

# Three new members of Characeae (Charales, Chlorophyta) from Taiwan, including one endangered monospecific genus

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**ABSTRACT.** From morphological and molecular analysis, three charalean species, *Lychnothamnus barbatus* (Meyen) Leonhardi, *Nitella japonica* Allen, and *N. inversa* Imahori are recorded for the first time to the freshwater algal flora of Taiwan. The endangered monospecific genus, *Lychnothamnus* (Ruprecht) Leonhardi, is the first genus recorded to the freshwater algal flora of Taiwan. In this study, we describe their morphological characters in detail and use *rbcL* and *atpB* sequences to confirm their phylogenetic relationships.

**Keywords:** *atpB* gene; Characeae; *Lychnothamnus*; *Nitella*; *rbcL* gene; Taiwan.

## INTRODUCTION

There are six genera in the Family Characeae. Both *Chara* Linnaeus and *Nitella* Agardh are widespread and abundant species; both *Lamprothamnium* J. Groves and *Tolypella* (A. Braun) A. Braun have a few species; and both *Nitellopsis* Hy and *Lychnothamnus* (Ruprecht) Leonhardi are represented each by only one extant species. The single extant species of *Lychnothamnus*, *L. barbatus* (Meyen) Leonhardi, is actually a rare charophyte species considered in decline (Casanova et al., 2003). The species is the first charophyte declared endangered in Australia (García, 2003).

In Taiwan, freshwater algal studies have mainly focused on microalgae (Yamagishi, 1992; Moriwaka and Chyi, 1996; Wang and Chen, 2000; Wang et al., 2002) and very little on macroalgae (Wu, 1999, 2001; Liu and Wang, 2004). Reports on Characeae from Taiwan seldom appear, except for some by Imahori (1951, 1953, 1954a, 1957) and Yang and Chiang (1978). In total, 21 species, 4 subspecies, and 10 varieties from two charalean genera have been recorded in Taiwan (Imahori, 1951, 1953, 1954a, 1954b, 1957). Recently, observations of oospore wall ornamentation using scanning electron microscopy (SEM) have been used to identify the species of the Order Charales (John and Moore, 1987; John et al., 1990; Leitch et al., 1990; Casanova, 1991, 1997). Furthermore, molecular phylogenetic analysis based either on the large subunit of Rubisco (*rbcL*) or 18S ribosomal DNA sequences has been carried out to resolve phylogenetic

relationships at the species or genera levels (McCourt et al., 1996, 1999; Meiers et al., 1999). In addition, the chloroplast-encoded *atpB* gene is a photosynthetic gene that has also been used in the phylogeny of certain algae and land plants (Lockhart et al., 1992; Wolf, 1997; Sakayama et al., 2004a). However, Wolf (1997) and Nozaki et al. (1999) pointed out similarities between the divergences of the *atpB* gene and the *rbcL* gene among the fern genera and the colonial Volvocales.

In this study, we collected three charalean algae from southeast, northeast, and northern Taiwan, respectively. We use SEM characters of oospores and the *rbcL*, *atpB* gene and combine data from sequences to define the species taxonomic relationships of our materials. Their morphological characters are also described in detail.

## MATERIALS AND METHODS

### Morphological observations

Specimens were collected from southeast, northeast, and northern Taiwan. Some specimen parts were preserved in 95% alcohol for molecular analysis while other parts were preserved in 4-5% formalin solution or dried as herbarium specimens for morphological observations. The liquid or cultural specimens were favored for detailed examination. In LM observations, the vegetative and reproductive structures were examined under a light microscope at 100x and 200x magnification (Zeiss Axioskop 2) and under a dissecting microscope (Zeiss Stemi SV11). In SEM observations, matured oospores of these species were cleaned before examination, and then the tube cells were removed from the mature oospores by a fine needle and forceps under a dissecting microscope.

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Selected oospores were placed for 12 h in a 10% solution of Tween-20 detergent in water at 60°C (Casanova, 1991). Then, they were sonicated for *ca.* 2 min (Casanova, 1997), washed several times with distilled water, dehydrated by Freeze Dryer (Eyela FDU-506), coated with Gold-Palladium by a Sputter coater (Hitachi E-1010), and finally observed with an SEM (Hitachi 2460N) at an accelerated voltage of 20 KV. The terminology used to describe the ornamentation patterns of charalean oospores followed that of Wood (1965) and John and Moore (1987). Voucher specimens have been deposited at Department of Biology, National Changhua University of Education, Taiwan.

