Botanica Marina 54 (2011): 159–167 © 2011 by Walter de Gruyter • Berlin • New York. DOI 10.1515/BOT.2011.017

Colpomenia claytonii sp. nov. (Scytosiphonaceae, Phaeophyceae) based on morphology and mitochondrial *cox*3 sequences

Sung Min Boo[1,*], Kyung Min Lee[1], Ga Youn Cho[2] and Wendy Nelson[3]

[1] Department of Biology, Chungnam National University, Daejon 305-764, Korea
[2] Non-vascular Plants Research Division, National Institute of Biological Resources, Incheon 404-708, Korea, e-mail: smboo@cnu.ac.kr
[3] National Institute of Water and Atmospheric Research Ltd, Private Bag 14-901, Wellington 6241, New Zealand

* Corresponding author

Abstract

Colpomenia, a small genus with 11 species of globular to bullate form, occurs in temperate to tropical waters worldwide. Because morphology is highly diverse, the species-level taxonomy requires re-evaluation. We analyzed the mitochondrial *cox*3 gene from 50 samples of the genus. A new species, *Colpomenia claytonii* sp. nov., is described based on samples collected from Korea, Japan, Hong Kong, Australia, New Zealand, South Africa, and the USA and compared with similar congeners. Compared to others, the new species is larger, and has a more irregular thallus often with a deeply infolded surface. It is usually epilithic in tide pools and in the lower intertidal and subtidal zones. In all phylogenetic analyses of *cox*3 sequences, *C. claytonii* was consistently distinct from congeners. *Colpomenia expansa* is closely related to *C. claytonii*, and the clade containing these two species is closely related to *C. peregrina*. A total of 14 *cox*3 haplotypes was found in *C. claytonii*, indicating high haplotype diversity and a recent dispersal pattern. The present study shows that *C. claytonii* is a widely distributed species across the Pacific Ocean to South Africa; it was previously misidentified as a variant of *C. peregrina*.

Keywords: *Colpomenia claytonii* sp. nov.; *cox*3; haplotype network; morphology; Phaeophyceae.

Introduction

Colpomenia (Endlicher) Derbès *et* Solier is a commonly found floristic component in habitats ranging from temperate to tropical waters. Eleven species are currently recognized in the genus: *C. bullosa* (De A. Saunders) Yamada, *C. durvillei* (Bory de Saint-Vincent) M.E. Ramírez, *C. ecuticulata* M.J. Parsons, *C. expansa* (De A. Saunders) Y.-P Lee, *C. mollis* R. Taylor, *C. nainativensis* Durairatnam, *C. peregrina* Sauvageau, *C. phaeodactyla* M.J. Wynne *et* J.N. Norris, *C. ramosa* W.R. Taylor, *C. sinuosa* (Mertens ex Roth) Derbès *et* Solier, and *C. tuberculata* De A. Saunders (Saunders 1898, Taylor 1945, Parsons 1982, Lee 2008). The genus is characterized by a globular to convoluted or bullate thallus with a continuous thin membrane, sori with 1–3-celled paraphyses, and a heteromorphic life history (Derbès and Solier 1851, Wynne and Norris 1976, Norris 2010). The distinctions between the species have been confusing and remain so.

Colpomenia peregrina occurs annually, appearing in early winter to early summer in Europe (Fletcher 1987); it is distributed in Europe (Fletcher 1987), North America (Scagel et al. 1989), Africa (Lawson and John 1987), Australasia (Womersley 1987, Adams 1994), and Asia (Kogame and Yamagishi 1997, Cho et al. 2005). Clayton (1975) described two morphotypes of *C. peregrina* from southern Australia: a relatively small globose form (thallus diameter 1–5 cm) and a larger irregular form (thallus diameter 7–10 cm) with a deeply infolded surface. The globose form is the type most commonly found in Europe (Blackler 1967), whereas the irregular form predominates during winter in southern Australia (Clayton 1975). However, Clayton (1975) suggested that variability in the two forms did not justify recognition of separate species.

During our previous investigation of *Colpomenia peregrina* using the large subunit of the protein-coding RuBisCO gene (*rbc*L) and the nuclear ribosomal DNA (nrDNA) internally transcribed spacer (ITS) region from specimens of the species collected throughout its range (Cho et al. 2005), we found a candidate for a species new to science and related to *C. peregrina.* Here, we describe the unidentified species as *C. claytonii* sp. nov. on the basis of its vegetative and reproductive morphology, and provide further evidence of its taxonomic distinctness based on mitochondrial protein-coding cytochrome c oxidase subunit III (*cox*3) gene sequences. *cox*3 is a fast-evolving gene that has proven suitable for unraveling hidden diversity (Kogame et al. 2005), revealing distribution patterns (Uwai et al. 2006), and identifying species (Lee et al. 2009) in brown algae.

Materials and methods

Sampling and morphological observations

Thalli of *Colpomenia claytonii* were collected from intertidal zones in Korea, Japan, Hong Kong, Australia, New Zealand, South Africa, and the USA. Material for observation was pressed onto herbarium sheets, whereas material used in molecular studies was desiccated in silica gel. Tissues were sectioned using a freezing microtome (FX-802A; Coper Electronics Co., Ltd., Kanagawa, Japan). Photographs were

taken with an FX-35 DX camera (Nikon, Tokyo, Japan). Voucher specimens are housed at the herbarium of Chungnam National University, Daejon, Korea (CNUK).

Analysis of the *cox*3 gene

Fifty specimens were available for our molecular studies (Table 1). Total DNA was extracted from approximately 5 mg of dried thallus ground in liquid nitrogen using a NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. The extracted DNA was stored at -20°C and used to amplify *cox*3.

