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Re-identifying *Grateloupia yangjiangensis* (Rhodophyta, Halymeniaceae) based on morphological observations, life history and *rbc*L sequence analyses

WANG Hongwei[1]*, GUO Shaoru[1], ZHANG Xiaoming[1], ZHAO Dan[1], ZHANG Wen[1], LUAN Rixiao[2]

[1] College of Life Sciences, Liaoning Normal University, Dalian 116081, China
[2] Dalian Natural History Museum, Dalian 116023, China

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Abstract

On the basis of morphological observations, life history and molecular phylogeny, *Grateloupia yangjiangensis*, which is similar to *G. filicina*, *G. orientalis*, *G. catenata*, and *G. ramosissima* in appearance, was re-examined. The results are as follows: (1) the auxiliary-cell ampullae of *G. yangjiangensis* were of *Grateloupia* type, thalli was fleshy and gelatinous in texture, and the erect axes were compressed; the cortex was 0.25–0.30 mm thick, consisting of five to seven outer layers, and there were five inner layers of triangular or stellate cells; (2) there was no filamentous stage in the development of the carpospores; (3) the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) sequence of four *G. yangjiangensis* examined showed that there was no intergeneric divergence among them, and for the phylogenetic tree, four sequences of *G. yangjiangensis* formed a single monophyletic subclade within the large *Grateloupia* clade of Halymeniaceae. In conclusion, *G. yangjiangensis* was a single species within the genus *Grateloupia*. This research provided criterion for identification and cultivation of *G. yangjiangensis*.

Key words: *Grateloupia yangjiangensis*, Halymeniaceae, Rhodophyta, morphological observations, life history, *rbc*L

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1 Introduction

Grateloupia yangjiangensis Li et Ding within the family Halymeniaceae of Rhodophyta was initially identified on the base of external morphological features, transverse view and tetrasporangia. The species was first recorded in Flora Algarum Marinarum Sinicarum (Xia, 2004) and has not been reported in any published papers. Furthermore, no descriptions currently exist about the reproductive structures and development of *G. yangjiangensis* sporelings.

Spore characteristics such as development type, form, and size can be used for classification of red algae (Shunpei, 1947). Spore development of most *Grateloupia* species was found to be "indirect discal type", with filamentous fronds sometimes appearing during early sporeling development (Shunpei, 1947). Sporogenesis modes of *G. filicina* collected from Xiamen and *G. ramosissima* were "indirect discal type"; however, the germinal form of their initial stage differed, which may be considered as a difference in species (Liu and Li, 1986). Filamentous fronds appeared in the early development of *G. asiatica* sporelings (Zhao et al., 2006). *Grateloupia* species have a typical triphasic life cycle with isomorphic gametophytes and tetrasporophytes (Xia, 2004).

In recent years, combining morphological observations with molecular methods has been used to research algae (Kimberly and Conklin, 2009; Milstein and Oliveira, 2012; Zuccarello et al., 2006). Genetic DNA barcoding studies, including the *rbc*L gene (De Clerck et al., 2005a, b; Fredericq et al., 1996; Gavio and Fredericq, 2002; Kawaguchi et al., 2001; Lin et al., 2008; Mateo-Cid et al., 2005; Moncalvo et al., 2000; Shimada et al., 1999; Wang et al., 2000, 2001; Wilkes et al., 2005; Zhao et al., 2012) have been increasingly performed. For example, Wang et al. (2000) conducted a critical reassessment of the morphological features of two closely related red algal genera, *Grateloupia* C. Agardh and *Prionitis* J. Agardh (Halymeniaceae), and clarified the taxonomic relationship between them, proposing that *Prionitis* should be synonymous under *Grateloupia*.

Xia (2004) confirmed that *G. yangjiangensis* was a new species in the genus *Grateloupia* according to the thallus texture, cortex thickness and tetrasporangia; however, this may be inaccurate. Thus, we re-examined *G. yangjiangensis* including morphological observations, life cycle, and *rbc*L sequence analyses.

2 Materials and methods

2.1 *Morphological observations*

Specimens of *G. yangjiangensis* used in the study were collected at Yangjiang, Yinggehai, and Lingshui in China. Voucher herbarium specimens were deposited in the Herbarium of the College of Life Sciences, Liaoning Normal University, Dalian, China (LNU).

G. yangjiangensis: (1) Yinggehai, Hainan Province, China (24 January 2009, leg. R.X. Luan; LNU20092062, LNU20092063,

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*Corresponding author, E-mail: kitamiwang@163.com

LNU20092064, LNU20092065). (2) Linshui, Hainan Province, China (30 January 2009, leg. R.X. Luan; LNU20092066, LNU20092067). (3) Yangjiang, Guangdong Province, China (20 March 2009, leg. H.W. Wang; LNU20092123, LNU20092124, LNU20092125).

Morphological observations were made on specimens preserved in 10% formalin/seawater, or rehydrated herbarium specimens. Sections were made using a hemotome, placed in water, and stained with 0.5% (v/v) cotton blue. Images were made using an Olympus BH2 digital camera mounted on a Nikon microscope.

2.2 *Culture of carpospore*

Fronds with mature cystocarps were selected, rinsed in filtered seawater and sterilized seawater and wiped with moistened cotton tissues to remove epiphytes. The carpospores were placed in Petri dishes containing 2–3 cover slips and sterilized seawater. After 24 h culture, carpospores were released and then attached to the slides at the bottom of the Petri dishes. When the density reached about 25 carpospores per microscopic field (magnification, 10×10), the carpospore attached slides were transferred into polyethersulphone (PES) culture medium and cultured under different light and temperature conditions. Low light conditions were provided by one fluorescent lamp (220 V, 30 W) and strong light conditions were provided by two fluorescent lamps (220 V, 30 W). The temperatures were 10°C, 15°C, and 20°C. Development from the original carpospores to juvenile seedlings was observed every day using a light microscope (Olympus BH2, Japan). Morphological features of carpospores were recorded with a digital microscopic camera (Olympus BH2, Japan).

