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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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7	Morphological and molecular assessment of Lithophyllum okamurae with
8	the description of <i>L. neo-okamurae sp. nov.</i> (Corallinales, Rhodophyta)
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28 RUNNING TITLE

29 *Lithophyllum okamurae* and *L. neo-okamurae*

30 ABSTRACT

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31	Lithophyllum okamurae has been widely reported in the Pacific Ocean with identification
32	based on morpho-anatomical observations. Two infraspecific taxa, L. okamurae f. okamurae
33	and f. angulare, described from Japan, have been recorded in the temperate region of Japan.
34	We assessed branched Lithophyllum samples morphologically referable to L. okamurae using
35	morpho-anatomical data and DNA sequences (psbA, rbcL and partial LSU rDNA) obtained
36	from herbarium specimens, including type material, as well as recently field-collected
37	material in Japan. The molecular analyses showed that these 'L. okamurae' samples
38	contained two species: L. okamurae and a cryptic new species which we describe as L. neo-
39	okamurae sp. nov. Because the holotype of L. okamurae f. angulare was conspecific with
40	original material cited in the protologue of L. okamurae, it is a heterotypic synonym of L.
41	okamurae f. okamurae. Lithophyllum okamurae and L. neo-okamurae were morphologically
42	similar in having warty, lumpy and fruticose thalli and in often forming rhodoliths.
43	Lithophyllum okamurae can be morpho-anatomically distinguished from L. neo-okamurae by
44	the thallus with tapering or plate-like protuberances (knobby protuberances in the latter) and
45	by having smaller tetrasporangial conceptacle chambers (167–314 μ m; 248–380 μ m in L.
46	neo-okamurae). Our LSU rDNA sequence data from L. okamurae f. angulare (=L. okamurae
47	f. okamurae) was identical to that of the type of L. margaritae, which has nomenclatural
48	priority over <i>L. okamurae</i> . However, considering that <i>psbA</i> and <i>rbcL</i> sequences of <i>L</i> .
49	margaritae type material could not be generated in the present study, we refrain, for the

- 50 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
- 54 algae; Northwestern Pacific Ocean; *psbA*; *rbcL*; Rhodolith

55 INTRODUCTION

56	Lithophyllum Philippi (Corallinales, Rhodophyta) is the largest genus of non-geniculate
57	coralline algae and currently includes 130 taxonomically accepted species names (Guiry &
58	Guiry 2021). However, most of these species have been described based only on morpho-
59	anatomical characters. An effective method to validate species identities, diversity and
60	distribution has been to combine DNA sequencing of freshly field-collected specimens and
61	type specimens with traditional morpho-anatomical characters (Gabrielson et al. 2011). This
62	approach has confirmed that there are many species of Lithophyllum in Europe (Hernández-
63	Kantún et al. 2015a; Peña et al. 2018; Pezzolesi et al. 2019; Caragnano et al. 2020), the
64	warm temperate-tropical western Atlantic ocean basin (Hernández-Kantún et al. 2016;
65	Richards et al. 2018), the western Indian ocean basin (Basso et al. 2015; Maneveldt et al.
66	2019), and the northwestern Pacific ocean basin (Kato & Baba 2019).
67	Lithophyllum okamurae Foslie (1900, 'okamurai'), described from Misaki, Kanagawa
68	Prefecture (previously as Sagami Province), Japan, has been reported worldwide based on
69	morpho-anatomical characters. This species is also known as one of major components of
70	rhodolith beds in the northwestern Pacific Ocean (Kato et al. 2017). Moreover, based on
71	growth-form and anatomical similarities, Basso et al. (1996) suggested that L. okamurae
72	could be the Indo-Pacific vicariant of the Mediterranean Lithophyllum racemus (Lam.)
73	Foslie. However, the application of the species name L. okamurae has not been confirmed
74	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. <i>japonicum</i> ; see Turland <i>et al.</i> 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

89 afterwards named f. angularis (Pl. XI, fig. 11–12)."

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90 Later, Foslie (1909, p. 30) used the designation '*L. okamurae* f. *typica*' for the typical

- 91 form of the species (not validly published; Turland et al. 2018, Art. 24.3, 26.2), and listed as
- 92 a synonym *L. okamurae* f. *japonicum*, thereby considering it to represent the typical form.
- 93 Herbarium specimens labeled *L. okamurae* f. *japonicum* should then be referred by the
- 94 autonym *L. okamurae* f. *okamurae*, as correctly proposed by Woelkerling *et al.* (2005).

