

## Molecular diversity of the calcified red algal genus *Tricleocarpa* (Galaxauraceae, Nemaliales) with the description of *T. jejuensis* and *T. natalensis*

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WIRIYADAMRIKUL J., GERALDINO P.J.L., HUISMAN J.M., LEWMANOMONT K. AND BOO S.M. 2013. Molecular diversity of the calcified red algal genus *Tricleocarpa* (Galaxauraceae, Nemaliales) with the description of *T. jejuensis* and *T. natalensis*. *Phycologia* 52: 338–551. DOI: 10.2216/13-155.1

*Tricleocarpa* (Nemaliales) is a calcified, dichotomously branched red algal genus currently with two recognized species, *T. cylindrica* and *T. fragilis*, both widely distributed in warm temperate and tropical seas. We evaluated the relationships of *Tricleocarpa* using molecular markers, including 35 *rbcL* and 32 *cox1* sequences, from specimens in the western Pacific, the Hawaiian Islands and South Africa. Our results demonstrated the presence of seven genetically distinct groups. The range of *T. cylindrica* was confirmed to include the West Indies, the Hawaiian Islands, and Asian waters, and specimens referable to *T. fragilis* were found in Asia. On the basis of our molecular analyses and detailed morphological examinations, we were able to discriminate two taxa that we here describe as new species: *T. jejuensis* sp. nov. and *T. natalensis* sp. nov. *Tricleocarpa jejuensis*, known from Korea, Japan, South Africa, and Taiwan, was distinguished by the presence of three to four cortical cell layers with elongated to compressed subsurface and innermost cells, monoecious sexuality, spherical cystocarps, and paraphyses intermixed with gonimoblast filaments. *Tricleocarpa natalensis* from Kwazulu-Natal, South Africa was characterized by three layers of elongate cortical cells, monoecious sexuality, hemispherical cystocarps, and paraphyses rarely intermixed with gonimoblast filaments. Our phylogenetic analyses indicated that three further clades were recognized at species level, but these were based on a few specimens and were not morphologically distinct; further sampling is needed in regions outside Asian waters before additional taxonomic revisions can be proposed.

KEY WORDS: *cox1*, Indo-Pacific Ocean, New species, *rbcL*, Rhodophyta, Systematics

### INTRODUCTION

The red algal family Galaxauraceae currently encompasses four calcified genera: *Actinotrichia* Decaisne, *Dichotomaria* Lamarck, *Galaxaura* J.V. Lamouroux, and *Tricleocarpa* Huisman & Borowitzka. These genera can be distinguished on the basis of life history patterns, postfertilization development, and phylogenetic relationships inferred from molecular analyses (Huisman *et al.* 2004; Wang *et al.* 2005; Huisman 2006). All four are widely distributed in warm temperate and tropical waters (Huisman & Womersley 1994; Littler & Littler 1997; Abbott 1999; Hwang & Kim 2011). Research interests on species diversity in the Galaxauraceae and distribution patterns may increase with ocean warming because members of this family are calcified organisms and possibly sensitive to ocean acidification (Nelson 2009).

Until relatively recently, the species assigned to *Tricleocarpa* had been included in *Galaxaura*. *Tricleocarpa* was segregated by Huisman & Borowitzka (1990) on the basis primarily of characteristics of life history and carposporophyte structure. Life histories in *Galaxaura* include conspicuous tetrasporophytes and gametophytes, these

differing subtly in habit and with different cortical structures. Huisman & Borowitzka (1990), however, considered the life history study of *G. oblongata* by Magruder (1984), which showed the species to have a heteromorphic life history with a filamentous tetrasporophyte, to be of generic significance and erected *Tricleocarpa* for *T. cylindrica* and *T. oblongata*. Papenfuss *et al.* (1982) had placed the former species in synonymy, but Huisman & Borowitzka (1990) recognized morphological and reproductive features that distinguished the two species. Subsequently, an examination of *Eschara fragilis* Linnaeus by Huisman & Townsend (1993) indicated conspecificity with *G. oblongata*, and the Linnaean name was resurrected as *T. fragilis* (Linnaeus) Huisman & Townsend. The autonomy of *Tricleocarpa* has subsequently been supported by numerous molecular analyses (Huisman *et al.* 2004; Wang *et al.* 2005), but the taxonomy of the species requires further study (Wang *et al.* 2005).

When first described, *Tricleocarpa* included only two species, one raised from synonymy (Huisman & Borowitzka 1990). As a result of the extensive synonymy proposed by Papenfuss *et al.* (1982), the genus essentially encompassed the entirety of Decaisne's Section *Eugalaxura*, which according to Kjellman (1900) included some 11 species. All of these species, plus another not treated by Kjellman, were

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considered by Papenfuss *et al.* (1982) to represent *G. oblongata*. *Tricleocarpa cylindrica*, the generitype, was described on the basis of a taxon from the West Indies, originally known under the name *Corallina cylindrica* J. Ellis & Solander (1786). The species usually occurs on rocks thinly covered with sand in intertidal pools and in the subtidal zone at a depth of about 15 m in all seasons (Huisman 2006); it is very widely distributed in Africa (Silva *et al.* 1996; De Clerck *et al.* 2005), Asia (Yoshida 1998; Boo & Ko 2012; Tsutsui *et al.* 2012), Australasia (Huisman & Womersley 1994), Europe (Haroun *et al.* 2002; John *et al.* 2004), North and South America (Wynne 2009; Creed *et al.* 2010), and the Pacific Islands (Skelton & South 2007). *Tricleocarpa cylindrica* is characterized by heavy calcification, cortical cell layers thicker than for *T. fragilis*, and carposporangia produced along the inner face of pericarps with paraphyses intermixing with gonimoblast filaments (Huisman & Borowitzka 1990). However, *rbcL* sequences from specimens attributed to this species from different regions are very divergent (Wang *et al.* 2005).

*Tricleocarpa fragilis* was described on the basis of specimens from Jamaica (Oceano Americano) originally as *E. fragilis* Linnaeus, a bryozoan genus (Linnaeus 1758), before being recognized as an earlier name for the well-known *T. oblongata* (Huisman & Townsend 1993). It is distributed worldwide, its range overlapping with that of *T. cylindrica*, in Africa (Silva *et al.* 1996; John *et al.* 2004), Asia (Yoshida 1998; Atmadja & Prud'homme van Reine 2012), Australasia (Huisman 2006), Europe (Haroun *et al.* 2002; John *et al.* 2004), North and South America (Creed *et al.* 2010; Wynne 2011), and the Pacific Islands (Skelton & South 2007). *Tricleocarpa fragilis* is distinguished by thick branches (up to 2.5 mm diameter), gonimoblast filaments arising from the base of the cystocarp cavity, and sterile paraphyses that are poorly developed (Huisman 2006). Despite its range spanning several continents, only a single *rbcL* sequence from a Taiwan specimen has been analyzed (Wang *et al.* 2005).

In the present study, we assessed the genetic diversity of *Tricleocarpa* specimens using both *rbcL* and *cox1* sequences from numerous fresh collections from northeastern and southeastern Asian waters and one each from Hawaii and South Africa. Our analysis of both *rbcL* and *cox1* revealed seven distinct groups in the genus, and, together with detailed descriptions of morphological features, led us to recognize two new species, *T. jejuensis* sp. nov. and *T. natalensis* sp. nov.

## MATERIAL AND METHODS

Specimens and their collection sites are listed in Table S1. Collections were made by snorkeling or scuba diving. Materials for morphological observations were pressed onto herbarium sheets, whereas those used in molecular studies were desiccated in silica gel. For morphological observation distal branch portions were decalcified in 1% HCl solution and sectioned by hand using a razor blade. The sectioned preparations were stained with 1% aqueous aniline blue. Photographs were taken with an FX-35DX camera (Nikon,

Tokyo, Japan) attached to a Vanox AHB3 microscope (Olympus, Tokyo, Japan). Voucher specimens are housed at the herbarium of Chungnam National University (CNUK), Daejeon, Korea.

