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Taxonomic revision of the genus *Lobophora* (Dictyotales, Phaeophyceae) based on morphological evidence and analyses *rbc*L and *cox3* gene sequences

Zhongmin Sun[1]*, Takeaki Hanyuda[1], Phaik-Eem Lim[3], Jiro Tanaka[2], Carlos F.D. Gurgel[4] and Hiroshi Kawai[1]

[1]*Kobe University Research Center for Inland Seas, Rokkodai, Nadaku, Kobe 657-8501, Japan*
[2]*Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7, Konan, Minatoku, Tokyo, 108-8744, Japan*
[3]*Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[4]*Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, University of Adelaide, SA 5005, Australia*

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A taxonomic revision of *Lobophora* based on molecular phylogenetic analyses of *rbc*L and *cox3* gene sequences as well as anatomical observations was carried out, mostly based on specimens collected from eastern Asia and southeastern Australia. In the molecular phylogenetic analyses, nine major clades supported by high bootstrap values were recognized. In combination with evaluation of morphological characters, four clades were concluded to be new species. The Australian species *L. australis* sp. nov. possessed erect thalli with sporangial sori scattered on the ventral surface and had a closer phylogenetic relationship with another Australian species, *L. nigrescens*, but it was distinguished from the latter in having fewer layers of cortical cells and smaller sporangia. The Asian species *L. crassa* sp. nov., *L. pachyventera* sp. nov. and *L. asiatica* sp. nov. possessed decumbent thalli with sporangia scattered on the dorsal surface. Among the three prostrate species, *L. crassa* had a considerably thicker thallus with four to five cortical layers, *L. asiatica* had a thinner thallus with two cortical layers, and *L. pachyventera* differed from another two species by its three-layered ventral cortex and well-developed anchoring rhizoids.

Key Words: *cox3*, *Lobophora asiatica*, *Lobophora australis*, *Lobophora crassa*, *Lobophora pachyventera*, New species, Phylogeny, *rbc*L, Taxonomy

INTRODUCTION

Lobophora J. Agardh (Dictyotales, Phaeophyceae) commonly occurs in tropical and subtropical seas, and currently five species are recognized in AlgaeBase (Guiry & Guiry 2011). They have relatively small, erect or prostrate thalli of relatively simple morphology and are important ecological elements of coral reefs (De Ruyter van Steveninck *et al.* 1988; Jompa & McCook 2002). However, there have been limited taxonomic studies, especially those employing genetic analyses, of the genus and related taxa. For the closely related genus *Padina* Adans, genetic analysis suggests that the species level divergence was considerably underestimated (Ni-Ni-Win *et al.* 2008, 2010, 2011).

There has been a rather complicated history of generic taxonomy surrounding *Lobophora*. Lamouroux (1809) first reported *Dictyota variegata* Lamouroux from the Antilles in the West Indies, and C. Agardh (1817) moved this species into *Zonaria* C. Agardh because of the presence of a series of apical cells at the thallus margin. Later, J. Agardh (1894) examined *Zonaria* species and moved *Z. variegata* (Lamouroux) C. Agardh, *Z. collaris* C. Agardh and *Z. nigrescens* Sonder into his new genus *Gymnosorus* J. Agardh, which was characterised by the absence of an indusium covering the sporangial sorus. J. Agardh (1894) also established the new genus *Lobophora* for his new species *Lobophora nigrescens* J. Agardh, which was collected from Dromana Bay, Victoria, Australia. *Lobophora* was morphologically similar to *Gymnosorus*. However, except for Okamura (1907, 1936), who used *Gymnosorus*, many subsequent researchers did not adopt either *Gymnosorus* or *Lobophora* (Weber-van Bosse 1913; Børgesen 1914, 1926; Taylor 1928).

Papenfuss (1943) re-examined *Gymnosorus* using Hawaiian materials and renamed the genus as *Pocockiella* Papenfuss because *Gymnosorus* was a later homonym of a red algal genus. He characterized *Pocockiella variegata* (Lamouroux) Papenfuss as having a single-layered large central medulla and asexual sori lacking paraphyses. Later, *P. papenfussii* Taylor from the Marshall Islands and *P. dichotoma* Simons from South Africa were added to the genus (Taylor 1950; Simons 1966). Womersley (1967) re-examined the type specimen of *L. nigrescens* and suggested the synonymy of J. Agardh's *Gymnosorus* and *Lobophora*. Therefore, Womersley (1987) recognized only the one species in the genus from southern Australia, *L. variegata* (Lamouroux) Womersley ex E. C. Oliveira, and commented that *P. papenfussii* and *P. dichotoma* needed further investigation (presumably before they could be confirmed as *Lobophora* species). Womersley defined *L. variegata* as distinct from *Zonaria* by having a single-layered large-celled medulla.

* Corresponding author (zhmsun@hotmail.com)

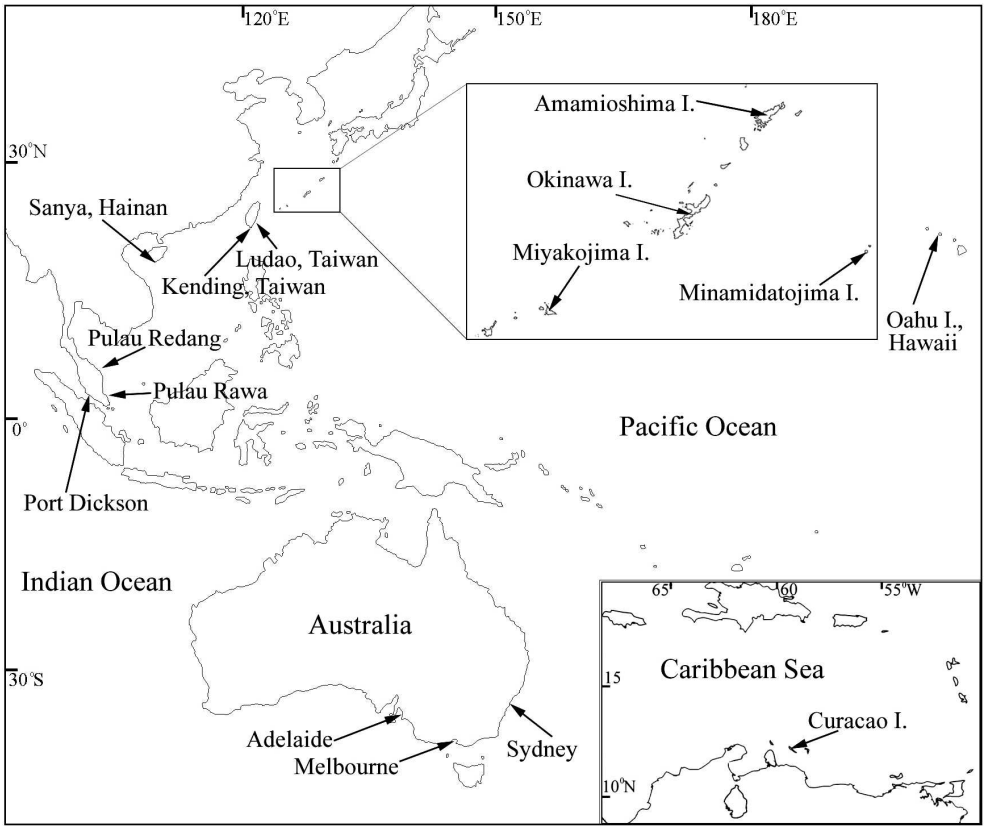


Fig. 1. Map showing the collection sites of the specimens used in the present study.