Specific primer pairs for amplification and sequencing of the gene were F49 and R20 (Boo et al. 2010). All PCR amplifications were carried out with a TechGene thermal cycler (Techne Ltd., Duxford, Cambridge, UK) using a TaKaRa ExTaq reaction kit (Takara Shuzo, Shiga, Japan). Reactions of 25 μl total volume consisted of 2.5 μl 10× ExTaq buffer, 2.5 μl 25 mM $MgCl_2$, 1.0 μM dNTP mixture, 0.15 μM of each primer, 0.625 U TaKaRa ExTaq, and 3.0 μM DNA solution (containing 0.5–1.0 μg DNA). PCR was performed with an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 30 s at 40°C, and 1 min at 72°C, with a final 10 min extension cycle at 72°C.

PCR products were purified using a High Pure PCR Product Purification kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions, and then sequenced commercially (Genotech, Daejeon, Korea). Both electropherograms from each sample were edited using Sequence Navigator ver. 1.0.1 software (Applied Biosystems, Foster City, CA, USA). Fifty new *cox*3 sequences were collated using Se-Al v.2.0a11 (Rambaut 2002) and aligned visually. *Scytosiphon lomentaria* (Lyngbye) Link and *Petalonia fascia* (O.F. Muller) Kuntze were used as outgroups.

Fifty *cox*3 sequences were used for the phylogenetic analyses. Maximum-likelihood (ML) phylogenetic analyses were performed using RAxML software (Stamatakis 2006) with the GTR+Γ+I model. We used 200 independent tree inferences using the default of automatically optimized SPR rearrangement and 25 distinct rate categories options of the program to identify the best tree. Bootstrap values were calculated using 1000 replicates with the same substitution model.

Bayesian analyses were conducted with MrBayes v.3.1 (Ronquist and Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo (MC^3) with the GTR+Γ+I model for the *cox*3 data. For each matrix, 1×10^6 generations of two independent runs were performed with four chains, and trees were sampled every 100 generations. The burn-in period was identified graphically by tracking the likelihoods at each generation to determine whether they had reached a plateau. The 12,491 trees sampled at stationarity were used to infer the Bayesian posterior probability (BPP). Majority-rule consensus trees were calculated using PAUP* (Swofford 2002).

A statistical parsimony network was drawn for *cox*3 haplotypes of *Colpomenia claytonii* using TCS version 1.21 software (Clement et al. 2000). This TCS program calculates the minimal number of mutational steps by which the sequences can be joined with >95% confidence interval.

Results

Colpomenia claytonii S.M. Boo, K.M. Lee, G.Y. Cho *et* W. Nelson. sp. nov. (Figure 1A–F)

Latin diagnosis *Thalli globulares vel vesiculati, aetate provecta irregulariter convolutascentes et expansi, usque as 30 cm diametro, plerumque epilithici; cortex laminam superficialem cubiformium cellularum parvarum pigmentosarumque constans; medulla cum 5–6 laminis cellularum gradatim majorium et irregulariter formatarum; paraphyses unicellulares; pili phaeophyceani ex cellulis medullaris oriundi; sporangia plurilocularia uniseriata vel biseriata, 6–8 loculata, irregulariter disposita in sori extensis in superficie thalli; sporangia unilocularia ignota.*

Thalli globular or vesicle-like, becoming irregularly convoluted and expanded with age, reaching a diameter of about 30 cm, usually epilithic; cortex composed of a surface layer of small pigmented cuboidal cells; medulla includes 5–6 layers of gradually larger, irregularly shaped cells; paraphyses unicellular; phaeophycean hairs arising from outer medullary cells; plurilocular sporangia arranged irregularly in extensive patches on thallus surface, uni- and biseriate, having 6–8 locules; unilocular sporangia unknown.

Holotype

SMB000001 (in CNUK, Herbarium of Chungnam National University, Daejeon, Korea), epilithic on rocks in the lower intertidal zone off Sangjokam (34°54′17.68 N, 128°08′57.50 E), Goseong, Korea, 12 January 2005 (Figure 1A). Isotypes SMB000002–7.

Etymology

The name *Colpomenia claytonii* is chosen to honor Prof. Margaret Clayton of Monash University, for her first discovery of this taxon in Australia (Clayton 1975) and her considerable contributions to the understanding of brown algal taxonomy.

Paratype specimens examined

Korea: Dolsando, Yeosu (24.vi, 2007, SMB000009–11), Jumunjin, Gangreung (22.iv, 2007, SMB000012), Homigot, Pohang (15.iv.2007, SMB000013), Sangjokam, Gosung (22.ii.2005, 12.iii.2005, 14.v.2010, SMB000014–20). Australia: Gellibrand Reserve, Melbourne (S.M. Boo, 7.viii.2001, CNUK, number not given). New Zealand: Portobello, Dunedin (S.M. Boo, 6.viii.2001, CNUK, number not given), Weller's Rock, Dunedin (S.M. Boo, 6.viii.2001, CNUK, number not given).

Morphology

Thalli are erect, globular, or vesicle-like hollow sacs becoming irregularly convoluted and expanded (Figure 1B), collap-

Table 1 Specimens and *cox*3 sequences of taxa included in this study.