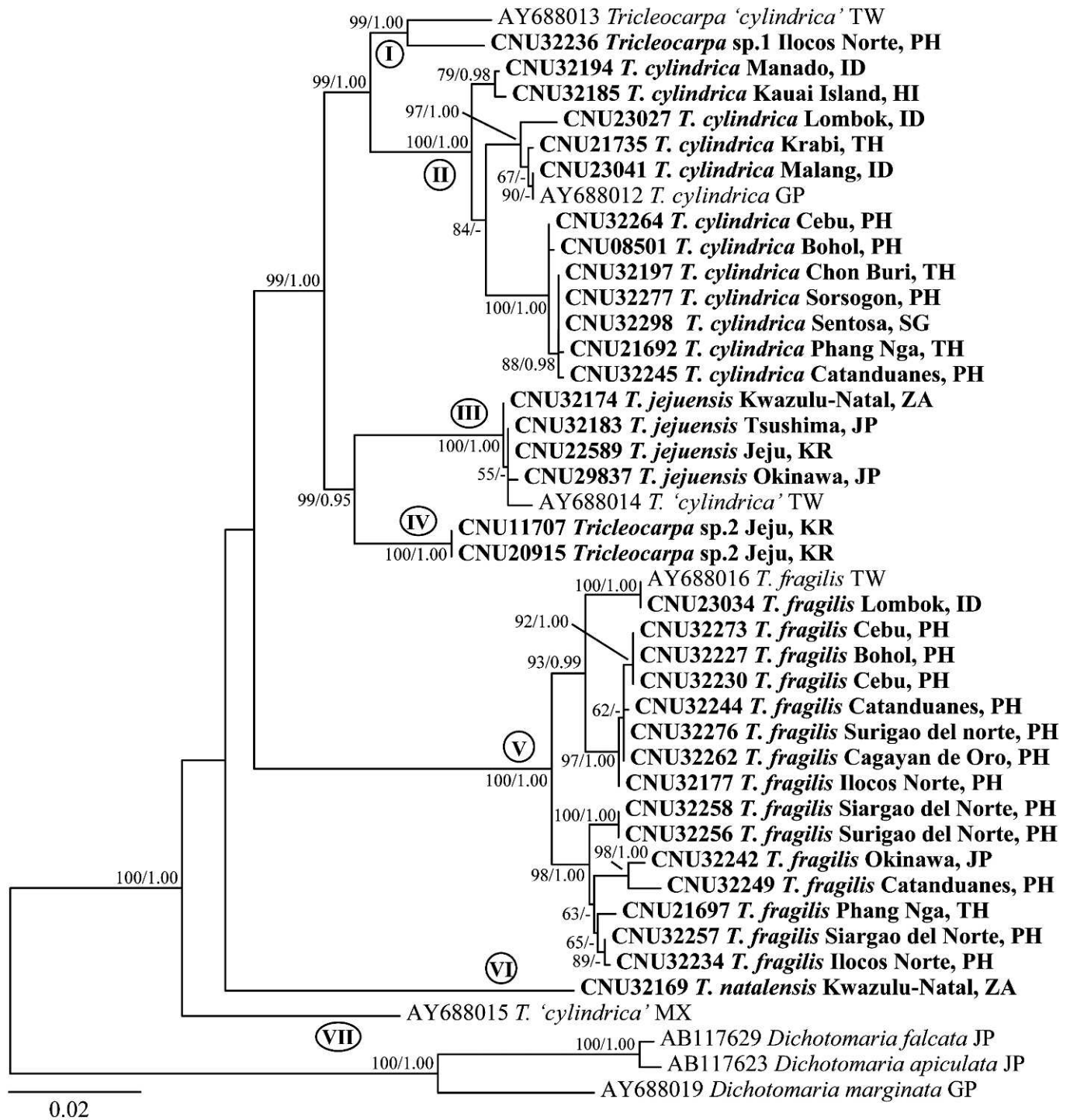
Information for specimens used for the molecular study is given in Table S1. DNA extraction, polymerase chain reaction amplification, and sequencing followed Geraldino *et al.* (2010). Specific primer pairs for the amplification and sequencing of each gene were as follows: for *rbcL*, newly designed forward primers F43 (5'-CGT TAY GAR TCT GGD GTA ATY CC-3') and F615 (5'-GAT GAT GAA AAY ATT AAY TC-3') and previously published reverse primers R753 and RrbcS start (Freshwater & Rueness 1994) and for *cox1*, *cox143F*, and C880R (Geraldino *et al.* 2006; Yang *et al.* 2008).

Forty-three *rbcL* sequences (including eight accessions from GenBank) and 37 *cox1* sequences (including three previously published, Sherwood *et al.* 2010) of *Tricleocarpa* plus three *Dichotomaria* sequences were collated using the multiple-sequence editing program Se-Al v.2.0a11 (Rambaut 1996) and aligned visually. Maximum-likelihood (ML) phylogenetic analyses were performed using RAxML software (Stamatakis 2006) using the GTR +  $\Gamma$  model of evolution. We used 200 independent tree inferences with the "number of run" option with default optimized subtree pruning and regrafting (SPR) rearrangement and 25 distinct rate categories to identify the best tree. Statistical support for each branch was obtained from 1000 bootstrap replications using the same substitution model and RAxML program settings.

Bayesian analyses of the sequence alignment for *rbcL* and *cox1* sequences were conducted with MrBayes v3.1 software (Ronquist & Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo method (MC<sup>3</sup>) with the GTR +  $\Gamma$  + I model and 2 million generations in two independent runs performed with four chains, respectively, and trees sampled every 100th generation. The 3390 sampled generation burn-in period for *rbcL* and 5580 sampled generation burn-in period for *cox1* were identified graphically by tracking likelihoods at each generation to determine whether the likelihood values had reached a plateau. The 16,611 trees for *rbcL* and 14,421 trees for *cox1* sampled at stationarity were used to infer the Bayesian posterior probabilities. Majority-rule consensus trees were calculated using PAUP\* 4.0b10 (Swofford 2002).

## RESULTS

Our multiple alignment [1404 base pairs (bp)] contained 43 *rbcL* accessions, including 35 newly generated sequences, from southeast (Indonesia, Philippines, Singapore, Thailand) and northeast Asia (Japan, Korea, Taiwan), the Hawaiian Islands, South Africa, northeast Pacific (Mexico), and Caribbean waters (Guadeloupe). Variable sites occurred at 466 positions (33.2%), and 384 positions (27.4%) were parsimony informative. Phylogenetic trees constructed with ML and Bayesian analyses were identical in topology. Only the ML tree is shown in Fig. 1. Despite the lack of support for the monophyly of the genus, *Tricleocarpa* consisted of



**Fig. 1.** Maximum likelihood tree of *Tricleocarpa* inferred from the phylogenetic analysis of *rbcL* sequences. Values shown near branches are bootstrap values (1000 iterations) and Bayesian posterior probabilities. Only bootstrap values > 50% and Bayesian posterior probabilities > 0.95 are shown. Roman numbers in circles denote clades as depicted in the text. Abbreviations: GP, Guadeloupe; HI, Hawaii; ID, Indonesia; JP, Japan; KR, Korea; MX, Mexico; PH, Philippines; SG, Singapore; TH, Thailand; TW, Taiwan; ZA, South Africa.

seven strongly supported groups (I–VII). Group I from Taiwan and Philippines formed a sister relationship to group II. True *T. cylindrica* was most likely represented in group II because it included a specimen from Guadeloupe, the type locality of the species. *Tricleocarpa cylindrica* contained specimens from the Hawaiian Islands, Indonesia, Philip-

pines, Singapore, and Thailand. Group III comprised five accessions from Korea, Japan, Taiwan, and South Africa. We described *T. jejuensis* on the basis of this group because the morphology was distinct (see description below). This species was sister to group IV from Korea. Groups I–IV formed a monophyletic clade (99% for ML and 1.0 for

**Table 1.** Pair-wise divergence (%), bp) of *Tricleocarpa* using *rbcL* sequence data. Each number indicates absolute distance among groups (above diagonal), uncorrected *p*-distance and absolute distance within each species (diagonal), and uncorrected *p*-distance among groups (below diagonal).

	<i>Tricleocarpa</i> sp. 1	<i>T. cylindrica</i>	<i>T. jejuensis</i>	<i>Tricleocarpa</i> sp. 2	<i>T. fragilis</i>	<i>T. natalensis</i>	<i>T. 'cylindrica'</i> Mexico
<i>Tricleocarpa</i> sp. 1	2.1, 29	38–54	57–61	47–51	87–97	77–86	74–78
<i>T. cylindrica</i>	2.8–4.0	2.2, 30	61–69	50–60	89–106	85–100	70–83
<i>T. jejuensis</i>	4.2–4.5	4.5–5.1	0.4, 5	45–46	88–97	96–97	76–78
<i>Tricleocarpa</i> sp. 2	3.5–3.7	3.7–4.4	3.3–3.4	0, 0	78–88	90	75
<i>T. fragilis</i>	6.4–7.1	6.5–7.8	6.5–7.1	5.7–6.5	2.6, 36	98–103	95–102
<i>T. natalensis</i>	5.7–6.3	6.2–7.3	7.1	6.6	7.2–7.6	-	99
<i>T. 'cylindrica'</i> Mexico	5.4–5.7	5.1–6.1	5.6–5.7	5.5	7.0–7.5	7.3	-

Bayesian posterior probability). *Tricleocarpa fragilis* consisted of two subgroups; one from Indonesia, Philippines, and Taiwan, and the other from Japan, Philippines, and Thailand. Group VI consisted of a specimen from South Africa, and we described it as *T. natalensis* because of its distinct morphology (see below). Group VII was represented by one specimen from Mexico, on the basis of an *rbcL* sequence sourced from GenBank.

The intra- and interspecific pair-wise genetic distances of *Tricleocarpa* for the *rbcL* marker are shown in Table 1. Intraspecific variation ranged on average from 0% to 2.2% (30-bp difference) in *T. cylindrica* and was up to 2.6% (36-bp difference) in *T. fragilis*. The average interspecific pair-wise divergence ranged from 2.8% (38-bp difference between *T. cylindrica* and *Tricleocarpa* sp. 1) to 7.8% (106-bp difference between *T. cylindrica* and *T. fragilis*).

