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## THE SYSTEMATIC SIGNIFICANCE OF LEAF EPIDERMAL MICROMORPHOLOGY OF TEN *NEPENTHES* SPECIES (NEPENTHACEAE) FROM PENINSULAR MALAYSIA

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### ABSTRACT

GHAZALLI, M. N., TAMIZI, A. A., ESA, M. I. M., BESI, E. E., NIKONG, D., NORDIN, A. R. M. & ZAINI, A. Z. 2019. The systematic significance of leaf epidermal micromorphology of ten *Nepenthes* species (Nepenthaceae) from Peninsular Malaysia. Reinwardtia 18(2): 81–96. — The pitcher plants of Malaysia belong to the genus *Nepenthes* and can be found thriving in swampy areas, along the roadside, on hillslopes and in mountainous terrains depending on species and their ecological preferences. In this study, cuticle micromorphology of ten species of *Nepenthes* (Nepenthaceae) collected from Peninsular Malaysia was intensively studied through scanning electron microscopy (SEM) to characterise and investigate diagnostic characters of cuticle micromorphology that can be useful in *Nepenthes* classification. A total of eleven characters from the inner and outer cuticles were enumerated in details and these characters have a value either for infrageneric classification or for diagnostic identification of the species. Characters observed and analysed were related to the epidermal cells, subsidiary cells, stomatal complex *i.e* type of waxes on both epidermal surfaces, abaxial and adaxial cuticular ornamentation, stomata characteristics, stomata formation, stomata frequency, cuticular ornamentation on stomata, shape of the stomata, stomata size, trichome existence and type of trichomes. *Nepenthes ampullaria* is clearly distinguished from the other species by markedly different types of tufted and multicellular trichomes of the epidermal cells on both leaf epidermal surfaces. For *N. alba*, its cuticular feature showed groovy cuticular pattern on the abaxial and adaxial surface, hence, can serve as a diagnostic cuticular pattern for this species. From these findings, the species delimitation based on cuticular features show a clear resolution, however some species might be individually distinct based on the combination of characters examined.

**Key Words:** Characters, classification, identifications, *Nepenthes*, Peninsular Malaysia,

### ABSTRAK

GHAZALLI, M. N., TAMIZI, A. A., ESA, M. I. M., BESI, E. E., NIKONG, D., NORDIN, A. R. M. & ZAINI, A. Z. 2019. Signifikansi sistematik mikromorfologi epidermis daun dari sepuluh jenis *Nepenthes* (Nepenthaceae) dari Semenanjung Malaysia. Reinwardtia 18(2): 81–96. — Kantong semar di Malaysia dimasukkan ke dalam marga *Nepenthes* dan dapat ditemukan tumbuh di rawa-rawa, tepian jalanan, tebing bukit dan tebing gunung tergantung jenis dan keperluan ekologi. Mikromorfologi kutikula sepuluh jenis *Nepenthes* (Nepenthaceae) yang diperoleh dari Semenanjung Malaysia telah diteliti secara intensif dengan menggunakan ‘scanning microscope electron’ (SEM).

Sebelas karakter kutikula bagian luar dan dalam telah diuraikan secara terperinci. Karakter-karakter ini mempunyai nilai untuk klasifikasi infragenerik dan identifikasi diagnostik jenis. Karakter yang diobservasi dan dianalisis berkaitan dengan sel epidermis, sel tetangga, kompleks stomata yaitu tipe lilin pada kedua permukaan epidermis, ornamentasi kutikula di abaksial dan adaksial, karakter, formasi dan frekuensi stomata, ornamentasi kutikula pada stomata, bentuk dan ukuran stomata, keberadaan dan tipe trikoma. *Nepenthes ampullaria* secara jelas berbeda dengan jenis-jenis *Nepenthes* yang lain dengan kehadiran variasi trikoma multiseluler dan trikoma berumpun pada kedua permukaan epidermis. Karakter kutikula yang bergerigi pada kedua permukaan abaksial dan adaksial pada *Nepenthes alba* dapat dijadikan panduan untuk ciri identifikasi diagnostik untuk jenis ini. Hasil penelitian ini menunjukkan pembatasan jenis berdasarkan ciri kutikula jelas menunjukkan resolusi yang bermakna, namun demikian sebagian jenis mempunyai kemungkinan untuk dibedakan secara individu berdasarkan kombinasi karakter-karakter yang diuji.

**Kata Kunci:** Identifikasi, karakter, klasifikasi, *Nepenthes*, Semenanjung Malaysia.

## INTRODUCTION

*Nepenthes* is one of the most abundant and diversified carnivorous plant groups. There are about 160 species recorded worldwide that distributed widely in the wet Old World from Madagascar, the Seychelles, Sri Lanka, India, across to China, the Philippines, New Guinea, Australia and New Caledonia (Adam, 1992; Alastair *et al.*, 2019; McPherson, 2009; Phillipps *et al.*, 2008; Shivas, 1984). In Malaysia, the genus is represented by 46 species; thriving in lowlands and mountain forests, also on disturbed land including the roadsides and old mining sites (Phillipps & Lamb, 1996; Saibeh *et al.*, 2016). To date, there are 11 species can be found in Peninsular Malaysia (*N. alba* Ridl., *N. albomarginata* T. Lobb. ex Lindl., *N. gracillima* Ridl., *N. ampullaria* Jack, *N. benstonei* C. Clarke, *N. gracilis* Korth., *N. macfarlanei* Hemsl., *N. rafflesiana* Jack., *N. mirabilis* (Lour.), Druce, *N. ramispina* Ridl. and *N. sanguinea* Lindl.) (Fig. 1); while the rest of 35 species are distributed randomly throughout Malaysian Borneo (Clarke, 2001; Clarke, 2002). As suggested by their vernacular name (pitcher plant or monkey cup), all *Nepenthes* species produce morphological variation of jug-shaped pitchers that function to attract, trap and digest small animals specifically the insect groups. However, some species have preferred dead leaves, and even animal excretions, over insects for nutrients as can be observed in *N. ampullaria* and a Bornean species *N. lowii* (Clarke, 1997 & 2001).

The plant cuticle is a technically important character for plants that basically functions as a protective layer for the plant and it imprints the sculptural layer of the epidermis (Barranova, 1992; Hu, 1986 & Fahn, 1990). Related research involving the study of cuticular layer of plants were initiated by several research projects that investigated the potential of epidermis cuticular study of the fossil conifers and several other species, which has sparked interests among plant taxonomists due to the stable chemical nature of the plant cuticle. This character is then utilized in spe-

cies enumerations and has gained more attention with the advent of SEM (Cantrill, 1989; Dilcher, 1974; Herbin & Sharma, 1969; Hill & Carpenter, 1991; Martin & Juniper, 1970; Marques, 2000; Stockey & Ko, 1986; Wells & Hill, 1989). As an example, recent microstructural research on the cuticular characteristics in leaf micromorphology of multiple plants including *Lepisanthes* (Sapindaceae), *Melastoma* (Melastomaceae) and *Parashorea* (Dipterocarpaceae) species in Malaysia (Mohd Norfaizal *et al.*, 2018; Noorma Wati *et al.*, 2016; Noraini & Cutler, 2009) had revealed relationship between cuticle characters and the currently accepted taxonomy classification, shedding light into the potential of utilizing these characters for taxonomic classification.