Species, collection sites and date	Voucher	GenBank accession no.
Colpomenia claytonii sp. nov.		
Korea: east coast		
Anin, Gangreung, 12 January 2002	PE018	HQ833787
Hupo, Uljin, 10 April 2001	PE020	HQ833783
Hupo, Uljin, 22 May 2005	PE579	HQ833814
Aninjin, Gangreung, 9 February 2006	PE898	HQ833803
Jumunjin, Gangreung, 20 February 2006	PE900	HQ833791
Jumunjin, Gangreung, 20 February 2006	PE901	HQ833792
Sacheon, Gangreung, 25 June 2009	PE1478	HQ833793
Korea: south coast		
Dolsando, Yeosu, 24 June 2007	PC1	HQ833788
Dolsando, Yeosu, 24 June 2007	PC2	HQ833790
Dolsando, Yeosu, 24 June 2007	PC3	HQ833789
Sangjokam, Goseong, 9 December 2003	PE122	HQ833794
Sangjokam, Goseong, 9 December 2003	PE123	HQ833796
Sangjokam, Goseong, 9 December 2003	PE124	HQ833797
Sangjokam, Goseong, 12 January 2005	PE372	HQ833785
Sangjokam, Goseong, 12 January 2005	PE373	HQ833786
Sangjokam, Goseong, 12 January 2005	PE374	HQ833784
Sangjokam, Goseong, 1 March 2006	PE904	HQ833795
Korea: Jejudo		
Hansuri, Hanrim, 4 December 2002	PE070	HQ833799
Sinsudo, Chujado, 24 May 2005	PE578	HQ833800
Shinyang, Seoguipo, 6 June 2009	PE1469	HQ833806
Japan		
Nakura, Ishigaki, 6 February 2005	PE1419	HQ833782
Hong Kong		
Stephenie Bay, 23 April 2008	PE754	HQ833804
Lobster Bay, 23 April 2008	PE755	HQ833805
Lobster Bay, 23 April 2008	PE758	HQ833802
South Africa		
Seaforth Road, Cape town, 3 February 2004	PE205	HQ833801
Australia		
Gellibrand Reserve, Melbourne, 7 August 2001	PE033	HQ833810
USA		
Coral St. Beach, California, 11 December 1999	PE047	HQ833811
San Pedro, California, 9 June 2010	PE2286	HQ833812
San Pedro, California, 9 June 2010	PE2417	HQ833813
New Zealand		
Island Bay, Wellington, 30 July 2004	PE348	HQ833798
Urupukapuka, Bay of Islands, 8 April 2006	ASG738	HQ833808
Wilson Bay, Coromandel, 10 September 2006	ASG832	HQ833807
Urquharts Bay, Whangarei Harbour, 28 September 2009	SS1008	HQ833809
Colpomenia bullosa (De A. Saunders) Yamada		
Mukri, Chujado, Korea, 26 April 2005	PE477	HQ833770
Kainga Reef, Burrewarra Point, Australia, 1 January 2009	JK3	HQ833769
Colpomenia ecuticulata M.J. Parsons		
Horseshoe Bay, Marlborough Sounds, New Zealand, 19 October 2005	ASF458	HQ833775
Marsden Point, Whangarei Harbour, New Zealand, 5 November 2009	SS1571	HQ833776
Colpomenia expansa (De A. Saunders) Y.P. Lee		
Damuraemi, Chujado, Korea, 26 May 2009	PE1465	HQ833780
Mukri, Chujado, Korea, 24 May 2009	PE1466	HQ833781
Seokduri, Chujado, Korea, 24 May 2009	PE1467	HQ833779
Colpomenia peregrina (Sauvageau) Hamel		
Sacheon, Gangreung, Korea, 23 February 1999	PE021	HQ833767
Jawbone Reserve, Melbourne, Australia, 7 April 2001	PE034	HQ833768
Colpomenia phaeodactyla M.J. Wynne *et* J.N. Norris		
Sangjokam, Goseong, Korea, 12 January 2005	PE375	HQ833772
Nagasaki, Kyushu, Japan, 26 February 2005	PE818	HQ833771

(Table 1 continued)

Species, collection sites and date	Voucher	GenBank accession no.
Colpomenia sinuosa (Martens ex Roth) Derbès *et* Solier		
Sacheon, Gangreung, Korea, 25 June 2009	PE1476	HQ833777
Black Bock, South Africa, 10 August 2005	PE702	HQ833778
Colpomenia tuberculata De A. Saunders		
El Sargento, Baja California, Mexico, 11 May 2009	PE1512	HQ833773
El Sargento, Baja California, Mexico, 11 May 2009	PE1513	HQ833774
Outgroups		
Petalonia fascia (O.F. Müller) Kuntze		
Munseom, Jejudo, Korea	PE627	HQ833766
Scytosiphon lomentaria (Lyngbye) Link		
Sormsangi, Chujado, Korea, 23 May 2005	PE582	HQ833765

sed and irregularly torn later with age, reaching a diameter of about 30 cm. Thalli are broadly attached at the base by localized patches of rhizoidal filaments produced from the surface cells. Surface cells are polygonal and irregularly arranged. Phaeophycean hairs are frequent, immersed in pits; each hair filament arises from an outer cortical cell (Figure 1C,D).

