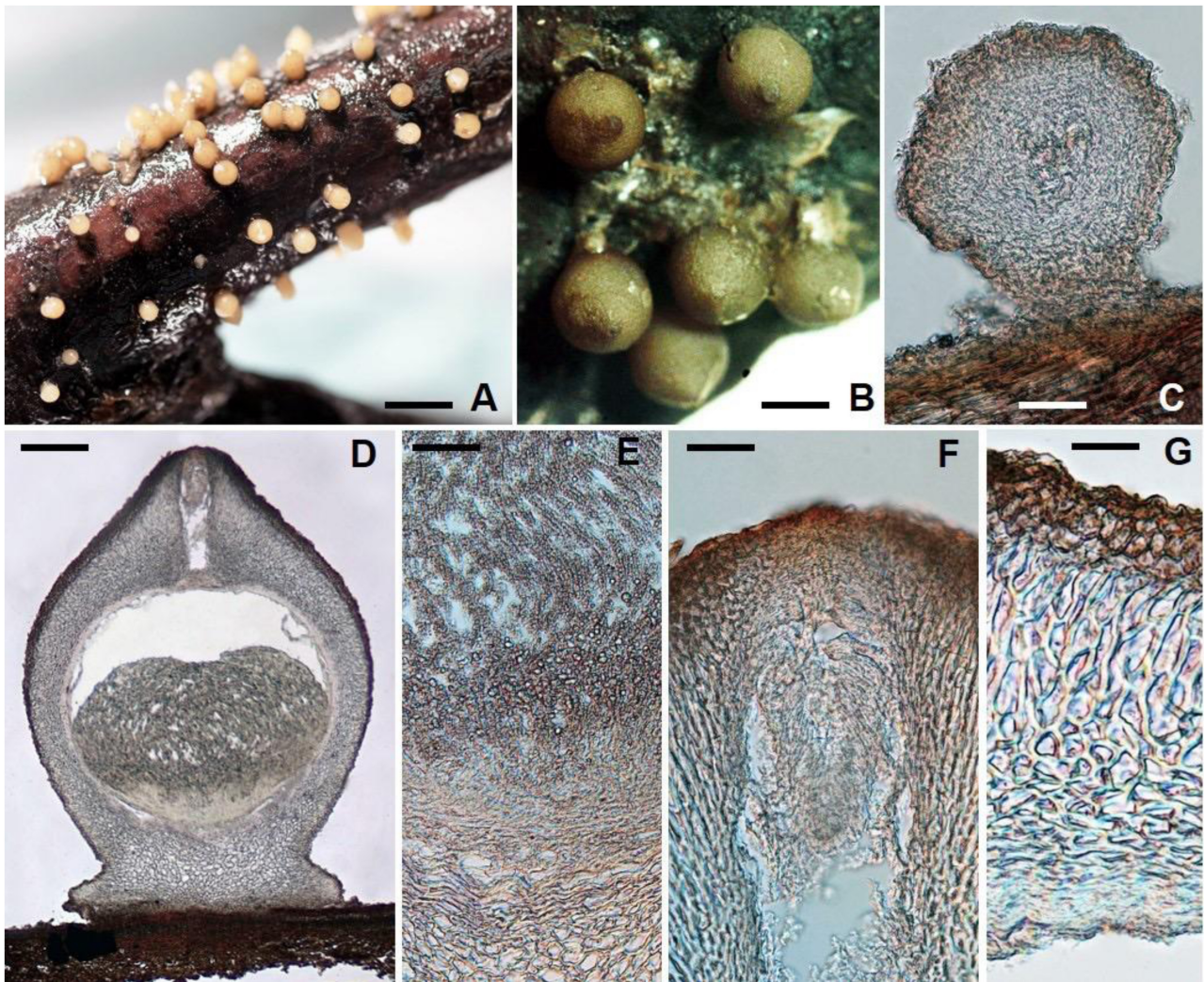


FIGURE 2. *Oceanitis abyssalis* (TNS-F-70722, holotype). A, B: Ascomata on wood. C: Vertical section through ascoma shows the polygonal pseudoparenchymatous tissue and the ascogonial cells in the center. D: Vertical section through mature ascoma. E: Vertical section through ostiole with periphyses. F: Magnified part of the ascogonial tissue and the developing asci. G: Vertical section through the peridium. Bars: A 4 mm, B 1 mm, C 170 μm , D 300 μm , E–G 50 μm .

