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D. D. Al Farishy, Nisyawati and D. Metusala



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# Characterization Anatomical Leaf Blade Five Species *Nepenthes* from Kerinci Seblat National Park, Kerinci Regency, Jambi Province

D. D. Al Farishy<sup>1</sup>, Nisyawati<sup>1, a)</sup>, and D. Metusala<sup>1, 2</sup>

<sup>1</sup>*Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA),  
Universitas Indonesia, Depok 16424, Indonesia*

<sup>2</sup>*Purwodadi Botanic Garden, Indonesian Institute of Sciences (LIPI),  
Purwodadi 67163, Indonesia*

<sup>a)</sup>Corresponding author: nsywt@yahoo.com

**Abstract.** *Nepenthes* is one of carnivorous plant genera which have key characters on leaf and pitcher as the modification. However, wide varieties of morphological features on pitcher intraspecies and between species could be tough for identification process. The objective was to provide alternative characters for identification process by anatomical features. Kerinci Seblat National Park was chosen because lack of update data on wild type of species there. Whole five species were collected at Lingkat Lake and Gunung Tujuh Lake as representative lowland and highland species. Leaves collected fresh, flawless, and has grown pitcher. Each leaf was separated into the paradermal and transversal section, dehydrated by series alcohol, and stained by safranin and fast green. Sections observed by light microscope. Result show there were specific differences between species that could be potential to be key characters. That features are stomatal density, stomatal length, sessile glands surface shaped, sessile glands density, trichome distribution, adaxial cuticle thickness, adaxial hypodermis thickness, and the number of layers of adaxial hypodermis

## INTRODUCTION

*Nepenthes* L. (Nepenthaceae) is a carnivorous plant genera that develop pitcher structure as a trap for insects. The structure of the pitcher is a modification from midrib which elongated and formed tendrils [1, 2]. *Nepenthes* species would be quite difficult to identify without the pitcher so that structure is very important for the identification process. However, wide variation in the morphology of pitcher could make difficulties and confusion for that process [2-4]. Therefore, it is necessary to characterize other various characters and structures that considered important for identification process in *Nepenthes* genera.

Radford [5] stated that plant anatomical structures could be useful for key identification. Several studies had been done in descriptive and quantitative observations of the anatomical leaf. Metcalfe and Chalk [6] first studied *Nepenthes* used slides collection at Kew Botanical Gardens. Toma *et al.* [7] used *N. maxima* from cultivation. Pavlovic [8] used *N. mirabilis* and *N. alata* from cultivation. Biati [9] used three species from species. Then, Paluvi *et al.* [10] used *N. gracilis* from the swamp forests of West Kalimantan. All those studies show lack of data on wild type species, especially from natural habitat of Sumatera where *Nepenthes* abundant [11, 12].

One of the locations at Sumatra where *Nepenthes* occur and growth is at Kerinci Seblat National Park (KSNP). KSNP has area 1,375,349.867 hectares and located in four provinces, namely South Sumatra, Bengkulu, Jambi and West Sumatra [13]. In Jambi province, ten species *Nepenthes* had been found and some of them are endemic [14, 15]. Limited collection and there is no up to date data on the species *Nepenthes* on KSNP became reasons had to do further characterization to complete the existing data. Aim of this research is to preserve and analyze anatomical description of *Nepenthes* genera from Kerinci Seblat National Park.

## MATERIALS AND METHODS

*Nepenthes* collected from two locations at KSNP in March 2016. Those locations represent lowland and highland altitude. Environment parameters also noted, such as humidity, light intensity, temperature, weather, and the coordinates. Leaf blade collected fresh, flawless, and had grown pitcher. Leaf blade collected one sheet of each individual and minimal two individuals of each species.

Leaf blade slices by the direct method (non-paraffin method) on the field, then placed on fixation solution alcohol 70 %. The paradermal section is used slices on both adaxial and abaxial sides. Transversal section is used slices on midrib at base, middle, and apex by about 1 cm wide. The tranverse section on tendril also had been made. Sections were dehydrated used alcohol series with safranin and fast green staining. Preservation was done wet in a modified solution of glycerin and alcohol (3:10) [16,17]. Slides preservation done contemporary only for observation.