In this study, we investigated the usefulness of leaf cuticle micromorphology of ten out of eleven *Nepenthes* species in Peninsular Malaysia in order to add the different cuticular characters for infrageneric classification of pitcher plants. This approach of using cuticular characters has not been enumerated and explored for *Nepenthes* species distinction; hence, it is thought this effort will be worth to be tested. The data obtained will also serve as reference material useful in identifying vegetative specimens of *Nepenthes* remains and affix more systematic evidences in *Nepenthes* genus for the identification purpose.

## MATERIALS AND METHODS

*Nepenthes* leaves used in this study were obtained from field collection and their identification were conducted in line with available herbarium specimens that are available at KEP; MDI and UKMB collections (see Table 1 for voucher details). In total, ten species with one to three replications of *Nepenthes* originating in Peninsular Malaysia were examined. Epidermis preparative fragments about 3 mm long were excised from the middle portion of approximately three mature leaves of each species. Cuticles part were prepared by soaking leaf blade fragments in 5–10% aqueous chromium trioxide until all



Fig. 1. Studied species of *Nepenthes*. A. *Nepenthes alba*. B. *N. albomarginata*. C. *N. ampullaria*. D. *N. benstonei*. E. *N. gracilis*. F. *N. ramispina*. G. *N. macfarlanei*, H. *N. mirabilis*. I. *N. sanguinea*. J. *N. rafflesiana*. (Photos by: Amin Asyraf & Mohd Norfaizal, 2019).

organic material except the cuticle was dissolved. Related SEM preparation procedures were conducted following Alvin & Boulter (1974); Halbritter (1997); Yoshie & Sakai (1985). Isolated *Nepenthes* epidermal cuticles were then rinsed in distilled water. Targeted leaves cuticles were mounted on aluminium stubs with double-sided adhesive tape and proceeded for air drying. The stubs were then sputter-coated with pure gold to a maximum thickness of 15 nm and examined with LEO (Model 1450 SEM)-Field-Emission Scanning Electron Microscope (FESEM). The remaining cuticles were soaked in 5% ammonia and mounted on microscope slides in Canada balsam. All the stubs and slides prepared are housed in the SEM Collection of Plant Anatomy and Palynology Laboratory, Agrobiodiversity and Environment Research Centre, MARDI Serdang, Selangor, Malaysia, while the SEM analysis was conducted at Microscopy Electron Unit, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi Campus, Selangor, Malaysia.

## RESULTS AND DISCUSSION

This study deals with *Nepenthes* cuticle micromorphology and has documented a new character set which will be useful in identification and classifying different *Nepenthes* species. Eleven important ultrastructural characters from both the inner and outer cuticular surfaces have been described, representing the first report of such characters for the Peninsular Malaysian *Nepenthes*. Enumeration of the *Nepenthes* micromorphological features of both abaxial and adaxial surfaces under scanning electron microscope are shown in Figs. 2–6. Examination of the leaves sculpturing, stomata and epidermal characters of the species showed interesting information-of which some of them can serve as diagnostic characteristics-specifically on the stomata features, stomata formation, trichome distribution and type of trichomes. The leaves of all species examined consisted of either hypostomatic or amphistomatic, the stomata were dispersed randomly all over the whole abaxial/

**Table 1.** *Nepenthes* species examined, their sources and voucher details.

Species	Localities
<i>Nepenthes alba</i>	Malaysia, Pahang, Gunung Tahan. Muhammad Ikhwanuddin M.E. MDI 12346, 8 February 2019 (MDI)
<i>N. albomarginata</i>	Malaysia, Kedah, Gunung Jerai FR, Compartment 20-23. Kamarul Hisham, M., Noorsiha, A. & Kueh, H.L, FRI 52039, 10 June 2006 (FRI) Malaysia, Pulau Pinang, Bukit Bendera, Mohd Norfaizal, G., MDI 12337, 16 December 2018 (MDI) Malaysia, Johor, Gunung Ledang. Ruth Kiew, RK1677, 8 May 1985 (FRI)
<i>N. ampullaria</i>	Malaysia, Johor, Mersing. Rohana M.S. RMS 081, 28 November 1989 (FRI) Malaysia, Terengganu, Sungai Tong. MDI 12341, Mohd. Norfaizal, G. Amin Asyraf, T., Dome, N. 31 January 2019 (MDI) Malaysia, Selangor, Sungai Karang FR, Tanjung Karang. FRI 38751. Lilian, C. 28 April 1993 (FRI)
<i>N. benstonei</i>	Malaysia, Kelantan, Machang, Bukit Bakar. FRI 53169. Yao, T.L. Duistermart, H. Kiew, R. Kueh, H.L. 27 September 2006 (FRI) Malaysia, Kelantan, Machang, Bukit Bakar Summit. MDI 12333. Mohd Norfaizal, G. & Salmaniza, S. 5 March 2019 (MDI) Malaysia, Terengganu, Gunung Tebu. FRI 13147. Mohd Shah, Ahmad Shukor, Mahmud Awang. 31 May 1974 (FRI)
<i>N. gracilis</i>	Malaysia, Pahang, Temerloh, Tasik Bera. FRI52720. Rafidah, A.R., Mohd. Nazri, A., Kueh, H.L. 10 July 2007 (FRI) Malaysia, Bangi, UKM Bangi. MDI 12336. Mohd. Norfaizal, G. & Amin Asyraf, T. 15 December 2018 (MDI) Malaysia, Kedah, Pulau Langkawi, FRI63422. Beentje, H.J., Rosdi, M., Angan. 18 February 2009 (FRI)
<i>N. macfarlanei</i>	Malaysia, Pahang, Bentong, Gunung Ulu Kali. FRI 52572. Nor Ezzawanis, A.T. 16 February 2007 (FRI) Malaysia, Pahang, Summit Ridge of Gunung Ulu Kali to Gunung Rajah. Lilian Chua. FRI 40516. 16 January 1995 (FRI) Malaysia, Pahang, Pathway to Telekom Telecommunication Tower, Genting Highlands. MDI 12331. Mohd Norfaizal, G. & Amin Asyraf, T. 9 December 2018 (MDI)
<i>N. mirabilis</i>	Malaysia, Selangor, Hulu Selangor, Bukit Tunggul FR. FRI 73129. Chew, M.Y., Mohd Hairul, M.A. & Mohd Afiq, K. 24 May 2016 (FRI) Malaysia, Selangor, Puncak Alam. MDI 12416. Mohd Norfaizal, G. Anuar Rasyidi, M.N., Ahmad Syahman, M.D.& Muhammad Syakir. 30 December 2018 (MDI)
<i>N. ramispina</i>	Malaysia, Selangor, Gunung Bunga Buah. FRI65498. Yao, T.L. Kiew, R. & Jun, W. 31 October 2010 (FRI) Malaysia, Pahang, Pathway to Telekom Telecommunication Tower, Genting Highlands. MDI 12332. Mohd Norfaizal, G. & Amin Asyraf, T. 9 December 2018 (MDI)
<i>N. sanguinea</i>	Malaysia, Terengganu, Hulu Terengganu, Gunung Padang. FRI70887. Mohd Hairul, M.A., Imin, K., Rafidah, A.R. Ummul Nazrah, A.R. Kueh, H.L. 21 March 2010 (FRI) Malaysia, Selangor, Gunung Ulu Semangkok. MDI 12418. Mohd Norfaizal, G. Anuar Rasyidi, M.N., Ahmad Syahman, M.D.& Muhammad Syakir. 13 January 2019 (MDI) Malaysia, Perak, Gunung Hijau summit plateau, FRI 40475. 6 September 1994. Lilian Chua (FRI)
<i>N. rafflesiana</i>	Malaysia, Johor, Kluang, Kluang FR, Gunung Belumut. FRI60328. Chew, M.Y. & Teo, Y.L. 10 August 2009 (FRI) Malaysia, Johor, Gunung Ledang, FRI33511. Lilian Chua. 4 June 1993 (FRI)

adaxial and abaxial surfaces, and the stomata were all of either slightly raised above, same level with the epidermis or sunken below the epidermis. Details on the species micromorphological features are described as below:

#### 1.1. NEPENTHES ALBA Ridl.

Type of waxes: film layer and crustose observed on the adaxial epidermal surface; crustose observed on the abaxial epidermal surface. Abaxial cuticular ornamentation: Type III, unclear – anticlinal wall unclear and periclinal wall sunken. Adaxial cuticular ornamentation: Type I, clear – anticlinal wall can be observed clearly, periclinal wall sunken. Cuticular striation exists on the adaxial surface. Stomata characteristics: hypostomatic. Stomata formation: superficial. Stomata frequency: exist with dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim raised and clearly noticeable. Shape: ellipse. Epidermal cell do not wrap the stomatal rim surface. Subsidiary and epidermal cells can be differentiated easily. Stomatal cuticular striation: absent. Stomatal size: L (13.50–16.89  $\mu\text{m}$ )  $\times$  W (8.01–9.07  $\mu\text{m}$ ). Trichome distribution: can be observed on the adaxial and adaxial epidermis surfaces. Trichome type: simple, unicellular trichome (short, bended) can be observed on the adaxial surface. Capitate glandular trichome (sessile) can be observed on the adaxial surface. Peltate glandular trichome observed with random formation on the abaxial surface.

#### 1.2. N. ALBOMARGINATA W.Lobb ex Lindl.

Type of waxes: film layer observed on the adaxial epidermal surface; film layer and granule observed on the abaxial epidermal surface. Abaxial cuticular ornamentation: Type III, unclear – anticlinal wall unclear and periclinal wall sunken. Adaxial cuticular ornamentation: Type III, unclear – anticlinal wall unclear and periclinal wall sunken. Cuticular striation observed clearly on the adaxial surface. Stomata characteristics: amphistomatic. Stomata formation: same level with the epidermal wall. Stomata frequency: exist with dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim raised and clearly noticeable. Shape: ellipse. Epidermal cell do not wrap the stomatal rim surface. Subsidiary and epidermal cells can be differentiated easily. Stomatal cuticular striation: absent. Stomatal size: L (14.29–18.12  $\mu\text{m}$ )  $\times$  W (7.01–10.07  $\mu\text{m}$ ). Trichome distribution: can be observed on the adaxial and adaxial epidermis surfaces. Trichome type: simple, unicellular trichome (short, bended, smooth) can be observed on the abaxial surface. Capitate glandular trichome (sessile) can be observed on the adaxial surface. Peltate glandular trichome (unicellular terminal) observed with random formation on the abaxial surface. Stellate

compound trichome observed on adaxial surface.

#### 1.3. N. AMPULLARIA Jack

Type of waxes: film layer observed on the abaxial epidermal surface; film layer and flakes observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type I, clear – anticlinal wall clear and distinct while periclinal wall sunken. No cuticular striation. Adaxial cuticular ornamentation: Type I, clear – anticlinal wall clear and distinct while periclinal wall sunken. No cuticular striation. Stomata characteristics: hypostomatic. Stomata formation: superficial, raised from the epidermal wall. Stomata frequency: very dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim raised and clearly noticeable. Stomatal rim and frame unclear. Epidermal cells do not wrap the stomatal surface. Shape: broad ellipse. Subsidiary cell and epidermal can be differentiated easily. Stomata semi-raised. Stomatal cuticular striation: absent. Stomatal size: L (11.48–16.12  $\mu\text{m}$ )  $\times$  W (9.01–10.02  $\mu\text{m}$ ). Trichome distribution: can be observed on the adaxial and adaxial epidermis surfaces. Trichome type: peltate glandular trichome (unicellular terminal) can be observed on the abaxial surface. Stellate rotate trichome can be observed on the adaxial surface. Peltate glandular trichome (unicellular; sessile). Simple, unicellular trichome (unicellular, bended, smooth surface) and be observed on abaxial surface. Scale trichome observed on the adaxial surface. Papillae ornamentation also can be seen covering the abaxial epidermal surface.

#### 1.4. N. BENSTONEI C. Clarke

Type of waxes: film layer observed on the abaxial epidermal surface; film layer, crustose, observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type I, clear – anticlinal wall clear while periclinal wall sunken. with cuticular striation. Adaxial cuticular ornamentation: Type II, unclear – anticlinal wall semi-raised while periclinal wall sunken. Cuticular striation exists on the adaxial surface. Stomata characteristics: hypostomatic. Stomata formation: parafacial, semi-raised from the epidermal wall. Stomata frequency: dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim not clearly noticeable. Stomatal rim and its frame unclear. Epidermal cells do not wrap the stomatal surface. Shape: rounded. Subsidiary cell and epidermal can be differentiated easily. Stomata semi-raised. Stomatal cuticular striation: absent. Stomatal size: L (10.61–13.17  $\mu\text{m}$ )  $\times$  W (12.89–14.63  $\mu\text{m}$ ). Trichome distribution: can be observed on the adaxial and adaxial epidermis surfaces. Trichome type: peltate glandular trichome (multicellular terminal, sessile) can be observed with scattered formation on the