95	The lectotype of <i>L. okamurae</i> , illustrated by Foslie (1904, pl. 11, fig. 11), corresponds
96	to Yendo specimen no. 408 (TRH A21-1318) while the rest of Yendo's specimens quoted in
97	the protologue were divided into TRH A21-1325 (no. 270) and TRH A21-1326 (no. 80, 120,
98	379, 382, 389). All these specimens were regarded as the main form (autonym) L. okamurae
99	f. okamurae (Woelkerling et al. 2005). In Foslie (1904), among figs 13–19 of pl. 11 of the
100	form <i>japonicum</i> (currently as f. <i>okamurae</i>), only fig. 13 was from the Pacific coast of Japan.
101	Unfortunately, Woelkerling et al. (2005) did not find the specimen corresponding to that
102	figure.
103	The holotype of L. okamurae f. angulare, indicated by Foslie (1904, pl. 11, fig. 12),
104	corresponds to specimen TRH A21-1327, quoted by Woelkerling et al. (2005). Foslie (1909)
105	mentioned that f. angulare was characterized by less closely spaced branches than the typical
106	form, and more or less angular branches. This holotype was not included in the material cited
107	in the protologue of L. okamurae (Foslie 1900).
108	Five more infraspecific taxa of L. okamurae have been described: Lithophyllum
109	okamurae f. ptychoides Foslie [currently as Lithophyllum ptychoides (Foslie) Foslie],
110	Lithophyllum okamurae f. trincomaliense Foslie ('trincomaliensis'), Lithophyllum okamurae
111	f. validum Foslie ('valida') [currently as Lithophyllum validum (Foslie) Foslie], Lithophyllum
112	okamurae f. subplicatum Foslie ('subplicata') [currently as L. subplicatum (Foslie) D.Basso,
113	Caragnano, L.Le Gall & Rodondi] and Lithophyllum okamurae f. contiguum Foslie
114	('contigua') [currently as Sporolithon gustralasicum (Foslie) Yam -Tomita ex M I Wynne]

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

117 Molecular analyses of Japanese coralline algae morpho-anatomically referable to *L*.

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

123 MATERIAL AND METHODS

124 Sample collection

125 *Lithophyllum* specimens used in this study (n = 37, Table S1) were collected at 18 sites from 126 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 127 128 Science, Hokkaido University, Japan (SAP); the remaining four specimens were used for 129 molecular studies and were deposited in the Herbaria of Muséum National d'Histoire 130 Naturelle, Paris, France (PC) and University of Santiago de Compostela, Spain (SANT). 131 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 132 133 Herbarium of Norwegian University of Science and Technology, Norway (TRH) and the 134 Herbarium of the Laboratory of Marine Botany, Faculty of Fisheries, Hokkaido University,

135	Hakodate, Hokkaido, Japan (HAK; Table S2). The lectotype of L. okamurae f. okamurae
136	(TRH A21-1318) was studied by T. Masaki in 1969–1970, who stored at HAK a small
137	fragment and two preparations of the lectotype as isolectotype (HAK M-179). Both the
138	lectotype (TRH A21-1318) and isolectotype (HAK M-179) were very small fragments, and
139	therefore no destructive investigation was undertaken following the recommendations of the
140	curators. The herbarium archival specimens of Lithophyllum okamurae are specimens
141	collected from the type locality in 1899 when the protologue specimens were collected by the
142	same collector, K. Yendo. The type specimens of Lithophyllum margaritae (Har.) Heydr. and
143	L. veleroae E.Y.Dawson (one of the synonyms of L. margaritae) were borrowed from PC
144	and University of California, USA (UC) for molecular studies (Table S2). In addition, nine
145	recent collections of <i>L. margaritae</i> preserved in GALW (National University of Ireland) and
146	FBCS (Universidad Autónoma de Baja California Sur, Mexico) were used for the molecular
147	studies (Table S1). Herbarium abbreviations follow Thiers (2021).

Molecular analyses 148

149	Total DNA	was extracted	from the	herbarium	specimens a	nd fi	ield-a	collected	specimens	dried
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- by silica gel, using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) or a 150
- NucleoSpin® 96 Tissue kit (Macherey-Nagel, GmbH and Co. KG, Germany), according to 151
- the manufacturer's instructions. PCR of the following gene fragments were carried out using 152
- a Blend Taq -Plus- or KOD FX NEO Reaction Kit (TOYOBO, Osaka, Japan), except for four 153
- vouchers (JHS0012, JHS0014, JHS0029B, VPF00887a) and herbarium specimens of L. 154

155	okamurae f. okamurae and type material of L. okamurae f. angulare, L. margaritae and L.
156	veleroae, which followed Basso et al. (2015) and Peña et al. (2015), and seven specimens
157	(E52, E57, E334, E108, E110, E116, E118) with previous GenBank records that followed
158	Hernández-Kantún et al. (2014, 2015b). The primer pairs for the PCR and sequencing of the
159	chloroplast <i>psb</i> A were psbA-F/psbA-R2 and psbA-F/psbA600R in addition to psbA-
160	F/psbA500F for sequencing (Yoon et al. 2002), while the primer pairs for PCR and
161	sequencing of the chloroplast <i>rbc</i> L were F-57/R-1150 and F-753/R-rbcS start (Freshwater &
162	Rueness 1994). When the <i>rbcL</i> primer pair F-57/R-1150 did not amplify, the F-57/R-753 pair
163	was used instead (Freshwater & Rueness 1994). The PCR and sequencing primer pairs for the
164	nuclear-encoded LSU (28S) rRNA gene were T01N (Harper & Saunders 2001) and TR273
165	(Basso et al. 2015) or T16N (Saunders & McDevit 2012) and the reverse primer designated
166	T99R (5'TGGTCCGTGTTTCAAGACGG3'). The PCR products were purified and
167	sequenced by Macrogen Japan (Kyoto, Japan) or by Eurofins (Eurofins Scientific, France).
168	Three data sets for phylogenetic analyses, psbA, rbcL and LSU sequences, were assembled,
169	which comprised novel sequences from this study and previously published sequences for the
170	genus <i>Lithophyllum</i> (Tables S1, S2, S3). The <i>rbc</i> L and LSU rDNA sequences of <i>L. okamurae</i>
171	and L. neo-okamurae were obtained from a subset of examined specimens in psbA analyses.
172	Specimens with identical sequences were represented by a single specimen in the data sets.
173	Moreover, sequences with less than 1% pairwise divergence estimated in MEGA X (ver.
174	10.1.8., Kumar et al. 2018) were also combined into a single sequence for the psbA dataset.
175	Sequences were aligned using MAFFT version 7 (Katoh & Standley 2013). Chamberlainium

