

# *Gracilariopsis nhatrangensis* (Gracilariaceae, Rhodophyta), a new marine red alga from Nhatrang, southern Vietnam

Le Nhu HAU<sup>1</sup> and Showe-Mei LIN<sup>2,\*</sup>

<sup>1</sup>*Institute of Materials Science, Nhatrang Branch, Nha Trang City, Khanh Hoa Province, Viet Nam*

<sup>2</sup>*Department of Natural Science Education, National Taitung University, Taitung 950, TAIWAN, Republic of China*

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**ABSTRACT.** A new species of *Gracilariopsis*, *Gp. nhatrangensis* sp. nov. Le et Lin, is described from Vietnam. *Gracilariopsis nhatrangensis* is unique in consisting of 1-15 main axes arising from a discoid holdfast and the main axes bearing numerous, racemose branchlets. The molecular analysis based on the *rbcL* gene of the species of *Gracilariopsis* sourced worldwide with the *chorda*-type spermatangial conceptacles supports the proposal of this new species.

**Keywords:** Gracilariaceae; *Gracilariopsis nhatrangensis*; Marine red alga; New species; Rhodophyta; Taxonomy; Vietnam.

## INTRODUCTION

The species of the family Gracilariaceae have economic importance to the agar and colloid industries, and their phylogenetic relationships have been extensively studied using DNA sequencing in recent years (Gurgel et al., 2003a, 2003b; Iyer et al., 2005a). Gurgel et al. (2003a) gave a comprehensive study on the molecular systematics of the five non-parasitic genera within the family Gracilariaceae (*Gracilaria* Greville, *Gracilariopsis* Dawson, *Curdiea* Harvey, *Hydropuntia* Montagne, and *Melanthalia* Montagne). *Gracilariopsis* was established by Dawson (1949) based on gonimoblast (=carposporophyte) development and the absence of any nutritive tubular cells connecting to the pericarp. Papenfuss (1966) sank *Gracilariopsis* into *Gracilaria* after comparing the cystocarp morphology of *Gracilariopsis sjoestedtii* Kylin [the generic type of *Gracilariopsis* at that time, currently known as *Gp. lemaneiformis* (Bory) Weber-van Bosse] with *Gracilaria verrucosa* (Hudson) Papenfuss [the generic type of *Gracilaria* at that time, currently recognized as *G. bursa-pastoris* (Gmelin) Silva].

Fredericq and Hommersand (1989) resurrected the genus *Gracilariopsis* using the type method and re-examined the generic type of *Gracilariopsis*, *Gp. lemaneiformis*, collected from California, USA. They emphasized the importance of the gonimoblast cells that linked to gametophytic cells in the floor of the cystocarp by means of secondary

pit connections and lack of multinucleate tubular cells in the cystocarp as well as the a continuous superficial layer of spermatangia as proposed by Dawson (1949). In recent years, several studies on the phylogenetic relationships between *Gracilaria* and *Gracilariopsis*, based on analyses of both chloroplast-encoded *rbcL* and nuclear small subunit (SSU) rRNA sequences, have show that all species of *Gracilariopsis* formed a distinct, monophyletic assemblage (Bird et al., 1994; Bellorin et al., 2002; Gurgel and Fredericq, 2004; Gurgel et al., 2003a; Iyer et al., 2005a).

Thirteen species of *Gracilariopsis* have been reported from the Pacific Ocean (see Gurgel et al., 2003b for a review). Gurgel et al. (2003b) added three new species into *Gracilariopsis* (*Gp. silvana* Gurgel, Fredericq et Norris, *Gp. hommersandii* Gurgel, Fredericq et Norris and *Gp. cata-luziana* Gurgel, Fredericq et Norris) from the western Atlantic Ocean. Later, Iyer et al. (2005b) described another new species of *Gracilariopsis*, *Gp. funicularis*, from southern Africa. Recent collections from Cua Be, Nha Trang, Vietnam, contain an unnamed species of *Gracilariopsis* found at a depth of 5-10 m in depth. The vegetative and reproductive morphology of *Gracilariopsis nhatrangensis* from Vietnam are documented in detail in this study and its taxonomic status is discussed based on its morphology and *rbcL* sequence analysis.

## MATERIALS AND METHODS

Collections were made by SCUBA at depths of 5-10 m. Algal samples for the morphological study were

\*Corresponding author: E-mail: smlin@nttu.edu.tw.

preserved in 3-5% formalin-seawater or pressed on herbarium sheets while materials used in the molecular study were desiccated in silica gel. Voucher specimens were deposited in the Herbaria of the National Taitung University (Taiwan) and Institute of Marine Science, Nha Trang Branch (Vietnam). Hand sections were stained with 1% aniline blue acidified with 1% HCl and mounted in 25-30% Karo<sup>®</sup> syrup (Englewood Cliffs, USA). Photomicrographs were taken on an Olympus BX51 microscope with a Q-imaging digital camera (Burnaby, BC, Canada) or an Epson scanner (Tokyo, Japan) for capturing habit pictures.