### Phylogenetic analysis

Preparation of total DNA, amplification of DNA by the polymerase chain reaction (PCR), and direct sequencing of the PCR products followed the methods described by Sakayama et al. (2002, 2004a). For phylogenetic analysis, *rbcL* (1169 bp), *atpB* (1020 bp), and the combined sequence data set (2189 bp) from 33 charalean operational taxonomic units (OTUs) and three related species as outgroup (Table 1) were each subjected to unweighted maximum parsimony (MP) analysis using PAUP\* 4.0b10 (Swofford, 2002). The MP trees were constructed using a heuristic search with the stepwise addition of 100 random replications and a tree bisection-reconnection (TBR) branch swapping algorithm. Support for nodes of the MP tree was assessed by calculating 1000 bootstrap resamplings of the heuristic searches based on random stepwise additions, MULTREES and TBR (Felsenstein, 1985). Based on the same alignment data, a maximum likelihood (ML) analysis with the Hasegawa-Kishino-Yano 85 model (Hasegawa et al., 1985) was carried out using PAUP\* 4.0b10 to estimate "quartet puzzling support (QPS) values," which has the same practical meaning as bootstrap values, for internal branches of the phylogenetic tree with 1000 "puzzling steps" (comparable to the number of bootstrap replicates) (Strimmer and von Haeseler, 1996). We compared the pairwise distance based on the Kimura 2-parameter method (Kimura, 1980) between the populations of *L. barbatus*, *N. mirabilis*, and *N. inversa* in Taiwan and those of other countries.

In order to test the congruence between the data sets of the *rbcL* gene sequences (1169 bp) and the *atpB* gene sequences (1020 bp), a partition-homogeneity test (Farris et al., 1994) was carried out for the two data sets based on 2000 replications of the heuristic search (with TBR branch-swapping algorithm) using PAUP\* 4.0b10. The *P*-value (0.4915) obtained here was not significant, suggesting no conflicting phylogenetic signals between the two data sets.

## RESULTS

### Morphological observations

*Lychnothamnus barbatus* (Meyen) Leonhardi, Lotos 13: 57, 1863. Figure 1

*Synonym.* *Chara barbata* Meyen, 1827; *Charopsis barbata* (Meyen) Kützing, 1843; *Nitella barbata* (Meyen) Rabenhorst, 1847.

*Specimen examined.* The specimens were collected by Liu, S. L. and Wang, W. L. from Lanyu Island (E121° 33' 2.10", N22°02' 19.38"), Taitung, Taiwan, on 11 July 2003, No: NCUE-JYC-920711-1, 920711-2, 920711-3.

*Diagnosis.* Plants are up to 30 cm in height and light green in color (Figure 1A). The stem axes are 550 µm in diameter. The internodes at the lower part of the plant are longer than the upper ones, which are longer than branchlets. Spine cells are absent on the stem axes. Stipulodes are in a single whorl and outnumber the branchlets twofold (Figure 1B). There are 6-7 branchlets in a whorl, and each branchlet is up to 1-3 cm high and with 2-3 articulations. In culture, several shoots rise from one starchy bulbil at the base of the axis. Plants are monoecious. Gametangia arise from all the nodes of the branchlets, except at the basal part of the whorls. At each node, one oogonium located between two antheridia are produced (Figure 1C). Antheridia are 200-230 µm in diameter. The largest polar axis (LPA) of mature oogonia are 950-1075 µm, and the largest equatorial diameter (LED) is 600-725 µm. Spiral cells show 8-10 convolutions and consist of 5 coronula cells (Figure 1D). Oospores are brown in color, 710-850 µm long and 500-580 µm in width with 8-9 striae (Figure 1E). SEM observation of the mature oospore wall showed verrucate ornamentation (Figure 1F).

*Nitella japonica* Allen, Bull. Torrey Bot. Club 20: 119-120, 1893. Figure 2

*Synonym.* *Nitella furcata* (Roxburgh ex Bruzelius) C. Agardh subsp. *furcata* var. *sieberi* (A. Braun) R. D. Wood f. *japonica* (Allen) R. D. Wood, 1965.

*Specimen examined.* The specimens were collected by Chou, J. Y. and Wu, S. Y. from Hsinchu (E121°08' 024", N24°50'565"), Taiwan on 21 September 2005, No: NCUE-JYC-940921-1, 940921-2, 940921-3.

*Diagnosis.* Plants are greenish in color and up to 15-25 cm in height (Figure 2A). The branchlets are once-forked with 2-3 2-celled dactyls (Figure 2B), gametangia conjoined at all the branchlet node. Thalli are monoecious. The antheridia are 250-280 µm in diameter (Figure 2C), and the oogonia are 500-600 µm in length (incl. coronula), 400-430 µm in width (Figure 2D). The oospores, 310-340 µm in length, 260-300 µm in width, are oval in face view and have 6-7 flanged ridges (Figure 2E). Under SEM observation, the oospore wall ornamentation makes a papillate pattern (Figures 2F-G). The papillae extend onto the spiral ridges and also are produced on the flanges.