176	tumidum (Foslie) Caragnano, Foetisch, Maneveldt & Payri and C. decipiens (Foslie)
177	Caragnano, Foetisch, Maneveldt & Payri were used as outgroups for <i>psbA</i> and <i>rbcL</i> data sets.
178	Phylogenetic relationships for <i>psbA</i> and <i>rbcL</i> data sets were inferred by maximum likelihood
179	(ML) using RAxMLGUI 1.5b1 (Silvestro & Michalak 2012), and Bayesian inference (BI)
180	using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001). ML analyses were performed using
181	the general-time-reversible model with gamma distribution and invariant sites (GTR+G+I)
182	and 1000 rapid bootstrap (BS) replicates. BI analyses were performed using the GTR+G+I
183	model. Four Markov chains were used. Analyses were run for 300,000 generations for the
184	psbA data set, for 1,000,000 generations for the rbcL data set, and sampling was performed
185	every 100 generations. The number of generations of run was chosen to ensure the attainment
186	of an average and standard deviation of split frequencies lower than 0.01. The burn-in was
187	determined after convergence of the tree samples using Tracer v1.7.1 (Rambaut et al. 2018)
188	after satisfactory convergences of the tree samples were obtained: 750 in <i>psbA</i> ; 2,500 in
189	rbcL. Consensus topology and posterior probability (PP) values were calculated using the
190	remaining trees. Neighbour-joining (NJ) analysis for the LSU data set was conducted in
191	MEGA X using the Maximum Composite Likelihood model with 1,000 BS replicates. In the
192	phylogenetic tree inferred from ML and NJ analysis, nodes with BS values ≥90% were
193	considered strongly supported; those between 89% and 70% moderately supported and those
194	<70% weakly supported. In the tree inferred from Bayesian analysis, nodes with PP values
195	\geq 0.95 were considered strongly supported and those between 0.95 and 0.90 moderately

196 supported. For all the genetic markers, the total number of pairwise sequence divergences197 (uncorrected, p distances) was calculated using MEGA X.

198 Morpho-anatomical observations

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

205 **RESULTS**

206 Molecular analyses

207 Recently collected specimens morpho-anatomically referable to Lithophyllum okamurae were 208 divided into two independent lineages (A and B) in the *psbA*, *rbcL* and LSU rDNA analyses 209 (Figs 1–3). The specimens in lineage B were confirmed to be conspecific with the holotype 210 of L. okamurae f. angulare (here regarded as L. okamurae, TRH A21-1327) by a match with the psbA sequence. The protologue and archival material of L. okamurae also split into two 211 212 separate species, which corresponded to the above two species, respectively, in the *psbA* and LSU rDNA analyses. In the present study, these taxa are treated as two independent species, 213 *L. neo-okamurae sp. nov.* (lineage A) and *L. okamurae* (part of lineage B; see Discussion) 214

215	In the <i>psbA</i> analyses (Fig. 1), <i>Lithophyllum neo-okamurae</i> was resolved as an
216	independent lineage (A) in a strongly-supported clade (100% in ML bootstrap, 1.00 in PP)
217	that included L. atlanticum Vieira-Pinto, M.C.Oliveira & P.A.Horta, 'L. margaritae', and
218	also 'L. okamurae' from China, the latter two specimens appearing distantly related to the
219	type specimens of both species. This lineage comprised 21 specimens of L. neo-okamurae
220	that shared nearly or completely identical sequences (0.0%-0.6% sequence divergences in
221	852 bp; Table S4) with an archival specimen of L. okamurae f. okamurae (TRH A21-1322)
222	from the type locality. <i>Lithophyllum neo-okamurae</i> formed a supported clade with L.
223	margaritae from the Gulf of California (E334) (90% in ML, 1.00 in PP) and the sequence
224	divergences between them were 1.3%–1.7%. However, the specimen of <i>L. margaritae</i> (E68)
225	from the type locality, which was nearly identical to three other specimens (E52, E59, E64)
226	from the Gulf of California (0.0%-0.7% sequence divergences), differed from the specimen
227	E334 by 3.1%–3.2%. Lineage B (Fig. 1) consisted of Lithophyllum okamurae, 'L.
228	margaritae' and the isotype of L. veleroae, which is a synonym of L. margaritae, with strong
229	support (100% in ML, 1.00 in PP) and the sequence divergences among them were 0.0% –
230	2.6% (Table S4). Lineage B was distantly related to other species in the northwestern Pacific
231	Ocean or the temperate regions. The total sequence divergences among the three archival
232	specimens (see below) and 16 recently collected ones of <i>L. okamurae</i> from Japan were 0.0%-
233	1.3 % (837–852 bp). The holotype of <i>L. okamurae</i> f. angulare (TRH A21-1327) and an
234	archival specimen from the type locality of L. okamurae f. okamurae (TRH A21-1321)
235	shared identical sequences, and differed from the nearest haplotype of recently collected L .

