



Species diversity and distribution of the genus *Colpomenia* (Scytosiphonaceae, Phaeophyceae) along the coast of China

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The marine brown algal genus *Colpomenia* has a worldwide distribution, with five species reported in Korea and Japan. However, no studies to date attempted to identify the number of species and geographical distribution of *Colpomenia* along Chinese coast. To fill the biodiversity knowledge gap, we analyzed 63 mitochondrial *cox3* and 62 mitochondrial *atp6* sequences of *Colpomenia* specimens collected from 30 localities along the Chinese coast. Maximum likelihood and Bayesian inference trees suggest the presence of at least three *Colpomenia* species (i.e., *C. peregrina*, *C. claytoniae*, and *C. sinuosa*) in China. *C. peregrina* and *C. claytoniae* are documented for the first time. *C. sinuosa* was only found in the South China Sea and its distribution didn't overlap with that of *C. peregrina* which was found in the Yellow-Bohai Sea and the East China Sea. *C. claytoniae* appears to be confined to three isolated islands in the East and the South China Sea, where it occurs in sympatry with, respectively, *C. peregrina* and *C. sinuosa*. Future study can focus on comparing eco-physiological differences of *Colpomenia* species in response to environmental variables and exploring possible genetic hybridization / introgression at inter-specific contact zones.

Key Words: distribution range; genetic variation; new record species; phylogenetic diversity

INTRODUCTION

Colpomenia (Endlicher) Derbès et Solier (Scytosiphonaceae, Phaeophyceae) is a cosmopolitan brown algal genus frequently found attached to rock with gentle slopes or attached to other marine macroalgae at middle

or lower intertidal zone. Due to developmental variation, *Colpomenia* has been traditionally divided into two morphological groups: (1) globular species producing plurilocular and unilocular zoidangia on crustose spo-



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rophytes, and (2) species with elongate or tubular thalli producing only unilocular zoidangia on sporophytes (Kogame et al. 1999, Boo et al. 2011, Lee et al. 2012). However, Santiañez et al. (2018) recently removed the tubular species [i.e., *Colpomenia bullosa* (D. A. Saunders) Yamada, *Colpomenia durvillei* (Bory) M. E. Ramírez, and *Colpomenia wynnei* K. M. Lee, R. Riosmena-Rodriguez, K. Kogame et S. M. Boo] from *Colpomenia* and proposed them as a new genus *Dactylosiphon* Santiañez, K. M. Lee, S. M. Boo et Kogame based on combined mitochondrial and chloroplast phylogenies.

The globular *Colpomenia* species usually contain oxygen generated by photosynthesis, making them buoyant. When oysters are the substrate, they can become buoyant and drift with the current, earning members of the globular species the moniker “oyster thieves” (Blackler 1967, Vandermeulen 1984). Adding to such mischief caused by the globular species, floating thalli can travel long distances and spread *Colpomenia* species in oyster mariculture (Lee et al. 2014a). Therefore, the behavior and mobility of globular *Colpomenia* species make them ideal for studying how maritime transportation affects biodiversity trends, population genetic structure and biogeographical patterns of seaweeds.

Ten *Colpomenia* species have been recognized (Guiry and Guiry 2019). Five species have been recorded from the coasts of Korea and Japan, i.e., *C. claytoniae* S. M. Boo, K. M. Lee, G. Y. Cho et W. Nelson (Boo et al. 2011), *C. ecuticulata* M. J. Parsons (Lee and Kang 2001), *C. expansa* (D. A. Saunders) Y. -P. Lee (Lee 2008, Lee et al. 2012), *C. peregrina* Sauvageau (Kogame et al. 1999, Boo 2010), and *C. sinuosa* (Mertens ex Roth) Derbès et Solier (Boo 2010, Yoshida et al. 2015). These studies indicate that *Colpomenia* species diversity in the northwest Pacific is rich. *C. sinuosa* is common in Korea, Japan, and China (e.g., Okamura 1936, Tseng 1983, Kogame 1997, Cho et al. 2005, Lee et al. 2013). Cho et al. (2009) presented a first glimpse at genetic diversity of *C. sinuosa* on a global scale. They analyzed nuclear internal transcribed spacers of the ribosomal cistron (ITS) and chloroplast encoded large subunit of the ribulose-1,5-bisphosphate carboxylase gene (*rbcL*) and found that the unique tandem repeats in the ITS region correspond to the geographical distribution of *C. sinuosa*. Lee et al. (2013) observed three genetic lineages within *C. sinuosa* around the world using mitochondrial cytochrome *c* oxidase subunit III (*cox3*) and chloroplast *rbcL*. They suggested that *C. sinuosa* had broad and complicated biogeographical patterns in the Indo-Pacific Ocean region where the Red Sea was likely a refugium before the Last Glacial Maximum (LGM), and

multiple introductions might occur among Australasia, Europe, and America.

Chinese coast, in particular the Yellow-Bohai Sea (YBS), geographically adjoins and shares homologous coastal marine environments with the Korean Peninsula and the Japanese Archipelago (Tyberghein et al. 2012). Similar coastal configurations in those neighboring areas during the LGM (Wang 1999), produced seaweed floras that are closely related taxonomically (Tseng 1983, Yoshida et al. 2015). In addition, the Kuroshio Current can connect populations of marine species, including seaweeds, along the coasts of East Asia (Barkley 1970, Hu et al. 2013, Li et al. 2017). The coastal currents in China (e.g., the Bohai Sea coastal current, the Yellow Sea coastal current and the East China Sea coastal current) in different seasons can also contribute to multiple dispersal to a great extent. Taken together, these historical climate change and oceanic currents can interplay and ultimately affect the richness, diversity and distribution patterns of present-day seaweeds in China, Korea, and Japan (e.g., *Chondrus ocellatus* Holmes, Hu et al. 2015, *Sargassum fusiforme* (Harvey) Setchel, Hu et al. 2017).