DNA samples were prepared using the DNeasy

Plant Mini Kit (Qiagen, Valencia, CA, USA) following the instructions of the manufacturer. DNA sequencing procedures were as described in Lin et al. (2001, 2004). New sequence data and those available from GenBank were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. Phylogenetic analyses were performed using the maximum parsimony method (MP) available in the computer program PAUP\* v4.0 (Swofford, 2003). An *rbcL* sequence for the new species *Gracilariopsis nhatrangensis* was newly generated in this study and compared with those of the named species of *Gracilariopsis* available from GenBank (see Table 1).

**Table 1.** List of species used in *rbcL* analysis and accession numbers in GenBank. The number after the accession number is the percentage of the gene sequenced.

Species	Collection information & GenBank accession number
<i>Curdia coriacea</i> (Hook. et Harv.) J. Agardh	AY049425*, 66.5%
<i>Curdia crassa</i> Millar	AY049427*, 98.1%
<i>Gracilaria bursa-pastoris</i> (Gmelin) Silva	AY049376*, 91.6%
<i>Gracilaria salicornia</i> (C. Agardh) Dawson	AY049385*, 98.0%
<i>Gracilaria tenuistipitata</i> Chang et Xia	NC_006137, 100% (Hagopian et al., 2004)
<i>Gracilariopsis andersonii</i> (Grunow) Dawson	AY049413*, 94.2%
<i>Gracilariopsis bailinae</i> Zhang et Xia	AY049411*, as <i>Gracilariopsis heteroclada</i> , 91.1%
<i>Gracilariopsis carolinensis</i> Liao et Hommersand in Gurgel et al.	AY049412*, 96.7%
<i>Gracilariopsis cata-luziana</i> Gurgel, Fredericq et J. Norris	AY049406*, 80.2%
<i>Gracilariopsis costaricensis</i> Dawson	AY049423*, 98.4%
<i>Gracilariopsis hommersandii</i> Gurgel, Fredericq et J. Norris	AY049405*, as " <i>Gp. aff. panamensis</i> ", 97.1%
<i>Gracilariopsis lemneiformis</i> (Bory de Saint-Vincent) E.Y. Dawson, Acleto et Foldvik	AY049415*, 97.6%
<i>Gracilariopsis longissima</i> (Stackhouse) Irvine, Steentoft et Farnham	AF527881*, 97.5%
<i>Gracilariopsis megaspora</i> Dawson	AY04922*, 97.8%
<i>Gracilariopsis funicularis</i> Iyer, Bolton et Coyne	AY049410*, 98.2% (Namibia), as <i>Gracilariopsis "lemneiformis"</i> from Namibia in Gurgel et al. (2003a). Note: Iyer et al. (2005b, p. 536) mentioned that the <i>Gracilariopsis "lemneiformis"</i> from Namibia in Gurgel et al. (2003a) is <i>Gracilariopsis funicularis</i> .
<i>Gracilariopsis nhatrangensis</i> Hau et Lin sp. nov.	Coll. Hau Le, 15.iii.2004, Cua Be, Nha Trang, Vietnam DQ119744, 78% (This study).
<i>Gracilariopsis silvana</i> Gurgel, Fredericq et J. Norris	AY049309*, 96.7%
<i>Gracilariopsis chorda</i>	AY049419*, 97.8% (Japan), as <i>Gracilariopsis</i> sp. 3 in Gurgel et al. (2003a)
<i>Gracilariopsis chorda</i>	AY049421*, 65% (China), as <i>Gracilariopsis</i> sp. 3 in Gurgel et al. (2003a)
<i>Gracilariopsis tenuifrons</i> (Bird et Oliveira) Fredericq et Hommersand	AY049418*, 97.8%
<i>Hydropuntia eucheumatoides</i> (Harvey) Gurgel et Fredericq	AY049389*, 93.3%
<i>Melanthalia obtusata</i> (Labillardière) J. Agardh	AY049431*, 99%

\*Refers to Gurgel et al. (2003a).