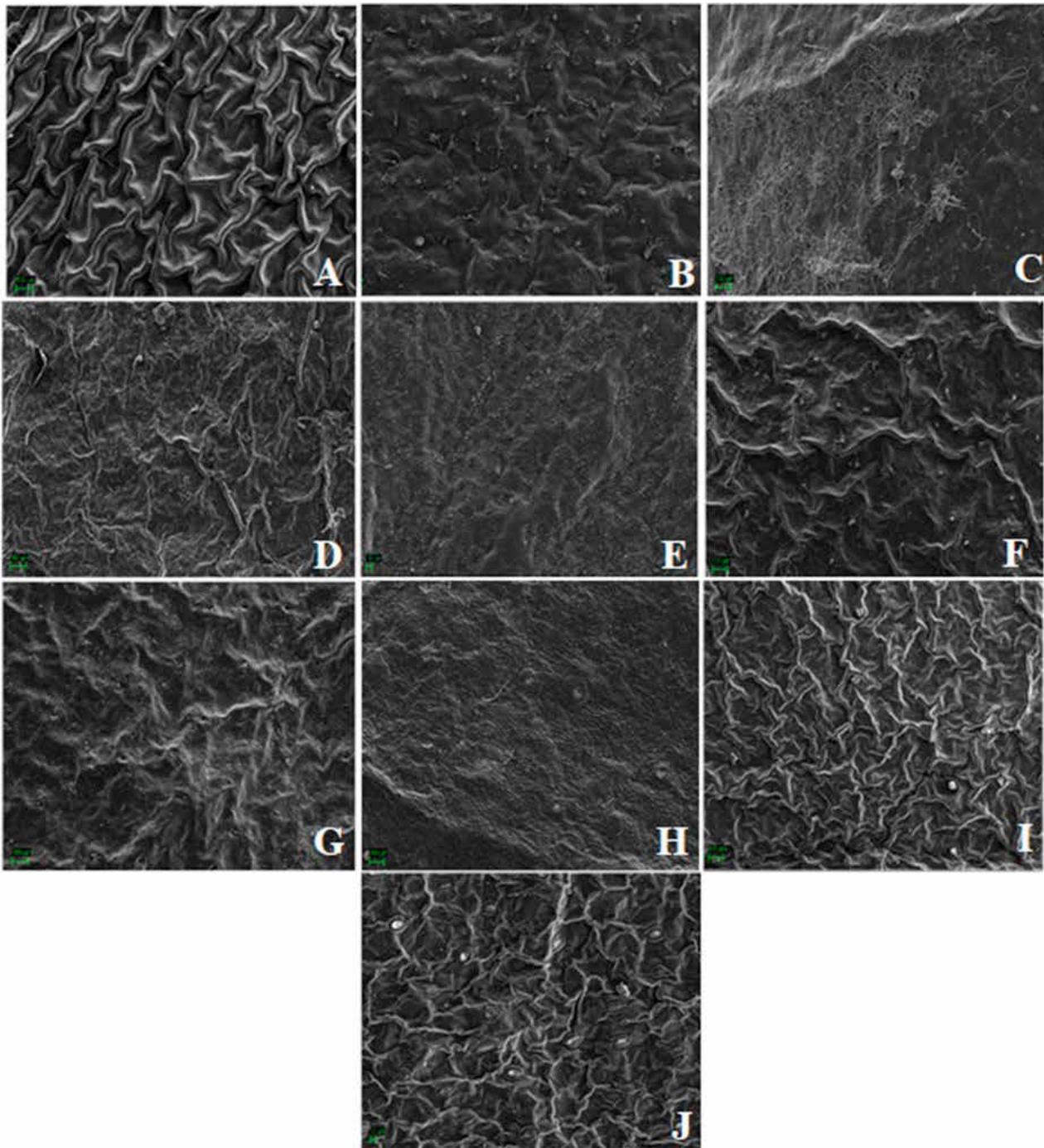


Fig. 2. SEMs of adaxial surfaces of leaf showing the surface sculpturing of A. *Nepenthes alba*. B. *N. albomarginata*. C. *N. ampullaria*. D. *N. benstonei*. E. *N. gracilis*. F. *N. macfarlanei*. G. *N. mirabilis*. H. *N. rafflesiana*. I. *N. ramispina*. J. *N. sanguinea*. (Magnification A-J: 100×). (Photos by: Mohd Norfaizal & Ahmad Zaki).

abaxial surface. Scale trichome observed on the adaxial surface.

#### 1.5. *N. GRACILIS* Korth.

Type of waxes: film layer and granule observed on the abaxial epidermal surface; film layer and flakes observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type III, unclear – anticlinal wall unclear while periclinal wall sunken. No cuticular striation. Adaxial cuticular ornamentation: Type II, unclear – anticlinal wall semi-raised while periclinal wall sunken. No cuticular striation exists on the adaxial surface. Stomata characteristics: hipostomatic. Stomata formation: superficial, in parallel formation, raised from the epidermal wall. Stomata frequency: very dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim not clearly noticeable. Stomatal rim and its frame unclear. Epidermal cells do not wrap the stomatal surface. Shape: ellipse. Subsidiary cell and epidermal not distinguishable. Stomata semi-raised. Stomatal cuticular striation: absent. Stomatal size: L (12.74–14.00  $\mu\text{m}$ )  $\times$  W (8.00–9.50  $\mu\text{m}$ ). Trichome distribution: can be observed on the adaxial epidermal surfaces. Trichome type: peltate glandular trichome (multicellular terminal) can be observed on the adaxial surface.

#### 1.6. *N. MACFARLANEI* Hemsl.

Type of waxes: film layer observed on the abaxial epidermal surface; film layer and flakes observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type II, unclear – anticlinal wall semi-raised while periclinal wall sunken. Cuticular striation observed on the abaxial surface. Adaxial cuticular ornamentation: Type II, unclear – anticlinal wall semi-raised while periclinal wall sunken. No cuticular striation exists on the adaxial surface. Stomata characteristics: hipostomatic. Stomata formation: superficial, in tangential parallel formation, raised from the epidermal wall. Stomata frequency: very dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim clearly noticeable. Stomatal rim and its frame clear. Epidermal cells do not wrap the stomatal surface. Shape: ellipse. Subsidiary cell and epidermal not distinguishable. Stomata raised on the epidermal surface. Stomatal cuticular striation: absent. Stomatal size: L (15.74–16.00  $\mu\text{m}$ )  $\times$  W (8.00–9.37  $\mu\text{m}$ ). Trichome distribution: can be observed on the abaxial and adaxial epidermal surfaces. Trichome type: peltate glandular trichome (multicellular terminal) can be observed on the abaxial surface. Simple, unicellular (smooth surface, pointed tip) can be observed randomly on the adaxial epidermal surface.

#### 1.7. *N. MIRABILIS* (Lour.) Druce

Type of waxes: film layer, sheet and granule observed on the abaxial epidermal surface; film layer and sheet observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type II, unclear – anticlinal wall semi-raised while periclinal wall sunken. Cuticular striation observed on the abaxial surface. Adaxial cuticular ornamentation: Type III, unclear – anticlinal wall unclear while periclinal wall sunken. Cuticular striation exists on the adaxial surface. Stomata characteristics: hipostomatic. Stomata formation: parafacial, in random formation, sunken in the epidermal wall. Stomata frequency: very dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim clearly noticeable. Stomatal rim and its frame clear. Epidermal cells wrapping the stomatal surface. Shape: ellipse. Subsidiary cell and epidermal not distinguishable. Stomata raised on the epidermal surface. Stomatal cuticular striation: present. Stomatal size: L (15.74–16.00  $\mu\text{m}$ )  $\times$  W (10.00–12.37  $\mu\text{m}$ ). Trichome distribution: can be observed on the abaxial and adaxial epidermal surfaces. Trichome type: peltate glandular trichome (multicellular terminal) can be observed on the abaxial and adaxial surfaces. Peltate glandular trichome (unicellular terminal, short-stalked) observed on the abaxial epidermal surface.

#### 1.8. *N. RAMISPINA* Ridl.

Type of waxes: film layer and granule observed on the abaxial and adaxial epidermal surfaces. Abaxial cuticular ornamentation: Type I, clear – anticlinal wall raised while periclinal wall sunken. No cuticular striation observed on the abaxial surface. Adaxial cuticular ornamentation: Type I, clear – anticlinal wall arise while periclinal wall sunken. Cuticular striation exists on the adaxial surface. Stomata characteristics: hipostomatic. Stomata formation: superficial, in random formation, raised on the epidermal wall. Stomata frequency: very dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim clearly noticeable. Stomatal rim and its frame clear. Epidermal cells do not wrap the stomatal surface. Shape: ellipse. Subsidiary and epidermal cells not distinguishable. Stomata raised on the epidermal surface. Stomatal cuticular striation: absent. Stomatal size: L (16.74–17.00  $\mu\text{m}$ )  $\times$  W (14.02–15.00  $\mu\text{m}$ ). Trichome distribution: can be observed on the abaxial and adaxial epidermal surfaces. Trichome type: peltate glandular trichome (multicellular terminal) can be observed on the abaxial surface. Peltate glandular trichome (unicellular terminal, short-stalked) observed on the abaxial epidermal surface. Simple, unicellular (pointed tip) can be observed on the abaxial surface. Papillae ornamentation can