Colpomenia species have broad latitudinal distributions in the West Pacific (Guiry and Guiry 2019). For example, *C. peregrina* appears in coastal temperate waters in the northern hemisphere of Japan, Korea, and Russia (e.g., Perestenko 1980, Lee et al. 2014a, Yoshida et al. 2015) and in the southern hemisphere of southern Australia and New Zealand (e.g., Harper et al. 2012, Nelson 2013, Scott 2017), respectively. Similar patterns have been reported for *C. claytoniae* (e.g., Boo and Ko 2012, Nelson 2013). China, which stretches over some 18,000 kilometers of coastlines and has considerable environmental shifts (e.g., the significantly different sea surface temperature, annual precipitation and solar radiation between northern and southern coast) (Ma et al. 2017), is the main component of the China Sea Coastal Province (Longhurst 2007) and geographically located near Japan and Korea. However, for the genus *Colpomenia*, only *C. sinuosa* has been reported in the past few decades (Tseng 1983, 2008, Liu 2008), and there has been no study of the quantity and distribution of *Colpomenia* species. We thus speculate that previous studies (Tseng 1983) may have underestimated the number of *Colpomenia* species along the coast of mainland China. Furthermore, climate change induced a northward shift of temperature zones in China, together with ocean acidification and coastal erosion, have exerted the necessary obligation of protecting seaweed species diversity (Zheng et al. 2018). The present study focuses on *Colpomenia* specimens collect-

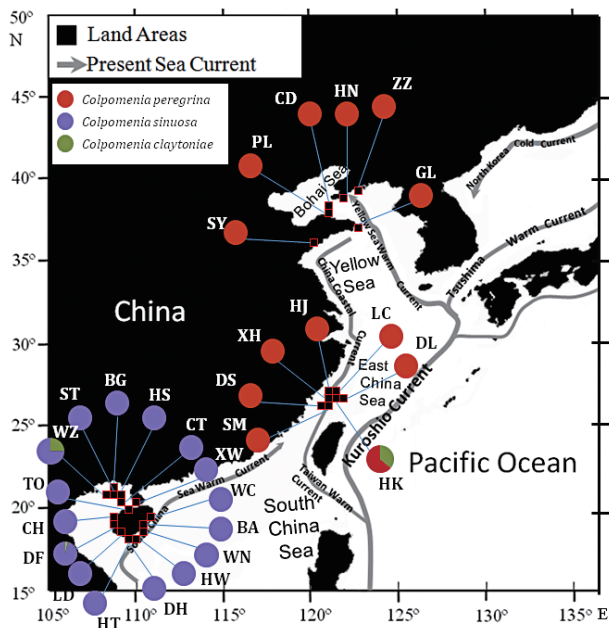


Fig. 1. Sampling sites and distribution of *Colpomenia* along the coast of China.

ed from the entire Chinese coast. We conducted morphological observations and phylogenetic reconstructions using mitochondrial *cox3* and *atp6* genes to determine species diversity and phylogenetic relationships in *Colpomenia* and range limits along the coast of China. This information may provide useful insights into discovering and conserving natural seaweeds resources in a time of climate change.

MATERIALS AND METHODS

Sampling and DNA extraction

Colpomenia specimens were collected from 30 coastal localities, from 18.21° N, 109.51° E to 39.02° N, 122.73° E (Fig. 1, Supplementary Table S1). At each site, 1-3 specimens were collected, with an interval transect ≥ 10 m to avoid sampling genetically homogeneous plants. Specimens were cleaned from epiphytes, washed and partially preserved in silica gel. Twenty specimens chosen randomly for morphological sections were kept in 4% formaldehyde and deposited in the Marine Biological Herbarium of the Institute of Oceanology, Chinese Academy of Sciences (voucher numbers 2017BC0015-2017BC0034).

Total genomic DNA was extracted using the DNAs-ecure Plant Kit (TIANGEN, Beijing, China) following the manufacturer's instructions. Purity and quality of ge-

nomeric DNA were checked by OD_{260} / OD_{280} ratios and 1% agarose gel electrophoresis.

Polymerase chain reaction amplification and DNA sequencing

Based on a recent molecular study of *Colpomenia* (Lee et al. 2014a), mitochondrial *cox3* and *atp6* were selected as gene markers. Primer sets F49 / R20 and F25P / R754P (Lee et al. 2014a) were used to amplify, respectively, *cox3* and *atp6* fragments from 63 specimens. Polymerase chain reaction (PCR) amplification was performed in 50 μ L volumes, each containing 25 μ L 2 \times Es Taq Master Mix (+dye) (CWBIO, Beijing, China), 10 pmol of each primer and 6 ng of template DNA. For the *cox3* gene, PCR reactions consisted of an initial denaturation at 95°C for 4 min, followed by 35 amplification cycles (denaturation at 94°C for 45 s, annealing at 53°C for 1 min, extension at 72°C for 1 min) with a final extension at 72°C for 10 min. For the *atp6* gene, PCR reactions consisted of an initial denaturation at 95°C for 4 min, followed by 35 amplification cycles (denaturation at 94°C for 30 s, annealing at 45°C for 30 s, extension at 72°C for 1 min) with a final extension at 72°C for 10 min. Quantity and quality of PCR products were checked using 1% agarose gel electrophoresis. Purification and sequencing of PCR products were performed using a BigDye Terminator Cycle sequencing kit and an ABI3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

Twenty-five recently published *cox3* sequences and 6 *atp6* sequences of *Colpomenia* (Boo et al. 2011, Lee et al. 2012, 2013, 2014a, 2014b) (Supplementary Table S2) were retrieved from GenBank to determine gene boundaries. Newly generated *cox3* and *atp6* fragments were aligned with referenced sequences using the MUSCLE model in MEGA v7.0 (Kumar et al. 2016). After editing and removal of excess bases, aligned sequences were adjusted manually to avoid false gaps.

