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## *Gracilaria parvispora* is the correct name of the species known as *G. bursa-pastoris* in Korea and Japan

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The incidence of *Gracilaria bursa-pastoris*, which is found mainly in Europe, in the northwest region of the Pacific Ocean was recently brought into question following the descriptions of *G. parvispora* and *G. chouae* from Hawaii and China, respectively. We examined the morphology of thalli previously identified as *G. bursa-pastoris* in Korea and Japan, and analyzed the plastid *rbcL* gene sequences of Korean and Japanese samples, and a *G. parvispora* sample from Hawaii. The morphological and sequence data both indicate identity between the taxon from Korea/Japan and *G. parvispora* from Hawaii, but significant differences exist between *G. parvispora* and *G. bursa-pastoris* from Europe. We propose that the thalli from Korea and Japan should be renamed *G. parvispora*. The *rbcL* tree grouped *G. parvispora* and *G. damaecornis* together, as a sister to the *G. tikvahiae*/*G. isabellana*/*G. cuneifolia* clade. This is the first recorded occurrence of *G. parvispora* outside Hawaii.

**KEYWORDS:** *Gracilaria parvispora*, *Gracilaria bursa-pastoris*, morphology, *rbcL*, Rhodophyta, taxonomy

### INTRODUCTION

*Gracilaria* is one of several expensive red alga genera used in food products and as a source of agar (Abbott, 1985), comprising more than 150 recognized species worldwide (Tseng & Xia, 1999). The members of the genus have been the focus of numerous systematic studies (e.g., Gurgel & Fredericq, 2004); however, classification of the genus based on the morphological and anatomical features of its species is difficult. One taxonomic problem is that species have been reported in both the Atlantic and Pacific oceans.

*Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva (1952: 265) is commonly reported in both the Atlantic and Pacific oceans (e.g., Yoshida, 1998; Hardy & Guiry, 2003). It was originally described as a member of *Fucus* based on specimens from the Mediterranean Sea by Gmelin (1768). The nomenclature of the species, however, was recently reviewed by Silva & al. (1996) and the name is now accepted as a conserved name for *G. compressa* (C. Agardh) Greville (Steentoft & al., 1991).

Abbott (1985) found that specimens previously identified as *G. bursa-pastoris* in Hawaii differed from European specimens of *G. bursa-pastoris* and a new name, *G. parvispora* Abbott, was assigned to thalli collected from Kaneohe Bay on Oahu Island in Hawaii. Compared to *G. bursa-pastoris* from Europe, *G. parvispora* is character-

ized by small pericarp cells, with star-shaped contents, and large gonimoblast cells. Zhang & Xia (1992) also studied specimens previously identified as *G. bursa-pastoris* from southern China and described the algae as a new species, *G. chouae* Chang & Xia, based on the small cystocarp and thin pericarp compared to *G. bursa-pastoris* from Europe and *G. parvispora* from Hawaii. They also reported that the taxon known as *G. bursa-pastoris* in Japan has higher similarity with *G. parvispora* than with *G. chouae*.

To date, no taxonomic studies of so-called *G. bursa-pastoris* in Korea and Japan have been conducted. In Korea, the species was identified in Busan by Okamura (1915) as *G. compressa*. Since then, its presence as *G. bursa-pastoris* has been reported along the entire Korean coastline (Lee & Kang, 2001). In Japan, *G. bursa-pastoris* is often reported from temperate region of Honshu, Shikoku, and Kyushu Island in papers on flora and taxonomy (e.g., Yoshida, 1998). In order to verify the taxonomy of *G. bursa-pastoris* from Korea and Japan, we examined the thalloid morphology and *rbcL* gene sequence of samples from Korea and Japan. In addition, we analyzed the *rbcL* gene sequence of *G. parvispora* from Hawaii and *G. bursa-pastoris* of a putative relative from Italy. To elucidate the phylogenetic relationships of the taxa from Korea and Japan, we downloaded the *rbcL* sequence data of 68 Gracilariales from GenBank.