Sections were observed qualitatively and quantitatively. Qualitative and quantitative observed were stomata, guard cells, subsidiary cell, sessile glands (hydathodes), trichomes, epidermal cell, hypodermic cell, vascular system, vascular bundles, and mesophyll. Observations were done by microscope LEICA DM500.

## RESULTS AND DISCUSSION

Five species of collected well from Kerinci Seblat National Park. *Nepenthes mirabilis* and *N. ampullaria* was found and collected on the swamp of Lingkat Lake with altitude 1019-1021 m asl. *Nepenthes pectinata* was found and collected on the swamp of Gunung Tujuh Lake with altitude 2000 m asl. *Nepenthes aristolochioides* and *N. singalana* was found and collected on the slope with estimated more than 45 degrees to one of the peaks of the seven mountains that surround the Gunung Tujuh Lake with altitude 2192 to 2317 m asl.

The result shows that some characters on one species different than others. Stomatal density and length divide to two groups by lowland and highland species. The groups were divided by density  $\geq 200 / \text{mm}^2$  and  $\leq 140 / \text{mm}^2$ . The stomatal density which has  $\geq 200 / \text{mm}^2$  are *N. ampullaria* and *N. mirabilis*. The stomatal density which has  $\leq 140 / \text{mm}^2$  are *N. pectinata*, *N. aristolochioides*, and *N. singalana*. In addition, stomatal length on *N. mirabilis* and *N. ampullaria* has range  $< 36 \mu\text{m}$  long, but stomatal length on *N. aristolochioides*, and *N. singalana* has range  $\geq 36 \mu\text{m}$ . Only on *N. pectinata* wich has overlapping range size from  $30.8 \mu\text{m}$  to  $38.7 \mu\text{m}$ .

Stomatal density on a many plants species much or less affected by environmental conditions. When conditions are drought, stomata tend to be denser [18]. In species *Cenchrus ciliaris*, Poaceae, stomatal density increases as the drought level rise. Then, the high number of stomatal density followed by a smaller size [19]. On *Nepenthes*, adaptation may also rule as that mechanism.

Sessile glands could be differentiated by the surface shape and density on abaxial, but not patterned based on habitat. Sessile glands *N. mirabilis* has plus-like shaped, while others have orbicular shaped. Then, sessile glands wich has density  $< 10 / \text{mm}^2$  are *N. aristolochioides* and *N. singalana* upper pitcher. Sessile glands which have density  $\geq 10 / \text{mm}^2$  are *N. ampullaria*, *N. mirabilis*, and *N. pectinata*. Other than that, Sessile glands which have density  $5 / \text{mm}^2$  to  $15 / \text{mm}^2$  is *N. singalana* lower pitcher. The overlap density was strengthen by data on *N. gracilis*, *N. x hookeriana*, *N. gymnamphora*, and *N. rafflesiana* [9].

Trichome on leaf blade only found on abaxial *N. ampullaria*, *N. mirabilis*, and adaxial *N. pectinata*. Trichome has not been found on both abaxial and adaxial sides *N. aristolochioides* and *N. singalana*. Trichome density on *N. ampullaria* even reach  $9 / \text{mm}^2$ , much different from *N. mirabilis* which only occasionally appear with a density of  $1 / \text{mm}^2$ . In *N. pectinata*, Trichome observed only in transversal section caused the presence only on adaxial midrib.

Adaxial cuticle tends to be thicker than abaxial side. Thickness varies from  $0.8 \mu\text{m}$  to  $11.8 \mu\text{m}$ . However, at the base of the leaf blade *N. mirabilis* occasionally has some part that was not covered by cuticle. A typical lowland species such as *N. ampullaria* and *N. mirabilis* has adaxial cuticle up to  $6.8 \mu\text{m}$ . Typical highland species such as *N. aristolochioides* and *N. singalana* has adaxial cuticle up to  $11.8 \mu\text{m}$ . Besides, there is different thickness on *N. pectinata* which is typical highland species that has adaxial cuticle up to  $5.4 \mu\text{m}$ . The difference may due to population of *N. pectinata* which grown in colder climate and nearby water resource. Therefore, there less effort to stabilize transpiration on the leaves.