Phylogenetic relationships were reconstructed based on the *cox3* alignment, the *atp6* alignment, and a concatenated *cox3* + *atp6* alignment, respectively using both maximum likelihood (ML) and Bayesian inference (BI). JModelTest v2.1.7 (Darriba et al. 2012) was used to determine the best-fit substitution model through Bayesian Information Criterion for each gene marker separately (*cox3*: TPM1uf + I + G [I = 0.35, G = 0.47], *atp6*: HKY + G [G = 0.14]). ML trees based on *cox3* and *atp6* were recon-

structured in PhyML v3.1 (Guindon and Gascuel 2003) using 1,000 bootstrap replicates with a Nearest-Neighbour-Interchange heuristic method. The ML tree based on concatenated *cox3* + *atp6* alignment was reconstructed in RAxML v8.0 (Stamatakis 2014). BI trees were reconstructed in MrBayes 3.2 (Ronquist et al. 2012), running four Markov chain Monte Carlo iteration chains simultaneously for 10^7 generations, keeping one tree every 100 generations, with the first 10^4 trees sampled discarded as ‘burn-in.’ Consensus topology and posterior probability (PP) values were calculated for the remaining trees. The *cox3* and *atp6* fragments of *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Cock et al. 2010) and *Scytosiphon lomentaria* (Lyngbye) Linkwern (Liu et al. 2016) were used as outgroup. Trees were visualized using FigTree v1.4.3, available at <http://tree.bio.ed.ac.uk/software/figtree/>.

Genetic variation

DnaSP v5.10 (Librado and Rozas 2009) was used to count variable sites and parsimony informative sites in each marker. Using 1,000 bootstrap replications, genetic distances among 30 sampling sites were computed using MEGA v7.0, with a *p*-distance substitution model including transitions and transversions. A heatmap and a box-plot were used to present intra- and inter-specific genetic distances, respectively.

Morphological observation

Multiple thalli were each cut at multiple parts and then were cut into thin slices of about 2–5 µm using a razor blade by hand and freezing microtome HM505E (MICROM, Neuss, Germany). Sections were stained in 1% aniline blue for 5–10 min, followed by decolorization with one or two drops of 1 M hydrochloric acid for 30 s and washed with seawater (Aisha and Shameel 2012). Sections were mounted and sealed in a mixture of 75% glycerin with aniline blue, observed and photographed under an optical microscope BX51TF (Olympus, Tokyo, Japan) and a fluorescence microscope Eclipse Ni (Nikon, Tokyo, Japan).

RESULTS

Molecular diversity of mitochondrial *cox3* and *atp6*

Sixty-three *cox3* sequences with an aligned length of

620 bp yielded 102 variable sites and 94 parsimoniously informative sites. Sixty-two *atp6* sequences with an aligned length of 611 bp contained 107 variable sites and 103 parsimoniously informative positions.

Species diversity of *Colpomenia* along the coast of China

The *Colpomenia* phylogenetic relationship based on the *cox3*, *atp6* and concatenated *cox3* + *atp6* datasets were congruent (Fig. 2, Supplementary Figs S1–S4). The specimens from China were represented in three distinct species clades. The ML and BI trees both indicated that five specimens from Weizhou Island, Guangxi (WZ), Huokun’ao, Zhejiang (HK), and Dongfang, Hainan (DF) grouped with referenced *C. claytoniae*, forming a unique clade with well-supported values [*cox3* + *atp6*: ML bootstrap percentage (BP) = 100, BI PP = 1.00; *cox3*: BP = 95, PP = 1.00; *atp6*: BP = 99, PP = 1.00]. Thirty-two sequences from Weizhou Island, Guangxi (BG, ST, HS, CT, and WZ), Guangdong (XW) and Hainan Island (WC, TO, CH, BA, DE, WN, LD, HW, HT, and DH) grouped with referenced *C. sinuosa*, forming another strongly supported clade (*cox3* + *atp6*: BP = 100, PP = 1.00; *cox3*: BP = 99, PP = 1.00; *atp6*: BP = 100, PP = 1.00). The remaining 26 sequences from Liaoning (ZZ and HN), Shandong (CD, PL, GL, and SY), and Zhejiang (DL, LC, HJ, XH, HK, DS, and SM) grouped with the referenced *C. peregrina* and constituted the third clade (*cox3* + *atp6*: BP = 76, PP = 1.00; *cox3*: BP = 94, PP = 1.00; *atp6*: BP = 98, PP = 0.94). It is therefore concluded that the three genetic clades found along the coast of China may correspond to *C. claytoniae*, *C. sinuosa*, and *C. peregrina*.

