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Morphological and molecular assessment of *Lithophyllum okamurae* with the description of *L. neo-okamurae sp. nov.* (Corallinales, Rhodophyta)

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RUNNING TITLE

Lithophyllum okamurae and *L. neo-okamurae*

ABSTRACT

Lithophyllum okamurae has been widely reported in the Pacific Ocean with identification based on morpho-anatomical observations. Two infraspecific taxa, *L. okamurae* f. *okamurae* and f. *angulare,* described from Japan, have been recorded in the temperate region of Japan. We assessed branched *Lithophyllum* samples morphologically referable to *L. okamurae* using morpho-anatomical data and DNA sequences (*psb*A, *rbc*L and partial LSU rDNA) obtained from herbarium specimens, including type material, as well as recently field-collected material in Japan. The molecular analyses showed that these '*L. okamurae*' samples contained two species: *L. okamurae* and a cryptic new species which we describe as *L. neo-okamurae sp. nov.* Because the holotype of *L. okamurae* f. *angulare* was conspecific with original material cited in the protologue of *L. okamurae,* it is a heterotypic synonym of *L. okamurae* f. *okamurae*. *Lithophyllum okamurae* and *L. neo-okamurae* were morphologically similar in having warty, lumpy and fruticose thalli and in often forming rhodoliths. *Lithophyllum okamurae* can be morpho-anatomically distinguished from *L. neo-okamurae* by the thallus with tapering or plate-like protuberances (knobby protuberances in the latter) and by having smaller tetrasporangial conceptacle chambers (167–314 µm; 248–380 µm in *L. neo-okamurae*). Our LSU rDNA sequence data from *L. okamurae* f. *angulare* (=*L. okamurae* f. *okamurae*) was identical to that of the type of *L. margaritae*, which has nomenclatural priority over *L. okamurae*. However, considering that *psb*A and *rbc*L sequences of *L. margaritae* type material could not be generated in the present study, we refrain, for the moment, from proposing the taxonomic synonymy between these two taxa until the status of *L. margaritae* and its synonyms from the type locality (Gulf of California) are clarified.

KEYWORDS

Lithophyllum margaritae; LSU rDNA*;* Morpho-anatomy; Non-geniculate coralline algae; Northwestern Pacific Ocean; *psb*A; *rbc*L; Rhodolith

INTRODUCTION

Lithophyllum Philippi (Corallinales, Rhodophyta) is the largest genus of non-geniculate coralline algae and currently includes 130 taxonomically accepted species names (Guiry & Guiry 2021). However, most of these species have been described based only on morpho-anatomical characters. An effective method to validate species identities, diversity and distribution has been to combine DNA sequencing of freshly field-collected specimens and type specimens with traditional morpho-anatomical characters (Gabrielson *et al.* 2011). This approach has confirmed that there are many species of *Lithophyllum* in Europe (Hernández-Kantún *et al*. 2015a; Peña *et al.* 2018; Pezzolesi *et al.* 2019; Caragnano *et al.* 2020), the warm temperate-tropical western Atlantic ocean basin (Hernández-Kantún *et al.* 2016; Richards *et al.* 2018), the western Indian ocean basin (Basso *et al.* 2015; Maneveldt *et al.* 2019), and the northwestern Pacific ocean basin (Kato & Baba 2019).

Lithophyllum okamurae Foslie (1900, '*okamurai*'), described from Misaki, Kanagawa Prefecture (previously as Sagami Province), Japan, has been reported worldwide based on morpho-anatomical characters. This species is also known as one of major components of rhodolith beds in the northwestern Pacific Ocean (Kato *et al.* 2017). Moreover, based on growth-form and anatomical similarities, Basso *et al*. (1996) suggested that *L. okamurae* could be the Indo-Pacific vicariant of the Mediterranean *Lithophyllum racemus* (Lam.) Foslie. However, the application of the species name *L. okamurae* has not been confirmed based on sequences from the type specimen.

Lithophyllum okamurae has a complicated taxonomic history. The species was described by Foslie (1900) from Japan based on material collected by K. Yendo (Yendo specimens no. 80, 120, 270, 379, 382, 389, 408). No infraspecific taxa were proposed in Foslie (1900). One year later, Foslie (1901) proposed in reference to *L. okamurae* the forms '*japonica*' and '*angularis*' as manuscript names ("Fosl. mscr."), without description or diagnosis; these names were therefore not validly published.

Foslie (1904) validated both *L. okamurae* f. *angulare* ('*angularis*' in the text, '*angulata*' in the legend of the figure) and *L. okamurae* f. *japonicum* ('*japonica*') by providing illustrations of specimens (Foslie 1904, pl. 11, fig. 12 for f. *angulare* and pl. 11, figs 13–19 for f. *japonicum*; see Turland *et al.* 2018, ICN, Art. 38.8 and 38.10); and his illustration of the species (Foslie 1904, legend to pl. 11, fig. 11) has been taken as indicating the illustrated specimen as the (lecto)type of *L. okamurae* (Woelkerling 1993, p. 163). However, in the text Foslie (1904, p. 60) states, concerning his original description of *L. okamurae*: "I then possessed specimens only of the form of the species which I have afterwards named f. *angularis* (Pl. XI, fig. 11–12)."

Later, Foslie (1909, p. 30) used the designation '*L. okamurae* f. *typica*' for the typical form of the species (not validly published; Turland *et al.* 2018, Art. 24.3, 26.2), and listed as a synonym *L. okamurae* f. *japonicum*, thereby considering it to represent the typical form. Herbarium specimens labeled *L. okamurae* f. *japonicum* should then be referred by the autonym *L. okamurae* f. *okamurae*, as correctly proposed by Woelkerling *et al.* (2005).

The lectotype of *L. okamurae*, illustrated by Foslie (1904, pl. 11, fig. 11), corresponds to Yendo specimen no. 408 (TRH A21-1318) while the rest of Yendo's specimens quoted in the protologue were divided into TRH A21-1325 (no. 270) and TRH A21-1326 (no. 80, 120, 379, 382, 389). All these specimens were regarded as the main form (autonym) *L. okamurae* f. *okamurae* (Woelkerling *et al.* 2005). In Foslie (1904), among figs 13–19 of pl. 11 of the form *japonicum* (currently as f. *okamurae*), only fig. 13 was from the Pacific coast of Japan. Unfortunately, Woelkerling *et al.* (2005) did not find the specimen corresponding to that figure.

The holotype of *L. okamurae* f. *angulare*, indicated by Foslie (1904, pl. 11, fig. 12), corresponds to specimen TRH A21-1327, quoted by Woelkerling *et al.* (2005). Foslie (1909) mentioned that f. *angulare* was characterized by less closely spaced branches than the typical form, and more or less angular branches. This holotype was not included in the material cited in the protologue of *L. okamurae* (Foslie 1900).

Five more infraspecific taxa of *L. okamurae* have been described: *Lithophyllum okamurae* f. *ptychoides* Foslie [currently as *Lithophyllum ptychoides* (Foslie) Foslie], *Lithophyllum okamurae* f. *trincomaliense* Foslie ('*trincomaliensis*'), *Lithophyllum okamurae* f. *validum* Foslie ('*valida*') [currently as *Lithophyllum validum* (Foslie) Foslie], *Lithophyllum okamurae* f. *subplicatum* Foslie ('*subplicata*') [currently as *L. subplicatum* (Foslie) D.Basso, Caragnano, L.Le Gall & Rodondi] and *Lithophyllum okamurae* f. *contiguum* Foslie ('*contigua*') [currently as *Sporolithon australasicum* (Foslie) Yam.-Tomita *ex* M.J.Wynne].

Of these, only f. *subplicatum* was revised using both DNA sequences and morpho-anatomical characters from the type material (Basso *et al.* 2015).

Molecular analyses of Japanese coralline algae morpho-anatomically referable to *L. okamurae* reveal that they belong to two distinct species: *L. okamurae* (including *L. okamurae* f. *angulare*, which is here demonstrated to be synonymous with the typical form) and *L. neo-okamurae sp. nov*. Here we report vegetative, sexual and asexual reproductive characters of these species, based on genetic and morpho-anatomical data of type and herbarium archival material, in addition to recently collected material from Japan.

MATERIAL AND METHODS

Sample collection

Lithophyllum specimens used in this study (n = 37, Table S1) were collected at 18 sites from less than 10 m depth in the temperate region of Japan. Voucher specimens (n = 33) used for morphological and molecular studies were deposited in the Herbarium of Graduate School of Science, Hokkaido University, Japan (SAP); the remaining four specimens were used for molecular studies and were deposited in the Herbaria of Muséum National d'Histoire Naturelle, Paris, France (PC) and University of Santiago de Compostela, Spain (SANT). Additionally, we borrowed and examined type specimens and herbarium archival specimens of *Lithophyllum okamurae* f. *okamurae* and the holotype of *L. okamurae* f. *angulare* from the Herbarium of Norwegian University of Science and Technology, Norway (TRH) and the Herbarium of the Laboratory of Marine Botany, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido, Japan (HAK; Table S2). The lectotype of *L. okamurae* f. *okamurae* (TRH A21-1318) was studied by T. Masaki in 1969–1970, who stored at HAK a small fragment and two preparations of the lectotype as isolectotype (HAK M-179). Both the lectotype (TRH A21-1318) and isolectotype (HAK M-179) were very small fragments, and therefore no destructive investigation was undertaken following the recommendations of the curators. The herbarium archival specimens of *Lithophyllum okamurae* are specimens collected from the type locality in 1899 when the protologue specimens were collected by the same collector, K. Yendo. The type specimens of *Lithophyllum margaritae* (Har.) Heydr. and *L. veleroae* E.Y.Dawson (one of the synonyms of *L. margaritae*) were borrowed from PC and University of California, USA (UC) for molecular studies (Table S2). In addition, nine recent collections of *L. margaritae* preserved in GALW (National University of Ireland) and FBCS (Universidad Autónoma de Baja California Sur, Mexico) were used for the molecular studies (Table S1). Herbarium abbreviations follow Thiers (2021).

