

MICRO-STRUCTURE STUDIES ON *CHIRITA* AND *UTRICULARIA* OF PENINSULAR MALAYSIA

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Abstract

Chirita Buch.-Ham. ex D. Don are herbs of limestone while *Utricularia* L. are small carnivorous herbs of wet habitats. Seed testa surface has proved to provide diagnostic characters for sections *Chirita* and *Microchirita* C.B. Clarke in the genus *Chirita*, replacing the stigma character previously used and showed less variation, therefore caused confusion in the past. *Chirita lacunosa* (Hook.f.) B.L. Burt from section *Chirita* (the only species in this section in Peninsular Malaysia) has a reticulate testa while other species from section *Microchirita* possess knobby or cannicolate testa. For the genus *Utricularia*, species from section *Phyllaria* (Kurz) Kamiński often show variable macro-characters, but micro-characters such as seed testa are less variable and provide diagnostic characters for species determination. For example, a specimen from the highlands of Kelantan was distinguished from the similar morphology but more common *Utricularia striatula* Sm. by its testa and could be identified as *U. furcellata* Oliv., a rare species formerly recorded only from North East India. The testa cells of *U. furcellata* have long processes with knobby tips, while those of *U. striatula* have glochidiate processes with stellate tips. SEM model JOEL and FEI were used to examine both genera.

Introduction

General characters of *Chirita* and *Utricularia*: *Chirita* Buch.-Ham. ex D. Don (Gesneriaceae) is a small herbaceous genus restricted to limestone hills (Taylor, 1989; Kiew, 2009). In Peninsular Malaysia, the genus can be found in wet and shady places on limestone hills, at the cliff base, on cliff walls in crevices and in cave mouths. *Chirita* is short-lived or perennial and the inflorescences are axillary or scapiform and sometimes epiphyllous, crested or cymose one- to many-flowered. There are species with bracts or without bracts.

Four sections are described in *Chirita* but only two sections occur in Peninsular Malaysia. Section *Microchirita* shows a characteristic pattern of inflorescence morphology where the peduncles are sometimes adnate to the petiole, and the anthers are joined by an apical ligature or are fused apically.

Utricularia L., (Lentibulariaceae) is a cosmopolitan genus of small carnivorous herbs of wet habitats with minute submerged or subterranean suction traps (Ridley, 1923). In Peninsular Malaysia, it can be found from sea level (Chew, 2009) to the highest peak of Gunung Tahan, usually in acidic conditions (Chew, 2010). Locally, two habits are observed, *i.e.*, the terrestrial, semi-aquatics with small linear or rosulate leaves and the free-floating aquatics with much-divided, floating leaf-masses. Both habits produce erect, racemose inflorescences held above the water or moist substrate surfaces (Ridley, 1923).

Seven sections of the genus *Utricularia* are found in Peninsular Malaysia. Species from the section *Phyllaria* (Kurz) Kamiński often show variable macro-characters such as corolla shape, size and colour, while leaves and fruits shapes are little differentiated. Micro-characters such as seed testa are less variable and provide relatively stable diagnostic character for species determination (Taylor, 1989).

Diagnostic micro-structures of *Chirita* and *Utricularia*:

For the genus *Chirita*, seed testa surface has been determined as a potential character for better delimitations

for sections *Chirita* and *Microchirita*, replacing the stigma character that was previously used but shows less variation hence causing confusion in the past.

Generally, the seeds of Gesneriaceae have not been studied very extensively and are usually incompletely described; possibly because of their small size (length ranges from a minimum of 0.2 mm to a maximum 4 cm). SEM techniques show that species of *Chirita* exhibit a trend from papillate, through mixed papillate and tuberculate to tuberculate (Beaufort-Murphy, 1983). Weber (2004) analysed the nature of the knobs in *Chirita* and found they represent concretions within the outer cell wall.

A specimen belonging to section *Phyllaria* of *Utricularia*, collected from a small patch of montane heath vegetation (1,500 m a.s.l.) in Kelantan, Peninsular Malaysia, appeared to have corolla characters that are slightly different from the known variations shown by the more common and widespread *Utricularia striatula* Sm., which is also known from the same area but occupying a slightly different niche. Its corolla structure most closely resembles that of *Utricularia furcellata* Oliv., a species previously known only from moist rocks on hills (1500–2700 m a.s.l.) of Sikkim, Darjiling and Meghalaya of North East India.

Robins & Subramanyam (1980) first highlighted the differences in testa micro-structure between *Utricularia furcellata* and *U. striatula* as seen by SEM. Taylor (1989) observed the same differences in the hooks at the end of the testal. However, he mentioned that the differences might not be consistent.