2.3 *DNA extraction and phylogenetic tree construction*

Total DNA was extracted from four collected *G. yangjiangensis* individuals (Table 1) using the Plant Genomic DNA kit following manufacturer's protocols (QIAGEN, Valencia, CA, Beijing). PCR amplification and sequencing were performed as described previously (Wang et al., 2000). The *rbc*L gene was PCR amplified using primers as per Wang et al. (2000). The *rbc*L sequences were aligned manually because no additional insertion-deletion mutations were detected. Sequences of 31 Halymeniaceae species were downloaded from GenBank and included in these alignments (Table 1). *Sebdenia monardiana* and *Gelidiella ligulata* were used as outgroup.

Table 1. Species used in *rbc*L gene analysis, collection location, GenBank accession number and references

Species	Collection data (location and site)	Reference	GenBank accession number
Grateloupia yangjiangensis Li et Ding	Yangjiang, Guangdong Province, China (LNU20092123)	this study	HQ324236
G. yangjiangensis Li et Ding	Yangjiang, Guangdong Province, China (LNU20092125)	this study	HQ324237
G. yangjiangensis Li et Ding	Lingshui, Hainan Province, China (LNU20092067)	this study	HQ324238
G. yangjiangensis Li et Ding	Yinggehai, Hainan Province, China (LNU20092064)	this study	HQ324239
G. filicina(Lamouroux) C. Agardh	Livorno, Italy	Wang et al. (2000)	AB055472
G. orientalis S.-M.Lin et H.-Y. Liang	Linyuan, southwestern Taiwan, China	Lin et al. (2008)	EU292744
G. catenata Yendo	Shimiao, Dalian, Liaoning Province, China	Wang et al. (2000)	AB038617
G. ramosissima Okamura	Ho Ping Island, Keelung, northern Taiwan, China	Gavio et al. (2002)	AF488810
G. carnosa Yamada et Segawa	Oryuzako, Miyazaki Prefecture, Japan	Wang et al. (2000)	AB038608
G. asiatica Kawaguchi et Wang	Qingdao, Shandong Province, China	Kawaguchi et al. (2001)	AB055488
G. livida (Harvey)Yamada	Izu-misaki, Miyake Island, Tokyo, Japan	Wang et al. (2000)	AB038610
G. acuminata Holmes	Katase, Fujisawa, Kanagawa Prefecture, Japan	Wang et al. (2000)	AB055480
G. americana Kawaguchi et Wang	Pigeon Point, San Matio County, California, USA	De Clerck et al. (2005a)	AY772037
G. patens (Okamura) Kawaguchi et Wang	Oohara, Chiba Prefecture, Japan	Wang et al. (2001)	AB061392
G. schmitziana (Okamura) Kawaguchi et Wang	Shikanoshima, Prefecture, Japan	Wang et al. (2000)	AB061398
G. divaricata Okamura	Oshoro, Hokkaido, Japan	Wang et al. (2000)	AB038609
G. cornea (Okamura) E.Y. Dawson	Oohara, Chiba Prefecture, Japan	Kawaguchi et al. (2001)	AB061382
G. elliptica Homles	Goshikinohama, Usa,Tosa, Kochi Prefecture, Japan	Wang et al. (2000)	AB055476
G. chiangii Kawaguchi et Wang	Izu-misaki, Miyake Island, Tokyo, Japan	Wang et al. (2001)	AB061387
G. kurogii Kawaguchi	Saikai-bashi,Nagasaki Prefecture, Japan	Wang et al. (2000)	AB038606
G. angusta (Okamura) Kawaguchi et Wang	Miyanoura, Hirado Island, Nagasaki Prefecture, Japan	Wang et al. (2001)	AB061380
G. imbricata Holmes	Tsuyazaki, Fukuoka Prefecture, Japan	Wang et al. (2000)	AB038607
G. dichotoma J. Agardh	Lugo, Galicia, Spain	De Clerck et al. (2005a)	AY772031
G. lanceola J. Agardh	Iberian Peninsula, Spain	Figueroa et al. (2007)	AM422894
G. somalensis Hauck	Plage de Monseigneur, Fort Dauphin, Madagascar	De Clerck et al. (2005a)	AY772021
G. capensis De Clerck	Kommetjie, Western Cape Province, South Africa	De Clerck et al. (2005a)	AJ868465
G. longifolia Kylin	Yzerfonteyn, Western Cape Province, South Africa	De Clerck et al. (2005a)	AY772023
G. taiwanensis Lin et Liang	Northeastern and southern Taiwan, China	Lin et al. (2008)	EU292742
G. phuquocensis Tanaka et Pham-Hoang	Kaalawai, Oahu, Hawaii, USA	De Clerck et al. (2005a)	AY772022
G. subpectinata Holmes	Irago-misaki, Atsumi, Aichi Prefecture, Japan	Faye et al. (2004)	AB114213
G. sparsa (Okamura) Chiang	Oohara, Chiba Prefecture, Japan	Wang et al. (2000)	AB055473
G. turuturu Yamada	Muroran, Hokkaido, Japan	Wang et al. (2000)	AB038611
G. belangeri (Bory) Setchell et Gardner	Platboom, Western Cape Province, South Africa	De Clerck et al. (2005a)	AY772027
Halymenia floresia (Clemente) C. Agardh	PulauRebak Besar, Langkawi, Kedah, Malaysia	Wang et al. (2000)	AB038603
Cryptonemia luxurians (C. Agardh) J. Agardh	Namikata, Ehime Prefecture, Japan	Wang et al. (2001)	AB061374
Polyopes constrictus (Turner) J. Agardh	Point Lonsdale, Victoria, Australia	Kawaguchi et al. (2001)	AB055468
Yonagunia formosana (Okamura) Kawaguchi et Masuda	Khanh Hoa, Nha Trang, Hon Miew, Viet Nam	Kawaguchi et al. (2004)	AB116241
Gelidiella ligulata Dawson	Miyake Island, Tokyo, Japan	Shimada et al. (1999)	AB017678
Sebdenia monardiana (Montagne) Berthold	Lachea Island, Catania, Italy	Fredericq et al. (1996)	U21600

Maximum likelihood analyses (ML) were used to construct a phylogenetic tree using a Dell D630 personal computer. All sites were treated as unordered and equally weighted. The ML analysis was implemented with PAUP 4.0b10 (Swofford, 2002). To seek optimal settings for ML analysis, a variety of increasingly complex models of molecular evolution were evaluated as outlined by Litaker et al. (1999) and Moncalvo et al. (2000).