Molecular analyses

Total DNA was extracted from the herbarium specimens and field-collected specimens dried by silica gel, using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) or a NucleoSpin® 96 Tissue kit (Macherey-Nagel, GmbH and Co. KG, Germany), according to the manufacturer’s instructions. PCR of the following gene fragments were carried out using a Blend Taq -Plus- or KOD FX NEO Reaction Kit (TOYOBO, Osaka, Japan), except for four vouchers (JHS0012, JHS0014, JHS0029B, VPF00887a) and herbarium specimens of *L.*

okamurae f. *okamurae* and type material of *L. okamurae* f. *angulare*, *L. margaritae* and *L. veleroae*, which followed Basso *et al.* (2015) and Peña *et al.* (2015), and seven specimens (E52, E57, E334, E108, E110, E116, E118) with previous GenBank records that followed Hernández-Kantún *et al.* (2014, 2015b). The primer pairs for the PCR and sequencing of the chloroplast *psb*A were psbA-F/psbA-R2 and psbA-F/psbA600R in addition to psbA-F/psbA500F for sequencing (Yoon *et al.* 2002), while the primer pairs for PCR and sequencing of the chloroplast *rbc*L were F-57/R-1150 and F-753/R-rbcS start (Freshwater & Rueness 1994). When the *rbc*L primer pair F-57/R-1150 did not amplify, the F-57/R-753 pair was used instead (Freshwater & Rueness 1994). The PCR and sequencing primer pairs for the nuclear-encoded LSU (28S) rRNA gene were T01N (Harper & Saunders 2001) and TR273 (Basso *et al*. 2015) or T16N (Saunders & McDevit 2012) and the reverse primer designated T99R (5′TGGTCCGTGTTTCAAGACGG3′). The PCR products were purified and sequenced by Macrogen Japan (Kyoto, Japan) or by Eurofins (Eurofins Scientific, France). Three data sets for phylogenetic analyses, *psb*A, *rbc*L and LSU sequences, were assembled, which comprised novel sequences from this study and previously published sequences for the genus *Lithophyllum* (Tables S1, S2, S3). The *rbc*L and LSU rDNA sequences of *L. okamurae* and *L. neo-okamurae* were obtained from a subset of examined specimens in *psb*A analyses. Specimens with identical sequences were represented by a single specimen in the data sets. Moreover, sequences with less than 1% pairwise divergence estimated in MEGA X (ver. 10.1.8., Kumar *et al*. 2018) were also combined into a single sequence for the *psb*A dataset. Sequences were aligned using MAFFT version 7 (Katoh & Standley 2013). *Chamberlainium* *tumidum* (Foslie) Caragnano, Foetisch, Maneveldt & Payri and *C. decipiens* (Foslie) Caragnano, Foetisch, Maneveldt & Payri were used as outgroups for *psb*A and *rbc*L data sets. Phylogenetic relationships for *psb*A and *rbc*L data sets were inferred by maximum likelihood (ML) using RAxMLGUI 1.5b1 (Silvestro & Michalak 2012), and Bayesian inference (BI) using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001). ML analyses were performed using the general-time-reversible model with gamma distribution and invariant sites (GTR+G+I) and 1000 rapid bootstrap (BS) replicates. BI analyses were performed using the GTR+G+I model. Four Markov chains were used. Analyses were run for 300,000 generations for the *psb*A data set, for 1,000,000 generations for the *rbc*L data set, and sampling was performed every 100 generations. The number of generations of run was chosen to ensure the attainment of an average and standard deviation of split frequencies lower than 0.01. The burn-in was determined after convergence of the tree samples using Tracer v1.7.1 (Rambaut *et al.* 2018) after satisfactory convergences of the tree samples were obtained: 750 in *psb*A; 2,500 in *rbc*L. Consensus topology and posterior probability (PP) values were calculated using the remaining trees. Neighbour-joining (NJ) analysis for the LSU data set was conducted in MEGA X using the Maximum Composite Likelihood model with 1,000 BS replicates. In the phylogenetic tree inferred from ML and NJ analysis, nodes with BS values ≥90% were considered strongly supported; those between 89% and 70% moderately supported and those <70% weakly supported. In the tree inferred from Bayesian analysis, nodes with PP values ≥0.95 were considered strongly supported and those between 0.95 and 0.90 moderately supported. For all the genetic markers, the total number of pairwise sequence divergences (uncorrected, p distances) was calculated using MEGA X.

Morpho-anatomical observations

Light microscopy followed either Kato *et al*. (2006) or Basso & Rodondi (2006) and SEM observation methods followed Basso & Rodondi (2006). Anatomical terminology followed Adey & Adey (1973) and Woelkerling (1988), and growth form terminology followed Woelkerling *et al.* (1993). Cell and conceptacle measurements followed Irvine & Chamberlain (1994). We observed the extent of the roof elevation of tetrasporangial conceptacles and followed the terminology of Adey *et al.* (1974).

RESULTS

Molecular analyses

Recently collected specimens morpho-anatomically referable to *Lithophyllum okamurae* were divided into two independent lineages (A and B) in the *psb*A, *rbc*L and LSU rDNA analyses (Figs 1–3). The specimens in lineage B were confirmed to be conspecific with the holotype of *L. okamurae* f. *angulare* (here regarded as *L. okamurae*, TRH A21-1327) by a match with the *psb*A sequence. The protologue and archival material of *L. okamurae* also split into two separate species, which corresponded to the above two species, respectively, in the *psb*A and LSU rDNA analyses. In the present study, these taxa are treated as two independent species, *L. neo-okamurae sp. nov.* (lineage A) and *L. okamurae* (part of lineage B; see Discussion)

In the *psb*A analyses (Fig. 1), *Lithophyllum neo-okamurae* was resolved as an independent lineage (A) in a strongly-supported clade (100% in ML bootstrap, 1.00 in PP) that included *L. atlanticum* Vieira-Pinto, M.C.Oliveira & P.A.Horta, '*L. margaritae*', and also '*L. okamurae*' from China, the latter two specimens appearing distantly related to the type specimens of both species. This lineage comprised 21 specimens of *L. neo-okamurae* that shared nearly or completely identical sequences (0.0%–0.6% sequence divergences in 852 bp; Table S4) with an archival specimen of *L. okamurae* f. *okamurae* (TRH A21-1322) from the type locality. *Lithophyllum neo-okamurae* formed a supported clade with *L. margaritae* from the Gulf of California (E334) (90% in ML, 1.00 in PP) and the sequence divergences between them were 1.3%–1.7%. However, the specimen of *L. margaritae* (E68) from the type locality, which was nearly identical to three other specimens (E52, E59, E64) from the Gulf of California (0.0%–0.7% sequence divergences), differed from the specimen E334 by 3.1%–3.2%. Lineage B (Fig. 1) consisted of *Lithophyllum okamurae*, '*L. margaritae*' and the isotype of *L. veleroae,* which is a synonym of *L. margaritae*, with strong support (100% in ML, 1.00 in PP) and the sequence divergences among them were 0.0%–2.6% (Table S4). Lineage B was distantly related to other species in the northwestern Pacific Ocean or the temperate regions. The total sequence divergences among the three archival specimens (see below) and 16 recently collected ones of *L. okamurae* from Japan were 0.0%–1.3 % (837–852 bp). The holotype of *L. okamurae* f. *angulare* (TRH A21-1327) and an archival specimen from the type locality of *L. okamurae* f. *okamurae* (TRH A21-1321) shared identical sequences, and differed from the nearest haplotype of recently collected *L.*

okamurae specimens from the type locality (HU39 and six specimens; Table S4) by 0.4%, whereas the holotype of *L. okamurae* f. *angulare* (TRH A21-1327) differed from the other archival specimen cited in the protologue of *L. okamurae* (TRH A21-1326) by 0.7%. A haplotype of *L. margaritae* from Taiwan was identical to the haplotype of *L. okamurae* (HU39 and six specimens), although it differed from specimens from the type locality (E57, E108, E110) and the other specimens (E116, E118) of *L. margaritae* from the Gulf of California by 0.2%–2.4%. The isotype of *L. veleroae* formed a moderate to strongly supported subclade with *L. margaritae* (E118) from the Gulf of California (73% in ML, 1.00 in PP) and the sequence divergence between them was 0.9% (524 bp). The subclade differed from other taxa in lineage B by 1.3%–2.6% sequence divergences.

In lineage A of the *rbc*L trees (Fig. 2), eight Japanese specimens of *L. neo-okamurae* showed very similar sequences with 0.0%–0.9% divergences, whereas they differed from '*L. okamurae*' from China by 4.7%–5.1% (1350 bp; Table S5). Lineage B consisted of Japanese *L. okamurae* specimens. The sequence divergences among six specimens of this species were 0.0%–2.2% (1301–1350 bp; Table S5).

In the LSU rDNA analysis (Fig. 3), the holotypes of *L. okamurae* f. *angulare* (TRH A21-1327) and *L. margaritae* shared identical sequences with recently collected specimens of these species and grouped with the isotype of *L. veleroae* with moderate support (87% in NJ). *Lithophyllum neo-okamurae* was distantly related to *L. okamurae, L. margaritae* and *L. veleroae*. The LSU rDNA sequence of the holotype of *L. neo-okamurae* was identical to an archival specimen of *L. okamurae* (TRH A21-1319) from the type locality and *L. margaritae* (E334) from the Gulf of California.

Lithophyllum okamurae **Foslie 1900, pp 4, 5**

Figs 4–24, S1–S4; Tables 1, S6

LECTOTYPE: TRH! A21-1318 (Yendo specimen no. 408), collected 1899 (no habitat data) by K. Yendo. Lectotype designated in Foslie (1904) (Woelkerling 1993, p. 163; Woelkerling *et al.* 2005, p. 178). Illustrated in Foslie (1904, pl. 11, fig. 11) and the present study (Figs 4, 5, S1).

ISOLECTOTYPE: HAK! M-179 (a fragment of Yendo specimen no. 408), present study, illustrated by Figs 6–10.

TYPE LOCALITY: Misaki, Miura City, Kanagawa Prefecture, Japan (as Marine Laboratory at Sagami Province; Woelkerling 1993).

