

Research Article

Anatomical Characters Used for Defining Five Species of *Nepenthes* from Bangka Belitung Islands, Indonesia

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ABSTRACT

Anatomical structures may vary among the species of *Nepenthes*. Therefore, the anatomical characters apparently could be used for supporting the morphological characters in identifying species. This study aimed to seek a valuable anatomical character for identifying and defining five species of *Nepenthes* occur in Bangka Belitung Islands. Samples of leaves, pitchers and its lids of *Nepenthes gracilis*, *Nepenthes mirabilis*, *Nepenthes ampullaria*, *Nepenthes rafflesiana*, and *Nepenthes reinwardtiana* were collected and prepared for paradermal and transverse section of microscope slides. The results showed that the anatomical characters of the leaves, pitchers and lids from these five species of *Nepenthes* could be used for differentiating five species *Nepenthes* observed. The absence of nectary gland at the abaxial of pitcher lids of *N. gracilis* and *N. ampullaria* separate them from others species. The leaf epidermis of these two species have different cell wall. The length of lunate cells at the inner surface of upper half pitchers are comparable for identifying the pitchers of *N. mirabilis* from those of *N. reinwardtiana*. The presence of crystal at the parenchymal tissue of pitcher differentiate *N. rafflesiana* from the four others. In this research, the identification key for *Nepenthes* from Bangka Belitung Islands based on the anatomical character was presented.

Keywords: Carnivorous plant, lunate cell, nectar gland, taxonomy, Nepenthaceae

Introduction

Floristic researches on the tropical pitcher plants (*Nepenthes*) in Indonesia were intensely conducted to explore the species diversity in the large islands such as Sumatra, Kalimantan, Sulawesi and Papua [1]. Since Indonesia has thousands of small islands where speciation may have occurred independently from the main islands; therefore, exploration work for documenting *Nepenthes* in small islands are challenging. Unfortunately, the sustainability many species of *Nepenthes* is currently threatened due to, among other things, habitat transformation by human activities. Some species of *Nepenthes*, for example, *Nepenthes rajah* are classified as an endangered plant and listed in the appendices I and II of Convention on International Trade of Endangered Species (CITES) of Wild Flora and Fauna [2].

Bangka Belitung Islands is a province located in the northeast of Sumatra, Indonesia. These Islands have various type of lowland habitat for *Nepenthes* comprise tropical heath forest and lowland tropical rainforest. Five species of *Nepenthes* occur in this island [3]. However, mining activities are intensively occurred in this island. These activities altered habitats and threatened the biodiversity [4].

Taxonomical studies on *Nepenthes* genus in Indonesia are more likely based on the morphological characters [5, 6, 7], only a few reports presented the anatomical structures of *Nepenthes* [8, 9, 10]. The anatomical characters are not widely used in the taxonomy of *Nepenthes*, yet the discussion on the anatomical characters of *Nepenthes* is generally associated with structure development, function, and adaptation [11, 12]. This study aims

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to provide information on the comparative anatomical character of *Nepenthes*, use these characters to construct key identification to the *Nepenthes* species of Bangka Belitung Islands and add this anatomical character to the morphological description of these species.

Material and Methods

Collecting plant materials

Five *Nepenthes* species (*Nepenthes gracilis*, *Nepenthes mirabilis*, *Nepenthes ampullaria*, *Nepenthes rafflesiana*, and *Nepenthes reinwardtiana*) were obtained from lowland habitats (0-1000 masl) at Bangka Belitung Islands, Indonesian in September 2016. The plant material (leaves, pitchers and lid of the pitchers) of those species were collected and fixed in 70% alcohol for the preparation of anatomical slide in the laboratory. Samples of the pitchers were divided into upper half and lower half. So that the parts sliced are divided into eight parts namely, abaxial leaf, adaxial leaf, abaxial pitcher's lid, adaxial pitcher's lid, inner surface upper half of the pitcher, outer surface upper half of the pitcher, inner surface lower half of the pitcher, outer surface lower half of the pitcher and inner surface lower half of the pitcher. All parts replicated three times. Observations were made on five field of view in 10× and 40× magnification for each section made.