Oceanitis scuticella-like organisms and phylogenetic analyses

Dupont *et al.* (2009) recorded twelve collections of *Oceanitis scuticella*-like fungus from trawled samples from the bottom of the South Pacific Ocean off Vanuatu from depths between 551 m and 1273 m. The authors identified their fungal collections as *O. scuticella*, although it has a significantly different morphological characteristics from the original collection of *O. scuticella* that Kohlmeyer described from the Gulf of Angola, Atlantic Ocean, by having stromatic ascomata vs. aggregated ascomata and a thinner walled peridium than the Angola's material (Dupont *et al.* 2009; Kohlmeyer 1977). Dupont & Schwabe (2016) also reported a collection of *Oceanitis scuticella*-like fungus from the sunken wood collected by trawling at abyssal depth from the Kuril-Kamchatka Trench in the Northwest Pacific Ocean. The authors identified their collection as *O. scuticella* although it has unicellular ascospores with much smaller polar appendages and smaller drop-shaped ascomata than the Angola's materials. We believe that both collections made by Dupont *et al.* (2009) and Dupont & Schwabe (2016) represent undescribed species different from *O. scuticella*, as the shape of ascomata and the spore septation are important characteristics for classifying fungal species. Detailed comparison of the morphology of *Oceanitis* species is summarized in Table 1.

TABLE 1. Comparison of the morphology of *Oceanitis* species.

Fungus	Habitat	Ascomata	Hypostroma	Neck	Ostioles	Hamathecium	Peridium	Asci	Ascospores	Appendages
<i>Oceanitis abyssalis</i> (this study)	Deep sea on sunken wood found at the bottom of the abyssal plain in the Northwest Pacific Ocean under the Kuroshio Extension.	1.2–1.6 mm high, 0.98–1.2 mm diam, drop-shaped, papillate, ostiolate, cream-colored, yellowish or brownish, subiculate, fleshy, single or gregarious.	Absent	Absent	470–545 µm in length, 90–110 µm diam; ostiolar canal periphysate; periphyses 21–39 µm in length, 1–2 µm wide.	Paraphyses absent; center of immature ascomata is filled with hyaline, thin-walled, polygonal pseudoparenchymatous cells which are eventually compressed by the asci.	290–400 µm thick at the upper part of the ascomata, 120–160 µm thick at the lower part of the ascomata, 3-layered, forming <i>textura angularis</i> .	83–115 × 15–20 µm, 8-spored, unitunicate, clavate or fusiform, thin-walled, with rounded apex, semi-persistent, ripening successively on an ascogenous tissue at the bottom of the ascomatal venter.	58–77 × 4–5 µm, elongate fusiform, curved or sigmoid, hyaline, with one apical appendage.	6–16 µm long, 0.5–2 µm diam, filamentous, uncoiling, tapering toward the apex, wavy, adhering with its base to the ascopore, of the ascospore, deciduous; occasionally ascospores develop tree-like appendages throughout its length while within the ascus or after its release.
<i>Oceanitis scuticella</i> (Kohlmeyer 1977)	Deep Sea, from wood collected by trawling in 3975 m. in the Gulf of Angola, the Atlantic Ocean.	1.4–2.03 mm high, 1.4–1.78 mm diam, subglobose to ellipsoidal, round at the top, flat at the base, seated on the surface of a thin hypostroma, ostiolate, epapillate, fleshy, brown to dull-orange colored, gregarious.	Present, 30–120 µm thick, light-colored, composed of small polygonal to rounded cells with large lumina; masses of lilac-colored hyphae can be found in the wood vessels under the ascomata.	25–30 µm diam; ostiolar canal periphysate.	Paraphyses absent; center of immature ascomata is filled with hyaline, thin-walled, polygonal pseudoparenchymatous cells which are eventually compressed by the asci.	450–470 µm thick at the apex, 240–260 µm at the sides, compressed of 17–35 layers of hyaline, thick-walled, polygonal cells with large lumina, forming <i>textura angularis</i> .	70–90 × 12–17 µm, 8-spored, clavate, unitunicate, thin-walled, without apical apparatus, ripening successively on an ascogenous tissue at the bottom of the ascomatal venter, becoming detached at maturity at the base and pushed upward by young asci, eventually dissolving in the upper part of the ascomatal venter.	60–80 × 4–6 µm, hyaline, filiform to elongate fusiform, one-septate, with one apical appendage.	32–50 µm long, 2–3 µm diam., filamentous, tapering toward the apex, wavy, whip-like, at first attached to the side of the ascospore, becoming detached from the wall, but adhering with its base to the apex of the ascospore.	