Cuticles and Trichome are modifications from epidermis [20]. Both of those modifications appear due to the influence of environmental stress [19, 21]. Trichome and cuticle function against abiotics response were not much different, such as control transpiration, temperature, gas exchange and water vapor, and sunlight intensity [22, 23].

Hypodermal layer on adaxial more varies rather than the abaxial side. Typical lowland species such as *N. ampullaria* and *N. mirabilis* has up to 2 layers with thickness up to 88.7 µm. Typical highland species such as *N. aristolochioides* and *N. singalana* has 2 to 5 layers with thickness up to 312.6 µm. However, *N. pectinata* which is typical highland species had 1 to 3 layers with thickness up to 168.2 µm.

Hypodermal thickness and layers look different between species. Biati [9] show that typical lowland species *N. gracilis* and *N. rafflesiana* has 1 layer, whereas *N. x hookeriana* has 1 to 3 layers. A typical highland species *N. gymnamphora* has 1 to 4 hypodermal layers. Toma *et al.* [7] also show that *N. maxima* which is typical highland species has 3 to 4 hypodermal layers. There is increase number of hypodermal layer and thickness when the altitude of natural habitat raises.

Cutler *et al.* [24] states that the existence of a hypodermic vascular plant at the species level can be an important character. *Hoya* genera, Apocynaceae, with succulent leaves could be distinguished from nonsucculent species based on the hypodermal presence and thickness [25,26]. *Pandanus labyrinthicus*, *P. amaryllifolius*, and *P. tectorius* cv. *sanderi* could be distinguished from each other by the first hypodermal thickness ratio [27]. Even, *Mapania* genera could be distinguished from other genus in family Cyperaceae by the absence of hypodermis [28]. Based on that evidence, hypodermis on *Nepenthes* should be also potentially used as distinguishing characteristic between species.

Other anatomical leaf features are fairly uniform. In mesophyll, symmetry always dorsiventral, palisade has 2 to 4 layers, and sponge layer had the extracellular room. Sessile glands tend to orbicular and flower-like form, spread on adaxial and abaxial, and the base buried to hypodermal layer. Vascular system of midrib from base to tendril composed of several vascular bundles and a large sclerenchyma ring. Each vascular bundles arrange collateral, then inner and outer side of sclerenchyma ring composed of parenchyma cells. That features also had been explained by Metcalfe and Chalk [6].

Anatomical leaf characters on *Nepenthes* species generally could be influenced by environmental conditions. Majority species are typical, so that could potentially limit the range of anatomical feature, or vice versa. Therefore, anatomical leaves on species could be worthy for identification character or even diagnostic character.

Overall, there are anatomical leaf features on *Nepenthes* that could be used for identification characteristic. That are stomatal density, stomatal length, trichome distribution, adaxial cuticle thickness, adaxial hypodermic thickness, and the number of layers of the adaxial hypodermic. In addition, almost no anatomical differences between positions of pitcher within a single species.

## Description

### 1. *Nepenthes ampullaria* Jack

Coll. number: DEE001; DEE002, in Fig. 1.