Although Papenfuss (1977, 1980) did not accept *Lobophora* and suggested retaining *Pocockiella*, other workers recognized the nomenclatural priority of *Lobophora* (Tseng 1983; Silva *et al.* 1996; Yoshida 1998; Littler & Littler 2000; Abbott & Huisman 2004, Guiry & Guiry 2011), and more recently *L. rickeri* Kraft was described from southern Great Barrier Reef (Kraft 2009). A recent summary of the genus recognised *L. variegata*, *L. nigrescens*, *L. papenfussii* (W.R. Taylor) Farghaly, *L. dichotoma* (Simons) P.C. Silva in Silva, Basson, & Moe and *L. rickeri* (Guiry & Guiry 2011).

As to the molecular phylogeny of *Lobophora* and its related taxa, Bittner et al. (2008) examined the 26S rDNA and *psa*A and *rbc*L gene sequence data of three to four *Lobophora* species (*L. variegata*, *L. papenfussii* and *L.* spp.) and indicated a sister relationship of the genus with *Newhousia* Kraft, Saunders, Abbott, & Haroun and *Zonaria*, but no species-level taxonomic studies that included genetic data have been reported. Therefore, in order to clarify the taxonomy of *Lobophora* species in the western Pacific region, we examined newly collected specimens using chloroplast *rbc*L and mitochondrial *cox3* gene sequences as well as combined anatomical features.

MATERIAL AND METHODS

Sampling and morphological analyses

Specimens of *Lobophora* spp. were collected from northwestern Pacific coasts and southeastern Australia (Fig. 1). Sampling was carried out by snorkeling or SCUBA diving, and the specimens were rapidly desiccated in silica gel for DNA extraction. All voucher specimens were deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP), and the herbarium of the Kobe University Research Center for Inland Seas. For anatomical studies, the dried thalli were put in water a few minutes, and then the middle and apical portions were sectioned manually using a razor blade and mounted on glass slides in Karo Syrup/seawater. Photographs were taken with a digital camera (Keyence VB-7010, Tokyo, Japan) attached to a compound microscope (Olympus BX-51, Tokyo, Japan). The size of inner cortical cells of the middle portion of thalli were measured, including the length and width from longitudinal sections and the width and height from transverse sections.

DNA sequencing and phylogenetic analyses

Genomic DNA was extracted from the specimens using a DNeasy Plant Minikit (Qiagen, Hiden, Germany), according to the manufacturer's instructions. The polymerase chain reaction (PCR) was carried out with a TaKaRa PCR Thermal Cycler Dice (Takara Shuzo, Shiga, Japan), using a TaKaRa ExTaq (Takara Shuzo) reaction kit (total volume of 20 µl composed of 2.0 µl 10× ExTaq Buffer, 5.0 µM dNTP mixture, 0.1 µM of each primer, 0.3 units TaKaRa ExTaq, and 0.5 µl DNA solution including 0.05–0.5 µg DNA). Primers used for PCR and sequencing are listed in Table 1. PCR was carried out with an initial denaturation

Table 1. List of primers used in this study.

Primer name	F(forward)/ R(reverse)	A(amplifying)/ S(sequencing)	Sequence(5′-3′)	Annealing position	References
*rbc*L-D1	F	A, S	TATTCCGAATCACACCTCAGC	*rbc*L (128–148)	Ni-Ni-Win *et al.* (2008)
U-rbc-R2.5	R	S	CCTTCATAAACAACACG	*rbc*L (587–571)	Kawai *et al.* (2008)
*rbc*L-Rh3	F	A, S	TTAAYTCTCARCCDTTYATGCG	*rbc*L (629–650)	Hanyuda *et al.* (2004)
Ral-R952	R	A, S	CATACGCATCCATTTACA	*rbc*L (969–952)	Kawai *et al.* (2007)
*rbc*L-P1	F	S	GKGTWATTTGTAARTGGATGCG	*rbc*L (944–965)	Kawai *et al.* (2007)
*rbc*L-D2	R	A	CDACRAARTCAGGWGTATCTG	*rbc*L (1444–1424)	Ni-Ni-Win *et al.* (2008)
*rbc*S-P1	R	A	GKGTWATTTGTAARTGGATGCG	*rbc*S (122–101)	Kawai *et al.* (2007)
trn Y-P2	F	A	GKCAGATTGTAAATCTGTTGG	*trn Y* (27–47)	present study
trn Y-P1	F	A, S	TCYATCRTAGGTTCGAATCC	*trn Y* (52–71)	Ni-Ni-Win *et al.* (2008)
cox3-D1	F	A, S	GTDGAYCCNAGYCCDTGGCC	*cox3* (46–65)	present study
cox3-P2	R	A, S	ACAAARTGCCAATACCAAGC	*cox3* (755–736)	Ni-Ni-Win *et al.* (2008)

at 95°C for 3 min, followed by 94°C for 0.5 min, annealing at 56°C (for *rbc*L) or 48°C (for *cox3*) for 0.5 min, extension at 72°C for 2 min for 28 cycles, and final extension at 72°C for 10 min. PCR products were purified by PEG purification (Lis 1980) and then were sequenced using the CE DTCS quick start kit (Beckman Coulter, Fullerton, CA, USA) and the CEQ8000 DNA analysis system (Beckman Coulter) according to the manufacturer's instructions. Sequences were aligned using the Clustal X program (Thompson et al. 1997) and then manually adjusted. Based on the molecular phylogenetic data (Bittner *et al.*2008), *Padina australis* Hauck was used as out-group to revise the relationships of *Newhousia*, *Zonaria* and *Lobophora* in the *rbc*L data set, and two *Zonaria* species were used as out-group in the *cox3* data set. With the aid of KAKUSAN4 (Tanabe 2011), the best-fit evolutionary model in each codon position of each gene was determined for each data set by comparing different evolutionary models via the corrected Akaike information criterion (Akaike 1974) for maximum likelihood (ML) analysis and the Bayesian Information Criterion (Schwarz 1978) for the Bayesian analysis. As the result, the following models were selected: ML (rbcL: GTR + G for all three codon positions; cox3: first codon: HKY + G, second codon: HKY + G, third codon: GTR + G and Bayes (rbcL: first codon: GTR + G, second codon: JC + G, third codon: GTR + G; cox3: first codon: K2P + G, second codon: F81 + G, third codon: GTR + G). ML analyses were conducted using the likelihood ratchet method (Vos 2003), 500 sets of 25% site-upweighted data were created using the pgresampleseq command in Phylogears 1.5.2009.12.29 (Tanabe 2009), and the ML trees with the upweighted data were estimated using Treefinder (Jobb et al. 2004) with application of the best-fit model. The robustness of the resulting phylogenies was tested by bootstrap analysis (Felsenstein 1985) using 100 replications in the ML analysis. Bayesian inference was conducted using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) with the selected evolutionary models. The Bayesian analyses were initiated with a random starting tree and ran four chains of Markov chain Monte Carlo iterations simultaneously for 10,000,000 generations, keeping one tree every 100 generations. The first 10,000 trees sampled were discarded as 'burn-in', based on the stationarity of ln *L* as assessed using Tracer version 1.4.1 (Rambaut & Drummond 2009); a consensus topology and posterior probability values were calculated with the remaining trees.

RESULTS

Molecular phylogenetic analyses

Twenty-five new *rbc*L and 25 *cox3* sequences of the examined specimens were obtained, and 10 *rbc*L sequences of *Lobophora* were obtained from DDBJ (Table 2). ML and BI analyses of the *rbc*L and *cox3* datasets yielded similar tree topologies (Figs 2, 3).

*rbc*L sequences

All specimens morphologically referable to *Lobophora* made a large clade (Fig. 2), and the maximum sequence diversity was 10.1% within the genus. In the *Lobophora* clade, about 12 well-supported clades (A–L) were recognized, and the sequences of newly collected specimens were included in nine clades (A–I).

cox3 sequences

Since no *cox3* sequences of *Lobophora* species were available in public databases, 25 new sequences based on the newly collected specimens were aligned. The tree topology based on *cox3* sequences was similar to that of *rbc*L data. The maximum sequence diversity among the *Lobophora* specimens included in the analyses was 15.8%.

