Ghazalli MN *et al.* (2021)
Notulae Scientia Biologicae
Volume 13, Issue 4, Article number 10980
DOI:10.15835/nsb13410980
Research Article

Comparative leaf anatomy of ten *Nepenthes* L. species (Nepenthaceae) from Peninsular Malaysia

Mohd N. GHAZALLI[1]*, Amin A. TAMIZI[2], Dome NIKONG[3], Muhammad I. MAT ESA[4], Edward E. BESI[4], Salmaniza SALLEH[1], Noraini TALIP[5], A. LATIFF[5]

[1]*Resource Utilisation and Agrobiodiversity Conservation Programme (BE2), Agrobiodiversity and Environment Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia; mnfaizal@mardi.gov.my (*corresponding author)*
[2]*Agri-Omics and Bioinformatics Programme (BN1), Biotechnology and Nanotechnology Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia; aminasyraf@mardi.gov.my*
[3]*Digital Dome Photography, 21500 Permaisuri, Terengganu, Malaysia; dome@digitaldome.org*
[4]*University Putra Malaysia (UPM), Faculty of Science, Department of Biology, 43400 Serdang, Selangor, Malaysia; m.ikhwan_wan@yahoo.com; edwardentalai92@gmail.com*
[5]*University Kebangsaan Malaysia, Faculty of Science and Technology, Department of Biological Sciences and Biotechnology, Bangi Campus, 43600 Bangi, Selangor, Malaysia; norainitalip@ukm.edu.my; pakteh48@yahoo.com*

Abstract

The genus *Nepenthes* (Nepenthaceae) in Peninsular Malaysia includes fourteen species that can be found from the sea-level to over 1000 m in the mountains. Our observation indicated that *Nepenthes* can be found in five major habitat types: specifically in tropical lowland evergreen rain forest, heath forest, peat swamp forest, montane forest, and limestone forest. Their leaves have several anatomical characters that remain underexplored. There were specific differences between species that could be potential identification characters. The following anatomical characteristics were explored for their diagnostic value and comprised of ten distinct anatomical characteristics in *Nepenthes*, viz., density and stomatal index (SI), hypodermis cell – cell layers, occurrence of fiber groups mixed with the hypodermis cells, adaxial cuticle thickness, vascular bundle arrangement, midrib outline shape, venation plasticity, druses appearance and appendage/trichome type.

Keywords: anatomy; identification; *Nepenthes*; Peninsular Malaysia; taxonomy

Introduction

There are fourteen species of *Nepenthes* L. recorded in Peninsular Malaysia and mainly distributed in heath forests, lowland area, including montane species up to 1000 m altitude and above (Table 1) (Ghazalli *et al.*, 2020; Tamizi *et al.*, 2020). The primary centers of diversity of *Nepenthes* are in Borneo, the Philippines and Sumatra, with updated enumeration of 170 species already recorded. It is also important to include their secondary centers of diversity that covers Peninsular Malaysia, Sulawesi and New Guinea (Jebb and Cheek, 1997; Cheek and Jebb, 2001; Christenhusz, 2016; Clarke, 2001 and 2002; McPherson, 2009; Robinson *et al.*, 2019). Out of the fourteen *Nepenthes* species recorded in Peninsular Malaysia, ten are considered endemic,

Received: 20 May 2021. Received in revised form: 28 Jun 2021. Accepted: 22 Oct 2021. Published online: 03 Nov 2021.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

one - *N. gracillima* seems to need to be further study and confirmation, and the other three annotated species are noted as common species with wider distribution records.

Table 1. Distribution of Peninsular Malaysia's *Nepenthes* species

No.	Species	Habitat
1.	*Nepenthes alba*	Mossy forest, open vegetation on ridge crests (≥1000-2000 m)
2.	*N. albomarginata*	Heath forest, summits and ridges of low mountains (0-1100 m)
3.	*N. ampullaria*	Heath forest, peat swamp forest, paddy fields (0-1100m)
4.	*N. benstonei*	Secondary vegetation, roadside, hilly area (450-600 m)
5.	*N. domei* sp. nov.	Upper hill forest habitat (850-1000 m)
6.	*N. gracilis*	Heath forest, open sunny areas (0-1100 m)
7.	*N. gracillima*	Mossy forest, open vegetation on ridge crests (≥1000-2000 m)
8.	*N. latiffiana* sp. nov.	Upper hill forest habitat (1000-1100 m)
9.	*N. macfarlanei*	Mossy forest (1000-2000 m)
10.	*N. malayensis* sp. nov.	Upper hill forest habitat (800 – 1000 m)
11.	*N. mirabilis*	Heath forest, swampy areas (0-1500 m)
12.	*N. ramispina*	Mossy forest, exposed ridges and summits of mountains (≥1000-2000 m)
13.	*N. rafflesiana*	Heath forest, peat swamp forest, seaside cliffs and wetlands (0-1200 m)
14.	*N. sanguinea*	Lower and upper montane forests (300-1800 m)

Pitcher's shape in morphological observation is the main character used for systematic identification of *Nepenthes* (Ridley, 1924; Corner, 1988). *Nepenthes* produces leaves that form into the shape of a jug with watery fluid inside intended to absorb and digest nutrients from the insects, organic matter or dead leaves trapped into the pitcher fluid, which are later transported to other parts of *Nepenthes* that promote growth and survival needs (Clarke, 2001 and 2002). Pitcher morphology is very important for the identification process as there is confusion due to the wider morphological variation between true species and their natural hybrids (Danser, 1928; Beveridge *et al.*, 2013). In view of this, is important to study other systematic characters of *Nepenthes* to assist alternative identification for this genus. Difficulties in identification of sterile *Nepenthes* specimen without lower or upper pitchers may also result in wrong identification and interpretation of the species.

Investigation of leaf anatomy provides as much information as alternative identification from the leaf lamina, venation and stomatal type, petiole, margin, appendages variation and vascular bundle patterns. de Candolle (1879) conducted a comprehensive study on petiole anatomy that enabled the description of several fundamental concepts of vascular bundles that can be implemented in other genus or family descriptions. Metcalfe and Chalk (1950) pioneered the study of *Nepenthes* anatomical characteristics using the slides collection obtained from Kew Botanic Gardens and successfully enumerated the basic qualitative observations from its basic anatomical description. Several other studies were conducted by Toma *et al.* (2002), Pavlovic *et al.* (2007), Biati (2012) and Paluvi *et al.* (2015) using cultivated *Nepenthes* specimens of *N. maxima, N. mirabilis, N. alata* and *N. gracilis*, but the studies are still incomplete due to the specimen collection restrictions and lack of wild specimen enumeration.

Limited knowledge and information on the anatomical descriptions of *Nepenthes* species, specifically the Peninsular Malaysia collections, prompted further evaluation and characterisation, complementing the existing type specimens in safekeeping. Thus, the aim of this research is to analyse the anatomical description of *Nepenthes* and its systematic information that can be utilised for alternative identification of the *Nepenthes* species studied.

Materials and Methods

Nepenthes specimen authentication and observation were conducted based on the available specimens from two herbaria (UKMB and KEP). All the ten *Nepenthes* species were collected in the field following the methods described by Martins and Filgueiras (2010). All examined specimens are listed in Table 2. Fresh collected specimens were removed from the middle third of a leaf segment, located in the median leaf region. For some species which required herbarium material observation, the sample was rehydrated in 50% (v/v) aqueous glycerine in a stove at c. 60 °C for 48 h, dehydrated in an ethanolic gradient and conserved in 70% ethanol. We analysed at least three individuals per species.

Table 2. *Nepenthes* species examined, their source and specimen details

Species	Localities
***Nepenthes alba* Ridl.**	Peninsular Malaysia, Pahang, Gunung Tahan. MDI 12346, Muhammad Ikhwanuddin M.E. 8.2.2019 (MDI)
***N. albomarginata* T. Lobb ex Lindl.**	Peninsular Malaysia, Kedah, Gunung Jerai FR, Compartment 20-23. FRI 52039, Kamarul Hisham, M., Noorsiha, A. & Kueh, H.L, 10.6.2006 (KEP)
	Peninsular Malaysia, Pulau Pinang, Bukit Bendera, MDI 12337, Mohd Norfaizal, G., 16.12.2018 (MDI)
	Peninsular Malaysia, Johor, Gunung Ledang. RK 1677, Ruth Kiew, 8.5.1985 (KEP)
***N. ampullaria* Jack**	Peninsular Malaysia, Johor, Mersing. Rohana M.S. RMS 081, 28.11.1989 (KEP)
	Peninsular Malaysia, Terengganu, Sungai Tong. MDI 12341, Mohd. Norfaizal, G. Amin Asyraf, T., Sairuzi, Z. 31.1.2019 (MDI).
	Peninsular Malaysia, Selangor, Sungai Karang FR, TanjungKarang. FRI 38751. Lilian, C. 28.4.1993.(KEP)
***N. benstonei* C. Clarke**	Peninsular Malaysia, Kelantan, Machang, Bukit Bakar. FRI 53169. Yao, T.L. Duistermart, H. Kiew, R. Kueh, H.L. 27.9.2006. (KEP).
	Peninsular Malaysia, Kelantan, Machang, Bukit Bakar Summit. MDI 12333. Mohd Norfaizal, G. & Salmaniza, S. 5.3.2019 (MDI)
	Peninsular Malaysia, Terengganu, Gunung Tebu. FRI 13147. Mohd Shah, Ahmad Shukor, Mahmud Awang. 31.5.1974. (KEP)
***N. gracilis* Korth.**	Peninsular Malaysia, Pahang, Temerloh, Tasik Bera. FRI 52720. Rafidah, A.R., Mohd. Nazri, A., Kueh, H.L. 10.7.2007. (KEP)
	Peninsular Malaysia, Selangor, Bangi, UKM Bangi. MDI 12336. Mohd. Norfaizal, G. & Amin Asyraf, T. 15.12.2018. (MDI)
	Peninsular Malaysia, Kedah, Pulau Langkawi, FRI63422. Beentje, H.J., Rosdi, M., Angan. 18.2.2009. (KEP)
***N. macfarlanei* Hemsl.**	Peninsular Malaysia, Pahang, Bentong, Gunung Ulu Kali. FRI 52572. Nor Ezzawanis, A.T. 16.2.2007. (KEP)
	Peninsular Malaysia, Pahang, Summit Ridge of Gunung Ulu Kali to Gunung Rajah. . FRI 40516, Lilian Chua, 16.1.1995 (KEP)
	Peninsular Malaysia, Pahang, Bukit Chin-Chin, Pathway to Telekom Telecommunication Tower, Genting Highlands. MDI 12331. Mohd Norfaizal, G. & Amin Asyraf, T. 9.12.2018. (MDI).
***N. mirabilis* (Lour.) Druce**	Peninsular Malaysia, Selangor, Hulu Selangor, Bukit Tunggal FR. FRI 73129. Chew, M.Y., Mohd Hairul, M.A. & Mohd Afiq, K. 24.5.2016. (KEP)
	Peninsular Malaysia, Selangor, Puncak Alam. MDI 12416. Mohd Norfaizal, G. AnuarRasyidi, M.N., Ahmad Syahman, M.D. & Muhammad Syakir. 30.12.2018. (MDI)
***N. ramispina* Ridl.**	Peninsular Malaysia, Selangor, Gunung Bunga Buah. FRI 65498. Yao, T.L. Kiew, R. & Jun, W. 31.10.2010. (FRI)
	Peninsular Malaysia, Pahang, Bukit Chin-chin, Pathway to Telekom Telecommunication Tower, Genting Highlands. MDI 12332. Mohd Norfaizal, G. & Amin Asyraf, T. 9.12.2018. (MDI).
***N. sanguinea* Lindl.**	Peninsular Malaysia, Terengganu, Hulu Terengganu, Gunung Padang. FRI 70887. Mohd Hairul, M.A., Imin, K., Rafidah, A.R. Ummul Nazrah, A.R. Kueh, H.L. 21.3.2010. (KEP)
	Peninsular Malaysia, Selangor, Gunung Ulu Semangkok. MDI 12418. Mohd Norfaizal, G. Anuar Rasyidi, M.N., Ahmad Syahman, M.D.& Muhammad Syakir.13.1.2019. (MDI)
	Peninsular Malaysia, Perak, Gunung Hijau summit plateau, FRI 40475. 6.9.1994. Lilian Chua (KEP)
***N. rafflesiana* Jack.**	Peninsular Malaysia, Johor, Kluang, Kluang FR, Gunung Belumut. FRI 60328. Chew, M.Y. & Teo, Y.L. 10.8.2009. (KEP)
	Peninsular Malaysia, Johor, Gunung Ledang, FRI 33511. Lilian Chua. 4.6.1993. (KEP)

