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NOTE

Description of a Newly Recorded *Nitella* species, *N. mirabilis* Nordstedt *ex* J. Groves (Charales, Charophyta), in Taiwan

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ABSTRACT: One newly recorded species of *Nitella*, *N. mirabilis* Nordstedt *ex* J. Groves (Charales, Charophyta) was found in Taiwan primarily based on the morphological features. This newly recorded species is characterized by being a monoecious plant, oospore surface appears an anastomosing network and possessing unicellular dactyls. Its monoecious nature and the characteristics of its reproductive organs separate it from other species of the genus. Its vegetative morphology is similar in many respects to that of the monoecious species belong to section *Nitella*, subgenus *Nitella*. However, the surface view of the oospores of *N. mirabilis* appears fibrillar based on the SEM observations due to the occurrence of the anastomosing network of fine fibrils. Such character is unique among the known species of the genus *Nitella*. *N. mirabilis* was found in a lake of Fushan Research Station, Yilan County, Taiwan in which the water depth range from a few centimeters to one meter.

KEY WORDS: Monoecious, newly recorded species, *Nitella mirabilis*, oospore, Taiwan.

INTRODUCTION

Nitella C. Agardh (Charales, Charophyta) is a genus with a lot of species within the Characeae. There are 895 species (and intra-specific) names in the database at present, of which 210 have been flagged as currently accepted taxonomically (AlgaeBase; searched on 12 September 2013). In common with all other charophytes, *Nitella* has a macroscopic thallus and complicated reproductive organs. The genus is characterized by its once or repeatedly forked branchlets with one or more-celled dactyls, two tiers of coronula cells in the female reproductive organ, oogonium. The oogonia are produced laterally to the nodes, and the antheridia are terminal. The structure and ornamentation on the outer organic wall of the oospores based on scanning electron microscope (SEM) observations showed the existence of distinct features and has been used as a taxonomic character. *Nitella* is worldwide distribution and mostly species are found in alkaline freshwater (Wood, 1965). Wood's worldwide monograph divides *Nitella* into three subgenera, *Nitella*, *Hyella* and *Tieffallenia*, with seventeen sections and modified the taxonomy of this group by recognized 53 defined species out of over 200 species previously described (Wood, 1965). However, recent studies based on SEM observations of oospores morphology have raised some of Wood's intraspecific taxa to species rank (Cáceres, 1975; John and Moore, 1987; Leitch et al., 1990; Casanova, 1991; Nozaki et al., 1998; Ray et al., 2001; Sakayama et al., 2002, 2004.).

Molecular analyses based on *rbc*L gene sequences support the subgenera *Hyella* and *Tieffallenia* are monophyletic, whereas the subgenus *Nitella* is paraphyletic (Sakayama, 2002, 2004). The subgenus *Nitella* is characterized by having unicellular dactyls. Within this subgenus, section *Nitella* can be distinguished from other five sections by its 1 (–3) celled obtuse, acute or apiculate dactyl apices. Four species included in this section, *Nitella flexilis* (Linnaeus) C. Agardh, *N. opaca* (C. Agardh *ex* Bruzelius) C. Agardh, *N. mirabilis* Nordstedt *ex* J. Groves, and *N. macounii* (T.F. Allen) T.F. Allen. *N. flexilis* and *N. opaca* are mainly distributed in north hemisphere but extends into South America and Africa. *N. mirabilis* is a southeastern Asiatic species, whereas *N. macounii* is endemic in central North America. Under SEM observations, the fossa wall of oospores appears vague reticulate appearance by having minute pits (0.43–0.85 μm) in *N. flexilis* (Ray et al., 2000), but it appears pitted with large pores in *N. opaca*. And it appears fibrous by having anastomosing network fibrils in *N. mirabilis* (Sakayama et al., 2004). In *N. macounii*, its fossa is strongly vermiferous and the irregular raised areas occasionally becoming imperfectly reticulate under LM observations (Wood, 1965). The last rare species have not yet had any SEM observations at present.

In this study, one newly recorded species of *Nitella*, *N. mirabilis* Nordstedt *ex* J. Groves is described. It is a monoecious species with once-forked branchlets and acute unicellular dactyl.

MATERIALS AND METHODS

Specimens were collected from a lake of Fushan Research Station, Yilan County, Taiwan (E121°35'042'', N24°45'587'') where the water depth ranges between a few centimeters and up to one meter (Fig. 1). Specimens were preserved in 95% alcohol and 4–5% formalin solution. Cultures have been kept at the Department of Biology, National Changhua University of Education, using soil-water medium in 1000 ml glass vessel. The glass vessel containing the soil layer and deionized water was autoclaved separately for 20 min before cultivation.