**Leaves anatomy** dorsiventral, total 304.0-443.1 µm thick; **Hypodermis** 1-2 layer on adaxial, total 13.0-68.2 µm thick; 1-2 layer on abaxial, total 9.7-46.6 µm thick; **Mesophyll** 207.4-306.2 µm thick; palisade parenchyma 3-4 layer, total 63.0-167.3 µm thick; spongy parenchyma total 104.3-148.8 µm thick, has many extracellular room; **Midrib** obvious, 1,421.4-2,132.8 µm thick, smaller toward apex leaves; vascular system collateral, widely spaced, sclerenchyma ring orbicular, flattened at adaxial, 822.9-1,438.7 µm diam. smaller toward apex leaves; vascular bundles several, larger at adaxial part, 47.2-198.6 µm diam.; **Adaxial epidermis** cuticle obvious, 3.0-6.9 µm thick; epidermis 1 layer, irregular, 4-6 sides, 20.0-56.7 µm x 7.1-28.7 µm, 6.5-18.1 µm thick; stomata absent; sessile glands flower-like, orbicular to elliptical, 26.5-37.0 µm x 26.5-35.4 µm, density 5-7 / mm<sup>2</sup>; trichome absent; **Abaxial epidermis** cuticle obvious, sometimes absent on leaves base, 0-3.4 µm thick; epidermis 1 layer, irregular, more than 6 sides, wavy margin, 22.0-58.3 µm x 7.2-40.9 µm, 6.4-14.9 µm thick; stomata anomocytic, kidney-like, 26.7-32.7 µm x 19.3-26.1 µm, density 200-360 / mm<sup>2</sup>, each guard cell width 6.9-10.0 µm; sessile glands flower-like, orbicular to elliptical, 42.0-69.4 µm x 43.3-58.2 µm, density 10-24 / mm<sup>2</sup>; trichome stellate, 40.7-1677.0 µm x 11.2-27.9 µm, density 4-9 / mm<sup>2</sup>; **Tendril** similar to midrib, 2,717.2 µm diam.; cuticle 1.2-1.3 µm thick; epidermis 1 layer, 11.4-25.1 µm thick; hypodermis 1 layer, 15.3-35.1 µm thick; vascular system collateral, widely spaced, sclerenchyma ring orbicular, 1,126.7-1,468.8 µm diam.; vascular bundles several, 110.9-160.0 µm diam.

### 2. *Nepenthes aristolochioides* Jebb & Cheek

Coll. number: DEE008; DEE010, in Fig. 2.

**Leaves anatomy** dorsiventral, total 584.5-935.6 µm thick; **Hypodermis** 3-4 layer on adaxial, total 87.6-234.7 µm thick; 1 layer on abaxial, 16.7-48.0 µm thick; **Mesophyll** 328.8-661.2 µm thick, palisade parenchyma 2-3 layer, total

67.1-203.9  $\mu\text{m}$  thick; spongy parenchyma total 274.9-490.8  $\mu\text{m}$  thick, has many extracellular room; **Midrib** obvious, 1,169.5-1,519.4  $\mu\text{m}$  thick, slightly smaller toward apex leaves; vascular system collateral, spaced, schlerenchyma ring horizontally elliptical, 633.2-1,500.0  $\mu\text{m}$  diam., smaller toward the apex leaves; vascular bundles several, larger at adaxial part, 108.1-175.5  $\mu\text{m}$  diam.; **Adaxial epidermis** cuticle obvious, 6.0-9.8  $\mu\text{m}$  thick; epidermis irregular, 4-6 sides, 26.2-72.4  $\mu\text{m}$  x 14.2-40.8  $\mu\text{m}$ , 8.9-21.4  $\mu\text{m}$  thick; stomata absent; sessile glands flower-like, orbicular to shortly elliptical, 56.3-88.2  $\mu\text{m}$  x 53.9-76.1  $\mu\text{m}$ , density 2-5 /  $\text{mm}^2$ ; trichome absent; **Abaxial epidermis** cuticle obvious, 2.3-7.8  $\mu\text{m}$  thick; epidermis irregular, 4-6 sides, 20.0-61.4  $\mu\text{m}$  x 6.3-35.3  $\mu\text{m}$ ; stomata anomocytic, kidney-like, 40-45.6  $\mu\text{m}$  x 34.8-41.7  $\mu\text{m}$ , density 80-100 /  $\text{mm}^2$ , each guard cell width 11.3-15.4  $\mu\text{m}$ ; sessile glands flower-like, orbicular, 59.0-69.1  $\mu\text{m}$  x 46.2-68.9  $\mu\text{m}$ , density 7-8 /  $\text{mm}^2$ ; trichome absent; **Tendrils** similar to midrib, 1,841.3-2,157.5  $\mu\text{m}$  diam.; cuticle 1.2-6.3  $\mu\text{m}$  thick; epidermis 1 layer, 10.0-23.9  $\mu\text{m}$  thick; hypodermis 1 layer, 21.1-37.5  $\mu\text{m}$  thick; vascular system collateral, spaced, schlerenchyma ring orbicular, 1,054.5-1,499.8  $\mu\text{m}$  diam.; vascular bundles several, 112.7-176.6  $\mu\text{m}$  diam.