Morphological analyses and taxonomy

Among the 12 major clades elucidated in the molecular phylogenetic analyses, three were assigned to existing taxa (i.e. *L. papenfussii and L. variegata*) based on the identification of the author who sequenced the specimen (Bittner et al. 2008) or by the morphological observations on representative specimens in the present study (i.e. *L. nigrescens*; for details, see below). However, the majority of the clades, which were considered to correspond to independent species, could not be assigned to any known taxon. Among them, in the present study, we described four new species, and we suspended the taxonomic treatment of the remaining taxa.

Table 2. Origin of the specimens used in this study and their GenBank accession numbers.

Species	Specimens		Voucher no.	DDBJ code	
	Code	Origin (collection sites, date, collector)		*rbc*L	*cox3*
L. asiatica sp. nov.	Hain4	Dadonghai, Sanya, Hainan, China, 15 Dec. 2008, J. Yao/Z. Sun	KU-d5130	AB665270	AB665365
	Taiw2	Ludao, Taiwan, China, 1 Mar. 2010, T. Kitayama	KU-d7621	AB665271	AB665366
	Okina	Ikejima Island, Okinawa, Japan, 10 Mar. 2010, Z. Sun	SAP109520	AB665272	AB665367
	Malay2	Port Dickson, Malaysia, 18 Aug. 2007, P. E. Lim	KU-d3965	AB665273	AB665368
	AB096897	Hoshina et al. (2004) [as *L. variegata*]	—	AB096897	—
L. australis sp. nov.	Aust3	Noarlunga, Adelaide, SA, Australia, 8 Oct. 2009, H. Kawai	SAP109517	AB665258	AB665369
L. crassa sp. nov.	Hawa1	Popukea Beach Park, Oahu Island, Hawaii, USA, 12 Jun. 2007, H. Kawai	KU-d3692	AB665260	AB665370
	Hawa2	Popukea Beach Park, Oahu Island, Hawaii, USA, 12 Jun. 2007, H. Kawai	KU-d3694	AB665261	AB665371
	Hain1	Xidao, Sanya, Hainan, China, 16 Dec. 2008, J. Yao/Z. Sun	SAP109518	AB665262	AB665372
	Miya1	Shimojishima, Miyakojima Island, Japan, 22 Nov. 2010, Z. Sun	KU-d9520	AB665264	AB665373
	EU579954	Bittner *et al.* (2008)	FRA0374	EU579954	—
L. nigrescens	Aust1	Long Leef, Sydney, VIC, Australia, 6 Nov. 2003, H. Kawai	KU-d271	AB646541	AB665374
	Aust2	Seaford, SA, Australia, 9 Oct. 2009, H. Kawai	KU-d6625	AB665257	AB665375
L. pachyventera sp. nov.	Hain2	Xidao Island, Sanya, Hainan, China, 16 Dec. 2008, J. Yao/Z. Sun	KU-d5110	AB665265	AB665376
	Hain3	Xidao Island, Sanya, Hainan, China, 16 Dec. 2008, J. Yao/Z. Sun	KU-d5112	AB665266	AB665377
	Taiw1	Kending, Taiwan, China, 31 May 2007, H. Kawai	KU-d3577	AB665267	AB665378
	Miya2	Sunayama Beach, Miyakojima Island, Japan, 9 May 2009, J. Tanaka/Z. Sun	SAP109519	AB665268	AB665379
	Malay1	Mak Kepit, Pulau Redang, Malaysia, 13 May 2008, P.E. Lim	KU-d5184	AB665269	AB665380
L. papenfussii	EU579953	Bittner *et al.* (2008)	FRA0458	EU579953	—
L. variegata	EU579956	Bittner *et al.* (2008)	FRA0367	EU579956	—
L. variegata	EU579957	Bittner *et al.* (2008)	FRA0375	EU579957	—
Lobophora spp.	EU579955	Bittner *et al.* (2008)	FRA0105	EU579955	—
	Mdai1	Kaigunbo, Minamidaitojima Island, Japan, 17 Feb. 2006, H. Kawai	KU-d1733	AB665263	AB665381
	Hain5	Dadonghai, Sanya, Hainan, China, 15 Dec. 2008, J. Yao/Z. Sun	KU-d5139	AB665274	AB665382
	Hain6	Xidao Island, Sanya, Hainan, China, 16 Dec. 2008, J. Yao/Z. Sun	KU-d5119	AB665275	AB665383
	Kaker	Kakeromajima Island, Japan, 15 May 2008, J. Tanaka/Z. Sun	KU-d4615	AB665276	AB665384
	Miya3	Shinshiro Beach, Miyakojima Island, Japan, 7 May 2009, J. Tanaka/Z. Sun	KU-d5807	AB665277	AB665385
	Miya4	Shinshiro Beach, Miyakojima Island, Japan, 7 May 2009, J. Tanaka/Z. Sun	KU-d5812	AB665278	AB665386
	Mdai2	Shioya, Minamidatojima Island, Japan, 17 Feb. 2006, H. Kawai	KU-d1727	AB665279	AB665387
	Malay3	Pulau Rawa, Terengganu, Malaysia, 16 Jun. 2008, P.E. Lim	KU-d5187	AB665280	AB665388
	Curaç	Curaçao Island, West Indies, S. Draisma	KU-d9471	AB665281	AB665389
	AB096898	Hoshina *et al.* (2004) [as *Lobophora* sp.]	—	AB096898	—
Newhousia imbricata		Phillips *et al.* (2008)	—	EF990240	—
Padina australis	AB096901	Hoshina *et al.* (2004)	—	AB096901	—
Zonaria angustata		Lee *et al.* (unpublished)	—	DQ866932	—
Z. diesingiana		Baten, Main Okinawa Island, Japan, 9 Mar. 2010, T. Hanyuda/Z. Sun	KU-d7720	AB665259	AB665390
Zonaria sp.		Melbourne, Australia, 21 Jul. 2004, H. Kawai	KU-d356	AB665282	AB665391