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TABLE 1. (Continued)

Fungus	Habitat	Ascomata	Hypostroma	Neck	Ostioles	Hamathecium	Peridium	Asci	Ascospores	Appendages
<i>Oceanitis cf. abyssalis</i> (described as <i>Oceanitis scuticella</i> in Dupont & Schwabe 2016)	Deep sea, on wood trawled from Kurile Kamchatka Abyssal Plain in the Northwest Pacific Oceans.	1.45 mm in length, 1.05 mm diam, spherical, ostiolate, apically rounded with side slopes straight to concave, fleshy, yellowish-beige with upper half dull orange to brown.	Absent	Absent	50 µm diam, ostiolar canal 335 µm in length.	Paraphyses absent	185 µm thick in apical region, 115 µm laterally, and 165 µm at the base, comprising 25 layers.	62 × 13 µm, clavate, eight-spored, thin-walled, unitunicate.	52 × 2.3 µm, unicellular, fusiform, with a single appendage.	coiled prior to maturity as a pad and strung out when mounted in sea water, pad measuring 17 µm × 1 µm, typically whip-like after uncoiling
<i>Unidentified Oceanitis sp.</i> (described as <i>Oceanitis scuticella</i> in Dupont et al. 2009)	Deep sea, on small twigs and sugar cane debris trawled from the bottom of the South Pacific Ocean off Vanuatu Islands.	Aggregated into a stroma, size is not given.	Not mentioned	Absent	Apical but lacking a neck.	Paraphyses absent	200 µm thick, composed of polygonal cells with large lumina forming a textura angularis, merging towards the center into flattened cells.	Not discernable and probably had deliquesced.	Size is not given, one-septate, with a single polar appendage.	Initially closely adpressed to the ascospore wall, but separating at maturity, composed of tightly coiled filaments arising as outgrowths from the mesosporium and retained along the ascospore wall by an outer elimitating membrane.
<i>Oceanitis cincinnatula</i> (Shearer & Crane 1980)	Littoral coastal habitats on mangroves, intertidal wood and culms of marsh plants.	68–248 µm high, 66–243 µm diam., globose to subglobose, hyaline, membranous, superficial or immersed, solitary and ostiolate.	Absent	113–465 µm long, 22–46 µm diam, hyaline, periphysate.	Not mentioned	Paraphyses absent	One-layered, composed of a few layers of elongate cells with large lumina.	41–62 × 11–16 µm, thin-walled, unitunicate, eight-spored, ellipsoidal to clavate, deliquescing.	34–60 × 4–5 µm, hyaline, cylindrical to fusiform, 5–11-septate, appendaged.	One polar appendage that uncoil into fine thread in water

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TABLE 1. (Continued)