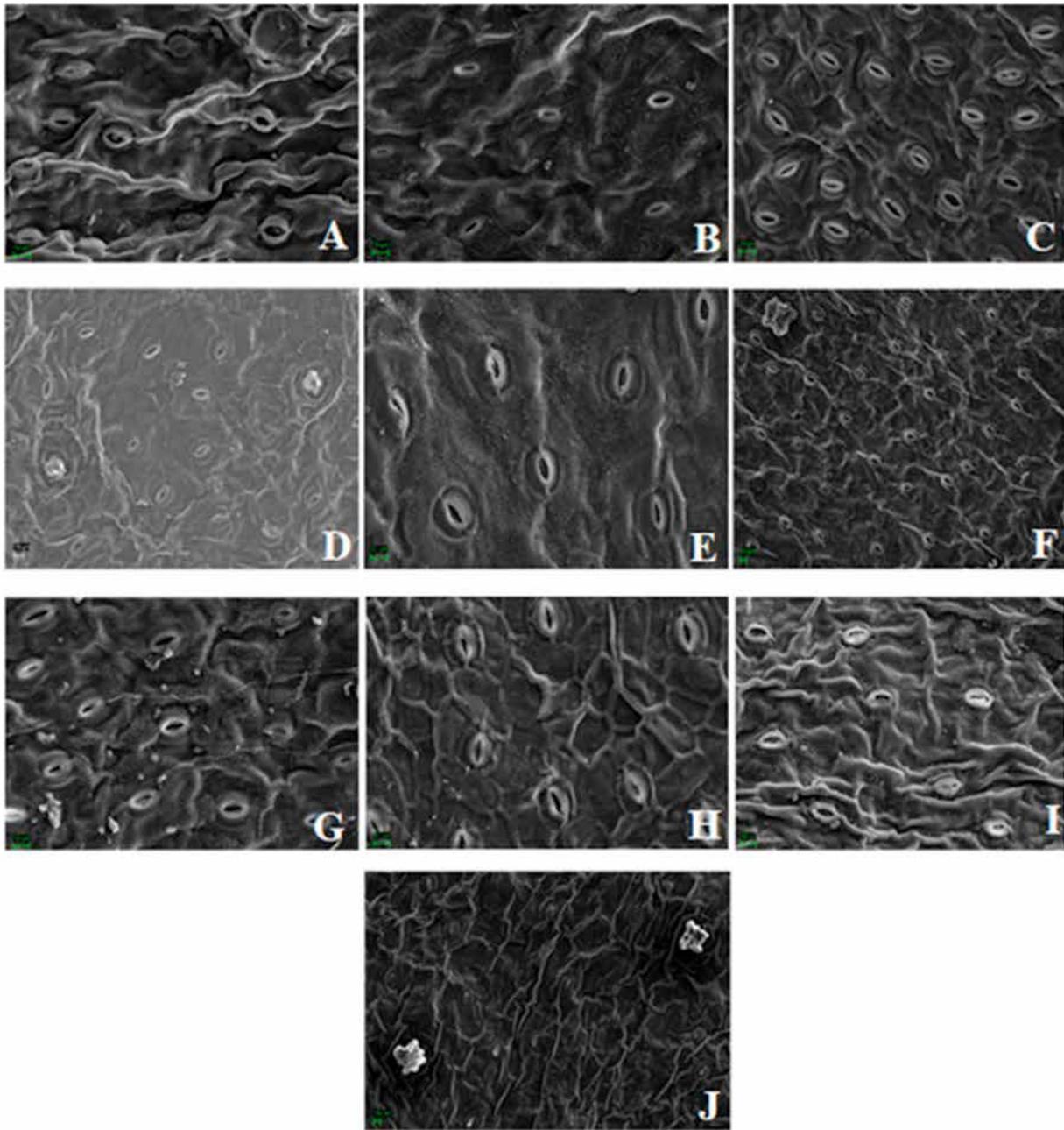


Fig. 3. SEMs of abaxial surfaces of leaf showing the surface sculpturing of A. *Nepenthes alba*. B. *N. albomarginata*. C. *N. ampullaria*. D. *N. benstonei*. E. *N. gracilis*. F. *N. macfarlanei*. G. *N. mirabilis*. H. *N. rafflesiana*. I. *N. ramispina*. J. *N. sanguinea*. (Magnification A-J: 100 $\times$ ). (Photos: Mohd Norfaizal & Ahmad Zaki).

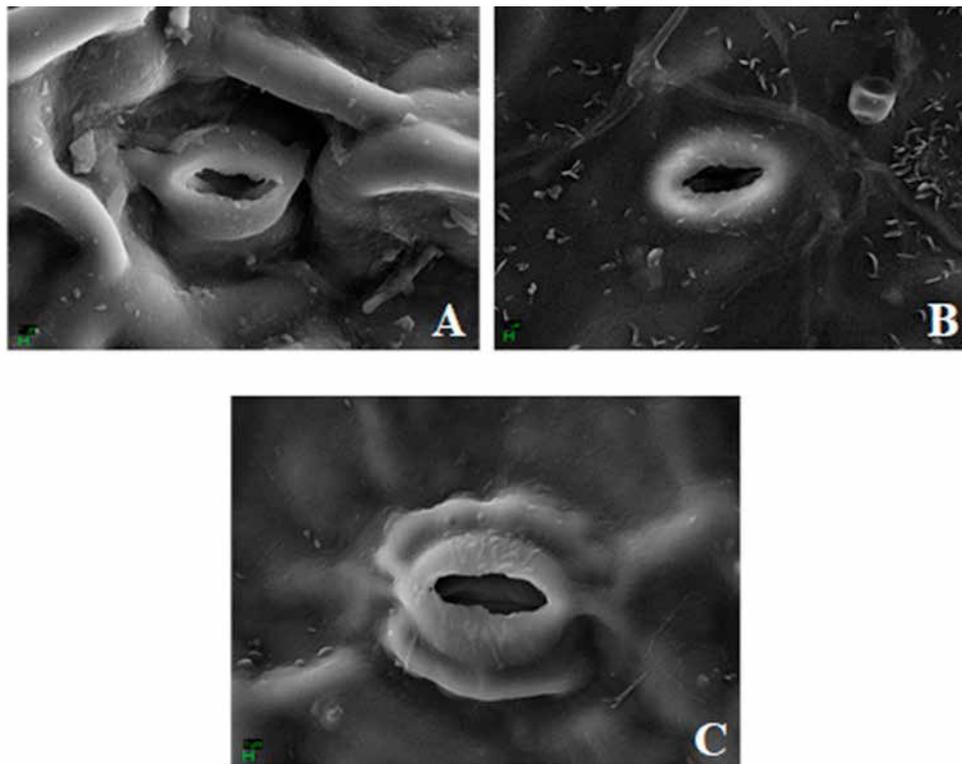


Fig. 4. Plate of diagnostic stomata formation in *Nepenthes*; A. *Nepenthes alba*, sunken stomata without striation. B. *N. albomarginata*, same level with the epidermal surface formation without striation, and C. *N. macfarlanei*, raised formation stomata as compared with the epidermal surface, with stomatal-rim formation. (Magnification A-C: 100 $\times$ ). (Photos: Mohd Norfaizal & Ahmad Zaki).