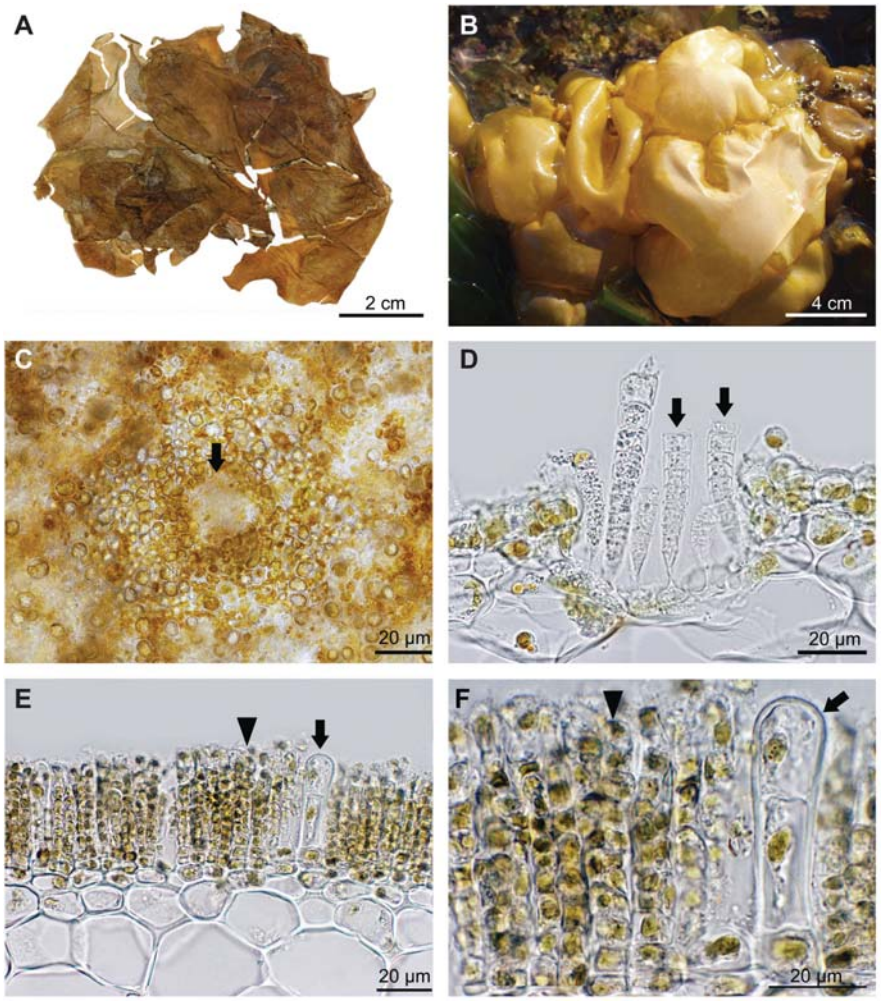

Figure 1 *Colpomenia claytonii*: morphology.
(A) Type specimen collected in Sangjokam, Goseong, Korea (12 January 2005). (B) Thallus growing in the lower tidal zone, Sangjokam, Goseong, Korea (12 January 2009). (C) Cryptostomata (arrow) in surface view. (D) Several phaeophycean hairs (arrows) arising from medullary cells. (E) Plurilocular sporangia (arrowhead) and ascocyst (arrow) in a cross-section of the thallus. (F) Close-up view of plurilocular sporangia (arrowhead) and ascocyst (arrow).

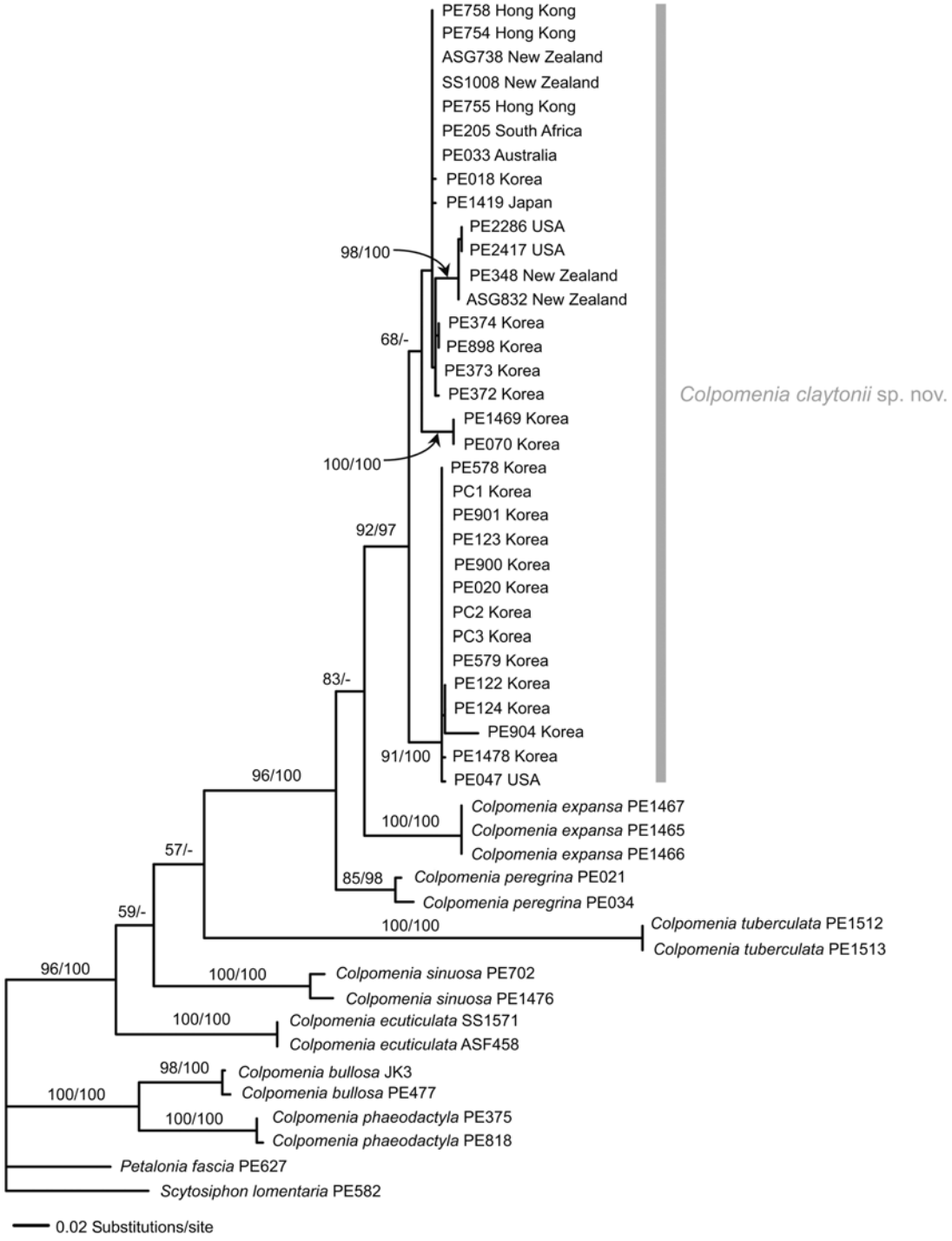

Figure 2 Maximum likelihood trees derived from analysis of *cox*3 sequences in the genus *Colpomenia* and putative relatives using the GTR+Γ+I evolution model [-lnL=3067.73; base frequencies πA=0.218901, πC=0.134380, πG=0.222512, πT=0.424207; shape parameter (α)=0.218070].
The numbers above or near the branches are bootstrap values from the maximum likelihood and Bayesian analyses (ML/BP).

