

Colpomenia durvillei (Scytosiphonaceae, Phaeophyceae): its distribution and relationships with other elongate species of the genus

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Abstract

The taxonomic distinctiveness and distribution of the brown alga *Colpomenia durvillei* are debatable; the species was first described from central Chile. We analyzed mitochondrial *cox3* and plastid *rbcL* sequences from specimens collected in central Chile and Sonora, Mexico, in combination with morphological observations of specimens collected in Chile, Peru, and Mexico. Compared with other elongate species of the genus, *C. durvillei* is distinguished by erect, elongate thalli arising from a wide colpomenioid base, up to eight layers of cortical and medullary cells, and plurilocular sporangia with many layers (up to 18 locules). In all phylogenetic analyses of *cox3* and *rbcL* sequences, all elongate species of the genus formed a clade, in which *C. durvillei* was consistently distinct from congeners. *Colpomenia bullosa* was closely related to *C. durvillei* in our *cox3* analyses, and the clade containing these two species was closely related to *Colpomenia phaeodactyla*. This is the first report to confirm *C. durvillei* by molecular data and also the first report on the occurrence of the species in Sonora, Mexico. It had previously been misidentified as a variant of *C. phaeodactyla* on the Pacific coast of South America, and the name *C. durvillei* was misapplied to *C. bullosa* in New Zealand.

Keywords: brown algae; *Colpomenia*; *cox3*; distribution; Ectocarpales; *rbcL*; systematics.

Introduction

Recent research on the family Scytosiphonaceae has revealed significant complexity in the phylogenetic relationships of genera and species, with paraphyly demonstrated in the genera *Colpomenia* (Endl.) Derbès et Solier, *Petalonia* Derbès et Solier, and *Scytosiphon* C. Agardh (Kogame et al. 1999,

Cho et al. 2006). Within the genus *Colpomenia*, there are two morphological groups, viz., (1) globose *Colpomenia*, which produce plurilocular and unilocular zoidangia on crustose sporophytes, and (2) elongate or tubular thalli, which produce only unilocular zoidangia as reproductive organs on sporophytes (Kogame et al. 1999, Boo et al. 2011a). Cho et al. (2006) pointed to the need for “an urgent revision of the family at species and genus level.”

Species belonging to the genus *Colpomenia* are found around the planet across a wide latitudinal range. Members of this genus are characterized by hollow, crisp thalli that are globular to convolute to elongate-ovoid in shape. There are currently twelve species recognized (Boo et al. 2011a, Guiry and Guiry 2012), including three species with an elongate, finger-like morphology, i.e., *Colpomenia bullosa* (D.A. Saunders) Yamada (type locality Pacific Grove, California, USA; Yamada 1948), *Colpomenia durvillei* (Bory de Saint-Vincent) M.E. Ramírez (type locality Concepcion, Chile; Ramírez and Rojas 1991), and *Colpomenia phaeodactyla* M.J. Wynne et J.N. Norris (type locality Puerto Peñasco, Sonora, Mexico; Wynne and Norris 1976).

The history and distinguishing features of *C. durvillei* were reviewed by Ramírez and Rojas (1991) when they established the combination (previously known as *Asperococcus durvillei* Bory de Saint-Vincent) and by Wynne (1999). The reported occurrence of *C. bullosa* in central Chile (Alveal 1970) and *C. phaeodactyla* in central Chile and Peru (Santelices et al. 1989, Hoffmann and Santelices 1997, Norris 2010) may be a result of misidentification of *C. durvillei*. The distribution of *C. durvillei* was thought to be very limited, extending from Peru to central Chile (Acleto 1973, Ramírez and Rojas 1991). In addition, it has been rarely reported in these areas since the morphological study of the species by Ramírez and Rojas (1991).

In 1982, Parsons reported the occurrence of an elongate *Colpomenia*, *C. bullosa*, for the first time in New Zealand waters. Parsons (1982) selected a lectotype for this species (from Pacific Grove, CA, USA) and suggested that the appearance of this species was an example of a human-mediated introduction, saying “this alga might have arrived in New Zealand associated with ships from either Japan or North America as the locality is not far from shipping lanes.” Adams (1994) re-determined the New Zealand specimens to be the Chilean species *C. durvillei*, considering that *C. bullosa* was a later name for this elongate species.

Our study was undertaken to clarify the identity of *C. durvillei*, characterizing specimens from the type region and comparing these with *C. bullosa*. Do these species differ? What are the relationships of the specimens found in New Zealand

Table 1 Specimens and *cox3* and *rbcL* sequences of the taxa included in this study.