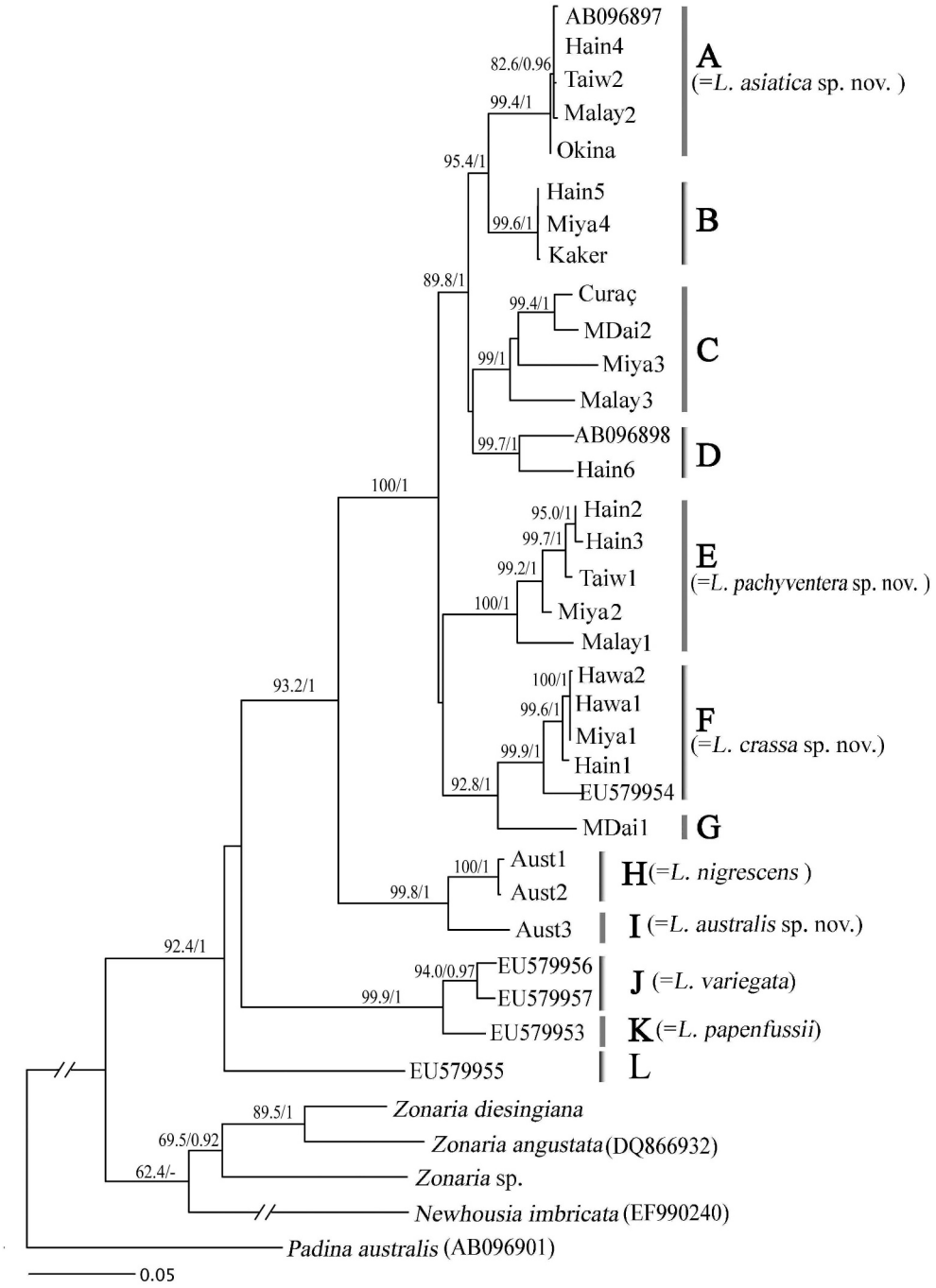


Fig. 2. Maximum likelihood tree based on *rbc*L gene sequences. The bootstrap values shown at each node were ML/BI (Bayesian analysis), ML > 50%, BI > 0.9. Scale bar = 0.05 substitutions per site.

Lobophora nigrescens J. Agardh

This species corresponds to clade H in Figs 2, 3.

TYPE LOCALITY: Dromana Bay, VIC, Australia.

HABITAT: Exposed reef face.

SPECIMENS EXAMINED: Sydney, NSW, Australia, 6 November 2003, H. Kawai (KU-d271); Seaford, SA, Australia, 9 October 2009, H. Kawai (KU-d6624–d6627); Sydney, NSW, Australia, 11 March 2010, H. Kawai (KU-d7067–d7070, d7072, d7074–d7077).

MORPHOLOGY: The thallus was erect, fan-shaped, 2–6 cm long, 3–7 cm wide, attached to the substrate by a basal holdfast with a mound of hairs (Fig. 4). The thallus was composed of a single layer of large medullary cells and five layers of cortical cells on both surfaces (Figs 5, 6), and was 160–210 μm thick at the middle part. The number of cortical layers was reduced in the distal part of thallus (Fig. 7). Sporangial sori were mainly scattered on the ventral surface of thallus. Paraphyses were absent. Sporangia were sessile and irregularly ovate to spherical and 80–95 μm in diameter (Fig. 8). Sexual reproductive structures were unknown. The

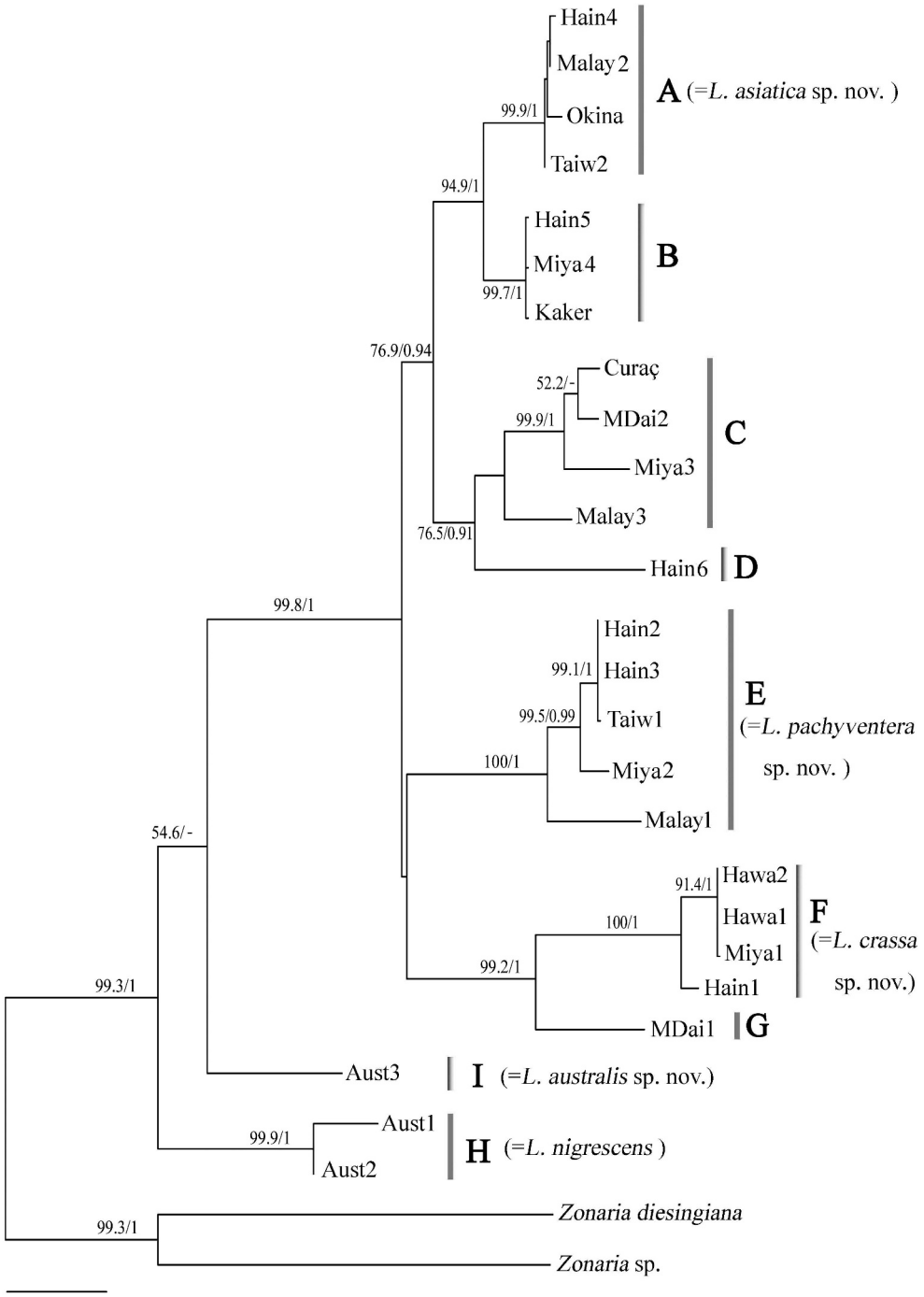


Fig. 3. Maximum likelihood tree based on *cox3* gene sequences. The bootstrap values shown at each node were ML/BI (Bayesian analysis), ML > 50%, BI > 0.9. Scale bar = 0.05 substitutions per site.