3 Results

3.1 *External morphology*

A single *Grateloupia yangjiangensis* plant consisted of multiple erect blades up to 12 cm high (Fig. 1), which arose gregariously from a small holdfast, which attaches to a rock with or without a short, stout stipe. Plants were fleshy-cartilaginous in texture and rusty red in color when living. The erect axes were terete below, becoming compressed above in branches bearing irregularly pinnate branchlets. Segments or blades were 0.5–0.7 cm (Fig. 2), broad near the base and gradually broadening upward, then narrowing toward blunt to blade-like apices and sickle-like apices, or frequently to fronds finely dissected terminally to 1 mm wide. Blades were furrowed and arose closely back to front when living. Proliferations were often on margins and surfaces on both young and older attached or drifting specimens.

Type locality: Yangjiang, Guangdong Province, China.

Habitat and seasonality: Plants were attached on middle-low tidal rock or rock pools. Plants were present year round but mature in February.

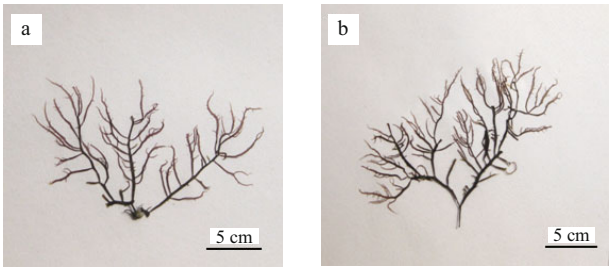

Fig.1. External morphology of *Grateloupia yangjiangensis*. a. Herbarium specimen from Yangjiang (LNU20092123). b. Female gametophyte of the *Grateloupia yangjiangensis* from Yinggehai (LNU20092063).

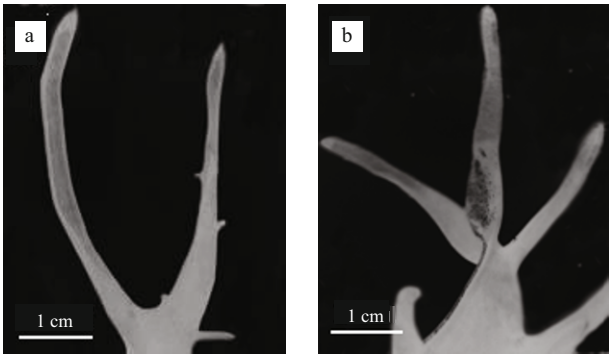

Fig.2. Upper portion of branchlet, showing sickle-like apex (a) and upper portion of branchlet, showing blade-like apex (b).

3.2 *Vegetative and reproductive structures*

The blades internally consisted of a dense filamentous medulla and a compact anticlinal cortex (Fig. 3a). Medullary filaments were short, densely packed and dispersed mostly periclinally and at right angles. The cortex was 65–80 μm thick, consisting of five to eight outer layers of increasingly smaller and slender cells, and two to three inner layers of triangular or stellate cells with dense contents (Fig. 3b).

Gametophytes were dioecious. Reproductive structures existed over the entire thallus except for the basal portions and holdfasts in female and male gametophytes. Carpogonial branches and auxiliary cells were formed singly in separate ampulla produced in the inner cortex. There were two or three secondary filaments and a two-celled carpogonial branch in each carpogonial branch ampulla (Fig. 3c). Each auxiliary cell ampulla possessed three or four simple secondary filaments that converged above and showed a narrowly flask-shaped outline. The auxiliary cell was the third or fourth cell of the primary filament. When mature, the auxiliary cell was oval in shape and slightly larger than other ampullary cells (Fig. 3d). Successive cystocarp development is shown in detail (Figs 3e–i). Cystocarps began to immerse in the medulla (Fig. 3e). Cystocarps were inconspicuous and scattered in patches, consisting of several gonimolobes with synchronously maturing carposporangia, maturing gonimoblasts in the process of becoming loose, and the auxiliary cell bore the primary gonimolobe (Fig. 3f). The cystocarp developed gradually (Fig. 3g). Cystocarps lodged in the outer medulla gonimoblasts and formed spherical cystocarps (Fig. 3h). Mature cystocarps were spherical to pear-shaped, and 150–180 μm across with a few involucral filaments (Fig. 3i). Spermatangia were scattered over the blade and were formed from the outermost cortical cells (Fig. 3j). Tetrasporangial initials were produced from the fourth or fifth cortical cells proximal to the thallus surface. The mature tetrasporangia were broadly ellipsoidal in shape and cruciately divided, and were 20–30 μm long by 10–17 μm wide (Fig. 3k). Cells of cortical filaments that cut off the tetrasporangial initials were typically more elongate than the same cells in ordinary vegetative filaments.

3.3 *Life history*

Liberated carpospores were 7–11 μm in diameter, round or oval, brown, and with a central nucleus (Fig. 4a). Released spores started to split after 24 h (Figs 4b–c), with one portion of the carpospores slightly expanded to triangle protuberance (Fig. 4d), and then developed to a germ tube. The germ tube cells gradually gave rise to multicellular, discoid sporeling cells, which started irregular division after three to four days (Figs 4e–f). Within fifteen to twenty days of development, sporeling cells directly divided and formed crusts (Figs 4g–h), which were mostly disc-shaped, round or oval, with a diameter of 25 μm to 40 μm. With another ten to thirteen days development, gemmules developed from the crust, and twenty days to one month later they developed to erect gemma with a branchlet at the terminal part (Figs 4i–k).

