



## NOTE

## Notes on the *Wiesnerella denudata* (Mitt.) Steph. (Wiesnerellaceae, Hepaticae) in Taiwan

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**ABSTRACT:** Description and photos of main characters of *Wiesnerella denudata* (Mitt.) Steph. in Taiwan are provided. Sequence of chloroplast DNA *rbcL* and SEM photos of spores and elaters from Taiwan's material are provided for the first time, which can be compared with materials from adjacent areas to show variation of the species. Three substitutions were found between plants of Taiwan and Nepal in *rbcL* sequences. Three morphological characters of spores are differentiated between the plants of Taiwan and mainland China. Spores are smaller in Taiwanese plant, 34-38 against 78.3-86.1  $\mu$  m in diameter. Spore equatorial belt does not have any opening in Taiwan's plant, but three openings are described in plant from mainland China. Exine is reticulate throughout the spore in Taiwanese plant, but it is restricted to the proximal surface of spore in plant from mainland China.

**KEY WORDS:** Elater, liverwort, *rbcL*, SEM, spore, Taiwan, *Wiesnerella denudata*.

### INTRODUCTION

*Wiesnerella* is a monotypic genus with *W. denudata* (Iwatsuki and Mizutani, 1972), belonging to the Wiesnerellaceae and Marchantiales (Candolle-Stotler et al., 2009). *Wiesnerella* is closely related to *Targionia* phylogenetically based on multiple genes (Forrest et al., 2006). This genus is distributed in the Himalayas, Java, Sumatra, China, Taiwan, Japan, Korea and Hawaii (Iwatsuki and Mizutani, 1972; Chang and Wu, 2006). Ever since Horikawa (1934) first reported the occurrence of this species in Taiwan, Lin (2000) has added more detailed descriptions of this species. Chang and Wu (2006) presented descriptions and scanning electro-microscope (SEM) photos of spores of this species from mainland China. In this paper, we described this species, presented photos of main characters of the plants, and provided description and SEM photos of spores and elaters prepared from fresh material. Differences between spore descriptions of Taiwan and mainland China were given. Sequence of chloroplast *rbcL* from Taiwan's material is also provided for comparison.

### MATERIALS AND METHODS

#### Preparation of SEM photos

Specimens were collected from Manyueyuan, New Taipei City, and the voucher (*C.H. Chang, 12*) was deposited in the Department of Applied Science, National Hsinchu University of Education (tentative acronym, NHCUE). Fresh materials were

collected and fixed with 70% alcohol, and then dehydrated in alcohol series, dried in a critical point dryer, coated with gold and were examined with Hitachi S-3000N, installed at the Department of Applied Science, National Hsinchu University of Education.

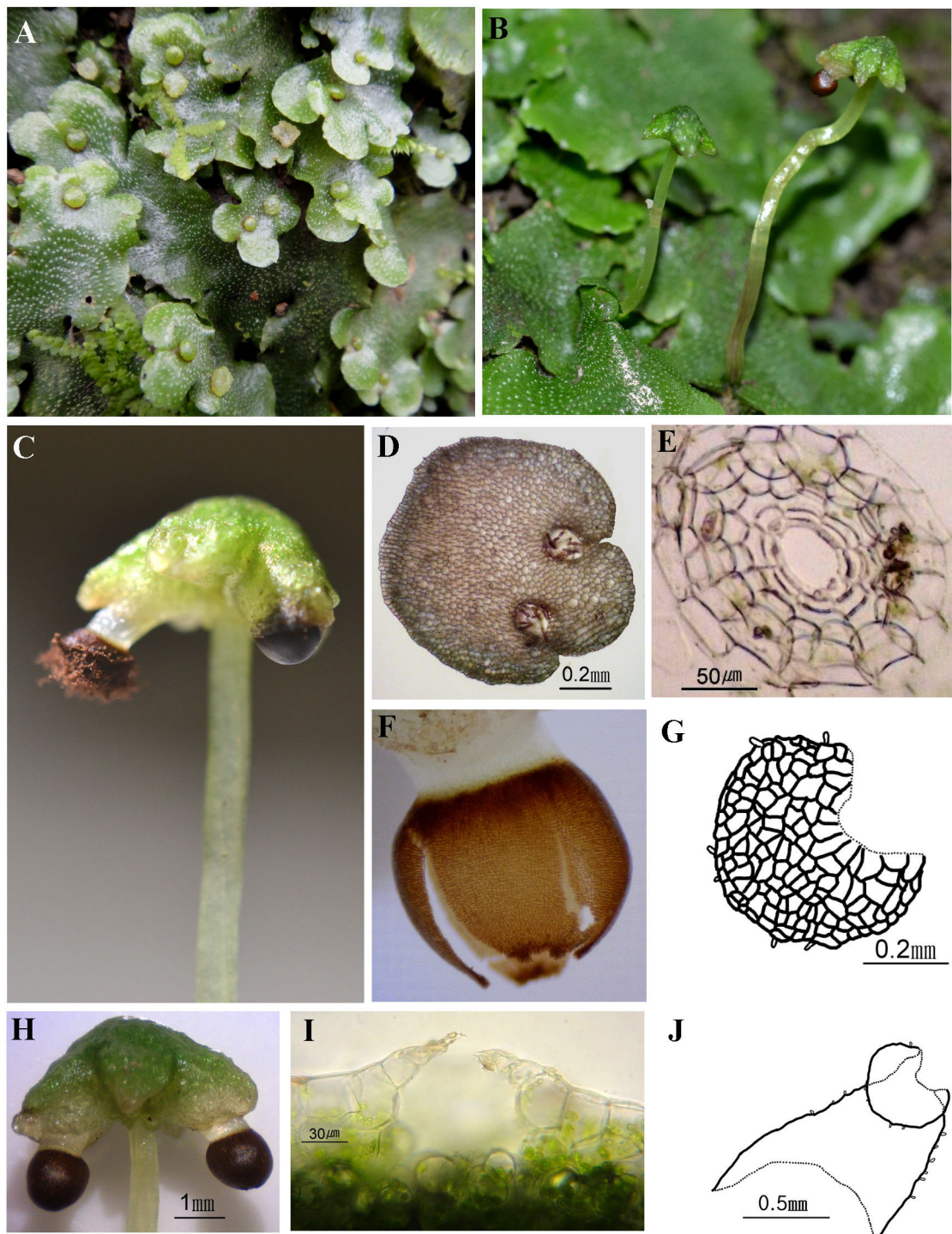
#### Obtaining *rbcL* sequence and analysis