Anatomical study

Cross-sections were made with a sliding microtome (Leica SM2500). Paradermal sections were made using acid dissociation (Krauss and Arduin, 1997). Standard plant anatomy procedures were used to make slides (Johansen 1940). At least three replicates were used per individual. The sections were analysed using a

Zeiss optical microscope and the images obtained with a Leica photomicroscope associated with the computer, using the LASEZ image capture system.

Terminology and data analysis

The characters were compiled from leaf anatomical literature on selected plant species (Glassman, 1972; Barfod, 1988; Moraes, 1996; Horn *et al.* (2009); Millan and Kahn, 2010; Alvarado and Jauregui, 2011; Tomlinson *et al.* (2011); Batagin-Piotto *et al.* (2012); Noraini *et al.* (2012); Noblick, 2013; Martins *et al.* 2015). For the quantitative analyses of the epidermis, hypodermis and mesophyll thickness, we used a mean of four measurements of two leaf segments of three individuals per species. The terminology of Turpe (1967) was used to distinguish the vascular bundles as complete or incomplete, depending on whether the union of the vascular bundle and sub-epidermal sclerenchyma tissue had occurred.

Results and Discussion

Transverse Sections (TS) of the leaf parts

Nepenthes albomarginata (Figure 1)

Leaf anatomy lamina thickness 180.70-197.68 µm (Figure 1H). **Hypodermis** 0-1 layer on adaxial, thickness 23.10-25.67 µm, 1-3 layer on abaxial, thickness 18.33-21.34 µm. Mesophyll 123.23-145.48 µm; palisade parenchyma 1-2 layer with total 38.51-42.79 µm thick; spongy parenchyma total 85.87- 89.85 µm, with more than ten extracellular spaces in between of spongy parenchyma (Figure 1G, H). **Midrib outline** Type 1(Figure 1A), vascular bundles arrangement outlines Type 5 (Figure 1A, B, C); midrib epidermis single layer, stomata not observed; (Figure 1C); crystal sand -druses occurring in masses specifically in parenchyma cells of the midrib (Figure 1E, F). **Margin** rounded with neck-like structure, pointing 90° downward to adaxial side (Figure 1G). **Adaxial epidermis** straight to curved, crystals sand observed on the adaxial surface, stomata absent, trichome absent (Figure 1K). **Abaxial epidermis** straight to curved, stomata ranunculaceous (Figure 1L), kidney-like, trichome absent. **Marginal venation** complete venation (Figure 1I). **Areolar venation** a mixture of closed and opened (Figure 1J), type of veinlet: simple uni-veinlet (Figure 1J).

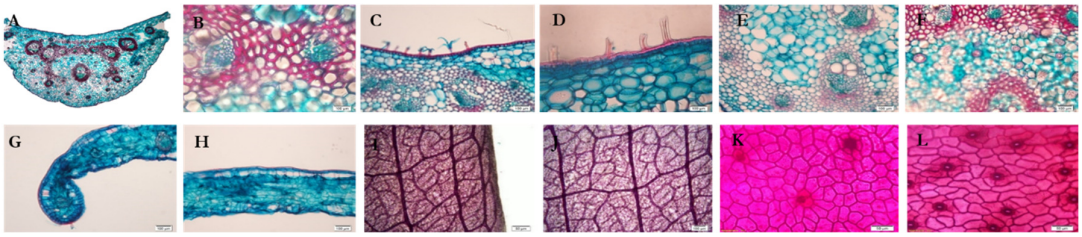

Figure 1. *Nepenthes albomarginata*
(A) Midrib TS. **(B)** Observation of solitary crystals. **(C)** Trichomes. **(D)** Simple unicellular trichomes. **(E)** Druses. **(F)** Vascular bundle and solitary crystals. **(G)** Margin TS. **(H)** Lamina TS. **(I)** Margin venation. **(J)** Lamina venation. **(K)** Adaxial epidermis. **(L)** Abaxial epidermis with stomata observation. Bars **A, I, K, L**: 50µm; **B, C, D, E, F, G, H, J:** 100 µm.

Nepenthes ampullaria (Figure 2)

Leaf anatomy lamina thickness 201.96- 206.22 µm (Figure 2H). **Hypodermis** 1 layer on adaxial, thickness 50.95-59.96 µm, none-1 layer on abaxial, thickness 14.55-16.75 µm. Mesophyll 127.51-139.59 µm; palisade parenchyma 1-3 layer with total 198.78-237.16 µm thick; spongy parenchyma total 36.8-56.48 µm, with more than 10 extracellular spaces in between of spongy parenchyma (Figure 2E, H, I). **Midriboutline** Type 2, vascular bundles arrangement Type 2B (Figure 2A, C, D), crystals sand -solitary prismatic crystals occurring in masses, specifically in parenchyma cells of the midrib (Figure 2 D, F). **Margin** rounded, pointing

45° downward to adaxial side (Figure 2I). **Adaxial epidermis** straight to curved, crystal sand observed on the adaxial surface, trichome absent (Figure 2J). **Abaxial epidermis** straight to curved, stomata ranunculaceous (Figure 2K), kidney-like, trichome absent. **Marginal venation** complete venation, no veinlet (Figure 2N). **Areolar venation** opened venation (Figure 2M), type of veinlet: simple veinlet (uni-bi) (Figure 2M, N).

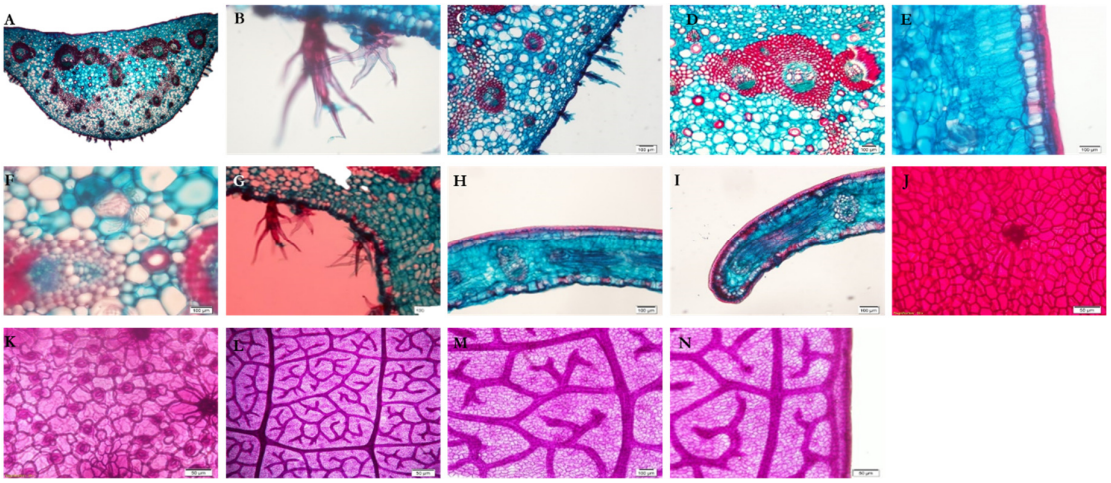

Figure 2. *Nepenthes ampullaria*
(A) Midrib TS. **(B)** Close up of multicellular trichomes. **(C)** Multicellular trichomes. **(D)** Vascular bundle. **(E)** Hypodermis. **(F)** Solitary crystals. **(G)** Multicellular trichomes on the midrib adaxial surface. **(H)** Lamina TS. **(I)** Margin TS. **(J)** Adaxial epidermis. **(K)** Abaxial epidermis with stomata observation. **(L)** Lamina venation. **(M)** Areolar venation. **(N)** Margin venation. Bars **A, B, J, K, L, N:** 50µm; **C, D, E, F, G, H, I, M:** 100µm.

Nepenthes benstonei (Figure 3)

Leaf anatomy lamina thickness 252.96-266.43µm (Figure 3G), **Hypodermis** 1-2 layers on adaxial, thickness 25.67-35.94µm, 1 layer on abaxial, thickness 16.26-27.38µm. Mesophyll 159.17-172.86µm; palisade parenchyma 1-3 layers with total 67.65-84.15µm thick; spongy parenchyma total 106.97-111.25µm, with plenty of extracellular space in between of spongy parenchyma (Figure 3g, H)). **Midrib** outline Type I, adaxial very slightly concave, abaxial prominent 'U-V' Shape (Figure 3A), 380.81-391.71µm thick, vascular system Type VI, outer ring present (rectangular in shape), central vascular bundle present (3 vascular bundles), additional vascular bundle present (several smaller size vascular bundles near abaxial epidermis) and 2 vascular bundles present on the adaxial side of outer ring vascular bundle on the left and right side, midrib epidermis single layered, 13.26-17.83µm at the abaxial and 15.59-16.42µm at the adaxial, irregular between each other; stomata not observed; trichome appendages not observed (Figure 3A and I); crystals sand -solitary sand crystals occurring in masses, specifically in parenchyma cells of the midrib (Figure 3B-D), druses observed (Figure 3E), starch grains observed (Figure 3B). **Margin** tapering, pointing 30° downward to adaxial side (Figure 3H). **Adaxial epidermis** straight to curved, crystals sand observed on the adaxial surface, stomata absent; sessile glands irregular in flower-like in 6-7 cells, elliptical, trichome absent (Figure 3J). **Abaxial epidermis** curved to wavy, stomata ranunculaceous (Figure 3K), kidney-like, trichome absent. **Marginal venation** closed venation, no veinlet (Figure 3M). **Areolar/lamina venation** mixture of opened and closed venation (Figure 3L), type of veinlets: simple veinlet (linear-curved) 1-2 branched (Figure 3L).