Cortices comprise a surface layer of small pigmented cuboidal cells (Figure 1E). Medulla consist of 5–6 layers of gradually larger, irregularly shaped cells. Paraphyses are unicellular, of the same height as plurilocular sporangia (Figure 1F).

Plurilocular sporangia are arranged irregularly in extensive patches on the thallus surface and are uni- and biseriate, with 6–8 locules and a length of 50–60 μm (Figure 1F). Uniloc-ular sporangia are unknown.

Erect thalli are usually epilithic on rock in tide pools and in the sublittoral zone.

Prostrate thalli (sporophytic thalli) are unknown.

Phylogeny of *cox*3

A 637-nucleotide portion of *cox*3 was aligned for all specimens analyzed here. The tree produced by the ML analysis based on the 50 *cox*3 sequences is illustrated in Figure 2. In total, 215 positions were variable (33.8%) and 197 positions (30.93%) were parsimoniously informative. Specimens of *Colpomenia claytonii* differed from one another by 0–29 bp (0–4.55%). *Colpomenia claytonii* differed by 39–51 bp (6.12–8.00%) from *C. expansa* and by 36–49 bp (5.65–7.69%) from *C. peregrina*. There was a 46 bp (7.22%) difference between *C. expansa* and *C. peregrina*. The trees consistently showed a clade of all 34 *C. claytonii* specimens. *C. expansa* was sister to *C. claytonii,* a relationship strongly supported by bootstrap values; the clades of both species clustered with *C. peregrina*. Each of the remaining five species of the genus was consistently distinct in the *cox*3 tree.

Fourteen *cox*3 haplotypes were found in *Colpomenia claytonii* (Figure 3). Haplotypes fell into five groups connected by many missing haplotypes. One group containing four haplotypes (C1–C4) was distributed in Korea and the USA. The second group comprising only C5 was connected with C4 but had nine missing haplotypes. The third group consisted of two haplotypes (C9–C10) from New Zealand and the USA. The fourth group included six haplotypes (C6–8 and C11–13). The last contained C14 from Korea.

Discussion

All phylogenetic analyses of *cox*3 sequences consistently demonstrated the distinctness of *Colpomenia claytonii* from congeners, as shown in previous ITS and *rbc*L data (Cho et al. 2005). *C. claytonii* is therefore well distinguished by nuclear ITS, plastid *rbc*L, and mitochondrial *cox*3 gene phylogenies. *C. claytonii* is diagnosed by thalli larger (approx. 30 cm) and more irregular than those of other species. *C. claytonii* frequently has a deeply infolded surface and irregular sori that lack cuticles. The species is epilithic and submerged in tide pools and subtidal zones (see Table 2). In addition, our study also reveals a wide distribution of the species from Korea and Hong Kong to Australasia, the USA, and South Africa. We also show that a common and morphologically and ecologically distinct species has been unrecognized until now; previously, it was incorrectly interpreted as a variant of *C. peregrina* in Korea and Australia (Clayton 1979, Cho et al. 2005).

We observed the type of *Colpomenia peregrina* in the herbarium of the Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle (PC), Paris (Womersley 1987, Yoshida 1998). It is small (up to 5 cm) and not convoluted. This species is always epiphytic on *Sargassum thunbergii* (Mertens ex Roth) Kuntze, *S. fusiforme* (Harvey) Setchell, *Neorhodomela aculeata* (L.P. Perestenko) Masuda, and articulated coralline algae in the intertidal zone of Korea (Cho et al. 2005). Although *C. peregrina* is reportedly epiphytic or epilithic (e.g., Parsons 1982, Womersley 1987), our study and that of Clayton (1975) demonstrate that epiphytic thalli

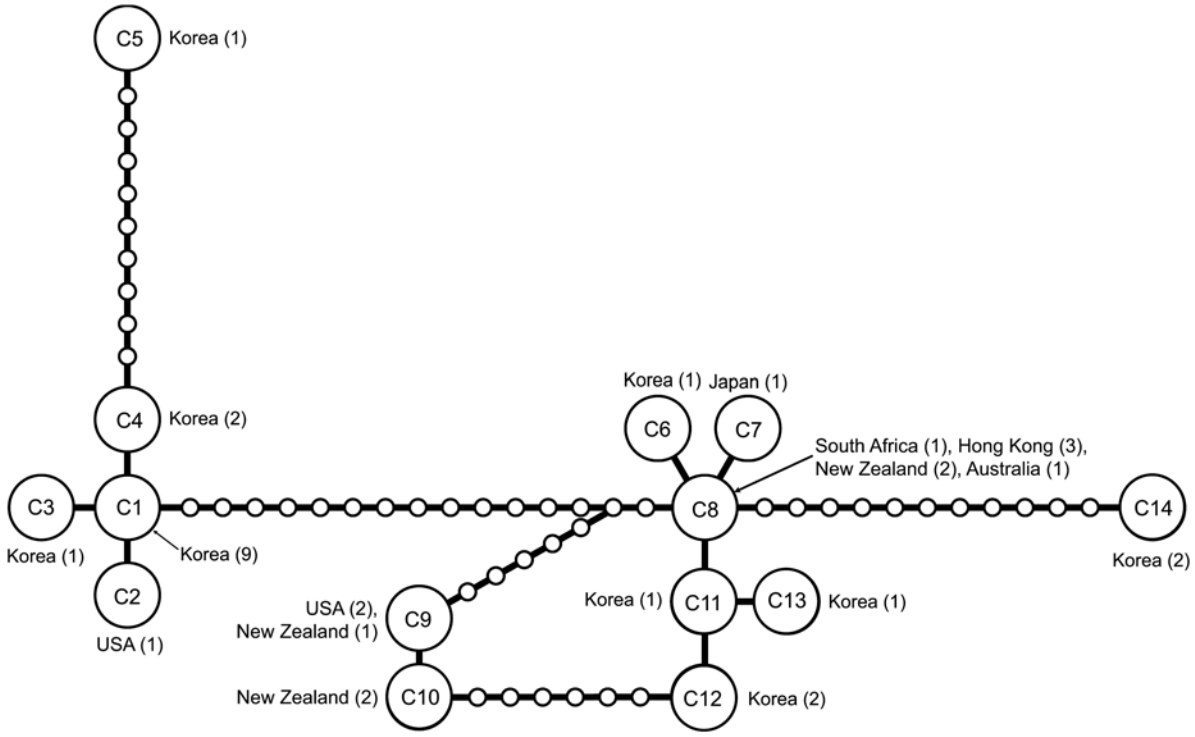

Figure 3 A statistical parsimony network of 14 haplotypes (C1–14) in *Colpomenia claytonii*. Small circles correspond to missing haplotypes.