236	okamurae specimens from the type locality (HU39 and six specimens; Table S4) by 0.4%,
237	whereas the holotype of L. okamurae f. angulare (TRH A21-1327) differed from the other
238	archival specimen cited in the protologue of L. okamurae (TRH A21-1326) by 0.7%. A
239	haplotype of L. margaritae from Taiwan was identical to the haplotype of L. okamurae
240	(HU39 and six specimens), although it differed from specimens from the type locality (E57,
241	E108, E110) and the other specimens (E116, E118) of L. margaritae from the Gulf of
242	California by 0.2%–2.4%. The isotype of <i>L. veleroae</i> formed a moderate to strongly
243	supported subclade with L. margaritae (E118) from the Gulf of California (73% in ML, 1.00
244	in PP) and the sequence divergence between them was 0.9% (524 bp). The subclade differed
245	from other taxa in lineage B by 1.3%–2.6% sequence divergences.
246	In lineage A of the <i>rbc</i> L trees (Fig. 2), eight Japanese specimens of <i>L. neo-okamurae</i>
247	showed very similar sequences with $0.0\%-0.9\%$ divergences, whereas they differed from 'L.
248	okamurae' from China by 4.7%–5.1% (1350 bp; Table S5). Lineage B consisted of Japanese
249	L. okamurae specimens. The sequence divergences among six specimens of this species were
250	0.0%–2.2% (1301–1350 bp; Table S5).
251	In the LSU rDNA analysis (Fig. 3), the holotypes of L. okamurae f. angulare (TRH
252	A21-1327) and L. margaritae shared identical sequences with recently collected specimens
253	of these species and grouped with the isotype of L. veleroae with moderate support (87% in
254	NJ). <i>Lithophyllum neo-okamurae</i> was distantly related to <i>L. okamurae, L. margaritae</i> and <i>L.</i>
255	veleroae. The LSU rDNA sequence of the holotype of L. neo-okamurae was identical to an

257 (E334) from the Gulf of California. 258 Lithophyllum okamurae Foslie 1900, pp 4, 5 259 Figs 4–24, S1–S4; Tables 1, S6 260 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. 261 262 178). Illustrated in Foslie (1904, pl. 11, fig. 11) and the present study (Figs 4, 5, S1). 263 ISOLECTOTYPE: HAK! M-179 (a fragment of Yendo specimen no. 408), present study, illustrated by 264 Figs 6–10. 265 TYPE LOCALITY: Misaki, Miura City, Kanagawa Prefecture, Japan (as Marine Laboratory at Sagami 266 Province; Woelkerling 1993). HETEROTYPIC SYNONYM: Lithophyllum okamurae f. angulare Foslie 1901 ('angularis') (Woelkerling 267 268 1993, p. 26). Holotype TRH A21-1327 (Woelkerling et al. 2005, p. 179). Illustrated in Foslie (1904, 269 pl. 11, fig. 12, as 'angulata' in the legend to figure), Printz (1929, pl. 64, fig. 7), and the present study 270 (Figs 11–15). 271 SPECIMENS EXAMINED: See Table S2. Sequences of two DNA markers were obtained from the 272 holotype of L. okamurae f. angulare, TRH A21-1327 (psbA, MZ128805; LSU rDNA, MZ129208). 273 DNA sequences obtained from two archival specimens of L. okamurae from the type locality, TRH 274 A21-1321 (Yendo specimen no. 327; Fig. S2) and TRH A21-1326 (Yendo specimen no. 120; Fig. 275 S4), the former not belonging and the latter belonging to protologue specimens, included two 276 respective *psbA* and two LSU rDNA sequences. 277 OTHER SPECIMENS EXAMINED: See Table S1. DNA sequences determined from recently collected

archival specimen of L. okamurae (TRH A21-1319) from the type locality and L. margaritae

278 Japanese material including specimens from the type locality contained 16 *psbA* sequences, six *rbcL*

279 sequences and two LSU rDNA sequences.

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

281 DISTRIBUTION: *Lithophyllum okamurae* occurs on the temperate coasts of the Pacific Ocean, the Japan

- 282 Sea and the Seto Inland Sea in Japan. Based on the *psbA* sequences, *L. margaritae* from Taiwan and
- some material from the Gulf of California are considered conspecific or very closely allied to *L*.
- 284 okamurae (Hernández-Kantún et al. 2015b; Liu et al. 2018).

285 Morphology and vegetative anatomy

Thalli ranged from encrusting, warty, lumpy, foliose to fruticose, and often formed free-286 287 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 288 289 diameter), and dichotomously branched or anastomosing. Colour of living plant was grayish-290 pink to light purple. Thalli were dimerous with unistratose hypothallus composed of 291 approximately isodiametric or slightly elongate or wide cells, non-palisade (Figs 7, 12, 18), 292 11–19 µm long and 7–19 µm in diameter. Cells of perithallial filaments were 7–19 µm long 293 and 5.5–11 µm in diameter. Secondary-pit connections were common. Cell fusions were not 294 observed. Subepithallial initials were 7–17 μ m long and 6–10 μ m in diameter. One to two 295 layers of epithallial cells were flattened, 2–4 µm long and 7.5–10 µm in diameter (Figs 8, 13, 296 19). Medullary regions in branches (protuberances) were coaxial (Fig. 20). Trichocytes were not observed. 297