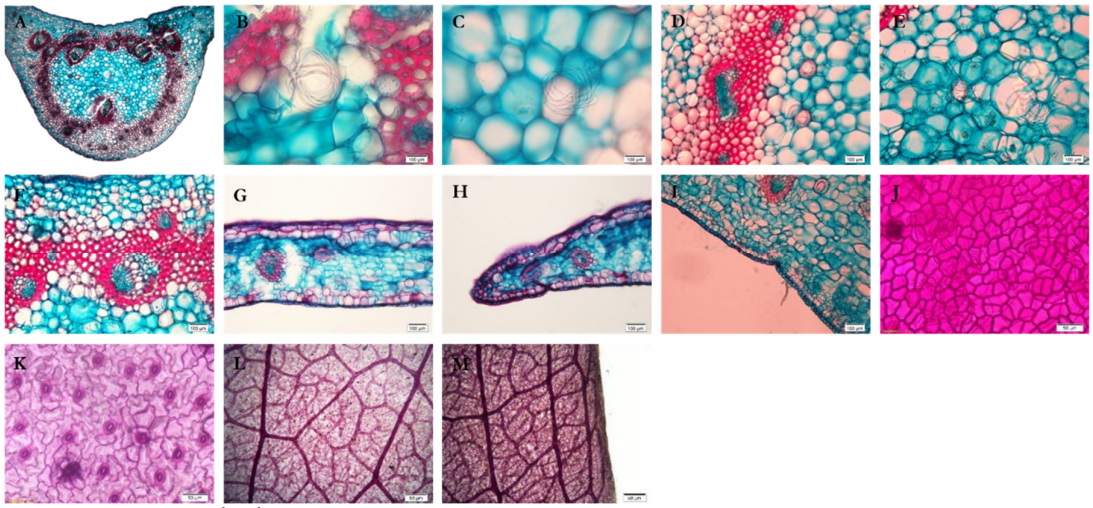

Figure 3. *Nepenthes benstonei*
(A) Midrib TS. **(B)** Observation of solitary crystals and starch grain. **(C)** Solitary crystals (arrow). **(D)** Solitary crystals. **(E)** Druses. **(F)** Vascular bundle. **(G)** Lamina TS. **(H)** Margin TS. **(I)** Simple, unicellular trichomes. **(J)** Adaxial epidermis. **(K)** Abaxial epidermis with stomata observation. **(L)** Lamina venation. **(M)** Margin venation. Bars **A, J, K, L, M:** 50µm; **B, C, D, E, F, G, H, I:** 100µm.

Nepenthes gracilis (Figure 4)

Leaf anatomy lamina thickness 341.44-395.35 µm, **Hypodermis** 1-2 layer on adaxial, thickness 50.49-59.98 µm, 1 layer on abaxial thickness 19.68-27.38 µm. Mesophyll 94.13-97.55 µm; palisade parenchyma 1-3 layer totally 278.98-302.06 µm thick; spongy parenchyma total 210.51-237.04 µm, with more than five extracellular spaces in between the spongy parenchyma (Figure 4E, F). **Midrib outline** Type 2; vascular bundle arrangement Type 4 (Figure 4A-D); 60.75-131.78 diameter; larger at the abaxial part, stomata not observed; single, unicellular, pointed tip trichome observed sparsely on the adaxial part of the midrib (Figure 4B, C); crystal sand - prismatic crystals occurring in masses specifically in parenchyma cells of the midrib (Figure 4B). **Margin** tapering-triangular and pointing 45° downward to adaxial side (Figure 4E). **Adaxial epidermis** straight to wavy, crystal sand observed on the adaxial surface, stomata absent; trichome absent (Figure 4H). **Abaxial epidermis** straight-wavy, stomata ranunculaceous (Figure 4H), kidney-like, trichome absent. **Marginal venation** complete venation, no veinlet (Figure 4L). **Areolar venation** closed and opened venation (Figure 4J), type of veinlets: simple veinlet (uni-veinlet) (Figure 4K).

Nepenthes macfarlanei (Figure 5)

Leaf anatomy lamina thickness 329.46-341.67 µm, **Hypodermis** 1-4 layer on adaxial, thickness 41.26-71.03 µm, 1 layer on abaxial, thickness 16.26-19.68 µm. Mesophyll 227.68-237.96 µm; palisade parenchyma 1-3 layer with total 80.44-90.71 µm thick; spongy parenchyma total 145.48-148.05 µm, with more than five extracellular spaces in between spongy parenchyma (Figure 5G, H). **Midrib outline** Type 4 (Figure 5A), vascular bundles arrangement Type 1 (Figure 5A and D); solitary crystal - prismatic crystals occurring in masses specifically in parenchyma cells of the midrib (Figure 5C). **Margin** lithop shaped, pointing at about 120° downward to adaxial side (Figure 5H). **Adaxial epidermis** straight to curved, crystals sand observed on the adaxial surface, trichome absent (Figure 5I). **Abaxial epidermis** straight-curved, stomata ranunculaceous (Figure 5J), kidney-like, trichome absent. **Marginal venation** complete venation, no veinlet (Figure 5L). **Areolar venation o**pened venation (Figure 5K), type of veinlets: simple veinlet (linear-uni) (Figure 5K).

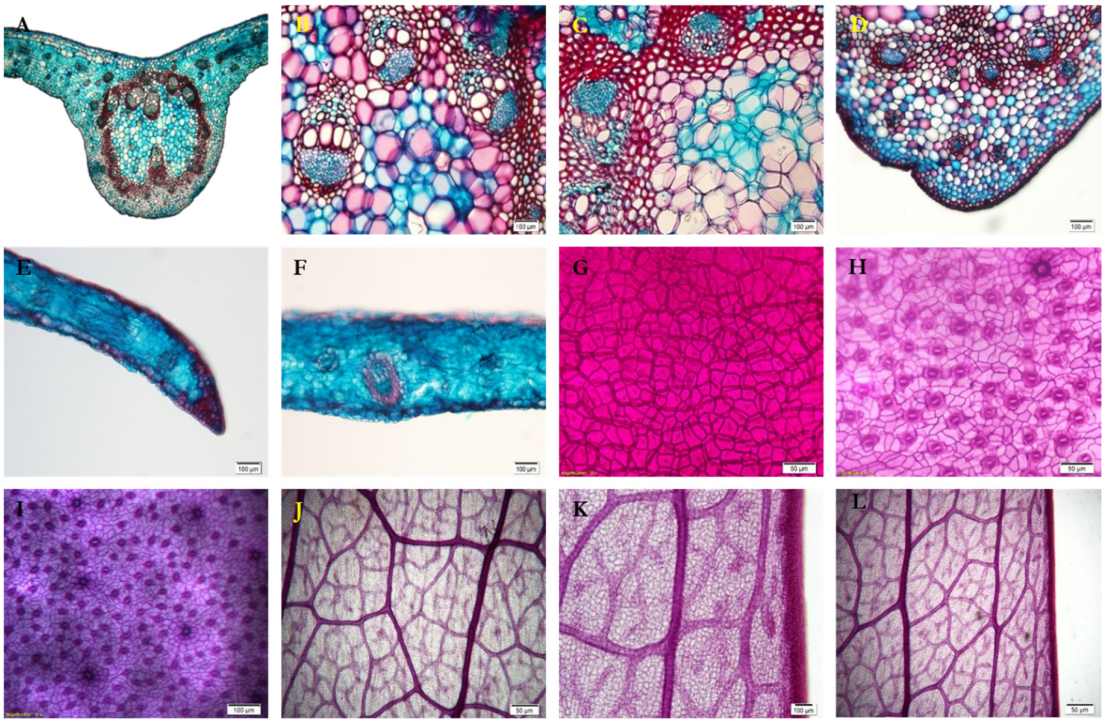

Figure 4. *Nepenthes gracilis*
(A) Midrib TS. **(B)** Observation of solitary crystals. (C) Close up of vascular bundle. **(D)** Midrib abaxial part. **(E)** Margin TS. **(F)** Lamina TS. **(G)** Adaxial epidermis. **(H)** Abaxial surface. **(I)** Sessile gland. **(J)** Lamina venation. **(K)** Areolar venation. **(L)** Margin venation. Bars **A, G, H, J, L:** 50μm; **B, C, D, E, F, I, K:** 100μm.

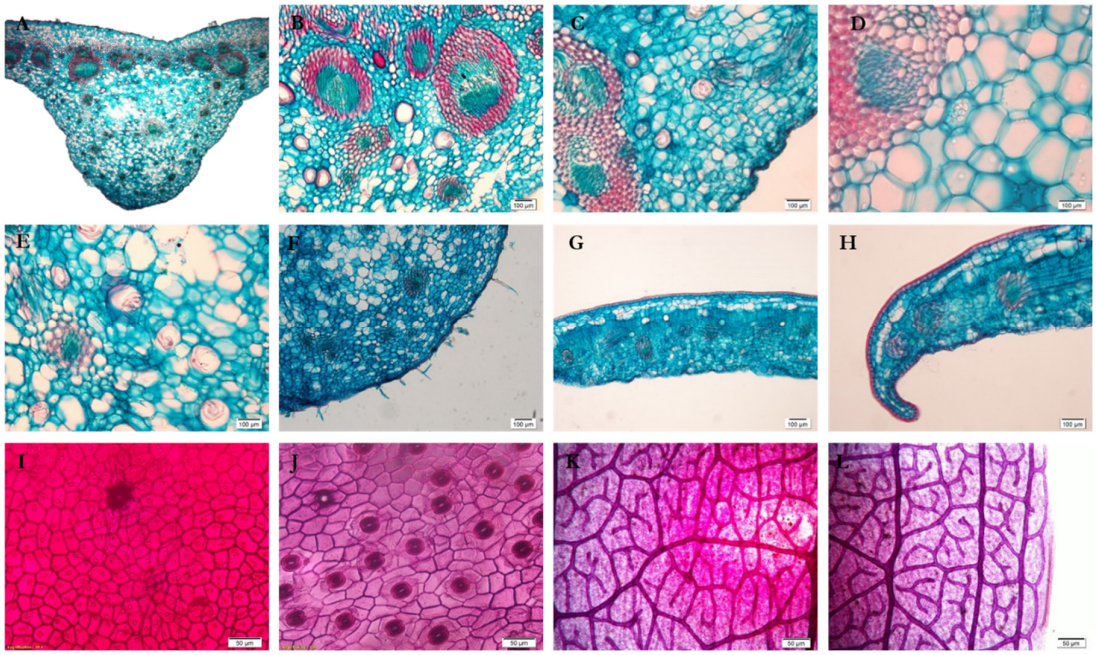

Figure 5. *Nepenthes macfarlanei*
(A) Midrib TS**. (B)** Close up of vascular bundle. **(C)** Solitary crystals. **(D)** Solitary crystals (sand-type). **(E)** Solitary crystals. **(F)** Simple, unicellular trichomes. **(G)** Lamina TS. **(H)** Margin TS. **(I)**. Adaxial epidermis. **(J)** Abaxial epidermis with stomata observation. **(K)** Lamina venation. **(L)** Margin venation. Bars **A, I, J, K, L:** 50μm; **B, C, D, E, F, G, H:** 100μm.