## RESULTS

### Species description

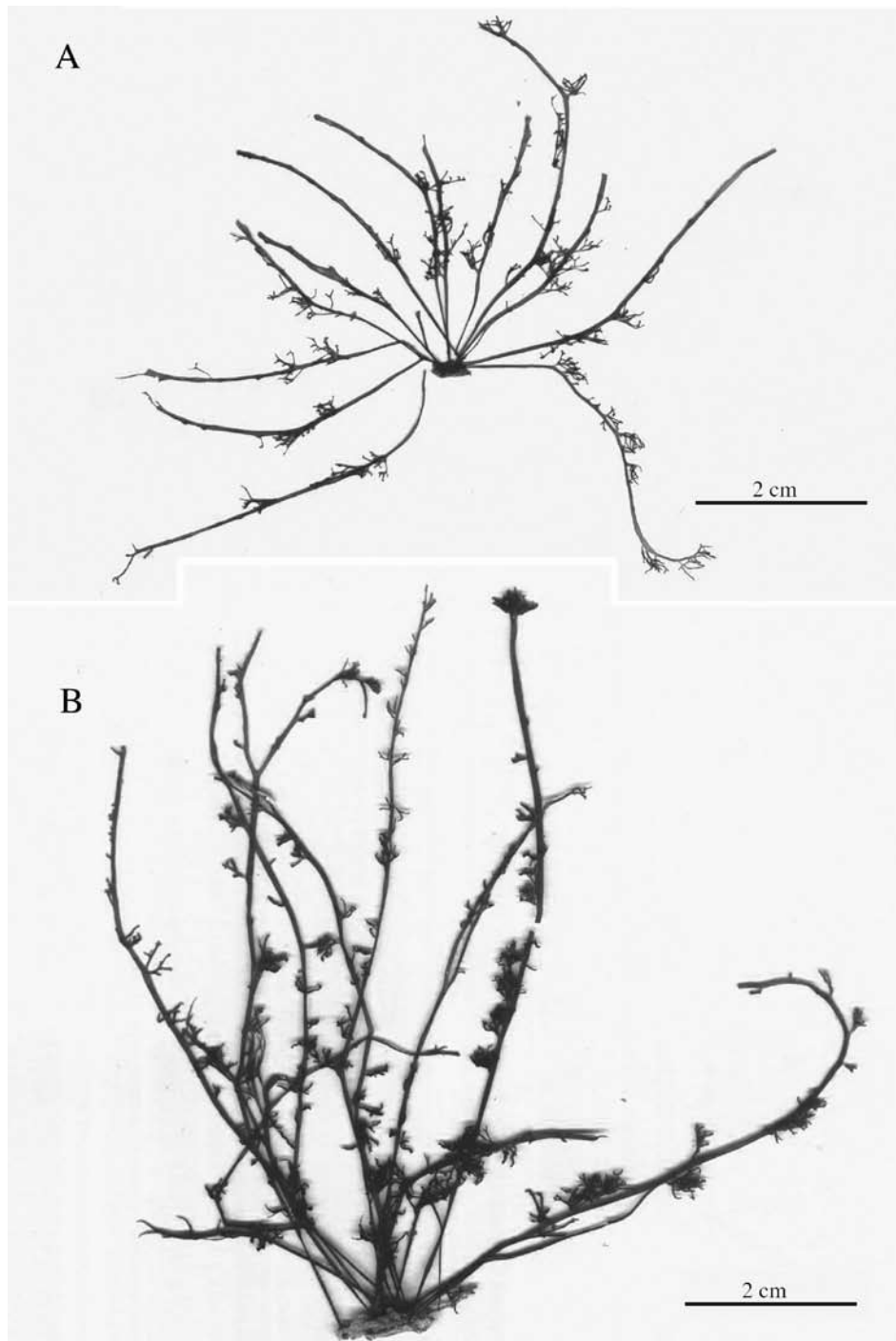
*Gracilariopsis nhatrangensis* Hau et Lin sp. nov.

Thallus erectus, cylindrical, usque ad 5-18 cm altum, puniceus ad rufus; constantes 1-15 stipes oriundus unico haptero parvo circularis, gestum plures ramificata, paululum termes; spermatia dispersa super laminam, valdosi *chorda*-typi; cystocarpia hemisphaerica, non

constricta basim, cellulae tubulosae nutriciae absentis, carposporangia portata in series cateniformis ramose; tetrasporangia superficialia, divisa cruciata.

*Etymology.* Named for the city, Nhatrang, where this alga was collected.

*Holotype.* In Institute of Marine Science, Nhatrang Branch, Vietnam. March 15, 2004, no. IMS 204610 (Figure 1A).



**Figure 1.** *Gracilariopsis nhatrangensis* Hau et Lin sp. nov. A, Holotype: female plant (#IMS204610); B, Isotype: tetrasporic plant (#IMS205102).