Thirty-seven *cox1* sequences, including three accessions from GenBank, were aligned using a 577-nucleotide portion of the *cox1* gene. Variable sites occurred at 179 positions (31.3%), and 155 positions (26.9%) were parsimony informative. Phylogenetic trees constructed with ML and Bayesian analyses were similar except for intraspecific relationships. Only the ML tree is shown in Fig. 2. The *cox1* tree revealed only six groups in *Tricleocarpa* because our group VII in *rbcL* (AY688015 from Mexico) was not available for the present study. The genetic distance of *cox1* between species ranged from 6.4% (37-bp difference between *T. cylindrica* and *Tricleocarpa* sp. 1) to 13.9% (80-bp difference between *T. cylindrica* and *T. fragilis*), whereas intraspecific divergences reached up to 7.3% (42 bp difference) in *T. cylindrica* and up to 8.2% (47 bp difference) for *T. fragilis* (Table 2).

### *Tricleocarpa cylindrica* (J. Ellis & Solander)

Huisman & Borowitzka 1990, p. 164

Figs 3–11

BASIONYM: *Corallina cylindrica* J. Ellis & Solander

LECTOTYPE: Ellis & Solander 1786: pl. 22, fig. 4 on the basis of a specimen from the West Indies (typified by Papenfuss *et al.* 1982).

GEOGRAPHICAL DISTRIBUTION: Indonesia, Hawaii, Philippines, Singapore, Thailand, and the West Indies; reported to be widespread in tropical seas, but many records require confirmation using molecular methods.

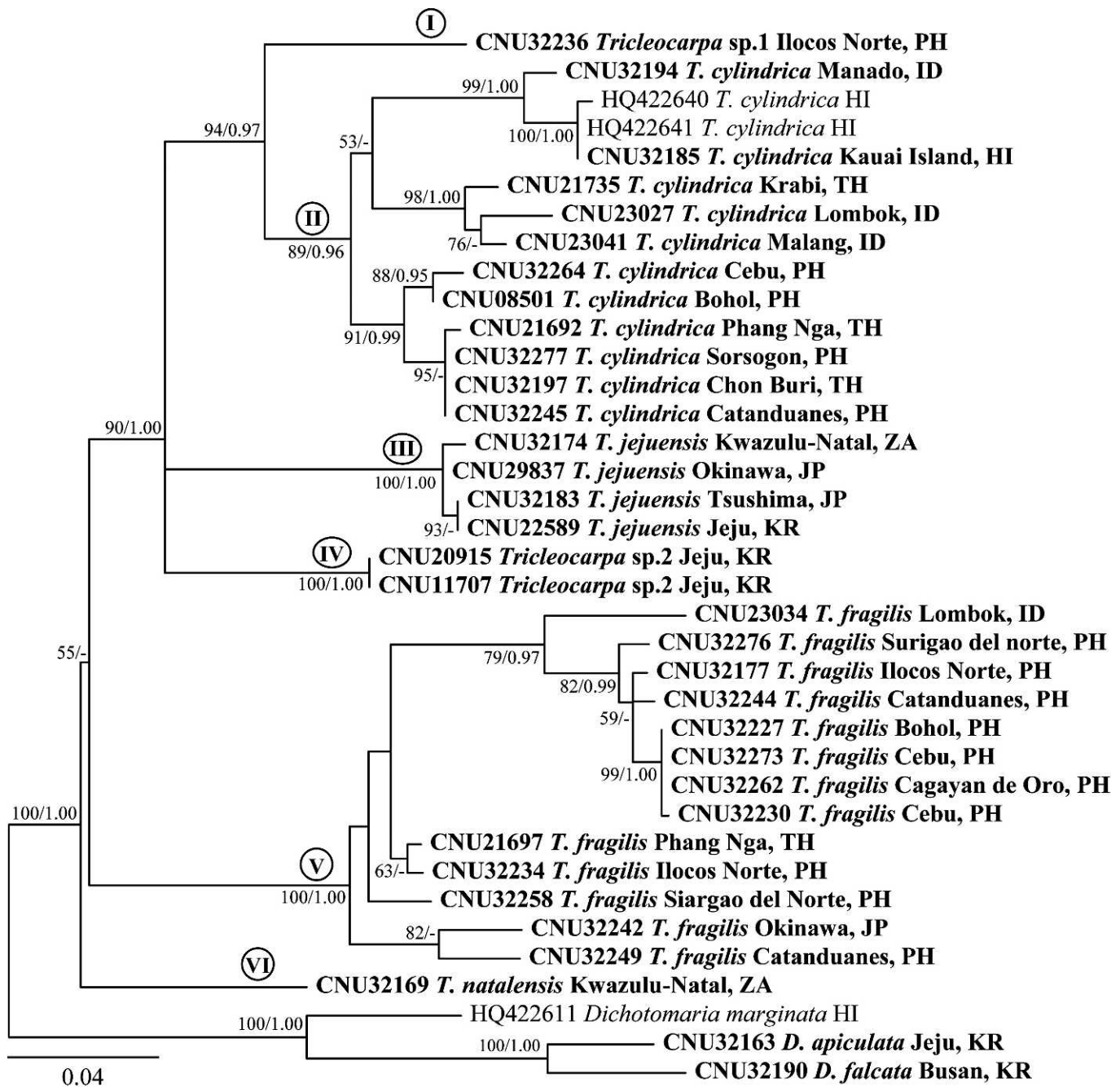
HABITAT: Occurring abundantly on rocky reefs, rocks, and shells of molluscs in the intertidal zones of coral reef communities (Fig. 3).

SPECIMENS EXAMINED: **Indonesia:** Are Goling, Lombok (*S.M. Boo*, 6 June 2012, CNU23032-33, CNU23035-36), Lombok (*S.M. Boo*, 6 June 2012, CNU23029-30). **Philippines:** Bulusan, Sorsogon (*P.J. Geraldino*, 11 January 2006, CNU32210), Gigmoto, Catanduanes (*S.M. Boo*, 18 April 2009, CNU32254), Nasugbu, Batangas (*P.J. Geraldino*, 28 May 2011, CNU08504), Olango Island, Cebu (*S.M. Boo*, 13 December 2003, CNU32233), Sulpa Island, Cebu (*P.J. Geraldino*, 7 January 2006, CNU32269). **Thailand:** Haad Nang Rong, Chon Buri (*T. Noiraksar*, 2 September 2012, CNU29845, CNU29848, CNU29853, CNU29857), Haad Phra Ae, Krabi (*K. Lewmanomont*, 16 April 2012, CNU21750, CNU21764).

Gametophytic plants were up to 7 cm in height (Fig. 4), heavily calcified, dichotomously branched, with branching intervals 0.2–1.2 cm long; branches were terete, glabrous, 0.5–1.5 mm in diameter (Fig. 5). The cortex was composed of three to four layers of compressed cells, innermost cells were 17–25 µm in diameter, grading to outermost cells of 7–15 µm in diameter, generally less pigmented than the layer supporting them (Fig. 6). Tetrasporophytes were not found in the present study.

Plants were dioecious. Spermatangial branch initials were derived from the apical portion of ordinary filaments (Fig. 7). They then formed a hemispherical conceptacle of 200–300 µm. The inner cortical cells of the conceptacles produced highly branched secondary spermatangial filaments that produced obovoid spermatangia, measuring 5–8 × 3–6 µm (Fig. 8).

Cystocarps were commonly found in distal parts of the thallus. Carpogonial branches were three celled, consisting of a carpogonium with elongated trichogyne, hypogynous cells that cut off four sterile branches, and basal cells with four to five involucreal filaments that ultimately formed the pericarp (Fig. 9). Cystocarps were hemispherical to slightly flattened, 250–350 µm in diameter. Carposporangia were ovoid to obovoid and 18–30 × 10–15 µm in size. They were produced singly and terminally from the gonimoblast filaments, which were distributed along the inner face of the pericarp. Three- to four-celled sterile paraphyses arising from the pericarp projected into the cystocarp cavity and rarely intermixed with gonimoblast filaments (Fig. 10). A multinucleate fusion cell was formed by the incorporation of carpogonium and hypogynous cell (Fig. 11).



**Fig. 2.** Maximum likelihood tree of *Tricleocarpa* inferred from the phylogenetic analysis of *cox1* sequences. Values shown near branches are bootstrap values (1000 iterations) and Bayesian posterior probabilities. Only bootstrap values > 50% and Bayesian posterior probabilities > 0.95 are shown. Roman numbers in circles denote clades as depicted in the text. Abbreviations: HI, Hawaii; ID, Indonesia; JP, Japan; KR, Korea; PH, Philippines; TH, Thailand; ZA, South Africa.

***Tricleocarpa fragilis* (Linnaeus) Huisman & Townsend 1993: 100**  
Figs 12–17

GEOGRAPHICAL DISTRIBUTION: Tropical to temperate waters.

HABITAT: Occurred abundantly on rocky reefs, rocks, and shells of molluscs in the lower intertidal and the upper subtidal zones.