Genetic variations of *cox3* and *atp6* along Chinese coast

Pairwise genetic distances among sampling sites along Chinese coast based on *cox3* and *atp6* showed low genetic differentiations at the intra-specific level in *Colpomenia* (Fig. 3, Supplementary Tables S3–S5). Mitochondrial *cox3* revealed a mean genetic distance of 0.5% within *C. peregrina*, 1.1% within *C. sinuosa*, and 1.9% within *C. claytoniae* (Supplementary Tables S3 & S5). Mitochondrial *atp6* showed a mean genetic distance of 0.4% within *C. peregrina*, 0.5% within *C. sinuosa*, and 2.7% within *C. claytoniae* (Supplementary Tables S4 & S5). Much higher genetic differentiations were detected at the inter-specific level (Fig. 3, Supplementary Tables S3–S5). The *cox3* gene showed a mean genetic distance of 10.3% between

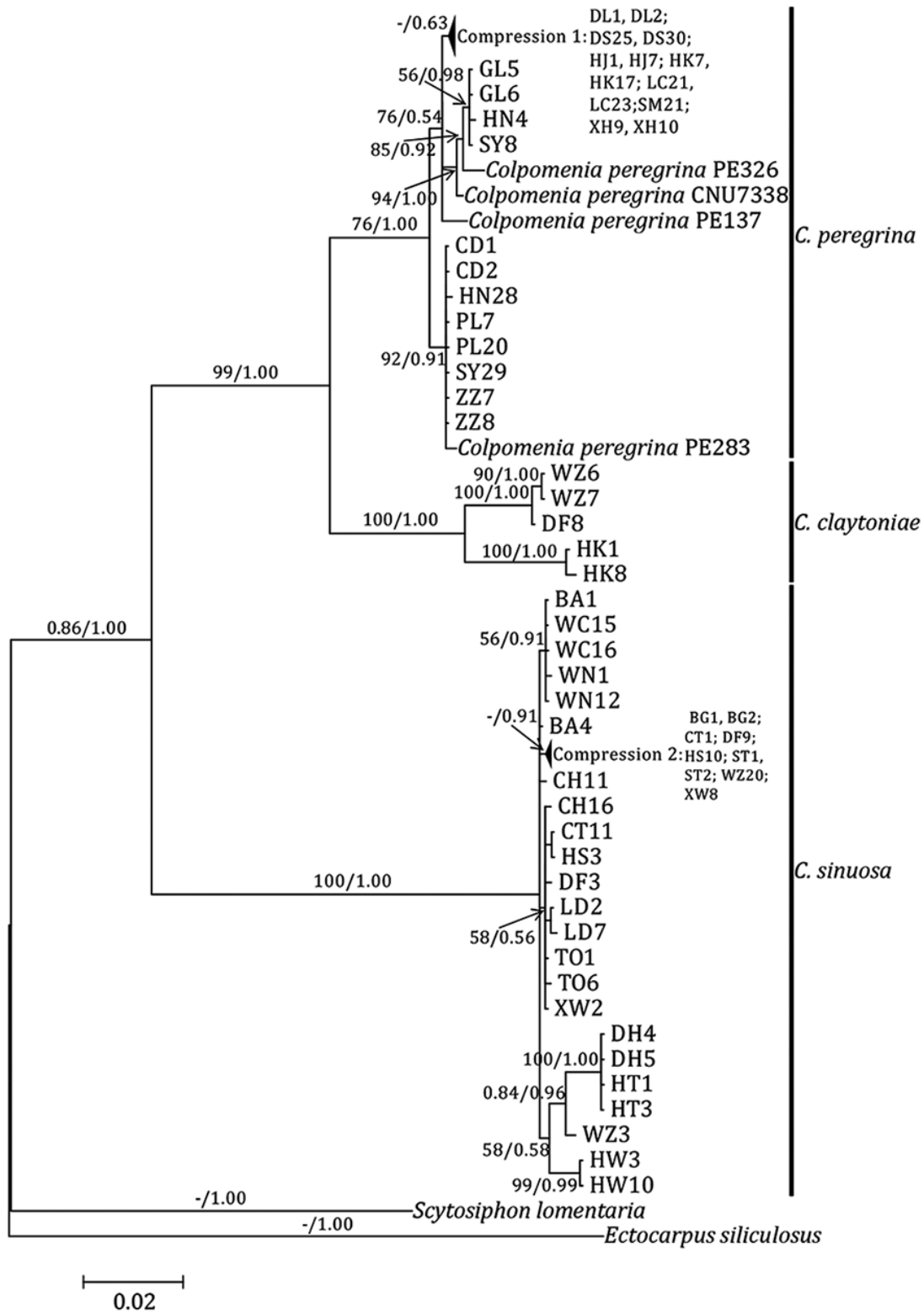


Fig. 2. Phylogenetic trees derived from concatenated *cox3* + *atp6* sequences of *Colpomenia*. Numbers above or near branches are maximum likelihood bootstrap values (left) / Bayesian inference posterior probabilities (right). Values <50% or <0.50 were not shown.