Different light and temperature affected growth conditions of the whole process. When optimal light intensity, 20°C was best for crusts and erect thalli, 15°C caused slow growth, and 10°C and below caused growth to cease. Under 18 to 25°C conditions, low light conditions were more suitable for crusts and

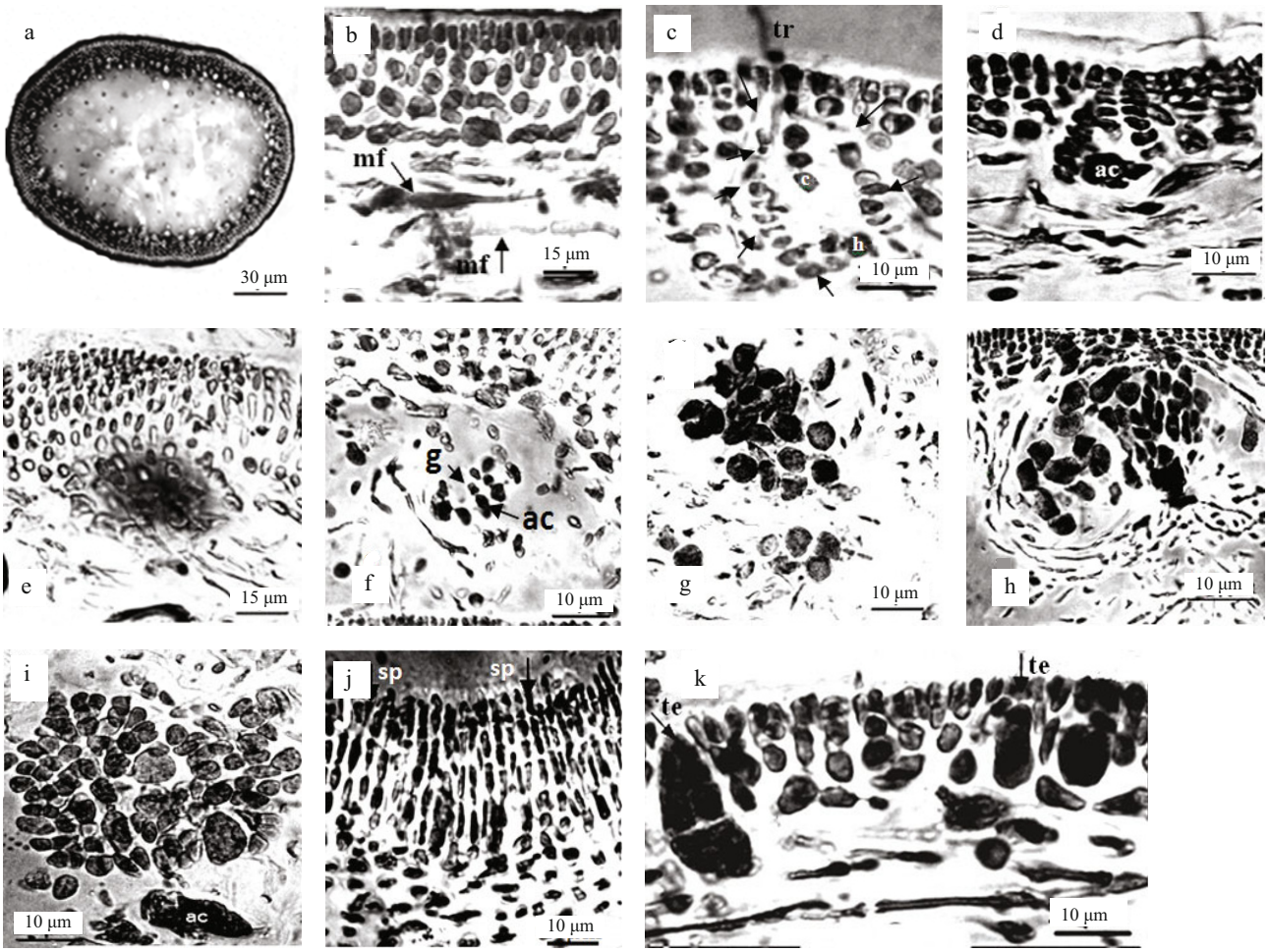

Fig.3. Vegetative anatomy of *Grateloupia yangjiangensis*. a. Cross-section through a branchlet showing cellular cortex and solid center. b. Cross-section showing gradual transition between the cortex and medullary layer and predominantly periclinal arrangement of the medullary filaments. c. Carpogonial-branch ampulla (arrows) with prominently stained carpogonium and hypogynous cell; the trichogyne extending beyond the blade surface. d. Lateral view of mature auxiliary ampulla. e–i. Successive stages of cystocarp development. e. Initial stage of cystocarp formation. f. Middle stage of cystocarp formation viewed in paradermal section from the medulla, with cystocarps consisting of several gonimolobes (arrows) with synchronously maturing carposporangia, auxiliary cell bore being the primary gonimolobe. g. Later stage of cystocarp formation. h. Mature cystocarp with auxiliary cell (arrows) in the bottom. i. Mature cystocarp immersed in the thallus interior. j. Spermatangia (arrows) produced from the outermost cortical cells. k. Mature, cruciately divided tetrasporangium (arrows). mf represents medullary filaments, tr trichogyne, c carpogonium, h hypogynous cell, ac auxiliary cell, g gonimolobe, sp spermatangia, and te tetrasporangium.

erect thalli growth, while strong light destroyed the formation of pigment and suppressed growth of thalli.

Carpospores were released from the mature cystocarp in the female gametophyte and then grew into tetrasporophytes. In the tetrasporophytes, tetraspores were formed through meiosis and developed into female or male gametophytes after liberation. The carpogonial branch ampulla and auxiliary cell ampulla were formed in the female gametophyte. The spermatangia were formed in male gametophytes. Fertilized carpogonia leaving carpogonial branch ampullae reached auxiliary cells through connecting filaments and developed into carpospores (Fig. 5).