Nepenthes mirabilis (Figure 6)

Leaf anatomy lamina thickness 221.64-240.43 µm, **Hypodermis** 1-2 layer on adaxial, thickness 11.12-12.84 µm, 2 layers on abaxial, thickness 18.83-23.96 µm. Mesophyll 169.44-208.81 µm; palisade parenchyma 1 layer with total 58.19-63.52 µm thick; spongy parenchyma total 96.7-121.54 µm, with less than four extracellular spaces in between spongy parenchyma (Figure 6E, F). **Midrib outline** Type 1 (Figure 6A), vascular bundle arrangement Type 5 (Figure 6A-D); crystals - prismatic crystals occurring in masses specifically in parenchyma cells of the midrib (Figure 6D). **Margin** tapering-triangular shaped, pointing 90° downward to adaxial side (Figure 6F). **Adaxial epidermis** straight to curved, crystals sand observed on the adaxial surface, stomata absent; trichome absent (Figure 6H). **Abaxial epidermis** straight-curved, stomata ranunculaceous (Figure 6I), kidney-like, trichome absent. **Marginal venation** complete venation, no veinlet (Figure 6L). **Areolar venation** mixture of opened and closed venation (Figure 6J), type of veinlets: simple veinlet (uni-veinlet) (Figure 6K).

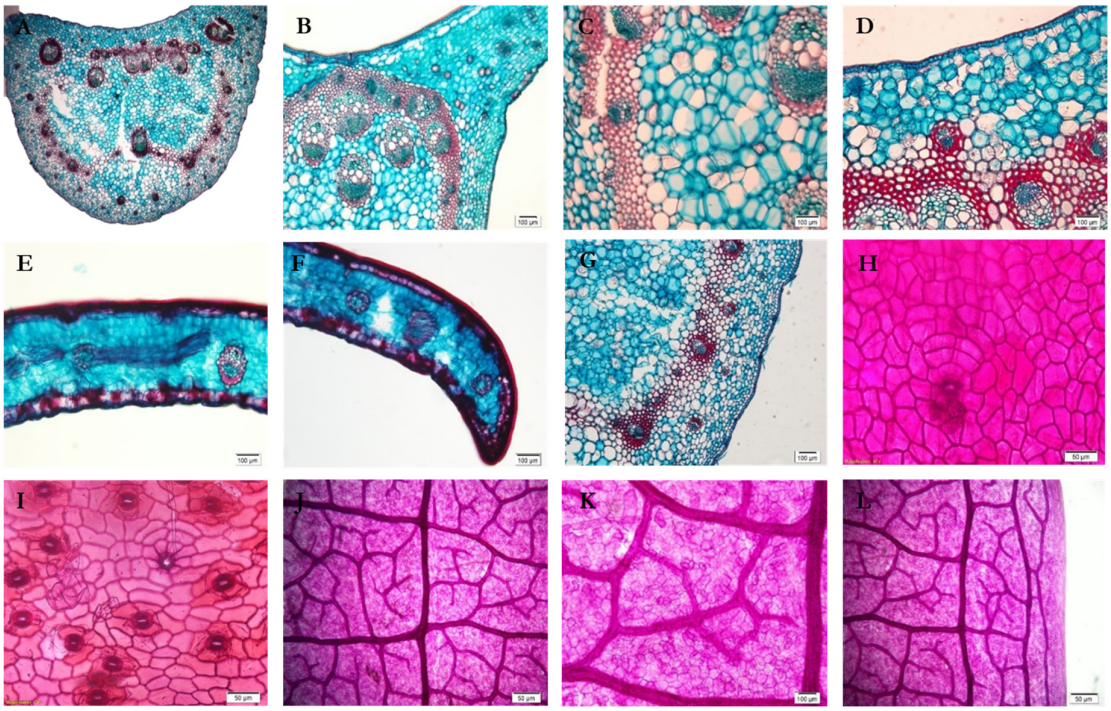

Figure 6. *Nepenthes mirabilis*
(A) Midrib TS. **(B)** Vascular bundles. **(C)** Solitary crystals. **(D)** Solitary crystals. **(E)** Lamina TS. **(F)** Margin TS. **(G)** Simple, unicellular trichomes. (**H**) Adaxial epidermis. **(I)**Abaxial epidermis with stomata observation. **(J)** Lamina venation. **(K)** Areolar venation. **(L)** Margin venation. Bars **A, H, I, J, L:** 50µm; **B, C, D, E, F, G, K:** 100µm.

Nepenthes rafflesiana (Figure 7)

Leaf anatomy lamina thickness 323.47-338.08 µm, **Hypodermis** 1 layer on adaxial, thickness 25.67-30.31 µm, 1 layer on abaxial, thickness 41.08-53.26 µm. Mesophyll 328.63-346.59 µm; palisade parenchyma 1-2 layer with total 159.17-227.63 µm thick; spongy parenchyma total 108.65-126.34 µm, with more than two extracellular spaces in between spongy parenchyma (Figure 7E, F). **Midrib outline** Type 3 (Figure 7A), vascular bundle arrangement Type 3 (Figure 7A, D); crystal - prismatic crystals occurring in masses specifically in parenchyma cells of the midrib (Figure 7C). **Margin** rounded, straight (Figure 7F). **Adaxial epidermis** straight to curved, crystal sand observed on the adaxial surface, stomata absent; elliptical, trichome absent (Figure 7G). **Abaxial epidermis** straight, stomata ranunculaceous (Figure 7H), kidney-like, trichome absent. **Marginal**

venation complete venation, no veinlet (Figure 7L). **Areolar venation** mixture of closed and opened venation (Figure 7J), type of veinlets: simple veinlet (uni-veinlet) (Figure I, K).

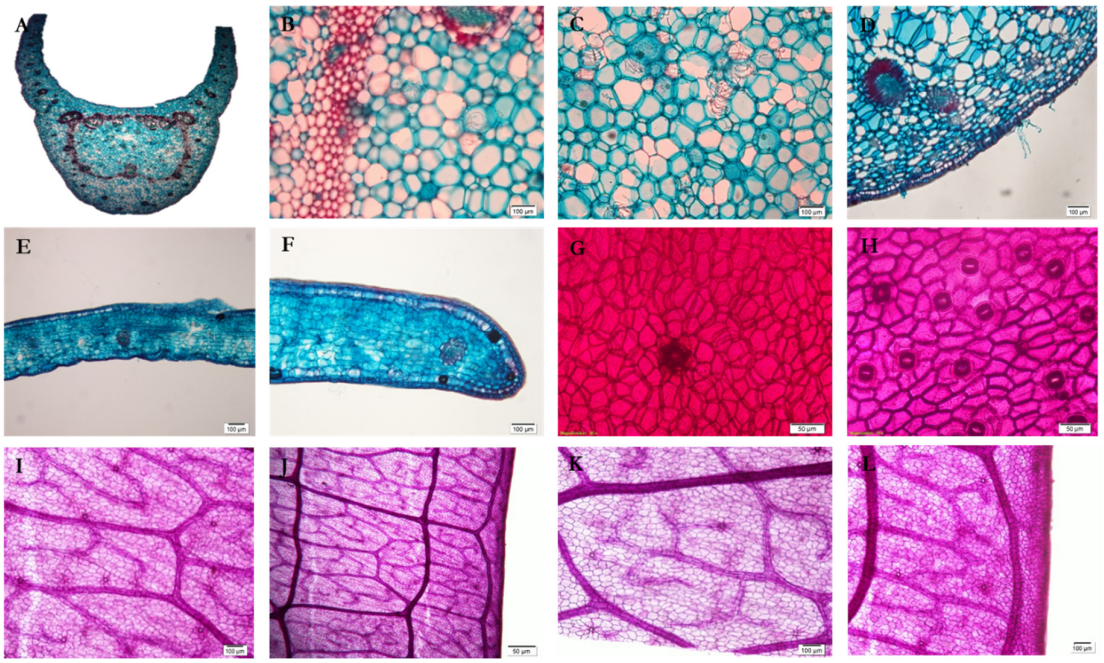

Figure 7. *Nepenthes rafflesiana*
(A) Midrib TS. **(B)** Observation of solitary crystals **(C)** Solitary crystals. **(D)** Simple, unicellular trichomes. **(E)** Lamina TS. **(F)** Margin TS. **(G)** Adaxial epidermis. **(H)** Abaxial epidermis with stomata observation. **(I)** Lamina venation. **(J)** Margin venation. **(K&L)** Close up of areolar venation. Bars **A, G, H, J:** 50µm; **B, C, D, E, F, I, K, L:** 100µm.

Nepenthes ramispina (Figure 8)

Leaf anatomy lamina thickness 420.17-449.26 µm, **Hypodermis** 1-3 layer on adaxial, thickness 48.78-73.59 µm, 1 layer on abaxial, thickness 25.67-59.76 µm. Mesophyll 303.56-376.67 µm; palisade parenchyma 1-4 layer with total 105.26-154.03 µm thick; spongy parenchyma total 180.56-183.14 µm, with more than three extracellular spaces in between spongy parenchyma (Figure 8D, E). **Midrib outline** Type 1 (Figure 8A), vascular bundle arrangement Type 2A (Figure 8A); crystal sand - prismatic crystals occurring in masses specifically in parenchyma cells of the midrib (Figure 8B). **Margin** rounded, pointing 45° downward to adaxial side (Figure 8E). **Adaxial epidermis** straight, crystal sand observed on the adaxial surface, stomata absent; trichome absent (Figure 8F). **Abaxial epidermis** straight, stomata ranunculaceous (Figure 8G), kidney-like, trichome absent. **Marginal venation** incomplete type, veinlet present (Figure 8I). **Areolar venation** mixture of closed and open systems (Figure 8H), type of veinlets: simple veinlet (uni-veinlet) (Figure 8H).