HETEROTYPIC SYNONYM: *Lithophyllum okamurae* f. *angulare* Foslie 1901 ('*angularis*') (Woelkerling 1993, p. 26). Holotype TRH A21-1327 (Woelkerling *et al.* 2005, p. 179). Illustrated in Foslie (1904, pl. 11, fig. 12, as '*angulata*' in the legend to figure), Printz (1929, pl. 64, fig. 7), and the present study (Figs 11–15).

SPECIMENS EXAMINED: See Table S2. Sequences of two DNA markers were obtained from the holotype of *L. okamurae* f. *angulare*, TRH A21-1327 (*psb*A, MZ128805; LSU rDNA, MZ129208). DNA sequences obtained from two archival specimens of *L. okamurae* from the type locality, TRH A21-1321 (Yendo specimen no. 327; Fig. S2) and TRH A21-1326 (Yendo specimen no. 120; Fig. S4), the former not belonging and the latter belonging to protologue specimens, included two respective *psb*A and two LSU rDNA sequences.

OTHER SPECIMENS EXAMINED: See Table S1. DNA sequences determined from recently collected Japanese material including specimens from the type locality contained 16 *psb*A sequences, six *rbc*L sequences and two LSU rDNA sequences.

HABITAT: Plants epilithic or epizoic, or free-living in the upper subtidal zone.

DISTRIBUTION: *Lithophyllum okamurae* occurs on the temperate coasts of the Pacific Ocean, the Japan Sea and the Seto Inland Sea in Japan. Based on the *psb*A sequences, *L. margaritae* from Taiwan and some material from the Gulf of California are considered conspecific or very closely allied to *L. okamurae* (Hernández-Kantún *et al.* 2015b; Liu *et al.* 2018).

Morphology and vegetative anatomy

Thalli ranged from encrusting, warty, lumpy, foliose to fruticose, and often formed free-living rhodoliths (Figs 5, 11, 16, 17, S2, S3). The protuberances were up to 10 mm long, tapering or plate-like (up to 10 mm wide, 1–2 mm thick) or apically enlarged (2–5 mm in diameter), and dichotomously branched or anastomosing. Colour of living plant was grayish-pink to light purple. Thalli were dimerous with unistratose hypothallus composed of approximately isodiametric or slightly elongate or wide cells, non-palisade (Figs 7, 12, 18), 11–19 µm long and 7–19 µm in diameter. Cells of perithallial filaments were 7–19 µm long and 5.5–11 µm in diameter. Secondary-pit connections were common. Cell fusions were not observed. Subepithallial initials were 7–17 µm long and 6–10 µm in diameter. One to two layers of epithallial cells were flattened, 2–4 µm long and 7.5–10 µm in diameter (Figs 8, 13, 19). Medullary regions in branches (protuberances) were coaxial (Fig. 20). Trichocytes were not observed.

Reproductive anatomy

Gametophytes are dioecious. Gametangial conceptacles were slightly raised above or flush with surrounding thallus surface. Spermatangial conceptacle chambers were 86–116 µm in diameter and 20–35 µm high, with roofs 18–30 µm thick. Simple spermatangial systems were restricted to the conceptacle floor (Fig. 21). Carpogonial conceptacle chambers were 94–139 µm in diameter and 18–30 µm high, with roofs 56–96 µm. Carposporangial conceptacle chambers were 207–243 µm in diameter and 76–109 µm high, with roofs 35–63 µm thick. Carposporangia were cut off from gonimoblast filaments borne at periphery of a large continuous flattened fusion cell (Fig. 22). Tetrasporangial conceptacles were uniporate with roofs raised above (Fig. 23) or flush with surrounding thallus surface or sunken below thallus surface. Buried conceptacles were observed (Figs 9, 14). Conceptacle chambers were 167–314 µm in diameter and 63–109 µm high. Pore canals were triangular and tapering towards surface, 28–61 µm long. Conceptacle roofs were 4–7 cell layers, 30–71 µm thick. Tetrasporangial conceptacle chamber floors were situated 8 to 13 cells below surrounding thallus surface. A central columella was present or absent; when present, it was comprised of sterile filaments. A calcified hump (two to five cell layers) below central columella was absent (Fig. 15) or present (Fig. 24). Tetrasporangia were zonately divided, 47–69 µm long and 20–30 µm in diameter, and peripherally arranged in the conceptacle chamber (Figs 10, 24). Data on measured vegetative and reproductive features in the above descriptions were based on recently collected specimens, because those of the holotype of *L. okamurae* f.

angulare and the isolectotype of *L. okamurae* were overlapping within the ranges of the recent material (summarized in Tables 1, S6).

Lithophyllum neo-okamurae **A.Kato, D.Basso, Caragnano, Rodondi, V.Peña & M.Baba** ***sp. nov.***

Figs 25–37, S5; Tables 1, S6

DIAGNOSIS: Thalli encrusting, warty, lumpy to fruticose, free-living rhodoliths (Figs 25, 29, 30, S5). Protuberances up to 12 mm long, knobby, columnar (up to 20 mm long) or apically enlarged (2–6 mm in diameter), dichotomously branched or anastomosing. Colour of living plant grayish-pink to light purple. Distinct from both *L. okamurae* and the related species *L. margaritae* by DNA sequences.

HOLOTYPE: SAP 115594, collected 9 June 2013 by A. Kato and M. Baba; epilithic on rock in the upper subtidal zone (Fig. 29).

TYPE LOCALITY: Misaki, Miura City, Kanagawa Prefecture, Japan.

ETYMOLOGY: Greek '*neos*' (in compounds '*neo-*'), new (Stearn 1992); '*okamurae*', dedicated to the Japanese phycologist Dr. Kintaro Okamura.

OTHER SPECIMENS EXAMINED: See Tables S1, S2. Sequences of three DNA markers were obtained from the holotype (*psb*A, LC620629; *rbc*L, LC624951; LSU, LC624957). In addition, DNA sequences determined from Japanese material contained 20 *psb*A sequences, seven *rbc*L sequences and two LSU rDNA sequences. DNA sequences obtained from two archival specimens of *L. okamurae* f. *okamurae* collected from the type locality, TRH A21-1319 (Yendo specimen no. 445; Figs 25–28) and TRH A21-1322 (Yendo specimen no. 377; Fig. S5), are an LSU rDNA and a *psb*A sequence, respectively. The respective sequence was identical to the holotype and recently collected specimens of *L. neo-okamurae* from the type locality.

DISTRIBUTION: Based on DNA sequences, *L. neo-okamurae* is widely distributed in the temperate coasts of the Pacific Ocean, the Japan Sea and the Seto Inland Sea in Japan.

HABITAT: Plants grow on bedrock, small stones, shells, or free-living in the upper subtidal zone.

Vegetative anatomy

Dimerous construction with unistratose hypothallus composed of approximately isodiametric or slightly elongate or wide cells, non-palisade, 8–22 µm long and 5.5–14 µm in diameter (Figs 26, 31). Cells of perithallial filaments were rectangular, 8–19 µm long and 6–9 µm in diameter. Secondary-pit connections were common. Cell fusions were not observed. Subepithallial initials were shortened to rectangular cells, 6–12 µm long and 7–10 µm in diameter. One to two layers of epithallial cells were flattened, 2–5 µm long and 7–10.5 µm in diameter (Figs 27, 32). Medullary regions in branches (protuberances) were coaxial (Fig. 33). Trichocytes were not observed.

Reproductive anatomy

Gametophytes are dioecious. Gametangial conceptacles were slightly raised above or flush with surrounding thallus surface. Spermatangial conceptacle chambers were 119–182 µm in diameter and 15–46 µm high, with roofs 25–46 µm thick. Simple spermatangial systems were restricted to the conceptacle floor (Fig. 34). Carpogonial conceptacle chambers were 94–200 µm in diameter and 23–53 µm high, with roofs 68–104 µm thick. Carposporangial conceptacle chambers were 218–339 µm in diameter and 73–144 µm high, with roofs 48–89 µm thick. Carposporangia were cut off from gonimoblast filaments borne at periphery of a large continuous flattened fusion cell (Fig. 35). Tetrasporangial conceptacles were uniporate with roofs flush with or raised above surrounding thallus surface (Fig. 36) or sunken below thallus surface. Buried conceptacles were observed. Conceptacle chambers were 248–380 µm in diameter and 86–121 µm high. Pore canals were triangular and tapering towards surface, 40–78 µm long. Conceptacle roofs comprised of 4–8 cell layers, 43–78 µm thick. Conceptacle chamber floors were situated 10 to 16 cells below surrounding thallus surface. A central columella was present or absent; when present, it was comprised of sterile cells. A calcified hump (four to seven cell layers) below central columella was present (Figs 28, 37). Tetrasporangia were zonately divided, 47–79 µm long and 20–34 µm in diameter, and peripherally arranged in conceptacle chamber. Data on measured vegetative and reproductive features in the above descriptions were based on recently collected specimens, because those of the archival material were overlapping within the ranges of the recent material (summarized in Tables 1, S6).

DISCUSSION

Our molecular analyses show that branched *Lithophyllum* specimens morphologically referable to *L. okamurae* belong in fact to two species: *Lithophyllum okamurae* and *L. neo-okamurae.* The two species sometimes occur together in the upper subtidal zone in the temperate region of Japan and often form rhodoliths. Our molecular analyses indicate that *L. okamurae* and *L. neo-okamurae* are distantly related to branched *Lithophyllum* species in the Western Pacific Ocean, namely *L. kaiseri* (Heydr.) Heydr., *L. kuroshioense* A.Kato & M.Baba, *L. longense* Hern.-Kant., P.W.Gabrielson & R.A.Town., and *L. subtile* (Foslie) A.Kato & M.Baba, which have been confirmed by their type sequences (Kato & Baba 2019;

Maneveldt *et al.* 2019). In contrast, *L. okamurae* and *L. neo-okamurae* are closely related to separate lineages of *L. margaritae* from its type locality, the Gulf of California. Each of these three species is discussed below.