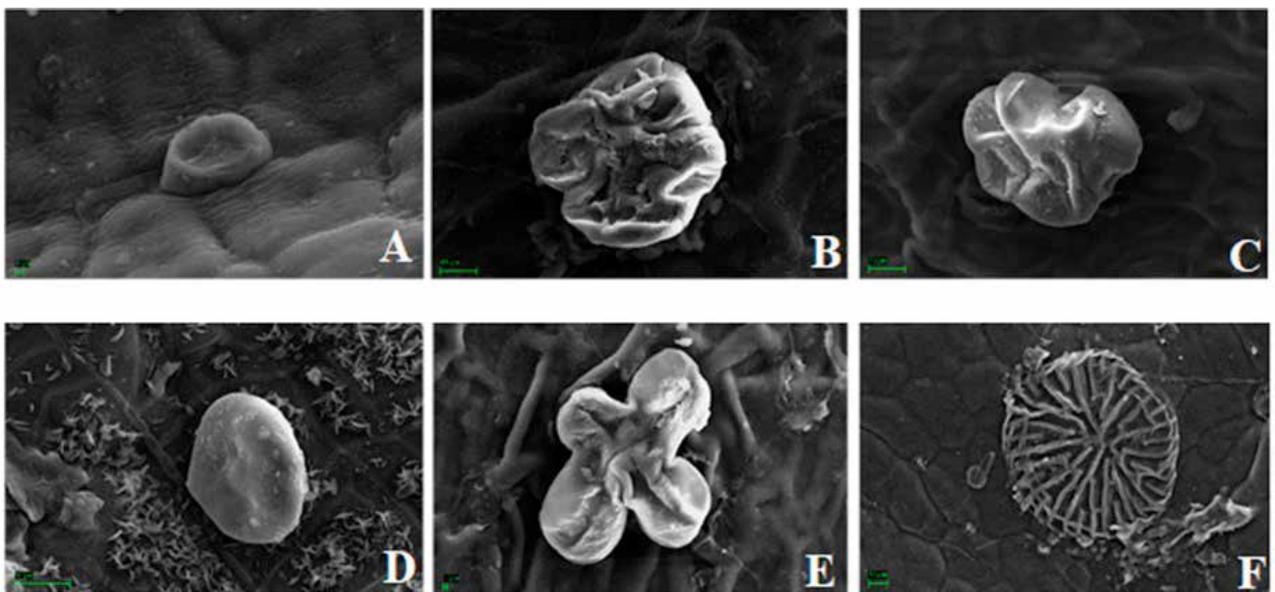


Fig. 5. A-E. Variation of peltate glandular trichomes (unicellular and multicellular) and F. scale trichome observed in *Nepenthes* genus (Magnification A-F: 1000 $\times$ ). (Photos: Mohd Norfaizal & Ahmad Zaki).

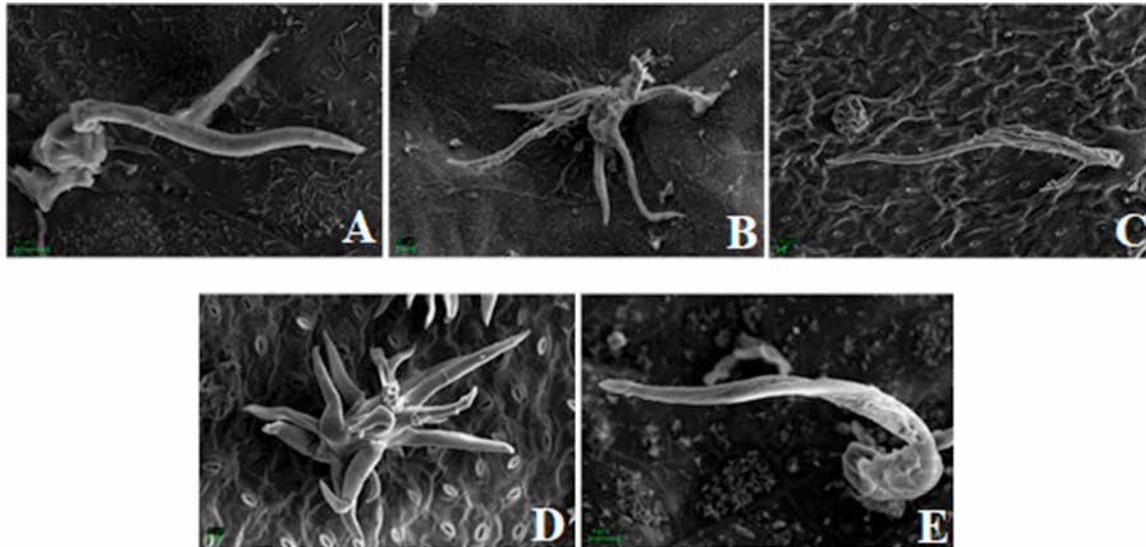


Fig. 6. A-E. Variation of non-glandular trichomes observed in *Nepenthes* genus. A. Simple, unicellular branched trichome. B. Tufted unicellular trichome. C. Simple, unicellular trichome with scales on the surface. D. Stellate rotate trichome. E. Simple, unicellular trichome, unbranched, smooth surface. (Magnification A-E: 1000 $\times$ ). (Photos: Mohd Norfaizal & Ahmad Zaki).

be observed on the abaxial surface. Peltate glandular trichome (multicellular terminal, short-stalked) observed on the abaxial epidermal surface.

#### 1.9. *N. RAFFLESIANA* Jack

Type of waxes: film layer observed on the abaxial epidermal surface; film layer and granule observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type II, unclear – anticlinal wall semi-arise while periclinal wall sunken. Cuticular striation observed on the abaxial surface. Adaxial cuticular ornamentation: Type III, unclear – anticlinal wall unclear while periclinal wall sunken. Cuticular striation exists on the adaxial surface. Stomata characteristics: hipostomatic. Stomata formation: parafacial, in random formation, raised on the epidermal wall. Stomata frequency: dense population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim clearly noticeable. Stomatal rim and its frame clear. Epidermal cells wrapping the stomatal surface. Shape: ellipse. Subsidiary cell and epidermal not distinguishable. Stomata raised on the epidermal surface. Stomatal cuticular striation: absent. Stomatal size: L (11.93–13.05  $\mu\text{m}$ )  $\times$  W (9.00–10.05  $\mu\text{m}$ ). Trichome distribution: can be observed on the abaxial and adaxial epidermis surfaces. Trichome type: peltate glandular trichome (multicellular terminal) can be observed on the abaxial surface. Peltate glandular trichome (unicellular terminal, short-stalked) observed on the abaxial epidermal surface. Simple, unicellular (pointed tip) can be observed

on the abaxial surface. Peltate glandular trichome (multicellular terminal, short-stalked) observed on the abaxial epidermal surface. Simple, unicellular (pointed tip) can be observed on the adaxial surface.

#### 1.10. *N. SANGUINEA* Lindl.

Type of waxes: film layer and granule observed on the abaxial epidermal surface; film layer observed on the adaxial epidermal surface. Abaxial cuticular ornamentation: Type I, clear – anticlinal wall raised while periclinal wall sunken. Cuticular striation observed on the abaxial surface. Adaxial cuticular ornamentation: Type I, clear – anticlinal wall arise while periclinal wall sunked. No cuticular striation exists on the adaxial surface. Stomata characteristics: hipostomatic. Stomata formation: parafacial, in random formation, raised on the epidermal wall. Stomata frequency: sparse population on the abaxial surface. Cuticular ornamentation on stomata: stomatal rim clearly noticeable. Stomatal rim and its frame clear. Epidermal cells wrapping the stomatal surface. Shape: ellipse. Subsidiary and epidermal cells not distinguishable. Stomata sunken in the epidermal surface. Stomatal cuticular striation: absent. Stomatal size: L (14.74–16.00  $\mu\text{m}$ )  $\times$  W (7.00–8.50  $\mu\text{m}$ ). Trichome existence: can be observed on the abaxial and adaxial epidermis surfaces. Trichome type: peltate glandular trichome (multicellular terminal) can be observed on the abaxial surface. Peltate glandular trichome (multicellular terminal, short-stalked) observed on the abaxial and abaxial epidermal surfaces.