Species, collection sites, and date	Voucher	GenBank accession no.	
		<i>cox3</i>	<i>rbcL</i>
<i>Colpomenia bullosa</i> (D.A. Saunders) Yamada			
Mukri, Chujado, Korea, 26 Apr. 2005		HQ833770	–
Mukri, Chujado, Korea, 26 Apr. 2005	PE478	JQ918792	–
Hakodate, Hokkaido, Japan, 8 Apr. 2004	PE297	JQ918791	–
Hakodate, Hokkaido, Japan, 27 Apr. 2005	PE503	JQ918790	–
Muroran, Hokkaido, Japan, 15 Feb. 1991		–	AB022236
Agate Beach, California, USA, 25 Feb. 2008	bB8	JQ918793	–
Agate Beach, California, USA, 25 Feb. 2008	CNU4104	JQ918796	–
Alaska, USA, collection date not given	SCL14083	JQ918794	–
San Juan Island, Seattle, USA, 10 Feb. 2010	CNU6605	JQ918795	–
Cattage, Melbourne, Australia, 18 Dec. 2010	CNU7360	JQ918797	–
Kainga Reef, Burrewarra Point, Australia, 1 Jan. 2009		HQ833769	GU014704
Kainga Reef, Burrewarra Point, Australia, 1 Jan. 2009		–	GU014705
Kainga Reef, Burrewarra Point, Australia, 1 Jan. 2009		–	GU014706
Whangarei Harbor, North Island, New Zealand, 3 Nov. 2009	WELT A030995	JQ918799	–
Wilson Bay, North Island, New Zealand, 10 Apr. 2006	WELT A028490	JQ918798	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-1	JQ918800	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-2	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-3	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-4	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-5	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-6	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-7	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-8	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-9	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-10	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-11	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-12	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-13	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-14	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-15	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-16	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-17	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-18	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-19	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-20	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-21	Same as above	–
Kaikoura, Canterbury, New Zealand, Jan. 2011	ASL063-22	Same as above	–
<i>Colpomenia claytoniae</i> S.M. Boo, K.M. Lee, G.Y. Cho et W. Nelson			
Dolsando, Yeosu, Korea, 24 Jun. 2007	PC1	HQ833788	–
Anin, Gangreung, Korea, 12 Jan. 2002	PE018	–	AY398435
<i>Colpomenia durvillei</i> (Bory de Saint-Vincent) M.E. Ramírez			
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12559	JQ918806	JQ918820
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12566	JQ918808	JQ918821
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12567	JQ918807	–
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12570	JQ918802	JQ918818
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12573	JQ918809	JQ918822
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12578	–	JQ918823
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12579	JQ918803	–
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12595	JQ918804	JQ918819
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12596	JQ918805	–
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12598	JQ918810	JQ918824
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12602	JQ918811	–
Los Molles, Coquimbo, Chile, 28 Oct. 2011	CNU12603	JQ918812	–
Las Cuevas, Sonora, Mexico, 31 Mar. 2009	PE2545	JQ918801	JQ918825
El Quisco, Valparaiso, Chile, Jul. 2007		GU252653	–
El Quisco, Valparaiso, Chile, Jul. 2007		GU252661	–
Las Cruces, Valparaiso, Chile, Jul. 2007		GU252658	–

(Table 1 continued)

Species, collection sites, and date	Voucher	GenBank accession no.	
		<i>cox3</i>	<i>rbcL</i>
Las Cruces, Valparaiso, Chile, Jul. 2007		GU252654	–
Las Cruces, Valparaiso, Chile, Jul. 2007		GU252652	–
Coquimbo, Chile, Jul. 2007		GU252663	–
Punta Choros, Coquimbo, Chile, Jul. 2007		GU252655	–
Punta Choros, Coquimbo, Chile, Jul. 2007		GU252662	–
Playa Los Verdes, Tarapacá Region, Chile, Aug. 2007		GU252657	–
Arica, Arica and Parinacota Region, Chile, Aug. 2007		GU252656	–
<i>Colpomenia ecuticulata</i> M.J. Parsons			
Marsden Point, Whangarei Harbour, New Zealand, 5 Nov. 2009	WELT A030993	HQ833776	–
<i>Colpomenia expansa</i> (D.A. Saunders) Y.P. Lee			
Mukri, Chujado, Korea, 24 May 2009	PE1466	HQ833781	JQ918816
<i>Colpomenia peregrina</i> Sauvageau			
Sacheon, Gangreung, Korea, 23 Feb. 1999		HQ833767	–
Monterey Bay, California, USA, 11 Dec. 1999		–	AY398464
<i>Colpomenia phaodactyla</i> M.J. Wynne et J.N. Norris			
Sangjokam, Goseong, Korea, 12 Jan. 2005		HQ833772	–
Sangjokam, Goseong, Korea, 21 Jan. 2011	CNU3	JQ918814	–
Sangjokam, Goseong, Korea, 21 Jan. 2011	CNU4	JQ918815	–
Hoedong, Jindo, Korea, 9 Mar. 2001		–	AY398467
Nagasaki, Kyushu, Japan, 26 Feb. 2005		HQ833771	–
Nagasaki, Kyushu, Japan, 26 Feb. 2005	PE819	JQ918813	–
Tsuyazaki, Fukuoka, Japan, 3 May 1989		–	AB022237
<i>Colpomenia sinuosa</i> (Mertens ex Roth) Derbès et Solier			
Sacheon, Gangreung, Korea, 25 Jun. 2009		HQ833777	–
Castillo san Cristobal, Gran Canaria, Spain, 25 Apr. 2004		–	AY875710
<i>Colpomenia tuberculata</i> D.A. Saunders			
El Sargento, Baja California, Mexico, 11 May 2009	PE1512	HQ833773	JQ918817
<i>Colpomenia ramosa</i> W.R. Taylor			
Punta La Esmeralda, Baja California, Mexico, 2 Dec. 2006	CNU7218	JQ918789	–
Outgroups			
<i>Petalonia fascia</i> (O.F. Müller) Kuntze			
Munseom, Jejudo, Korea, 15 Jan. 1997		HQ833766	–
<i>Scytosiphon lomentaria</i> (Lyngbye) Link			
Sormsangi, Chujado, Korea, 23 May 2005		HQ833765	–
Oshoro, Hokkaido, Japan, 1 May 1989		–	AB022238