Lithophyllum okamurae

The protologue of *L. okamurae* cited seven specimens, none of which was designated as the holotype (Foslie 1900). Subsequently, a lectotype (TRH A21-1318) was set apart from the other specimens (TRH A21-1325 and A21-1326) (Woelkerling *et al.* 2005, pp 176–179). In the present study, the gross morphology of the lectotype (TRH A21-1318) and isolectotype (HAK M-179) of *L. okamurae* could not be confirmed because of their very small sizes. However, a photograph of the lectotype taken in 1969–1970 by T. Masaki (Fig. 5) showed that the specimen was sparsely branched and had somewhat pointed apices, consistent with fig. 11 (TRH A21-1318) and fig. 12 (the holotype of *L. okamurae* f. *angulare,* TRH A21-1327) in Foslie (1904, pl. 11). The voucher collection TRH A21-1325 (Fig. S3) represented a single 3-cm-diameter specimen and had sharp-pointed protuberances. The collection TRH A21-1326 (Fig. S4) contains six small fragments (less than 2 cm in diameter) which were mutually similar encrusting to warty thalli. One of them (specimen no. 120) was conspecific with *L. okamurae* f. *angulare* based on the similarities to *psb*A sequences of the holotype of this species. Considering these results, we concluded that all specimens in the *L. okamurae* protologue were conspecific with *L. okamurae* f. *angulare* which we regard as a heterotypic synonym of *L. okamurae* f. *okamurae.*

Verheij (1994) observed old buried male and female conceptacles of the lectotype of *L. okamurae*. However, the isolectotype of *L. okamurae* (HAK M-179) has only tetrasporangial conceptacles. Verheij (1994, figs 10, 11) showed remnants of spermatangia remaining both on a wall and a floor of the male conceptacle, whereas spermatangia were formed only on the conceptacle floor in our observation of *L. okamurae* and *L. neo-okamurae* (Figs 21, 34). Therefore, we think that Verheij's (1994) observations of the old buried conceptacles are doubtful.

The holotype of *L. margaritae,* a species name with nomenclatural priority over *L. okamurae*, was identical to the holotype of *L. okamurae* f. *angulare* in the short LSU rDNA sequence (214 bp), indicating that *L. okamurae* was closely related to *L. margaritae,* not to *L. neo-okamurae*. However, we consider that the conspecificity between *L. okamurae* and *L. margaritae* is not convincingly demonstrated solely on the basis of this short and weakly variable LSU rDNA sequence. We refrain from proposing the taxonomic synonymy between these two taxa because more variable *psb*A and *rbc*L sequences of the *L. margaritae* type material could not be generated.

The minimum threshold of the interspecific divergences of *psb*A sequences among closely related *Lithophyllum* species was less than 2%; e.g. 1.7%–2.2% in *psb*A between *L. platyphyllum* (Foslie) Foslie and *L. pseudoplatyphyllum* Hern.-Kant., W.H.Adey & P.W.Gabrielson (Hernández-Kantún *et al.* 2016); 1.5%–3.0% between *L. racemus* and *L. pseudoracemus* Caragnano, Rodondi & Rindi (Caragnano *et al.* 2020); and mostly 2%–5% among 13 phylogenetic species of *L. stictiforme* (Aresch.) Hauck (Pezzolesi *et al.* 2019). In the present study, the pairwise divergences of *psb*A sequences of *L. margaritae* in lineage B including the isotype of *L. veleroae*, one of synonyms of *L. margaritae,* were 0.0%–2.6%, indicating that it is likely to contain some cryptic species. A haplotype of *L. okamurae* (HU39 and six specimens) showed up to 0.5% (4 bp out of 849 bp) sequence divergence among two haplotypes of *L. margaritae* from the Gulf of California (E108) and Taiwan, which were similar to the intraspecific sequence divergence of *L. longense* (up to 0.59%, representing 5 bp out of 841 bp; Maneveldt *et al.* 2019). This means that at least one genetically circumscribed species is widely distributed in the North Pacific Ocean.

The pairwise divergences of *psb*A sequences of *L. okamurae* used in the present study (up to 1.3%) were similar to or lower than the above minimum threshold of the species-level divergence. In contrast, the pairwise divergence of *rbc*L sequences of *L. okamurae* (2.2%) was similar to the divergence between *L. platyphyllum* and *L. pseudoplatyphyllum* (1.9%; Hernández-Kantún *et al.* 2016). The pairwise sequence divergences of *L. okamurae* are at or near the minimum threshold of the species level. Therefore, species delimitation analyses using multiple genes are needed to confirm whether more than one species are included under a similar morphology.

Lithophyllum neo-okamurae

Lithophyllum neo-okamurae differs from *L. okamurae* in having mostly knobby protuberances, not tapering nor plate-like (foliose thalli), while *L. okamurae* shows a wide range of morphologies including these protuberances. Although *L. okamurae* can also be anatomically distinguished from *L. neo-okamurae* by smaller tetrasporangial conceptacle chambers (167–341 µm vs 248–380 µm; Table 1), the conceptacle chamber sizes overlap considerably between two species. Thus, DNA sequences are needed for reliable identification.

In the *psb*A analyses of the present study, *L. neo-okamurae* was closely related to the other two '*L. margaritae*' lineages from the type locality (E334 and E68). The intraspecific divergence of *L. neo-okamurae* (up to 0.6%, representing 5 bp out of 852 bp) in *psb*A was similar to that of *L. longense* (up to 0.59%, representing 5 bp out of 841 bp; Maneveldt *et al.* 2019). In contrast, the sequence divergences between '*L. margaritae*' and *L. neo-okamurae* were 1.3%–3.2%, which means that '*L. margaritae*' includes at least one species different from *L. neo-okamurae*.

Taxonomic relationships among *Lithophyllum okamurae*, *L. margaritae* and related species

Riosmena-Rodríguez *et al.* (1999) merged five species described from La Paz, BCS, Mexico, namely *Lithophyllum diguetii* (Har.) Heydr., *L. lithophylloides* Heydr., *L. margaritae, L. pallescens* (Foslie) Foslie and *L. veleroae*, within *L. margaritae* based on their morpho-anatomical similarity. Subsequently, Schaeffer *et al.* (2002) indicated that foliose and fruticose growth forms of *L. margaritae* were genetically distinct using amplified fragment length polymorphism (AFLP) analyses. Following Schaeffer *et al.* (2002), Norris (2014) recognized three species out of the five synonyms of *L. margaritae*: *L. diguetii* for the foliose form; *L. pallescens* for the fruticose form; and *L. margaritae* for the intermediate form. Based on the morphological similarities, Norris (2014) tentatively treated *L. veleroae* and *L. lithophylloides* as synonyms of *L. diguetii* and *L. pallescens*, respectively, until molecular analyses could be done on the type material of these species. Furthermore, it should be noted that, although the species epithet *diguetii* has the same taxonomic priority as *margaritae,* Riosmena-Rodríguez *et al.* (1999) chose the species epithet *margaritae* for that species because the type material is in better condition and shows the characteristics of the species more clearly. Therefore, in addition to type material of *L. margaritae* [DNA sequences of material ascribed to it have also been reported from Brazil and Taiwan (Vieira-Pinto *et al.* 2014; Liu *et al.* 2018)] and its synonyms, fresh material of each species is required to assess the genuine boundary of *L. margaritae* and the phylogenetic relationships among the taxa that were proposed to be synonyms of this species.

In conclusion, the present study reassessed the complicated taxonomic history of *L. okamurae* and showed that *L. okamurae* f. *angulare* is a synonym of the autonym *L. okamurae* f. *okamurae*, which takes precedence according to the rules of nomenclature. It also indicated that the diversity of species morphologically referable to *L. okamurae* is underestimated in the northwestern Pacific Ocean, as the present study described a new species, *L. neo-okamurae sp. nov.*, and also showed that *L. okamurae* and *L. neo-okamurae* were genetically different entities from other specimens reported as '*L. okamurae*' from the tropical region of China (Hu *et al.* 2020). Further taxonomic revisions of *L. okamurae* and *L.*

margaritae are needed in order to reveal the species diversity and distribution of their related species.

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LEGENDS FOR FIGURES

Fig. 1. ML phylogeny inferred from the *psb*A sequences of *Lithophyllum* spp. In bold face names of species sequenced in the present study. Species denoted by single quotes have not been confirmed by comparison with the DNA sequences of type specimens. GenBank accession or specimen numbers and collection sites provided. Numbers at nodes represent bootstrap values >70% and Bayesian posterior probabilities >0.90.

Fig. 2. ML phylogeny inferred from the *rbc*L sequences of *Lithophyllum* spp. In bold face names of species sequenced in the present study. Species denoted by single quotes have not been confirmed by comparison with the DNA sequences of type specimens. GenBank accession or specimen numbers and collection sites provided. Numbers at nodes represent bootstrap values >70% and Bayesian posterior probabilities >0.90.

Fig. 3. Neighbour-joining phylogeny inferred from the LSU rDNA sequences of *Lithophyllum* spp. In bold face names of species sequenced in the present study. Species denoted by single quotes have not been confirmed by comparison with the DNA sequences of type specimens. GenBank accession or specimen numbers and collection sites provided. Numbers at nodes represent bootstrap values >70%.

Figs 4–10. Morphology and anatomy of herbarium specimens of *Lithophyllum okamurae.* Figures 4–5 were taken by T. Masaki in 1969–1970. The isolectotype *L. okamurae* f. *okamurae* (HAK M-179) is a fragment of the lectotype of *L. okamurae* f. *okamurae* (TRH A21-1318).

Fig. 4. Box of the lectotype of *L. okamurae* f. *okamurae* (TRH A21-1318). Note the label ‘No. 5’ placed on the box by T. Masaki for his reference.

Fig. 5. Habit of the lectotype of *L. okamurae* f. *okamurae* (TRH A21-1318). Scale bar = 5 mm.

Fig. 6. Habit of the isolectotype of *L. okamurae* f. *okamurae* (HAK M-179). Scale bar = 3 mm.

Fig. 7. Vertical section of inner thallus with dimerous construction comprised of non-palisade cells (HAK M-179). Arrows indicate secondary pit-connections. Scale bar = 20 µm.

Fig. 8. Vertical section of outer thallus showing secondary pit-connections (arrows) between cells of adjacent filaments (HAK M-179). Scale bar = 20 µm.

Fig. 9. Vertical section of protuberance showing buried conceptacles (HAK M-179). Scale bar = 200 µm.

Fig. 10. Vertical section through tetrasporangial conceptacle with peripherally arranged, divided tetrasporangium (HAK M-179). Scale bar = 20 µm.