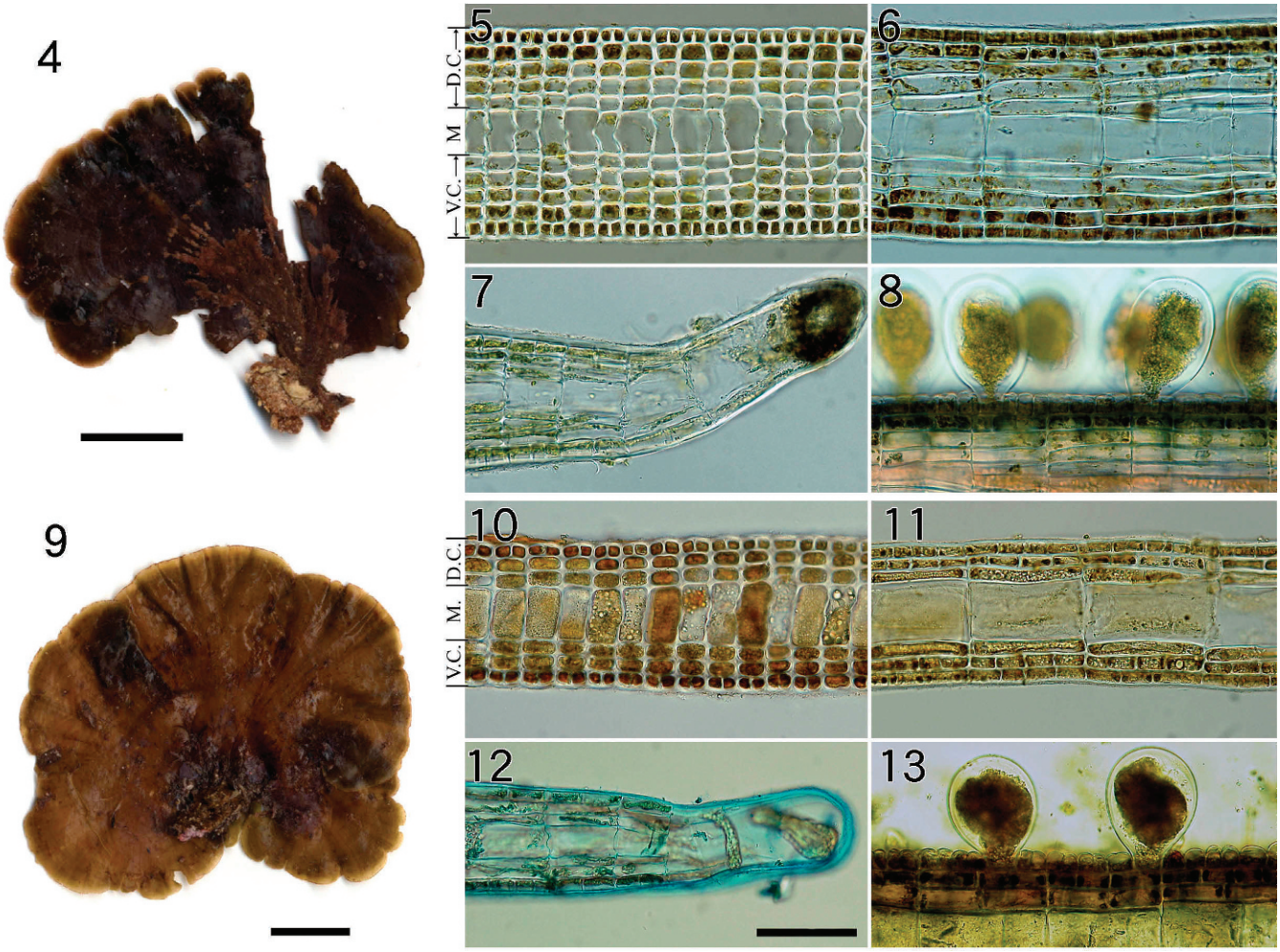
species was distinguished from its related species in the erect fan-shaped thallus, more than four layers in the cortex and the distinctive DNA sequences AB646541, AB665257, AB665374 and AB665375.

***Lobophora australis* sp. nov. Z. Sun, F. C. Gurgel & H. Kawai**

This species corresponds to clade I in Figs 2, 3.

DESCRIPTION: *Thallus erectus vel prostratus, flavus vel atro-brunneus, 2–5 cm longus, 3–6 cm latus ad partem mediam 120–200 µm crassus, e medulla unius strati cellularum et cortice trium stratorum cellularum utraque facie thalli constans, substrato affixus per hapteron basale vel rhizoidea in facie ventrali. Sori sporangiales dispersi in facie ventrali thalli erecti. Sporangia sessilia subrotunda 60–70 µm diametro. Partes reproductionis sexualis ignotae. Species a taxis affinibus cortice trium stratorum cellularum per totum thallum et sequentiis nucleotidorum ADN propriis AB665258 and AB665369 distincta.*

Thallus erect or prostrate, yellow to dark brown, 2–5 cm long, 3–6 cm wide and 120–200 µm thick at the middle part,



Figs 4–13. Morphology of *Lobophora nigrescens* and *L. australis* sp. nov. M., medullary cells; D.C., dorsal cortical cells; V.C., ventral cortical cells. Scale bar in Fig. 12 applies to Figs 5–8 and 10–13.

Figs 4–8. Morphology of *Lobophora nigrescens*.

Fig. 4. Erect fan-shaped thallus with obvious basal holdfast (KU-d6625). Scale bar = 1 cm.

Fig. 5. Transverse medial section of a thallus, showing single-cell-layered medulla and five-cell-layered cortex on both sides of the medullary layer (KU-d6625).

Fig. 6. Longitudinal section corresponding to Fig. 5.

Fig. 7. Longitudinal section of the apical portion, showing the development of the cortical layer (KU-d6625).

Fig. 8. Longitudinal section of a sporangial sorus, showing absence of paraphyses and stalk cells (KU-d7077).

Figs 9–13. Morphology of *Lobophora australis* (SAP109517, holotype).

Fig. 9. Erect thallus with basal holdfast. Scale bar = 1cm.

Fig. 10. Transverse medial section of a thallus, showing single-cell-layered medulla and three-cell-layered cortex on both sides of the medullary layer.

Fig. 11. Longitudinal section corresponding to Fig. 10.

Fig. 12. Apical segmentation of a thallus. Scale bar = 100 μm.

Fig. 13. Longitudinal section of a sporangial sorus, showing absence of paraphyses and stalk cells.

composed of single-cell-layered medulla and three-cell-layered cortical layer on both side of thallus, attached to the substrate by basal holdfast or rhizoids on the ventral surface. Sporangial sori scattered on ventral surface of erect thallus. Sporangia sessile, roundish, 60–70 μm in diameter. Sexual reproductive structures unknown. The species was distinguished from its related taxa in having three-cell-layered cortical layer through the thallus and the distinctive DNA sequences AB665258 and AB665369.

HOLOTYPE: SAP109517. Coobowie, South Australia, Australia. 9 October 2009. H. Kawai.

HABITAT: Intertidal zone of moderate wave-action to sheltered coasts.

ETYMOLOGY: The specific epithet derived from its principal distributional range.

SPECIMENS EXAMINED: Noarlunga, SA, Australia, 8 October 2009, H. Kawai (KU-d6667, d6668, d6670); Coobowie, SA, Australia, 9 October 2009, H. Kawai (KU-d6644–d6648).