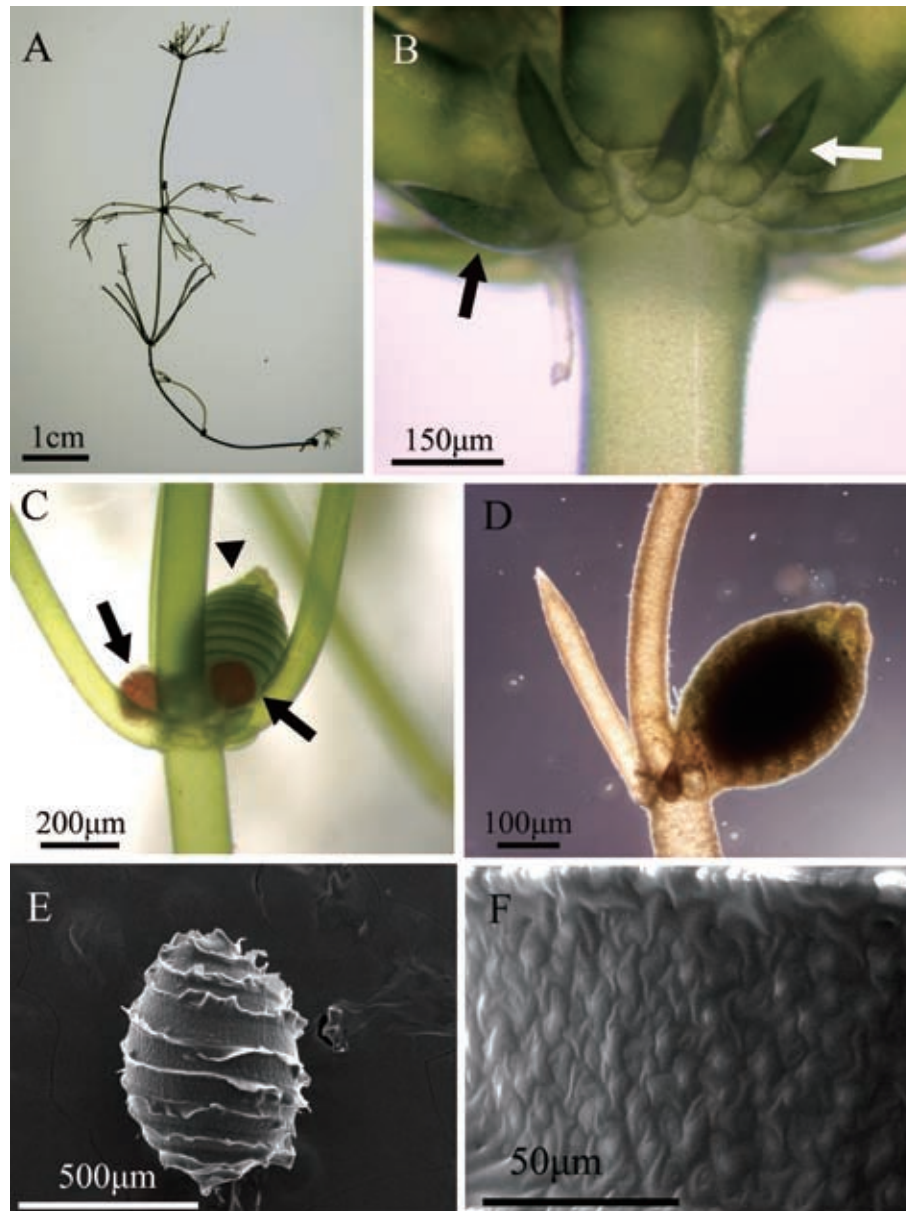
*Nitella inversa* Imahori, Kanazawa University Press, Japan 234 pp, 1954b. Figure 3

*Synonym.* *Nitella furcata* (Roxburgh ex Bruzelius) C. Agardh subsp. *furcata* var. *Sieberi* (A. Braun) R. D. Wood f. *inversa* (Imahori) R. D. Wood, 1965.

**Table 1.** List of the charalean species or strains and related species and GenBank accession numbers used for the present phylogenetic analysis.