Nepenthes sanguinea (Figure 9)

Leaf anatomy lamina thickness 224.20- 235.23µm (Figure 9E), **Hypodermis** 1 layer on adaxial, thickness 20.54-37.65µm, 1 layer on abaxial, thickness 29.15-32.52µm. Mesophyll 145.87-166.45µm; palisade parenchyma 2 layers with total 53.06-63.32µm thick; spongy parenchyma total 90.71-94.99µm, with plenty of extracellular spaces in between spongy parenchyma (Figure 9E & F). **Midrib** outline Type I, adaxial slightly flattened and abaxial arched, (Figure 9A), 383.37-445.89µm thick, vascular system Type VI, sclerenchyma ring orbicular upwards, slightly curved at adaxial, outer ring present, central or medullary vascular bundle present (4 VB), 1 additional vascular bundle present (smaller size vascular bundles observed near abaxial epidermis), 367.11-413.32µm diameter (Figure 9A-C); diameter up to 40.35µm; irregular between each other; stomata not

observed; trichome appendages not observed (Figure 9A); crystal sand - solitary crystals and druse occurring in parenchyma cells of the midrib (Figure 9B-D). **Margin** tapering, pointing 90° downward to adaxial side, roughly creased surface (Figure 9F). **Adaxial epidermis** straight to curved, druses not observed on the adaxial surface, 3.8-7.8µm thick; epidermis 1 layer, 3-5 sides; stomata absent; sessile glands irregular in rounded up to 5 cells, elliptical, trichome absent (Figure 9I). **Abaxial epidermis** straight to curved, 2.4-4.9 µm thick; epidermis 1 layer, irregular, more than 4-6 sides; stomata anomocytic (Figure 9J), 95.47-117.74µm × 105.54-147.65µm, sessile glands not observed, trichome absent. **Marginal venation** incomplete type, uni-veinlet (Figure 9H). **Areolar/lamina venation** mixture of closed and open system (Figure 9I), type of veinlets – simple uni veinlet (slight curved) (Figure 9I).

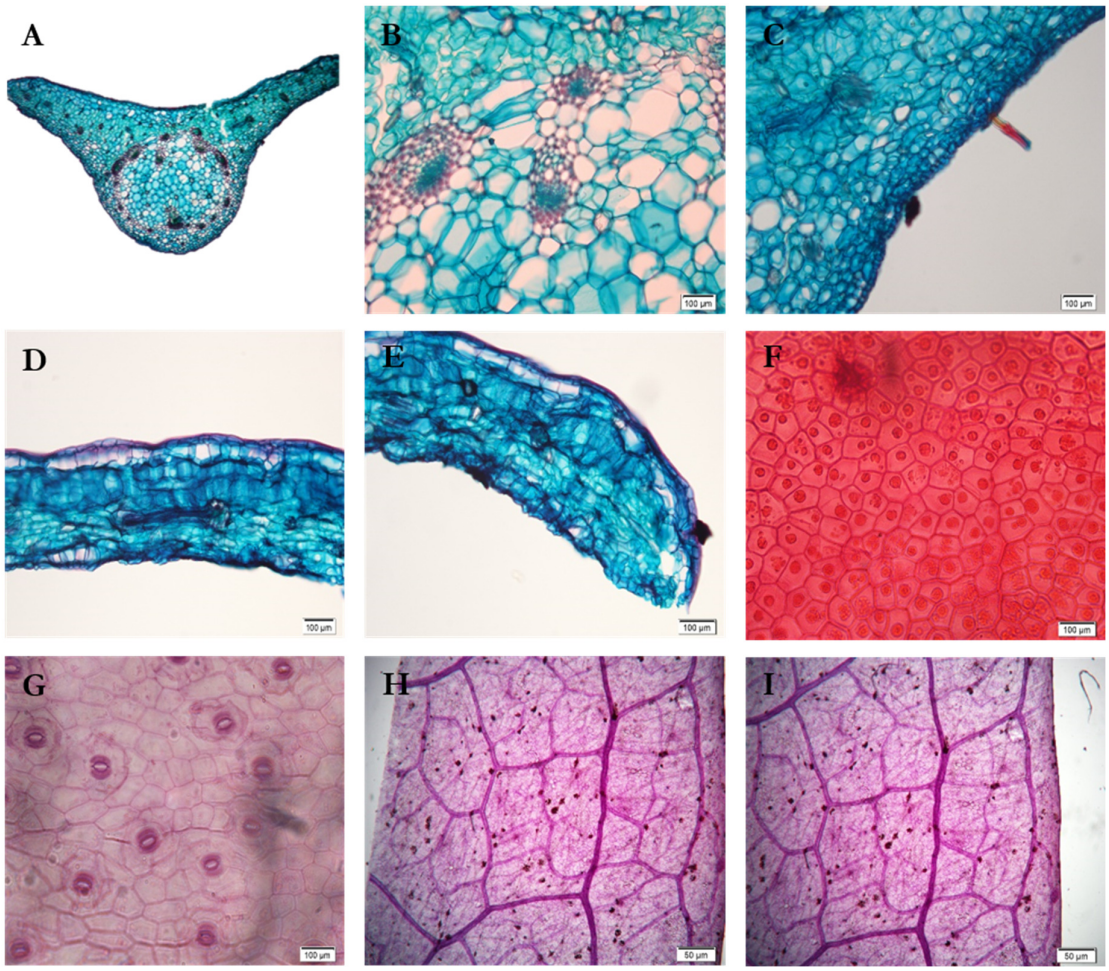

Figure 8. *Nepenthes ramispina*
(A) Midrib TS. **(B)** Observation of solitary crystals **(C)** Simple, unicellular trichomes. **(D)** Lamina TS. **(E)** Margin TS. **(F)** Adaxial epidermis. **(G)** Abaxial epidermis with stomata observation. **(H)** Lamina venation. **(I)** Marginal venation. Bars **A, H, I:** 50µm; **B, C, D, E, F, G:** 100µm.

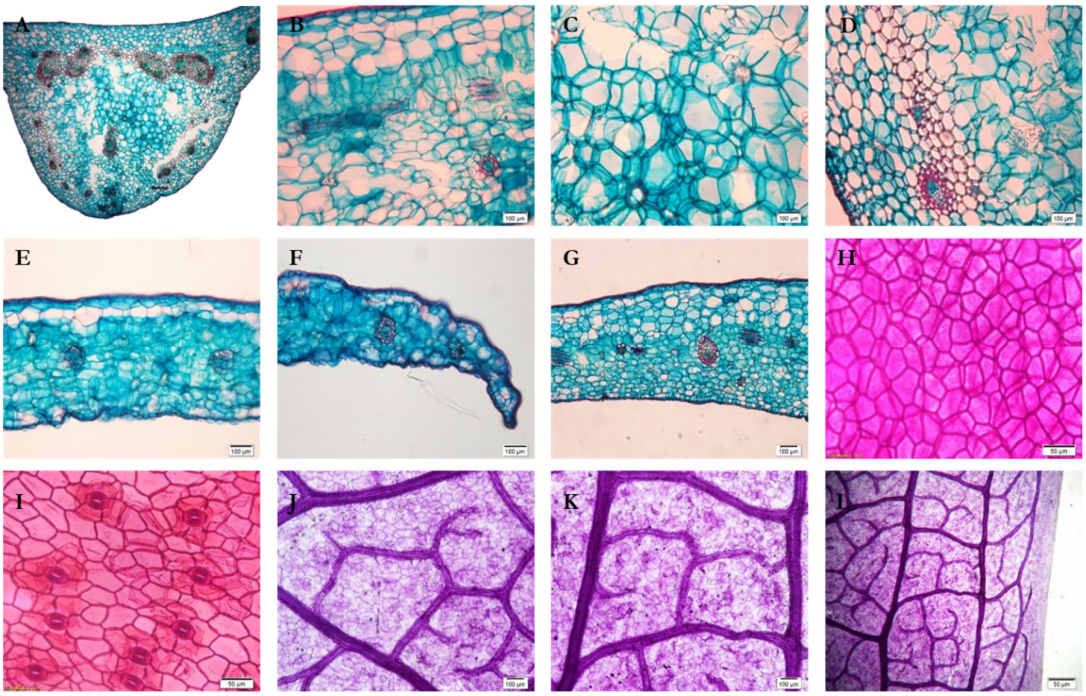

Figure 9. *Nepenthes sanguinea*
(A) Midrib TS. **(B)** Hypodermis cells, **(C)** Solitary crystals **(D)** Solitary crystals **(E)** Lamina TS. **(F)** Margin TS **(G)** Druses, **(H)** Adaxial epidermis, **(I)** Abaxial epidermis with stomata observation, **(J)** Areolar venation, **(K)** Areolar venation, **(L)** Marginal venation. Bars **A, H, I, L:** 50µm**; B, C, D, E, F, G, J, K:** 100µm.

Nepenthes alba (Figure 10)

Leaf anatomy lamina thickness 254.15-306.35 µm, **Hypodermis** 1 layer on adaxial, thickness 46.21-56.48 µm, 1-3 layer on abaxial, thickness 15.41-29.11 µm. Mesophyll 195.96-225.21 µm; palisade parenchyma 1-3 layer with total 59.91-84.7 µm thick; spongy parenchyma total 115.52-154.89 µm, with more than 16 extracellular spaces in between spongy parenchyma (Figure 10D, E). **Midrib** outline Type 4; vascular bundles arrangement 2B (Figure 10A); crystal sand -prismatic crystals occurring in masses specifically in parenchyma cells of the midrib (Figure 10B). **Margin** tapering, triangular and pointing 90° downward to adaxial side, (Figure 10E). **Adaxial epidermis** straight to curved, crystal sand observed on the adaxial surface, stomata absent, trichome absent (Figure 10F). **Abaxial epidermis** straight to curved, stomata ranunculaceous (Figure 10G), kidney-like, trichomes absent. **Marginal venation** incomplete venation (Figure 10I). **Areolar venation** closed venation (Figure 10H), type of veinlets: no-veinlet (Figure 10H).

Our study shows that there is significant anatomical variation among the ten species of *Nepenthes* studied. Some anatomical leaf characters in the *Nepenthes* species could be generally influenced by environmental conditions; however, some characters are stable and have taxonomic significance i.e. venation pattern plasticity, druse observation, midrib outlines, vascular bundle arrangements and also leaf margin outlines. In some species, these characters could be further analysed due to the uniqueness of the characteristics that can serve as diagnostic characteristics specifically involving taxa having leaf anatomical characters influenced by environmental conditions such as *N. ampullaria, N. macfarlanei* and *N. ramispina* in which the analysed features of appendages type, layer of hypodermis are varied.