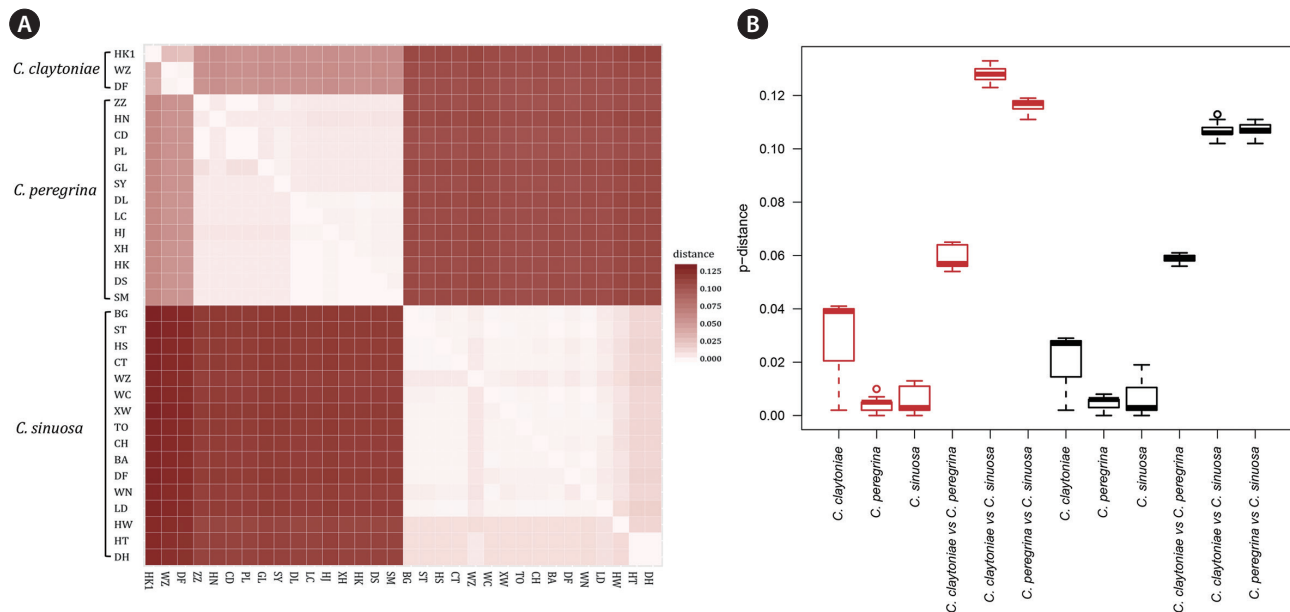


Fig. 3. Heatmap (A) and boxplot (B) showing average genetic distances using *cox3* and *atp6* genes among sampling sites, calculated by *p*-distance model (1,000 bootstrap replications). Each value is visualized as a square color-block in the heatmap, varying from 0 to 0.125. Lower-left blocks represent values of *cox3* and upper-right blocks represent those of *atp6*. The red boxes in the boxplot represent genetic distances using *cox3* gene while the black boxes represent genetic distances using *atp6* gene. *C.*, *Colpomenia*.

C. peregrina and *C. sinuosa*, 6.0% between *C. peregrina* and *C. claytoniae*, and 8.9% between *C. sinuosa* and *C. claytoniae* (Supplementary Table S3). *Atp6* showed a mean genetic distance of 11.4% between *C. peregrina* and *C. sinuosa*, 6.0% between *C. peregrina* and *C. claytoniae*, and 12.8% between *C. sinuosa* and *C. claytoniae* (Supplementary Table S4).

Morphological characters of *Colpomenia* species

Table 1 indicates that morphological characters of the three *Colpomenia* species reported here (Fig. 4).

***Colpomenia sinuosa* (Mertens ex Roth) Derbès et Solier (Fig. 4A-D).** Thalli attached erectly to substratum on rocks at intertidal and subtidal zone, irregularly hollow, saccate, 3-15 cm in diameter and yellowish brown in color (Fig. 4A). Phaeophycean hairs unicellular, about 100 µm long (Fig. 4B). Cortex consists of 4-6 layers of small polygonal cells and phaeoplasts (Fig. 4C). Medulla has five or more layers of irregular and large colorless cells (Fig. 4C). Sori discrete and punctate on thallus surface, with cuticle stained blue on plurilocular sporangia (Fig. 4C). Plurilocular sporangia cylindrical, uniseriate and biseriate, with cuticle stained blue, about 50 µm long (Fig. 4D); paraphysis brown in color, about 30 µm long (Fig. 4D). Unilocular sporangia were not observed.

***Colpomenia claytoniae* S. M. Boo, K. M. Lee, G. Y. Cho**

et W. Nelson (Fig. 4E & F). Thalli attached erectly on rocks at subtidal zone, globular or vesicle-like hollow sacs, irregularly convoluted and expanded or collapsed, 3-30 cm in diameter and yellowish green in color (Fig. 4E). Cortices have 1-2 layers of small polygonal cells with phaeoplasts (Fig. 4F). Medulla has 5-6 layers of increasingly larger, irregularly shaped cells (Fig. 4F). Sporangia and paraphyses were not observed.

***Colpomenia peregrina* Sauvageau (Fig. 4G-L).** Thalli epilithic, thin, globular, 2-10 cm in diameter and greenish brown in color (Fig. 4G). Phaeophycean hairs start from medulla, 50-100 µm long (Fig. 4H). Cortical cells polygonal and irregularly arranged, 2-5 layers (Fig. 4I & J). Medullary cells large, irregular, and colorless, 2-4 layers (Fig. 4I & J). Plurilocular sporangia biseriate, club-shaped or elongate, about 30 µm long (Fig. 4K). Paraphysis club-shaped, as tall as plurilocular sporangia (Fig. 4L). Unilocular sporangia were not observed.

DISCUSSION

Genetic variations of *cox3* and *atp6* in *Colpomenia*

Mitochondrial *cox3* and *atp6* have been previously used to identify new species in *Colpomenia* and examine genetic variation at inter- and intra-specific levels.