## MATERIALS AND METHODS

Thalli of *G. bursa-pastoris* were collected in the littoral zone of Korea and Japan: in Korea, Sinyang (10 March 2005, KMR, R04013; 7 June 2005, KMR, R03854), Jongdal (8 April 2005, KMR, R04046), Kimnyoung (7 June 2005, KMR, R03745; 19 July 2005, KMR, R04080), Nando (23 June 2001, KMR, R01301-306), Dallyeodo (5 July 2001, KMR, R01382), Hamo (6 June 2005, KMR, R03763), and Haengwon (15 October 2005, KMR, R03746); in Japan, Kagoshima (11 April 1996, Terada, 412), Kochi (27 June 1999, Terada, 956), Awaji Island (30 March 1997, Terada, 719). The specimens were collected at Honolulu, Hawaii (3 May 2004, Terada, 1618 & 1623) as *G. parvispora* and in Italy, at Sicily, Messina (28 June 2006) as a putative relative of *G. bursa-pastoris*. The thalli were fixed in 5% formalin/seawater, pressed to form herbarium sheets, and deposited in the herbarium of Chungnam National University in Korea. Cross sections of the thalli were made by hand using a stainless steel razor blade. Photographs of the sections were taken under a light microscope (Vanox AHB3; Olympus Corp., Tokyo, Japan). The habits of the specimens were documented using a digital camera (Canon Corp., Tokyo, Japan), and the images were edited and assembled on plates using Photoshop 5.5 (Adobe, San Jose, CA, U.S.A.).

For the *rbcL* analysis, five silica-gel-dried samples were used: three *G. bursa-pastoris* (Jongdal [G425, EF434942] and Sinyang [G435, EF434943] from Korea and one from Kamo Bay [G280, EF434944] in Japan), one *G. parvispora* from Hawaii (G671, EF434945), and one putative relative from Italy (G672, EF434946). Extraction of the DNA and PCR were performed as described by Kim & al. (2006). The forward and reverse sequences were determined for the *rbcL* genes from all of the taxa using an ABI PRISM™ 377 DNA Sequencer (Applied Biosystems, Foster City, CA, U.S.A.) at the Research Center, Chungnam National University, Daejeon, Korea. The electropherogram of each specimen was edited using Sequence Navigator® v. 1.0.1 (Applied Biosystems). The sequence alignment was based on the inferred amino acid sequence of each gene, and this was refined visually.

For the phylogenetic analysis, 75 *rbcL* gene sequences (1,425 bp each) were aligned; 5 of the sequences in this study, 68 gracilarioid sequences from GenBank, and 2 outgroups, *Chondrus crispus* (U02984) and *Hypnea japonica* (DQ095829). Maximum likelihood analysis was performed using PAUP\* with a general time reversible (GTR) model and proportion of invariable sites. This model of sequence

evolution was selected using Modeltest v3.6 (Posada & Crandall, 1998). The Akaike information criterion (AIC) selected GTR+I as the best-fitting model for the data. Tree likelihoods were estimated using a heuristic search with 100 random addition sequence replicates and TBR branch swapping. To test the stability of the monophyletic groups, Bayesian and bootstrap analyses were performed. Bayesian analysis was conducted with MrBayes v.3.1 (Ronquist & Huelsenbeck, 2003) GTR+I as in the ML analysis. The GTR rates and the proportion of invariable sites value were not fixed. For the data matrix, 1.3 million generations were performed with four chains and trees sampled every 100 generations. The burn-in period was identified graphically by tracking the likelihood at each generation. Based on our preliminary analyses, a burn-in period of 300,000 generations was determined to be appropriate for the data. The 10,000 trees sampled at stationarity were used to infer the Bayesian posterior probability (Bp). Majority-rule consensus trees were constructed using PAUP\*. Bootstrap values (Bt<sub>MP</sub>) from the parsimony analysis were calculated from 1,000 replicates with the following options selected: heuristic search, TBR branch swapping, collapse of zero-length branches, and random sequence addition with one replicate. Bootstrap values from the neighbor-joining analysis were calculated using 1,000 replicates with the Kimura two-parameter model.