Preparing paradermal and transverse section

Samples of the plant materials were prepared for the abaxial and adaxial (leaves and pitcher's lids), outer and inner (pitchers) paradermal section by using wholemount method that has been modified [13]. The fixed samples were immersed in HNO₃ 20% solution for 25 – 40 minutes to allow the epidermal layer to be easily released from the mesophyll tissues. A layer of the epidermis scraped using a razor blade. Furthermore, the epidermal layer was immersed in chlorox to clear off any remaining dirt or tissue. The epidermal layer was placed on a petri dish and stained with 1% safranin for 2 – 4 minutes, then the epidermal layer was washed with aquades, then placed on a glass object, added with 1 to 2 drops of 30% glycerin and covered with a glass cover.

The transverse section was made by following the frozen microtome methods [13]. The fresh samples were cut to 0.5 × 0.5 cm then fixed in FAA solution for 24 hours. The fixed samples

were cut with the Yamato RV-240 frozen microtome for obtaining a thickness of 18 to 20 μm. The sliced samples were subsequently soaked in water to separate the transverse section and then stained with 0.5% safranin solution for ± 1 minute. The stained samples then washed with water and placed on a glass object and glued with a drop of 30% glycerin and covered with a glass cover.

Characters observation

Microscope slides of paradermal and transverse section of leaves, pitcher, and lid of the pitchers were observed under compound microscope Olympus CX21 with 10 × 10 and 10 × 40 magnification. Photographs of the anatomical structure were taken using Optilab Viewer® v.2.1. The characters of epidermal cells, stomata, and trichome were observed at the abaxial and adaxial of leaves and lids as well as the outer surface of pitchers; the epidermal cells, lunate cells, nectar glands and digestive glands were observed at the inner pitchers. The structure and characters of epidermis, hypodermis, mesophyll, and others were surveyed at transverse section of leaves, pitchers and the lids

Results and Discussion

Paradermal structures

The leaves of five *Nepenthes* species collected from Bangka Belitung Islands have polygonal epidermal cells with either straight or sinuous walls (Figure 1). The cells shape of epidermal leaves was previously considered as a good diagnostic character for identifying species [14]. It is applicable in some case such as *Pandanus* [15]. In the case of *Nepenthes*, this genus could be described as having the polygonal epidermal cells with either straight or sinuous walls. However, this character is not specific for the genus *Nepenthes*. Many other plants, such as *Solanum* [16], have similar characters to the epidermal cells of *Nepenthes* leaves. Therefore, this anatomical character cannot be used alone as a diagnostic character of this taxon, but it can be used for supporting the morphological diagnostic characters.

The character of epidermal cell leaves that could be used for identifying the *Nepenthes* species of Bangka Belitung is whether the character of epidermal cell walls at adaxial surface of the leaves is similar or different from that at abaxial surface of the leaves. The *Nepenthes* species col-

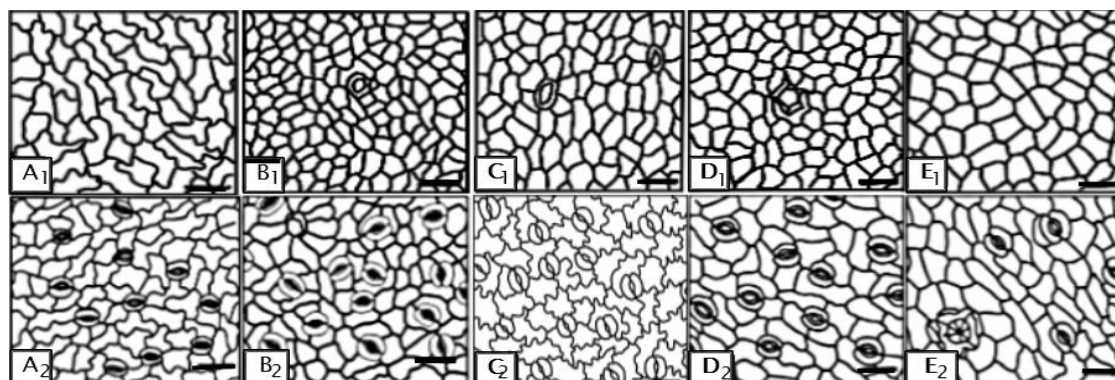


Figure 1. Paradermal section of adaxial (top row) and abaxial (bottom row) leaves of five species *Nepenthes* of Bangka Belitung (Note: *N. gracilis* (A), *N. mirabilis* (B), *N. ampullaria* (C), *N. rafflesiana* (D) and *N. reinwardtiana* (E) (scale bar = 50 µm))