Figs 11–15. Morphology and anatomy of herbarium specimens of *Lithophyllum okamurae.*

Fig. 11. Habit of the holotype of *L. okamurae* f. *angulare* (TRH A21-1327). Scale bar = 1 cm.

Fig. 12. Vertical section of thallus showing dimerous construction (TRH A21-1327). Note non-palisade basal layer of cells. Scale bar = 60 µm.

Fig. 13. Vertical section of outer thallus with secondary pit-connections (arrows) between cells of adjacent filaments (TRH A21-1327). Scale bar = 30 µm.

Fig. 14. Vertical fracture face of inner thallus showing ascending filaments and buried conceptacles (TRH A21-1327). Scale bar = 200 µm.

Fig. 15. Vertical section through tetrasporangial conceptacle with central columella (c) (TRH A21-1327). Note absence of calcified hump below the central columella. Scale bar = 50 µm.

Figs 16–24. Morphology and anatomy of recently collected specimens of *Lithophyllum okamurae*. Figures 16, 21 and 22 taken from specimens from the type locality.

Fig. 16. Habit of a warty specimen (SAP 115621). Scale bar = 1 cm.

Fig. 17. Habit of a rhodolith-shaped specimen (SAP 115616). Scale bar = 1 cm.

Fig. 18. Vertical section of inner thallus with dimerous construction comprised of non-palisade cells (SAP 115608). Scale bar = 50 µm.

Fig. 19. Vertical section of outer thallus showing secondary pit-connections (arrows) between cells of adjacent filaments (SAP 115608). Scale bar = 50 µm.

Fig. 20. Longitudinal section of a branch showing a coaxial medulla (SAP 115615). Scale bar = 50 µm.

Fig. 21. Vertical section through a spermatangial conceptacle with simple spermatangial systems restricted to conceptacle floor (SAP 115621). Scale bar = 50 µm.

Fig. 22. Section through carposporangial conceptacle with large continuous flattened fusion cell (fc) with peripheral gonimoblast filaments (gf) bearing terminal carposporangia (ca). (SAP 115621). Scale bar = 50 µm.

Fig. 23. Surface view of tetrasporangial conceptacles with raised roofs (arrows) (SAP 115612). Scale bar = 500 µm.

Fig. 24. Vertical section through a tetrasporangial conceptacle with peripherally arranged tetrasporangia around a central columella (c) with calcified hump (arrowhead) (SAP 115608). Scale bar = 50 µm.

Figs 25–28. Morphology and anatomy of herbarium specimens of *Lithophyllum neo-okamurae.*

Fig. 25. Habit of warty specimens (TRH A21-1319). Scale bar = 2 cm.

Fig. 26. Vertical section of thallus showing dimerous construction (TRH A21-1319). Note non-palisade basal layer of cells. Scale bar = 60 µm.

Fig. 27. Vertical section of outer thallus with secondary pit-connections (arrows) between cells of adjacent filaments (TRH A21-1319). Scale bar = 30 µm.

Fig. 28. Vertical section through a tetrasporangial conceptacle and a central columella (c) with calcified hump (arrowhead) (TRH A21-1319). Scale bar = 60 µm.

Figs 29–37. Morphology and anatomy of recently collected specimens of *Lithophyllum neo-okamurae.* Specimens from Misaki, Kanagawa, Japan (type locality), except for Fig. 30.

Fig. 29. Habit of the warty holotype specimens (SAP 115594). Scale bar = 2 cm.

Fig. 30. Habit of a rhodolith-shaped specimen (SAP 115601). Scale bar = 2 cm.

Fig. 31. Vertical section of inner thallus with dimerous construction comprised of non-palisade cells (SAP 115599). Scale bar = 50 µm.

Fig. 32. Vertical section of outer thallus showing secondary pit-connections (arrows) between cells of adjacent filaments (SAP 115599). Scale bar = 20 µm.

Fig. 33. Longitudinal section of a branch showing a coaxial medulla (SAP 115593). Scale bar = 50 µm.

Fig. 34. Vertical section through a spermatangial conceptacle with simple spermatangial systems restricted to conceptacle floor (SAP 115595). Scale bar = 50 µm.

Fig. 35. Section through carposporangial conceptacle with large continuous flattened fusion cell (fc) with peripheral gonimoblast filaments (gf) bearing terminal carposporangia (ca). (SAP 115598). Scale bar = 50 µm.

Fig. 36. Surface view of tetrasporangial conceptacles with raised (arrows) and flush (arrowheads) roofs (SAP 115594). Scale bar = 500 µm.

Fig. 37. Vertical section through a tetrasporangial conceptacle with peripherally arranged tetrasporangia around a central columella (c) with calcified hump (arrowhead) (SAP 115596). Scale bar = 50 µm.

Table 1 Comparison of the vegetative and tetrasporangial anatomy of *Lithophyllum okamurae* and *L. neo-okamurae*. Measurements are presented as means ± standard deviation, followed by ranges in brackets.

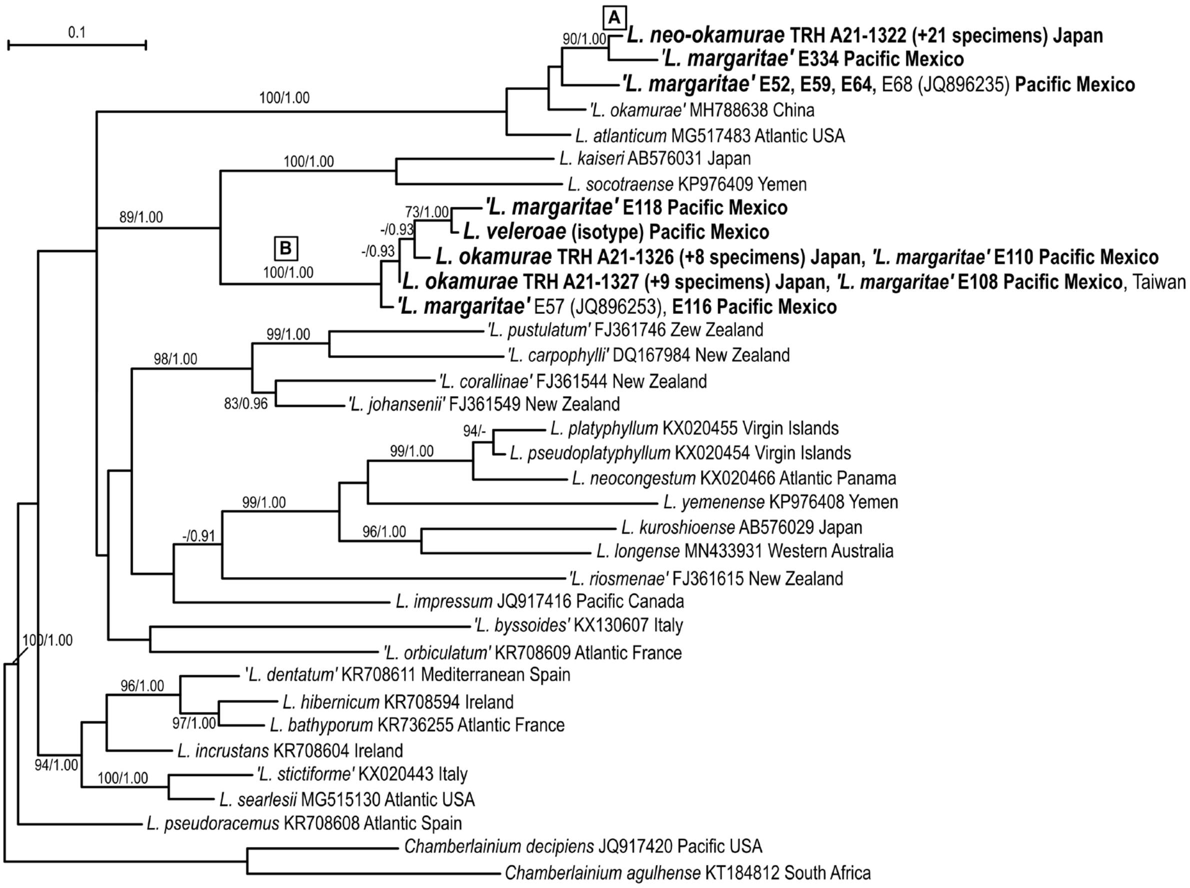
	L. okamurae (new collections from Japan including the type locality)	*L. okamurae* (isolectotype of *L. okamurae* f. *okamurae,* HAK M-179)	*L. okamurae* (holotype of *L. okamurae* f. *angulare,* TRH A21-1327)	*L. neo-okamurae* (holotype and new collections from Japan including the type locality)	*L. neo-okamurae* (as *L. okamurae* f. *okamurae,* TRH A21-1319)
Vegetative structures	(5 specimens, 30 cells)	(1 specimen, 20 cells)	(1 specimen, 30 cells)	(6 specimens, 30 cells)	(1 specimen, 30 cells)
Epithallial cell					
Shape	flattened	flattened	flattened	flattened	flattened
Number	1–2	1	1	1–2	1

Length (µm)	3.1 ± 0.4 [2–4]	2.2 ± 0.5 [1.5–3]	3.7 ± 1 [2–6]	3.3 ± 0.6 [2–5]	4.4 ± 1.2 [3–6]
Diameter (µm)	8.8 ± 0.6 [7.5–10]	8.3 ± 1.7 [6.5–14.5]	9.8 ± 1.7 [6–12]	8.5 ± 0.7 [7–10.5]	7.8 ± 1.1 [6–10]
Subepithallial initial			(10 cells)		(10 cells)
Length (µm)	10.5 ± 2.1 [7–17]	6.3 ± 1.1 [5–9]	11.9 ± 2.5 [7–15]	8.3 ± 1.5 [6–12]	8.7 ± 1.7 [6–12]
Diameter (µm)	8.5 ± 0.8 [6–10]	6.4 ± 0.9 [5–8]	9.8 ± 1.5 [7–12]	8.2 ± 0.9 [7–10]	8.8 ± 1.2 [6.5–10]
Perithallial cell					
Length (µm)	11.1 ± 2.7 [7–19]	9.4 ± 2.2 [6.5–14.5]	12.6 ± 2.8 [7–19]	12.3 ± 3.4 [8–19]	13.2 ± 4.3 [6–20.5]
Diameter (µm)	8.5 ± 1.2 [5.5–11]	6.4 ± 0.8 [5–7]	11 ± 1.5 [9–13]	8.2 ± 0.7 [6–9]	8.8 ± 1.5 [6–12]
Hypothallial cell					(20 cells)
Length (µm)	14.3 ± 2.2 [11–19]	12.7 ± 2.0 [9–15]	16.1 ± 3.3 [12–25]	13.4 ± 2.7 [8–22]	13.1 ± 3.8 [9–19]
Diameter (µm)	13.8 ± 3.7 [7–19]	9.7 ± 1.9 [6.5–13.5]	9.8 ± 2.5 [6–15]	8.9 ± 1.9 [5.5–14]	12.7 ± 3.6 [9–19]