to the specimens collected from the native ranges of *C. bullosa* and *C. durvillei*, and to the species known as *C. phaodactyla* from Korea and Japan? In the present study, we investigated morphology and phylogenetic relationships of *C. durvillei* based on mitochondrial *cox3* and plastid *rbcL* sequences, and compared these with those of other species within the genus.

Materials and methods

Sampling and morphological observations

Thalli of *Colpomenia durvillei* were collected from intertidal zones in Chile and Mexico. 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-801; Yamato Kohki Industrial Co. Ltd., Saitama, Japan). Photographs were taken with a DP-71 camera (Olympus, Tokyo, Japan) attached to a microscope (BX 51; Olympus). Voucher specimens

were deposited at the herbarium in the Chungnam National University, Daejeon, Korea. We also observed 13 herbarium specimens of *C. durvillei* deposited in the Natural History Museum of San Carlos University in Lima, Peru: three specimens collected on July 1973 at Chala, Camana, were mounted on a sheet with a label of Acleto 1777, and 10 specimens collected on August 1978 at Lagunillas, Pisco, were mounted on three sheets with a label marked Acleto 1924.

DNA extraction and analysis

Specimens used for the molecular study are summarized in Table 1. DNA extraction, PCR amplification, and sequencing followed Boo et al. (2011a). Primer pairs for the amplification and sequencing of each gene were as follows: for *cox3*, F49–R20 (Boo et al. 2010, 2011b); for *rbcL*, RLrbcLF (5′-CCA CGG CCT TAC CAC TTG GC-3′)–RL960R (5′-GAC GNG CAT ATG TWG ART THC C-3′) and RL814F (5′-CGA TTT AGT TRT YGG TTA YAC WG-3′)–RLrbcSR (5′-GYT CAT CNS TTA ART CTG G-3′).

We newly updated 48 *cox3* and 10 *rbcL* sequences in the GenBank database of the genus *Colpomenia* during the present study. Including published data from *Colpomenia* and, as outgroups, *Petalonia fascia* (O.F. Müller) Kuntze and *Scytosiphon lomentaria* (Lyngbye) Link, a total of 70 *cox3* and 20 *rbcL* sequences were collated using Se-AL v.2.0a11 (Rambaut 2002) and aligned visually.

Maximum likelihood (ML) phylogenetic analyses were performed with RAxML software (Stamatakis 2006) using the GTR+C model. We used 200 independent tree inferences with the “number of run” option, with default optimized 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.

Maximum parsimony (MP) trees were constructed for each data set with PAUP* v.4.0b.10 (Swofford 2002) using a heuristic search algorithm with the following settings: 1000 random sequence additions, tree bisection-reconnection (TBR) branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed. Bootstrap values for the resulting nodes were assessed using 1000 bootstrapping replicates with 10 random sequence additions, TBR, and MulTrees.

A statistical parsimony network of *cox3* haplotypes was created using TCS v. 1.21 software (Clement et al. 2000). Haplotype and nucleotide diversity measurements were performed using DnaSP software v.5 (Librado and Rozas 2009).

Results

Morphological observation

Specimens of *Colpomenia durvillei* from central Chile and Peru were consistent in their habit and structure of cortex and medulla, and plurilocular sporangia, although Mexican specimens were very small and unsuitable for morphological observations.

A colpomenioid-base thallus (Figure 1A) measures 2–5 mm in thickness and about 18 mm in diameter. Erect thalli (Figure 1B–C) are oval to fusiform or sac, occasionally divided, hollow, and up to 16 sacs arise from each colpomenioid base. Sacs are about 4.4 cm long and about 0.8 cm wide. Cortical cells on the surface of thalli are polygonal and irregularly arranged (Figure 1D). In cross section, the thallus measures 48–128 µm thick and consists of one layer of angular cells of 7–8 µm in diameter, two layers of round subcortical cells 13–54 µm in diameter, and five layers of cuboidal and colorless medullary cells that are larger and more variable in size than the subcortical layer (Figure 1E–F). Phaeophyceyan hairs are occasionally present, immersed in pits; each hair filament arises from an outer cortical cell (Figure 1G). Paraphyses are 34–46 µm long and 5–8 µm in width, club-shaped, up to two-celled, of the same height or longer than plurilocular sporangia. Plurilocular sporangia (Figure 1H) are in continuous or extensive sori, distributed across the surface of the thallus, uni- and biserial, 31–41 µm long, and 4–5 µm in diameter.