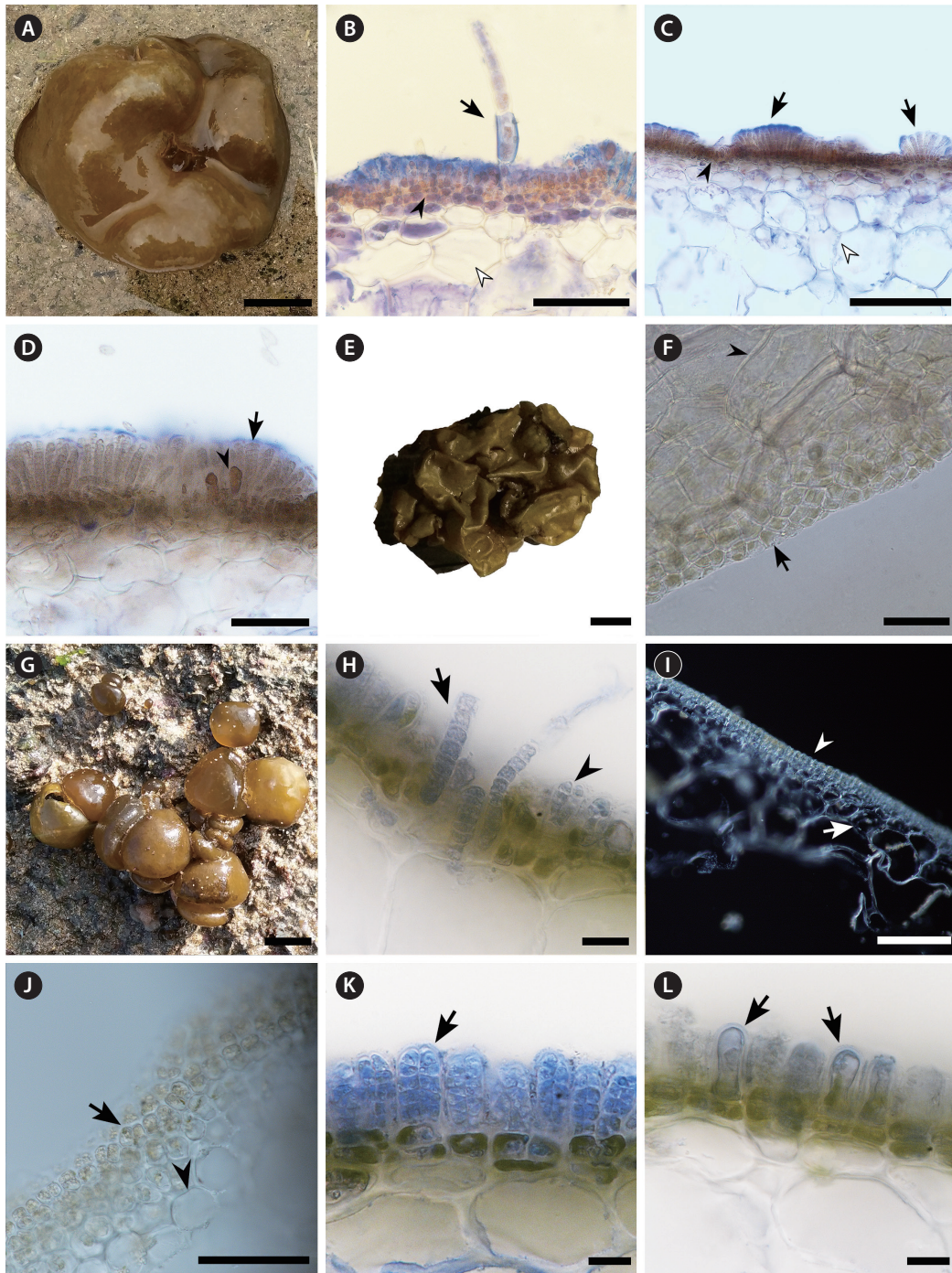


Fig. 4. Morphology of *Colpomenia* species. (A) *Colpomenia sinuosa* thallus growing attached to rocks above the lowest tide (collected from BA in Hainan, Apr 14, 2018). (B) Phaeophyceae hairs (black arrow) arising from cortical cells (black arrowhead), and medulla (white arrowhead) of *C. sinuosa* (sample collected from CH in Hainan, Mar 31, 2019). (C) Sori growing on the surface (black arrows) with cuticle (stained blue), cortex (black arrowhead), and medulla (white arrowhead) of *C. sinuosa*. (D) Close-up view of plurilocular sporangia (arrow) and paraphysis (arrowhead). (E) *Colpomenia claytoniae* thallus growing attached to rocks in subtidal zone, about 5 m below the lowest tide (collected from HK in Nanji islands, May 15, 2018). (F) Cortex (arrow) and medulla (arrowhead) of *C. claytoniae*. (G) *Colpomenia peregrina* thallus growing attached to rocks about 1.5 m above the lowest tide (collected from DS in Nanji islands, May 16, 2018). (H) Paraphysis (arrow) arising from medullary cells, and nascent plurilocular sporangia (arrowhead) of *C. peregrina* (sample collected from SY in Shandong, May 20, 2019). (I) Cortex (arrowhead) and medulla (arrow) of *C. peregrina*. (J) Close-up view of cortex (arrow) and medulla (arrowhead) of *C. peregrina*. (K) Plurilocular sporangia stained blue (arrow) of *C. peregrina*. (L) Paraphysis in blue (arrows) of *C. peregrina*. Scale bars represent: A, E & G, 1 cm; B, D, F & J, 50 µm; C & I, 100 µm; H, K & L, 10 µm. [Colour figure can be viewed at <http://www.e-algae.org>].

