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Stomata as a Differentiator of Nepenthes Type in North Sumatera

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Abstract. Research conducted in plant Systematics Laboratory of the Department of Biology University of North Sumatera. Semi-permanent preparation method for paradermal layer with a simple scraping method that modified. Samples were obtained by conducting a collection into the habitat and using *Nepenthes* specimens in the Herbarium Medanense (MEDA). Observation of anatomical characteristics conducted in plant structure Laboratory of North Sumatera University. Stomata observed in 32 *Nepenthes*, 4 of which have a companion cell and epidermis cell whose cell walls are wavy. These types are *Nepenthes* sp., *N. pectinata*, *N. rhombicaulis*, and *N. ampullaria*. Some other species have crystals in the companion cells and epidermis cells, such as *N. lingulata* and *N. mirabilis*. The size of the stomata varies greatly among all types found. *N. rafflesiana* has the smallest size of 34.43 μm^2 , while the largest size can found in the *N. ovata* type with 165.78 μm^2 and *N. spectabilis* x *N. Above* with 160.48 μm^2 . The stomata frequency per unit area also varies and shows the correlation between the stomata size and frequency. *N. x hookeriana* and *N. x trichocarpa* are two types with the highest stomata frequency while *Nepenthes* sp. has the least number of frequencies.

1. Introduction

Indonesia has high biodiversity, one of which is the diversity of the family *Nepenthaceae*. The family consists of only one genus, *Nepenthes*. *Nepenthes* is often referred to as insect-eating plants, having a unique form. The uniqueness can see from a variety of pockets shapes and colors [1]–[3]

Nepenthes most commonly found on the island of Sumatra and Borneo Island. There are 30 types in Kalimantan [3], [4]. Sumatra Island has a diversity of the second-highest type after the island of Borneo, found 29 species, and 12 hybrid natural spread ranging from the coast to the mountain forest area [5]. The region with the highest value of *Nepenthes* diversity in Sumatra Island is North Sumatra province. There are 26 types and 6 types of natural cruciferous found in the area of North Sumatra [1], [6], [7].



The main character used for the identification of the *Nepenthes* family is the pocket character. The sac in *Nepenthes* is the result of differentiation from the leaves. In addition to these morphological characters, anatomical characteristics also used to be a differentiator of the *Nepenthes* family. However, not all leaves have a pitcher, so it is required anatomical character that can used as a differentiator. Anatomical characters that can used are stomata characters [7], [8][9]. Besides stomata, trichome and cuticle observation can also be done [10], [11]. The size of the stomata can also be seen as a level of plant stress on the availability of water in its habitat [12]

2. Methods

Research conducted in plant Systematics Laboratory of the Department of Biology University of North Sumatra. Semi-permanent preparation method for Paradermal layer with a simple scraping method (Metcalf, 1960) that has been modified [13]. Samples were obtained by conducting a collection into the habitat and using *Nepenthes* specimens in the Herbarium Medanense (MEDA). Observation of anatomical characteristics conducted in plant structure Laboratory of North Sumatera University.

Paradermal observation

The leaves are cut to a length of $\pm 1-1.5$ cm, heated in HNO_3 (50%) Approximately 5 – 10 minutes (depending on the thickness of the leaves). The boiled leaf pieces are placed on glass objects and washed with aquades until clean, epidermis surfaces colored with Safranin 1%. Specimens are marked with Glyserin as a medium and covered with glass cover, further observed under the microscope to determine anatomical characters such as 1) type stomata, 2) size stomata, 3) Form epidermis and 4) crystalline form.

3. Results and Discussion

Paradermal preparation done to see the shape of the cover cell, the number of stomata, the number of epidermis cells, the shape of the herding cells, the length and width of the stomata, and the stomata type based on the Guards cell. Observations carried out, on the *Nepenthes* stomata in North Sumatra, generally have anomocytic type, where the herding cells can not distinguished with the epidermis cells surrounding it. Stomata on *Nepenthes* only found at the bottom of the leaves. Research conducted by [14] mentions that stomata only found on the part of the abaxial leaves.

Research on Stomata on *Nepenthes* has also conducted in other areas in West Kalimantan [8]. Observations conducted on five types. This type of *N. Bicalcarata* has the most extended length of the epidermis and the longest stomata size with the type of aktinositic stomata. *Nepenthes clipeata*, *N. veitchii*, *N. hirsuta*, and *N. neglecta* have anomositic stomata types. Stomata is only found on the bottom surface of the leaf except on the *N. neglecta* where the stomata is also on the top surface of the leaf. The highest density and stomata indices are owned by *N. Neglecta*. In addition, the number of neighboring cells varies from 4 – 5 neighboring cells.