Molecular analyses

All 70 *cox3* sequences including 22 from *Colpomenia durvillei* (12 from Chile and Mexico in the present study and 10 in GenBank under the name of *Colpomenia bullosa* from Chile; see Table 1); 34 from *C. bullosa* from Korea, Japan, USA, Australia, and New Zealand; and five *Colpomenia phaeodactyla* from Korea and Japan were aligned with a size of 456 bp. Variable sites occurred at 169 positions (37.1%), and 129 positions (28.3%) were parsimoniously informative. *Colpomenia durvillei* from Chile and Mexico differed by 35–40 bp (7.90–8.77%) from *C. phaeodactyla* from the north-west Pacific and by 26–33 bp (5.70–7.24%) from *C. bullosa*. There was a 38–43 bp (8.33–9.21%) difference between *C. phaeodactyla* and *C. bullosa*. Within *C. durvillei*, the pairwise divergence was in a range of up to 14 bp (0–3.07%). In the *cox3* tree (Figure 2), *C. durvillei* formed a monophyletic group with strong support values (100% for MP and ML), and, within the species, there were two separated sub-clades. *Colpomenia durvillei* was sister to *C. bullosa*, and the clade of *C. durvillei* and *C. bullosa* clustered with *C. phaeodactyla*. Each of the remaining seven species of the genus was consistently distinct in the *cox3* tree.

A 1084-nucleotide portion of the *rbcL* gene was compared across 19 sequences from 12 species of *Colpomenia* and outgroups. Variable sites occurred at 96 positions (8.9%), and 51 positions (4.7%) were parsimoniously informative. *C. durvillei* differed by 4–5 bp (0.37–0.46%) from *C. phaeodactyla* and by 2–3 bp (0.19–0.28%) from *C. bullosa*. There was a difference of 4 bp (0.37%) between *C. phaeodactyla* and *C. bullosa*. The *rbcL* sequences of *C. durvillei* were identical, with a minor difference of up to 2 bp (0.19%). In the *rbcL* tree (Figure 3), *C. durvillei*, *C. bullosa*, and *C. phaeodactyla* formed a clade, but there was no resolution within this clade.

Discussion

Taxonomy of *Colpomenia durvillei*

Specimens of *Colpomenia durvillei* from central Chile and Peru corresponded in their habit and reproductive structures to the description of Ramírez and Rojas (1991). *Colpomenia durvillei* is distinguished by erect, elongate thalli arising from a wide colpomenioid base (up to 3 cm in width, Ramírez and Rojas 1991), up to eight layers of cortical and medullary cells, and many layers (up to 18 locules) of plurilocular sporangia. Sacs in our specimens were very small (about 4.4 cm long and 0.8 cm wide), but large sacs are reported to reach up to 15 cm in length and 0.8 cm in width (Ramírez and Rojas 1991). However, crusts bearing unilocular sporangia were not collected, and it is not known whether they occur in other seasons and/or in other places. In *Colpomenia*, the plurilocular-sporangial thallus is the macroscopic gametophyte, which is morphologically distinct from the crustose sporophytes bearing unilocular sporangia (Clayton 1979, Kogame and Yamagishi 1997). *C. durvillei* is separable from the other elongate species of the genus, *Colpomenia*