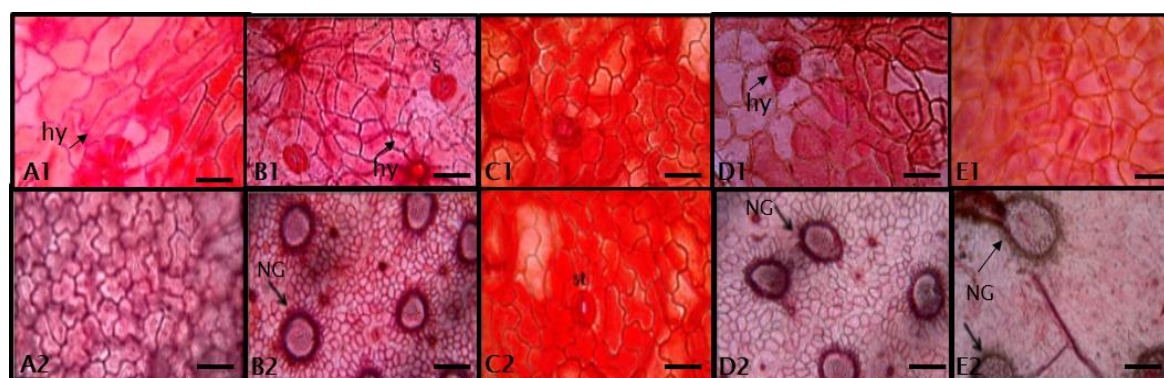


Figure 2. Paradermal section of adaxial (top rows) and abaxial (bottom rows) pitcher lid of five *Nepenthes* species of Bangka Belitung. (Note: *N. gracilis* (A), *N. mirabilis* (B), *N. ampullaria* (C), *N. rafflesiana* (D), *N. reinwardtiana* (E). hy: hydathode; NG: nectary gland; st: stoma. Scale bar abaxial (A1-E1) = 100 µm; adaxial (A2-E2) = 30 µm)

Table 1. Average length of the leaf epidermal cells of five species *Nepenthes* from Bangka Belitung Islands

Species	The Average Length of Epidermal Cells	
	Adaxial (µm)	Abaxial (µm)
<i>N. gracilis</i>	44 ± 5.47	43 ± 3.77
<i>N. mirabilis</i>	23 ± 4.10	38 ± 4.24
<i>N. ampullaria</i>	25 ± 6.27	27 ± 3.26
<i>N. rafflesiana</i>	36 ± 8.21	42 ± 4.10
<i>N. reinwardtiana</i>	46 ± 3.79	47 ± 3.27

lected from Bangka Belitung Islands have similar type of epidermal cells in both adaxial and abaxial side of the leaves, except for *N. ampullaria*, which has epidermal cells with a sinuous wall on the abaxial and straight wall on the adaxial leaf surface (Figure 1). However, this case is also found in the leaves of *Nepenthes bicalcarata* from West Borneo, which also have epidermal cells with a straight wall in the adaxial leaf and sinuous wall in the abaxial side of the leaf [17]. The species *N. gracilis* has a slightly sinuous wall in the epider-

mal cells in both abaxial and adaxial surface of the leaves; the other three species have a straight wall of epidermal cell in both abaxial and adaxial surface of the leaves (Figure 1).