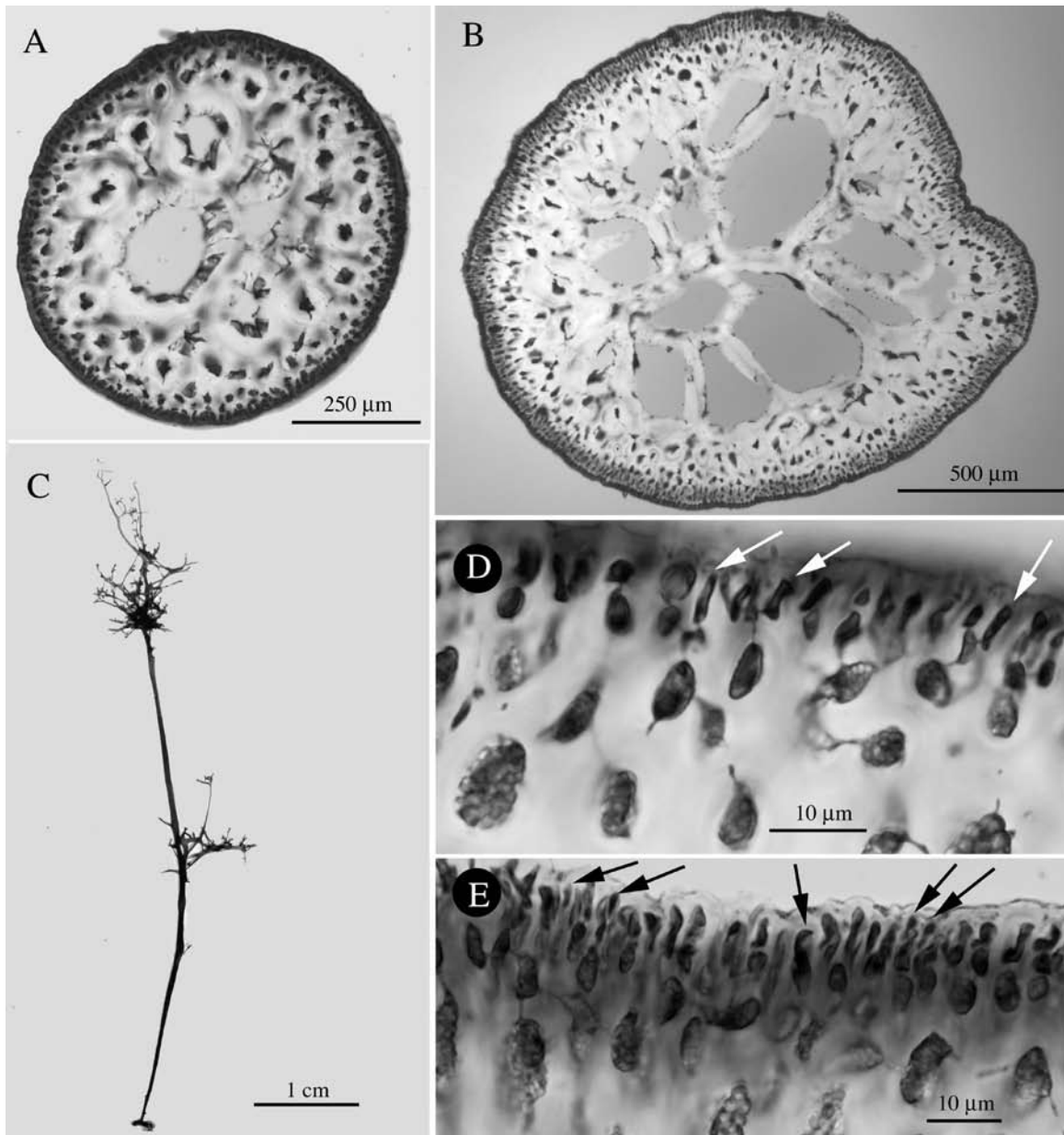
*Type locality.* Cua Be, Nhatrang, Vietnam (12°09'N, 109°13'E).

*Distribution, Habitat and seasonality.* Restricted to the coast of Cua Be, Nhatrang, Vietnam, growing on living shells (*Conus tessulatus*) at 5-10 m deep. The collections were made seasonally from November 2002 through June 2005.

*Specimens examined.* Cua Be, Nhatrang, Vietnam: coll. Hau L. N., IMS 202 400, 20.xi.2002 (female and male); IMS 203 606, 1.xi.2003 (male); 8.xi.2003 (tetrasporic);

IMS203610, 15.iii.2004 (female); IMS204611, 1.iv.2004 (female); 2.iv.2004 (female and tetrasporic); IMS205102, 1.vi.2005 (tetrasporic); IMS204111, 2.iv.2004 (tetrasporic); IMS 205125, 23.VII.2005 (female).

*Habit and vegetative structure.* Thalli (Figures 1A, 1B, 2C) are erect, terete to cylindrical, cartilaginous, 5-18 cm in height, light to purplish red in color, consisting of 1-15 main axes, 1.2-2.2 mm in diameter, arising from a discoid holdfast, 2-5 mm in diameter. Main axes are usually branched 1-2 orders from the base, composed of 3-4