Trichocytes	Absent	Absent	Absent	Absent	Absent
Reproductive structures					
Tetrasporangial conceptacle	(5 specimens, 24 conceptacles)	(1 specimen, 9 conceptacles)	(1 specimen, 4 conceptacles)	(7 specimens, 35 conceptacles)	(1 specimen, 2 conceptacles)
Chamber diameter (μm)	225 ± 41 [167–314]	228 ± 9 [213–238]	[235–295 (341)]	309 ± 33 [248–380]	[270–341]
Chamber height (μm)	85 ± 9 [63–109]	92 ± 11 [76–109]	[94–105]	104 ± 10 [86–121]	[94–129]
Roof elevation (% of examined roofs)[7]	Raised, flush to sunken	flush	Weakly raised, flush	Flush, raised to sunken	Raised, flush
Pore canal length	45 ± 8 [28–61]	39 ± 9 [23–56]	53	57 ± 10 [40–78]	53 (1 measure)
Shape of pore channel	Triangular	Triangular	Triangular	Triangular	Triangular
Roof thickness (μm)	46 ± 10 [30–71]	37 ± 8 [23–53]	48	58 ± 10 [43–78]	53

Number of roof cells	5 ± 1 [4–7]	4 ± 1 [4–6]	4–5	6 ± 1 [4–8]	4–6
Depth from chamber floor to surface (no. of cells)	10 ± 2 [8–13]	12 ± 1 [11–14]	8–12	13 ± 2 [10–16]	12
Depth from chamber floor to surface (µm)	120 ± 24 [89–175]	120 ± 12 [101–137]	88–141	156 ± 22 [114–202]	135
Occurrence of calcified hump below central columella	Present	Present	Present	Present	Present
Number of cells forming calcified hump below central columella	3 ± 1 [2–5]	2 ± 1 [1–3]	2–4	5 ± 1 [4–7]	Not visible
Tetra (bi-) sporangium	Tetrasporangial	Tetrasporangial	-	Tetrasporangial	Tetrasporangial

Length (µm)	60 ± 7 [47–69]	[43–44] (3 measures)	-	65 ± 8 [47–79]	(damaged)
Diameter (µm)	25 ± 3 [20–30]	[15–26] (3 measures)	-	28 ± 4 [20–34]	(damaged)
Buried conceptacles	Present	Present	-	Present	Present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A
90/1.00
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'L. margaritae' E52, E59, E64, E68 (JQ896235) Pacific Mexico
100/1.00
'L. okamurae' MH788638 China
L. atlanticum MG517483 Atlantic USA
100/1.00
L. kaiseri AB576031 Japan
L. socotraense KP976409 Yemen
89/1.00
73/1.00
'L. margaritae' E118 Pacific Mexico
-/0.93
L. veleroae (isotype) Pacific Mexico
B
-/0.93
L. okamurae TRH A21-1326 (+8 specimens) Japan, 'L. margaritae' E110 Pacific Mexico
100/1.00
L. okamurae TRH A21-1327 (+9 specimens) Japan, 'L. margaritae' E108 Pacific Mexico, Taiwan
'L. margaritae' E57 (JQ896253), E116 Pacific Mexico
99/1.00
'L. pustulatum' FJ361746 Zew Zealand
98/1.00
'L. carpophylli' DQ167984 New Zealand
'L. corallinae' FJ361544 New Zealand
83/0.96
'L. johansenii' FJ361549 New Zealand
94/-
L. platyphyllum KX020455 Virgin Islands
99/1.00
L. pseudoplatyphyllum KX020454 Virgin Islands
L. neocongestum KX020466 Atlantic Panama
99/1.00
L. yemenense KP976408 Yemen
96/1.00
L. kuroshioense AB576029 Japan
-/0.91
L. longense MN433931 Western Australia
'L. riosmenae' FJ361615 New Zealand
L. impressum JQ917416 Pacific Canada
'L. byssoides' KX130607 Italy
100/1.00
'L. orbiculatum' KR708609 Atlantic France
96/1.00
'L. dentatum' KR708611 Mediterranean Spain
L. hibernicum KR708594 Ireland
97/1.00
L. bathyporum KR736255 Atlantic France
L. incrustans KR708604 Ireland
94/1.00
100/1.00
'L. stictiforme' KX020443 Italy
L. searlesii MG515130 Atlantic USA
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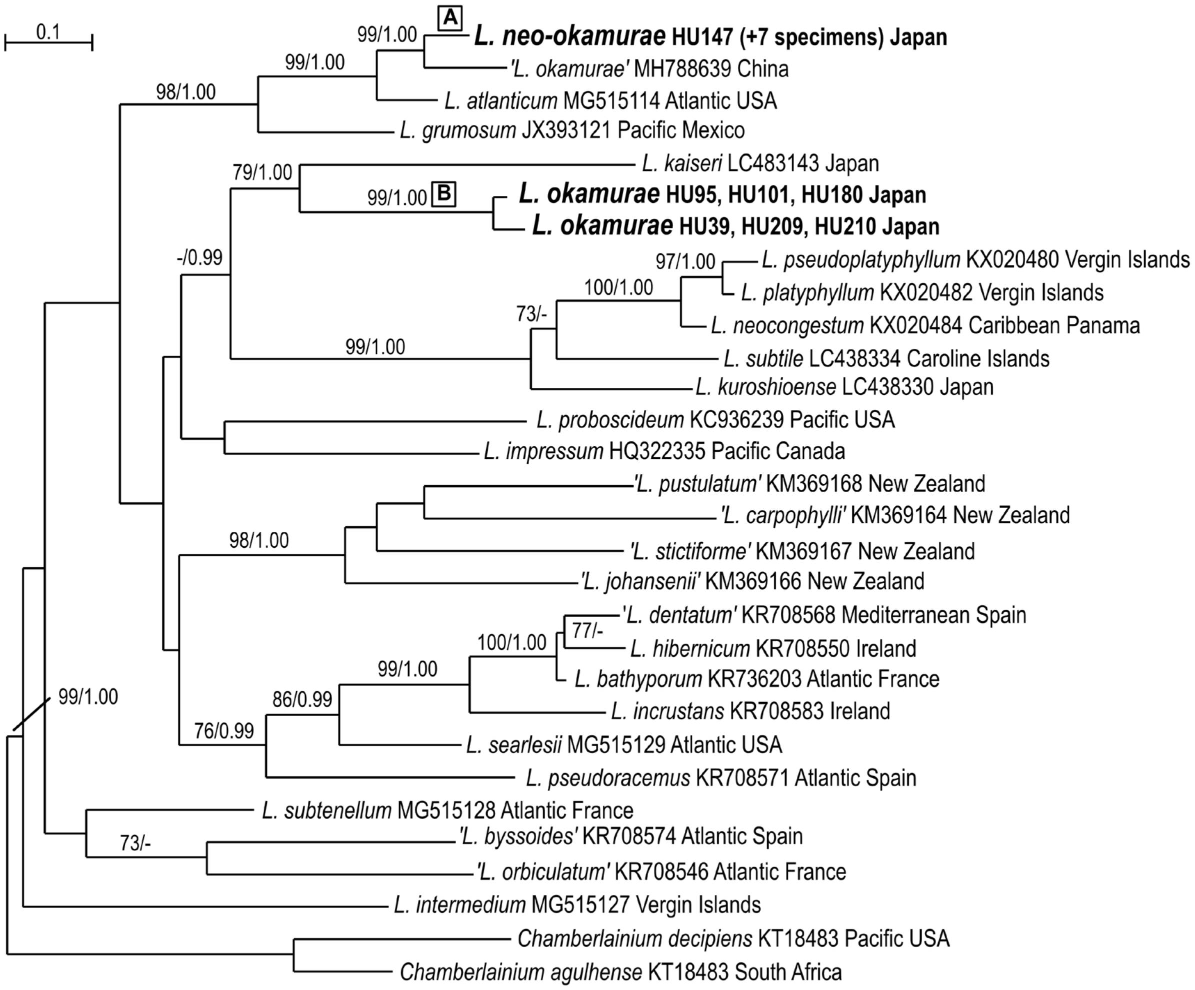
Chamberlainium decipiens JQ917420 Pacific USA
Chamberlainium agulhense KT184812 South Africa