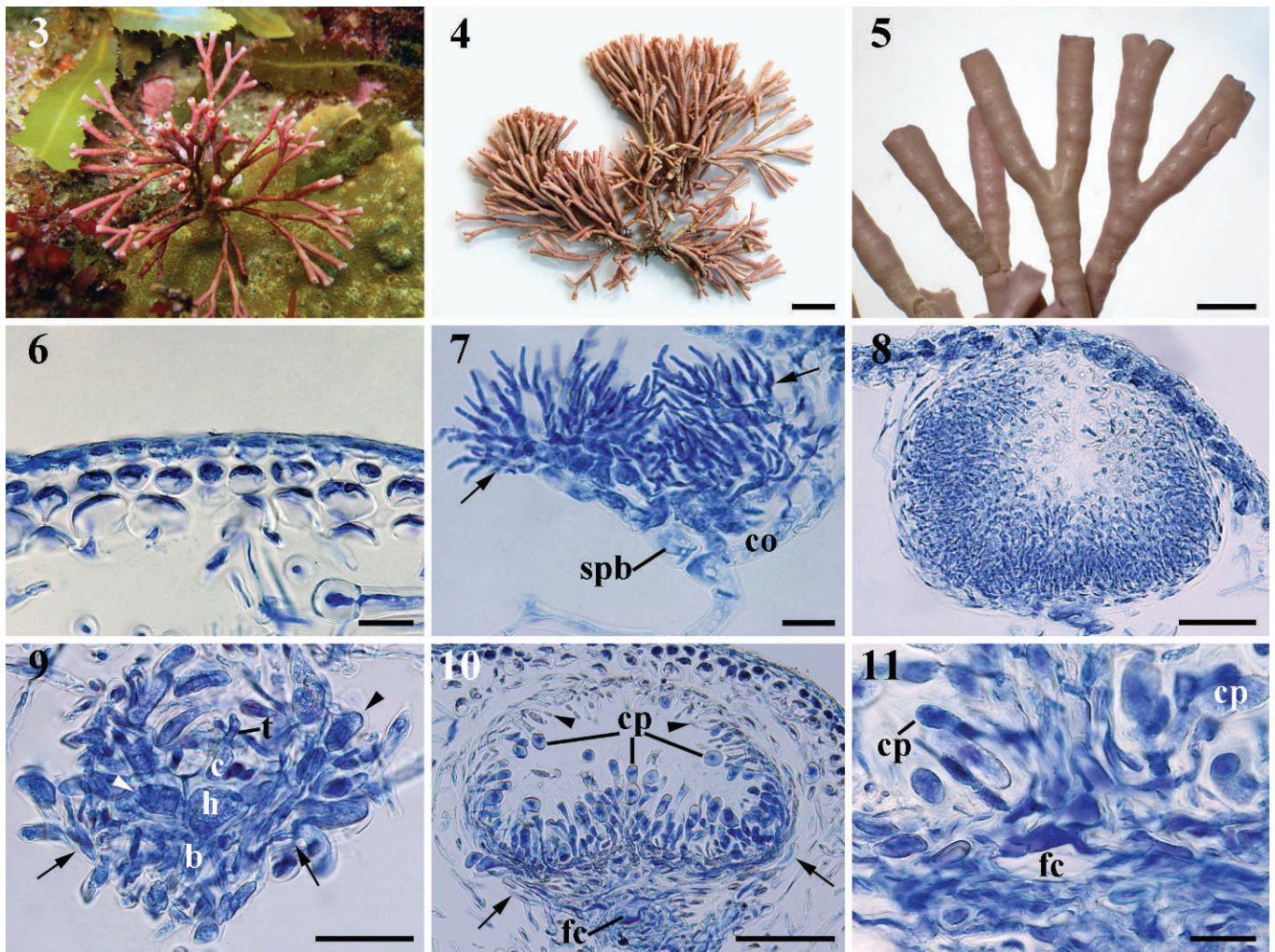
BASIONYM: *Eschara fragilis* Linnaeus

SPECIMENS EXAMINED: **Philippines:** Bonbon, Cagayan de Oro, (*P.J. Geraldino*, 20 January 2006, CNU32263), Del Carmen, Siargao del Norte (*P.J. Geraldino*, 23 January 2006, CNU32259, CNU32271), Gigmoto, Catanduanes (*S.M. Boo*, 18 April 2009, CNU32247-48,

LECTOTYPE: LINN sheet 1297.1, upper specimen from “Oceano Americano” (Jamaica) (Huisman & Townsend 1993).

**Table 2.** Pair-wise divergence (% , bp) of *Tricleocarpa* using *cox1* sequence data. Each number indicates absolute distance among groups (above diagonal), uncorrected *p*-distance and absolute distance within each species (diagonal), and uncorrected *p*-distance among groups (below diagonal).

	<i>Tricleocarpa</i> sp. 1	<i>T. cylindrica</i>	<i>T. jejuensis</i>	<i>Tricleocarpa</i> sp. 2	<i>T. fragilis</i>	<i>T. natalensis</i>
<i>Tricleocarpa</i> sp.1	-	37–50	47–48	45	57–75	49
<i>T. cylindrica</i>	6.4–8.7	7.3, 42	47–60	42–53	54–80	44–58
<i>T. jejuensis</i>	8.1–8.3	8.1–10.4	0.9, 5	48–49	54–68	53–55
<i>Tricleocarpa</i> sp.2	7.8	7.3–9.2	8.3–8.5	0, 0	52–70	44
<i>T. fragilis</i>	9.9–13.0	9.4–13.9	9.4–11.8	9.0–12.1	8.2, 47	49–63
<i>T. natalensis</i>	8.5	7.6–10.1	9.2–9.5	7.6	8.5–10.9	-



**Figs 3–11.** *Tricleocarpa cylindrica*: Vegetative and reproductive structures.

**Fig. 3.** A thallus growing on rocky reefs in Krabi, Thailand (16 April 2012).

**Fig. 4.** Morphology of herbarium specimen. Scale bar = 1 cm.

**Fig. 5.** Dichotomous branches. Scale bar = 2 mm.

**Fig. 6.** Section of cortex of gametophyte. Scale bar = 20  $\mu$ m.

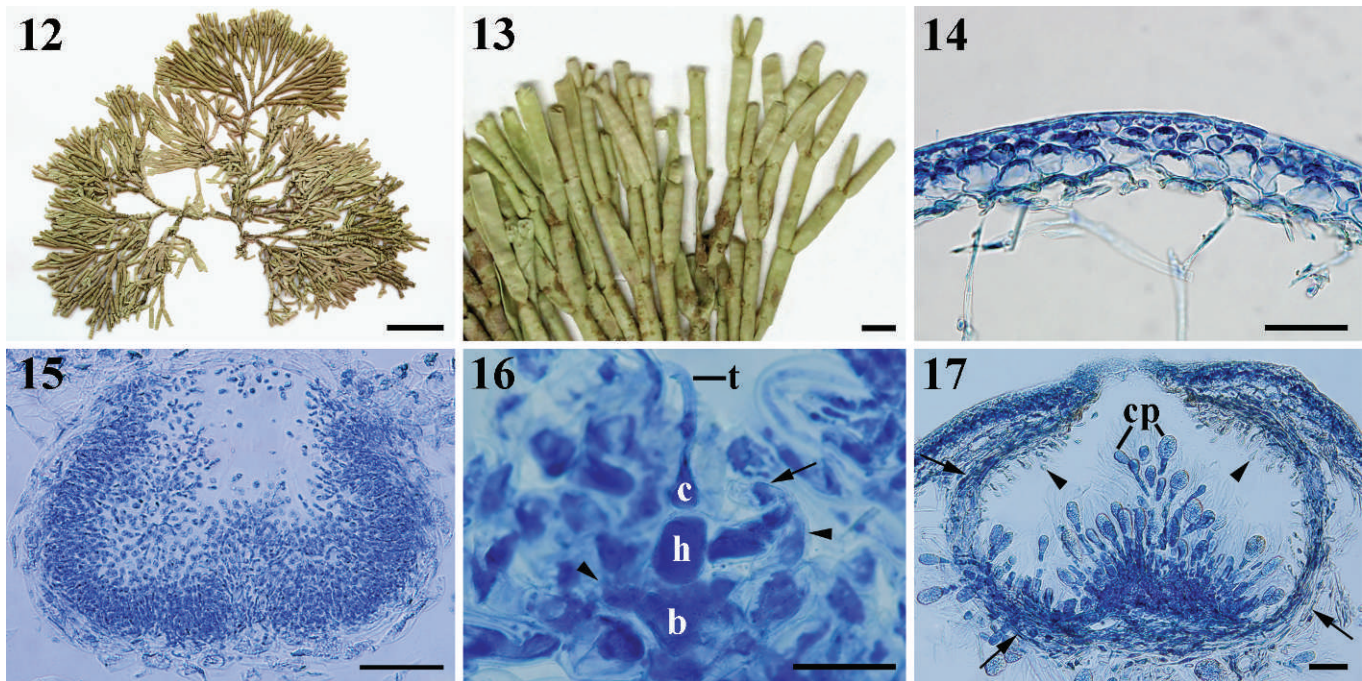
**Fig. 7.** Immature spermatangial branch (spb) with abundant secondary spermatangial filaments (arrows) (co, cortex). Scale bar = 20  $\mu$ m.

**Fig. 8.** Section of spermatangial cavity. Scale bar = 50  $\mu$ m.

**Fig. 9.** Young carpegonial branch showing carpegonium (c) with trichogyne (t), hypogynous cell (h) bearing sterile branches (arrowheads), and basal cell (b) bearing sterile filaments (arrows). Scale bar = 50  $\mu$ m.

**Fig. 10.** Section of cystocarp with carposporangia (cp) arising from inner face of pericarp (arrows) and sterile paraphyses (arrowheads) intermixing with gonimoblast filaments (fc, fusion cell). Scale bar = 100  $\mu$ m.