3.4 *rbcL analysis*

In the phylogenetic tree, including the two outgroups (*Sebdenia monardiana* and *Gelidiella ligulata)*, sequences of 29 *Grateloupia* species were used along with 28 species downloaded from GenBank (Table 1). Because many sequences were incomplete at the 5′ and 3′ ends, 1 259 sites were used for alignment in the study. Tissue samples were collected from three localities in China (Yangjiang, Yinggehai, and Lingshui). Methods for *rbc*L gene sequencing were as described by Wang et al. (2000). Sequences of the *G. yangjiangensis* samples from different localities in China were found to be identical.

The *rbc*L analysis (Fig. 6) revealed the phylogenetic relationships of *G. yangjiangensis* and other species within Halymeniaceae. From the phylogenetic tree obtained by maximum likelihood (ML) analysis, the presence of a large *Grateloupia* monophyletic clade was evident. Most of the terminal and subterminal clades were supported by high bootstrap values.

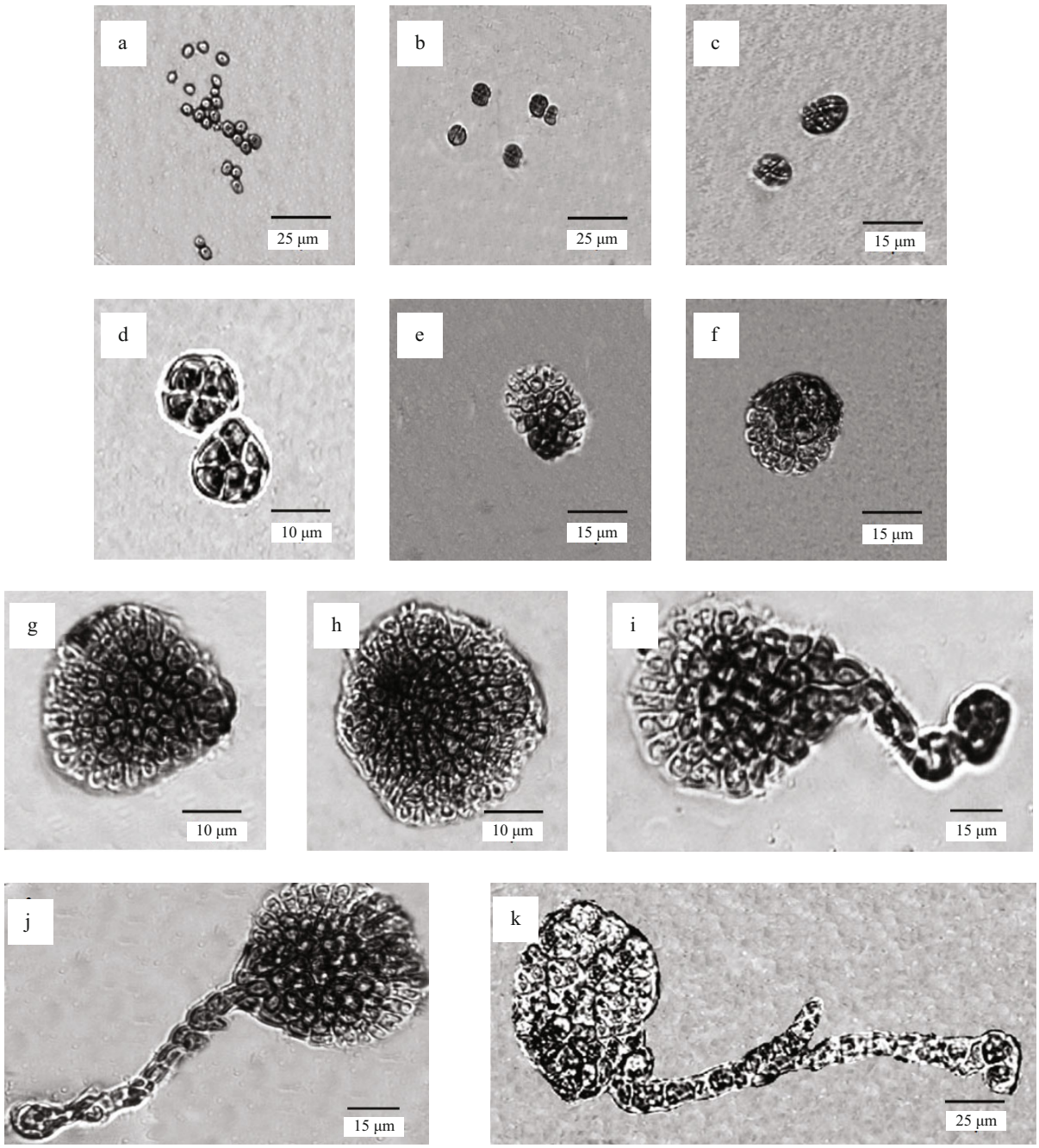

Fig.4. Development of carpospores in culture of (water temperature 21–23°C). a. Carpospores, b–c. carpospores dividing in two or three parts, d–f. carpospores dividing into more parts, g–h. development of crust in culture: sporeling cells directly divided and formed crusts, i–j. gemmule developed from the crust, and k. gemmule developed to erect gemma with branchlet at the terminal part.

Within the *Grateloupia* clade, four samples of *G. yangjiangensis* and *G. hawaiiana* from Hawaii formed a single monophyletic subclade with high bootstrap support (100%). The *G. orientalis* from Taiwan was a sister taxon to the above species with 74% bootstrap support. Although the phylogenetic position of *G. orientalis* was supported only moderately, the remaining clades had 100% bootstrap support.