Material and Methods

Seeds of *Chirita* and *Utricularia* were mounted using double-sided carbon tape on aluminium stubs to show the proximal polar, distal polar and lateral surfaces. They were then coated with gold under sputter coater at 20 mA for 90 seconds. SEM model JOEL and FEI were used to

examine the samples, micrographs were taken of the whole structure, anterior, posterior, polar and lateral views and details of specific surface structures. All specimens examined are listed in Table 1.

Table 1. Specimens examined.

Species	Collection No.	Parts examined
<i>Chirita lacunosa</i>	RK2304	Seeds
<i>Chirita sericea</i>	FRI64347	Seeds
<i>Utricularia furcellata</i>	FRI53603	Seeds
<i>Utricularia striatula</i>	FRI60185	Seeds

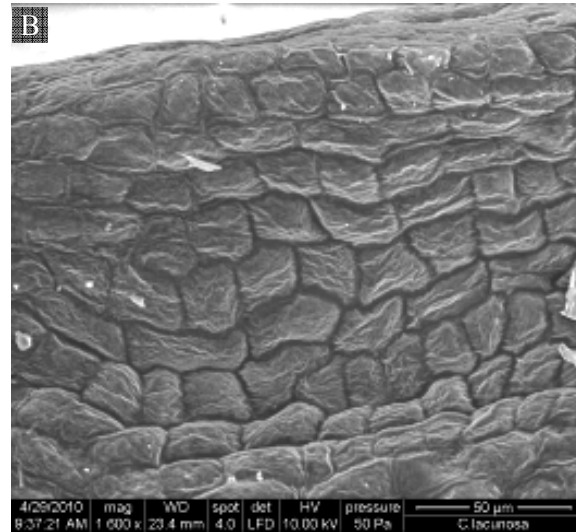
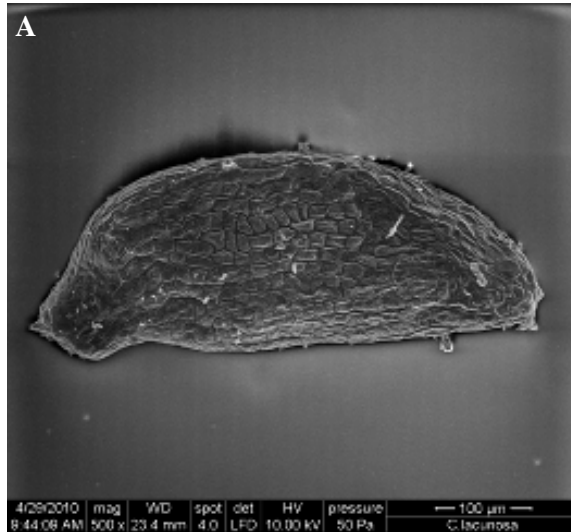


Fig. 1. A. *Chirita lacunosa* seed testa. B. The reticulate surface of seed testa in *C. lacunosa*.

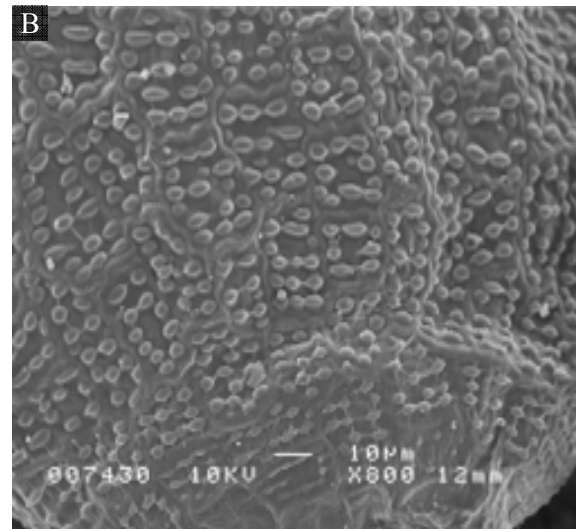
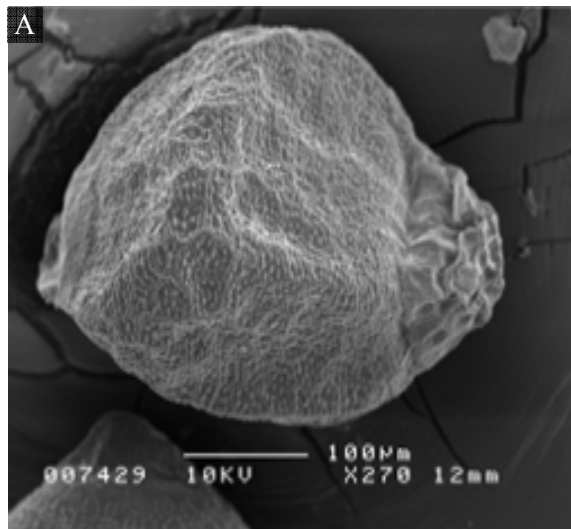


Fig. 2. A. *Chirita sericea* seed testa. B. The knobby or cannicolate surface of seed testa in *C. sericea*.