Fresh material (voucher: *C.H. Chang s.n.* Jan. 18, 2012) was washed with distilled water and DNAs were extracted with commercial extraction kit (Biokit, Miaoli, Taiwan). Conditions of Polymerase Chain Reaction (PCR) for amplifying *rbcL* gene, followed Miwa et al. (2003). The PCR products were purified with commercial kit and sequenced with a model ABI3700 automatic sequencer (Applied Biosystems) using Big Dye terminator. Sequence was adjusted manually using Chromas version 2.22 (Technelysium). The sequence was then compared with published ones from NCBI GenBank using BLAST.

### TAXONOMIC TREATMENT

*Wiesnerella denudata* (Mitt.) Steph., Bull. Herb. Boissier 7 : 382, 1899; Iwatsuki and Mizutani, Colored Illustration of Bryophytes of Japan 364, Pl. 48; Lin, The Liverwort Flora of Taiwan 362, 2000.  
魏氏蘚 Figs. 1 & 2

Thalloid plants, green and shining, divaricate, wavy at margin, 2-5 cm long, about 1 cm wide; air pores simple, protruding, 30-50  $\mu$  m across, chamber 1-layered, with green cells; ventral scales in 2 rows,



**Fig. 1.** Morphology of *Wiesnerella denudata* (Mitt.) Steph. **A & B:** Habits. **C:** female receptacle with sporophyte that is releasing spores. **D:** cross section of stalk. **E:** air pores. **F:** 4-valved capsule. **G:** appendage of ventral scale. **H:** receptacle with sporophytes. **I:** cross section of the air pore. **J:** ventral scale.

lunular, with single orbicular appendages. Dioicous; archegoniophores at the upper part of the plant, receptacles green, 4-7-valved, stalks whitish green, with 2 rhizoid furrows, 1-5 cm long; antheridiophores

protruding, about 6-valved. Capsules brownish black, globular; seta less than 1 mm long. Elaters 2-spiralled, glabrous, attenuate to both ends, 272-495  $\mu$  m long. Spores dark brown, spherical, wavy, with large waves at