Table 2 Morphological comparison of species in the genus *Colpomenia*.

	C. bullosa	*C. claytonii*	*C. durvillei*	*C. ecuticulata*	*C. expansa*	*C. mollis*	*C. nainativensis*	*C. peregrina*	*C. phaeodactyla*	*C. ramosa*	*C. sinuosa*	*C. tuberculata*
Thallus shape	Finger-like, branched at the base	Globose or vesicle-like, irregularly convoluted	Finger-like, branched at the base	Globose or vesicle-like, slightly folded	Globose or vesicle-like, very tiny clumps of hairs on the surface	Compressed sacs with short branches and spine-like projections	Irregularly lobed hollow sac with spine-like projections	Globose or vesicle-like	Finger-like, wth many sacs arising from a single base	Adherent clumps with short peglike branches, crisp	Globose or vesicle-like, firm, deeply folded	Brain-like, with blunt tubercles on the surface
Thallus size	Usually up to 30 cm in length, 3 cm in width	Up to 30 cm in diameter	Up to 15 cm in length, 0.8 cm in width	Up to 50 cm or more in length, 2.5 cm in diameter	Up to 6 cm in diameter	Up to 25 cm tall, 2.5 cm in diameter	5–6 cm tall, 4–7 cm in diameter	3–7 (–9) cm tall, about 0.8 cm in diameter	Up to 25 cm tall, about 2.5 cm in width	About 4 cm in diameter, about 2 cm tall	About 14 cm in diameter	About 15 cm in diameter
Cortex	2–3 layers of angular cells	A single layer of angular cells	1–3 layers of angular cells	1–2 layers of angular cells	1–3 layer of angular cells	A single layer of sub-quadrangular cells	A single layer of angular cells	1–3 cell layers	A single layer of small cells	Small celled	1–2 layers of cuboidal cells	4–5 layers of cuboidal cells
Medullary structure	3–5 layers of cuboidal cells	5–6 layers of cuboidal cells	About 5 layers of cuboidal cells	3–4 layers of cuboidal cells	5–7 layers of cuboidal cells	A single layer of angular cells	About 3 layers of cells	3–4 layers cuboidal cells	2–3 layers of cuboidal cells	6–8 layers of cuboidal cells	4–6 layers of large cells	4–5 layers of large cells
Paraphysis	1–2 celled, same height or longer than plurilocular sporangia	One-celled, same height as plurilocular sporangia	Often present	Up to 3 celled, longer than plurilocular sporangia	One-celled, same height as plurilocular sporangia	–	–	One celled, same height as plurilocular sporangia	Rare, shorter or same height as plurilocular sporangia	–	Longer than plurilocular sporangia	Slightly shorter than plurilocular sporangia
Phaeophycean hairs	Hair pits scattered	Arising from cortical cells	Occur occasionally	Hair pits scattered, hair arising from medullary cell	Arising from cortical cells	–	–	Arising from basal meristem	Arising from cortical cells	–	Tufts arising from medullary cells	Arising from medullar cells, long
Plurilocular sporangia	Uni- to biseriate, 12–15 locules	Uni- and biseriate, 6–8 locules	Uni- to biseriate, about 18 locules	Biseriate, about 4–6 locules	Multiseriate, 7–8 locules	–	–	Uni- to multiseriate, up to 12 locules	Uni- and biseriate, 6–8 locules	Uniseriate, 10–12 locules	Uni- and biseriate, 8–12 locules, covered with cuticle	Uniseriate (occasionally biseriate), 6–8 locules
Ecology	Epilithic, intertidal	Epilithic, usually low tidal	Epilithic, intertidal, semiexposed	Epiphytic, subtidal	Epilithic, intertidal	Epilithic, intertidal	Epilithic, intertidal	Usually epiphytic, rarely epilithic, intertidal to subtidal	Epilithic, mid to lower intertidal	Entangled with other algae, lower intertidal to subtidal	Epilithic or occasionally epiphytic, lower intertidal to subtidal	Epilithic to epiphytic, mid to lower intertidal
Type locality	Pacific Grove, California, USA	Sangjokam, Gosung, Korea	Concepcion, Chile	Takatu Peninsula, North Island, New Zealand	Avalon Bay, Santa Catalina, USA	Isla Gorgona, Valle, Colombia	Nainativu Island, Sri Lanka	Morbihan, France	Puerto Peñasco, Sonora, Mexico	Isla Cedros, Baja California, Mexico	Cadiz, Spain	Near San Pedro, California
Geographical distribution	Temperate waters of the Pacific	Korea, Hong Kong, Oceania, South Africa, USA	Concepcion, Chile	Australia and New Zealand	Santa Catalina, California, USA, Korea	Colombia	Sri Lanka	Temperate waters worldwide	Temperate waters of the Pacific Ocean	Baja California, Mexico to Costa Rica	Warm waters worldwide	California, USA to Baja California, Mexico
References	Saunders 1898, Yoshida 1998	This study	Ramírez and Rojas 1991	Parsons 1982, Womersley 1987	Saunders 1989, Lee 2008	Taylor 1945	Durairatnam 1962	Fletcher 1987	Wynne and Norris 1976, Yoshida 1998, Norris 2010	Taylor 1945, Wynne and Norris 1976, Norris 2010	Clayton 1975, Norris 2010	Saunders 1898, Wynne and Norris 1976, Norris 2010

belong to *C. peregrina*, while epilithic thalli belong to *C. claytonii*. It would be difficult to conclude that this is also the case for *C. peregrina* from the UK, where the species is reported to be epiphytic or rarely epilithic (Fletcher 1987). *C. peregrina* is also small in Korea, with a diameter of about 9 cm. However, Hamel (1937) reported that *C. peregrina* in France was abundant from November to May, and reached about 35 cm in diameter. Re-examination of specimens identified as *C. peregrina* in algal herbaria throughout the world is absolutely necessary to provide a more realistic distribution in temperate regions of the world.