MORPHOLOGY: This species was composed of both erect and prostrate thallus, attached to the substrate by an obvious basal holdfast or rhizoids issuing from the ventral

surface. Thallus was fan-shaped, golden brown in color, 2–5 cm long, 3–6 cm wide and 120–200 μm thick at the middle part (Fig. 9). The thallus was composed of a single layer of large medullary cells and three layers of cortical cells on both surfaces (Figs 10, 11). Cortical layers were three-cell layered throughout (Fig. 12). Sporangial sori were scattered on the ventral surface of the erect thallus. Paraphyses were absent. Sporangia were sessile, irregularly ovate when young, and becoming spherical and measuring 60–70 μm in diameter when mature (Fig. 13).

***Lobophora crassa* sp. nov. Z. Sun, P. E. Lim & H. Kawai**

This species corresponds to clade F in Figs 2, 3.

DESCRIPTION: *Thallus plerumque prostratus, brunneus vittis radiantibus griseis, substrato affixus in facie ventrali, e medulla unius strati cellularum et cortice 4 stratorum cellularum in latere ventrali, 5 stratorum cellularum in latere dorsali constans. Sori sporangiales dispersi in facie dorsali thalli. Paraphyses nullae. Sporangia subrotunda 45–60 μm diametro. Organa reproductionis sexualis ignotae. Species haec a speciebus affinibus thallo plerumque prostrato, stratis corticalibus crassis, et sequentiis nucleotidorum ADN propriis AB665262 and AB665372 distinguenda.*

Thallus predominantly prostrate, brown with radial gray stripes, attached on the substrates at the ventral surface. Thallus composed of a single-cell-layered medulla and four- and five-cell-layered cortex on the ventral and dorsal side, respectively. Sporangial sori scattered on dorsal surface of thallus. Paraphyses absent. Sporangia roundish, 45–60 μm in diameter. Sexual reproductive organs unknown. The species was distinguished from its related species in the predominantly prostrate thallus, thick cortical layers and the distinctive DNA sequences AB665262 and AB665372.

HOLOTYPE: SAP109518. Xidao Island, Hainan, China. 16 December 2008. J. Yao & Z. Sun.

HABITAT: Grew abundantly on the exposed reef face.

ETYMOLOGY: The specific epithet refers to the thick thallus.

SPECIMENS EXAMINED: Dadonghai, Hainan, 15 December 2008, J. Yao & Z. Sun (Xidao Island, Hainan, 16 December 2008, J. Yao & Z. Sun (KU-d5104–d5105, d5083, d5091, d5111); Shimojishima, Miyakojima Island, 22 November 2010, Z. Sun (KU-d9514–d9516, d9518–d9522); Popukea Beach Park, Oahu Hawaii, 12 June 2007, H. Kawai (KU-d3692, d3694).

MORPHOLOGY: The thallus was predominantly prostrate, attached to the substrate by the entire ventral surface (Fig. 14). The thallus became as large as 10 cm in width but tended to become fragmented when detached from the substrate, and each fragment measured 1.5–3 cm long and 2–4 cm wide. The middle part of thallus was 160–250 μm thick and was composed of a single-layered medulla and four- and five-cell-layered cortex on the ventral and dorsal side, respectively (Figs 15, 16). Peripheral cells of the dorsal cortex often divided into dark-colored small cells. The number of cells composing the cortex was reduced at the distal end of the thallus near the margin but to no fewer than three (Fig. 17). Sporangial sori were scattered on the dorsal surface of the thallus. Paraphyses were absent. Sporangia were sessile, spherical and 45–60 μm in diameter (Fig. 19). Sexual reproductive organs were unknown.

***Lobophora pachyventera* sp. nov. Z. Sun, P.-E. Lim, J. Tanaka & H. Kawai**

This species corresponds to clade E in Figs 2, 3.

DESCRIPTION: *Thallus prostratus, flavobrunneus sed in sicco atrobrunnescens, saxis vel algis crustosis corallinis affixus per rhizoidea evoluta, e medulla unius strati cellularum et cortice 2 stratorum cellularum in latere dorsali, 3 stratorum cellularum in latere ventrali constans. Sori sporangiales dispersi in facie dorsali thalli. Organa reproductionis sexualis ignota. Species haec thallo plerumque prostrato cortice 2 stratorum cellularum in latere dorsali et 3 stratorum cellularum in latere ventrali, et sequentiis nucleotidorum ADN propriis AB665268 and AB665379 distincta.*

Thallus prostrate, yellow brown but turning dark brown when dried, attached on rocks or crustose coralline algae by developed rhizoids. Thallus composed of single-cell-layered medulla and two- and three-cell-layered cortex on the dorsal and ventral sides, respectively. Sporangial sori scattered on dorsal surface of thallus. Sexual reproductive organs unknown. The species was distinctive in the predominantly prostrate thallus with two- and three-cell-layered cortex on the dorsal and ventral sides, respectively, and the distinctive DNA sequences AB665268 and AB665379.

HOLOTYPE: SAP109519. Sunayama Beach, Miyakojima Island, Okinawa, Japan. 9 May 2009. J. Tanaka & Z. Sun.

HABITAT: Frequently grew on crustose coralline algae of exposed reef faces.

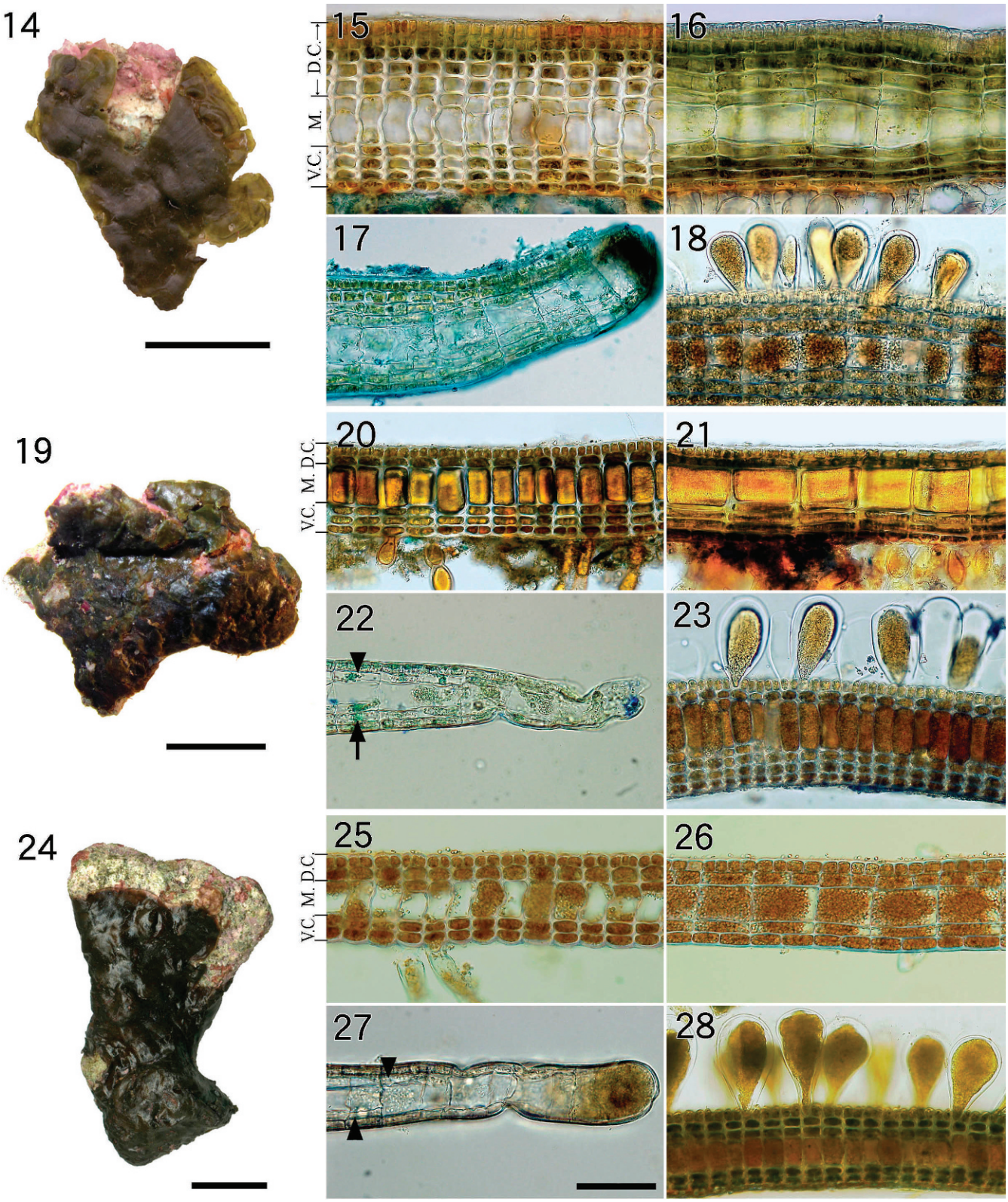
ETYMOLOGY: The specific epithet refers to the ventral cortex being thicker than the dorsal one.