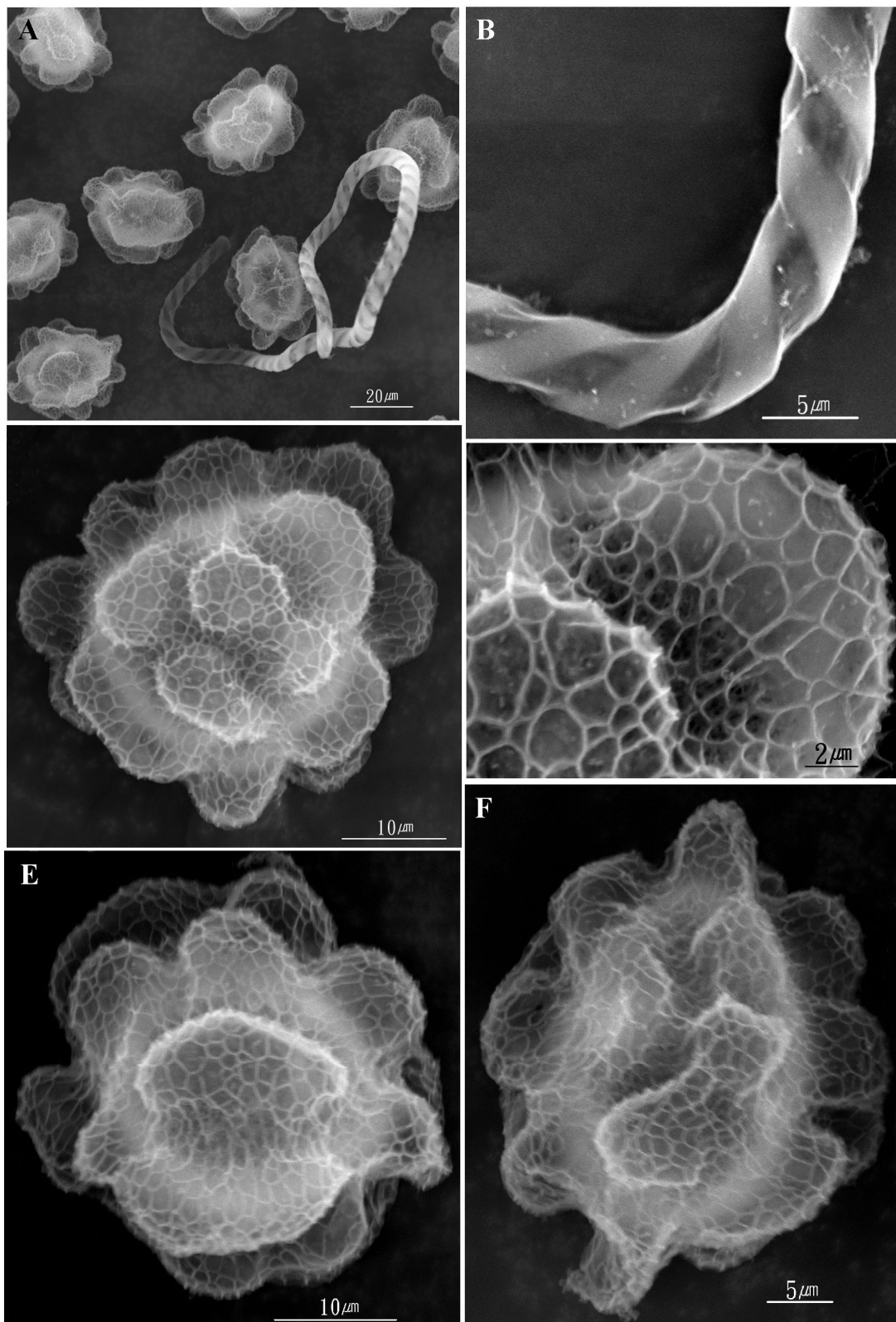


Fig. 2. Scanning electro-microscope photos of spores and elaters of *Wiesnerella denudata* (Mitt.) Steph. A: Spores and elater. B: portion of elater showing two spirals. C: proximal surface of the spore. D: reticulate ornamentation of exine. E: distal surface of the spore. F: equatorial view of the spore.



the distal surface, reticulate, 34-38  $\mu$  m across.

Phenology: Gametophytes seen all the year around; archegoniophores seen in February and March; capsules seen in April.

Specimens examined: TAIWAN, New Taipei City: Manyueyuan, C. H. Chang s. n., Jan. 18, 2012; Taoyuan County: Lalashan, C. C. Liu 446, 680.

Notes: Chang and Wu (2006) presented descriptions and SEM photos of spores based on a specimen (Without locality, Wang s.n. s.d.). Differences in characters between spores of the species from Taiwan and mainland China are described as follows. Spores are smaller in Taiwanese plant, 34-38 against 78.3-86.1  $\mu$  m in diameter. Equatorial belt lacks opening in Taiwan's plant, but three openings are described in plant from mainland China. Exine is reticulate throughout the entire spore in Taiwanese plant, but it is restricted to the proximal surface in plant from mainland China. Further studies are needed to clarify the relationship between these areas.

#### Comparison of *rbcL* sequence

The sequence obtained is 947 base pairs in length, which corresponds to *rbcL* gene base pairs from 153 to 1099 of *Wiesnerella denudata* from Nepal, deposited in NCBI with accession number DQ286027 (Forrest et al., 2006). No indels were found, and three substitutions, namely sites 206 (G to C), 237 (T to G) and 317 (T to A), are between the two sequences. The next closely similar sequences are 29-30 substitutions belonging to *Targionia* and other genera. Thus Taiwanese plants should be the same species with that from the Himalayas compared to the variation of *rbcL* sequences of *Conocephalaum* (Miwa et al., 2003)

#### 台灣產魏氏蘚的觀察

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摘要：本文除描述台灣產魏氏蘚之外部特徵，提供主要特徵之圖片以供鑑定外，主要目的在比對台灣產本種的葉綠體*rbcL*基因序列，並提供孢子，彈絲的掃描式電子顯微鏡相片及他們的描述，以與中國大陸之種類比對。結果發現台灣產的*rbcL*基因序列與尼伯爾產的序列有三個替代突變差異。台灣產的孢子則有三個特徵與中國大陸者不同：前者孢子較小，直徑34-38  $\mu$  m 對比後者78.3-86.1  $\mu$  m 長；赤道環帶無開口對比後者具有三個開口；前者外壁全部為網狀，對比後者之網狀僅限於近極面。

關鍵詞：彈絲、蘚類、*rbcL*、掃描式顯微鏡、孢子、台灣、魏氏蘚。

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