The *rbc*L sequences of *G. yangjiangensis* showed there was no intergeneric divergence among them from the three sites. The pairwise distance in *rbc*L sequences between *G. yangjiangensis* and the generitype *G. filicina* was 5.75% (38 bp changes); the pairwise distances between *G. yangjiangensis* and *G. orientalis*, *G. catenata*, and *G. ramosissima* were 28 bp (4.98%), 34 bp (5.41%), and 36 bp (5.53%), respectively; the pairwise distances between *G. yangjiangensis* and other related species ranged from 43 to 63 bp (6.49%–7.50%); and the pairwise distances

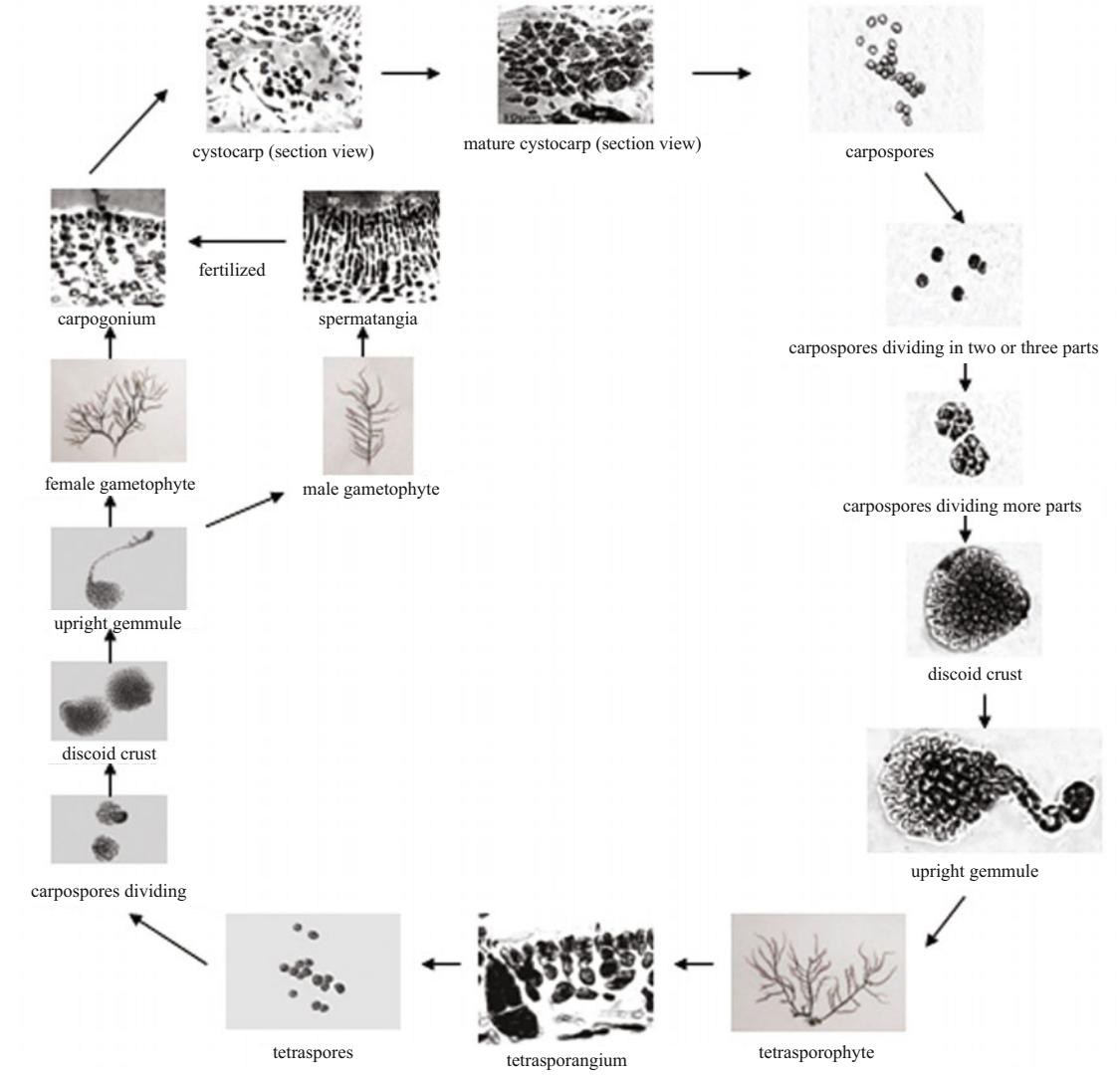

Fig.5. Life history of *G. yangjiangensis*.

Table 2. Comparison of morphological features between *Grateloupia yangjiangensis* and other related species

Morphological feature	Thallus habit	Thallus texture: medulla	Thickness (T) and cortex (C)	Distribution	Reference
G. yangjiangensis	bushy, composed of several slightly compressed branches bearing irregularly pinnate branchlets with the blade-like or sickle-like tip; up to 12 cm high	fleshy, cartilaginous; solid	T: 0.25–0.38 mm, C: 5–10 layers	China	Xia (2004) and this study
G. catenata	composed of terete to compressed branches bearing irregularly pinnate branchlets; 7–35 cm high, 2–3 mm wide	cartilaginous; hollow	T: 0.16–0.22 mm, C:6–14 layers	Japan, China, Korea	Wang et al. (2000) and Lee et al. (2009)
G. ramosissima	composed of simple or linear to several lanceolate blades with irregular branchlets; 13–22 cm high and up to 1 mm wide	cartilaginous; solid	T: 0.1–0.12 mm, C: 8–9 layers	Japan; Korea; Vietnam; Taiwan, China; China	Lin et al. (2008)
G. filicina	composed of several terete below to flattened blades above with irregularly pinnate branchlets; 9–12 cm high and up to 2.5 mm wide	mucilaginous and hard; solid	T: 1–1.2 mm, C: 5–8 layers	France, Spain, Italy	Kawaguchi et al. (2001)
G. orientalis	bushy, composed of terete to slightly compressed branches bearing irregularly pinnate branchlets; up to 16 cm high	gelatinous to cartilaginous; solid	T: 0.2–0.5 mm, C: 6–9 layers	Taiwan, China	Lin et al. (2008)