The paradermal leaf section of five *Nepenthes* species of Bangka Belitung cannot be separated on the basis of the average length of epidermal cells since it is a continue character. Although the adaxial epidermal cells of *N. mirabilis* are shorter compare with those of *N. reinwardtiana* and *N. gracilis*, they are not significantly distinct from those

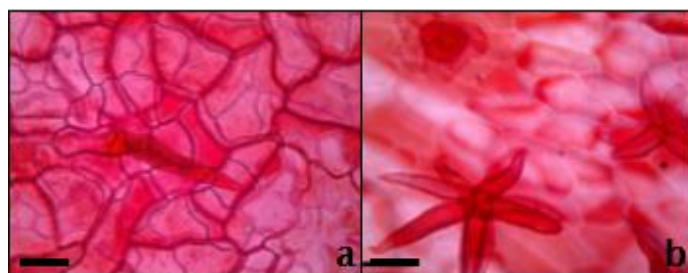


Figure 3. Glandular trichome (A) and Non-glandular trichome (B) (scale bar = 50 µm)

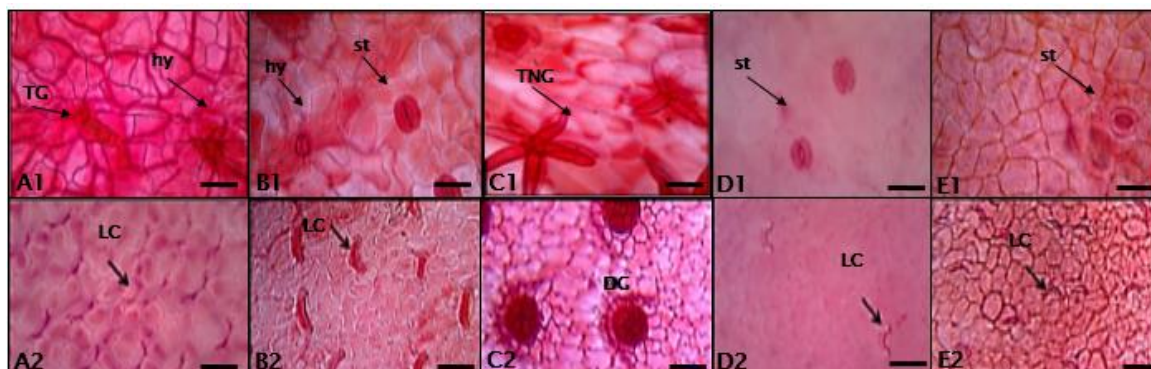


Figure 4. Paradermal sections of the outer (top rows) and inner (bottom rows) surface of upper half pitchers of five *Nepenthes* species of Bangka Belitung. (Note: *N. gracilis* (A), *N. mirabilis* (B), *N. ampullaria* (C), *N. rafflesiana* (D), *N. reinwardtiana* (E). DG: digestive gland; Hy: hydatoda; LC: lunate cell; st: stoma; TG: glandular trichome; TNG: non glandular trichome (Scale bar = 40 µm))

of *N. ampullaria* and *N. rafflesiana* (Table 1). The epidermal cells of abaxial surface tends to be longer than those of the adaxial surface (Table 1), this is similar to the case of *Nepenthes* species found in West Borneo (*N. veitchii*, *N. bicalcarata*, *N. clipeata*, *N. neglecta*, and *N. hirsuta*) [17].

A spherical channel-like structure of hydathodes are sparsely distributed on the epidermis of adaxial leaves (Figure 2). This structure is present not only in leaves but also on the epidermal section of the outer surface of the pitchers and stems [14]. Hydathode is a type of pore, that secrete water or salt out of the leaf through an active process [18].

Stomata are absent on the adaxial leaf surfaces of five *Nepenthes* species from Bangka Belitung. However, anomostic stomata that have subsidiary cells similar to the epidermal cells are found on abaxial leaf surfaces of those species (Figure 1). *Nepenthes* do not specifically have anomostic stomata, another stomata type occur in this genus. The species of *N. bicalcarata* has actinocytic stomata, the guard cells are surrounded by a circle of radiating subsidiary cells [17]. Multicellular non-glandular and multicellular glandular trichomes

are found on both abaxial and adaxial leaf surfaces of the *Nepenthes* species from Bangka Belitung Islands (Table 2). The glandular trichomes are uniseriate, while the non-glandular trichomes are stellated (Figure 3).