Measurements of the vegetative and reproductive structures were taken from living specimens using light microscope (Zeiss Axioskop 2) and stereomicroscope (Zeiss Stemi SV11). For SEM imaging, fully mature oospores were collected from the detritus deposited on the surface of the soil layer of the soil-water medium. 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 subjected to sonicate for *ca*. 2 min (Casanova, 1997), and washed several times with distilled water, dehydrated by Freeze Dryer (Eyela FDU-506), coated with Gold-Paladium 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.

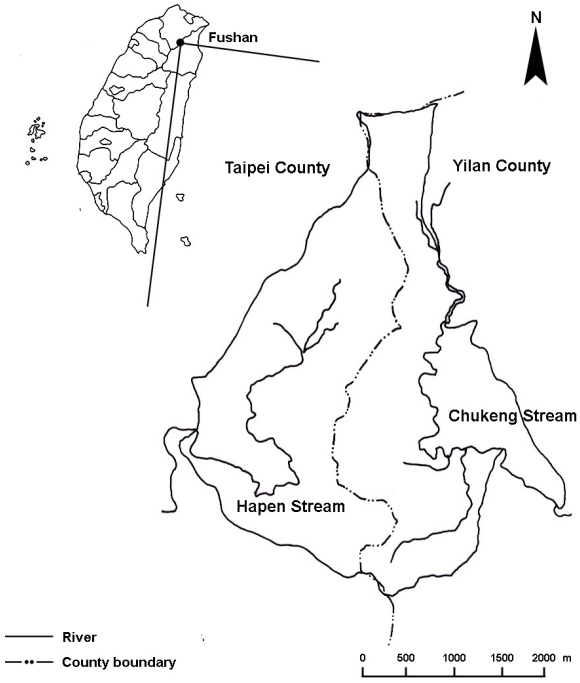

Fig. 1. Map showing the location of the collection site of *Nitella mirabilis*Nordstedt *ex* J. Groves, Fushan Research Station, Yilan County, Taiwan.

RESULTS

Nitella mirabilis Nordstedt *ex* J. Groves

Figs 2A–G

Plants are monoecious and greenish in color and around 3–25 cm in height, not enveloped in mucus (Fig. 2A). The branchlets are once-forked with unicellular dactyl (Fig. 2B). Gametangia are solitary or aggregate at the branchlet node or at the basal part of the whorl. There are 1–3 antheridia at one node, 500–620 μm in diameter (Fig. 2C). There are 1–3 oogonia at one node, 550–610 μm in length (excl. coronula), 510–590 μm in width with 8–9 circumvolutions (Fig. 2D). The oospores are 490–560 μm in length, 410–460 μm in width (Fig. 2E) and oval in face view and have six to seven robust and strongly flanged spiral ridges (Fig. 2E). The walls of the mature oospores are brown to yellowish brown. Under SEM observation, the fossa wall has fibrous pattern (Fig. 2F), in which the fibrils form an anastomosing network (Fig. 2G). Fibrils are absent from both the spiral ridges and laterally extended walls (called flanges).

Specimens examined: NCUE-Nisp-JYC-920126 (collected by Jui-Yu Chou, Shao-Lun Liu and Wei-Lung Wang, 26 January 2003), NCUE-Nisp-JYC-930908-1, NCUE-Nisp-JYC-930908-2 (collected by Jui-Yu Chou and Ching-Lin Huang, 8 September 2004) from Fushan Research Station, Yilan County, Taiwan (E121°35'042'', N24°45'587''). The specimens consist of fluid-preserved specimens (95% alcohol or 4–5% formalin solution).

Distribution：India, Japan and Taiwan.

Habitat：*Nitella mirabilis* is found in the lake of the Fushan Research Station, Yilan County, Taiwan, in having shallow but moving water (Fig. 1). Plants there were associated with *Chara braunii* Gmelin and many aquatic plants, such as *Nuphar shimadae* Hayata, *Hydrilla verticillata* (L. f.) Royle, *Ludwigia ovalis* Miq. and *Callitriche verna* L.. It is located near the border between Taipei County and Yilan County, and this region is a moist subtropical forest at elevations of *ca*. 400 m. From 1998 to 2004, the average air temperature is 17.5ºC, the average relative humidity is 93.6%, and the precipitation per year is 4494.6 mm in this region (Taiwan Ecological Research Network; http://211.20.146.134/).