298 **Reproductive anatomy**

299 Gametophytes are dioecious. Gametangial conceptacles were slightly raised above or flush 300 with surrounding thallus surface. Spermatangial conceptacle chambers were 86–116 µm in 301 diameter and 20–35 µm high, with roofs 18–30 µm thick. Simple spermatangial systems were 302 restricted to the conceptacle floor (Fig. 21). Carpogonial conceptacle chambers were 94-139 303 μm in diameter and 18–30 μm high, with roofs 56–96 μm. Carposporangial conceptacle 304 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 305 306 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 307 308 surface. Buried conceptacles were observed (Figs 9, 14). Conceptacle chambers were 167-309 314 µm in diameter and 63–109 µm high. Pore canals were triangular and tapering towards 310 surface, 28–61 µm long. Conceptacle roofs were 4–7 cell layers, 30–71 µm thick. 311 Tetrasporangial conceptacle chamber floors were situated 8 to 13 cells below surrounding 312 thallus surface. A central columella was present or absent; when present, it was comprised of 313 sterile filaments. A calcified hump (two to five cell layers) below central columella was 314 absent (Fig. 15) or present (Fig. 24). Tetrasporangia were zonately divided, 47-69 µm long 315 and 20-30 µm in diameter, and peripherally arranged in the conceptacle chamber (Figs 10, 316 24). Data on measured vegetative and reproductive features in the above descriptions were

317 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.*

322 Figs 25–37, S5; Tables 1, S6

323 DIAGNOSIS: Thalli encrusting, warty, lumpy to fruticose, free-living rhodoliths (Figs 25, 29, 30, S5).

324 Protuberances up to 12 mm long, knobby, columnar (up to 20 mm long) or apically enlarged (2–6 mm

325 in diameter), dichotomously branched or anastomosing. Colour of living plant grayish-pink to light

326 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).

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

330 ETYMOLOGY: Greek 'neos' (in compounds 'neo-'), new (Stearn 1992); 'okamurae', dedicated to the

- 331 Japanese phycologist Dr. Kintaro Okamura.
- 332 OTHER SPECIMENS EXAMINED: See Tables S1, S2. Sequences of three DNA markers were obtained
- from the holotype (*psbA*, LC620629; *rbcL*, LC624951; LSU, LC624957). In addition, DNA

334 sequences determined from Japanese material contained 20 psbA sequences, seven rbcL sequences

and two LSU rDNA sequences. DNA sequences obtained from two archival specimens of *L*.

336 *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 *psbA*

- 338 sequence, respectively. The respective sequence was identical to the holotype and recently collected
- 339 specimens of *L. neo-okamurae* from the type locality.
- 340 DISTRIBUTION: Based on DNA sequences, L. neo-okamurae is widely distributed in the temperate
- 341 coasts of the Pacific Ocean, the Japan Sea and the Seto Inland Sea in Japan.

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

343 Vegetative anatomy

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

352 **Reproductive anatomy**

353	Gametophytes are dioecious. Gametangial conceptacles were slightly raised above or flush
354	with surrounding thallus surface. Spermatangial conceptacle chambers were 119–182 μm in
355	diameter and 15–46 μ m high, with roofs 25–46 μ m thick. Simple spermatangial systems were
356	restricted to the conceptacle floor (Fig. 34). Carpogonial conceptacle chambers were 94-200
357	μm in diameter and 23–53 μm high, with roofs 68–104 μm thick. Carposporangial
358	conceptacle chambers were 218–339 μm in diameter and 73–144 μm high, with roofs 48–89
359	μ m thick. Carposporangia were cut off from gonimoblast filaments borne at periphery of a
360	large continuous flattened fusion cell (Fig. 35). Tetrasporangial conceptacles were uniporate
361	with roofs flush with or raised above surrounding thallus surface (Fig. 36) or sunken below

362	thallus surface. Buried conceptacles were observed. Conceptacle chambers were 248–380 μ m
363	in diameter and 86–121 μ m high. Pore canals were triangular and tapering towards surface,
364	40–78 μ m long. Conceptacle roofs comprised of 4–8 cell layers, 43–78 μ m thick.
365	Conceptacle chamber floors were situated 10 to 16 cells below surrounding thallus surface. A
366	central columella was present or absent; when present, it was comprised of sterile cells. A
367	calcified hump (four to seven cell layers) below central columella was present (Figs 28, 37).
368	Tetrasporangia were zonately divided, 47–79 μ m long and 20–34 μ m in diameter, and
369	peripherally arranged in conceptacle chamber. Data on measured vegetative and reproductive
370	features in the above descriptions were based on recently collected specimens, because those
371	of the archival material were overlapping within the ranges of the recent material
372	(summarized in Tables 1, S6).

DISCUSSION

374	Our molecular analyses show that branched Lithophyllum specimens morphologically
375	referable to L. okamurae belong in fact to two species: Lithophyllum okamurae and L. neo-
376	okamurae. The two species sometimes occur together in the upper subtidal zone in the
377	temperate region of Japan and often form rhodoliths. Our molecular analyses indicate that L .
378	okamurae and L. neo-okamurae are distantly related to branched Lithophyllum species in the
379	Western Pacific Ocean, namely L. kaiseri (Heydr.) Heydr., L. kuroshioense A.Kato &
380	M.Baba, L. longense HernKant., P.W.Gabrielson & R.A.Town., and L. subtile (Foslie)
381	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.