The lamina surface has been the subject of investigations compared other plant micromorphology characteristics that often have taxonomic significance (Stace, 1969; Rudall, 1992). Through observation of studied *Nepenthes* lamina surfaces, the anticlinal wall of the adaxial and abaxial epidermal cells was either straight-wavy, straight or groovy which therefore appears to be a common character for this genus. According to Stace (1965), Wilkinson (1979) and Wu *et al.* (2005), cuticular sculpturing may be a diagnostic character of some species. In fact, several related study of the character of cuticular sculpturing in selected genera e.g. *Parnassia* and *Pinus* could serve as a diagnosis note to species level (Price 1998; Wu *et al.*, 2005; Yoshie & Sakai 1985).

In this study, the *Nepenthes* epidermal surfaces revealed a number of important micromorphological characters and these characters exhibited interesting interspecific variations that are of significance for species identification. Further observation under SEM of the adaxial lamina surfaces showed a few taxonomically significant characteristics, *i.e.* the abaxial and adaxial surface being groovy (*Nepenthes alba*), possibly owing to the thick cuticle, lack of stomata on the surfaces and type of trichomes. On the other hand, when the leaf abaxial surface was viewed under the SEM at low magnification, a high diversity in the 'cellular patterns' and the distribution of idioblastic elements such as trichomes were readily recognised. The appearance of the cuticular wax - film layer, granular or crustose - provides much information for classification and observation that may be useful as an additional systematic evidence together with morphological and anatomical observations.

All of the species examined showed characteristics of amphistomatic and hypostomatic, agreeable with preliminary study of Nepenthaceae characters by Metcalfe and Chalk (1950). In this study, amphistomatic stomata features could be only observed in *N. albomarginata*, while the other nine *Nepenthes* species showed hypostomatic stomata feature; hence such a unique trait can give an affirmative diagnostic feature for *N. albomarginata*. Metcalfe and Chalk (1950) also reported the type of stomata in Nepenthaceae as being of ranunculaceous. However, our finding concludes *Nepenthes* from Peninsular Malaysia are characterized by not only ranunculaceous but also paracytic stomatal type, adding a new information that is useful in defining *Nepenthes* species. Further observation also showed that stomata in *Nepenthes* were either raised or same level, and sunken in some species (Fig. 4). In general, sunken and raised stomata are related to habitat preference of the species and environmental adaptations. The presence of sunken

stomata is usually an ecological adaptation related to control of water loss under direct exposure to wind and solar radiation. In the case of sunken stomata, guard cells are placed at the base of a stomatal pit and this arrangement prevents water loss through the stoma (Das & Ghose 1997; Mbagwu & Edeoga 2006). Nevertheless, our study indicates the correlation between sunken, parafacial and superficial stomata and their potential taxonomic and ecological significance for Peninsular Malaysian *Nepenthes* still needs to be further assessed and established in order to see the physiological adaptation factors that influenced these characters. All of the studied *Nepenthes* species also possessed guard cell pairs with either an elliptical, broadly-elliptical or rounded outline.

Photomicrographs of stomata also have revealed an interesting key feature that may be of diagnostic value (Fig. 3). From series of observations, stomatal features as seen under the SEM could be useful for identification especially at the species level as observed in *Nepenthes alba* that possesses sunken stomata without striation, *N. albomarginata* with its stomata same level with the epidermal surface formation without striation, and *N. macfarlanei* with raised stomata from the epidermal surface together with stomatal-rim formation (Fig. 4). Detailed observation of the characters from stomatal microstructure and type of trichome also has succeeded in adding three more diagnostic characters (stomatal formations, trichome distribution and observation of the epidermal surface) that are unique to certain taxa thus becoming important for systematic significance of *Nepenthes* species. Based on stomatal formations – that can be classified as superficial, same level or parafacial – *N. albomarginata* herein could be clearly separated from the rest of the species for having same-level-with-the-epidermal surface stomatal feature. Based on trichome distribution on the epidermal surfaces, *N. gracilis* it the only species showing trichome distribution on the adaxial epidermal surface while the other nine species possess trichome distribution on both sides of epidermal. This finding is coherent with a previous study by Xiang *et al.* (2010) that reported the morphology and distribution of trichomes have valuable systematics significance at species level in *Chelonopsis* (Lamiaceae). Factor that delimiting trichome distribution on the adaxial surface can be compensated by thick epicuticular waxes (film layer and flakes) on the adaxial leaf surface and delimiting the species description in the genus *Nepenthes*. However, further evaluation is relevantly recommended to determine whether these features are influenced by environmental factors due to its habitat preferences.

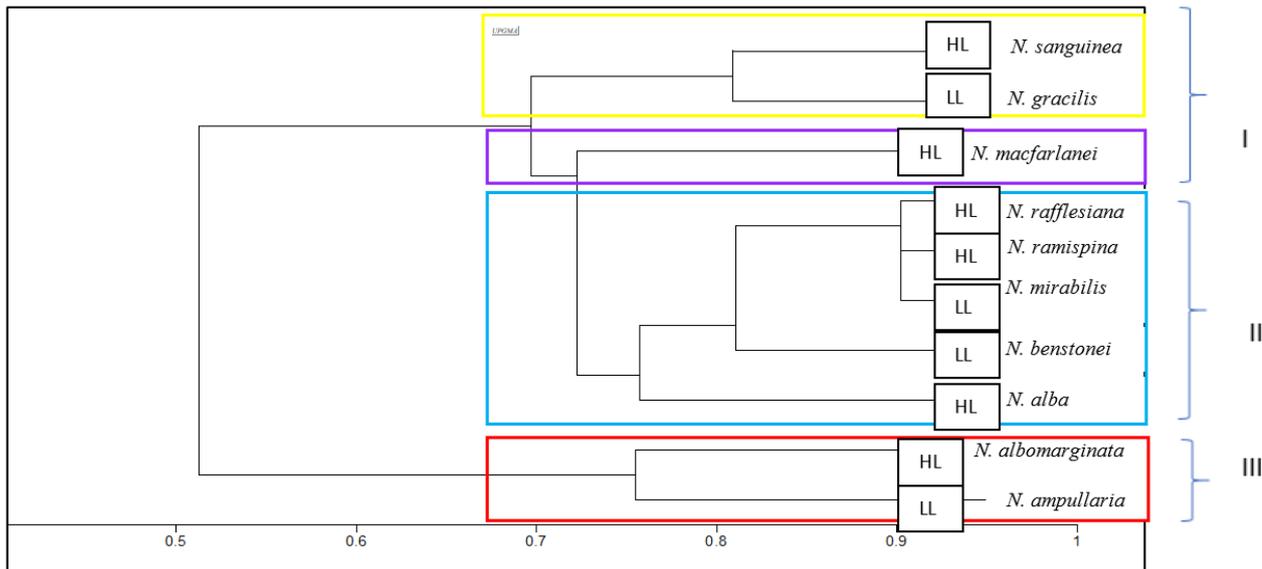


Fig. 7. UPGMA analysis of micromorphology characteristics of Peninsular Malaysia *Nepenthes* using Gower General Similarity Coefficient (HL: highland species; LL: lowland species); (Yellow and Purple Lines: Cluster 1; Blue Line: Cluster II; Red Line: Cluster III).