In previous studies of the genus *Colpomenia*, *cox3* indicated that *C. peregrina* differentiated from *C. claytoniae* by 4.40-7.69% (Boo et al. 2011, Lee et al. 2014a) and from *C. expansa* by 7.22% (Boo et al. 2011). Subsequently, *cox3* was reported to differentiated by 12.9% between *C. claytoniae* and *C. sinuosa* (Lee et al. 2013). At the intra-specific level, *cox3* shows 0-4.55% sequence divergence within *C. claytoniae* (Boo et al. 2011), 0-7.4% within *C. sinuosa* (Lee et al. 2013), and 0-3.7% within *C. peregrina* (Lee et al. 2014a). As for the *atp6*, the sequences up to 2.4% pairwise divergence within *C. peregrina*, and 4.8% between *C. peregrina* and *C. claytoniae* (Lee et al. 2014a). Furthermore, concatenation of *cox3* and *atp6* fragments shows average inter-specific divergence of 11.4% in *Colpomenia* and 0-2.9% in *C. peregrina* (Lee et al. 2014a).

In this study, mitochondrial *cox3* and *atp6* not only revealed diverse genetic distances between inter- and intra-clades in *Colpomenia* along the coast of China (Fig. 3, Supplementary Tables S3-S5), but also presented divergence values similar to the previous studies described above. This combined evidence further indicates that *cox3* and *atp6* are valuable markers for identifying hidden diversity in *Colpomenia*. Noticeably, the genetic distances of *C. peregrina* and *C. sinuosa* in *cox3* were higher than those in *atp6*, while that of *C. claytoniae* in *cox3* was lower than *atp6*. This kind of difference may result from the unequal evolutionary rate of *cox3* and *atp6*. Addition-

ally, the insufficiency of *C. claytoniae* sampling quantity could affect the accuracy of the results to some extent.

Colpomenia species along the coast of China

Widely distributed in temperate coastal waters of Korea and Japan (Lee and Kang 2001, Lee 2008, Boo 2010, Yoshida et al. 2015), *C. peregrina* Sauvageau was initially recognized as *C. sinuosa* var. *peregrina* due to its thin form, smooth bladders that are not folded or wrinkled and phaeophycean hairs more deeply seated than in *C. sinuosa* (Sauvageau 1927). Later, Hamel (1937) lifted it to species level. *C. peregrina* has extensive irregular sori without cuticles and thin thalli with 3-4 layers of colorless medullary cells (Clayton 1975, Cho et al. 2005). Nevertheless, distinguishing *C. peregrina* from *C. sinuosa* is challenging due to striking morphological similarity. In this study, morphological evidence allows us to distinguish three *Colpomenia* species mainly through the cuticle on the surface of plurilocular sporangia, the number of layers of outer cortex cells and medullary cells (Table 1).

C. claytoniae was newly identified based on congruent genetic evidence of *rbcL* and ITS regions and its distribution was considered to be restricted to Korea and Japan, and recently in Australia and New Zealand (Boo et al. 2011, Nelson 2013, Yoshida et al. 2015). It was characterized by a more irregular thallus with a deeply infolded

Table 1. Morphological characteristics of three *Colpomenia* species along the coast of China

| | <i>C. peregrina</i> | <i>C. sinuosa</i> | <i>C. claytoniae</i> |
|---------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------|
| Color | Greenish brown | Yellowish brown | Yellowish green |
| Form | Globular | Irregularly saccate | Irregularly saccate |
| Surface configuration | Smooth | Infolded | Deeply infolded |
| Diameter (cm) | 5-10 | 3-15 | 3-30 |
| Wall thickness (µm) | Up to 300 | No less than 500 | 100-300 |
| Cortex | 2-5 layers of small polygonal cells at outer cortex | 4-6 layers of polygonal cells at outer cortex | 1-2 layers of polygonal cells at outer cortex |
| Medulla | 2-4 layers of large colorless, thin-walled cells lacking pits | 5 or more layers of large colorless, thin-walled cells | 5-6 layer of large, irregular cells |
| Sori shape | Extensive | Discrete, punctate | Irregularly in extensive patches |
| Length of plurilocular sporangia (µm) | 20-30 | Up to 50 | 50-60 |
| Cuticle on sorus | Absent | Present | Absent |
| Habitat | Epilithic or epiphytic on the macroalgae in the middle or lower intertidal zone | Epilithic in the lower intertidal and subtidal zone | Epilithic in the subtidal zone |
| Distribution in China | From the Bohai Sea, south to Nanji islands in Zhejiang | Guangxi, Guangdong and Hainan (the South China Sea) | Nanji islands in Zhejiang and Weizhou Island in Guangxi |
| Reference | Clayton (1975), Vandermeulen (1984), this study | Clayton (1975), Kogame (1997), Aisha and Shameel (2012), this study | Boo et al. (2011), this study |

surface. This species often appears epilithically in the lower intertidal, subtidal zones and tide pools (Boo et al. 2011).