**Fig. 11.** Detail of cystocarp showing the large fusion cell (fc) formed from carpegonium, hypogynous cell, and basal cell (cp, carpospores). Scale bar = 20  $\mu$ m.



**Figs 12–17.** *Tricleocarpa fragilis*. Vegetative and reproductive structures.

**Fig. 12.** Morphology of herbarium specimen. Scale bar = 2 cm.

**Fig. 13.** Dichotomous branches. Scale bar = 2 mm.

**Fig. 14.** Section of cortex of gametophyte. Scale bar = 50  $\mu$ m.

**Fig. 15.** Section of spermatangial cavity. Scale bar = 50  $\mu$ m.

**Fig. 16.** Young carpogonial branch showing carpogonium (c) with trichogyne (t), hypogynous cell (h) bearing sterile branches (arrowheads), and basal cell (b) bearing sterile filaments (arrow). Scale bar = 20  $\mu$ m.

**Fig. 17.** Section of cystocarp with pericarp (arrows) showing carposporangia (cp) arising from the base of cystocarp and sterile paraphyses (arrowheads), which do not intermix with gonimoblast filaments. Scale bar = 50  $\mu$ m.

CNU32250-53), Pangil, Ilocos Norte (S.M. Boo, 11 March 2005, CNU32238), Panglao Island, Bohol (P.J. Geraldino, 29 January 2006, CNU32224-25, CNU32274-75), San Roque, Surigao del Norte (P.J. Geraldino, 23 January 2008, CNU32243).

Gametophytic plants were up to 10 cm in height, lightly calcified (Fig. 12), subdichotomously branched, with branching intervals 0.4–1.2 cm long; branches were terete, glabrous, 1.2–2.0 mm in diameter (Fig. 13). The cortex was composed of three- to four-cell layers, innermost cells were inflated and colorless, 15–20  $\times$  20–26  $\mu$ m, grading to pigmented outermost cortical cells of 6–15  $\mu$ m in diameter (Fig. 14).

Plants were dioecious or rarely monoecious. Spermatangia were produced in hemispherical conceptacles identical to those of *T. cylindrica*. Mature conceptacles were 200–250  $\mu$ m in diameter with obovoid spermatangia measuring 5–8  $\times$  3–6  $\mu$ m (Fig. 15). Cystocarps were commonly found in the distal parts of the thallus. Carpogonial branches consisted of three cells (basal cell, hypogynous cell, and distal carpogonium) and arose near the apex of the plant in place of a normal vegetative filament (Fig. 16). Mature cystocarps were spherical to slightly flattened, 400–500  $\mu$ m in diameter. Obovoid carposporangia (55–70  $\times$  25–40  $\mu$ m) were produced singly and terminally from the secondary gonimoblast filaments. Two- to three-celled sterile paraphyses arising from the pericarp projected into the cystocarp cavity but did

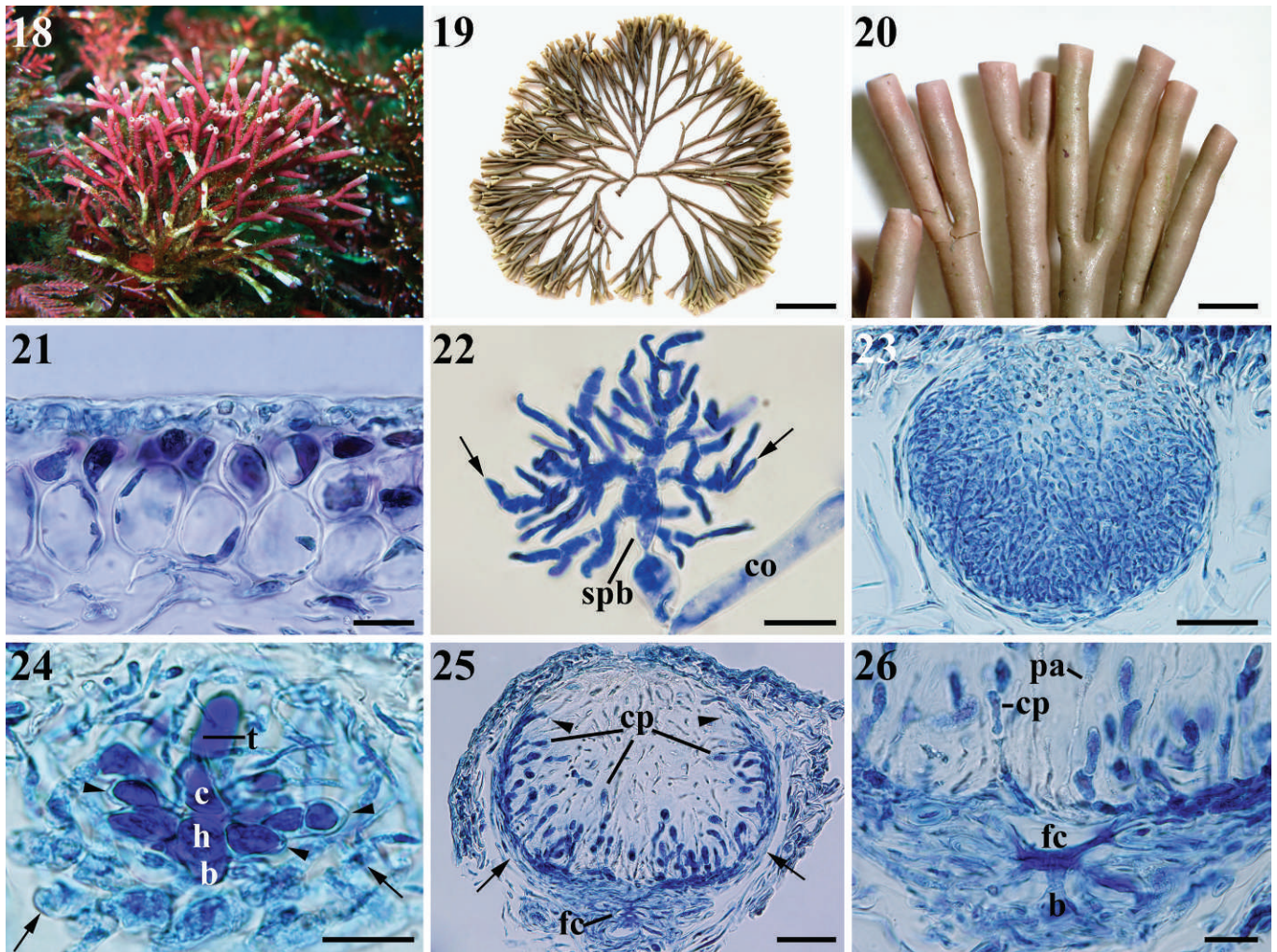
not intermix with gonimoblast filaments. The terminal cells of the paraphyses were slightly inflated (Fig. 17).

***Tricleocarpa jejuensis* J. Wiryadamirikul, Geraldino, Huisman, Lewmanomont & Boo *sp. nov.***

Figs 18–26

**DESCRIPTION:** Thallus to 8.7 cm high, heavily calcified, dichotomously branched; branching intervals 0.4–2.2 cm long; branches terete, glabrous, 0.8–2.0 mm in diameter. Cortex with three to four layers of elongate to compressed cells. Monoecious. Spermatangial cavities 200–300  $\mu$ m in diameter; spermatangia obovoid, 5–8  $\times$  3–6  $\mu$ m, terminal and lateral on filaments projecting into cavity. Basal cells of carpogonial branch with four small-celled branches; hypogynous cell with four branches of nutritive cells. Cystocarps spherical, 250–400  $\mu$ m in diameter. Gonimoblast filaments lining the pericarp and mixing with pericarp filaments. Carposporangia 20–30  $\times$  10–15  $\mu$ m, terminal on gonimoblast filaments along the inner face of the pericarp. Paraphyses produced by the pericarp, projecting into the cavity and mixing with gonimoblast filaments.

**HOLOTYPE:** CNU22589 (Herbarium of CNUK, Daejeon, Korea), attached on rocks in the subtidal zones of Munseom, Jeju Island, Korea collected by J. Wiryadamirikul and Y. D. Ko (13 May 2012) (image shown in Fig. 18).



**Figs 18–26.** *Tricleocarpa jejuensis* J. Wiryadamrikul, Geraldino, Huisman, Lewmanomont & Boo *sp. nov.*: vegetative and reproductive structures.

**Fig. 18.** A thallus attached to a rock on Jeju Island, Korea (10 July 2011).

**Fig. 19.** Morphology of the holotype specimen (CNU22589) collected by J. Wiryadamrikul and Y. D. Ko (13 May 2012). Scale bar = 2 cm.

**Fig. 20.** Dichotomous branches. Scale bar = 2 mm.

**Fig. 21.** Section of cortex of gametophyte. Scale bar = 20  $\mu$ m.

**Fig. 22.** Immature spermatangial branch (spb) with abundant secondary spermatangial filaments (arrows) (co, cortex). Scale bar = 20  $\mu$ m.

**Fig. 23.** Section of spermatangial cavity. Scale bar = 50  $\mu$ m.

**Fig. 24.** Young carpogonial branch showing carpogonium (c) with trichogyne (t), hypogynous cell (h) bearing sterile branches (arrowheads), and basal cell (b) bearing sterile filaments (arrows). Scale bar = 20  $\mu$ m.