### 3. *Nepenthes mirabilis* (Lour.) Druce

Coll. number: DEE004; DEE005; DEE006; DEE007, in Fig. 3.

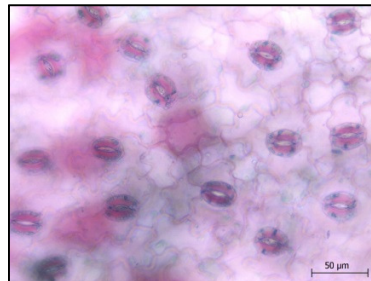
**Leaves anatomy** dorsiventral, total 271.6-554.7  $\mu\text{m}$  thick; **Hypodermis** 1-2 layer on adaxial, total 5.0-88.7  $\mu\text{m}$  thick; 1 layer on abaxial, total 11.6-47.5  $\mu\text{m}$  thick; **Mesophyll** 198.7-554.7  $\mu\text{m}$  thick; palisade parenchyma 2-3 layer, 56.8-267.2  $\mu\text{m}$  thick; spongy parenchyma total 94.3-272.7  $\mu\text{m}$  thick, has many extracellular room; **Midrib** obvious, 1,348.6-3,066.9  $\mu\text{m}$  thick, smaller toward apex leaves; vascular system collateral, widely spaced, schlerenchyma ring horizontally elliptical, flattened at adaxial, 755.8-1,399.6  $\mu\text{m}$  diam. smaller toward apex leaves; vascular bundles several, larger at adaxial part, 92.0-282.1  $\mu\text{m}$  diam.; **Adaxial epidermis** cuticle obvious, 0.8-5.5  $\mu\text{m}$  thick; epidermis 1 layer, irregular, 4-6 sides, 11.1-63.9  $\mu\text{m}$  x 6.0-31.9  $\mu\text{m}$ , 7.1-18.8  $\mu\text{m}$  thick; stomata absent; sessile glands flower-like, orbicular to elliptical, sometimes plus-shaped 27.9-46.4  $\mu\text{m}$  x 22.2-40.0  $\mu\text{m}$ , density 9-12 /  $\text{mm}^2$ ; trichome absent; **Abaxial epidermis** cuticle obvious, sometimes absent on leaves base, 0.7-4.7  $\mu\text{m}$  thick; epidermis 1 layer, irregular, 4-6 sides, or more than 6 sides with wavy margin, 18.1-64.6  $\mu\text{m}$  x 4.5-34.9  $\mu\text{m}$ , 5.3-18.3  $\mu\text{m}$  thick; stomata anomocytic, kidney-like, 24.3-35.0  $\mu\text{m}$  x 20.9-29.2  $\mu\text{m}$ , density 220-340 /  $\text{mm}^2$ , each guard cell width 8.3-12.5  $\mu\text{m}$ ; sessile glands plus-shaped, rarely orbicular, 51.6-71.5  $\mu\text{m}$  x 41.5-63.4  $\mu\text{m}$ , density 11-17 /  $\text{mm}^2$ ; trichome rarely present, stellate, 45.5-208.0  $\mu\text{m}$  x 5.5-7.5  $\mu\text{m}$ , 0-1 /  $\text{mm}^2$ ; **Tendrils** similar to midrib, 2,043.8-2,597.1  $\mu\text{m}$  diam.; cuticle 2.0-2.9  $\mu\text{m}$  thick; epidermis 1 layer, 12.7-18.3  $\mu\text{m}$  thick; hypodermis 1 layer, 10.7-27.2  $\mu\text{m}$  thick; vascular system collateral, widely spaced, schlerenchyma ring orbicular, 1,431.2-1,852.3  $\mu\text{m}$  diam.; vascular bundles several, 117.9-225.2  $\mu\text{m}$  diam.

### 4. *Nepenthes pectinata* Danser

Coll. number: DEE014; DEE015, in Fig. 4.