Our combined morphological and phylogenetic evidence indicates that three *Colpomenia* species (i.e., *C. peregrina*, *C. claytoniae*, and *C. sinuosa*) occur in China. In addition, it is worthy to note that *C. peregrina* and *C. claytoniae* are recorded for the first time in China. Geographically, *C. peregrina* is distributed in the warm-temperate waters of the YBS and subtropical waters of the East China Sea; *C. sinuosa* is only distributed in the tropical coastal South China Sea, but the specific boundary between these two species has not been clear up to now. *C. claytoniae* may only appear in isolated islands in southern China. *C. sinuosa* and *C. claytoniae* overlap in distribution in WZ (Dishui village, Weizhou Island) and DF (Dongfang, Hainan Island); *C. peregrina* and *C. claytoniae* co-occur in HK (Huokun'ao, Nanji islands), but *C. claytoniae* grows at subtidal whereas *C. peregrina* grow at intertidal zone. Therefore, the geographical-scale distribution boundaries among *Colpomenia* species along the coast of China have not been delineated yet. Interestingly, only limited samples of *C. claytoniae* are reported in this study. But recent studies indicate that *C. claytoniae* occurs in the northern and southern West Pacific (e.g., Australia and New Zealand) (Boo et al. 2011, Nelson 2013), suggesting that extensive sampling is needed to identify the complete distribution range of each *Colpomenia* species along the Chinese coast. Furthermore, concatenated *cox3* + *atp6* revealed three genetic lineages in *C. sinuosa* (Fig. 2). Considering that Lee et al. (2013) reported multiple hidden lineages in this species, *C. sinuosa* may have cryptic diversity patterns and complicate trans-oceanic dispersal routes and evolutionary history.

Biogeographic processes contributing to *Colpomenia* diversity

The close relationships and co-distribution patterns of seaweed floras among the Yellow Sea, the South China Sea, the East Sea (Japan Sea) and the Pacific coast of Japan have been studied by Tseng and Chang (1963) over five decades ago. Such patterns can be ascribed to two main abiotic factors. First, LGM climate change caused sea levels to drop approximately 120 m and marginal seas in East Asia (e.g., the YBS and the East China Sea) disappeared completely (Guo 1979, Liu 1995, Wang 1999), which considerably impacted local natural coastal landscapes and resource allocation (Xie et al. 1996, Li and Chang 2009). Nevertheless, sea surface tempera-

ture, a vital factor shaping diversity and distribution patterns of seaweed (Müller et al. 2009, Tittensor et al. 2010), maintained the similar level in these marginal seas (Wang 1999). Consequently, seaweed floras in the YBS, the ECS and the Pacific coast of Japan underwent similar contraction-expansion processes induced by glacial and inter-glacial cycles, leading to many seaweed species co-distributed in China, Korea and Japan (Tseng and Chang 1959, van den Hoek 1984, Keith et al. 2014).

Second, oceanic current systems are of important factors in driving spatial dispersal and migration of seaweed (Tseng and Chang 1959). As illustrated in Fig. 1, the Kuroshio Current, which is the main system connecting the northern coast of China to other areas in the northwest Pacific, has been known to have a significant impact on species diversity and population connectivity (Barkley 1970, Hu et al. 2013, Lin et al. 2018). Therefore, the water flow driven by the Kuroshio Current can possibly promote trans-regional dispersal of *Colpomenia* species, expanding the geographical range from China to Korea and Japan.

Anthropogenic influence can also lead to seaweed introduction. Fouling of ships' hulls, ballast water carried by trading vessels, and oyster mariculture are considered to be important pathways for the introduction of algae (Schaffelke et al. 2006). For example, Lee et al. (2014a) reported that *C. peregrina* was likely to be introduced from the NE Atlantic to the NW Atlantic via oyster mariculture and vessel hulls. Thus, maritime trade may contribute to the distributing expansion of *Colpomenia* species.

In summary, this study shows new occurrences of two *Colpomenia* species (*C. claytoniae* and *C. peregrina*) in China, with a clearly separate distribution between *C. peregrina* and *C. sinuosa*. The distribution range of subtidal *C. claytoniae* partially overlaps with intertidal *C. peregrina* and *C. sinuosa* in the East and South China Sea (Fig. 1). These contrasting distribution patterns inspire further exploration of how eco-physiological differences of *Colpomenia* species respond to environmental shifts, population-level phylogeographical diversification and evolutionary history, including possible genetic hybridization/introgression at inter-specific contact zones.

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SUPPLEMENTARY MATERIALS

Supplementary Table S1. Sampling details of *Colpomenia* in this study (<https://www.e-algae.org>).

Supplementary Table S2. Mitochondrial *cox3* and *atp6* sequences retrieved from GenBank in this study (<https://www.e-algae.org>).

Supplementary Table S3. MtDNA *cox3* based mean genetic divergences among three *Colpomenia* species along the coast of China (<https://www.e-algae.org>).

Supplementary Table S4. MtDNA *atp6* based mean genetic divergences among three *Colpomenia* species along the coast of China (<https://www.e-algae.org>).

Supplementary Table S5. MtDNA *cox3*- and *atp6*-based genetic divergences among sampling sites of *Colpomenia* along the coast of China (<https://www.e-algae.org>).

Supplementary Fig. S1. The maximum likelihood (ML) tree derived from *cox3* sequences of *Colpomenia*. Numbers above or near branches are ML bootstrap values. Values <50% were not shown (<https://www.e-algae.org>).

Supplementary Fig. S2. The Bayesian inference (BI) tree derived from *cox3* sequences of *Colpomenia*. Numbers above or near branches are BI posterior probabilities. Values <0.50 were not shown (<https://www.e-algae.org>).

Supplementary Fig. S3. The maximum likelihood (ML) tree derived from *atp6* sequences of *Colpomenia*. Numbers above or near branches are ML bootstrap values. Values <50% were not shown (<https://www.e-algae.org>).

Supplementary Fig. S4. The Bayesian inference (BI) tree derived from *atp6* sequences of *Colpomenia*. Numbers above or near branches are BI posterior probabilities. Values <0.50 were not shown (<https://www.e-algae.org>).

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