**Fig. 25.** Section of cystocarp with carposporangia (cp) arising from inner face of pericarp (arrows) and sterile paraphyses (arrowheads) intermixing with gonimoblast filaments (fc, fusion cell). Scale bar = 50  $\mu$ m.

**Fig. 26.** Detail of cystocarp showing the large fusion cell (fc) formed from carpogonium and hypogynous cell (b, basal cell; cp, carpospores; pa, paraphyses). Scale bar = 20  $\mu$ m.

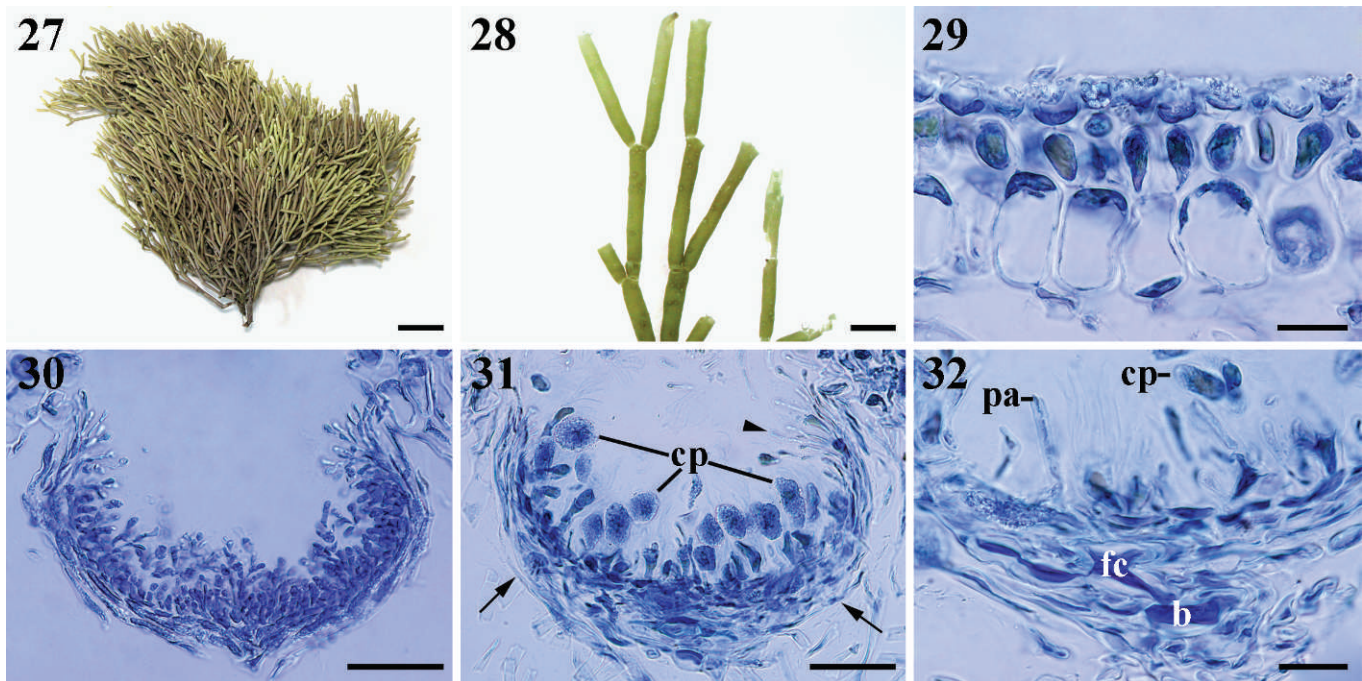
ISOTYPES: CNU22568-69, CNU22571-72, CNU22575.

ETYMOLOGY: The species epithet *jejuensis* refers to Jeju Island, Korea, where the type specimen was collected.

SPECIMENS EXAMINED: **Korea:** Munseom, Jeju Island (Y.D. Ko, 28 January 2012, CNU20916), Munseom, Jeju Island (Y.D. Ko, 15 January 2012, CNU20918), Munseom, Jeju Island (Y.D. Ko, 13 May 2012, CNU22577, CNU22580-81, CNU22591, CNU22595), Seopseom, Jeju Island (J. Wiryadamrikul and Y. D. Ko, 10 July 2011, CNU11767).

Gametophytic plants were up to 8.7 cm in height (Fig. 19), heavily calcified, richly dichotomously branched, with branching intervals 0.4–2.2 cm long. Branches were terete, glabrous, 0.8–2.0 mm in diameter (Fig. 20), structurally multiaxial with a pseudoparenchymatous cortex and a medulla of longitudinally aligned filaments. The cortex was composed of three to four layers of elongate to compressed subsurface and innermost cells. Innermost cortical cells were 23–33  $\mu$ m long by 16–25  $\mu$ m in diameter, grading to pigmented outermost cortical cells of 9–15  $\mu$ m in diameter,





**Figs 27–32.** *Tricleocarpa natalensis* J. Wiriyadamirikul, Geraldino, Huisman, Lewmanomont & Boo *sp. nov.*: vegetative and reproductive structures.

**Fig. 27.** Morphology of the holotype specimen (CNU32169) collected by S.M. Boo (9 August 2005). Scale bar = 1 cm.

**Fig. 28.** Dichotomous branches. Scale bar = 2 mm.

**Fig. 29.** Section of cortex of gametophyte. Scale bar = 20  $\mu$ m.

**Fig. 30.** Section of spermatangial cavity. Scale bar = 50  $\mu$ m.

**Fig. 31.** Section of cystocarp with carposporangia (cp) arising from inner face of pericarp (arrows) and sterile paraphyses (arrowhead) intermixing with gonimoblast filaments. Scale bar = 50  $\mu$ m.

**Fig. 32.** Detail of cystocarp showing the large fusion cell (fc) formed from carpogonium and hypogynous cell (b, basal cell; cp, carpospores; pa, paraphyses). Scale bar = 20  $\mu$ m.

generally less pigmented than the subcortical cell layer (Fig. 21).

Plants were monoecious. Spermatangial branch initials formed in place of ordinary filaments near the apex of the cylindrical branches and divided laterally and transversely into primary spermatangial filaments (Fig. 22) that formed a hemispherical conceptacle 200–300  $\mu$ m in diameter. Obovoid spermatangia (5–8  $\times$  3–6  $\mu$ m) were produced either terminally or laterally from filaments projecting into the cavity (Fig. 23).

Carpogonial branch initials consisted of three cells (basal cell, hypogynous cell, and distal carpogonium) and arose near the apex of the plant in place of a normal vegetative filament. Before fertilization, the basal cell produced four small-celled branches that were the precursors of the pericarp. The hypogynous cell cut off four branches of larger cells and the carpogonium produced an elongate trichogyne (Fig. 24). After fertilization, the pericarp developed further and was well developed before the production of gonimoblast initials. Gonimoblast initials arose directly from the carpogonium and grew distally and laterally into gonimoblast filaments that intermixed with the sterile paraphyses produced by the pericarp (Fig. 25). As cystocarp development progressed, the pit connections linking the carpogonium and the hypogynous cell broke down to form a

multinucleate fusion cell. The pit connection linking hypogynous cell and basal cell broadened but the basal cells remained distinct throughout cystocarp development (Fig. 26). Mature cystocarps were spherical, 250–400  $\mu$ m in diameter. Ovoid to obovoid carposporangia (20–30  $\times$  10–15  $\mu$ m) were produced singly and terminally from the secondary gonimoblast filaments on the inner face of the pericarp and were directed toward the center of the cavity (Figs 25, 26).

***Tricleocarpa natalensis* J. Wiriyadamirikul, Geraldino, Huisman, Lewmanomont & Boo *sp. nov.***

Figs 27–32

**DESCRIPTION:** Thallus to 5.5 cm high, heavily calcified, dichotomously branched; branching intervals 0.2–0.8 cm long; branches terete, glabrous, 0.5–1.1 mm in diameter. Cortex with three layers of elongate cells. Monoecious. Spermatangial cavities 200–250  $\mu$ m in diameter; spermatangia obovoid, 5–7  $\times$  3–5  $\mu$ m, terminal and lateral on filaments projecting into cavity. Basal cell of carpogonial branch with four small-celled branches; hypogynous cell with four branches of nutritive cells. Cystocarps hemispherical, 200–250  $\mu$ m in diameter. Gonimoblast filaments lining the pericarp and mixing with pericarp filaments. Carposporangia 15–30  $\times$  10–15  $\mu$ m, terminal on gonimoblast filaments along the inner face of the

pericarp. Paraphyses produced by the pericarp, projecting into the cavity and rarely mixing with gonimoblast filaments.

**HOLOTYPE:** CNU32169 (Herbarium of CNUK, Daejeon, Korea), growth on rocks in the subtidal zones of Lala Neck, Kwazulu-Natal, South Africa, collected by S.M. Boo (9 August 2005) (image shown in Fig. 27).

**ISOTYPE:** CNU32170.

**ETYMOLOGY:** The species epithet *natalensis* refers to the province, Kwazulu-Natal in South Africa, where this new species was collected.