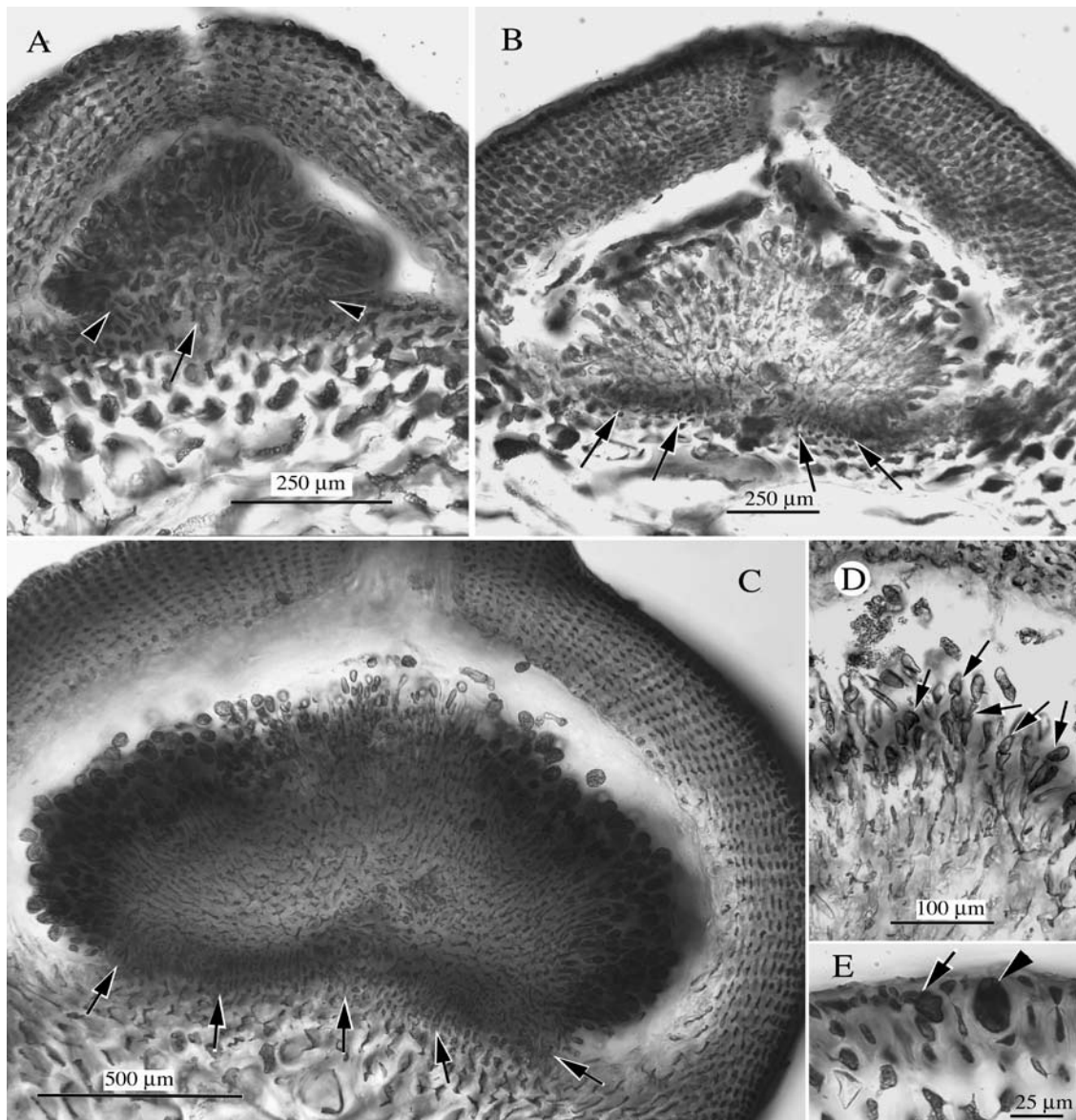


**Figure 2.** *Gracilariopsis nhatrangensis* Hau et Lin sp. nov. A, Transverse section through a racemose branchlet showing small-celled cortex and vacuolated cells of the medulla in the center; B, Transverse section through a main branch showing small-celled cortex and vacuolated and large cells of the medulla; C, Habit of a male plant; D, Transverse section of a fertile branch of a male plant showing spermatangial mother cells (arrows) resulting from oblique longitudinal cell divisions; E, Transverse section through a fertile branch of a male plant showing newly formed spermatangia (arrows), a chorda-type spermatangial conceptacle.

layers of pigmented, ovoid cortical cells, 6–7  $\mu\text{m}$  in diameter, 1–3 layers of subcortical cells, 10–18  $\mu\text{m}$  in diameter, and a large, thin-walled medulla, 180–250  $\mu\text{m}$  in diameter (Figure 2B). Numerous, racemose branchlets, 2–5 mm in length and 0.3–0.7 mm in diameter, are borne at middle to upper parts of the main axes (Figure 2A). Thalli are imperfectly adhered to the herbarium papers when dried. In general, male plants (Figure 2C) are smaller than the female gametophytes and tetrasporophytes.