385 Lithophyllum okamurae

386 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 387 388 other specimens (TRH A21-1325 and A21-1326) (Woelkerling et al. 2005, pp 176-179). In 389 the present study, the gross morphology of the lectotype (TRH A21-1318) and isolectotype 390 (HAK M-179) of L. okamurae could not be confirmed because of their very small sizes. 391 However, a photograph of the lectotype taken in 1969–1970 by T. Masaki (Fig. 5) showed 392 that the specimen was sparsely branched and had somewhat pointed apices, consistent with 393 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 394 395 single 3-cm-diameter specimen and had sharp-pointed protuberances. The collection TRH 396 A21-1326 (Fig. S4) contains six small fragments (less than 2 cm in diameter) which were 397 mutually similar encrusting to warty thalli. One of them (specimen no. 120) was conspecific with L. okamurae f. angulare based on the similarities to psbA sequences of the holotype of 398 399 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 400 401 synonym of L. okamurae f. okamurae.

402	Verheij (1994) observed old buried male and female conceptacles of the lectotype of L.
403	okamurae. However, the isolectotype of L. okamurae (HAK M-179) has only tetrasporangial
404	conceptacles. Verheij (1994, figs 10, 11) showed remnants of spermatangia remaining both
405	on a wall and a floor of the male conceptacle, whereas spermatangia were formed only on the
406	conceptacle floor in our observation of <i>L. okamurae</i> and <i>L. neo-okamurae</i> (Figs 21, 34).
407	Therefore, we think that Verheij's (1994) observations of the old buried conceptacles are
408	doubtful.
409	The holotype of <i>L. margaritae</i> , a species name with nomenclatural priority over <i>L</i> .
410	okamurae, was identical to the holotype of L. okamurae f. angulare in the short LSU rDNA
411	sequence (214 bp), indicating that L. okamurae was closely related to L. margaritae, not to L.
412	neo-okamurae. However, we consider that the conspecificity between L. okamurae and L.
413	margaritae is not convincingly demonstrated solely on the basis of this short and weakly
414	variable LSU rDNA sequence. We refrain from proposing the taxonomic synonymy between
415	these two taxa because more variable <i>psbA</i> and <i>rbcL</i> sequences of the <i>L. margaritae</i> type
416	material could not be generated.
417	The minimum threshold of the interspecific divergences of <i>psbA</i> sequences among
418	closely related <i>Lithophyllum</i> species was less than 2%; e.g. 1.7%–2.2% in <i>psbA</i> between <i>L</i> .
419	platyphyllum (Foslie) Foslie and L. pseudoplatyphyllum HernKant., W.H.Adey &
420	P.W.Gabrielson (Hernández-Kantún et al. 2016); 1.5%–3.0% between L. racemus and L.
421	pseudoracemus Caragnano, Rodondi & Rindi (Caragnano et al. 2020); and mostly 2%-5%
422	among 13 phylogenetic species of L. stictiforme (Aresch.) Hauck (Pezzolesi et al. 2019). In

423	the present study, the pairwise divergences of <i>psbA</i> sequences of <i>L. margaritae</i> in lineage B
424	including the isotype of <i>L. veleroae</i> , one of synonyms of <i>L. margaritae</i> , were 0.0%–2.6%,
425	indicating that it is likely to contain some cryptic species. A haplotype of L. okamurae (HU39
426	and six specimens) showed up to 0.5% (4 bp out of 849 bp) sequence divergence among two
427	haplotypes of <i>L. margaritae</i> from the Gulf of California (E108) and Taiwan, which were
428	similar to the intraspecific sequence divergence of <i>L. longense</i> (up to 0.59%, representing 5
429	bp out of 841 bp; Maneveldt et al. 2019). This means that at least one genetically
430	circumscribed species is widely distributed in the North Pacific Ocean.
431	The pairwise divergences of <i>psbA</i> sequences of <i>L. okamurae</i> used in the present study
432	(up to 1.3%) were similar to or lower than the above minimum threshold of the species-level
433	divergence. In contrast, the pairwise divergence of <i>rbc</i> L sequences of <i>L. okamurae</i> (2.2%)
434	was similar to the divergence between L. platyphyllum and L. pseudoplatyphyllum (1.9%;
435	Hernández-Kantún et al. 2016). The pairwise sequence divergences of L. okamurae are at or
436	near the minimum threshold of the species level. Therefore, species delimitation analyses
437	using multiple genes are needed to confirm whether more than one species are included under
438	a similar morphology.

439 Lithophyllum neo-okamurae

440 *Lithophyllum neo-okamurae* differs from *L. okamurae* in having mostly knobby

441 protuberances, not tapering nor plate-like (foliose thalli), while *L. okamurae* shows a wide

442 range of morphologies including these protuberances. Although *L. okamurae* can also be

443	anatomically distinguished from <i>L. neo-okamurae</i> by smaller tetrasporangial conceptacle
444	chambers (167–341 μ m vs 248–380 μ m; Table 1), the conceptacle chamber sizes overlap
445	considerably between two species. Thus, DNA sequences are needed for reliable
446	identification.
447	In the psbA analyses of the present study, L. neo-okamurae was closely related to the
448	other two 'L. margaritae' lineages from the type locality (E334 and E68). The intraspecific
449	divergence of <i>L. neo-okamurae</i> (up to 0.6%, representing 5 bp out of 852 bp) in <i>psbA</i> was
450	similar to that of L. longense (up to 0.59%, representing 5 bp out of 841 bp; Maneveldt et al
451	2019). In contrast, the sequence divergences between 'L. margaritae' and L. neo-okamurae
452	were 1.3%-3.2%, which means that 'L. margaritae' includes at least one species different
453	from <i>L. neo-okamurae</i> .