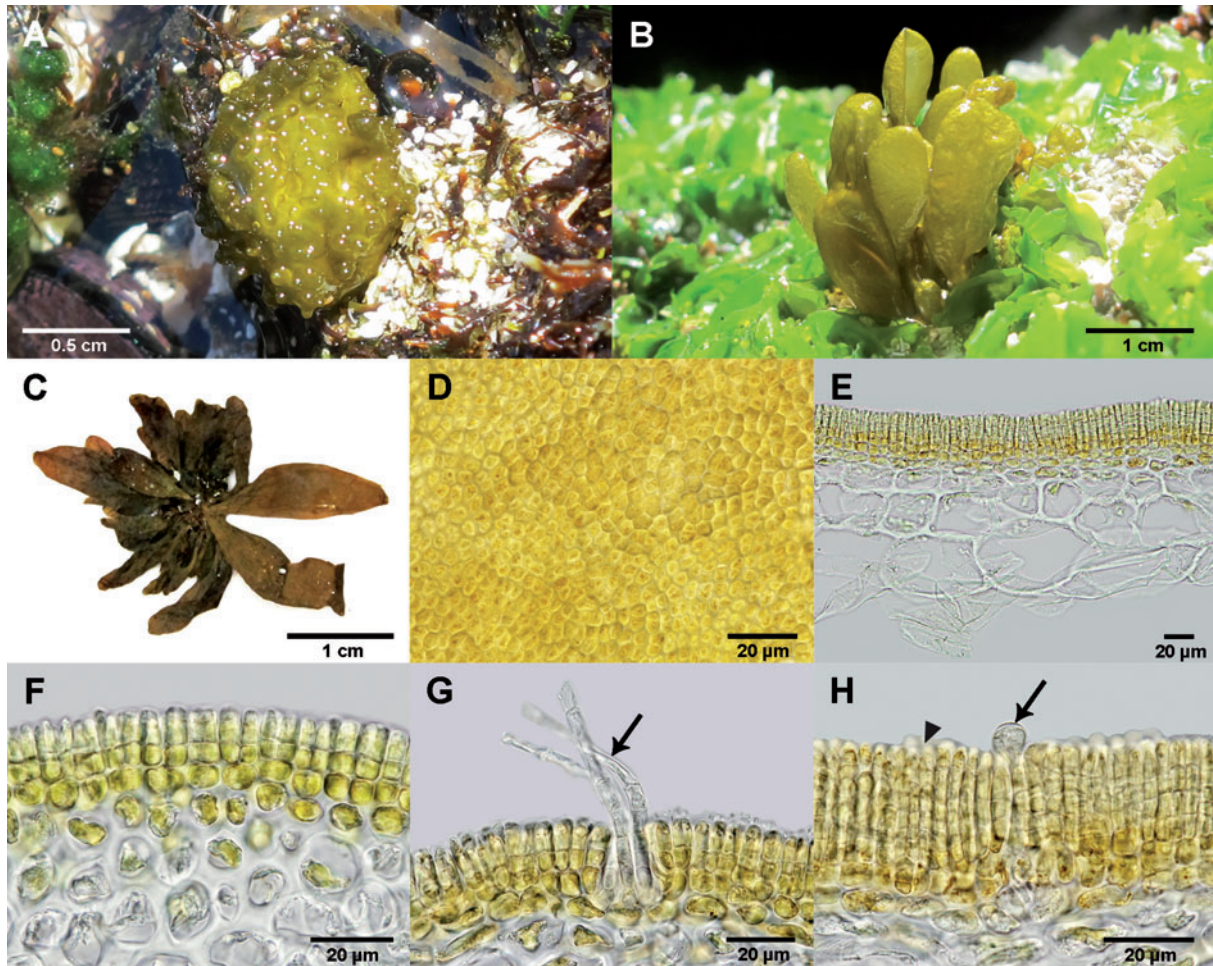


Figure 1 *Colpomenia durvillei*. (A) A colpomenioid base in Los Molles, Coquimbo, Chile (October 28, 2011). (B) Erect thalli arising from a crustose base at Los Molles, Coquimbo, Chile. (C) A herbarium specimen collected at Lagunillas, Pisco, Peru, on August 18, 1978, by Cesar Acleto, deposited in the Natural History Museum of San Carlos University at Lima, Peru. (D) Surface view of B. (E) Cross section of thallus with plurilocular sporangia. (F) Cortical layer. (G) Phaeophyceyan hairs (arrow) arising from outer cortical cells. (H) Plurilocular sporangia (arrowhead) and paraphyses (arrow).

bullosa and *Colpomenia phaeodactyla*, in morphology as seen in Table 2, and by interspecific divergences in the *cox3* (5.7–7.2% divergence from *C. bullosa* and 7.9–8.8% divergence from *C. phaeodactyla*). Although the specimens of *C. durvillei* were not clustered into a clade in *rbcL* tree, the pairwise divergences were 0.2–0.3% from *C. bullosa* and 0.4–0.5% from *C. phaeodactyla* sequences, respectively.

The reported occurrence of *C. phaeodactyla* in central Chile and Peru (Santelices et al. 1989, Hoffmann and Santelices 1997, Norris 2010) and *C. bullosa* in central Chile (Alveal 1970) may be a result of misidentification of *C. durvillei*. Based on material of *C. phaeodactyla* from Puerto Peñasco, Sonora, Mexico, the type locality of the species, Ramírez and Rojas (1991) reported that *C. phaeodactyla* has abundant paraphyses and hairs on plurilocular reproductive tissue, whereas these structures are almost entirely absent or occur only occasionally in *C. durvillei*. In addition, *C. phaeodactyla* is distinguished by cylindrical thalli consisting of clusters of long, hollow sacs arising from an adherent base,

with flaccid and thin thallus walls four to five cell layers thick (Wynne and Norris 1976, Yoshida 1998, Oak et al. 2002). Compared to the smooth and entire habit of sacs of specimens from Mexico (Wynne and Norris 1976), Korean and Japanese thalli when mature are occasionally contorted with adventitious branchlets. In our study, both *cox3* and *rbcL* sequences for *C. phaeodactyla* were from Korea and Japan, part of the distributional range described for this species by Wynne and Norris (1976).