Fungus	Habitat	Ascomata	Hypostroma	Neck	Ostioles	Hamathecium	Peridium	Asci	Ascospores	Appendages
<i>Oceanitis unicaudatum</i> (Jones & Le Campion-Alsumard 1970a)	Littoral coastal habitats.	400–700 µm diam, globose, immersed or superficial, ostiolate, papillate, coriaceous, brown to black, solitary, staining the substrate.	Absent	200–400 × 50–100 µm, ostiolar canal periphysate.	Not mentioned	Paraphyses absent	Not mentioned	60–75 × 15–20 µm, 8-spored, clavate, thin-walled, aphysoelastate, early deliquescing; developing at the base of the ascocarp venter on an ascogenous tissue.	36–60 × 2.5–5 µm, cylindrical or fusiform, 3–5 septa, hyaline, appendaged.	15–36 µm long, terminal or subterminal, more or less lateral, forming an irregular sheath around the upper tip or along the upper side of the ascospore.
<i>Oceanitis viscidula</i> (Kohlmeyer & Kohlmeyer 1965)	Littoral coastal habitats.	120–240 µm high, 100–252 µm in diam., globose or subglobose, ellipsoidal or depressed, immersed, ostiolate, papillate, coriaceous, cream-colored, yellowish or brownish, solitary, occasionally staining the substrate cardinal to maroon red.	Absent	160–250 × 25–36 µm, cylindrical, ostiolar canal with short periphyses.	Not mentioned	Paraphyses absent; thin-walled cells filling venter of young ascocarps; deliquescing at ascospore maturity.	13–25 µm thick, hyaline or pale cream-colored, composed of four to six layers of thickwalled, roundish or elongate cells, merging toward the center into the pseudoparenchyma.	8-spored, clavate or ellipsoidal, unitunicate, thin-walled, aphysoelastate, without apical apparatuses, early deliquescing, developing at the base of the ascocarp venter.	37.5–89 × 3–6.5 µm, arranged in parallel in the ascus, long cylindrical or fusiform, straight or slightly curved, 5–16 septa, hyaline, appendaged.	at both ends a subterminal, caplike, mucilaginous appendage, 6–10 µm long, eventually becoming viscous and forming a long thread.