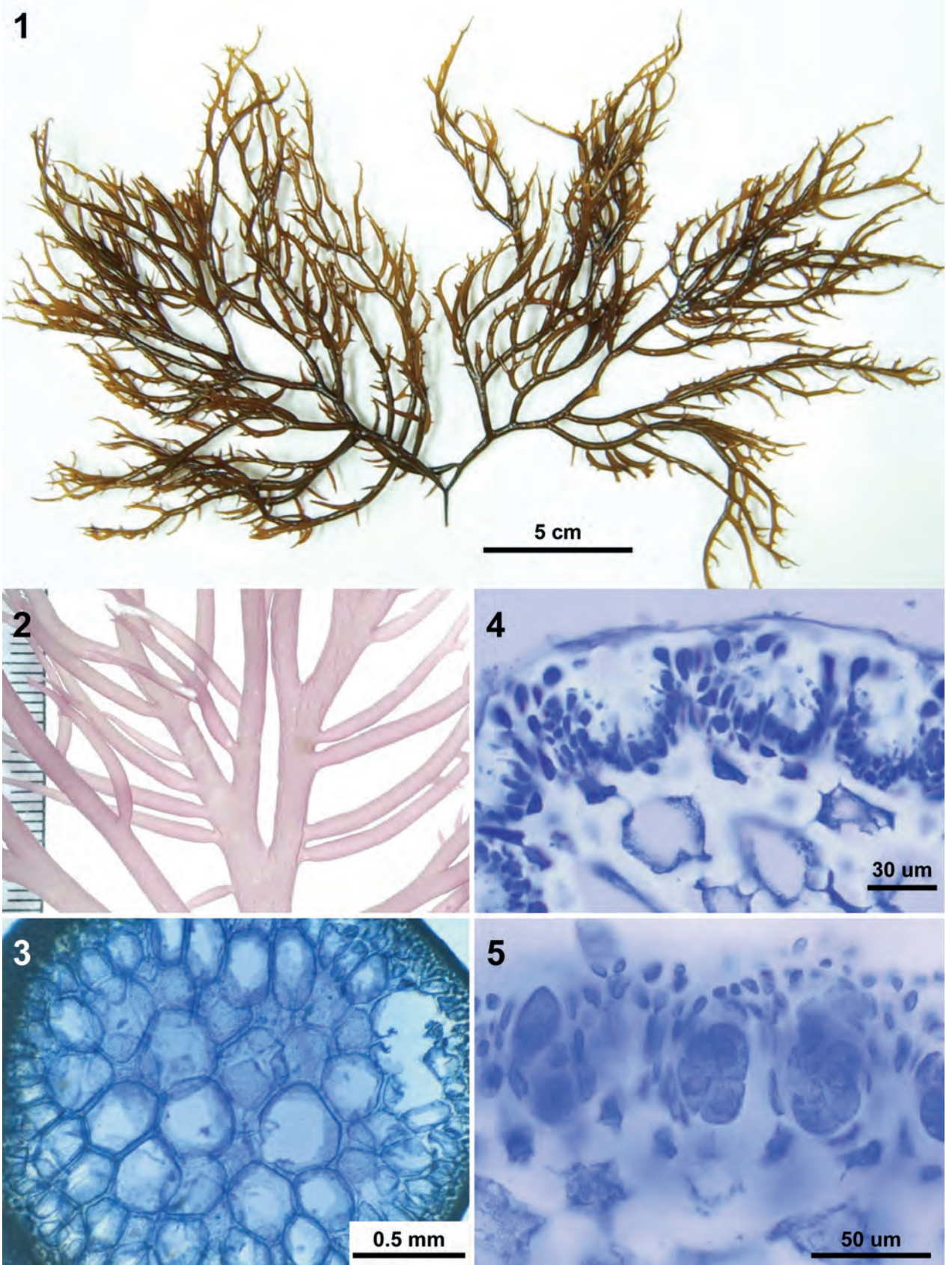
## RESULTS

**Habitat and phenology.** — Thalli of so-called *Gracilaria bursa-pastoris* occurs on rocks and stones with sand in the sublittoral zone of calm areas near the open sea. The species grows underwater at low tide, and in places with flowing water. Reproductive thalli predominate from June to July, while vegetative thalli are abundant from September to May.