Gametophytic plants were up to 5.5 cm in height (Fig. 27), heavily calcified, dichotomously branched, with branching intervals 0.2–0.8 cm long. Branches were terete, glabrous, 0.5–1.1 mm in diameter (Fig. 28), structurally multiaxial, with a pseudoparenchymatous cortex and medulla of longitudinally aligned filaments. The cortex was composed of three-cell layers of elongate cells except in reproductive regions. The innermost cortical cells were 28–34  $\mu\text{m}$  long by 17–26  $\mu\text{m}$  in diameter, grading to outermost cortical cells of 7–15  $\mu\text{m}$  in diameter, generally less pigmented than the subcortical cell layer (Fig. 29).

Plants were monoecious. Spermatangia were produced in hemispherical conceptacles identical to those of other *Tricleocarpa* species. Mature conceptacles measured 200–250  $\mu\text{m}$  in diameter with obovoid spermatangia measuring  $5\text{--}7 \times 3\text{--}5 \mu\text{m}$  (Fig. 30). The formation of cystocarps agreed with the original description of the genus *Tricleocarpa* (Huisman & Borowitzka 1990). Carposporangia were produced singly and terminally from the secondary gonimoblast filaments on the inner face of the hemispherical cystocarp. Three- to four-celled sterile paraphyses arose from the pericarp and projected into the cystocarp cavity, but rarely intermixed with gonimoblast filaments. Mature cystocarps measured 200–250  $\mu\text{m}$  in diameter with ovoid to obovoid carposporangia measuring  $15\text{--}30 \times 10\text{--}15 \mu\text{m}$  (Fig. 31). As cystocarp development progressed, the pit connections linking the carpogonium and hypogynous cells broke down to form a multinucleate fusion cell. The pit connection linking the hypogynous cell and basal cell broadened, but the basal cell remained distinct throughout cystocarp development (Fig. 32).

## DISCUSSION

Our analyses of *rbcL* and *cox1* sequences derived from numerous specimens of *Tricleocarpa* from Asia, the Hawaiian Islands, South Africa, and West Indies revealed very high genetic diversity within the genus. The *rbcL* sequences provided sufficient resolution to recognize seven genetic groups that can be considered as distinct species. Although four groups [*T. 'cylindrica'* (AY688015), *T. natalensis*, and *Tricleocarpa* sp. 1 and sp. 2] were represented by only one or two specimens, the pair-wise divergences between these groups ranged from 3.5% to 7.3%, and were comparable with or higher than those in other congeneric species of red algae: e.g. 0.5% between *Dichotomaria apiculata* (Kjellman) Kurihara & Masuda and *D. falcata*

(Kjellman) Kurihara & Masuda in the present study and 0.2–2% between *Pterocladia capillacea* (Gmelin) Santelices & Hommersand, and *P. tenuis* (Okamura) Shimada, Horiguchi & Masuda (Boo *et al.* 2010). All seven groups in the *rbcL* data are consistently supported by *cox1*. However, *cox1* genetic diversities were very high within *T. cylindrica* and *T. fragilis* due to its evolution rate being faster than *rbcL* in red algae. One group missing in the *cox1* analysis is the Mexican taxon (GenBank accession AY688015) because sequences were unavailable.

Our recent collections of *Tricleocarpa cylindrica* from Indonesia, Hawaii, Philippines, Singapore, and Thailand correspond in their habit and vegetative and reproductive morphology to the description of this species by Huisman & Borowitzka (1990). On the basis of both *rbcL* and *cox1* gene sequences, *T. cylindrica* is confirmed to occur from the West Indies (the type locality) to the Hawaiian Islands and southeastern Asian waters. The genetic connectivity of the species between the Caribbean Sea and Asian waters suggests that *T. cylindrica* was possibly dispersed via the Panama Strait before its closure (4.6–3.6 million years ago; Haug & Tiedemann 1998). Our concept of *T. cylindrica* is the same as that of Wang *et al.* (2005), with the exception of a sample from Mexico (Gulf of California) that was consistently placed outside *T. cylindrica* in our analysis (Fig. 1). We are unable to make any taxonomic conclusions regarding this entity, which is demonstrably different from *T. cylindrica* and *T. fragilis*, but it might represent Kjellman's *Galaxaura constipata* (1900: 63), the only species of *Eugalaxaura* with a Mexican type locality (Veracruz) and currently treated as a synonym of *T. fragilis*. However, the genetic diversity of *T. cylindrica* is still high (2.2% for *rbcL* and 7.3% for *cox1*) and more sampling is necessary from the range reported in the literature and not represented in our analyses, including Africa (Silva *et al.* 1996; De Clerck *et al.* 2005), Australia (Huisman & Womersley 1994), Europe (Haroun *et al.* 2002; John *et al.* 2004), and other Pacific Islands (Skelton & South 2007).

The morphology of specimens attributed here to *Tricleocarpa fragilis* agrees well with the description of the species by Huisman & Townsend (1993), which was based on an examination of the Linnaean type specimen. The *T. fragilis* clade is well supported in both *rbcL* and *cox1* analyses. All samples representing *T. fragilis* in the present study were from Indonesia, Japan (Okinawa), the Philippines, Taiwan, and Thailand, suggesting that *T. fragilis* is a warm-water species compared with the eurythermal *T. cylindrica*. Our analysis, however, does not include samples from Jamaica (Oceano Americano), the type locality of the species (Huisman & Townsend 1993); thus our attribution of this clade to *T. fragilis* is based on morphology and should be confirmed by inclusion of additional specimens from the West Indies. The *T. fragilis* clade contained two subgroups, one from Indonesia and the Philippines, and the other from Japan, the Philippines, and Thailand. Both subgroups were very similar in morphology, and their ranges overlapped at Ilocos Norte and Surigao del Norte, the Philippines. As with *T. cylindrica*, further sampling of *T. fragilis* is necessary to confirm its presence in other areas, including Africa (Silva *et al.* 1996; John *et al.* 2004), Australasia (Huisman 2006), Europe (Haroun *et al.* 2002; John *et al.* 2004), North and South

America (Creed *et al.* 2010; Wynne 2011), and the Pacific Islands (Skelton & South 2007).

Of the seven taxonomic groups indicated by our molecular analyses, we therefore attribute two to the currently recognized species *Tricleocarpa cylindrica* and *T. fragilis*. Two further clades (groups III and VI) can be distinguished morphologically and undoubtedly represent independent species. The choice of names to apply to these clades is somewhat complex. When first recorded, *Tricleocarpa* included only two species, one raised from synonymy (Huisman & Borowitzka 1990). This concept of the species diversity was primarily from that of Papenfuss *et al.* (1982), who reduced 11 species to synonymy with *T. oblongata*, these essentially the entirety of Decaisne's Section *Eugalaxaura*. According to Kjellman (1900), this section included 11 species, several newly described by himself. All of these species, plus another not treated by Kjellman, were considered by Papenfuss *et al.* (1982) to represent *G. oblongata*.

As with *Galaxaura* and the recently resurrected *Dichotomaria*, the species included in what is now recognized as *Tricleocarpa* have therefore undergone a period of expansion (Kjellman 1900), then considerable contraction (Papenfuss *et al.* 1982). Our molecular analyses have suggested that most certainly the Papenfuss *et al.* (1982) approach was excessive. Huisman & Borowitzka (1990) redressed the balance only slightly by recognizing two species, but the true diversity of the genus probably approaches the 11 or 12 species of Kjellman (1900). This leads us to our possibly intractable situation, one faced by most taxonomists attempting to marry earlier, poorly defined names to DNA-based clades. We can discern several species on the basis of morphological and molecular analyses, but we cannot be sure whether these species correspond to those recognized historically without considerable further study and collection. The species recognized by Kjellman (1900) could possibly provide names for several of the clades, but the marrying of the two is a difficult process.

Nevertheless, on the basis of geography and morphology we feel that we can exclude many possible names. *Galaxaura pilifera* Kjellman is the only species with a western Indian Ocean type locality (Mauritius). The epithet, suggesting a pilose surface, would seemingly exclude this species from consideration as both *Tricleocarpa jejuensis* and *T. natalensis* are glabrous. However, surface filaments could not be found in the type specimen by Svedelius (1945: 46, commenting that "the whole matter seems to me strange"), which would accord with the glabrous nature of all other *Tricleocarpa*. Svedelius (1945) regarded *G. pilifera* to be identical to his concept of *G. fastigiata* Decaisne. Huisman & Borowitzka (1990: 169) examined the type specimen and found it to have cystocarps partially lining the pericarp, essentially intermediate between those of *T. cylindrica* and *T. fragilis* (as *T. oblongata*), whereas in *T. jejuensis* and *T. natalensis* the cystocarp essentially lines the pericarp surface. On the basis of this feature, *G. pilifera* can be removed from consideration. Of the remaining species, none has type localities in the northwest Pacific, where *T. jejuensis* is known to occur. Although this does not unequivocally exclude them from consideration, we feel that attributing these names to our clades without sequence data from authentic specimens