Remarks: *Nitella mirabilis* found in Taiwan has several distinctive characters. (1) monoecy, i.e. the oogonia and antheridia occur on the same thalli. (2) The

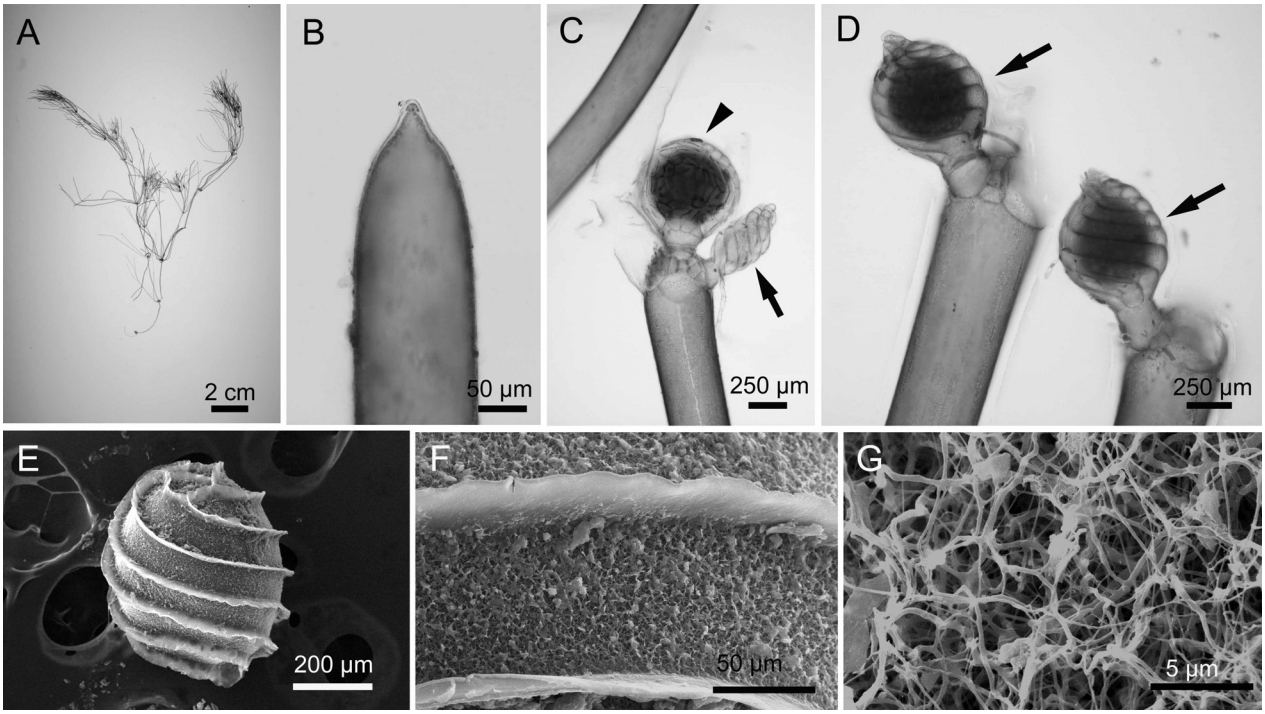

Fig. 2. *Nitella mirabilis* Nordstedt *ex* J. Groves. A. Habit. B. The dactyl is unicellular. C. Branchlet node with conjoined antheridium (arrow) and immature oogonium (arrowhead). D. Mature oogonium on thalli (arrow). E. Oospore with six flanged spiral ridges under SEM observation. F. Part of fossa wall showed fibrous pattern with strongly flanged spiral ridges lacking fibrils on the surface under SEM observation. G. Detail of fossa wall showed fibrous pattern in which the fibrils formed an anastomosing network under SEM observation.

oospore surface appears fibrillar due to an anastomosing network of fine fibrils that not extend to the flanges under SEM. (3) The female branchlets are 1(–2)-furcate with solitary or aggregate gametagia.

The features shared by species of the section *Nitella*, subgenus *Nitella* are: dactyls apices obtuse, acute or apiculate (not acuminate), oogonial convolutions swelling at maturity, and 1(–3)-celled dactyls. In Taiwan, only one species of the section *Nitella* has been previously described, *N. flexilis* (Imahori, 1951; Imahori, 1954a; Imahori, 1957; Yang and Chiang, 1978). *N. flexilis* is also a monoecious species, but mainly different from *N. mirabilis* in oospores wall ornamentation.