Species	Strain designation and collection information	Accession number	
		rbcL gene	atpB gene
<i>Chara connivens</i> Salzmänn ex A. Braun	F140, Spain	AF097161 <sup>1</sup>	AF408782 <sup>9</sup>
<i>Lamprothamnium macropogon</i> (A. Braun) Ophel	X695, Australia	U27534 <sup>2</sup>	AF408783 <sup>9</sup>
<i>Nitellopsis obtusa</i> (Desvaux in Loiseleur-Deslongchamps) J. Groves	F131B, Germany	U27530 <sup>2</sup>	AF408785 <sup>9</sup>
<i>Lychnothamnus barbatus</i> (Meyen) Leonhardi	Croa	U27533 <sup>1</sup>	-
	Aus	AF097171 <sup>1</sup>	AF408784 <sup>9</sup>
	Ger	AF097172 <sup>1</sup>	-
	Lanyu Island, Taiwan	AY707914 <sup>3</sup>	DQ076313 <sup>3</sup>
<i>N. gracilens</i> Morioka	S017, Japan	AB076061 <sup>4</sup>	AB110847 <sup>5</sup>
	S018, Japan	AB076062 <sup>4</sup>	AB110848 <sup>5</sup>
	KINU, Japan	AB076063 <sup>4</sup>	AB110849 <sup>5</sup>
	S049, Japan	AB110870 <sup>5</sup>	AB110850 <sup>5</sup>
	S050, Japan	AB110871 <sup>5</sup>	AB110851 <sup>5</sup>
<i>N. furcata</i> (Roxburgh ex Bruzelius) C. Agardh	S003, Japan	AB076058 <sup>4</sup>	AB110842 <sup>5</sup>
	S037, Japan	AB076059 <sup>4</sup>	AB110843 <sup>5</sup>
	S074, Japan	AB169966 <sup>6</sup>	AB169958 <sup>6</sup>
<i>N. inversa</i> Imahori	S035, Japan	AB076060 <sup>4</sup>	AB110844 <sup>5</sup>
	Hsinchu, Taiwan	AY804256 <sup>3</sup>	DQ119289 <sup>3</sup>
<i>N. tumulosa</i> Zaneveld	S058, Thailand	AB110868 <sup>5</sup>	AB110845 <sup>5</sup>
	S060, Malaysia	AB110869 <sup>5</sup>	AB110846 <sup>5</sup>
<i>N. japonica</i> Allen	S077, Japan	AB169959 <sup>6</sup>	AB169967 <sup>6</sup>
	S083, Japan	AB169960 <sup>6</sup>	AB169968 <sup>6</sup>
	S077, Japan	AB169961 <sup>6</sup>	AB169969 <sup>6</sup>
	Hsinchu, Taiwan		
<i>N. pseudoflabellata</i> A. Braun	S031, Japan	AB076064 <sup>4</sup>	AB110852 <sup>5</sup>
	S032, Japan	AB076065 <sup>4</sup>	AB110853 <sup>5</sup>
	S016, Japan	AB076066 <sup>4</sup>	AB110854 <sup>5</sup>
<i>N. hyalina</i> (De Candolle) C. Agardh	S012, Japan	AB076067 <sup>4</sup>	AB110856 <sup>5</sup>
	S061, unknown	AB110873 <sup>5</sup>	AB110857 <sup>5</sup>
<i>N. spiciformis</i> Morioka	S015, Japan	AB076068 <sup>4</sup>	AB110859 <sup>5</sup>
	S055, Japan	AB110875 <sup>5</sup>	AB110860 <sup>5</sup>
<i>N. moriokae</i> R.D. Wood	S004, Japan	AB076069 <sup>4</sup>	AB110861 <sup>5</sup>
	S052, Japan	AB110876 <sup>5</sup>	AB110862 <sup>5</sup>
<i>Tolypella prolifera</i> (Ziz ex A. Braun) Leonhardi	F150, France	AF097175 <sup>1</sup>	AF408787 <sup>9</sup>
<i>Coleochaete orbicularis</i> Pringsheim	UTEX LB 2651	L13477 <sup>7</sup>	AF408788 <sup>9</sup>
<i>Zygnema peliosporum</i> Wittrock	UTEX LB 45	U38701 <sup>8</sup>	AF408799 <sup>9</sup>
<i>Klebsormidium flaccidum</i> (Kützinger) P.C. Sliva et al.	UTEX LB 2017	L13478 <sup>7</sup>	AF408801 <sup>9</sup>

<sup>1</sup>McCourt et al., 1999; <sup>2</sup>McCourt et al., 1996; <sup>3</sup>This study; <sup>4</sup>Sakayama et al., 2002; <sup>5</sup>Sakayama et al., 2004a; <sup>6</sup>Sakayama et al., 2004b; <sup>7</sup>Manhart, 1994; <sup>8</sup>McCourt et al., 1995; <sup>9</sup>Karol et al., 2001; <sup>10</sup>Cimino and Delwiche, 2002.



**Figure 1.** *Lychnothamnus barbatus* (Meyen) Leonhardi. A, Habit; B, Part of branchlets showed the single whorl stipulodes (arrow); C, One oogonium (arrowhead) between two antheridia (arrow) arose from the node of branchlet; D, Mature oogonium arose from the node; E, Oospore with 8-10 flanged spiral ridges under SEM observation; F, Oospore wall showed verrucate ornamentation.

*Specimen examined.* The specimens were collected by Chou, J. Y. and Wu, S. Y. from Hsinchu (E121°08'024", N24°50'565"), Taiwan on 14 May 2004, No: NCUE-JYC-930514-1, 930514-2, 930514-3.

*Diagnosis.* Plants are greenish in color and up to 8-20 cm in height (Figure 3A). Dactyls are predominantly abbreviated and 2 (-3) celled (Figure 3B). Gametangia are conjoined at each branchlet node, except the ultimate node. The antheridia are 190-260  $\mu\text{m}$  in diameter (Figure 3C), and the oogonia are 440-500  $\mu\text{m}$  in length (incl. coronula), 300-400  $\mu\text{m}$  in width (Figure 3D). The oospores, 302-310  $\mu\text{m}$  in length, 279-311  $\mu\text{m}$  in width, are orbicular in face view and have 6-7 weakly flanged ridges

(Figure 3E). Under SEM observation, the oospore wall ornamentation makes a papillate pattern (Figures 3F-G).