Fig. 1.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99/1.00
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L. grumosum JX393121 Pacific Mexico
L. kaiseri LC483143 Japan
79/1.00
99/1.00
B
L. okamurae HU95, HU101, HU180 Japan
L. okamurae HU39, HU209, HU210 Japan
-/0.99
97/1.00
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100/1.00
L. platyphyllum KX020482 Vergin Islands
73/-
L. neocongestum KX020484 Caribbean Panama
99/1.00
L. subtile LC438334 Caroline Islands
L. kuroshioense LC438330 Japan
L. proboscideum KC936239 Pacific USA
L. impressum HQ322335 Pacific Canada
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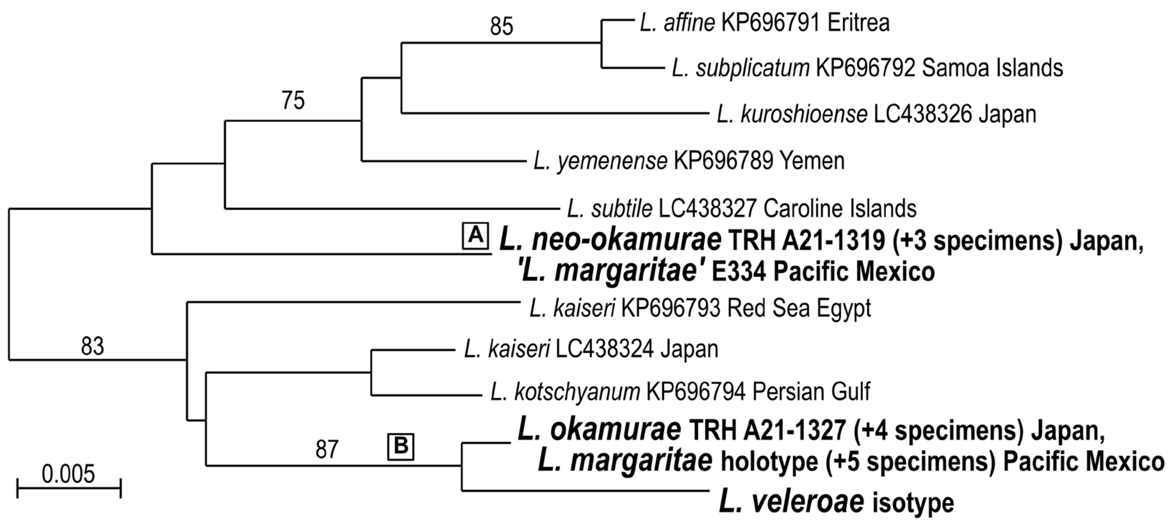
'L. carpophylli' KM369164 New Zealand
98/1.00
'L. stictiforme' KM369167 New Zealand
'L. johansenii' KM369166 New Zealand
'L. dentatum' KR708568 Mediterranean Spain
100/1.00
77/-
L. hibernicum KR708550 Ireland
99/1.00
L. bathyporum KR736203 Atlantic France
99/1.00
86/0.99
L. incrustans KR708583 Ireland
76/0.99
L. searlesii MG515129 Atlantic USA
L. pseudoracemus KR708571 Atlantic Spain
L. subtenellum MG515128 Atlantic France
73/-
'L. byssoides' KR708574 Atlantic Spain
'L. orbiculatum' KR708546 Atlantic France
L. intermedium MG515127 Vergin Islands
Chamberlainium decipiens KT18483 Pacific USA
Chamberlainium agulhense KT18483 South Africa

Fig. 2.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L. subplicatum KP696792 Samoa Islands
L. kuroshioense LC438326 Japan
75
L. yemenense KP696789 Yemen
L. subtile LC438327 Caroline Islands
A
***L. neo-okamurae* TRH A21-1319 (+3 specimens) Japan,**
'*L. margaritae*' E334 Pacific Mexico
L. kaiseri KP696793 Red Sea Egypt
83
L. kaiseri LC438324 Japan
L. kotschyanum KP696794 Persian Gulf
87
B
***L. okamurae* TRH A21-1327 (+4 specimens) Japan,**
***L. margaritae* holotype (+5 specimens) Pacific Mexico**
***L. veleroae* isotype**
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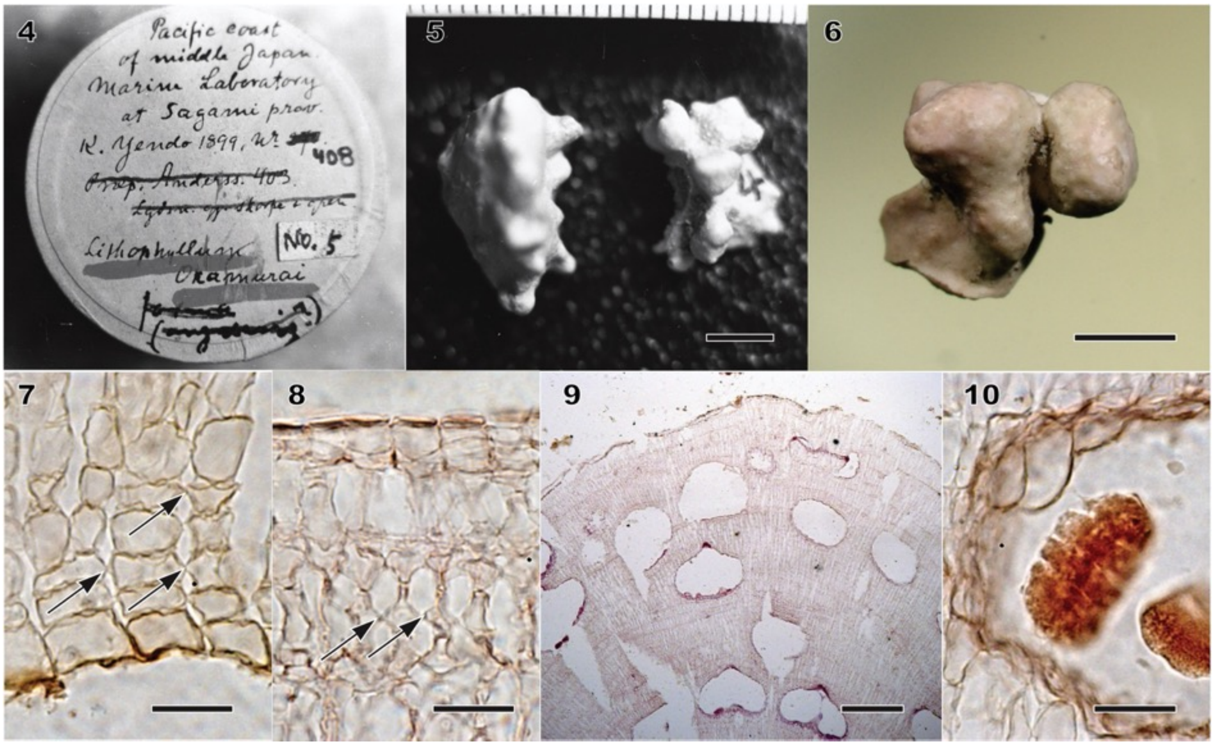
Fig. 3.