Our finding of *C. durvillei* in Las Cuevas, Sonora, Mexico, close to the type locality of *C. phaeodactyla*, indicates that either these species may have an overlapping distribution or that *C. phaeodactyla* is conspecific with *C. durvillei*, making this a highly morphologically and anatomically variable species. If these species are conspecific, taxonomic priority rests with *C. durvillei*, described by Bory de Saint-Vincent (as *Asperococcus durvillei*) in 1827–1829 rather than *C. phaeodactyla* described by Wynne and Norris in 1976. The elongate species from the northwestern Pacific, identified here as

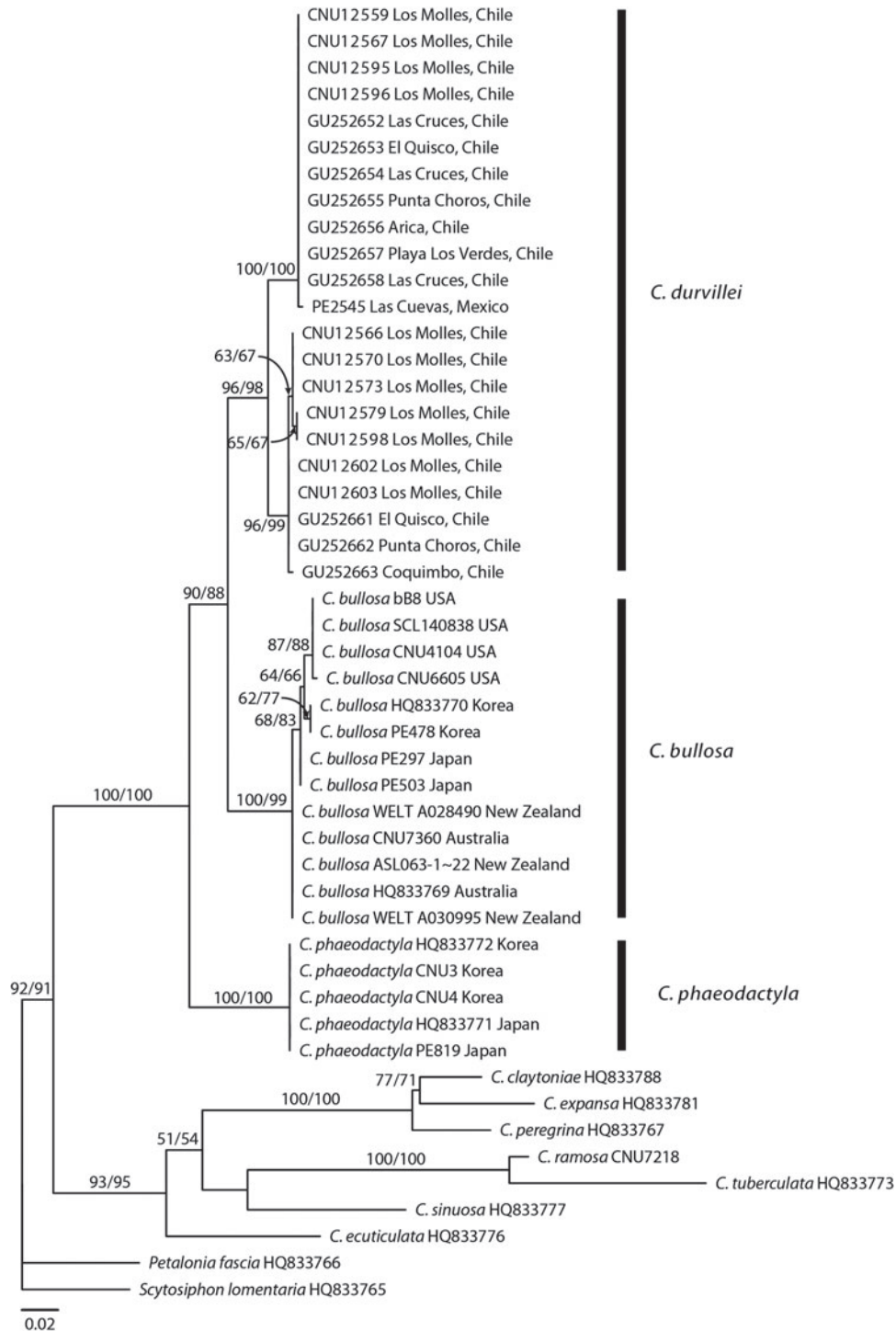


Figure 2 Maximum likelihood tree derived from analysis of *cox3* sequences in the genus *Colpomenia* (*C.*) and putative relatives using the GTR+C evolution model [-ln L=3067.73; base frequencies $\pi_A=0.208483$, $\pi_C=0.160038$, $\pi_G=0.205791$, $\pi_T=0.425688$; shape parameter (α)=0.251180]. The numbers above or near the branches are bootstrap values from the MP and ML.