Utricularia: Both *Utricularia furcellata* and *U. striatula* have minute seeds that are less than half a millimetre long (excluding the processes). Their dimensions are shown in Table 2. Under a 40× or 100× light microscope, the seeds

Results and Discussion

Chirita: *Chirita lacunosa* from section *Chirita* (the only species in this section in Peninsular Malaysia) has a distinctly reticulate testa (Fig. 1). The seeds of six species from section *Microchirita* have been investigated; here *Chirita sericea* serves as an example (Fig. 2). The species possesses uneven knobby or cannicolate testa, not in regular rows. The seed shape of *Chirita lacunosa* is narrowly ellipsoid with 480-650 µm length while *C. sericea* possesses a narrowly broadly ellipsoid seed 300-380 µm long.

of both species appear obliquely ovoid. The proximal tips closest to the hilum are obtuse or conical with striate but process-free testa surfaces, while the distal ends are rounded with many stiff, clavate processes or papillae.

Compared to *U. striatula*, the seeds of *U. furcellata* are almost double in size (1.8× larger), are more densely papillate with longer processes. The micro-structures of the testa surfaces, however, are not readily observable. The higher magnifications and resolution provided by SEM are therefore crucial to reviewing the diagnostic micro-characters.

Table 2. Dimensions of *Utricularia furcellata* and *U. striatula* seeds.

Species	<i>U. furcellata</i>	<i>U. striatula</i>
Average seed length (µm)		
without process	455	251
with process	539	295
Process length (µm)	(up to) 100	(up to) 56

When viewed under 1,600× magnification, the densely packed long processes with knobby, clavate tips on the testa cells of *U. furcellata* (Fig. 3) are clearly differentiated from the sparse and short processes with glochidiate (having hooks), almost stellate tips belonging to *U. striatula* (Fig. 4).

The processes of both species are situated in the middle of the elongate testa cells. The anticlinal boundaries of the testa cells are minutely sinuate and sunken. For *U. furcellata*, the periclinal testa cells walls are microscopically and uniformly verrucose; whereas for *U. striatula*, the periclinal walls are relatively smooth with the verrucae (wart-like projections) occurring only along the sinuate boundaries.

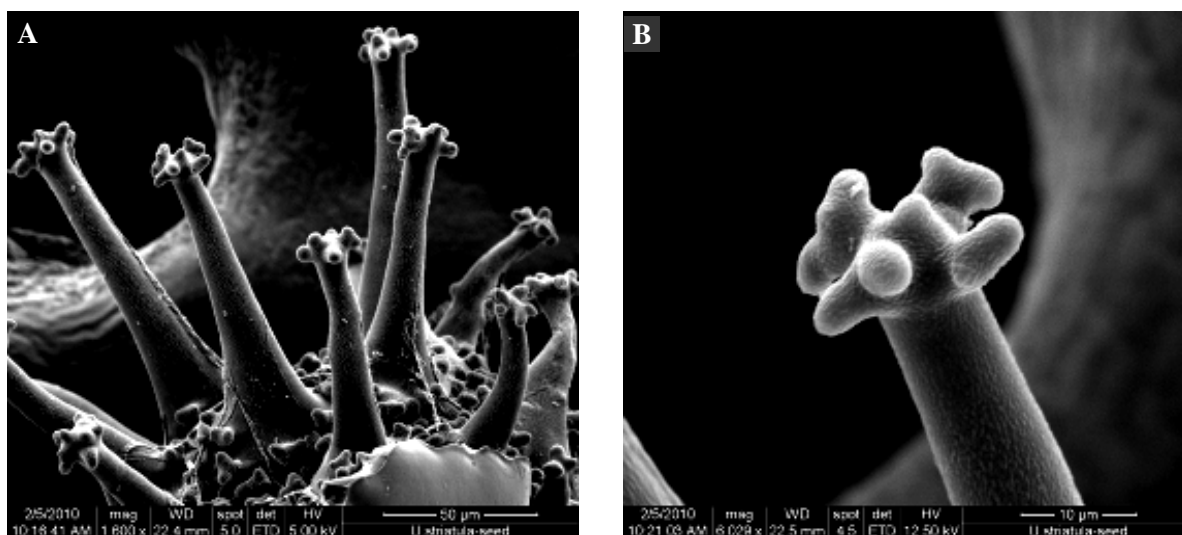


Fig. 3. *Utricularia furcellata* seed testa. A. The densely packed and long processes on the highly verrucose periclinal walls. B. The knobby, clavate tips.