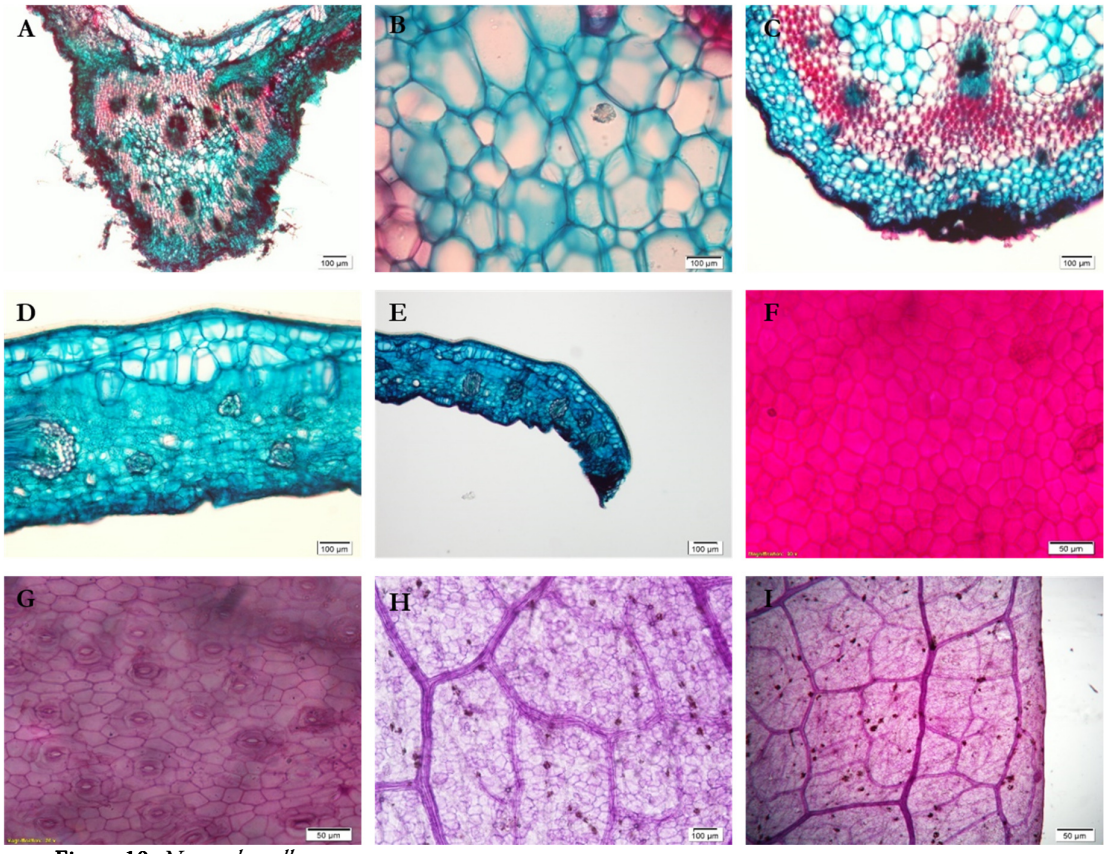

Figure 10. *Nepenthes alba*
(A) Midrib TS**. (B)** Observation of solitary crystals, **(C)** Vascular bundle, **(D)** Lamina TS **(E)** Margin TS, **(F)** Adaxial epidermis, **(G)** Abaxial epidermis with stomata observation, **(H)** Lamina venation. **(I)** Margin venation. Bars **F, G, I:** 50µm**; A, B, C, D, E, H:** 100µm.

Five types of vascular bundle arrangements combined with four types of midrib outer shape were identified (Figures 11, 12, 13). The vascular bundle is arranged in collateral, which too had been explained by Metcalfe and Chalk (1965). The results have shown that all the *Nepenthes* species studied have a complex vascular structure with closed or open ring system and consist of additional and medullary vascular bundles. This result supported the findings by Metcalfe and Chalk (1979). The complex vascular structure present in all the species studied consists of outer and medullary vascular bundles with closed or open ring system. Specifically, this is the first effort in determining and describing the complex structure of plant vascular tissues in *Nepenthes* , observation of Type 1 (adaxial VB: five VB in stacks; medullary VB: absent, additional VB: present as several smaller size VB near abaxial epidermis); Type 2A (outer ring present: circular in shape; medullary VB present: one to three scattered VB; additional VB: absent); Type 2B (outer ring present: triangular in shape; central or medullary VB present: one to three scattered VB; additional VB: present – several smaller size VB near abaxial epidermis); Type 3 (outer ring present (rectangular in shape); medullary VB: absent; additional VB present: several smaller size VB near epidermis abaxial and two VB present on the adaxial side of outer ring VB at the left and right side); Type 4 (outer ring present: triangular in shape; medullary VB: one to three scattered VB; additional VB: present as several smaller size VB near abaxial and adaxial epidermis) and Type 5 (outer ring present: triangular, elliptic or rectangular in shape; medullary VB: one to three scattered VB; additional VB: several smaller size VB near abaxial epidermis and two VB present on the adaxial side of outer ring VB on the left and right side). Type 1 was present in *Nepenthes macfarlanei*, Type 2A in *N.*

ramispina, Type 2B in *N. alba* and *N. ampullaria*, Type 3 in *N. rafflesiana*, Type 4 in *N. gracilis* and Type 5 in *N. benstonei, N. mirabilis, N. sanguinea* and *N. albomarginata* (Figure 11).

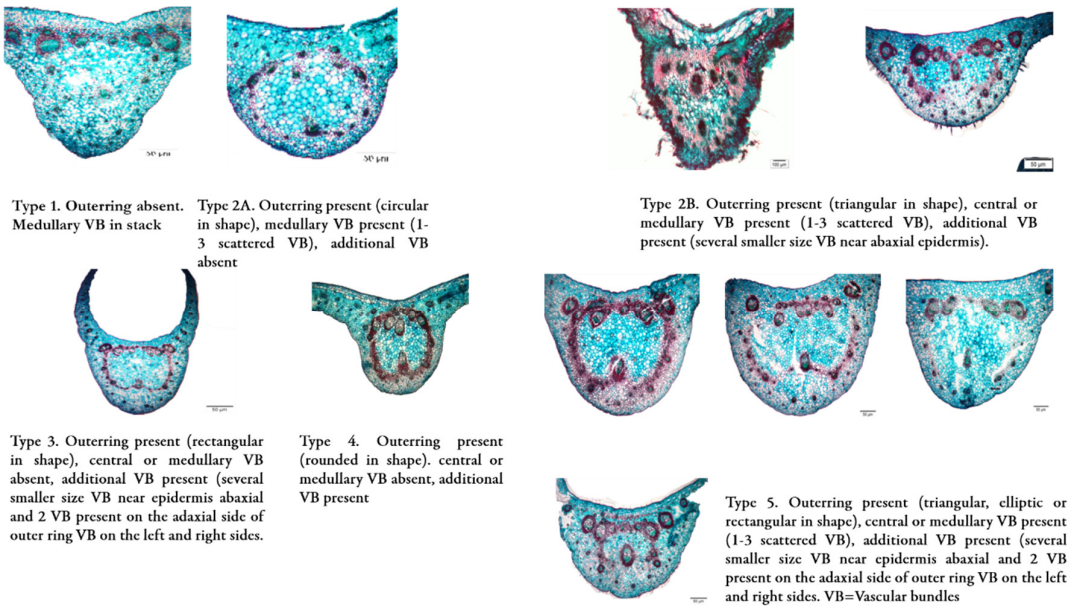

Figure 11. Variation and classification of *Nepenthes* vascular bundle arrangement

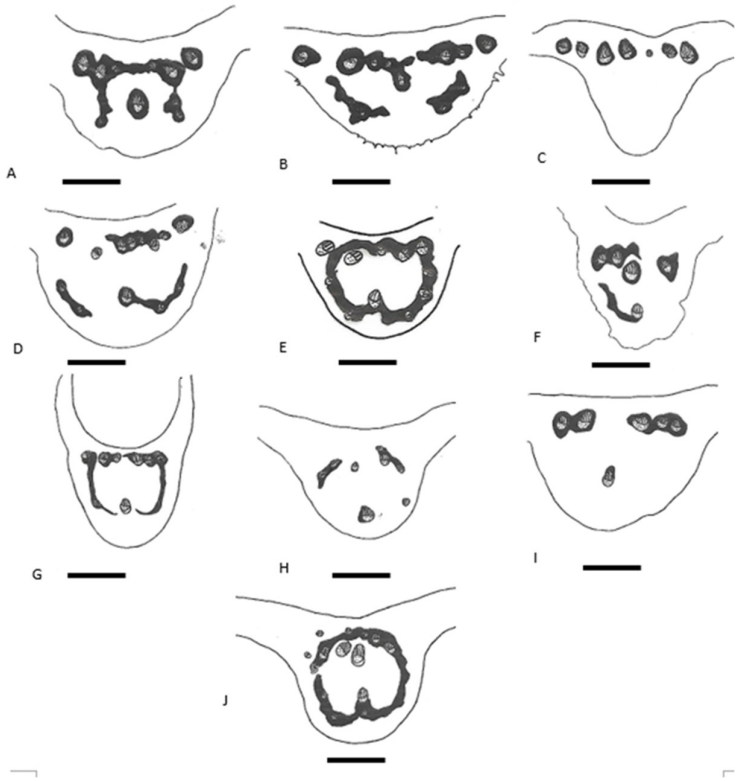

Figure 12. Transverse sections of midribs of *Nepenthes* species
A, *Nepenthes albomarginata*. B, *N. ampullaria*. C, *N. macfarlanei*. D, *N. mirabilis*. E, *N. benstonei*. F, *N. alba*, G. *N. rafflesiana*. H, *N. ramispina*. I, *N. sanguinea*. J, *N. gracilis*

Type 1. Abaxial: prominent UV-shaped; Adaxial: very slightly concave
Nepenthes benstonei, N. mirabilis, N. sanguinea, N. albomarginata& N. ramispina.

Type 2. Abaxial: arch shaped; Adaxial: slightly flattened
Nepenthes gracilis& N. ampullaria

Type 3. Abaxial: prominent UV-shaped; Adaxial: prominent U-shaped
Nepenthes rafflesiana

Type 4. Abaxial: prominent V-shaped; Adaxial: prominent wide V-shaped
Nepenthes macfarlanei & N. alba

Figure 13. Variation and classification of *Nepenthes* midrib outer shape

Based on the vascular bundle arrangement in the petiole, it is clearly shown that this character can be useful in species differentiation, especially for the ten studied species. It is also clearly shown that the types of vascular bundle systems can be used for species grouping in the genus *Nepenthes*. These findings agree with the study of Mohamad Ruzi (2007), who found that the types of vascular bundle based on the arrangement and system in the vascular bundle strands can be used for grouping of species in *Dipterocarpus* and help in species identification. As Metcalfe (1942) stated, the anatomy of both leaf and stem gives numerous characteristics, which in combination are reliable for diagnostic purposes; some species can be identified with certainty based on the vascular bundle arrangement (Figure 11). Therefore, anatomical characteristics could be valuable for taxa identification and differentiation.