SPECIMENS EXAMINED: Xidao Island, Hainan, 16 December 2008, J. Yao & Z. Sun (KU-d5110, d5112, d5114); Kending, Taiwan, 31 May 2007, H. Kawai (KU-d3577); Sunayama Beach, Miyakojima Island, 9 May 2009, J. Tanaka & Z. Sun (KU-d7036–d7043). Mak Kepit, Pulau Redang, Malaysia, 13 May 2008, P. E. Lim (KU-d5184).

MORPHOLOGY: The thalli were predominantly prostrate, firmly attached to rocks or crustose coralline algae by the ventral surface (Fig. 20). The thallus tended to be fragmented when removed from the substrate, and the thallus fragments normally measured 2–3 cm long and 3–4 cm wide. The thallus was 100–140 μm thick in the middle part and was normally composed of a single-cell-layered medulla and a two- and three-cell-layered cortex on the dorsal and ventral sides, respectively (Figs 21, 22). Moniliform or tubular rhizoids issued from the ventral surface (Fig. 23). The ventral cortex was always thicker than the dorsal one. Sporangial sori were scattered on the dorsal surface of the thallus. Paraphyses were absent. Sporangia were sessile and irregularly ovate to spherical and 70–80 μm in diameter (Fig. 24).

***Lobophora asiatica* sp. nov. Z. Sun, J. Tanaka & H. Kawai**

This species corresponds to clade A in Figs 2, 3.



Figs 14–28. Morphology of *Lobophora crassa* sp. nov., *L. pachyventera* sp. nov. and *L. asiatica* sp. nov. D.C., dorsal cortical cells, V.C., ventral cortical cells. Scale bar in Fig. 27 applies to Figs 15–18, 20–23 and 25–28.

Figs 14–18. Morphology of *Lobophora crassa* (SAP109518, holotype).

Fig. 14. Prostrate thallus. Scale bar = 1cm.

Fig. 15. Transverse section, showing five-cell-layered dorsal cortex and four-cell-layered ventral cortex.

Fig. 16. Longitudinal section corresponding to Fig. 15.

Fig. 17. Apical segmentation, showing the development of the cortical layer.

DESCRIPTION: *Thalli prostrati vel erecti, inter se imbricati, in vivo flavobrunnei vel brunnei, in sicco fuscantes, e medulla unius strati cellularum et cortice ad partem mediam 2 vel 3 stratorum cellularum in latere dorsali, 2 stratorum cellularum in latere ventrali constantes. Sori sporangiales dispersi in facie dorsali thalli. Partes reproductionis sexualis ignotae. Species haec thallo decumbenti, stratis corticalibus tenuibus, et sequentiis nucleotidorum ADN propriis AB665272 and AB665367 distincta.*

Thallus prostrate or erect, overlapping each other, yellow brown to brown when alive and becoming darker when dried. Thallus composed of single-cell-layered medulla and two- to three-cell-layered dorsal cortex and two-cell-layered ventral cortex at the middle part. Sporangial sori scattered on dorsal surface of thallus. Sexual reproductive structures unknown. The species was distinctive in the decumbent thallus, thin cortical layers and the distinctive DNA sequences AB665272 and AB665367.

HOLOTYPE: SAP109520. Ikeijima Island, Okinawa, Japan. 10 March 2010. Z. Sun.

HABITAT: Grew on hard substrates or unhealthy corals.

ETYMOLOGY: The specific epithet derived from the principal distributional range of the species.

SPECIMENS EXAMINED: Dadonghai, Hainan Island, China, 15 December 2008, J. Yao & Z. Sun (KU-d5130, d5132-5137); Ludao, Taiwan, China, 1 March 2010, T. Kitayama (KU-d7620–d7622); Shinshiro Beach, Miyakojima Island, 7 May 2009, J. Tanaka & Z. Sun (KU-d5805–d5828); Ikejima Island of Okinawa, 10 March 2010, Z. Sun (KU-d7781, d7802, d7799). Port Dickson, Malaysia, 18 August 2007, P. E. Lim (KU-d3965).

MORPHOLOGY: The thalli were prostrate or erect, and attached to substrates on the entire ventral surface (Fig. 25). The thalli were fan-shaped and measured 0.5–3 cm long and 0.5–4 cm wide, 90–130 μm in the middle portion, and composed of single-cell-layered large medulla and two-cell-layered cortex on both surfaces (Figs 26, 27). Sometimes the dorsal cortex was composed of three cell layers but always two-cell layered at the apical part (Fig. 28). Sporangial sori were scattered on the dorsal surface. Paraphyses were absent. The sporangia were sessile, irregularly ovate when young, becoming spherical when mature, 80–100 μm in diameter.

DISCUSSION

Molecular phylogenetic analyses of *Lobophora* species were focused on the western Pacific Ocean using *rbcL* and *cox3* gene sequences. Most of the described taxa could be identified by morphological features using newly collected materials in the present study (i.e. *L. nigrescens*) or by the available genetic information (i.e. *L. papenfussii* and *L. variegata*). Although we could not include the other two described species, *L. dichotoma* and *L. rickeri*, in the genetic analyses, they are morphologically different from the specimens included in the present analyses (Table 3). We could not find described *Lobophora* species corresponding to most of the northwestern Pacific specimens, and hence they were considered to represent undescribed taxa. Previously, there have been no molecular data for the type species *L. nigrescens*, but we identified clade H in the present analyses as corresponding to the type species; even though our specimens were collected from southern Australia, they agreed well with the basic morphological features (J. Agard 1894).

Of the nine major *Lobophora* clades revealed in the present analyses based on the *rbc*L and *cox3* analyses, we clarified characteristic morphological features for five. For the rest, unfortunately, there were a limited number of available specimens, or there was a lack of distinctive morphological features to characterize the taxa. Therefore, at present we suggest the establishment of four new species (i.e. *L. asiatica* sp. nov., *L. australis* sp. nov., *L. crassa* sp. nov. and *L. pachyventera* sp. nov.), but we suspend taxonomic treatment of the others. Although it is difficult to find a phylogenetic criterion to delimit each species of *Lobophora*, we suggest that the sequence diversity within the species may not exceed 5% in *rbc*L and 7% in cox3. For example, the sequence diversity within *L. pachyventera* (= clade E) was 4.6% in *rbc*L and 6.3% in cox3. On the other hand, it is clear that the sequence diversity within *L. asiatica* (= clade A) has been underestimated, but we suspend the taxonomic treatment of the sister clades until further research can be conducted.