454 Taxonomic relationships among *Lithophyllum okamurae*, *L. margaritae* and 455 related species

456 Riosmena-Rodríguez et al. (1999) merged five species described from La Paz, BCS, Mexico,

457 namely Lithophyllum diguetii (Har.) Heydr., L. lithophylloides Heydr., L. margaritae, L.

- 458 pallescens (Foslie) Foslie and L. veleroae, within L. margaritae based on their morpho-
- 459 anatomical similarity. Subsequently, Schaeffer et al. (2002) indicated that foliose and
- 460 fruticose growth forms of *L. margaritae* were genetically distinct using amplified fragment
- 461 length polymorphism (AFLP) analyses. Following Schaeffer *et al.* (2002), Norris (2014)
- 462 recognized three species out of the five synonyms of *L. margaritae*: *L. diguetii* for the foliose

463	form; L. pallescens for the fruticose form; and L. margaritae for the intermediate form. Based
464	on the morphological similarities, Norris (2014) tentatively treated <i>L. veleroae</i> and <i>L.</i>
465	lithophylloides as synonyms of L. diguetii and L. pallescens, respectively, until molecular
466	analyses could be done on the type material of these species. Furthermore, it should be noted
467	that, although the species epithet diguetii has the same taxonomic priority as margaritae,
468	Riosmena-Rodríguez et al. (1999) chose the species epithet margaritae for that species
469	because the type material is in better condition and shows the characteristics of the species
470	more clearly. Therefore, in addition to type material of L. margaritae [DNA sequences of
471	material ascribed to it have also been reported from Brazil and Taiwan (Vieira-Pinto et al.
472	2014; Liu et al. 2018)] and its synonyms, fresh material of each species is required to assess
473	the genuine boundary of <i>L. margaritae</i> and the phylogenetic relationships among the taxa
474	that were proposed to be synonyms of this species.
475	In conclusion, the present study reassessed the complicated taxonomic history of L .
476	okamurae and showed that L. okamurae f. angulare is a synonym of the autonym L.
477	okamurae f. okamurae, which takes precedence according to the rules of nomenclature. It
478	also indicated that the diversity of species morphologically referable to L. okamurae is
479	underestimated in the northwestern Pacific Ocean, as the present study described a new
480	species, L. neo-okamurae sp. nov., and also showed that L. okamurae and L. neo-okamurae
481	were genetically different entities from other specimens reported as 'L. okamurae' from the
482	tropical region of China (Hu et al. 2020). Further taxonomic revisions of L. okamurae and L.

483 *margaritae* are needed in order to reveal the species diversity and distribution of their related484 species.

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657 LEGENDS FOR FIGURES

Fig. 1. ML phylogeny inferred from the *psbA* 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.

668 Fig. 3. Neighbour-joining phylogeny inferred from the LSU rDNA sequences of

669 *Lithophyllum* spp. In bold face names of species sequenced in the present study. Species

670 denoted by single quotes have not been confirmed by comparison with the DNA sequences of

671 type specimens. GenBank accession or specimen numbers and collection sites provided.

672 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.

675 okamurae (HAK M-179) is a fragment of the lectotype of L. okamurae f. okamurae (TRH

676 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.

681 682	Fig. 6 . Habit of the isolectotype of <i>L. okamurae</i> f. <i>okamurae</i> (HAK M-179). Scale bar = 3 mm.
683	Fig. 7. Vertical section of inner thallus with dimerous construction comprised of non-
684	palisade cells (HAK M-179). Arrows indicate secondary pit-connections. Scale bar = 20
685	μm.
686	Fig. 8. Vertical section of outer thallus showing secondary pit-connections (arrows)
687	between cells of adjacent filaments (HAK M-179). Scale bar = $20 \ \mu m$.
688	Fig. 9. Vertical section of protuberance showing buried conceptacles (HAK M-179). Scale
689	$bar = 200 \ \mu m.$
690	Fig. 10. Vertical section through tetrasporangial conceptacle with peripherally arranged,
691	divided tetrasporangium (HAK M-179). Scale bar = $20 \ \mu m$.
692	Figs 11–15. Morphology and anatomy of herbarium specimens of <i>Lithophyllum okamurae</i> .
693	Fig. 11 . Habit of the holotype of <i>L. okamurae</i> f. <i>angulare</i> (TRH A21-1327). Scale bar = 1
694	cm.
695	Fig. 12. Vertical section of thallus showing dimerous construction (TRH A21-1327). Note
696	non-palisade basal layer of cells. Scale bar = $60 \ \mu m$.
697	Fig. 13. Vertical section of outer thallus with secondary pit-connections (arrows) between
698	cells of adjacent filaments (TRH A21-1327). Scale bar = $30 \mu m$.
699	Fig. 14. Vertical fracture face of inner thallus showing ascending filaments and buried
700	conceptacles (TRH A21-1327). Scale bar = 200 μ m.
701	Fig. 15. Vertical section through tetrasporangial conceptacle with central columella (c)
702	(TRH A21-1327). Note absence of calcified hump below the central columella. Scale bar
703	$= 50 \ \mu m.$