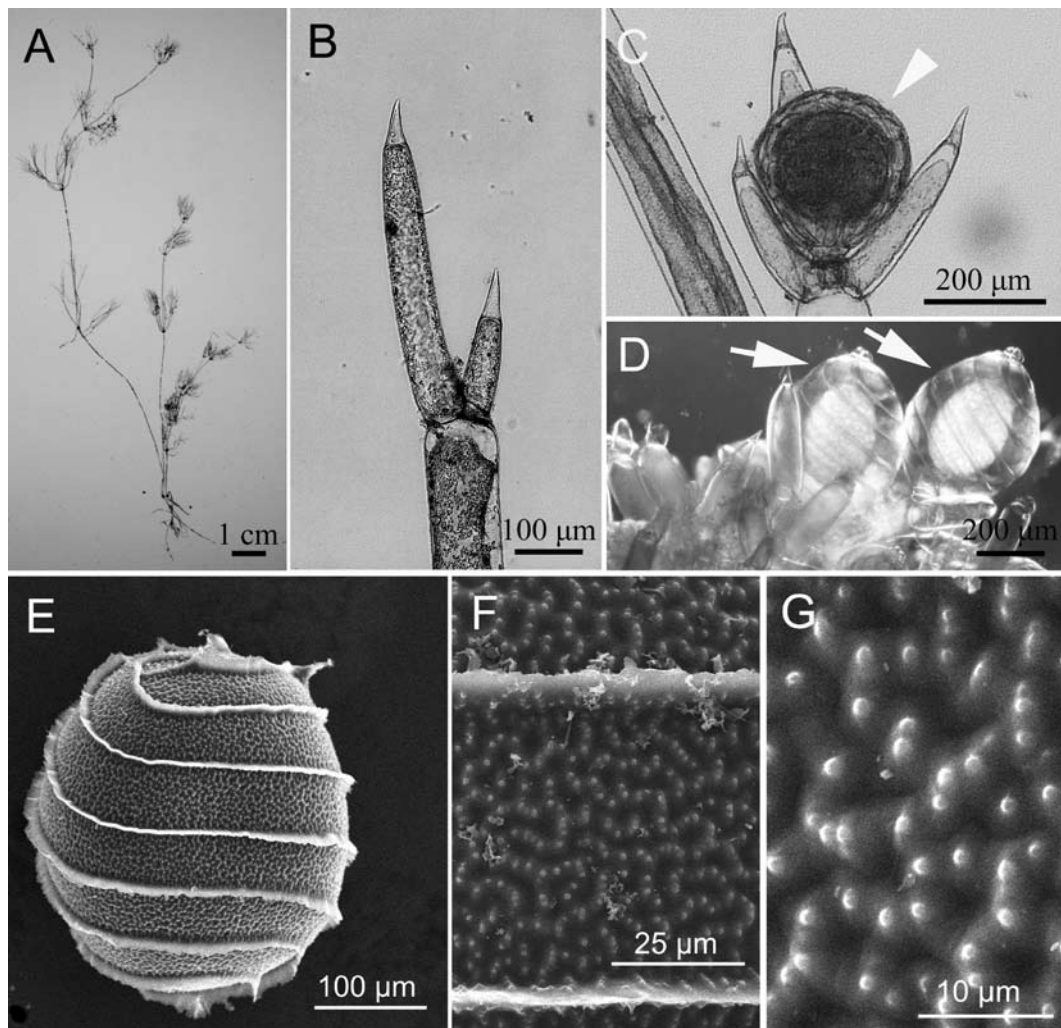
### Molecular analyses

The topologies resolved in the *rbcL-atpB* combined gene analysis were essentially the same as those in the *rbcL* gene analysis (Figure 4, the tree is 767 steps long, with CI=0.6845 and RI= 0.8328), except for some detailed phylogenetic relationships that did not affect our conclusion. Only one of six equally maximum parsimonious trees (based on *rbcL* sequence data set's 1169 aligned characters with 271 potentially parsimony-informative characters), was found in MP analyses, based