Nitella mirabilis has some similarities with other monoecious species that have 1-celled dactyls (Table 1). One of these resembles is *N. flexilis*, but can be distinguished by (1) less secondary rays. (2) the oogoinia being larger (750–850 µm in length and 580–650 µm in width) (excl. coronula). (3) the oospore membrane appears vague reticulate by having minute pits. Another species with which *N. mirabilis* can be confused is *N. macounii*. It differs from *N. macounii* in that the latter species has (1) smaller oogoinia (345–360 µm in length and 255–270 µm in width) (excl. coronula). (2) the smaller oospores (225–240 µm in length and 180–225 µm in width). (3) the antheridia are much smaller (195–225 µm in diameter). Compared with the specimens in previous studies (Wood, 1965; Sakayama et al., 2004), *N. mirabilis* found in Taiwan shows some unique features in having (1) the bigger oospores (490–560 µm in length and 410–460 µm in width). (2) the oospore membrane appears fibrous by having anastomosing network of coarse fibrils. (3) both male and female organs in the same individual (monoecious instead of dioecious).

In terms of dioecious species, in addition to sexual state, *N. opaca* can be distinguished from *N. mirabilis* by (1) less secondary rays. (2) the longer oogoinia (650–700 µm in length) (excl. coronula). (3) the smaller oospores (375–425 µm in length and 350–400 µm in width). (3) the antheridia are larger (650–775 µm in diameter). (4) the oospore membrane appears pitted with large pores (Table 1).

DISCUSSION

Whether monoecy/dioecy is an important characters for species separation has been a controversy. Wood (1965) pointed the features of monoecy/dioecy was really intraspecific variations rather than species level. But Proctor (1975, 1980) considered monoecy/dioecy as

a good taxonomic character in the Characeae and rejected all polyecious species (*i.e*. a species including both monoecious and dioecious individuals). McDonald *et al*. (1966) and Proctor (1971) processed the hybridization experiments of Characeae and concluded that monoecy and dioecy were critical importance in recognizing species of Characeae, and suggested that it should be given more taxonomic weight than a morphological character. It was based on the fact that the components of morphologically similar monoecios-diecious species pairs were always reproductively isolated. Besides, the ecological tolerances and requirements of the monoecios and diecious species are also different (*cf*. García and Casanova, 2003). Proctor (1980) conjectured that monoecious forms of charophytes are dominant in islands, especially remote oceanic islands. Totally around 26 species known so far are all monoecious species occurred in Taiwan (Imahori, 1951; Imahori, 1954a; Imahori, 1957; Yang and Chiang, 1978; Chou, 2006; this study) supports Proctor's hypothesis.

However, some taxonomists suggest that the reproduction mode can not be a reliable character for the species delimitation. For example, *Schisandra chinensis* (Schisandraceae), a woody vine distributed in northeast China, was traditionally considered both monoecious and dioecious (Ueda, 1988). In Zhao et al. (2013), they found the gender system in this species is environmentally-induced unstable phenotypes. Gender in flowering plant is regulated by a complex interplay of genetic and environmental elements (Cipollini and Whigham, 1994; Wolfe and Shmida, 1996). In some species, monoecious individuals may represent one of several gender morphs in a population, including genetic males and femals (Glawe and de Jong, 2005). In Serrão et al. (1999), they also concluded that the character ''monoecious/dioecious'' has changed several times during the evolutionary history of the Fucaceae (Phaeophyceae), and the same conclusion in Desmarestiaceae (Peters et al., 1997). This suggests that in brown algae, monoecy/dioecy can be changed by relatively simple mechanisms and thus is not a good indicator of evolutionary relationships.

The morphological features of *N. mirabilis* found in Taiwan are highly congruent with the specimens in other countries, except for the reproduction mode (Table 1). Most of the morphological features overlap between these specimens. The only two differences are the reproduction mode and the surface structure of oospores. We have discussed the usage of the reproduction mode for the species delimitation. In terms of the second morphological evidence, except for the oogoinial length and width, the oospores of both species have anastomosing network. Moreover, *N. mirabilis* is a southeastern Asiatic species. It has been recorded in countries near Taiwan (India and Japan). Based on the reasons mentioned above, we propose a new record instead of proposing a new species in this study.

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LITERATURE CITED

Cáceres, E. J. 1975. Novedades carologicas Argentinas I. Una nueva especie de *Nitella* y tres andiciones al genero para la flora Argentina. Kurtziana **8**: 105–125.