4
Pacific coast
of middle Japan.
Marine Laboratory
at Sagami prov.
K. Yendo 1899, Nr. 408
No. 5
Lithophyllum
Okamurai
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6
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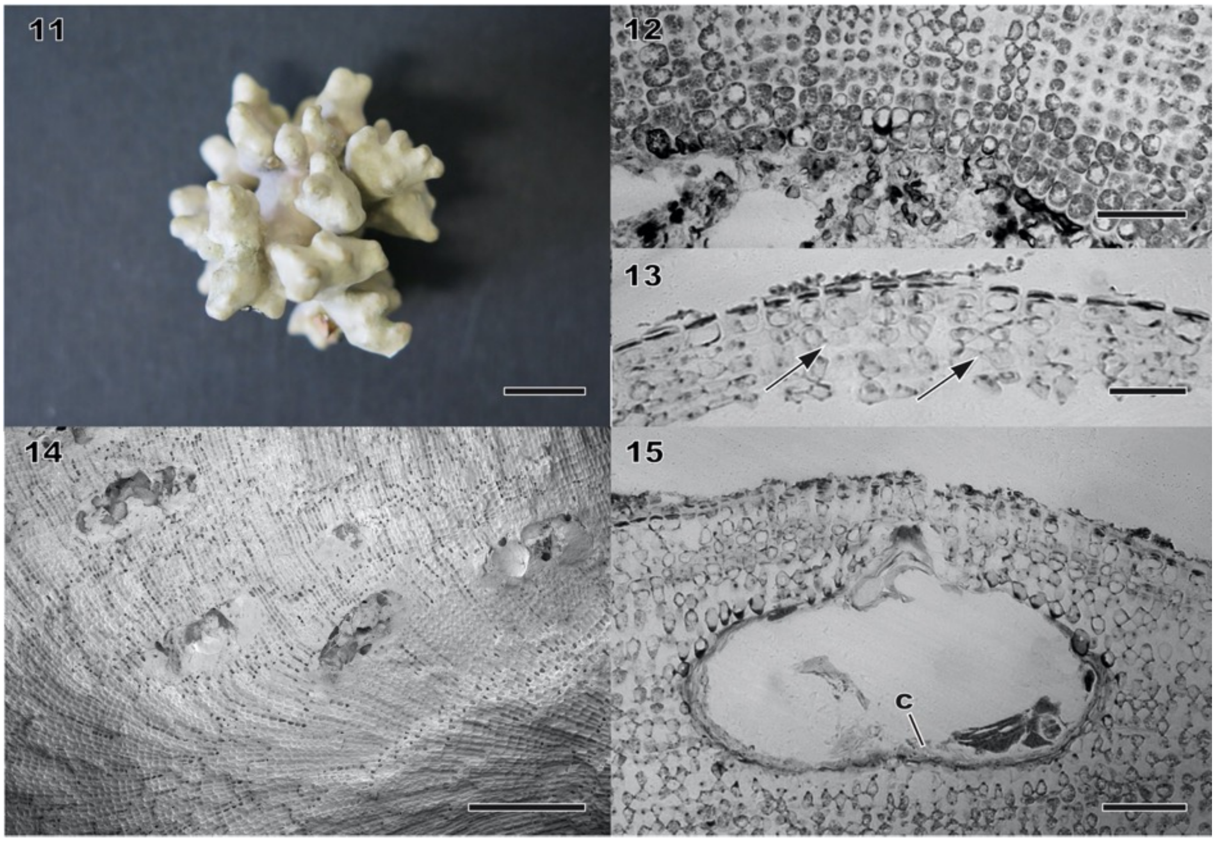
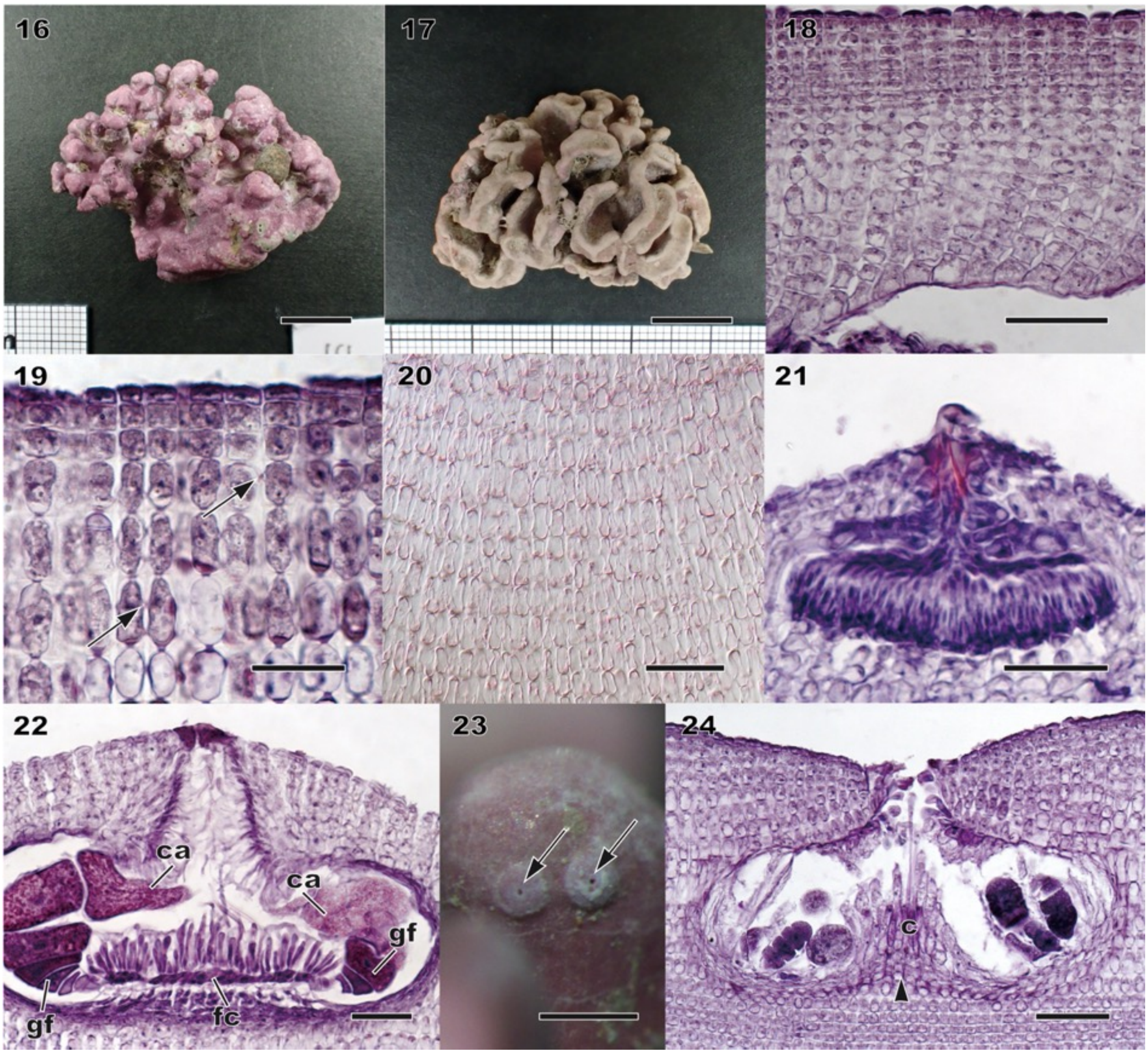
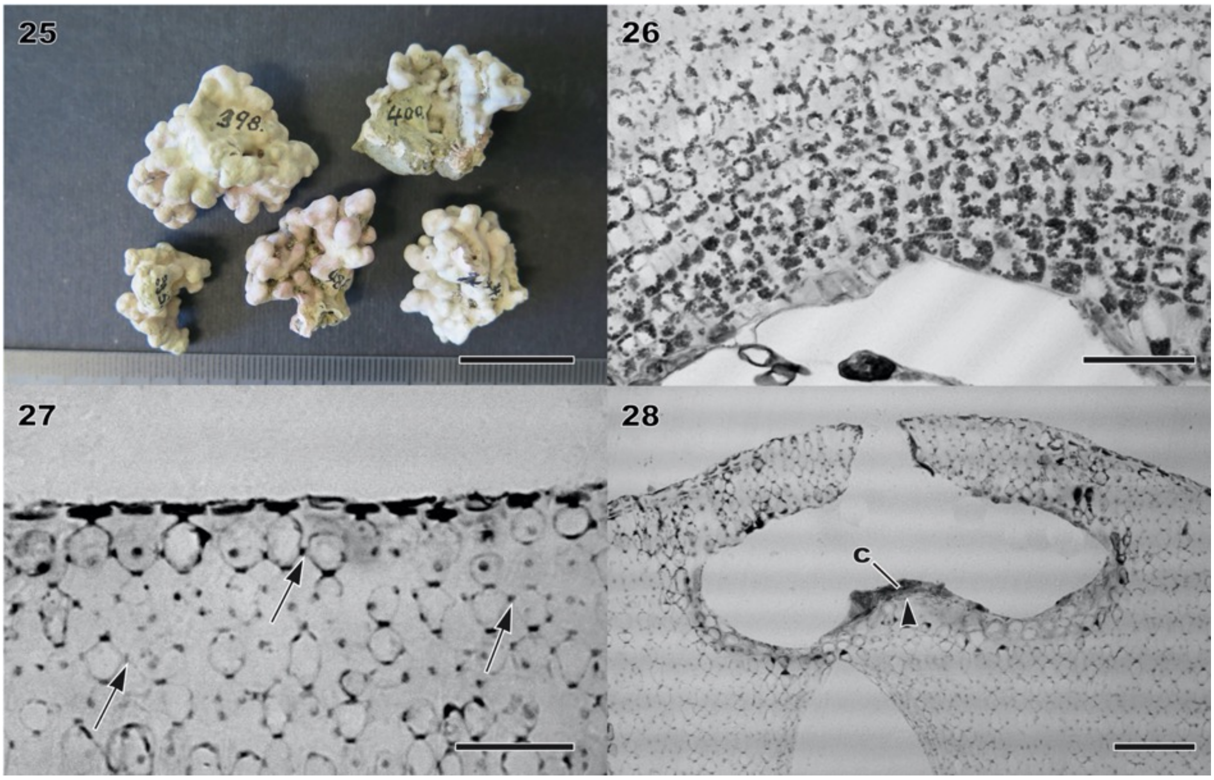
Figs 4-10.



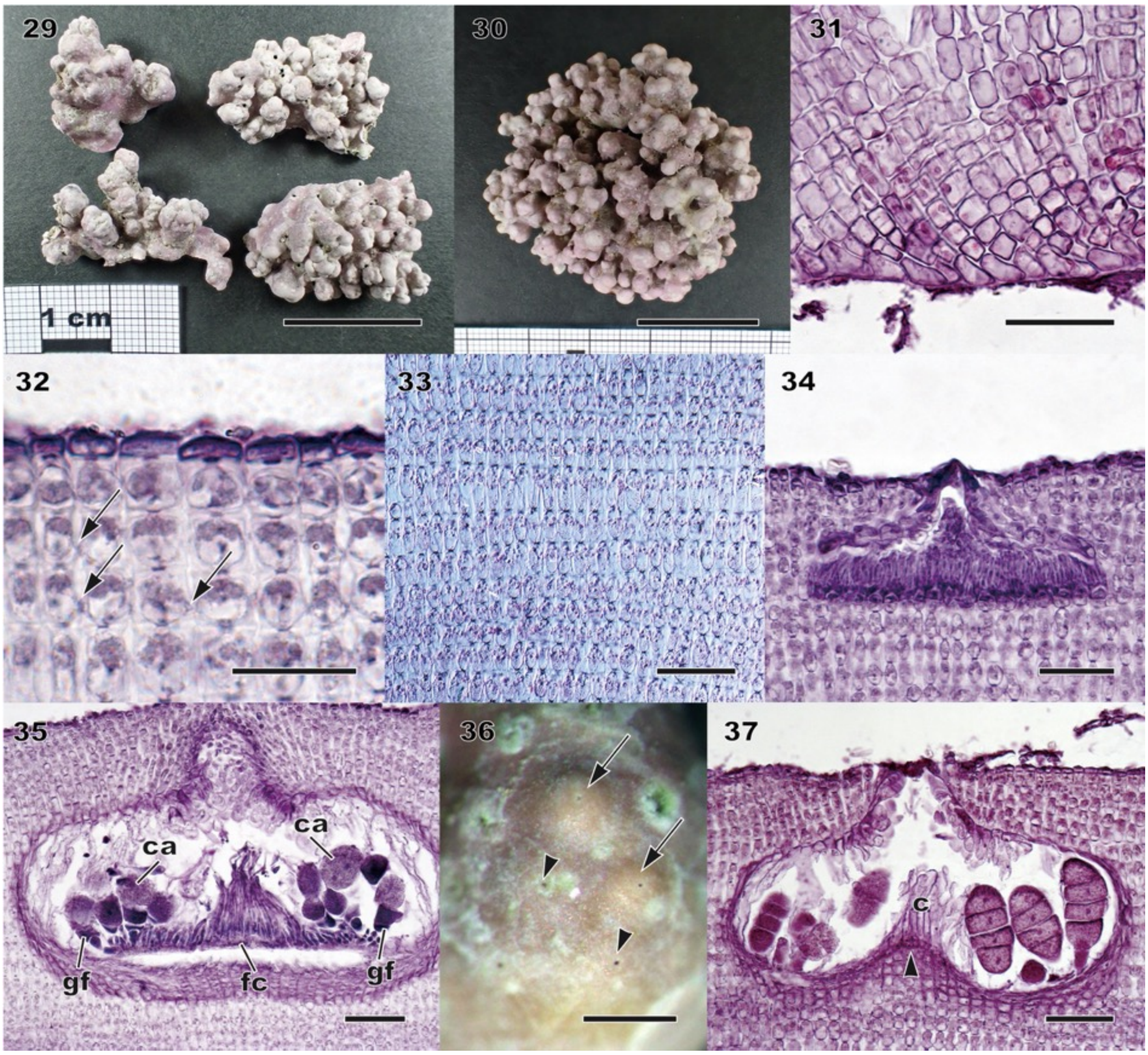
Figs 11-15.



Figs 16-24.



Figs 25-28.



Figs 29-37.