704	Figs 16–24. Morphology and anatomy of recently collected specimens of Lithophyllum
705	okamurae. Figures 16, 21 and 22 taken from specimens from the type locality.
706	Fig. 16. Habit of a warty specimen (SAP 115621). Scale bar = 1 cm.
707	Fig. 17. Habit of a rhodolith-shaped specimen (SAP 115616). Scale bar = 1 cm.
708	Fig. 18. Vertical section of inner thallus with dimerous construction comprised of non-
709	palisade cells (SAP 115608). Scale bar = 50 μ m.
710	Fig. 19. Vertical section of outer thallus showing secondary pit-connections (arrows)
711	between cells of adjacent filaments (SAP 115608). Scale bar = 50 μ m.
712	Fig. 20. Longitudinal section of a branch showing a coaxial medulla (SAP 115615). Scale
713	$bar = 50 \ \mu m.$
714	Fig. 21. Vertical section through a spermatangial conceptacle with simple spermatangial
715	systems restricted to conceptacle floor (SAP 115621). Scale bar = 50 μ m.
716	Fig. 22. Section through carposporangial conceptacle with large continuous flattened
717	fusion cell (fc) with peripheral gonimoblast filaments (gf) bearing terminal carposporangia
718	(ca). (SAP 115621). Scale bar = 50 μ m.
719	Fig. 23. Surface view of tetrasporangial conceptacles with raised roofs (arrows) (SAP
720	115612). Scale bar = 500 μ m.
721	Fig. 24. Vertical section through a tetrasporangial conceptacle with peripherally arranged
722	tetrasporangia around a central columella (c) with calcified hump (arrowhead) (SAP
723	115608). Scale bar = 50 μ m.
724	Figs 25–28. Morphology and anatomy of herbarium specimens of Lithophyllum neo-
725	okamurae.

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

- 727Fig. 26. Vertical section of thallus showing dimerous construction (TRH A21-1319). Note728non-palisade basal layer of cells. Scale bar = $60 \ \mu m$.
- Fig. 27. Vertical section of outer thallus with secondary pit-connections (arrows) between cells of adjacent filaments (TRH A21-1319). Scale bar = $30 \mu m$.
- 731 **Fig. 28**. Vertical section through a tetrasporangial conceptacle and a central columella (c)
- with calcified hump (arrowhead) (TRH A21-1319). Scale bar = $60 \mu m$.
- 733 Figs 29–37. Morphology and anatomy of recently collected specimens of *Lithophyllum neo-*
- 734 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 nonpalisade cells (SAP 115599). Scale bar = $50 \mu m$.
- 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.
- 745 Fig. 35. Section through carposporangial conceptacle with large continuous flattened
- fusion cell (fc) with peripheral gonimoblast filaments (gf) bearing terminal carposporangia
- 747 (ca). (SAP 115598). Scale bar = $50 \mu m$.
- 748 Fig. 36. Surface view of tetrasporangial conceptacles with raised (arrows) and flush
- (arrowheads) roofs (SAP 115594). Scale bar = $500 \mu m$.

- 751 tetrasporangia around a central columella (c) with calcified hump (arrowhead) (SAP
- 752 115596). Scale bar = 50 μ m.
- 753

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

Table 1 Comparison of the vegetative and tetrasporangial anatomy of Lithophyllum okamurae and L. neo-okamurae. Measurements are

presented as means \pm standard deviation, followed by ranges in brackets.

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)	$8.8 \pm 0.6 \; [7.5 {-} 10]$	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)	$8.5 \pm 0.8 \; [6 10]$	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]

Absent	Absent	Absent	Absent	Absent
(5 specimens, 24	(1 specimen, 9	(1 specimen, 4	(7 specimens, 35	(1 specimen, 2
conceptacles)	conceptacles)	conceptacles)	conceptacles)	conceptacles)
225 ± 41 [167–314]	228 ± 9 [213–238]	[235–295 (341)]	309 ± 33 [248–380]	[270–341]
85 ± 9 [63–109]	92 ± 11 [76–109]	[94–105]	104 ± 10 [86–121]	[94–129]
Paised flush to			Flush raised to	

Reproductive structures

Chamber diameter (µm)

Trichocytes

Tetrasporangial

conceptacle

Chamber height (µm)	85 ± 9 [63–109]	92 ± 11 [76–109]	[94–105]	104 ± 10 [86–121]	[94–129]
Roof elevation (% of	Raised, flush to	fluch	Weakly raised, flush	Flush, raised to	Raised, flush
examined roofs) ⁷	sunken	nusn		sunken	
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	10 ± 2 [8–13]	12 ± 1 [11–14]	8–12	13 ± 2 [10–16]	12
to surface (no. of cells)					
Depth from chamber floor	120 + 24 [89_175]	120 + 12 [101_137]	88_141	156 + 22 [114_202]	135
to surface (µm)	120 - 24 [07 175]	120 ± 12 [101 157]	00 141	100 ± 22 [114 202]	155
Occurrence of calcified					
hump below central	Present	Present	Present	Present	Present
columella					
Number of cells forming					
calcified hump below	3 ± 1 [2–5]	2 ± 1 [1-3]	2–4	5 ± 1 [4–7]	Not visible
central columella					
Tetra (bi-) sporangium	Tetrasporangial	Tetrasporangial	_	Tetrasporangial	Tetrasporangial

Buried conceptacles	Present	Present	_	Present	Present
Diameter (µm)	25 ± 3 [20–30]	measures)	-	28 ± 4 [20–34]	(damaged)
		[15–26] (3			
Length (µm)	60 ± 7 [47–69]	measures)	-	65 ± 8 [47–79]	(damaged)
		[43–44] (3			



Fig. 1.



Fig. 2.



Fig. 3.



Figs 4-10.



Figs 11-15.



Figs 16-24.



Figs 25-28.



Figs 29-37.