C. phaeodactyla, is consistent with the description by Wynne and Norris (1976) and clearly distinct morphologically from *C. durvillei*, based on material we examined from Chile, Peru, and Mexico. Further study of elongate *Colpomenia* in the type region of *C. phaeodactyla* is clearly required, but is beyond

the present report. However, if future research establishes that *C. durvillei* and *C. phaeodactyla* from Puerto Peñasco, Sonora (and surroundings), Mexico, are conspecific, the elongate *Colpomenia* from the northwest Pacific would require a new name.

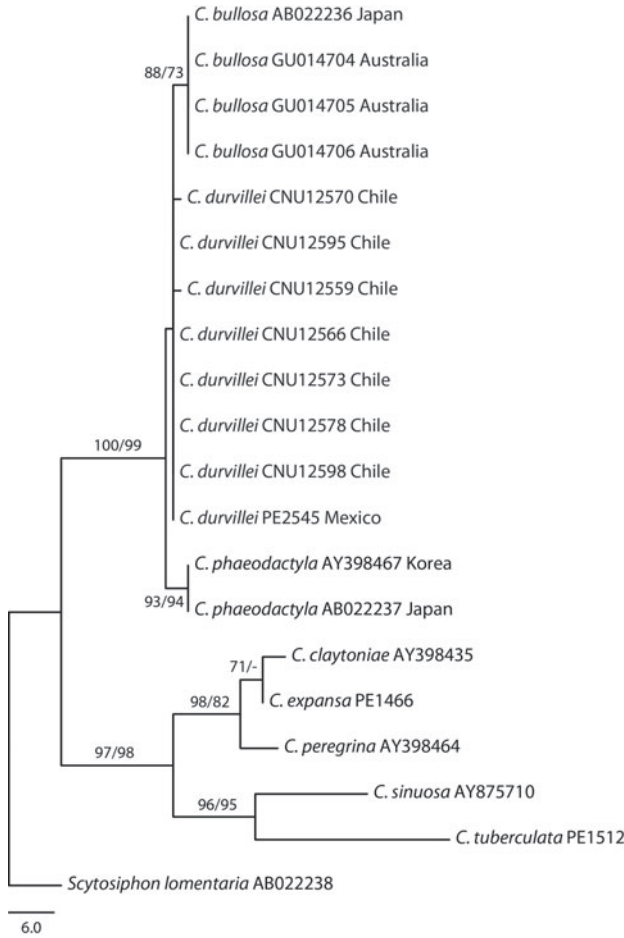


Figure 3 Maximum likelihood tree derived from analysis of *rbcL* sequences in the genus *Colpomenia* (*C.*) and putative relatives using the GTR+C evolution model [-ln L=2360.06; base frequencies $\pi A=0.293404$, $\pi C=0.161900$, $\pi G=0.218404$, $\pi T=0.326292$; shape parameter (α)=0.010014]. The numbers above or below the branches are bootstrap values from the MP and ML.

The occurrence of *Colpomenia durvillei* in New Zealand reported by Adams (1994) is not supported by *cox3* and *rbcL* markers in the present study or by ITS in the study of Cho et al. (2005). Instead, all elongate *Colpomenia* from New Zealand are confirmed to be *C. bullosa* by *cox3* in the present data, in agreement with ITS data of Cho et al. (2005). All *cox3*, *rbcL*, and ITS data support the report by Parsons (1982) that *C. bullosa* occurs in New Zealand. The New Zealand specimens showed the typical characters of *C. bullosa* such as solitary hollow thalli and firmer texture of the balloon-like sacs (Yamada 1948, Wynne and Norris 1976, Kain et al. 2010). From our current findings we conclude that *C. durvillei* is a naturally distinct species, and its occurrence in New Zealand is not supported by our study.

Distribution of *Colpomenia durvillei*

Although our collection of *Colpomenia durvillei* in central Chile is limited to Los Molles, we found that 11 *cox3* sequences

Table 2 Morphological comparisons of elongate species of *Colpomenia*.

	<i>C. bullosa</i>	<i>C. durvillei</i>	<i>C. phaodactyla</i>
Thallus shape	Finger-like, branched at the base	Finger-like, branched at the colpomenioid base of up to 3 cm in diameter	Finger-like, arising from a colpomenioid base of up to 2 cm in diameter, contorted sacs with adventitious branchlets
Thallus size	Usually up to 30 cm in length, 3 cm in width	Up to 15 cm in length, 0.8 cm in width	Up to 25 cm tall, about 2.5 cm in width
Cortex	2–3 layers of angular cells	1–3 layers of angular cells	A single layer of small cells
Medullary structure	3–5 layers of cuboidal cells	About 5 layers of cuboidal cells	2–3 layers of cuboidal cells
Paraphyses	1–2 celled, same height or longer than plurilocular sporangia	Rare, same height or longer than plurilocular sporangia	Rare, shorter or same height as plurilocular sporangia
Phaeophyceae hairs	Hair pits scattered	Occur occasionally	Arising from cortical cells
Plurilocular sporangia	Uni- to biseriolate, 12–15 locules	Uni- to biseriolate, about 18 locules	Uni- and biseriolate, 6–8 locules
Habitat	Epilithic, intertidal	Epilithic, intertidal, semiexposed	Epilithic, mid to lower intertidal
Type locality	Pacific Grove, CA, USA	Concepcion, Chile	Puerto Peñasco, Sonora, Mexico
Geographical distribution	Temperate waters of the Pacific	Sonora, Mexico Central to northern Chile	Temperate waters of the Pacific Ocean
References	Saunders (1898) This study	Ramírez and Rojas (1991) This study	Wynne and Norris (1976) This study