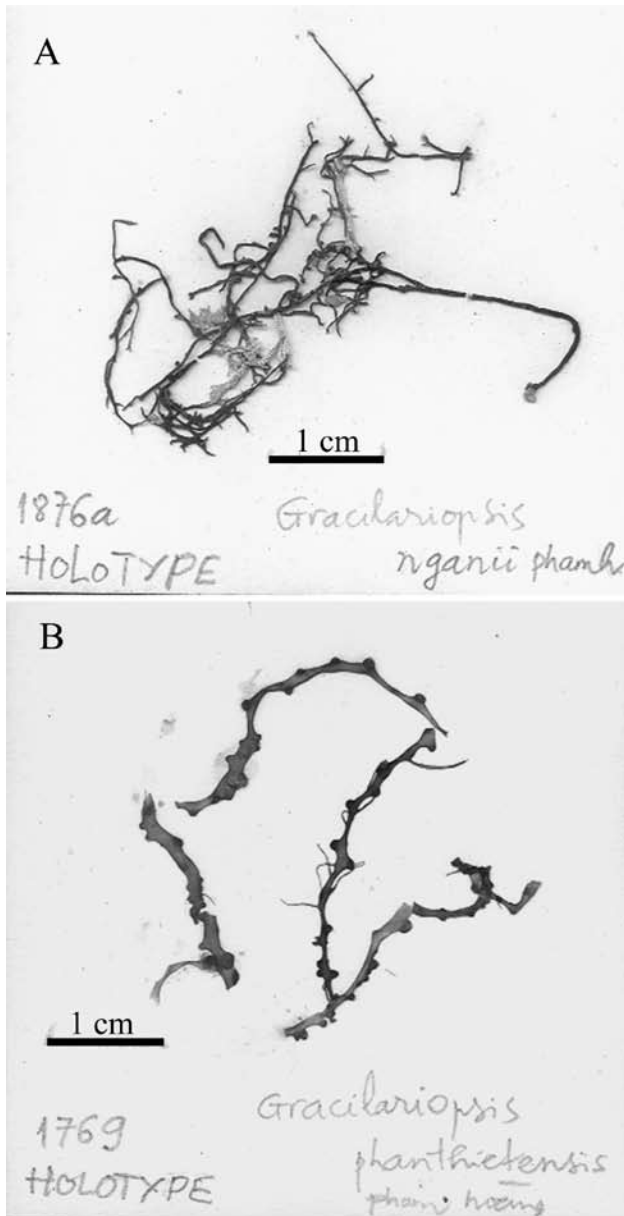
**Reproductive structures.** The tetrasporophytes and gametophytes are isomorphic and the gametophytes are dioecious. Reproductive structures are scattered over on the

fertile branches. Spermatangial parent cells are initiated from outer cortical cells (Figure 2D, arrows) by oblique longitudinal cell divisions, followed by unequal oblique or transverse cell division to produce spermatangia (Figure 2E, arrows) distally. Cystocarps are scattered over the fertile branches, usually at middle portions of the main branches. Carpogonial branches and very early post-fertilization stages were not found in the examined materials. At an early stage of gonimoblast development, the fusion cell cuts off gonimoblast cells in dense clusters, and the innermost cells of the gonimoblasts soon become vacuolate (Figure 3A). Later, the vacuolated cells between different

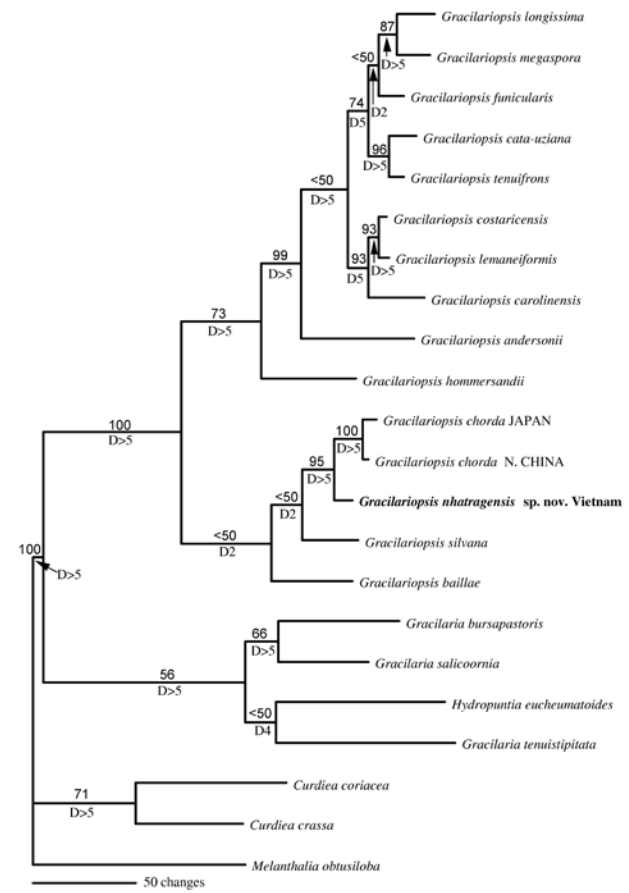


**Figure 3.** *Gracilariopsis nhatrangensis* Hau et Lin sp. nov. A, An early post fertilization stage showing a fusion cell (arrow) bearing dense gonimoblast clusters (arrowheads); B, Cross-section of immature cystocarp showing vacuolated inner gonimoblast cells and some pit-connecting (arrows) to vegetative cells on the floor; C, Transverse section of a mature cystocarpic showing dense, small-sized gonimoblast filaments and pit-connections (arrows) between lower gonimoblast cells and vegetative cells in the floor; D, Close-up of carposporangia (arrowheads); E, Cross-section through a tetrasporic plant showing a mature tetrasporangium (arrowhead) and an immature one (arrow).