Casanova, M. T. 1991. An SEM study of developmental variation in oospore wall ornamentation of three *Nitella* species (Charophyta) in Australia. Phycologia **30**: 237–242. doi: 10.2216/i0031-8884-30-3-237.1

Casanova, M. T. 1997. Oospore variation in three species of *Chara* (Charales, Chlorophyta). Phycologia **36**: 274–280. doi: 10.2216/i0031-8884-36-4-274.1

Chou, J. Y. 2006. The taxonomy of the extant Characeae (Charales, Chlorophyta) from Taiwan. Unpublished Master's degree thesis. National Changhua University of Education, Changhua, Taiwan. 115 pp.

Cipollini, M. L. and D. F. Whigham. 1994. Genderual dimorphism and cost of reproduction in the dioecious shrub *Lindera benzoin* (Lauraceae). Am. J. Bot. **80**: 769–777. doi: 10.2307/2445564

García, A. and M. T. Casanova. 2003. *Lamprothamnium heraldii* sp. nov.: a unique dioecious representative from Australia. Phycologia **42**: 622–628.

Guiry, M. D. and G. M. Guiry. 2013. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 12 September 2013.

Imahori, K. 1951. Studies on the Charophyta in Formosa I. Science Reports of the Kanazawa University **1**: 201–221.

Imahori, K. 1954a. Similarity between the Characeae of Formosa and Philippine islands. Acta Phytotaxonomica et Geobotanica **15**: 123–128.

Imahori, K. 1954b. Ecology, Phytogeography and Taxonomy of Japanese Charophyta. Kanazawa University Press, Kanazawa, Japan. 234 pp.

Imahori, K. 1957. Similarity between the Characeae of Formosa and the Philippines. Proceedings of the Eighth Pacific Science Congress **4**: 563–568.

John, D. M. and J. A. Moore. 1987. An SEM study of the oospore of some *Nitella* species (Chlorophyta, Charales) with descriptions of wall ornamentation and an assessment of its taxonomic importance. Phycologia **26**: 334–355. doi: 10.2216/i0031-8884-26-3-334.1

Leitch, A. R., D. M. John, and J. A. Moore. 1990. The oosporangium of the Characeae (Chlorophyta, Charales). Progress in Phycological Research **7**: 213–268.

McDonald, M. B., V. W. Proctor and A. T. Hotchkiss.

Table 1. Comparison of vegetative and reproductive characteristics of *Nitella mirabilis* and three similar species.

	N. flexilis[A]	*N. opaca* (C. Agardh *ex* Bruzelius) C. Agardh[A]	*N. mirabilis*[BC]	*N. mirabilis*[D]	*N. macounii*[B]
Sexual state	Monoecious	Dioecious	Dioecious	Monoecious	Monoecious
Mucus	Absent	Absent	Present or absent	Absent	Absent
Whorl branchlets	6–8, 1-furcate	6–7, 1-furcate	6–8, 1(–2)-furcate	6–9, 1(–2)-furcate	6–8, (1–)2(–3)-furcate
Rays	Secondaries 1–3	Secondaries 2–3	Secondaries 2–4	Secondaries 3–6	Fertile branches：secondaries 5–6 Sterile branches：secondaries 4–5
Dactyls	1–3, 1-celled	2–3, 1-celled	2–4, 1-celled	2–4, 1-celled	2–4, 1(–2–3)-celled
End cell	Apex abruptly pointed	Apex abruptly pointed	Tapering sharply to acute or apiculate tip	Acute or tapering to abrupt sharp point	Acuminate, deciduous leaving branchlet with blunt tip
Oogoinia length (μm) (excl. coronula)	750–850	650–700	450–570	550–610	345–360(–720)
Oogoinia width (μm)	580–650	500–565	390–570	510–590	255–270(–600)
Circumvolutions	8–9	7–9	7–8	8–9	7–8
Oospores length (μm)	500–600	375–425	330–475	490–560	225–240
Oospores width (μm)	440–570	350–400	300–425	410–460	180–225
striae	5–6	7	5–6	6–7	5–8
Oospore membrane	Vague reticulate appearance by having minute pits[E]	The fossa wall is pitted with large pores[E]	Fibrillar by having anastomosing network of coarse fibrils	Fibrillar due to an anastomosing network of fine fibrils	Strongly vermiferous and the irregular raised areas occasionally becoming imperfectly reticulate (LM observations)
Antheridial diameter (μm)	500–600	650–775	450–600	500–620	195–225

[A] Imahori, 1954b;[B] Wood, 1965;[C] Sakayama et al., 2004; [D] This study; [E] Ray et al., 2001.