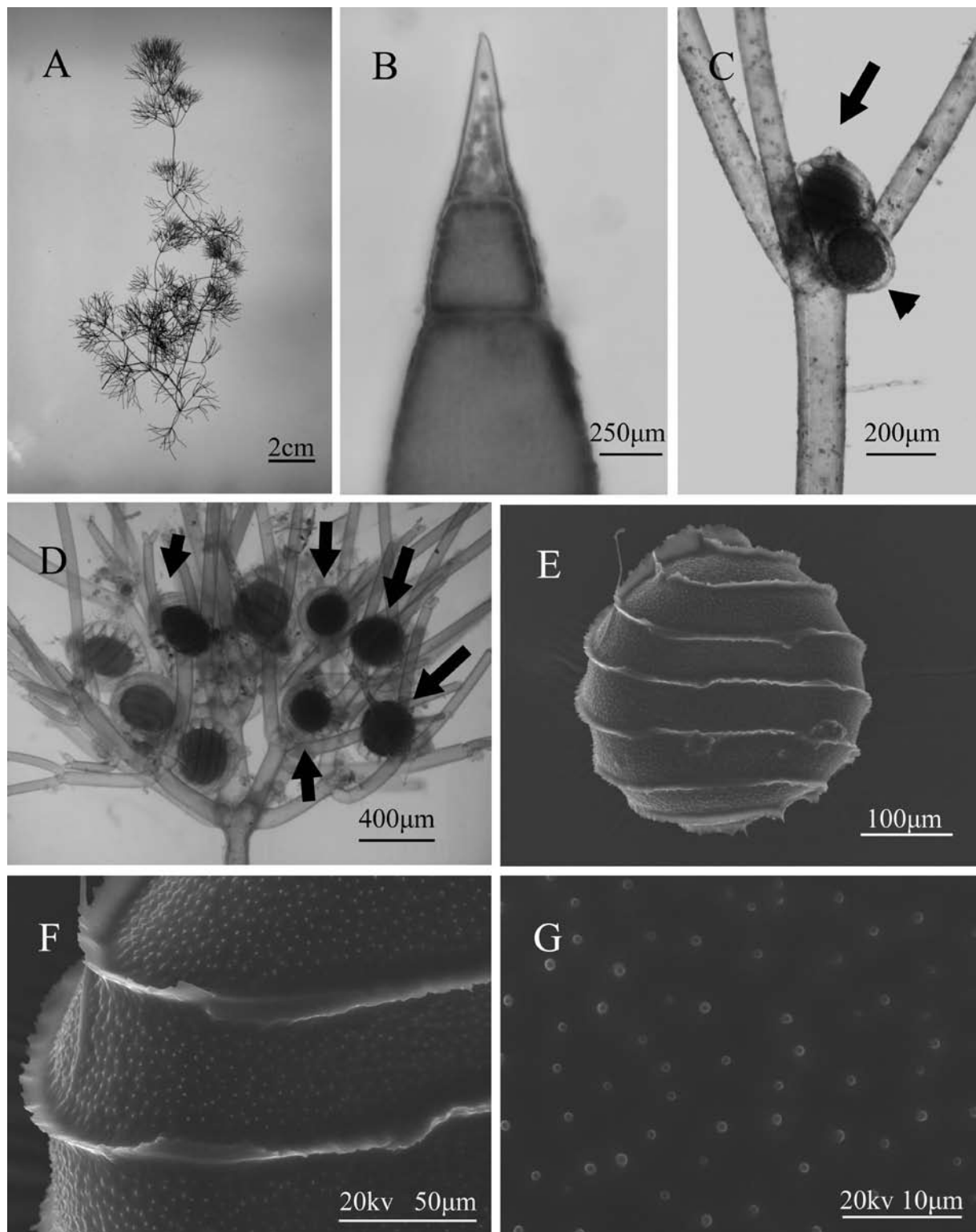
on a heuristic search using the stepwise addition of 100 random replications, and it is shown in Figure 4, in which we show the branches supported by  $\geq 50\%$  bootstrap values in MP analysis and the QPS value. In our molecular analysis, we compared the pairwise distance based on the Kimura 2-parameter method between the populations of *L. barbatus*, *N. japonica* and *N. inversa* in Taiwan and those of other countries. All of them were below 1% in the *rbcL* gene, *atpB* gene, and combined sequence (Table 2). In the *L. barbatus* pair, the pairwise distance of the *rbcL* gene, *atpB* gene, and combined sequence between the populations in Taiwan and Australia was 0.086%, 0.197%, and 0.046%, respectively. In the *N. japonica* pair, no difference between the pairwise distances of the *rbcL* gene, *atpB* gene, or the populations in Taiwan and Japan emerged. In the *N. inversa* pair, the pairwise distance of the *rbcL* gene, *atpB* gene, and combined sequence between the populations in Taiwan and Japan was 0.086%, 0.197%, and 0.138%, respectively.