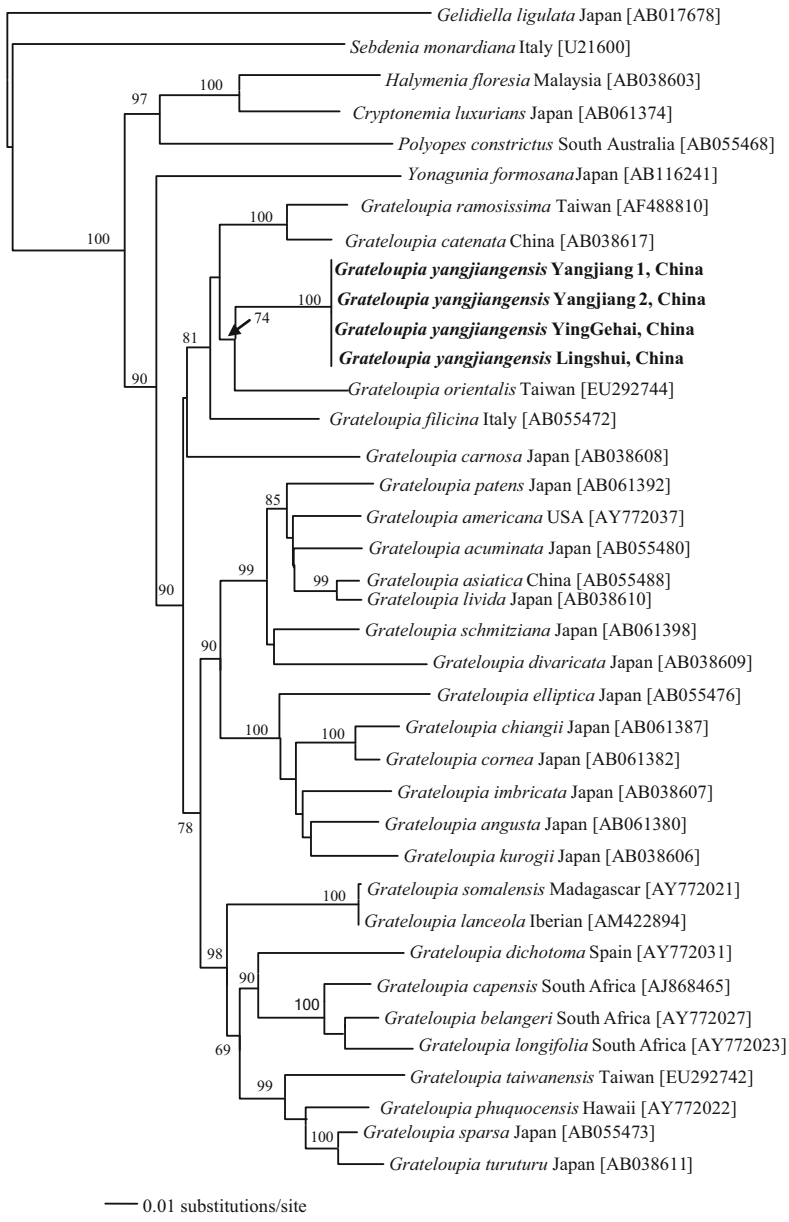

Fig.6. Maximum likelihood tree based on *rbc*L sequence data, showing phylogenetic relationships of *G. yangjiangensis* and related genera within *Grateloupia* inferred from *rbc*L gene sequences (1 259 bp). *Sebdenia monardiana* (Italy) and *Gelidiella ligulata* (Japan) were used as outgroups. Numerals at internal nodes (percentages) are bootstrap value (100 replicates); only values above 50% bootstrap support are shown. Scale bar is 0.01 substitutions per site.

between *G. yangjiangensis* and the outgroup ranged from 97 to 101 bp (9.13%–9.35%).

4 Discussion

The red algal genus *Grateloupia* (Halymeniaceae, Rhodophyta) is characterized by non-procarpic thalli in which auxiliary cells and two-celled carpogonial branches are situated in separate accessory branch systems, termed ampullae. The auxiliary cell ampullae of *Grateloupia* are simple, composed of a primary filament and two to three unbranched secondary filaments (Sjostedt, 1926; Kylin, 1930; Chiang, 1970; Kawaguchi et al., 2004). Table 2 compares the morphological structures among *G. yangjiangensis*, *G. hawaiiana* and other related species in the phylogenetic tree. By contrast, obvious differences between *G. yangjiangensis* and other species were found in their vegetative and reproductive features. These differences are the same as described in Flora Algarum Marinarum Sinicarum (Xia, 2004), and are the sole characteristic used for distinguishing *G. yangjiangensis* from other related species.

We described the life cycle of *G. yangjiangensis*, which had a typical triphasic life history with isomorphic gametophyte and tetrasporophyte. This indicated that *G. yangjiangensis* was cer-

tainly a single species. In addition, our *rbc*L sequence analysis also strongly supported that *G. yangjiangensis* was a new species.

Xia (2004) confirmed *G. yangjiangensis* as a new species only according to traditional morphological observations in Flora Algarum Marinarum Sinicarum (Xia, 2004). We found that *G. yangjiangensis* was indeed a single species within the genus *Grateloupia* on the basis of morphological observations, life cycle and *rbc*L sequence analysis. This conclusion supports and complements the description of *G. yangjiangensis* in Flora Algarum Marinarum Sinicarum. Our research also provides further criteria for the identification and cultivation of *G. yangjiangensis*.