1966. Attempted hybridization between monoecious and dioecious clones of *Chara*. Australian Journal of Botany **53**: 937–940. doi: 10.2307/2439818

Nozaki, H., M. Kodo, M. Miyaji, M. Kato, M. M. Watanabe and H. Kasaki. 1998. Observations on the morphology and oospore wall ornamentation in culture of the rediscovered Japanese endemic *Nitella gracilens* (Charales, Chlorophyta). Eur. J. Phycol. **33**: 357–359. doi: 10.1017/S0967026298001863

Peters, A., M. J. H. van Oppen, C. Wiencke, W. T. Stam and J. L. Olsen. 1997. Phylogeny and historical ecology of the Desmaresiaceae (Phaeophyceae) support a southern hemisphere origin. J. Phycol. **33**: 294–309. doi: 10.1111/j.0022-3646.1997.00294.x

Proctor, V. W. 1971. Taxonomic significance of monoecism and dioecism in the genus *Chara*. Phycologia **10**: 299–307. doi: 10.2216/i0031-8884-10-2-299.1

Proctor, V. W. 1975. The nature of chrophyte species. Phycologia **14**: 97–113. doi: 10.2216/i0031-8884-14-2-97.1

Proctor, V. W. 1980. Historical biogeography of *Chara* (Charophyta): an appraisal of the Braun-Wood classification plus a falsifiable alternative for future consideration. Journal of Phycology **16**: 218–233. doi: 10.1111/j.1529-8817.1980.tb03023.x

Ray, S., S. Pekkari and P. Snoeijs. 2001. Oospore dimensions and wall ornamentation patterns in Swedish charophytes. Nord. J. Bot. **21**: 207–224. doi: 10.1111/j.1756-1051.2001.tb01359.x

Sakayama, H., H. Nozaki, H. Kasaki and Y. Hara. 2002. Taxonomic re-examination of *Nitella* (Charales, Charophyceae) from Japan, based on microscopical studies of oospore wall ornamentation and *rbc*L gene sequences. Phycologia **41**: 397–408. doi: 10.2216/i0031-8884-41-4-397.1

Sakayama, H., Y. Hara and H. Nozaki 2004. Taxonomic re-examination of six species of *Nitella* (Charales, Charophyceae) from Asia, and phylogenetic relationships within the genus based on *rbc*L and *atp*B gene sequences. Phycologia 43: 91–104. doi: 10.2216/i0031-8884-43-1

-91.1

Serrão, E. A., A. A. Lawrence and S. H. Brawley. 1999.Evolution of the Fucaceae (Phaeophyceae) inferred from nrDNA-ITS. J. Phycology 35: 382–394.

Ueda, K. 1988. Sex change in a woody vine species, *Schisandra chinensis*, a preliminary note. J. Jap. Bot. **63**: 319-321.

Wood, R. D. 1965. Monograph of the Characeae. In Wood R.D. and Imahori K. (eds.), A revision of the Characeae, vol. I. J. Cramer, Weinheim. 904 pp.

Yang, Y. P. and T. N. Chiang. 1978. A synopsis of Taiwan aquatic plants. Quarterly Jounal of Chinese Forestry **11**: 115−122.

Zhao, X. N., S. J. Huang, J. M. Zhao and Y. M. Zhang. 2013. Gender variation in a monoecious woody vine *Schisandra chinensis* (Schisandraceae) in northeast China. Ann. Bot. Fennici **50**: 209−219. doi: 10.5735/086.050.0402

臺灣產輪藻目麗藻屬新紀錄種奇異麗藻

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摘要：本研究報導臺灣產輪藻目麗藻屬一新紀錄種奇異麗藻，依據其雌雄同體、單細胞末枝及合子外部微細構造，可明顯與本屬其他已知種類區分。尤其在掃瞄式電子顯微鏡下觀察到其合子外壁呈現纖細纖維狀，此特徵在本屬其他種類是很獨特的。本新紀錄種奇異麗藻發現於台灣宜蘭縣福山植物園內水池，分布水深約幾公分到最高一公尺環境中。文中對臺灣麗藻進行描述，並提供照片及與其他相近種類之比較。

關鍵詞：雌雄同體、新紀錄種、奇異麗藻、合子、台灣。