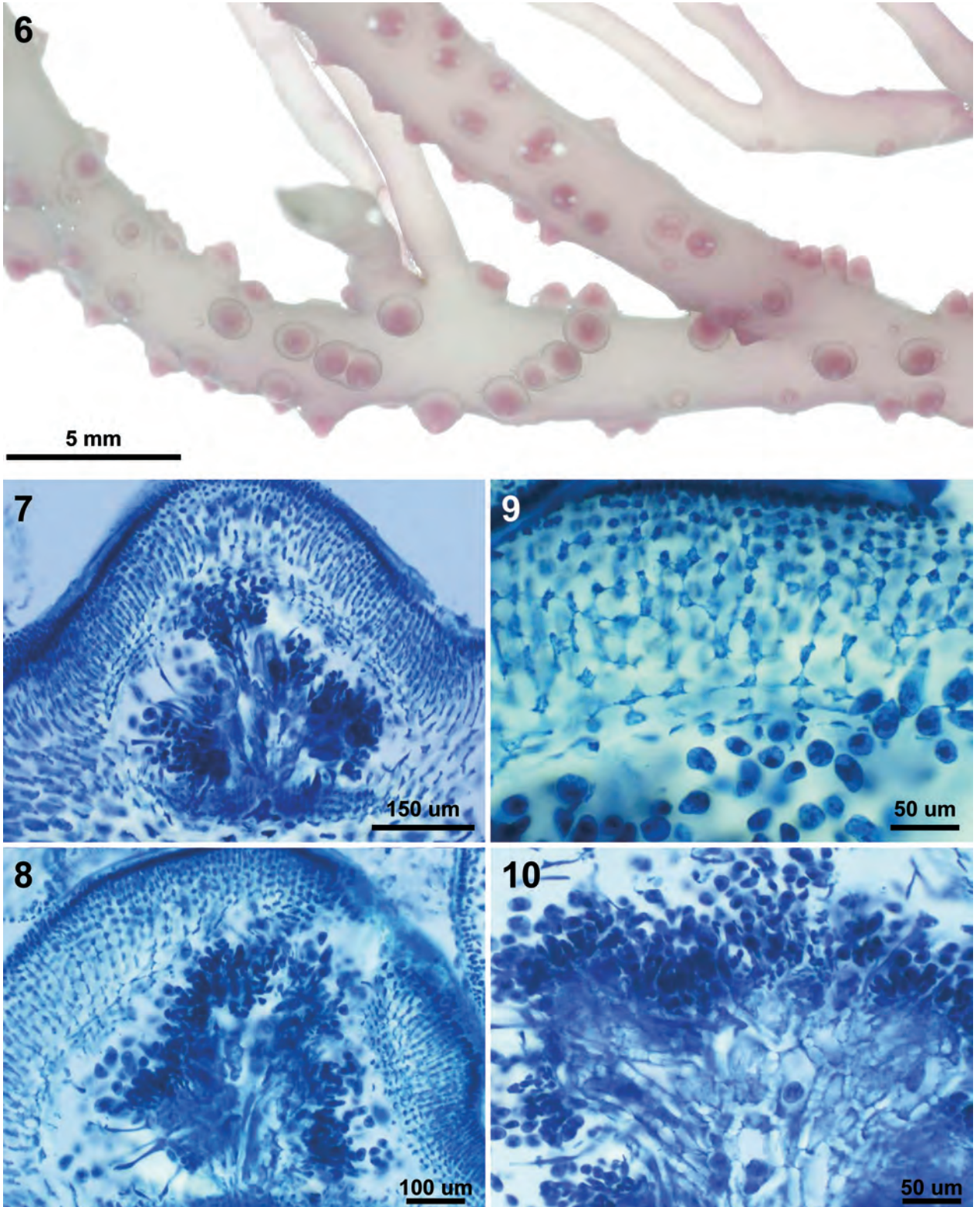
**Description.** — Thalli are erect, coarse, compressed, and solitary or caespitose; they arise from a small disk, usually 15–23 cm long and 2–3.5 mm in diameter, with two to four orders of branches, the last order being short, slender, and spinelike (Fig. 1). The alga is succulent, percurrent, easily broken, and brittle when dry, thalli are purplish red or sometimes greenish red when fresh and they do not adhere well to paper during drying; branches are irregularly alternate, occasionally furcate, and strongly mixed with unilateral or secund branching in whole or in part (Fig. 2); they gradually taper toward the apices and are not constricted where at-