Colpomenia expansa from Korea is closely related to *C. claytonii.* Both species are also similar in being epilithic. However, *C. expansa* usually occurs intertidally and has minute clumps of hair pits on the surface of the thallus (Lee 2008). In *C. claytonii,* no clumps of hair pits are present when thalli are mature and have plurilocular sporangia.

Despite the inclusion of more species (*Colpomenia claytonii*, *C. ecuticulata*, *C. expansa*, and *C. tuberculata*) of the genus than in previous studies, our *cox*3 tree revealed that *Colpomenia* consists of at least two groups: one for *C. bullosa* and *C. phaeodactyla*, and the other for remaining species of the genus (Figure 2). *Colpomenia* has been repeatedly paraphyletic in previous studies using different genes: SSU rDNA and *rbc*L (Kogame et al. 1999, Kain et al. 2010 for *rbc*L) and *psa*A (Cho et al. 2006). However, *C. claytonii* formed a close relationship with *C. expansa* and *C. peregrina*. The one-celled paraphysis which is the same height as plurilocular sporangia may be a synapomorphic character for these species (Table 2). Hence, the morphological evolution of the genus *Colpomenia* requires additional study.

Network analysis of 14 *cox*3 haplotypes of *Colpomenia claytonii* revealed five clusters, representing high haplotype diversity. It is interesting that haplotype 1 from Korea is connected to haplotype 2 from the USA, and haplotype 8, occurring from Hong Kong to Australasia and South Africa, is nested within haplotypes from Korea and Japan, without missing haplotypes. This pattern of haplotype distribution may be interpreted as a recent dispersal history of haplotypes, probably in the last glacial period, as suggested in other brown algae (Coyer et al. 2001). Again, haploype 8 may be a consequence of recent invasion because of its occurrence in these different marine ecoregions (Spalding et al. 2007). In *Colpomenia*, human-mediated introduction may be a common phenomenon. For example *C. bullosa* and *C. peregrina* have been introduced from Japan to Australasia (Parsons 1982, Kain et al. 2010) and to Europe (Fletcher 1987, Cho et al. 2005), and *C. tuberculata*, mostly occurring in southern California to Baja California (Norris 2010), has been reported recently in Korea (Lee 2008). However, we believe that the large number of missing haplotypes between haplotypes 4 and 5, haplotypes 1 and 14, and haplotypes 14 and 8 may be an artifact of sampling and that an increase of sampling effort from areas with isolated haplotypes will significantly reduce the number of steps linking the clusters and lead to a more realistic interpretation.

The present study shows that two morphologically similar species of brown algae can be easily distinguished using evidence of morphology, ecology, and *cox*3 sequences, together with the protein-coding *rbc*L gene and the nrDNA ITS region sequences. Despite its large size (compared to *C. peregrina*) and epilithic occurrence, failure to recognize *C. claytonii* has led to underestimation of brown algal diversity. The present study also demonstrates the suitability of *cox*3 for identifying haplotypes and interpreting the distribution patterns within species of scytosiphonacean algae.

Acknowledgments

We thank Il Ki Hwang and Jeong Kwang Park for assistance in morphological observations, John Bolton, Put Ang, and Rafael Riosmena-Rodriguez for help in collection trip in South Africa, Hong Kong, and Mexico, respectively, Bruno de Reviers for his hospitality during the first author's visit to Paris, Richard More for the Latin description and reading the first draft, James Coyer for discussion on haplotype network, and Margaret Clayton for reading the manuscript. This work was supported by a Korean Research Foundation (KRF) grant (MEST; 2009-0077972) and a grant from Marine Biotechnology Program funded by Ministry of Land, Transport and Maritime Affairs, Korea, and a New Zealand Foundation for Research, Science and Technology grant (CO1X0502).

References

Adams, N.M. 1994. *Seaweeds of New Zealand: an illustrated guide*. Canterbury University Press, Christchurch, New Zealand. 360 pp.

Blackler, H. 1967. The occurrence of *Colpomenia peregrina* (Sauv.) Hamel in the Mediterranean (Phaeophyta, Scytosiphonales). *Blumea 15*: 5–8.

Boo, S.M., H.S. Kim, W. Shin, G.H. Boo, S.M. Cho, B.Y. Jo, J.H. Kim, E.C. Yang, P.A. Siver, A.P. Wolfe, D. Bhattacharya, R.A. Andersen and H.S. Yoon. 2010. Complex phylogeographic patterns in the freshwater alga *Synura* provide new insights on ubiquity versus endemism in microbial eukaryotes. *Mol. Ecol. 19*: 4328–4338.

Cho, G.Y., S.M. Boo, W. Nelson and M.N. Clayton. 2005. Genealogical partitioning and phylogeography of *Colpomenia peregrina* (Scytosiphonaceae, Phaeophyceae) based on plastid *rbc*L and nuclear ribosomal DNA internal transcribed spacer sequences. *Phycologia 44*: 103–111.

Cho, G.Y., K. Kogame and S.M. Boo. 2006. Molecular phylogeny of the family Scytosiphonaceae (Phaeophyceae). *Algae 21*: 175–183.