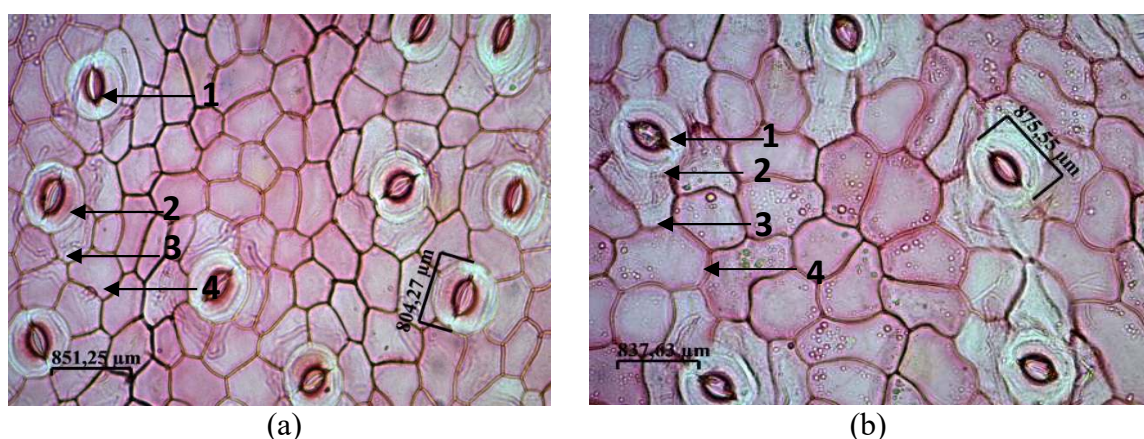


Figure 1. Type of anomositic stomata with *Nepenthes*, (a) *N. diatas*, (b) *N. diatas x N.*

spectabilis, (1) Porus, (2) cell cover, (3) companion cell, (4) Epidermis cell (magnification 10 x 40)

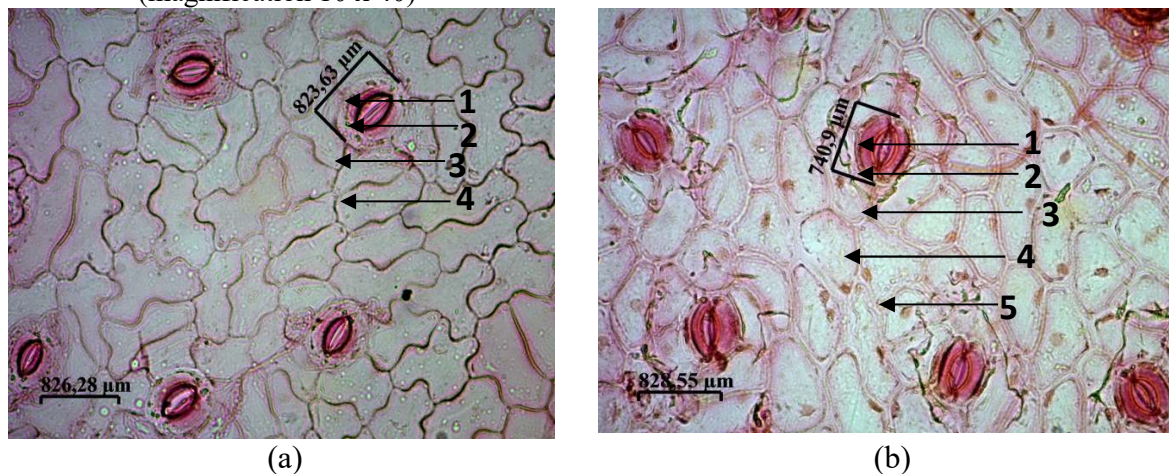


Figure 2. Epidermis cell and closing cell (a) *Nepenthes* sp. has wavy cells, (b) *N. lingulata*, (1) Porus, (2) cell cover, (3) companion cell, (4) Epidermis cell, (5) Crystal (magnification 10 x 40)