**Figs. 1–5. Vegetative structure of *Gracilaria parvispora* Abbott from Jeju Island, Korea. 1. Fresh thallus from Sinyang on Jeju Island. 2. Branching pattern with conspicuous unilateral branching at the lower part. 3. Cross section showing the gradual transition from the medulla cells to the smaller cortical cells. 4. Vertical section of the spermatangial conceptacles (*textorii*-type). 5. Vertical section showing cruciately divided tetrasporangia.**









Figs. 6–10. Reproduction of *Gracilaria parvispora* Abbott from Jeju Island, Korea. 6. The cystocarp on the cylindrical middle part of the branches. 7–8. Cross section of a mature cystocarp showing tubular nutritive filaments that connect the gonimoblast with the pericarp. 9. Vertical section of pericarp with star-shaped cells and prominent secondary pit connections. 10. Vertical section of the cystocarp showing the gonimoblast consisting of large cells and tubular nutritive cells.

tached. In transverse section, the medulla of the branches consists of large pseudoparenchymatous cells 380–500  $\mu\text{m}$  in diameter, with cell walls that are 10–15  $\mu\text{m}$  thick; the medulla is surrounded by two layers of cortical cells, which are small, somewhat round, and pigmented at the outermost layer; the transition from the medulla to the cortex is gradual (Fig. 3).

*Textorii*-type spermatangial conceptacles are scattered over the surface of the fronds in small, shallow, well-defined depressions that are 45–55  $\mu\text{m}$  deep and 40–50  $\mu\text{m}$  in diameter; they are separated by modified, elongated cortical cells, which are characteristic of this type of conceptacle (Fig. 4). Cystocarps, which protrude prominently, are 1.5–2 mm long and conical or hemispherical, slightly rostrate, and not constricted at the base (Fig. 6). The pericarp is 200–260  $\mu\text{m}$  thick and consists of 10 to 12 layers of cells; the outermost layer of the pericarp is pigmented and its cells are star-shaped; the pericarp of small cells is composed of conspicuous lateral and vertical pit connections (Fig. 9). The gonimoblast is composed of large pseudoparenchymatous cells developing from a fusion cell (Fig. 10), with many nutritive tubular absorbing filaments that connect the gonimoblast to the pericarp (Figs. 7–8). Carpospores are round or ovoid. Tetrasporangia are scattered among the cortical cells, which are ovoid or subspherical in surface view, and ovoid or oblong in transverse section (30–40  $\mu\text{m}$  by 18–25  $\mu\text{m}$  in diameter), surrounded by slightly modified cortical cells; furthermore, they are cruciately divided (Fig. 5).

**Molecular phylogeny.** — Seventy-five *rbcL* gene sequences (1,425 bp each) including two outgroups were aligned. Variations at 610 positions (42.8%) and 522 positions (36.6%) were parsimony informative. The consistency index (CI) and retention index (RC) of 32 maximum parsimonious trees were 0.324 and 0.656, respectively. The three *G. bursa-pastoris* sequences from Korea and Japan showed 100% identity while *G. parvispora* from Hawaii differed by 4 bp (0.281%) (Fig. 11). The monophyly of *G. bursa-pastoris* and *G. parvispora* was strongly supported (100% Bt<sub>MF</sub> and Bt<sub>NJ</sub>, and 1.0 Bp), and they clustered with *G. damaecornis* J. Agardh, the sister clade to *G. tikvahiae* McLachlan, *G. isabellana* Gurgel, Fredericq & J. Norris in Gurgel & al. (2004) (as *G. lacinulata* (Vahl) Howe in Gurgel & Fredericq, 2004), and *G. cuneifolia* (Okamura) I. K. Lee & Kurogi. The putative relative from Italy was related to *G. gracilis* from England (100% Bt<sub>MF</sub> and Bt<sub>NJ</sub>, and 1.0 Bp) (Fig. 11).

## DISCUSSION

It is clear that the thalli described as *G. bursa-pastoris* in Korea and Japan were misidentified. The morphological and *rbcL* sequence data revealed significant

differences between our specimens and *G. bursa-pastoris* from Italy. Based on our results, we propose that some *G. bursa-pastoris*-like thalli from Korea and Japan should be renamed *G. parvispora* Abbott (1985). This is the first report of *G. parvispora* from outside Hawaii.