For outer shape of the midrib, four outline types were characterised in this study, which is Type 1 (Abaxial: prominent UV-shaped; Adaxial: very slightly concave) found in *Nepenthes benstonei, N. mirabilis*, *N. sanguinea, N. albomarginata* and *N. ramispina*; Type 2 (Abaxial: arch shaped; Adaxial: slightly flattened) characterized in *N. gracilis* and *N. ampullaria,* Type 3 (Abaxial: prominent UV-shaped; Adaxial: prominent U-shaped) in *N. rafflesiana* and Type 4 (Abaxial: prominent V-shaped; Adaxial: prominent wide V-shaped) which

is showed in *N. macfarlanei* & *N. alba*). This characters clearly distinguished *N. rafflesiana* from other *Nepenthes* taxa and can be used in diagnostic charaterisation (Figures 12 and 13).

Selected anatomical characters observed in the *Nepenthes* species studied are stomatal density and length, trichome types and distribution, cuticle thickness, thickness of adaxial hypodermis, and the number of adaxial hypodermis layers (Table 3). Some characters are present in all the species studied and considered common characters that enumerate this carnivorous plant genus; for example, all the species studied have a single layer of palisade cells with variable height.

Druses are present in enlarged idioblast cells in palisade layers of most of the studied species. Other anatomical leaf features are also fairly uniform. Two to four palisade layers are found with extracellular spaces in spongy cell layers. Sessile glands tend to be orbicular and flower-like in shape, spread on adaxial and abaxial surfaces, and the base is buried up to the hypodermal layer. In *N. alba* and *N. albomarginata,* druses are not only confined to the idioblast cells, but also occur in the palisade and spongy cell layers and in the vascular tissue, that can be observed in the parenchyma cells of the midrib, and in the phloem cells. However, most of the species showed the occurrence of solitary crystals in the palisade and spongy mesophyll cells, also in the marginal and in the leaf lamina. Anomocytic stomata were present in all species, which is considered as characteristic for Nepenthaceae (Metcalfe and Chalk, 1950). Anomocytic stomata are also known as ranunculaceous type of stomata (Metcalfe and Chalk, 1965). This feature also in line with the finding involving new taxa of *N. domei*, *N. latiffiana* which also characterized the same anomocytic stomata features (Ghazalli *et al*., 2020).

Stomata anatomical characteristics can be used in species identification and also in dividing the species based on its ecological habitat. Stomatal Index (SI) divides the stomatal density index within three categories: **Index I = ≥10%, Index II = ≥15%** and **Index III = ≥20%.** It is interesting to note that the stomatal index can be used in dividing the *Nepenthes* species based on their ecological and habitat preferences. Lowland species *N. gracilis* has stomatal index of 12.25% (Index I); lowland-intermediate is placed in Index II which consists of *Nepenthes ampullaria* (16.93%), *N. mirabilis* (18.75%), *N. rafflesiana* (15.38%) and *N. benstonei* (17.79%), while Index III which is more than 20% associated with highland species (viz., *N. albomarginata* (26.67%), *N. macfarlanei* (23.71%), *N. ramispina* (27.29%), *N. sanguinea* (22.25%) and *N. alba* (31.23%). Mensah (2012) noted that stomatal density can be affected by environmental and climatic conditions such as drought and will influence stomatal count in plant species. Tomlinson et al. (2011) also stated that stomata in pitcher plants do not necessarily occur in continuous regular longitudinal rows.

Hypodermis cells are the cells like those found in Poaceae that have the same function as bulliform cells (adaxially occurring in grasses species leaf lamina) (Turpe, 1967; Oliveira *et al.* (2015); Reis *et al.* 2015). The hypodermis cells in *Nepenthes* are present on both abaxial and adaxial surfaces. Variations between species in number of cell layers and the occurrence of fibre groups mixed with the hypodermis cells were significant for species differentiation in *Nepenthes*. Hypodermal cells layer on the adaxial side of *Nepenthes* are varied, compared to the abaxial side. This study supported the previous study by Biati (2012) which found *N. gracilis* and *N. rafflesiana* having a single layer of hypodermal cells, while typical highland species such as *N. gymnamphora* have between 3 and 4 variable layers. It demonstrates that hypodermal layers and their thickness will increase according to the altitude or elevation of the natural habitat (Toma *et al*., 2002). Analysis showed that adaxial hypodermal layer varies within species – *N. alba, N. sanguinea, N. mirabilis,N. rafflesiana, N. ampullaria* and *N. albomarginata* with single layer, *N. gracilis* and *N. benstonei* with 1-2 layers, *N. macfarlanei* with 1-4 layers) and *N. ramispina* with 1-3 layers (Table 3).

In this study, it is noted that the adaxial cuticle thickness varies from 0.8-12.5 µm, tending to be thicker than the cuticle on the abaxial side. However, at the base of *N. mirabilis* the leaf blade occasionally has some part not covered by cuticle. Riederer (2006) and Javelle (2010) stated that trichome and cuticle function against abiotics response were not much different, such as control transpiration, temperature, gas exchange and sunlight intensity. Lowland associated species such as *N. ampullaria* and *N. mirabilis* have adaxial cuticle to 5.9µm thick (Table 3). Typical highland species such as *N. albomarginata*, *N. macfarlanei*, *N. ramispina*, *N. sanguinea* and *N. alba* have adaxial cuticle to 12.5µm thick. The differences may be due to the population of

these species, which grow in colder climate and nearby water resources. Therefore, there is less effort to stabilize transpiration on the leaves, considering that cuticles and trichome are modifications from epidermis (Haron, 2008) and both of those modifications appear due to the influence of environmental stress (Esau, 1965).

Table 3. Comparison anatomical characterisation of studied *Nepenthes* specimens

Species / characters	Midrib outline	Vascular bundles arrangement	Stomatal Index	Hypodermis cells characters	Occurrence of fibre groups mixed with the hypodermis cells	Cuticle Thickness	Margin outline
Nepenthes alba	Type 4	Type 2B	31.23% (Index III)	Single layer	Not observed	Up to 12.5µm thick.	tapering, triangular and pointing 90° downward to adaxial side.
N. albomarginata	Type 1	Type 5	26.67% (Index III)	Single layer	Not observed	Up to 12.5µm thick.	rounded with neck-like structure, pointing 90° downward to adaxial side.
N. ampullaria	Type 2	Type 2B	16.93% (Index II)	Single layer	Not observed	Up to 5.9µm thick.	rounded, pointing 45° downward to adaxial side.
N. benstonei	Type 1	Type 5	17.79% (Index II)	1-2 layers	Not observed	Up to 12.5µm thick.	tapering, pointing 30° downward to adaxial side.
N. gracilis	Type 2	Type 4	12.25% (Index 1)	1-2 layers	Not observed	Up to 5.9µm thick.	tapering-triangular and pointing 45° downward to adaxial side.
N. macfarlanei	Type 4	Type 1	23.71% (Index III)	1-4 layers	Not observed	Up to 12.5µm thick.	lithop shaped, pointing at about 120° downward to adaxial side.
N. mirabilis	Type 1	Type 4	18.75% (Index II)	Single layer	Observed	Up to 5.9µm thick.	tapering-triangular shaped, pointing 90° downward to adaxial side.
N. ramispina	Type 1	Type 2A	27.29% (Index III)	1-3 layers	Not observed	Up to 12.5µm thick.	rounded, pointing 45° downward to adaxial side.
N. rafflesiana	Type 3	Type 3	15.38% (Index II)	Single layer	Not observed	Up to 12.5µm thick.	rounded, straight.
N. sanguinea	Type 1	Type 5	22.25% (Index III)	Single layer	Not observed	Up to 12.5µm thick.	tapering, pointing 90° downward to adaxial side, roughly creased surface.

Prominent leaf characters play an important role in supporting identification and classification; for example, the number of nerves and secretory cells are usually diagnostic, especially when complete specimens are available for observation (Dickinson, 2000). Combinations of marginal and areolar venation patterns for certain taxon are unique, the veinlet endings vary greatly among them, namely uni-, simple and linear, bi-, with their ultimate marginal venation complete or incomplete (Table 4). The abundance of druses on the lamina surface of *N. alba* and *N. ramispina* can be a diagnostic character for them (Figure 8 H, I, Figure 10 H, I). The presence of swollen veinlets or swollen tracheids can also be used as additional characters for differentiation. For example, swollen veinlets are present in *N. ampullaria* (Figure 2M) and *N. macfarlanei* (Figure 5 L). Three veinlet patterns were observed in this study, namely simple and linear veinlets, uni-veinlets, bi-veinlets. In particular, *N. alba* showed no ending veinlets and this is diagnostic for this taxon. Others showed either uni-, or simple and linear, or bi-veinlets and these can be the diagnostic characters for taxa identification. Only one type of ultimate marginal venation was observed in this study, which is closed ultimate marginal venation, which refers to higher vein order fused into a vein running just inside the margin.

Table 4. Variation in the areolar / marginal venation pattern & druses observation in studied *Nepenthes* specimens

Taxa	Type of marginal venation	Type of veinlets		
	Closed	Simple (linear to curved)	Uni-veinlet	Druse observation
N. albomarginata	+	-	+	-
N. ampullaria	+	-	+	-
N. benstonei	+	+	-	-
N. gracilis	+	-	+	-
N. macfarlanei	+	-	+	-
N. mirabilis	+	-	+	-
N. rafflesiana	+	-	+	-
N. ramispina	+	-	+	+
N. sanguinea	+	-	+	-
N. alba	+	+	-	+

+ : Present, - : absent

Another systematic evidence is the variation in the appendages between *Nepenthes* taxa (Table 5). Combination of tufted and simple unicellular trichome on the abaxial leaf blade surface can be observed in *N. ampullaria* and simple unicellular trichome on the abaxial leaf blade surface in *N. albomarginata, N. benstonei, N. mirabilis, N. rafflesiana* and *N. ramispina,* while both abaxial and adaxial parts of the leaf surface were demonstrated in *N. macfarlanei* and *N. alba*, which is supported by the studies of Metcalfe and Chalk (1950, 1965 and 1979) and Farishy (2017) on the variation of appendage type and dispersion on the leaf surfaces of *Nepenthes*.

Table 5. Variation of appendages morphology observed in studied *Nepenthes* specimens

Species / characters	Simple unicellular trichomes (on abaxial surface only)	Simple unicellular trichomes (on both abaxial and adaxial surfaces)	Tufted trichomes
Nepenthes alba	-	+	-
N. albomarginata	+	-	-
N. ampullaria	+	-	+
N. benstonei	+	-	-
N. gracilis		-	-
N. macfarlanei	-	+	-
N. mirabilis	+	-	-
N. ramispina	+	-	-
N. rafflesiana	+	-	-
N. sanguinea		-	-

+ : Present, - : absent

Conclusions

There are ten distinguishing anatomical characteristics of *Nepenthes* that combine the features of density and stomatal index (SI), hypodermis cell – cell layers, occurrence of fibre groups mixed with the hypodermis cells, adaxial cuticle thickness, vascular bundle arrangement, midrib outline shape, venation plasticity, druses appearance and appendage/ or trichomes type. Some of these characters such as stomatal density, hypodermis cell analysis and cuticle thickness can be influenced by environmental conditions specifically in altitudinal changes but some characters are worth interpretated in taxonomic significance that involves venation pattern plasticity, druse observation, midrib outlines, vascular bundle arrangements and also leaf margin outlines. These characters are important and can serve as systematically related features or diagnostic characters for Peninsular Malaysia's *Nepenthes* species interpretation, as well as an additional identification evidence in supporting morphological observation.