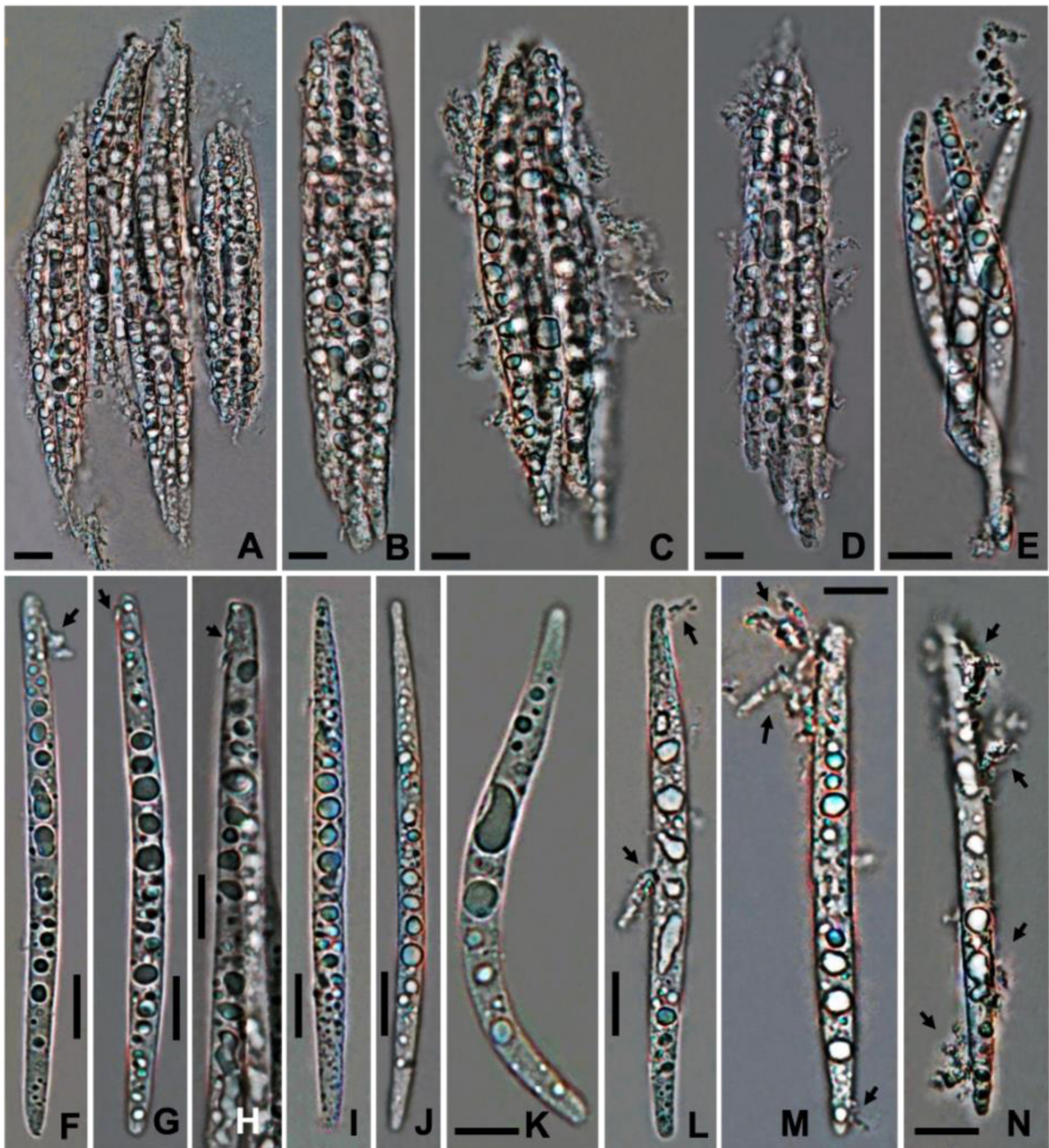


FIGURE 3. *Oceanitis abyssalis* (TNS-F-70722, holotype). A–E: Variously shaped asci at different stages of maturity. F–N: Variously shaped ascospores. Polar uncoiling appendage is arrowed in figures F–H. Tree-like appendages along the ascospore length is arrowed in figures L–N. Bars: A–N 10 μ m.

In order to clarify the phylogenetic position of *O. abyssalis* and *Oceanitis scuticella*-like organisms, the LSU rDNA region of *O. abyssalis* was aligned with other *Oceanitis* sequences and other appropriate sequences retrieved from GenBank (Fig. 4). The new species, *O. abyssalis* is well placed in the genus *Oceanitis* clade with 100% support in the ML phylogenetic analysis. *Oceanitis abyssalis* was placed with the Kuril-Kamchatka Trench material (M0229768) but located separately from other *Oceanitis* taxa. The LSU and ITS rDNA sequences of *O. abyssalis* showed 100% match with the Kuril-Kamchatka Trench material (M0229768). Both *Oceanitis abyssalis* and the Kuril-Kamchatka Trench material (M0229768) have drop-shaped ascomata and unicellular ascospores. They were collected from a similar environment, the abyssal plain of the North Pacific Ocean. From a morphological perspective, as well as considering the 100% similarity in ITS rDNA sequences and ecological aspects of the collected material, we inclined

to consider that M0229768 reported as *O. scuticella* in Dupont & Schwabe (2016) is a representative of *O. abyssalis*. Although there are slight morphological differences between M0229768 and *O. abyssalis* (Table 1), specifically, they differ in the dimensions of the ascospores ($58\text{--}77 \times 4\text{--}5 \mu\text{m}$ for *O. abyssalis* vs. $52 \times 2.3 \mu\text{m}$ for M0229768), and ascospores of *Oceanitis abyssalis* are larger in size with a thicker, three-layer peridial wall (Dupont & Schwabe 2016). Also, ascospores of *Oceanitis abyssalis* have two types of appendages, while the collection of the Kuril-Kamchatka Trench has one polar uncoiling appendage. Some of these differences may result from a lack of detailed comprehensive observation and further findings of the specimens and detailed analyses will be needed to determine their precise identification. Therefore, we concluded in the present study that the Kuril-Kamchatka Trench material (M0229768) should be treated as *Oceanitis* cf. *abyssalis* (Table 1 and Fig. 4).