*Gracilaria parvispora* from Korea has several distinguishing characteristics, including compressed thalli with prominent unilateral branching (Fig. 2), conspicuous absorbing filaments in the cystocarps (Figs. 7–8), small and star-shaped cells in the pericarp (Fig. 9), and *textorii*-type (i.e., shallow depressions) spermatangia (Fig. 4). Even though the spermatangial conceptacles of the Korean *G. parvispora* are deeper (about 50  $\mu\text{m}$  deep) than those of the Hawaiian specimen (35  $\mu\text{m}$  deep) with shallow *textorii*-type spermatangial conceptacles, the samples from Jeju Island share the features of *G. parvispora* from Hawaii. Our molecular data revealed few sequence differences (0.281%) in the *rbcL* genes of the Korean and Japanese specimens and the specimen of *G. parvispora* from Hawaii. Although same values (0.281%) have been reported in the *Gracilaria* species, *G. textorii* (Kim & al., 2006), some intraspecific *rbcL* divergences of *Gracilaria bursa-pastoris* and *G. gracilis* from the Mediterranean Sea and Ireland were up to 1.22%, respectively (Gargiulo & al., 2006). Accordingly, we conclude that *G. parvispora* is the correct name for the species known in Korea and Japan as *G. bursa-pastoris*.

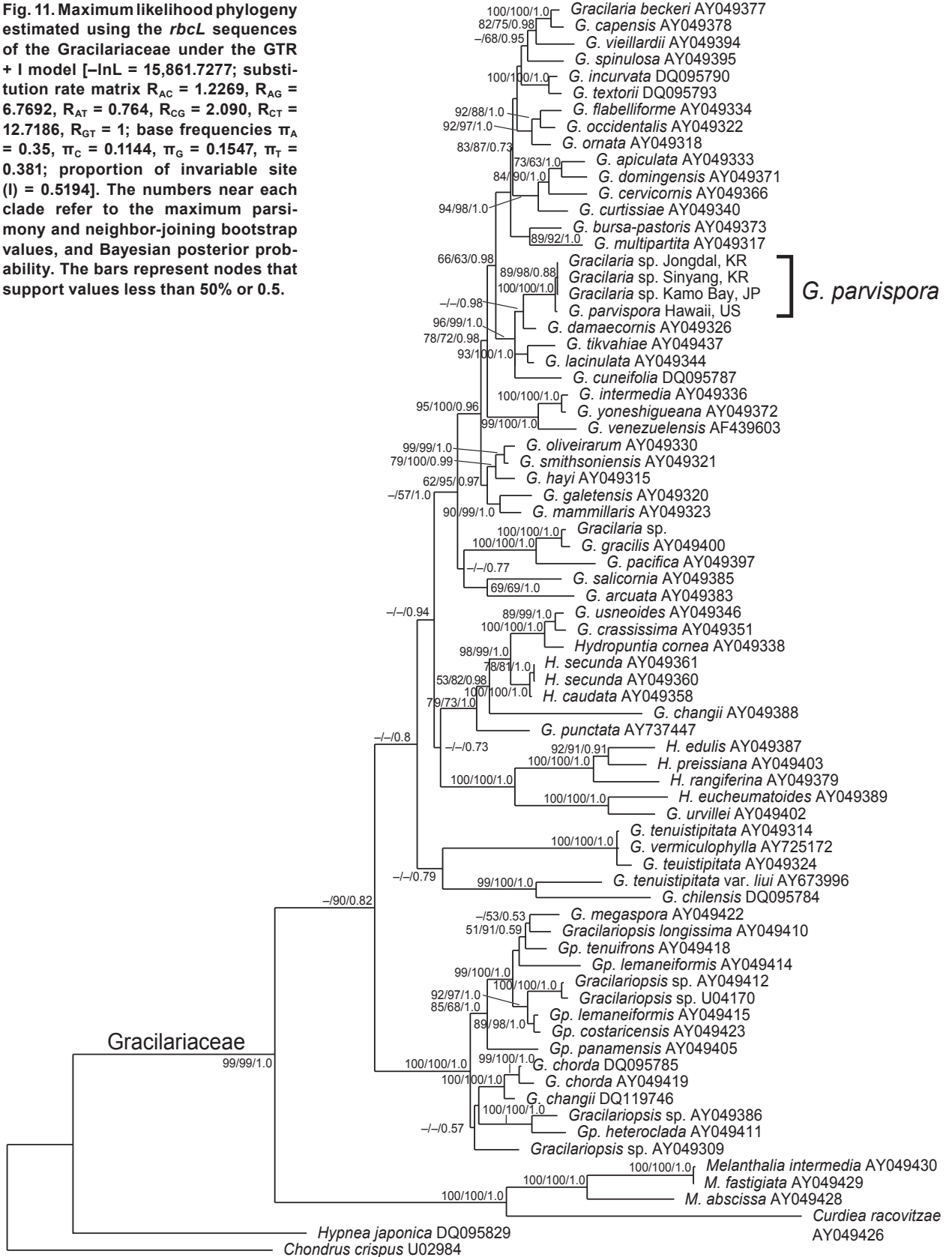
Morphologically, *G. parvispora* can be readily distinguished from the Atlantic *G. bursa-pastoris* by the small cells in its pericarp and their star-shaped contents, together with the large cells of the gonimoblast (Abbott, 1985). Zhang & Xia (1984) stated that the pericarp could be used as a primary means of distinguishing the species, and they pointed out differences among several species (Zhang & Xia, 1985, 1988, 1992). In Korean and Hawaiian *G. parvispora*, the pericarp is 200–300  $\mu\text{m}$  thick and has distinct star-shaped cellular contents (Fig. 9).

