

SIMPLE TREATMENT TO INVESTIGATE SPORE ORNAMENTATION OF FERNS FOR SEM OBSERVATION FROM HERBARIUM SPECIMENS

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Abstract

Fern spore surface from herbarium specimens is usually difficult to study because of the presence of dust or debris. After being soaked in ethanol (70%) and shaken in an ultrasonic wave bath (50-60Hz, 90W), the treated spore surfaces become clearer than untreated ones under scanning electron microscopy. This is an easy method that represents an improvement to commonly use techniques for the preparation of fern spores for SEM observation. As a case study, we observed the spore morphology of *Bolbitis changjiangensis*, *Humata henryana* and *Hypodematium hirsutum*. Micromorphological data for *Hypodematium hirsutum* are reported for the first time.

Introduction

Sample preparation of specimens for scanning electron microscopy (SEM) is regularly applied for spore morphological observations of ferns and non-vascular plants (Erkara & Savaroglu, 2007; Gerald & Rolla, 1976; Lu, 2007; Tryon & Lugardon, 1991; Zhang *et al.*, 1976). This is a required procedure for taxonomic studies of ferns. Spores from herbarium specimens of ferns are always covered by appendages, which affect the observation of their ultra-morphological surfaces. The most common technique for preparation of spores for electron microscopy is based on the use of glacial acetic acid and Vitriol (Zhang *et al.*, 1976), however, this is a lengthy procedure which involves handling toxic reagents.

During taxonomic study of the fern families Davalliaceae, Bolbitidaceae and Hypodematiaceae, we developed an alternative treatment for cleaning spores prior to SEM observation. This new technique is presented in this paper; including SEM results obtained with three species from Davalliaceae, Bolbitidaceae and Hypodematiaceae.

Material and Methods

Spores of *Bolbitis changjiangensis*, *Humata henryana* and *Hypodematium hirsutum* were removed from herbarium specimens (Table 1) and subjected to a three steps process. The initial step consisted in placing the spores inside 1.5 ml microtubes with 70% ethanol. This initial treatment was followed by placing the microtubes inside an ultrasonic wave bath (1510 BRANSON, 50-60Hz, 90W) for 10 min. This second step was followed by 4 min. centrifugation (6000 rpm). This three steps protocol was repeated three times, each washing step (70% ethanol) required a re-suspension of the pellet of spores. Subsequent to this treatment, the spores and washing 70% ethanol solution were transferred to a double sided adhesive tape with a micro-pipette. Once the 70% ethanol solution vaporized the dried spores were gold coated using standard electron microscopy techniques. These samples were subsequently observed and photographed under a Hitachi JSM-6360LV Scanning Electron Microscope. Untreated spores were compared to the ones that were prepared following the protocol presented in this study.

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Table 1. Data of voucher specimens studied.

Species	Locality	Voucher specimen	Herbarium	Collection date
<i>Hypodematium hirsutum</i> (D. Don) Ching (Hypodematiaceae)	Guangdong Province: Huaiji	Wang Faguo 671b	IBSC	Jan.12, 2005
<i>Bolbitis changjiangensis</i> F.G. Wang & F.W. Xing (Bolbitidaceae)	Hainan Province: Changjiang county	Wang Faguo 051	IBSC	Jul. 14, 2004
<i>Humata henryana</i> (Baker) Ching (Davalliaceae)	Yunnan province: Lufeng county	Zhu Weiming 06	PE	1955

Results

Spores of *Bolbitis*, *Hypodematium* and *Humata* are clean and with clear surface morphological construction after using the method of ultrasonic wave bath in Micro Movie-making of spore. (Fig. I.1, 2, 3, 4, 5, 6). *Hypodematium hirsutum*, spore ellipsoid, monolete, which perispore is lophate with drape (Fig. I.1, 2). *Bolbitis changjiangensis* (Wang & Xing, 2008), spore ellipsoid, monolete, which perispore is cristate-undulate, with dense, irregular and thin wing (Fig. I. 3, 4). *Humata henryana*, spore ellipsoid, monolete, without perispore, exine with obvious verrucate and reticulate in equatorial view (Fig. I. 5, 6). However, untreated spores of species of *Bolbitis*, *Hypodematium* and *Humata* have more impurities with unclear ornamentation on their surfaces (Fig. I. 7, 8, 9, 10, 11, 12), which make taxonomy study more difficult.

Discussion

Seeds from herbarium specimens are usually large and easy to be cleaned for SEM studies (Hu *et al.*, 2004). In contrast, spores are small in size and are more difficult to be prepared for SEM studies. Available techniques to clean spores for ultrastructure observations require the follow lengthy procedures that involve using toxic compounds. As an alternative protocol Wang *et al.*, (2006) and Dai *et al.*, (2005) tried to study fern spores by means of SEM without any pretreatment; however, they found that preparations following this procedure yielded poor results. Most of the material had many impurities such as scale and glandular hairs. This initial treatment was followed by placing the microtubes inside an ultrasonic wave bath (1510 BRANSON, 50-60Hz, 90W) for 10 min. Among the three steps of the protocol presented in this paper we found that the time to be critical when the microtubes were placed inside the ultrasonic wave bath. Time much longer than 10 min. can easily destroy the surface structures of the spores. Time much shorter than 10 min. will not allow for the complete removal of impurities. 70% Ethanol could fix as well as clean the spore. Spore surface of contaminated small spores collected from herbarium specimens is unclearly observed, after being soaked in 70% Ethanol, shaken in the ultrasonic wave bath, the treated spore surface becomes clear than untreated ones under SEM. This method is easy to operate and obviously effective than routine methods.