## DISCUSSION

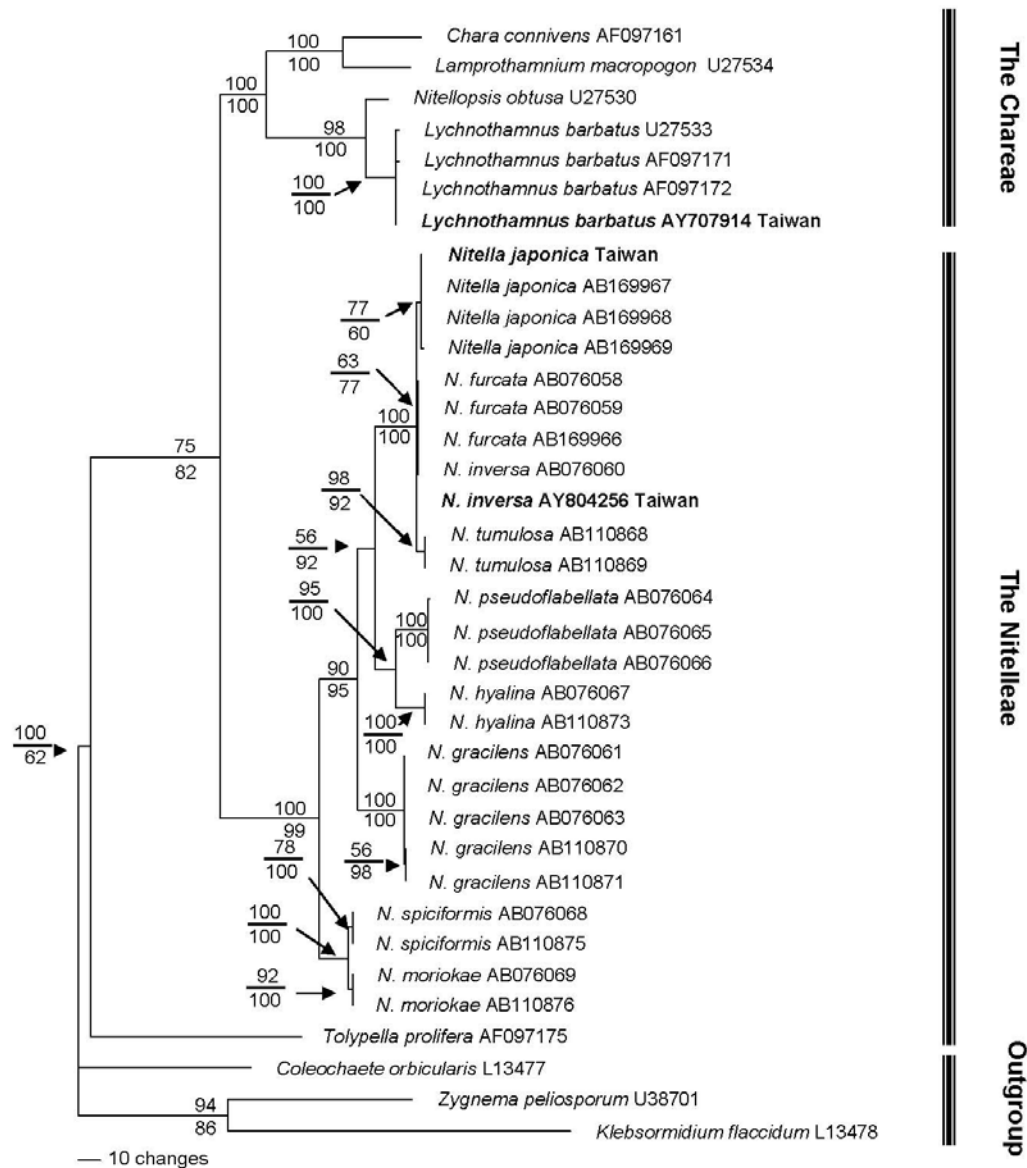
The Characeae comprise about 80 species in the world (Wood, 1965). A total of three genera have been recorded in Taiwan (Imahori, 1951, 1953, 1954a, 1957; Yang and Chiang, 1978; this study). The occurrences of some species, such as *L. barbatus*, are really very rare. After an extensive survey from Taiwan and its offshore island, three new members of Characeae, including one new recorded genus, *Lychnothamnus*, can be added to the algal flora of Taiwan. Imahori (1954a, 1957) pointed out that the affinities between the floras of Taiwan and the Philippines are 61.2%. Although these countries are not continuous geographically, from the standpoint of phytogeography, both belonging to the Paleotropical Floral Zone. Imahori identified those species, however, based only on morphological characters. Taxonomic re-examination of Characeae in Taiwan is carried out based on SEM characters and molecular analysis. In this study,



**Figure 2.** *Nitella japonica* Allen. A, Habit; B, The 2-celled dactyl; C, The antheridium; D, The oogonium; E, Oospore with six to seven flanged spiral ridges under SEM observation; F, Under SEM observation, the oospore wall ornamentation makes a papillate pattern that extends onto the spiral ridges and is also produced on the flanges; G, Detail of fossa wall showed it has more than 22 papillae across the fossa.



**Figure 3.** *Nitella inversa* Imahori. A, Habit; B, The dactyl is three-celled; C, Branchlet node with conjoined antheridium (arrowhead) and immature oogonium (arrow); D, Immature oogonia and mature oogonia (arrow); E, Oospore with six to seven flanged spiral ridges on the surface under SEM observation; F, Part of fossa wall showed papillate pattern, with flanged spiral ridges on the surface under SEM observation; G, Detail of fossa wall showed papillate pattern under SEM observation.



**Figure 4.** One of the six equally maximum parsimonious (MP) trees based on 1169 bp in the coding regions of the *rbcL* gene sequence data using 33 OTUs of the Characeae as ingroup and three related species, *Coleochaete orbicularis*, *Zygnuma peliosporum*, and *Klebsormidium flaccidum* as outgroup (tree length = 767 steps, CI = 0.6845, RI = 0.8328). The numbers on the branches are bootstrap values ( $\geq 50\%$ ) in MP analysis (above the branch) or QPS value (below the branch).

we found molecular analysis to also be a fast and accurate way to identify taxonomic relationships.

Among the charalean algae, the genus *Lychnothamnus* was erected based on the species *Chara barbata* Meyen (1827) (Leonhardi, 1863; cf. Wood, 1965). Fossil records make clear this genus occurred widely from the Late Eocene to the Holocene. It was particularly divergent and widespread in the Pliocene, but has declined since then (Casanova et al., 2003). For the last few decades, *Lychnothamnus barbatus* could not be found in Europe or Asia, except for a population in Wallace Creek, Queensland, Australia (McCourt et al., 1999) and one in Lithuania (Balevicius, personal communication). It has

been enrolled in the protected list by the government of Australia since 1997 (García, 2003). Currently, *L. barbatus* is considered a "Rare and Endangered Species" in Australia (Casanova et al., 2003). Today, only five different populations (in Croatia, Australia, Germany, Lithuania, and Taiwan) are extant, and very few samples are used for molecular study (McCourt et al., 1999; this study). In this study, we found that the *rbcL* sequences of *L. barbatus* from Taiwan are nearly identical to those of specimens from Croatia, Australia, and Germany (data not shown). When we compare the *rbcL* gene, *atpB* gene, and combined sequence between the populations in Taiwan and Australia, their sequences are closely related. The

**Table 2.** Pairwise distance between the populations in Taiwan and other countries of *L. barbatus*, *N. japonica*, *N. inversa* in the *rbcL* (1169 bp), *atpB* (1020 bp) genes and combining (2189 bp) sequence. Each number indicates the absolute distances and sequence divergence in parentheses based on Kimura 2-parameter method (%).