(a)



(b)

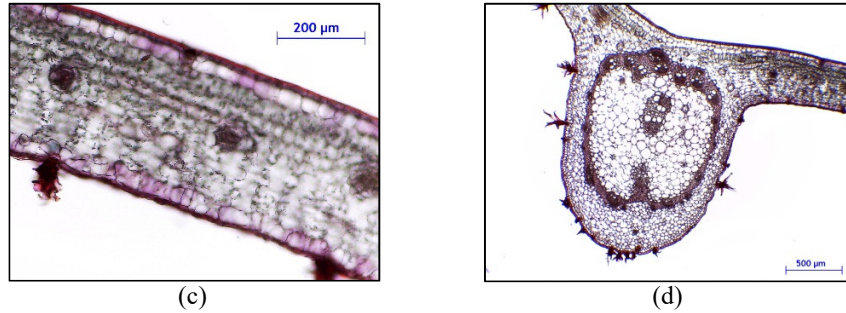


FIGURE 1. *N. ampullaria* Jack (a) abaxial leaf with trichome (b) stomata (c) midrib (d) leaf blade

**Leaves anatomy** dorsiventral, total 326.0-863.7 µm thick; **Hypodermis** 1-3 layer on adaxial, total 23.9-168.2 µm thick; 1-2 layer on abaxial, total 8.4-52.1 µm thick; **Mesophyll** 228.0-707.9 µm thick; palisade parenchyma 3 layer, total 66.2-247.4 µm thick; spongy parenchyma total 160.1-537.8 µm thick, has many extracellular room; **Midrib** obvious, 1,427.6-2,960.1 µm thick, smaller toward apex leaves; vascular system collateral, widely spaced, schlerenchyma ring orbicular, 818.2-1821.4 µm diam. smaller toward apex leaves; vascular bundles several, larger at adaxial part, 47.0-266.0 µm diam.; **Adaxial epidermis** cuticle obvious, 1.9-5.4 µm thick; epidermis 1 layer, irregular, 4-6 sides, wavy margin, 40.5-69.3 µm x 25.5-48.2 µm, 7.3-18.7 µm thick; stomata absent; sessile glands flower-like, orbicular, 60.0-83.3 µm x 56.7-76.9 µm, density 3-5/mm<sup>2</sup>; trichome stellate, 13.9-331.4 µm x 8.6-19.2 µm, numerous only on midrib; **Abaxial epidermis** cuticle obvious, 0.9-2.2 µm thick; epidermis 1 layer, irregular, more than 6 sides, wavy margin, 41.1-85.1 µm x 16.6-39.4 µm, 4.6-19.5 µm thick; stomata anomocytic, kidney-like, 30.8-38.7 µm x 23.5-31.8 µm, density 40-120 / mm<sup>2</sup>, each guard cell width 8.9-14.3 µm; sessile glands flower-like, orbicular 95.2-115.6 µm x 93.9-113.6 µm, density 13-15 / mm<sup>2</sup>; trichome absent; **Tendrils** similar to midrib, 1,550.6-2,026.9 µm diam.; cuticle 1.5-2.6 µm thick; epidermis 1 layer, 9.8-19.0 µm thick; hypodermis 1 layer, 11.3-30.1 µm thick; vascular system collateral, widely spaced, schlerenchyma ring orbicular, 1,020.0-1,291.3 µm diam.; vascular bundles several, 76.3-87.0 µm diam.