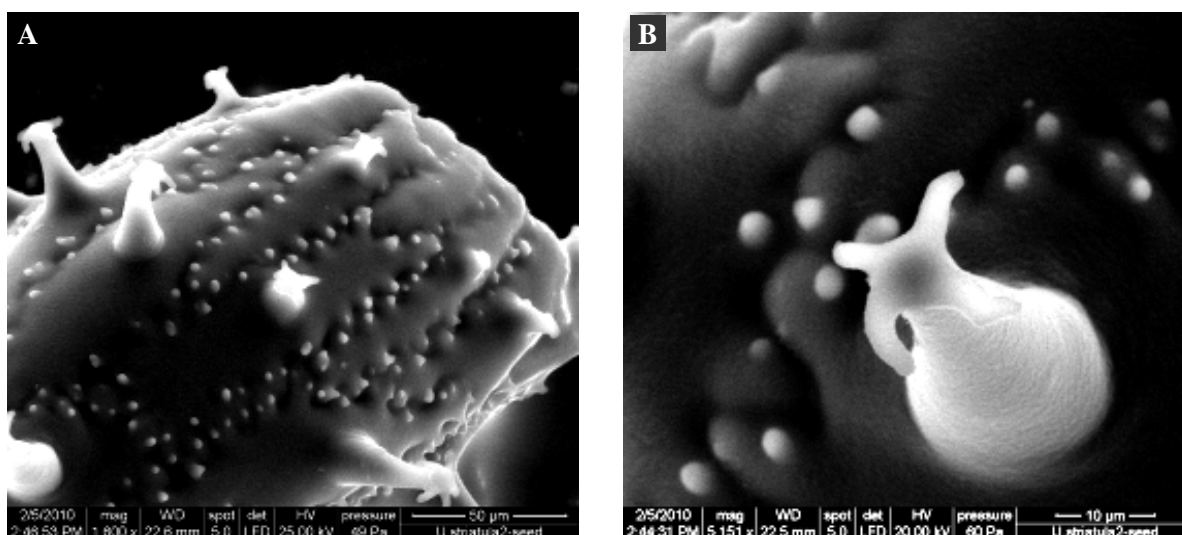


Fig. 4. *Utricularia striatula* seed testa. A. The sparse and short processes on the smooth periclinal walls with the verrucose boundaries. B. The glochidiate tips.

Conclusion

A SEM study of seeds has discovered a new character that has the potential to distinguish sections and species of *Chirita*. The present study has provided additional evidence on the importance of micromorphological characters in solving various taxonomic problems (Abid & Ali, 2010; Rajbhandary & Shrestha, 2010; Abid *et al.*, 2011).

With the confirmation of diagnostic micro-characters using SEM, *U. furcellata* could be established as a new *Utricularia* species record for Peninsular Malaysia. Its disjunctive distribution in two different floristic regions that are almost 3,000 km apart could suggest successful long distance dispersal or the possibility of specimens from localities in between having been misidentified as the similar looking *U. striatula*, which, at the moment, is reported from Africa to New Guinea. *Utricularia furcellata* is currently given a Critically Endangered status in the Plant Red List for Peninsular Malaysia because of its extremely restricted population and its sensitive nature to disturbance. This further distinguishes it from *U. striatula*, a fairly common montane species with a Near Threatened status.

Acknowledgement

The authors would like to thank the following institutions for financial support pertaining to the study: Forest Research Institute Malaysia (FRIM), Research and Pre-commercialisation grant (GPP-TFBC-1208-001); Ministry of Science, Technology and Innovation, Flora of Peninsular Malaysia Project (01-04-01-0000 Khas 2); University of Malaya (UM), Postgraduate Research Grant (PS169/2008B / PS235/2009C and PS171/2008B) and Ministry of Natural Resource and Environment, RMK9 Masters and PhD Scholarship. Sincere gratitude is also due to curators and keepers of the herbaria of BKF, C, K, KEP, KLU, L and SING, and the technical staff of SEM and anatomy laboratories of FRIM and Institute of Biological Sciences, UM. Last but not least to Dr. R.

Kiew for editing help, Dr. L.S.L. Chua for reviewing the conservation status assessments, and Dr. L.G. Saw, Dr. R.C.K. Chung and Dr. E. Soepadmo for their advice and to the staff of Forest Biodiversity Division, FRIM, for assistance and support.

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(Received for publication 2 November 2011)