Through this study, it is also clearly indicated that *N. ampullaria* can be differentiated from other studied *Nepenthes* species with variation in the types of trichomes for having combination of simple, glandular and scale trichome, and we have recorded six types *i.e.* peltate glandular (unicellular terminal, short-stalked), stellate rotate (on adaxial surface), peltate glandular (unicellular, sessile), simple unicellular trichome (smooth surface), scale trichome and combination of papillae formation. This observation of trichomes are agreeable with related research involving different taxa by Chen *et al.* (2013) and Guimares *et al.* (1999) that refer and employed the trichome characteristics in systematic studies of Melastomaceae family.

Finally, we incorporated the features of the *Nepenthes* leaf epidermal micromorphology to infer the relationships among the ten studied species. In this study, *N. ampullaria*, *N. alba*, *N. gracilis* and *N. albomarginata* are clearly distinguished from the other species with the observation of the epidermal ornamentation, stomata features as well as trichome morphology. UPGMA (unweighted pair group method with arithmetic mean) groupings analysis using cuticular micromorphology, however, gave poor resolution. As illustrated, the typical highland *Nepenthes* species namely *N. sanguinea*, *N. macfarlanei*, *N. ramispina*, *N. alba* and *N. benstonei* are not clustered as monophyletic, while subsection III occupying the basal position and comprised of *N. ampullaria* and *N. albomarginata* are not related to each other in terms of ecology and morphology (Fig. 7). For the remaining *Nepenthes* species (*N. gracilis*, *N. rafflesiana* and

*N. mirabilis*), they are considered as monophyletic due to their lowland habitat preference but poorly resolved in this analysis as cuticular study does not support that, due to only one systematic evidence analysis conducted in this study. It is suggested that other systematic evidences – using thorough morphological, anatomical and molecular analyses – should be conducted and incorporated in future research to complement this microstructural section analysis.

All features of the *Nepenthes* leaf epidermal micromorphology analysed in this study are produced in Appendix I and II.

## CONCLUSION

Leaf micromorphology analyses have shown a significant taxonomic value in the genus *Nepenthes* (Nepenthaceae) through the surface sculpturing ornamentation, trichome morphology and its distribution as well as the stomatal feature. It is concluded that this study has added new systematics evidences that are useful in defining the *Nepenthes* species, hence it can be postulated that the micromorphology of the leaf surfaces varies greatly between species.

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Appendix 1. Summary of *Nepenthes* leaf epidermal micromorphology characteristics (Epidermal, stomata)

Species	Sinuosity of		Sinuosity of anticlinal cell wall (adax)	Radiate cuticular striation	Type of stomata	Shape of stomata in outline	Stomatal distribution	Pattern of stomatal distribution	Occurrence of	
	anticlinal cell wall (abax)	trichome on abax							trichome on adax	
<i>Nepenthes ampullaria</i>	Undulate-straight	Straight	Straight	Striated	Ranunculaceus	Rounded	Hipostomatic	Random	No	Low
<i>N. alba</i>	Groovy	Groovy	No	No	Paracytic	Rounded	Hipostomatic	Regular	Low	No
<i>N. albomarginata</i>	Straight	Straight-wavy	No	No	Ranunculaceus	Rounded	Amphistomatic	Random	Low	Low
<i>N. benstonei</i>	Straight	Straight-slightly wavy	No	No	Ranunculaceus	Rounded	Hipostomatic	Regular	Low	Low
<i>N. gracilis</i>	Straight-wavy	Straight	No	No	Ranunculaceus	Broadly-elliptic	Hipostomatic	Regular	No	No
<i>N. macfarlanei</i>	Straight-wavy	Wavy	Striated	Striated	Ranunculaceus	Broadly-elliptic	Hipostomatic	Regular	Medium	Low
<i>N. mirabilis</i>	Straight-wavy	Straight-wavy	No	No	Ranunculaceus	Elliptic	Hipostomatic	Regular	Low	High
<i>N. ramispina</i>	Straight-wavy	Groovy	No	No	Ranunculaceus	Elliptic	Hipostomatic	Random	Low	Medium
<i>N. rafflesiana</i>	Straight-wavy	Straight-wavy	No	No	Ranunculaceus	Elliptic	Hipostomatic	Regular	Low	Low
<i>N. sanguinea</i>	Wavy	Straight	No	No	Ranunculaceus	Broadly-elliptic	Hipostomatic	Random	No	No

Appendix II. Summary of *Nepenthes* leaf epidermal micromorphology characteristics (Trichome)

Species	Frequency of simple trichome	Frequency of tufted trichome	Frequency of stellate trichome	Frequency of cushioned stellate trichome	Occurrence of glandular trichome on abax	Occurrence of glandular trichome on adax	Bodystalk of glandular trichome
<i>Nepenthes ampullaria</i>	Medium-frequency	High	Medium	High	Medium	Low	Sessile
<i>N. alba</i>	Low- frequency	No	No	Low	Medium	High	Sessile
<i>N. albomarginata</i>	Low- frequency	Medium	High	Low	Medium	Low	Shortly-stalked
<i>N. benstonei</i>	Low- frequency	No	No	No	Medium	Low	Shortly-stalked
<i>N. gracilis</i>	No	No	No	No	No	Low	Shortly-stalked
<i>N. macfarlanei</i>	Low- frequency	No	No	No	Low	Low	Shortly-stalked
<i>N. mirabilis</i>	Low- frequency	No	No	No	Low	High	Shortly-stalked
<i>N. ramispina</i>	Low- frequency	No	No	No	Medium	High	Shortly-stalked
<i>N. rafflesiana</i>	Low- frequency	No	No	No	Low	High	Shortly-stalked
<i>N. sanguinea</i>	No	No	No	No	High	Low	Shortly-stalked

## INSTRUCTION TO AUTHORS

**Scope.** *Reinwardtia* is a scientific regular journal on plant taxonomy, plant ecology and ethnobotany published in June and December. Manuscript intended for a publication should be written in English.

**Titles.** Titles should be brief, informative and followed by author's name and mailing address in one-paragraphed.

**Abstract.** English abstract followed by Indonesian abstract of not more than 250 words. Keywords should be given below each abstract.

**Manuscript.** Manuscript is original paper and represent an article which has not been published in any other journal or proceedings. The manuscript of no more than 36 pages by using Times New Roman 11, MS Word for Windows of A4 with double spacing, submitted to the editor through <reinwardtia@mail.lipi.go.id>. New paragraph should be indented in by 5 characters. For the style of presentation, authors should follow the latest issue of *Reinwardtia* very closely. Author(s) should send the preferred running title of the article submitted. Every manuscript will be sent to two blind reviewers.

**Identification key.** Taxonomic identification key should be prepared using the aligned couplet type.

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**Map/line drawing illustration/photograph.** Map, line drawing illustration, or photograph preferably should be prepared in landscape presentation to occupy two columns. Illustration must be submitted as original art accompanying, but separated from the manuscript. The illustration should be saved in JPG or GIF format at least 350 pixels. Legends or illustration must be submitted separately at the end of the manuscript.

**References.** Bibliography, list of literature cited or references follow the Harvard system as the following examples.

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