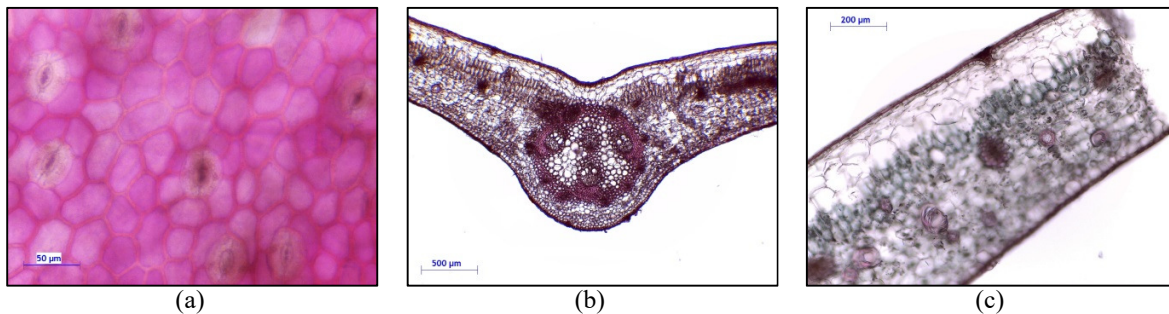
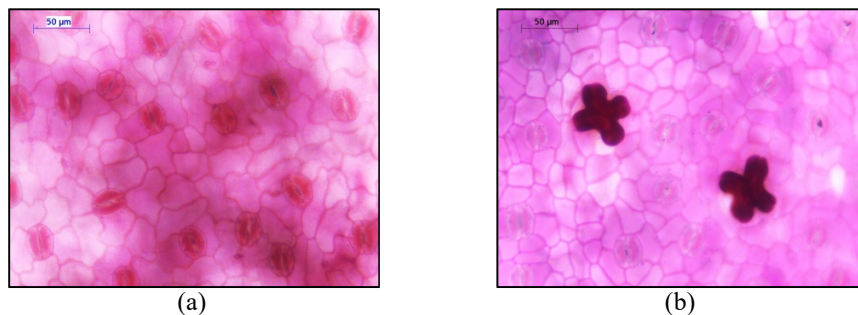


FIGURE 2. *N. aristolochioides* Jebb and Check (a) stomata (b) midrib (c) leaf blade



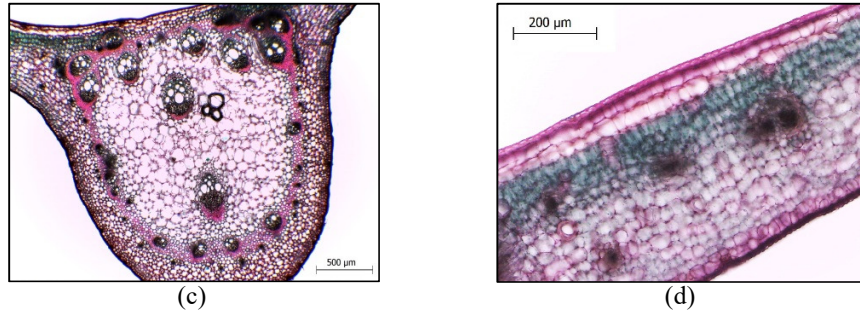


FIGURE 3. *N. mirabilis* (Lour.) Druce (a) stomata (b) sessile glands abaxial (c) midrib (d) leaf blade