The epidermal characters of adaxial pitcher lid surfaces are similar to those of the adaxial leaf surface, in terms the shape of epidermal cells, type of stomata, hydathodes, and trichomes. The epidermal cells of the abaxial lid surface are similar to that of the adaxial lid surface is found in *N. gracilis* and *N. ampullaria*. However, the epidermis of abaxial pitcher lid surface of the three others *Nepenthes* differs from their adaxial lid surface. It consists of nectary glands and smaller polygonal cells (Figure 2). This extra floral nectary glands of *Nepenthes* are oval and consist of many well-ordered cells. The largest nectary glands among the *Nepenthes* of Bangka-Belitung was found at the lid of *N. rafflesiana*, while those the smallest occurred at the lid of *N. mirabilis* (Table 3). Nectary glands produce nectar used to attract prey such as invertebrate, insect and arthropod [20]. The nectary glands have a solid cytoplasm and a small va-

Table 2. Comparison of anatomical structures occur on the epidermal layer of leaf, pitcher, and lid among the five species of *Nepenthes* of Bangka Belitung.

Organ	Surface	Structure	<i>N.</i>				
			<i>gracilis</i>	<i>mirabilis</i>	<i>ampullaria</i>	<i>rafflesiana</i>	<i>reinwardtia</i>
Leaf	Adaxial	1. Trichomes	Absent	Absent	Absent	Absent	Absent
		2. Hydathodes	Present	Present	Present	Present	Present
	Abaxial	3. Stomata	Present	Present	Present	Present	Present
Pitcher lid	Adaxial	4. Trichomes	Absent	Absent	Absent	Absent	Absent
		5. Hydathodes	Present	Present	Present	Present	Present
	Abaxial	6. Nectary glands	Absent	Present	Absent	Present	Present
Pitcher	Outer	7. Trichomes	GT	NGT	NGT	NGT	Absent
		8. Hydathodes	Present	Present	Present	Present	Present
	Inner	9. Lunate cells	Present	Present	Absent	Present	Present
		10. Digestive glands	Present	Present	Present	Present	Present

Abbreviation: NGT = Non glandular trichome, GT= Glandular trichome

Tabel 3. Average length and density of nectary glands on the abaxial lid surfaces of five *Nepenthes* species of Bangka Belitung.

Species	Length (μm)	Density per mm^2
<i>N. gracilis</i>	-	-
<i>N. mirabilis</i>	123.2 ± 6.7	18.2 ± 0.7
<i>N. ampullaria</i>	-	-
<i>N. rafflesiana</i>	198.3 ± 3.4	3.10 ± 0.3
<i>N. reinwardtiana</i>	138.0 ± 9.5	8.50 ± 0.5

cuole containing tannin [21].

Nectary glands are absent at the lids of *N. gracilis* and *N. mirabilis*. It was reported that the extra floral nectary glands may occurs on the other part of pitchers, such as it is found in peristome of *N. fusca* pitchers [19]. The pitchers of *N. fusca* morphologically resemble those of *N. gracilis*.

The paradermal section of the outer surface of both the upper and lower half part of pitchers are homogenous to those of the adaxial surface of the leaves and pitcher lids. Anomositic stomata, uniseriate glandular or stellate non-glandular trichomes, and hydathodes are present among the polygonal epidermal cells. The pitchers and its lids of *Nepenthes* are modified leaves, thereby the anatomical structures of the outer surface of pitchers, the adaxial surface of lids and leaves are similar [9].

The paradermal structure of inner surface of upper half pitchers are composed of small polygonal cells and semicircular sickle (lunate) cells. Such a structure is found in *N. gracilis*, *N. mirabilis*, *N. rafflesiana*, and *N. reinwardtiana* but absence in *N. ampullaria* (Figure 4). A report on

pitcher plants stated that the presence of lunate cell related to the presence of crystalline wax at the inner surface of its pitchers; therefore, it is called waxy zone. The lunate cell has a function in denying traction to the claws of invertebrates travelling in an upward direction forming a smooth and wavy surface of the pitcher [22]. The waxy zone serves as a trap for prey since the adhesive waxy surface prevent the prey from escaping [19].