(GU252652–9, GU252661–3) registered under the name of *Colpomenia bullosa* are not comparable to those of the species but belong to *C. durvillei*. These samples are from five different locations in central Chile: Arica, Playa Los Verdes, Punta Choros, El Quisco, and Las Cruces. *Colpomenia durvillei* is thus now confirmed to occur in six different locations in central Chile, implying its common occurrence in central Chile, contrary to current knowledge. According to Acleto (1973) and Ramírez and Rojas (1991), *C. durvillei* commonly grows attached to rocks, gravel, and other hard substrata in the low intertidal of exposed and semiexposed areas in central Chile and Peru. This is the first report to confirm the distinctiveness of *C. durvillei* using molecular data and the first report on the occurrence of the species in Sonora, Mexico. Given the finding of this species in Sonora, Mexico, more detailed observations of field-collected material are needed to evaluate the distribution of *C. durvillei* on the Pacific coast of Latin America.

Despite its unambiguous occurrence in central Chile, Peru, and Sonora, Mexico, *C. durvillei* has not been reported in its range since its description by Ramírez and Rojas (1991). However, it appears that *C. durvillei* has been misidentified as *C. bullosa* in Chile (e.g., identifications of specimens used for sequences deposited in GenBank).

Our *cox3* and *rbcL* data provide evidence of the distribution of *Colpomenia durvillei* in central Chile and Sonora, Mexico, showing a discontinuous distribution pattern. We confirmed its occurrence in Peru based on observations of herbarium specimens in the Natural History Museum of San Carlos University in Lima, Peru. There are several different possible explanations for the discontinuous distribution of *C. durvillei* between Sonora, Mexico, and central Chile. The disjunct distribution of *C. durvillei* could be the result of extinction in the tropical area, i.e., that it was more widely distributed previously and then unable to survive in part of its range. An alternate scenario is that the distribution of *C. durvillei* in these two regions is the result of anthropogenic transportation between central Chile and Sonora, Mexico. This species may be also highly seasonal, and, as a consequence, under-represented in collections. Based on its occurrence mostly during September to October and disappearance after the austral summer (Santelices et al. 1989), *C. durvillei* may be a winter species. Our collection of *C. durvillei* was in October in central Chile. Although we failed to collect the species in February 2012 in Peru, the Peruvian specimens deposited in the Natural History Museum of San Marcos and University in Lima, Peru, had been collected in July 1973 and August 1978, respectively.

Although we did not find the identical *cox3* haplotype present in both Sonora, Mexico, and central Chile, at least 11 missing haplotypes were found in a haplotype network (not shown). This result indicates low taxon sampling and a high genetic diversity of the species, likely suggesting a wide distribution of the species. Further sampling of *Colpomenia* both geographically and seasonally is needed to establish a more complete picture of *C. durvillei* in the eastern Pacific Ocean from Mexico to central Chile.

The distribution of *C. phaeodactyla* is now reported to be limited to California, Baja California, and Costa Rica in the

eastern Pacific, and in Korea and Japan in the western Pacific (Chihara 1975, 2002, Wynne and Norris 1976, Oak et al. 2002, Norris 2010). Our molecular study has confirmed the occurrence of the species in Korea and Japan. However, we could not find any samples of *C. phaeodactyla* in California, USA, or Baja California. Based on personal communications with Chihara, Wynne and Norris (1976) reported that *C. phaeodactyla* occurs in southern and central Japan, whereas *C. bullosa* occurs in northern Japan. According to our observations, *C. phaeodactyla* predominates in February to March in the intertidal areas mostly on the southwestern coast, Korea.

Our *cox3* and *rbcL* data have confirmed the occurrence of *C. bullosa* in Korea, Japan, Australia, New Zealand, Alaska, Seattle, and California. This is supported by previous ITS data by Cho et al. (2006). It is therefore concluded that *C. bullosa* may be more widespread on both sides of the North Pacific Ocean region. *C. bullosa* also occurs in temperate waters of Australasia. In a recent study, Kain et al. (2010) established the presence of the crustose phase of *C. bullosa* in eastern Australia, previously mistakenly identified as *Ralfsia verrucosa* (Areschoug) Areschoug.

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