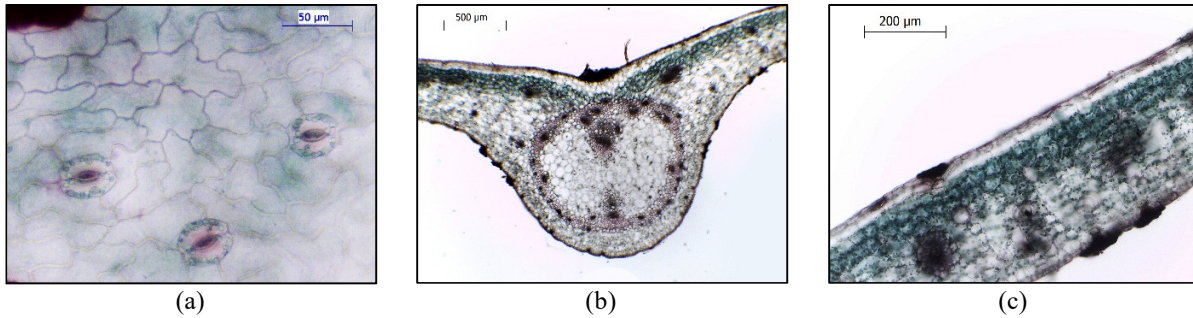


FIGURE 4. *N. pectinata* Danser (a) stomata (b) midrib (c) leaf blade

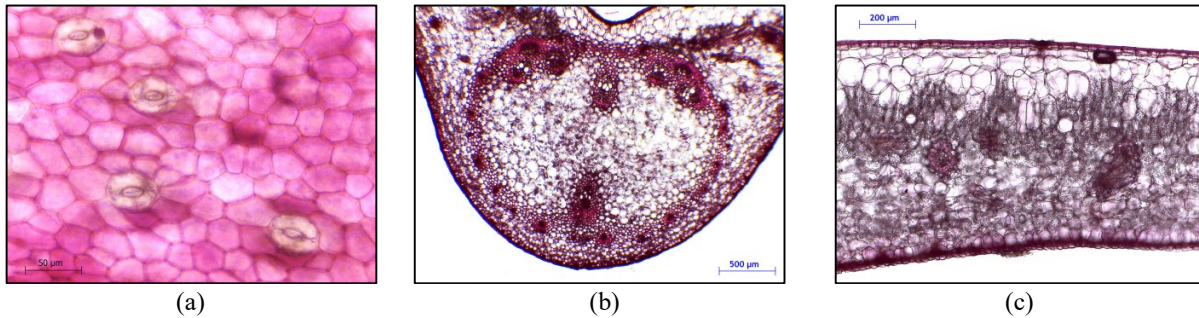


FIGURE 5. *N. singalana* Becc. (a) stomata (b) midrib (d) leaf blade

5. *Nepenthes singalana* Becc.

Coll. number: DEE009; DEE011; DEE012; DEE013, in Fig. 5.

**Leaves anatomy** dorsiventral, total 548.7-1,104.9 µm thick; **Hypodermis** 2-4 layer on adaxial, total 89.2-312.6 µm thick; 1 layer on abaxial, total 11.9-59.2 µm thick; **Mesophyll** 316.6-640.8 µm thick; palisade parenchyma 2-3 layer, total 63.0-267.4 µm thick; spongy parenchyma total 271.0-411.8 µm thick, has many extracellular room; **Midrib** obvious, 1,220.2-2,228.0 µm thick, smaller toward apex leaves; vascular system collateral, spaced, schlerenchyma ring horizontally elliptical to orbicular, 793.1-2,126.1 µm diam. smaller toward apex leaves; vascular bundles several, larger at adaxial part, 82.5-247.6 µm diam.; **Adaxial epidermis** cuticle obvious, 2.9-7.7 µm thick; epidermis 1 layer, irregular, 4-6 sides, 32.0-65.5 µm x 12.0-41.4 µm, 9.4-23.4 µm thick; stomata absent; sessile glands flower-like, orbicular, 78.3-92.3 µm x 77.0-85.6 µm, density 0-5 / mm<sup>2</sup>; trichome absent; **Abaxial epidermis** cuticle obvious, 1.2-3.5 µm thick; epidermis 1 layer, irregular, 4-6 sides, 21.6-68.1 µm x 6.6-34.1 µm, 9.2-29.2 µm thick; stomata anomocytic, kidney-like, 36.3-41.3 µm x 29.5-36.0 µm, density 60-140 / mm<sup>2</sup>, each guard cell width 10.3-13.8 µm; sessile glands flower-like, orbicular, 68.0-87.5 µm x 61.5-83.2 µm, density 5-15 / mm<sup>2</sup>; trichome absent; **Tendrils** similar to midrib, 1,236.8-2,703.8 µm diam.; cuticle 1.5-6.9 µm thick; epidermis 1 layer, 15.1-19.2 µm thick; hypodermis 1 layer, 20.7-31.7 µm thick; vascular system collateral, spaced, schlerenchyma ring orbicular, 675.6-1,897.7 µm diam.; vascular bundles several, 75.1-254.4 µm diam.

## CONCLUSIONS

There are eight distinguish characteristics based on anatomical leaf of *Nepenthes*. Characters are stomatal density, stomatal length, sessile glands surface shaped, sessile glands density, trichome distribution, adaxial cuticle thickness, adaxial hypodermic thickness, and the number of layers of adaxial hypodermis. That characters quite eligible as a key or additional character in the future.

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