Table 1. Frequency, Indices and Size of Stomata on *Nepenthes* In North Sumatera

No	Species	Frequency	Index	Size (μm^2)
1	<i>N. ampullaria</i>	352,047	22,60	63,83
2	<i>N. beccariana</i>	81,871	5,13	65,53
3	<i>N. densiflora</i>	164,912	7,23	85,57
4	<i>N. diatas</i>	128,655	7,25	113,95
5	<i>N. dubia</i>	111,111	7,29	118,45
6	<i>N. eustachya</i>	102,924	4,49	61,23
7	<i>N. flava</i>	49,123	5,18	114,09
8	<i>N. gracilis</i>	350,877	13,70	117,10
9	<i>N. izumiea</i>	92,398	6,34	95,55
10	<i>N. jamban</i>	149,708	11,26	105,96
11	<i>N. jacquelineae</i>	72,515	5,77	51,54
12	<i>N. lingulata</i>	51,462	4,62	115,09
13	<i>N. miki</i>	91,228	6,44	142,94
14	<i>N. mirabilis</i>	140,351	10,63	79,84
15	<i>N. naga</i>	57,310	5,27	143,95
16	<i>N. ovata</i>	74,854	6,93	165,78
17	<i>N. pectinata</i>	90,058	5,30	90,09
18	<i>N. rafflesiana</i>	180,117	12,75	34,43
19	<i>N. reinwardtiana</i>	107,602	7,26	78,65
20	<i>N. rhombicaulis</i>	54,971	4,05	118,41
21	<i>N. spectabilis</i>	101,754	7,53	136,77
22	<i>N. sumatrana</i>	76,023	6,74	118,48
23	<i>Nepenthes</i> sp.	29,240	2,64	91,45
24	<i>N. tobaica</i>	169,591	9,73	108,49
25	<i>N. x hookeriana</i>	356,725	22,58	90,73
26	<i>N. x trichocarpa</i>	377,778	15,27	69,83
27	<i>N. eustachya x N. ampullaria</i>	343,860	16,14	50,73
28	<i>N. eustachya x N. beccariana</i>	94,737	7,26	120,78
29	<i>N. eustachya x N. gracilis</i>	130,994	5,95	82,23

30	<i>N. sumatrana x N. mirabilis</i>	99,415	6,09	104,71
31	<i>N. spectabilis x N. diatas</i>	97,076	4,89	160,48
32	<i>N. spectabilis x N. mikei</i>	92,328	7,41	92,04

Stomata observed in 32 *Nepenthes*, 4 of which have a companion cell and epidermis cell whose cell walls are wavy. These types are *Nepenthes* sp. (Fig. 2a), *N. pectinata*, *N. rhombicaulis*, and *N. ampullaria*. Some other types have crystals in the companion cells and epidermis cells such as *N. lingulata* (Fig. 2b) and *N. mirabilis*.

The size of the stomata varies greatly among all types found. *N. rafflesiana* has the most small size of 34.43 μm^2 , while the largest size can found in the *N. ovata* type with 165.78 μm^2 and *N. spectabilis x N. diatas* with 160.48 μm^2 (table 1). Generally known leaves with a larger stomata have a smaller stomata size and vice versa. The stomata frequency per unit area also varies and shows the correlation between the stomata size and frequency. *N. x hookeriana* and *N. x trichocarpa* are two types with the greatest stomata frequency while *Nepenthes* sp. has the least number of frequencies.

Pasaribu [13] can interpreted that the frequency of stomata nudge is higher in the types that grow in open areas such as secondary forest when compared with in the collapsed or closed area. The types that grow on dry soils and regions with low humidity generally have a higher frequency of stomata compared to the types that grow in damp soil with high humidity. This theory is in line with the results of the research.

In some plants such as *Acacia mearnsii* [15] The size difference of this stomata is used to see the ploidi nature of the plant. While some other plants, the size of the stomata can be used to see the intensity of light and moisture content that exists around the plant habitat [11], [12], [16]

Pasaribu [13] stated that on some research, the stomata index is relatively constant on many plants with a humid condition when compared to the frequency of stomata. In some types of stomata numbers are characteristic of a type and varieties. While Ghazali [9] mentioned that the epidermis cells, neighboring cells, stomata complex i.e. wax type on both surface epidermis, ornamentation cuticle in abaxial and adaxial, stomata character, stomata formation, frequency stomata, ornamentation cuticle on stomata, stomata shape, stomata size, presence and type of trichoma used to see ecological factors and variation of the type of *Nepenthes* in Peninsular Malaya.

4. Conclusions

Based on the results obtained in this research, it can concluded that the type stomata in 32 *Nepenthes* type found in North Sumatera is type anomosistic. The epidermis around the stomata has a cell wall that is wavy and straight. Some types have a stomata and an epidermis network, the cell of the dribble and the cover cell has crystals. *Nepenthes* Habitat also affects the size of the stomata. Stomata found in coastal areas have a larger Stomata size or more amount.

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