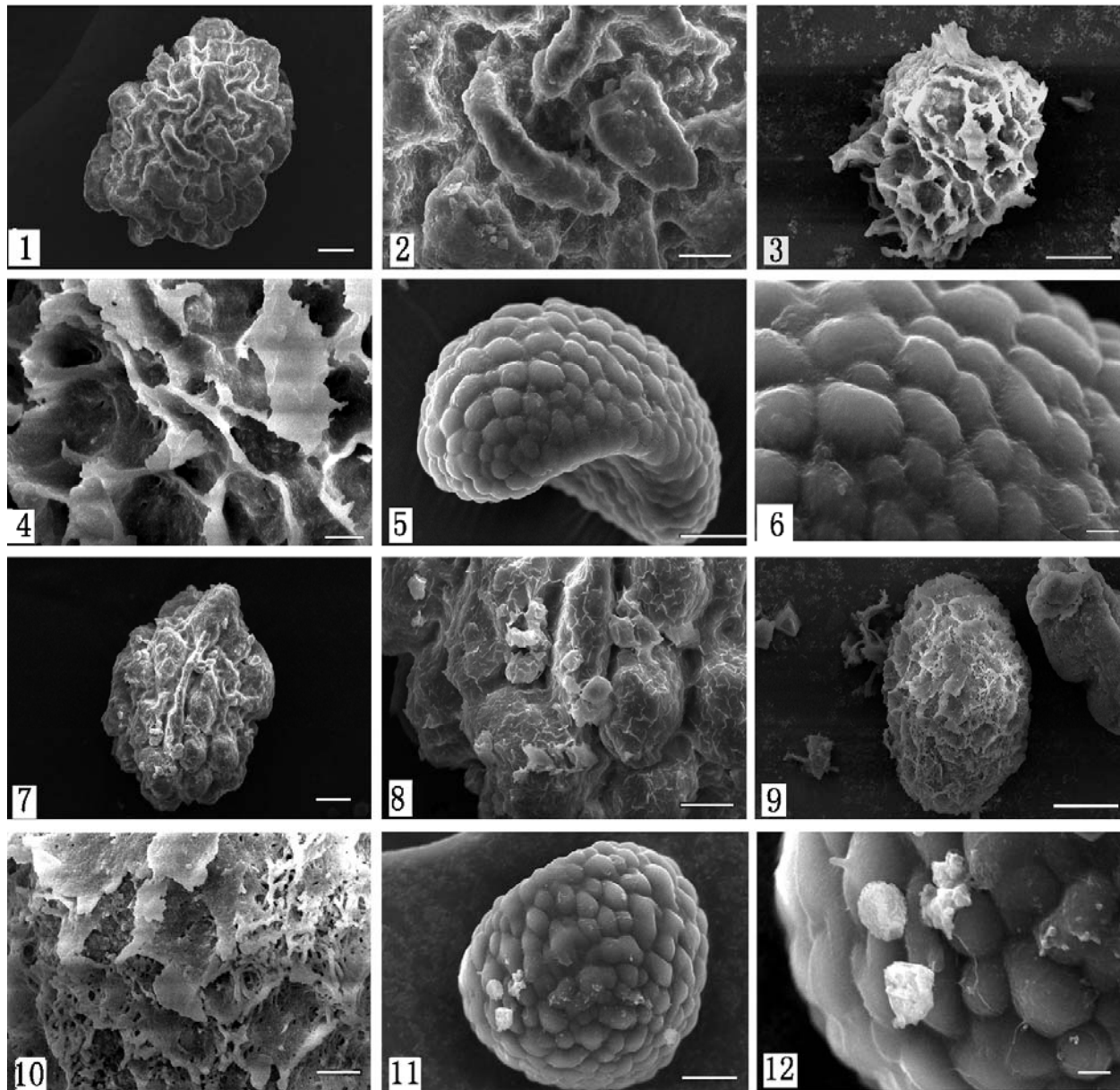


Fig. I. Surfaces of entire (1, 3, 5) and magnification part (2, 4, 6) of treated spore. Surfaces of entire (7, 9, 11) and magnification part (8, 10, 12) of untreated spore; *Hypodematium hirsutum* (Fig. I. 1, 2, 7, 8); *Bolbitis changjiangensis* (Fig. I. 3, 4, 9, 10); *Humata henryana* (Fig. I. 5, 6, 11, 12) Fig. I. 1, 3, 5, 7, 9, 11 Bar=10 μ m; Fig. I. 2, 6 Bar=5 μ m; Fig. I. 4, 8, 10, 12 Bar=2 μ m.

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