gonimoblast clusters are united by numerous secondary pit-connections while the vegetative cells in the floor of the cystocarp cavity are secondarily pit-connected with the inner cells of the gonimoblasts near the floor (Figure 4B). Tubular nutritive cells are totally absent in the cystocarp cavity. Mature cystocarps are dome-shaped, broad based, and not constricted at the point of attachment with the pericarps' 11-17 cell layers (Figure 4C). Carposporangia are borne in branched chains, 15-20  $\mu\text{m}$  wide by 20-25  $\mu\text{m}$  long (Figure 4D). Tetrasporangia are initiated superficially from outer cortical cells through an oblique, longitudinal cell division of terminal cells, and then expand and divided twice to produce four cruciately arranged tetraspores at maturity, 20-30  $\times$  10-20  $\mu\text{m}$  in diameter (Figure 4E).



**Figure 4.** The two species of *Gracilariopsis* previously described from Vietnam. A, Holotype of *Gracilariopsis nganii*; B, Holotype of *Gracilariopsis phanthietensis*.



**Figure 5.** One of two most parsimonious trees from analysis of the *rbcL* sequence data. Bootstrap proportion values are shown above nodes and thick bold branches correspond to 100% support; decay indices are shown below nodes. Branch lengths are proportional to the amount of sequence change.

### Molecular analysis

Fifteen *rbcL* sequences of species of *Gracilariopsis*, sourced worldwide and possessing the *chorda*-type of spermatangial conceptacles, and a set of four additional representative species belonging to the genera *Gracilaria* and *Hydropuntia* were selected for analysis, together with two species of *Curdiea* and one species of *Melanthalia*, which served as the outgroup (see Table 1, Figure 5). No insertion or deletion mutations were found in the *rbcL* sequences used in this study, allowing for unambiguous alignment of all sequences. The final *rbcL* data matrix was restricted to 1407 sites as the first 60 bases of the sequenced gene at 5' end were not complete. Parsimony analysis revealed two most parsimonious trees with a length of 1111 steps, CI = 0.577 and RI = 0.545; there were 339 informative characters out of 1407 included sites (24.1%). Bootstrap proportion values (1000 replicates, above) and decay indices (below) derived from maximum parsimony analysis are shown on the nodes. The proposed new species, *Gracilariopsis nhatrangensis*, from Vietnam and two collections of *Gp. chorda* from China and Japan

**Table 2.** Morphological comparison of all the recognized species of *Gracilariopsis*.

Recognized species	Maximum height (cm)	Main axes (mm in diameter)	Branching	Pericarp (cell layers)
<i>G. andersonii</i> (Grunow) Dawson <sup>A,D</sup>	200	Cylindrical, 0.5-1.5	Sparsely & irregularly with numerous short proliferations.	9-11
<i>G. bailiniiae</i> (Zhang et Xia) Zhang et Xia <sup>C</sup>	70	Cylindrical, 1-2 (-3)	Irregularly alternate or unilateral.	7-10
<i>G. carolinensis</i> Liao et Hommersand in Gurgel et al. <sup>A</sup>	200	Terete, 0.5-2	Sparse, irregular, radial & short proliferations.	8-10
<i>G. cata-luzitana</i> Gurgel, Fredericq et J. Norris <sup>B</sup>	25 (-36)	Terete to slightly compressed, 0.37-0.55	Elongate, alternate to linear.	Unknown
<i>G. chorda</i> (Holmes) Ohmi <sup>E</sup>	150 (-200)	Cylindrical to compressed, up to 5	Alternately or irregularly with long or short filiform.	6-8
<i>G. costaricensis</i> Dawson <sup>F,A</sup>	25	Cylindrical, 0.8	Sparsely, few or absent.	8-15
<i>G. hommersandii</i> Gurgel, Fredericq et J. Norris <sup>B</sup>	45	Terete, 1-2.3	Stringy, slender, cartilaginous to profuse with terminal uncinat branchlets.	9-10
<i>G. lemaneiformis</i> (Bory de Saint-Vincent) Dawson <sup>A,D</sup>	100	Terete, flagelliform, 0.5-1.3	Sparsely & irregularly.	14-18
<i>G. longissima</i> (Gimelin) Steentoft, Irvine et Farnham <sup>G,K</sup>	90 (-135)	Terete, 1-3	Slender, densely irregularly.	Up to 18
<i>G. megaspora</i> Dawson <sup>P</sup>	10	Cylindrical, <0.5	Abundantly sub-determinate.	Not stated.
<i>G. nganii</i> Pham <sup>H,I</sup>	3-5	Terete, 0.2-0.3	Subdichotomous.	15-18
<i>G. nhatrangensis</i> Hsu et Lin sp. nov. (herein) <sup>I</sup>	5-18	Terete, 1.2-2.2	Numerous, racemose branchlets.	11-17
<i>G. panamensis</i> (W. Taylor) Dawson <sup>B,D</sup>	165	Terete & slender, 0.15-0.3	Simple or alternately near the base.	Not stated.
<i>G. phanhiensis</i> Pham <sup>H,I</sup>	20	Terete, 2-3	Alternately or unilaterally.	7-10
<i>G. rhodotricha</i> Dawson <sup>P</sup>	20	Cylindrical & slender, seldom exceeding 0.7	Remote, indeterminate, long-attenuate.	Not stated. <sup>L</sup> Note that the spermatangia are borne in deep pockets, unlike in <i>Gracilariopsis</i> .
<i>G. silvana</i> Gurgel, Fredericq et J. Norris <sup>B</sup>	20	Flattened, strap-shaped.	Sparingly or profusely sub-dichotomously or irregularly branched.	12-14
<i>G. tenuifrons</i> (Bird et Oliveira) Fredericq et Hommersand <sup>F,J</sup>	40	Cylindrical, up to 1.	Loosely, branched, sometimes curly and tangled with short filiform ultimate branchlets.	8-11