	<i>L. barbatus</i>	<i>N. japonica</i>	<i>N. inversa</i>
<i>rbcL</i> gene	0.2 (0.086)	0 (0)	1 (0.086)
<i>atpB</i> gene	2 (0.197)	0 (0)	2 (0.197)
Combining sequence	1 (0.046)	0 (0)	3 (0.138)

origin of the population in Taiwan can not be resolved using present molecular data. We also compared the mean oospore isopolarity index (ISI = LPA × 100/LED) of those extant and fossil populations by measuring the largest polar axis (LPA), the largest equatorial diameter (LED) of oospores. We found the ISI index of the population of Taiwan (ISI = 135-166) was close to the population of Australia (ISI = 140-171), but quite different from the population of Europe (ISI = 115-140). We suggest the distribution of *L. barbatus* in Taiwan was dispersed from Australia. Furthermore, we will try to use some appropriate gene marker, such as the ITS regions of nuclear ribosomal DNA, to support our hypothesis.

In this study, the materials of *N. japonica* agree with the descriptions of Wood (1965) in having 2-4 forked branchlets with 2-celled dactyl, gametangia conjoined at all the branchlet nodes. Based on the similarity in vegetative characters, Wood (1965) reduced *N. japonica* to a form of *N. furcata*. However, the two species can be clearly distinguished by differences in their oospore wall ornamentation under SEM (Sakayama et al., 2002, 2005). The ornamentation of the former species is in a papillate pattern (Sakayama et al., 2005; this study, Figure 2E-G) while the later species exhibits an imperfect reticulate ornamentation (Caceres, 1975; Mandal et al., 1995; Sakayama et al., 2002). Though the oospore wall ornamentation of *N. japonica* is similar to *N. inversa*, it can be distinguished based on the number of papillae across the fossa. The former species has more than 22 papillae across its fossa (Sakayama et al., 2005; this study, Figure 2F) while the later species has up to 20 (Sakayama et al., 2002; this study, Figure 3F). Additionally, we can use phylogenetic analyses to separate these two species (Figure 4).

The oospore wall ornamentation of *N. furcata* exhibits an imperfect reticulate ornamentation (Mandal et al., 1995; Sakayama et al., 2002), and *N. inversa* exhibits papillate ornamentation (Sakayama et al., 2002). According to Wood (1965), *N. furcata* f. *megacarpa* (Allen) R. D. Wood (= *N. megacarpa* Allen) has the same papillate ornamentation on the fossa wall as *N. inversa*. However, the positioning of the antheridia in *N. inversa* is unique in the infraspecific taxa of *N. furcata*. They are predominantly lateral on the branchlet nodes and borne terminally in the other taxa (Wood, 1965). Therefore, *N. inversa* can be clearly distinguished from *N. furcata* and its related taxa. John and Moore (1987), however,

suggested that the morphology of oospores should be used to delineate the species of *Nitella*, due to its stability under SEM observations. Both *N. inversa* and *N. furcata* exhibit nearly identical *rbcL* gene sequences (Sakayama et al., 2002). The fossa wall of *N. furcata* shows an imperfect or irregular reticulate pattern formed by swollen waved and occasionally fused ridges (Sakayama et al., 2002), but the fossa wall of *N. inversa* has a papillate pattern, with flanged spiral ridges on the surface under SEM observation. The algae are clearly distinguished from each other in oospore wall ornamentation, as in the position of antheridia on the branchlet nodes (Wood, 1965; Sakayama et al., 2002). The characteristics of *N. inversa* collected from Taiwan agreed well with those morphological characteristics, and we suggest that they are different species.

Many charophyte species have declined in response to the changes in their habitats (Moore, 1991; Blindow and Langangen, 1995; Simons and Nat, 2000). Given the apparent pattern of decline in this family and genus, further decline could be prevented by conserving the natural water-regimes of the extant populations. Secondly, conservation strategies based on protection of endangered habitat and ecosystems are likely to benefit these species, as they have *L. barbatus* in its current habitat in Australia

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## 臺灣產輪藻科三種新紀錄種—包含一瀕臨絕種的單種屬

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本文藉由形態特徵及分子序列分析，報導臺灣產輪藻科燈枝藻屬一新紀錄種 *Lychnothamnus barbatus* (Meyen) Leonhardi 及麗藻屬兩種新紀錄種 *Nitella japonica* Allen 和 *N. inversa* Imahori。燈枝藻屬亦為臺灣藻類相中一新紀錄屬，並為瀕臨絕種之單種屬。本文除對此三藻種形態作詳細描述外，同時以葉綠體二磷酸核酮糖羧化酵素 (*rbcL*) 及葉綠體 ATP 合成酵素之 B 次單位 (*atpB*) 基因序列確定其親緣關係的分類地位。

**關鍵詞：**葉綠體 ATP 合成酵素之 B 次單位基因；輪藻科；燈枝藻屬；麗藻屬；葉綠體二磷酸核酮糖羧化酵素基因；臺灣。