The South China Sea material (CP4157) was closely related to *O. abyssalis* and Kuril-Kamchatka Trench material (M0229768) but placed independently within the *Oceanitis scuticella*-like clade and showed 3 nucleotide differences in the LSU rDNA sequence and one nucleotide difference in the SSU rDNA sequence from *O. abyssalis* (ITS rDNA sequence data is not available). As the detailed morphological information of the South China Sea material (CP4157) is not available, it cannot be discussed further. The Vanuatu materials (CP2457b, CP2429, CP2421) formed a clade being separate from CP4157 and *O. abyssalis*. There are 3 to 4 nucleotide differences in the LSU rDNA sequence and 11 to 14 bases (2.5–3.0%) differences in ITS rDNA sequence between the Vanuatu materials and *O. abyssalis*. Morphological and phylogenetic analyses suggested that the Vanuatu materials (CP2457b, CP2429, CP2421) are likely another undescribed *Oceanitis* species different from the original *O. scuticella* and *O. abyssalis*. Therefore, we concluded in the present study that the Vanuatu materials (CP2457b, CP2429, CP2421) should be treated as unidentified *Oceanitis* spp. (Table 1 and Fig. 4). The South China Sea material (CP4157), of which details are unknown, might be an another unidentified *Oceanitis* sp. because of its independent phylogenetic position. To clarify the taxonomy of these collections and to understand the diversity within the genus *Oceanitis* more accurately, additional collections, including *O. scuticella sensu stricto* from the type locality, are needed. Furthermore, comprehensive morphological and phylogenetic studies are essential to formally describe these species.

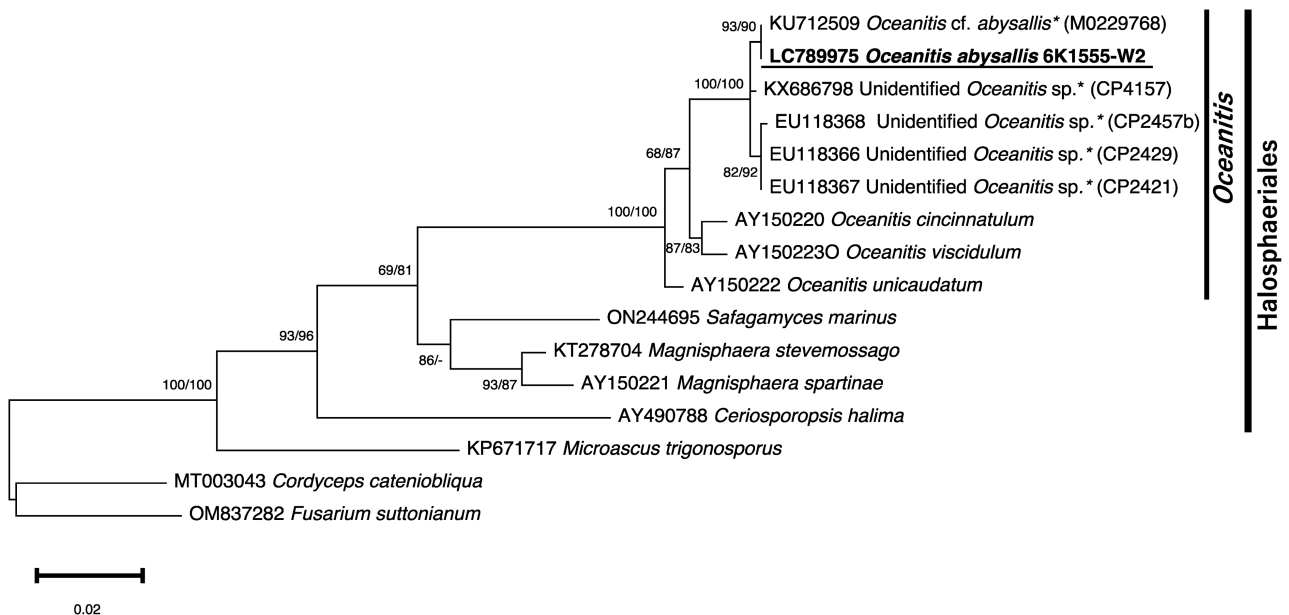


FIGURE 4. Phylogenetic relationships of *Oceanitis abyssalis* and other *Oceanitis* species analyzed by the maximum likelihood tree (ML), based on the nucleotide sequences of the LSU rDNA. Numbers at nodes are bootstrap indices of support (ML/MP), and only branches with bootstrap values above 50% from an analysis of 1,000 bootstrap replicates are indicated. New species are highlighted in bold and underlined. The collections previously identified as *O. scuticella* and newly proposed as *Oceanitis* cf. *abyssalis*, or unidentified *Oceanitis* species in this study, are marked with an asterisk.