Authors' Contributions

Conceptualization: MNFG, AAT, DN; Data curation: MNFG, AAT, DN, MIME, EEB; Formal analysis: MNFG, NT; Funding acquisition: MNFG, AAT; Investigation: MNFG, AAT, DN, MIME, EEB; Methodology: MNFG, NT, DN; Project administration: MNFG, AAT, DN; Resources: DN, AL; Software: MNFG, AAT, NT; Supervision: NT, AL; Validation: NT, AL; Visualization: MNFG, AL; Writing - original draft: MNFG, AAT, AL; Writing - review and editing: MNFG, NT, AL. All authors read and approved the final manuscript.

Acknowledgements

This exploration study was carried out under permit ref. JH/100 Jld. 22 (41) of the Forestry Department of Peninsular Malaysia (Ministry of Water, Land and Natural Resources). We are grateful to Dr. Richard Chung Cheng Kong (Forest Research Institute Malaysia, FRIM) for his guidance and help in obtaining permission involving herbarium records and observation of the pitcher plant herbarium specimens (KEP). Our special thanks go to Madam Salmaniza Salleh and Miss Nurshahidah Mohd Rusli for their technical assistance in the anatomical works as well as in herbarium curations. We are also grateful to Muhammad Ikhwan Affandi Md Daud for his assistance during the field work.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Alvarado G, Jauregui D (2011). Anatomıa del raquisy la lamina foliar de *Attalea butyracea* (Mutis ex Lf) Wess. Boer y *Attalea maripa* (Aubl.) Mart. (Arecaceae, Arecoideae). Pittieria 35:71-83.

Barfod A (1988). Leaf anatomy and its taxonomic significance in phytelephantoid palm (Arecaceae). Nordic Journal of Botany 8:341-348. https://doi.org/10.1111/j.1756-1051.1988.tb00509.x

Batagin-Kiotto KD, Almaeida CVD, Piotto FA, Almaeida MD (2012). Anatomical analysis of peach palm (*Bactris gasipaes*) leaves cultivated *in vitro*, *ex vitro* and *in vivo*. Brazilian Journal of Botany 35:71-78. https://doi.org/10.1590/S0100-84042012000100008

Beveridge NGP, Rauch C, Kebler PJAK, Van Vugt RR, van Welzen PC (2013). TITLE OF THE ARTICLE.Carnivorous Plant Newsletter 42:122-128.

Biati NYK (2012). Studi Anatomi dan Struktur sekretori Tanaman Kantong Semar (*Nepenthes* spp.). Master thesis, Bogor Agricultural University, Bogor.

Corner EJH (1988). Wayside Trees of Malaya. 3rd Edition. Malayan Nature Society, Kuala Lumpur.

Cheek M, Jebb M (2001). Nepenthaceae. Flora Malesiana 15. Netherlands, Leiden.

Clarke CM (2001). Nepenthes of Sumatra and Peninsular Malaysia. Natural History Publications, Kota Kinabalu.

Clarke C (2002). A guide to the pitcher plants of Peninsular Malaysia. Natural History Publications (Borneo).

Christenhusz MJM, Byng JW (2016). The number of known plants species in the world and its annual increase. Phytotaxa 261(3):201-217. *https://doi.org/10.11646/phytotaxa.261.3.1*

Danser BH (1928). TITLE OF THE ARTICLE. Bulletin du Jardin Botanique de Buitenzorg 9:249-438.

de Candolle CDE (1879). Anatomie compare des feulles chez quelques familles de Dicotyledones. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 26:427-480.

Dickinson WC (2000). Integrative Plant Anatomy. Harcourt Academic Press, San Diego, pp 56.

Esau K (1965). Plant Anatomy, Second Ed. John Wiley & Sons, Hoboken, New Jersey.

Farishy-Al DD, Nisyawati, Metusala D (2017). Characterization anatomical leaf blade five species *Nepenthes* from Kerinci Seblat National Park, Kerinci regency, Jambi Province. *A*IP Conference Proceedings 1862:030115. *https://doi.org/10.1063/1.4991219*

Ghazalli MN, Tamizi AA, Nikong D, Besi EE, Mat Esa MI, Mohd Nordin AR, Latiff A, Zaini AZ, Shakri MA (2020). *Nepenthes latiffiana* and *N. domei* (Nepenthaceae), two new species of pitcher plants from Terengganu, Peninsular Malaysia. Webbia 75(1):5-28. *https://doi.org/10.36253/jopt-7950*.

Glassman SF (1972). Systematic studies in the leaf anatomy of palm genus *Syagrus*. American Journal of Botany 59:775-788.

Haron N, David DM (2008). The taxonomic significance of leaf micromorphology in the genus *Eugenia* L. (Myrtaceae). Botanical Journal of the Linnean Society 120(3):265-277. *https://doi.org/10.1111/j.1095-8339.1996.tb00776.x*

Horn JW, Fisher JB, Tomlinson PB, Lewis CE, Laubengayer K (2009). Evolution of lamina anatomy in the palm family (Arecaceae). American Journal of Botany 96:1462-1486. *https://doi.org/10.3732/ajb.0800396*

Javelle M, Vernoud V, Depege-Fargeix N, Arnould C, Oursel D, Domergue F, Sarda X, Rogowsky PM (2010). Over-expression of the epidermis-specific HD-ZIP IV transcription factor OCL1 in maize identifies target genes involved in lipid metabolism and cuticle biosynthesis. Plant Physiology 154:273-286. *https://doi.org/10.1104/pp.109.150540*

Jebb M, Cheek M (1997). A skeletal revision of *Nepenthes* (Nepenthaceae). Blumea: Biodiversity, Evolution and Biogeography of Plants 42(1):1-106.

Johansen DA (1940). Plant Microtechnique. New York: Mc Graw-Hill Book.

Krauss JE, Arduin M (1997). Manual basico de metodos em morfologia vegetal. Seropedica, Edur.

Moraes M (1996). Allagoptera (Palmae). Flora Neotropica 73:1-34.

Martins RC, Filgueiras TS (2010). Roteiro morfologico para a coleta de palmeiras (Arecaceae) no Cerrado. Heringeriana 4:51-59.

Martins RC, Filgueiras TS, Graciano-Ribeiro D, Somavilla NS (2015). A new species of *Allagoptera* (Arecaceae) from the Cerrado of central Brazil. Phytotaxa 197:115-124. *https://doi.org/10.11646/phytotaxa.197.2.4*

McPherson S (2009). Pitcher plants of the Old World (Vol. 1). Redfern Natural History Productions.

Mensah DB (2012). Leaf anatomical variation in relation to stress tolerance among some woody species on the Accra plains of Ghana. Journal of Plant Development 19:13-22.

Metcalfe CR (1942). On the taxonomic value of the anatomical structure of the vegetative organs of the dicotyledons: An introduction with special reference to the anatomy of the leaf and stem. Proceedings of the Linnean Society of London 155(3):211-223.

Metcalfe CR, Chalk L (1950). Anatomy of the Dicotyledons: leaves, stem and wood in relation to taxonomy with notes on economic uses. Volume I. The Clarendron Press, Oxford.

Metcalfe CR, Chalk L (1979). Anatomy of the Dicotyledons. Vol I. Systematic anatomy of leaf and stem, with a brief history of the subject. Anatomy of the dicotyledons. Vol I. Systematic anatomy of leaf and stem, with a brief history of the subject (Ed. 2).

Millan B, Kahn F (2010). Characterization of leaf anatomy in species of *Astrocaryum* and *Hexopetion* (Arecaceae). Revista Peruana de Biologia 17:81-94. https://doi.org/10.15381/rpb.v17i1.55

Mohamad Ruzi AR (2007). Anatomi daun *Dipterocarpus* Gaertn. *f.* dan *Shorea* Roxb. (Dipterocarpaceae). Master thesis. Universiti Kebangsaan Malaysia (UKM), Bangi.

Noblick LR (2013). Leaflet anatomy verifies relationships within *Syagrus* (Arecaceae) and aid in identification. PhytoKeys 26:75-99. https://doi.org/10.3897/phytokeys.26.5436

Noraini T, Ruzi AR, Nurnida MK, Hajar NR (2012). Systematic significance of leaf anatomy in *Johannesteijsmannia* HE Moore (Arecaceae). Pertanika Journal of Tropical Agricultural Science 35:223-235.

Oliveira RC, Valls JFM, Gomes SM, Fagg CW, Pozzobon MT (2015). A new species of *Paspalum*, Notata group (Poaceae, Paspaleae), from the Cerrado biome, Brazil: description, chromosome number, and leaf blade anatomy. Phytotaxa 203:159-168. https://doi.org/10.11646/phytotaxa.203.2.5

Paluvi N, Mukarlina RL (2015) Struktur Anatomi Daun, Kantung dan Sulur Nepenthes gracilis Korth. yang Tumbuh di Area Intensitas Cahaya Berbeda. Protobiont 4(1):103-107.

Pavlovic A, Masarovicova E, Hudak J (2007). Carnivorous syndrome in Asian pitcher plants of the genus *Nepenthes*. Annals of Botany 100:527-536. https://doi.org/10.1093/aob/mcm145

Reis PA, Silva ALH, Silva AS, Valls JFM, Gomes SM, Oliveira RC (2015). *Paspalum minutispiculatum* (Poaceae, Panicoideae): a new annual species from central Brazil. Systematic Botany 40:746-754. https://doi.org/10.1600/036364415X689203

Ridley HN (1924). Nepentheaceae. The Flora of the Malay Peninsula 3:21-25.

Riederer M, Muller C (2006). Biology of the Plant Cuticle. Blackwell Publishing, Oxford.

Tamizi AA, Ghazalli MN, Nikong D, Edward EB, Muhamad Ikhwanuddin ME, Anuar Rasyidi MN, A. Latiff, Mohamad Alias S (2020). *Nepenthes malayensis* (Nepenthaceae), a new species of carnivorous pitcher plant from Peninsular Malaysia. Kew Bulletin 75:63. https://doi.org/10.1007/s12225-020-09918-z

Toma I, Toma C, Stanescu I (2002). Histo – anatomical aspects of the *Nepenthes maxima* Reinw. Ex Ness metamorphosed leaf. Revue Roumaine de Biologie Série de Biologie Vegetale 47:3-7.

Tomlinson PB, Horn JW, Fisher JB (2011). The anatomy of palms: Arecaceae-Palmae. Oxford University Press, Oxford. https://doi.org/10.1093/aob/mcr265

Turpe AM (1967). Histotaxonomıa de las especiesargentinas del genero *Paspalum*. Lilloa 32:35-299.