<sup>A</sup>Gurgel et al. (2003a); <sup>B</sup>Gurgel et al. (2003b); <sup>C</sup>Zhang et Xia (1999); <sup>D</sup>Dawson (1949); <sup>E</sup>Yamamoto (1978); <sup>F</sup>Bird & de Oliveira (1986); <sup>G</sup>Steenstoff et al. (1995); <sup>H</sup>Pham (1969); <sup>I</sup>This study.; <sup>J</sup>Fredericq & Hommersand (1989); <sup>K</sup>Iyer et al. (2005b); <sup>L</sup>Professor Max H. Hommersand (personal communication).

were closely associated and clustered in a single clade with a strong bootstrap support (95% out of 1000 re-samplings). The remaining species of *Gracilariopsis* included in the dataset were distantly related to the species from the western Pacific. Interspecific *rbcL* sequence divergences (pairwise distance) among the analyzed species with the *chorda*-type clade varied from 0.63 to 7.48%.

## DISCUSSION

The *rbcL*-based phylogenetic analysis of the 13 species of *Gracilariopsis* with the *chorda*-type spermatangial conceptacles collected worldwide resulted in a single clade (see Figure 5). This result is similar to the tree topology shown in Gurgel et al. (2003a, Figure 1) and is based on the same molecular marker, the *rbcL* gene. The *rbcL* topology shown in this study is also congruent to Iyer et al. (2005a). *Gracilariopsis nhatrangensis* from Vietnam is closely related to *Gp. chorda* from Japan and China and is distinct from the other named species within the genus based on plastid *rbcL* DNA sequence analysis.

Most species of *Gracilariopsis* are up to 70 (-100-200) cm in height (Dawson, 1949; Gurgel et al., 2003a, 2003b; also see Table 2 for a brief description of each described species.). Pham (1969) described two new species of *Gracilariopsis*, *Gp. nganii* and *Gp. phanthietensis*, originally from Vietnam. However, since that time, Pham's two species of *Gracilariopsis* have never been re-collected. The holotype of *Gracilariopsis nganii* is a slender, cylindrical plant about 6.5 cm in height with 2-3 orders of sub-dichotomous branches, 0.2-0.3 mm in diameter (see Figure 4A) while the holotype of *Gp. phanthietensis* is a coarser, terete plant with main branches 2-3 mm in diameter (see Figure 4B). On the other hand, *Gracilariopsis nhatrangensis* is characterized mainly by 1-15 main branches (up to 18 cm tall), arising from a discoid holdfast. The main axes bear numerous, racemose branchlets, and its unique branching pattern distinguishes this new species easily from the other named species around the world.

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## 來自越南南部芽莊地區擬龍鬚菜（紅藻門，龍鬚菜科）海洋紅藻一新種：芽莊擬龍鬚菜

Le Nhu HAU<sup>1</sup> 林綉美<sup>2</sup>

<sup>1</sup>越南芽莊分所材料科學研究所

<sup>2</sup>國立台東大學自然科學教育學系

本文發表來自越南南部芽莊地區擬龍鬚菜一新種：芽莊擬龍鬚菜。此新種外形與其它已知的擬龍鬚菜藻種十分不同，藻體具有 1-15 的主軸枝，叢生自於一圓盤狀的固著器，且具有許多圍繞主軸繁生有如稻穗短小分枝。分析與比較來自世界各地的擬龍鬚菜藻種的葉綠體二磷酸核酮糖羧化酶的大次單元基因（Chloroplast-encoded gene, *rbcL*）序列，結果亦支持芽莊擬龍鬚菜為一世界新種。

**關鍵詞：**分類；越南；紅藻門；龍鬚菜科；芽莊擬龍鬚菜；新種；海洋紅藻。