The three southern-hemispheric species, *Lobophora nigrescens*, *L. australis* and *L. dichotoma*, are distinguished easily from other *Lobophora* species by erect thalli and obvious basal holdfasts. These three species are also distinguished from each another by morphological

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Fig. 18. Transverse section of a sporangial sorus, showing absence of paraphyses and stalk cells.

Figs 19–23. Morphology of *Lobophora pachyventera*.

Fig. 19. Decumbent thallus (SAP109519, holotype). Scale bar = 1cm.

Fig. 20. Transverse section of holotype, showing two layers of dorsal cortical cells and three layers of ventral cortical cells.

Fig. 21. Longitudinal section, corresponding to Fig. 20.

Fig. 22. Apical segmentation of holotype, showing two-cell-layered dorsal cortex (arrowhead) and three-cell-layered ventral cortex (arrow).

Fig. 23. Transverse section of a sporangial sorus without paraphyses and stalk cells (KU-d5114).

Figs 24–28. Morphology of *Lobophora asiatica*.

Fig. 24. Decumbent thallus (SAP109520, holotype). Scale bar = 1cm.

Fig. 25. Transverse section of holotype, showing two layers of cortical cells on both surfaces (SAP109520).

Fig. 26. Longitudinal section, corresponding to Fig. 25.

Fig. 27. Apical segmentation of a thallus, showing two-cell-layered dorsal cortex on both sides of the medullary layer (arrowheads) (KU-d5130). Scale bar = 100 μm.

Fig. 28. Transverse section of a sporangial sorus without paraphyses and stalk cells (KU-d5130).

Table 3. Comparison of morphological characters among species of *Lobophora*.

Characters	*L. asiatica* sp. nov.	*L. australis* sp. nov.	*L. crassa* sp. nov.	*L. dichotoma*	*L. nigrescens*	*L. nigrescens*	*L. pachyventera* sp. nov.	*L. papenfussii*	*L. richeri*
Thallus									
Developed holdfast	absent	present	absent	present	present	present	absent	present	absent
Erect or prostrate	prostrate	erect, prostrate	prostrate	erect	erect	erect	prostrate	semierect	prostrate
Shape	unbranched	fan-shaped, unbranched	unbranched	dichotomously branched	fan-shaped, 1 obed	fan-shaped, lobed	unbranched	fan-shaped, unbranched	unbranched
Thickness	108.2 ± 12.9	152.1 ± 28.6	195.4 ± 23.8	unknown	150–200	187.9 ± 14.3	112.7 ± 12.7	385–640	110–185
Inner cortical cells									
Length	80.1 ± 10.1	97.5 ± 11.8	80.8 ± 17.3	unknown	80–100	95.2 ± 14.7	59.6 ± 11.9	57–70	40–50
Width	25.8 ± 4.1	27.2 ± 3.1	28.6 ± 3.2	unknown	20–30	24.5 ± 2.7	28.7 ± 5.2	28–30	25–35
Height	11.4 ± 1.5	13.0 ± 1.8	18.6 ± 3.0	unknown	15–20	16.5 ± 1.6	10.4 ± 0.8	14–23	15–20
Cortical layers number									
Dorsal layers	2(–3)	3	(3–)4(–6)	3	(3–)4(–6)	(4–)5	2	8–11	4(–5)
Ventral layers	2(–1)	3	(3–)4	3(–4)	(3–)4(–6)	(4–)5	(2–)3(–4)	8–11	3
Diameter of unilocular sporangia	85–95	60–70	45–60	unknown	85–95	80–95	70–80	80–100	45–60
References	this study	this study	this study	Simons (1966)	J. Agardh (1894), Womersley (1987)	this study	This study	Taylor (1950)	Kraft (2009)

characters: *L. dichotoma* has dichotomous branching, but *L. nigrescens* and *L. australis* do not, and *L. australis* has fewer cortical cell layers and smaller sporangia than *L. nigrescens*.

Among the prostrate *Lobophora* species, the three new species are distinctive in morphology: *L. crassa* has a thick cortex (more than three layers), *L. pachyventera* has a three-layered ventral cortex and well-developed rhizoids issuing from the ventral surface and *L. asiatica* possesses a thin thallus. Another described decumbent species, *L. rickeri,* is similar to *L. crassa*, but the former is distinguished from the latter by its thinner thallus. One specimen from Minamidaito Island (MDai1) in clade G is similar in morphology to *L. rickeri*, and the Minamidaito specimen is sister to the clade of *L. crassa* in our phylogenetic trees. We suggest that it is necessary to re-examine *L. rickeri* and clade G in further studies. *Lobophora papenfusii* is a semierect species attached to the substrate by the basal part, and its thallus is thicker and larger than other species of *Lobophora*.

Our specimens were identified as *L. nigrescens*, the type species, and they were collected near the type locality. The morphology of our specimens is similar to that described by J. Agardh (1894) and Womersley (1967, 1987). On the other hand, the Caribbean species *L. variegata* described by Lamouroux (1809) is probably different from *L. nigrescens*, and we do not consider *L. variegata* to be synonymous with *L. nigrescens*. Although we obtained one specimen from Curaçao (Curaç in clade C), it did not resemble the *L. variegata* specimen (= *Dictyota variegata* Lamouroux) in Lamouroux's herbarium that had a large and erect thallus. Littler and Littler (2000) described three forms of *L. variegata* depending on depth and habitat in the Caribbean Sea. We suggest that at least two species occur in the Caribbean Sea based on the molecular phylogenetic analysis, but neither is synonymous with *L. nigrescens*.

The *Lobophora* species previously reported from the northwestern Pacific region (Okamura 1907, 1936; Tseng 1983; Yoshida 1998) are probably not *L. variegata*, which was described from the Caribbean Sea based on morphology only (Lamouroux 1809). The Hawaiian *Lobophora* species (Papenfuss 1943; Abbott & Huisman 2004) are also not *L. variegata*. We collected a few specimens from Hawaii and identified them as *L. crassa* by morphological and genetic evidence. Papenfuss (1943) characterized *Pocockiella* (replacement name for *Gymnosorus*) by the presence of a large medulla and absence of pharaphyses of sporangial sori; however, Womersley (1967, 1987) suggested redefining *Lobophora* only by the presence of a large medulla, being aware of *Zonaria angustata* (Kützing) Papenfuss, which lacks paraphyses. In our phylogenetic tree, *Lobophora* is monophyletic, and *Z. angustata* also did not cluster with the *Lobophora* clade, and this result agrees with Bittner et al. (2008). We suggest that the presence of a large medulla is a valuable morphological feature to define *Lobophora*. On the other hand, the absence of paraphyses is a morphological feature shared by *Lobophora* and some species of *Zonaria*. The illegitimate *Gymnosorus* was originally separated from *Lobophora*, but currently only *G. variegata* has been moved into *Lobophora* [*L. variegata* (Lamouroux) Womersley ex Oliveira]. *Gymnosorus collaris* has never been treated taxonomically since it was moved from *Zonaria* to *Gymnosorus* (J. Agardh 1894). We suggest that this

Zonaria-like species without paraphyses should be re-examined.

A biogeography of *Lobophora* was reflected in the phylogenetic tree: an Australian clade composed of clades H and I was shown clearly. Clades E, F and G shared a tropic-Pacific distribution, and the large clade composed of clades A–D showed a cosmopolitan distribution. Although we could not clarify the distributional history of *Lobophora*, we assume that the ancestral group that had *Zonaria*-like erect thalli perhaps spread from the ancient Tethys Sea and their descendants, mainly remained in the southern Pacific and the West Indies. On the other hand, the northern Pacific group, which was related to the Australian group, evolved into several decumbent species and dispersed widely into tropical seas around the world.

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