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References

Chiang Y M. 1970. Morphological studies of the red algae of the family Cryptonemiaceae. University of California Publications in Botany, 58: 1–83

De Clerck O, Gavio B, Fredericq S, et al. 2005a. Systematics of *Grateloupia filicina* (Halymeniaceae, Rhodophyta), based on *rbc*L sequence analyses and morphological evidence, including the reinstatement of *G. minima* and the description of *G. capensis* sp. nov. Phycologia, 41: 391–410

De Clerck O, Gavio B, Fredericq S, et al. 2005b. Systematic reassessment of the red algal genus *Phyllymenia* (Halymeniaceae, Rhodophyta). European Journal of Phycology, 40: 169–178

Faye E T, Wang Hongwei, Kawaguchi S, et al. 2004. Reinstatement of *Grateloupia subpectinata* (Rhodophyta, Halymeniaceae) based on morphology and *rbc*L sequences. Phycological Research, 52: 59–67

Figueroa F L, Korbee N, De Clerck O, et al. 2007. Characterization of *Grateloupia lanceola* (Halymeniales, Rhodophyta), an obscure foliose *Grateloupia* from the Iberian peninsula, based on morphology, comparative sequences and mycosporine-like amino acid composition. European Journal Phycology, 42: 231–242

Fredericq S, Hommers M H, Freshwater D W. 1996. The molecular systematics of some agar and carrageenan-containing marine red algae based on *rbc*L sequence analysis. Hydrobiologia, 326/327: 125–135

Gavio B, Fredericq S. 2002. *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. European Journal of Phycology, 37: 349–360

Kawaguchi S, Wang Hongwei, Horiguchi T, et al. 2001. A comparative study of the red alga *Grateloupia filicina* (Halymeniaceae) from the northwestern Pacific and Mediterranean with the description of *Grateloupia asiatica* sp. nov. Phycologia, 37: 433–442

Kawaguchi S, Shimada S, Wang Hongwei, et al. 2004. The new genus *Yonagunia* Kawaguchi et Masuda (Halymeniaceae, Rhodophyta), based on *Y. tenuifolia* Kawaguchi et Masuda sp. nov. from southern Japan and including *Y. formosana* (Okamura) Kawaguchi et Masuda comb. nov. from Southeast Asia. Phycologia, 40: 180–192

Kimberly Y, Conklin A K. 2009. A molecular method for identification of the morphologically plastic invasive algal genera *Eucheuma* and *Kappaphycus* (Rhodophyta, Gigartinales) in Hawaii. Journal of Applied Phycology, 21: 691–699

Kylin H. 1930. Uber die Entwicklungsgeschichte der Florideen. Lunds Univ Arsskr NFAvd 2, 26: 1–103

Lee J I, Kim H G, Geraldino P, et al. 2009. Molecular classification of the genus *Grateloupia* (Halymeniaceae, Rhodophyta) in Korea. Algae, 24(4): 231–238

Lin Xiumei, Liang Hongyiang, Max H Hommersand. 2008. Two types of auxiliary cell ampullae in *Grateloupia* (Halymeniaceae, Rhodophyta), including *G. taiwanensis* sp. nov. and *G. orientalis* sp. nov. from Taiwan based on *rbc*L gene sequence analysis and cystocarp development. Phycologia, 44: 196–214

Litaker R W, Tester P A, Colorni A, et al. 1999. The phylogenetic relationship of *Pfiesteria piscicida*, cryptoperidiniopsoid sp. *Amyloodinium ocellatum* and a *Pfiesteria*-like dinoflagellate to other dinoflagellates and apicomplexans. Phycologia, 35: 1379–1389

Liu Fengxian, Li Weixin. 1986. A comparative study of the mode of sporogenesis of *Grateloupia filicina* C. AG. and *Grateloupia ramosissima* Okam. Journal of Fisheries of China (in Chinese), 10: 281–287

Mateo-Cid L E, Mendoza-González A C, Gavio B, et al. 2005. *Grateloupia huertana* sp. nov. (Halymeniaceae, Rhodophyta), a peculiar new prostrate species from tropical Pacific Mexico. Phycologia, 44: 4–16

Milstein D, Oliveira E C. 2012. Will a DNA barcoding approach be useful to identify *Porphyra* species (Bangiales, Rhodophyta)?. Journal of Applied Phycology, 24: 837–845

Moncalvo J M, Lutzoni F M, Rehner L S, et al. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systems Biology, 49: 278–305

Shimada S, Horiguchi T, Masuda M. 1999. Phylogenetic affinities of the genera *Acanthopeltis* and *Yatabella* (Gelidiella. Rhodophyta) inferred from molecular analyses. Phycologia, 38: 528–540

Shunpei I. 1947. Kaiso No Hassei (in Japanese). Tokyo: Hokuryukan, 95–223

Sjostedt L G. 1926. Floridean Studies. Lunds Univ Arsskr NFAvd 2, 22: 1–95

Swofford D L. 2002. Paup. Phylogenetic Analysis Using Parsimony, v 4. Sunderland, Massachusette: Sinauer Associates

Wang Hongwei, Kawaguchi S, Horiguchi T, et al. 2000. Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and *rbc*L sequences. Phycologia, 39: 228–237

Wang Hongwei, Kawaguchi S, Horiguchi T, et al. 2001. A morphological and molecular assessment of the genus *Prionitis* J. Agardh (Halymeniaceae, Rhodophyta). Phycological Research, 49: 251–261

Wilkes R J, McIvor L M, Guiry M D. 2005. Using *rbc*L sequence data to reassess the taxonomic position of some *Grateloupia* and *Dermocorynus* species (Halymeniaceae, Rhodophyta) from the north-eastern Atlantic. European Journal of Phycology, 40: 53–60

Xia Bangmei. 2004. Flora Algarum Marinarum Sinicarum (in Chinese). Beijing: Science Press, 1: 139–140

Zhao Fengjuan, Wang Aihua, Liu Jidong, et al. 2006. New phenomenon in early development of sporelings in *Gracilaria asiatica* Chang et Xia (Gracilariaceae, Rhodophyta). Chinese Journal of Oceanology and Limnology, 24: 364–369

Zhao Dan, Wang Hongwei, Sheng Yingwen, et al. 2012. Morphological observation and *rbc*L gene sequences studies of two new species, *Grateloupia dalianensis* H.W.Wang et D.Zhao, sp. nov. and *G. yinggehaiensis* H.W.Wang et R.X.Luan, sp. nov. (Halymeniaceae, Rhodophyta) from China. Acta Oceanol Sin, 31(2): 109–120

Zuccarello G C, Critchley A T, Smith J, et al. 2006. Systematics and genetic variation in commercial *Kappaphycus* and *Eucheuma* (Solieriaceae, Rhodophyta). Journal of Applied Phycology, 18: 643–651