Key to *Oceanitis* species

1. Ascospores are perithecioid2
1. Ascospores are stromatic *Oceanitis* sp. (Dupont *et al.* 2009)
2. Ascospores are septate3
2. Ascospores are unicellular6
3. Ascospores are one-septate..... *O. scuticella*
3. Ascospores with more than one septum.....4

4.	Ascospores with less than 5 septa	<i>O. unicaudatum</i>
4.	Ascospores with more than 5 septa	5
5.	Ascospores with one polar appendage	<i>O. cincinnatula</i>
5.	Ascospores with two polar appendages	<i>O. viscidula</i>
6.	Ascospores with one type of appendages	<i>Oceanitis</i> cf. <i>abyssalis</i> (Dupont & Schwabe 2016)
6.	Ascospores with two types of appendages	<i>O. abyssalis</i>

Ecology of Oceanitis abyssalis

Both *O. abyssalis* and the Kuril-Kamchatka Trench materials were collected from abyssal depths of deep-sea and both had drop-shaped ascomata with unicellular ascospores on intact bark with absence of wood-boring bivalves (Dupont & Schwabe 2016). In contrast, the original *O. scuticella* and the Vanuatu materials were collected from bathyal depths of deep-sea and they had ascomata with one-septate ascospores on barkless wood along with wood-boring bivalves (Kohlmeyer 1977; Dupont *et al.* 2009). It would be interesting to disclose their ecological characteristics and phylogenetic relationship within the genus *Oceanitis*. It was previously believed that the presence of bark inhibits the colonization of deep-sea fungi on wood (Dupont *et al.* 2009). However, recent observations have shown that some deep-sea fungal species, such as *O. abyssalis*, are capable of colonizing wood with intact bark. In addition, tree-like appendages have been observed in *O. abyssalis*. Tree-like appendages have also been observed in some marine fungi and aquatic fungi (Abdel-Wahab *et al.* 2012; Noureldin *et al.* 2022). These structures may help these fungi to settle on sunken wood. Also, it is very interesting to see if there is any habitat segregation between the deep-sea fungi and wood-boring bivalves. However, the presence/absence of wood-boring bivalves may be just related to their habitable water depths or the dimension of wood materials, as it is known that marine wood-degrading fungi are believed to contribute to the settlement and growth of wood-borers in shallow marine environments (Vishwakiran *et al.* 2001).

The genus *Oceanitis* occurs on various plant species with or without bark in various aquatic environments, littoral to deep-sea areas. It is also widely distributed in geologically separated deep-sea regions as one of the most successful fungal taxa in the environment. Dupont *et al.* (2009) suggested that the thick peridium of *O. scuticella* could be an adaptation to the extreme conditions of the deep sea. A thick three layered peridial walled of *O. abyssalis* is consistent with this hypothesis. Since there are shallow marine species and deep-sea species within the *Oceanitis*, these organisms are useful subjects to study the mechanism of adaptations from the littoral to the deep areas. Comparative genome analysis within the *Oceanitis* may provide key insights into the adaptation, evolution and ecology of deep-sea fungi. It would also be interesting to investigate its wood-degrading enzymes, as they degrade sunken wood in a completely different environment from terrestrial.

CONCLUSIONS

The present study described the new *Oceanitis* species, namely *O. abyssalis*. It was suggested that the Kuril-Kamchatka Trench material (M0229768), which was previously identified as *O. scuticella*, is also be a representative of *O. abyssalis*. The study highlights the limited data available on the ecology and physiology of deep-sea fungi, including *Oceanitis*, due to their rarity and the difficulty in culturing them. Despite attempts, culturing *O. abyssalis* was not successful in this study. Given that no deep-sea endemic fungal taxa have been successfully cultured to date, developing an effective long-term culturing system involving high-pressure conditions would significantly enhance our understanding of their ecology. This study contributed to the limited baseline knowledge of deep-sea fungal diversity and ecology. Further and broader research on the distribution, physiology, and genomics of *Oceanitis*, including the newly described *O. abyssalis*, will provide a better understanding of deep-sea fungi.

DISCLOSURE

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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