*Gracilaria parvispora* from Korea differs from *G. chouae* from China in the following respects: the medulla cells of *G. chouae* are thinner, a gradual transition occurs from the cortex to the medulla in *G. parvispora* compared to an abrupt transition in *G. chouae*, the cystocarps in *G. chouae* are smaller, the pericarp in *G. chouae* is thinner, the pericarp cells in *G. parvispora* are star-shaped while those in *G. chouae* are rounded, and the pit connections in the pericarp of *G. parvispora* run in the anticlinal/peri-clinal direction while those in *G. chouae* are strictly anticlinal. *Gracilaria chouae* occurs frequently in southern China, where the algal flora is largely tropical, while the flora in Korea is largely temperate. A molecular comparison of *G. chouae* with *G. parvispora* should produce interesting results.

*Gracilaria parvispora* belongs to subgroup VIII of the genus *Gracilaria* s.str. Gurgel & Fredericq, in spite



Fig. 11. Maximum likelihood phylogeny estimated using the *rbcL* sequences of the Gracilariaceae under the GTR + I model [–lnL = 15,861.7277; substitution rate matrix  $R_{AC} = 1.2269$ ,  $R_{AG} = 6.7692$ ,  $R_{AT} = 0.764$ ,  $R_{CG} = 2.090$ ,  $R_{CT} = 12.7186$ ,  $R_{GT} = 1$ ; base frequencies  $\pi_A = 0.35$ ,  $\pi_C = 0.1144$ ,  $\pi_G = 0.1547$ ,  $\pi_T = 0.381$ ; proportion of invariable site ( $I$ ) = 0.5194]. The numbers near each clade refer to the maximum parsimony and neighbor-joining bootstrap values, and Bayesian posterior probability. The bars represent nodes that support values less than 50% or 0.5.



— 0.01 substitution/site

of its *textorii*-type spermatangial conceptacle. Gurgel & Fredericq (2004) reported that Asian cylindrical species with *textorii*-type spermatangial pits may be part of subgroup I. The western Atlantic species *G. damaecornis*, *G. tikvahiae*, *G. isabellana* (also known as *G. lacinulata*), and *G. cuneifolia* form a sister group within *G. parvispora* in subgroup VIII. *Gracilaria parvispora* and *G. cuneifolia* are restricted to the Pacific, while the other three species are restricted to the Atlantic (Kim & al., 2006). However, *G. bursa-pastoris* from Italy belongs to *Gracilaria* subgroup IX, which consists mostly of derived species with flat or compressed thalli and *textorii*-type spermatangial conceptacles. To determine how many times *textorii*-type spermatangial conceptacles within the viewpoint of the evolutionary history have arisen in *Gracilaria* s.str., further molecular analyses with additional *Gracilaria* taxa from various ocean regions are needed.

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