could confuse the taxonomy. Given, however, that *T. jejuensis* is also known from South Africa, its range could encompass much of the Indo-Pacific. If true, six other presently synonymous species have type localities at least feasibly within its range (*G. conglutinata* Kjellman 1900: 62, eastern Australia; *G. dactylophora* Piccone & Grunow, in Piccone 1884: 312, Ethiopian Red Sea coast; *G. dimorpha* Kjellman 1900: 63, Timor; *G. eburnea* Kjellman 1900: 62, Queensland, Australia; *G. fastigiata* Decaisne 1842: 116, Timor; *G. schimperi* Decaisne 1842: 116, Red Sea). Of these, the types of *G. eburnea* and *G. conglutinata* were examined by Huisman & Borowitzka (1990: 169) and found to correspond to those of *T. oblongata* and *T. cylindrica*, respectively. Thus we also exclude these species from consideration. *Galaxaura dactylophora* is not known from type material (Papenfuss *et al.* 1982: 416), and its status cannot be evaluated here. Papenfuss *et al.* (1982) treated it as a synonym of their broadly conceived *G. oblongata*. On the basis of the descriptions provided by Kjellman (1900), *G. dimorpha* can be excluded on the basis of its habit, which as depicted by Kjellman (1900: tab. 2, fig. 3) approaches that of *T. fragilis* and is unlike that of both our new species. Moreover, Kjellman gave the dimension of outer cortical cells as 16–18  $\mu\text{m}$ , which is larger than the 7–15  $\mu\text{m}$  of *T. natalensis* and *T. jejuensis*. The remaining two species (*G. fastigiata* and *G. schimperi*) were both described by Decaisne (1842). In Svedelius' appraisal of species attributed to *Eugalaxaura* (Svedelius 1945), he examined the type of *G. fastigiata*, concluding that it was distinguishable from *G. oblongata*, being a slender species, "hardly 1 mm in breadth, at the base at most 1.3 mm". This species is similar to our concept of *T. cylindrica* and possibly similar in habit to *T. natalensis*. Svedelius (1945) provided an illustration of the cortex from the lectotype (1945: fig. 14c), which seemingly differs from that of *T. natalensis* in having subsurface cells broader than long, whereas those of *T. natalensis* are elongate (Fig. 29). Unfortunately the type locality of *G. fastigiata* is uncertain. The "syntype" specimen examined and figured by Svedelius (1945: pl. 6, fig. 2) was labelled as being from the Philippines, but Papenfuss *et al.* (1982) noted some discrepancies and suggested that the specimen was presumably from Timor. The type of *G. schimperi*, from the Red Sea, was also examined by Svedelius (1945), who concluded it to be synonymous with *G. oblongata* (= *T. fragilis*). As we do not have fresh material from the Red Sea and no authentic sequences are available, we cannot assess this species. For the present, we accept the opinion of Svedelius. Interestingly, Svedelius (1945) believed that *G. schimperi* as known from Japan differed from *G. schimperi* from the Red Sea, and depicted the cortex of a Japanese specimen (1945: fig. 14f) with at least some elongate subsurface cells. Thus this apparently misidentified entity in Japan may be the same as our *T. jejuensis*.

Several Kjellman species with western Indian Ocean type localities are presently included as synonyms in *Galaxaura* or *Dichotomaria*. *Galaxaura breviararticulata* Kjellman (1900: 84, Port Natal, South Africa), *G. effusa* Kjellman (1900: 84, Port Natal, South Africa), and *G. insignis* Kjellman (1900: 87, Nosy Bé Madagascar) were included as synonyms of *G. obtusata* by Papenfuss *et al.* (1982: 418), itself now a synonym of *D. obtusata* (see Huisman *et al.* 2004). Each of

**Table 3.** Comparison of morphological characters of *Tricleocarpa* species.

	<i>T. cylindrica</i>	<i>T. jejuensis</i>	<i>T. natalensis</i>	<i>T. fragilis</i>
Plant height (cm)	c. 7.0	c. 8.7	c. 5.5	c. 10.0
Branch length (cm)	0.2–1.2	0.4–2.2	0.2–0.8	0.4–1.2
Branch width (mm)	0.5–1.5	0.8–2.0	0.5–1.1	1.2–2.0
Degree of calcification	strong	strong	strong	light
Cortical cell layers	three to four	three to four	three	three to four
Innermost cortex shape	compressed	elongate to compressed	elongate	compressed
Plant sexuality	dioecious	monoecious	monoecious	dioecious or monoecious
Cystocarp diam. (µm)	250–350	250–400	200–250	400–500
Cystocarp shape	hemispherical to slightly flattened	spherical	hemispherical	spherical to slightly flattened
Gonimoblast position	distributed along the inner face of cystocarp	distributed along the inner face of cystocarp	distributed along the inner face of cystocarp	compact in the basal part of cystocarp
Paraphyses	rarely intermixed with gonimoblast filaments	intermixed with gonimoblast filaments	rarely Intermixed with gonimoblast filaments	not intermixed with gonimoblast filaments
Habitat	intertidal zones	subtidal zones	intertidal zones	intertidal zones

these species has features precluding their inclusion in *Tricleocarpa* and can be removed from consideration, in the case of the first two the presence of stalked cortical cells (Kjellman 1900: tab. 18, figs 1, 14) and in the last the apparent fusion of cortical cells (Kjellman 1900: tab. 19, figs 18, 20). In addition, *G. insignis* is distinctly geniculate with branches up to 3 mm in diameter (Kjellman 1900: 87), larger than both *T. jejuensis* and *T. natalensis*. Two additional non-Kjellman species from South Africa, *G. beckeri* Schmitz ex Mazza (1900: 3) and *G. natalensis* Kylin (1938: 6), were considered synonymous with *G. diesingiana* Zanardini by Papenfuss *et al.* (1982: 405), the former with a question mark. *Galaxaura diesingiana* has also been transferred to *Dichotomaria*, as *D. diesingiana* (Zanardini) Huisman, J.T. Harper & G.W. Saunders (2004: 230). *Dichotomaria diesingiana* has flattened branches and is therefore morphologically distinct from our new species of *Tricleocarpa*. We cannot judge the validity of the synonymy proposed by Papenfuss *et al.* (1982), but on the basis of the descriptions and illustrations of both *G. natalensis* and *G. beckeri* by Kylin (1938: 5, 6), who also examined the type of latter, both are compressed species and therefore unlike *Tricleocarpa*.

From our molecular analyses and detailed morphological examinations, compared with authentic specimens and literature reports, we conclude that two of our distinguishable clades should be described as new species, which we have named *Tricleocarpa jejuensis* sp. nov. and *T. natalensis* sp. nov. *Tricleocarpa jejuensis* is based on specimens from Korea, Japan, and South Africa and an *rbcL* sequence from Taiwan, and is distinguished by three to four cortical cell layers with elongate to compressed subsurface and innermost cells, monoecious sexuality, spherical cystocarps, and paraphyses intermixed with gonimoblast filaments. It formed a sister relationship with *Tricleocarpa* sp. 2 from Jeju Island, Korea. It is likely that the ancestor of *T. jejuensis* and *Tricleocarpa* sp. 2 arose from northeast Asian populations of the widespread *T. cylindrica*. This could be explained by the founder effect and subsequent speciation model applied to speciation of intertidal benthos distributed in Indo-Pacific tropical waters such as the brown alga *Padina* and the hermit crab (Malay & Paulay 2009, Silberfeld *et al.* 2013).

*Tricleocarpa jejuensis* occurs mainly in the subtidal zone, whereas *T. cylindrica* occurs primarily in the intertidal zone. *Tricleocarpa jejuensis* and *Tricleocarpa* sp. 2 are morphologically similar but occur in separate clades. As we are unable to distinguish these clades morphologically, we defer any taxonomic proposals for the latter taxon until more samples can be examined. Both taxa occur in Jeju Island, Korea: *T. jejuensis* occurs in southern parts and *Tricleocarpa* sp. 2 can be found from the western to the eastern parts of the island.

*Tricleocarpa natalensis* is distinct in morphology and molecular phylogeny. Its position outside the remaining groups of *Tricleocarpa* in both *rbcL* and *cox1* trees indicates that *T. natalensis* likely diverged first within the genus. A morphological comparison of four species within *Tricleocarpa* is given in Table 3. The remaining three genealogical groups are based on only a few specimens, and further sampling is necessary before assessing their positions.

We can now update the concept of *Tricleocarpa* to recognize four named species in the genus (*T. cylindrica*, *T. fragilis*, *T. jejuensis*, and *T. natalensis*), and at least three genealogical groups that may be described as species in the future. This is the first study of *Tricleocarpa* that has incorporated analyses of both *rbcL* and *cox1* marker data from a wide taxon sampling in Northeast and Southeast Asian waters.

#### ACKNOWLEDGEMENTS

We gratefully acknowledge Yong Deok Ko for collections in Jeju; John Bolton, Grevo S. Gerung, and Cai Yixiong for their help in collecting in Kwazulu-Natal, Java, and Singapore; other colleagues listed in Table S1 for the provision of samples; Ga Hun Boo for designing *rbcL* forward primers; and Antonella Bottalico for the Latin grammar of the specific epithets. This work was financially supported by a Korean Research Foundation grant (2012-002579) and a Korean government's marine biotechnology grant from the Ministry of Oceans and Fisheries to SMB, the CHED-Biodiversity and Database Program (CEB 307-2009)

and IAPT Research Grants Program to PJLG, and the Australian Biological Resources Study to JMH.

## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at <http://dx.doi.org/10.2216/13-155.1.s1>.

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Received 16 March 2013; accepted 13 April 2013