Clayton, M.N. 1975. A study of variation in Australian species of *Colpomenia* (Phaeophyta, Scytosiphonales). *Phycologia 14*: 187–195.

Clayton, M.N. 1979. The life history and sexual reproduction of *Colpomenia peregrina* (Scytosiphonaceae, Phaeophyta) in Australia. *Br. Phycol. J. 14*: 1–10.

Clement, M.D., D. Posada and K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol. 9*: 1657–1669.

Coyer, J.A., G.J. Smith and R.A. Andersen. 2001. Evolution of *Macrocystis* spp. (Phaeophyceae) as determined by ITS1 and ITS2 sequences. *J. Phycol. 37*: 574–585.

Derbès, A.A. and A.J.J. Solier. 1851. *Algues. In*: (J.L.M. Castagne, ed), *Supplément au catalogue des plantes qui croissent naturel-*

lement aux environs de Marseille. Nicot et Pardigon, Aix. pp. 93–121.

Durairatnam, M. 1962. Some marine algae from Ceylon – 1. *Fish. Res. Station, Ceylon 15:* 3–16.

Fletcher, R.L. 1987. *Seaweeds of the British Isles. Vol. 3. Fucophyceae (Phaeophyceae). Part I.* British Museum (Natural History), London. 359 pp.

Hamel, G. 1937. *Phaeophyceae de France*. Fasc. III. Wolf, Paris. pp. 177–240.

Kain (Jones), J.M., J. Buchanan, S.M. Boo and K.M. Lee. 2010. *Colpomenia bullosa* crust masquerading as *Ralfsia verrucosa* (Phaeophyceae) in southern Australia. *Phycologia 49*: 617–627.

Kogame, K. and Y. Yamagishi. 1997. The life history and phenology of *Colpomenia peregrina* (Scytosiphonales, Phaeophyceae) from Japan. *Phycologia 36*: 337–344.

Kogame, K., T. Horiguchi and M. Masuda. 1999. Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbc*L, partial *rbc*S, and partial LSU nrDNA. *Phycologia 38*: 496–502.

Kogame, K., S. Uwai, S. Shimada and M. Masuda. 2005. A study of sexual and asexual populations of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) in Hokkaido, northern Japan, using molecular markers. *Eur. J. Phycol. 40*: 313–322.

Lawson, G.W. and D.W. John. 1987. The marine algae and coastal environment of tropical West Africa. *Beihefte Nova Hedwigia 93:* 1–415.

Lee, Y.P. 2008. *Marine algae of Jeju.* Academic Publishers, Seoul. 477 pp.

Lee, K.Y., G.H. Boo, R. Riosmena-Rhodriquez, J.A. Shin and S.M. Boo. 2009. Classification of the genus *Ishige* (Ishigeales, Phaeophyceae) in the north Pacific Ocean with recognization of *I. foliacea* based on plastid rbcL and mitochondrial *cox*3 gene sequences. *J. Phycol. 45*: 906–913.

Norris, J.N. 2010. Marine algae of the Northern Gulf of California: Chlorophyta and Phaeophyceae. *Smithson. Contrib. Bot. 94*: 1–276.

Parsons M.J. 1982. *Colpomenia* (Endlicher) Derbès et Solier (Phaeophyta) in New Zealand. *New Zealand J. Bot. 20*: 289–301.

Rambaut, A. 2002. *SeAl, v. 2.0*. Institute of Evolutionary Biology, Ashworth Laboratories, Edinburgh.

Ramìrez, M.E. and V.G. Rojas. 1991. El genero *Colpomenia* (F.C. Mertens ex Roth) Derbès et Solier (Phaeophyceae), en Chile. *Bol. Museo Nacional Hist. Nat. Chile 42*: 11–24.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics 19*: 1572–1574.

Saunders, De A. 1898. Phycological memoirs. *Proc. Calif. Acad. Sci. Ser. 3. Bot. 1*: 147–168.

Scagel, R.F., P.W. Gabrielson, D.J. Garbary, L. Golden, M.W. Hawkes, S.C. Lindstrom, J.C. Oliviera and T.B. Widdowson. 1989. *A synopsis of the benthic marine algae of British Columbia, southeast Alaska, Washington and Oregon.* Phycological contribution no. 3. Department of Botany, University of British Columbia, British Columbia, Canada. 532 pp.

Spalding, M.D., H.E. Fox, G.R. Allen, N. Davidson, Z.A. Ferdana, M. Finlayson, B.S. Halpern, M.A. Jorge, A. Lombana, S.A. Lourie, K.D. Martin, E. McManus, J. Molnar, C.A. Recchia and J. Robertson. 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience 57*: 573–583.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics 22*: 2688–2690.

Swofford, D.L. 2002. *PAUP*: phylogenetic analysis using aarsimony (*and other methods).* Verison 4.0b10. Sinauer Associates, Saunderland, MA.

Taylor, W.R. 1945. Pacific marine algae of the Allan Hancock expeditions to the Galapagos Islands. *Allan Hancock Pacif. Exped. 12*: 1–528.

Uwai, S., W.A. Nelson, K. Neill, W.D. Wang, L.E. Aguilar-Rosas, S.M. Boo, T. Kitayama and H. Kawai. 2006. Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondrial genes – origin and succession of introduced populations. *Phycologia 46*: 687–695.

Womersley, H.B.S. 1987. *The marine benthic flora of southern Australia. Part II.* South Australian Government Printing Division, Adelaide. 484 pp.

Wynne, M.J. and J.N. Norris. 1976. The genus *Colpomenia* Derbès et Solier (Phaeophyta) in the Gulf of California. *Smithson. Contrib. Bot. 35*: 1–18.

Yoshida, T. 1998. *Marine algae of Japan*. Uchida Rokakuho Publ. Ltd., Tokyo. 1222 pp.

Received 4 November, 2010; accepted 3 January, 2011; online first 1 April, 2011