The lunate cell at inner surface of upper half pitchers were found at the species that have elongated cylindrical pitchers. These pitchers can be divided clearly into waxy and digestive zones (Figure 5). Despite that the lunate cells at the inner pitcher surface of *N. ampullaria* is absent, this inner surface is composed by epidermal cells and the digestive glands. This species has a short cup-shape pitcher that cannot be morphologically divided into two different zone. This absence of waxy zone in the pitcher of *N. ampullaria* is in line with other report [23].

The presence or absence of the lunate cell is a distinct character that can be used in distinguishing a species in the genus *Nepenthes*. The platelet-

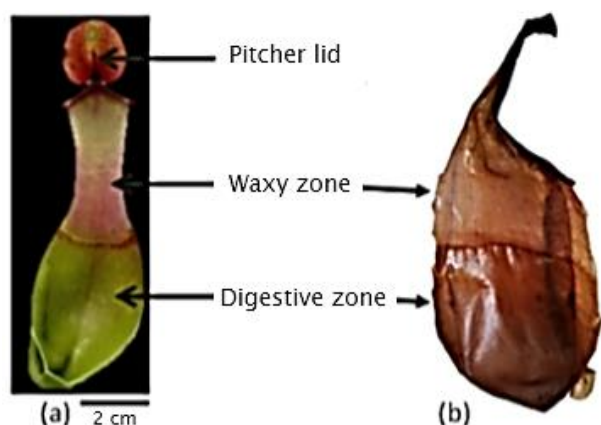


Figure 5. The pitcher of *Nepenthes* comprises of waxy zone at the upper half and digestive zone at lower half. (Note: *N. gracilis* (A) and *N. rafflesiana* (B))

Table 4. Average length and density of lunate cells on the inner surface of upper half pitchers of five species *Nepenthes* species of Bangka Belitung

Species	Length (μm)	Density (per mm^2)
<i>N. gracilis</i>	52.7 ± 2.0	49.2 ± 0.5
<i>N. mirabilis</i>	42.7 ± 2.0	28.6 ± 2.0
<i>N. ampullaria</i>	-	-
<i>N. rafflesiana</i>	38.0 ± 5.7	5.0 ± 0.0
<i>N. reinwardtiana</i>	59.0 ± 3.2	9.8 ± 0.9

Table 5. Average length and density of digestive glands at the inner surface of lower half pitchers of five species *Nepenthes* species of Bangka Belitung.

Species	Length (μm)	Density (per mm^2)
<i>N. gracilis</i>	193.5 ± 1.8	6.8 ± 0.9
<i>N. mirabilis</i>	27.0 ± 1.7	5.6 ± 2.1
<i>N. ampullaria</i>	73.7 ± 3.3	60.8 ± 2.6
<i>N. rafflesiana</i>	55.3 ± 2.8	96.3 ± 0.6
<i>N. reinwardtiana</i>	56.3 ± 3.5	24.6 ± 0.6

Table 6. Leaf thickness of the five *Nepenthes* species of Bangka Belitung

Species	Thickness (μm)
<i>N. gracilis</i>	323.64 ± 3.26
<i>N. mirabilis</i>	234.51 ± 1.27
<i>N. ampullaria</i>	212.68 ± 0.24
<i>N. rafflesiana</i>	249.37 ± 2.30
<i>N. reinwardtiana</i>	412.23 ± 2.40

shape of lunate cells is not various among the species of *Nepenthes* [19, 24, 25, 26]. However, the size and density of lunate cells are various among five *Nepenthes* species from Bangka Belitung. The species *N. reinwardtiana* has the largest size ($59.0 \pm 3.2 \mu\text{m}$) but the lowest density (9.8 per mm^2) of lunate cell. The smallest ($38.0 \pm 5.7 \mu\text{m}$) lunate cells are found in *N. rafflesiana* and the highest density (49.2 per mm^2) are found in *N. gracilis* (Table 4).

The paradermal structure of the inner lower half pitchers which comprise small polygonal cells and digestive glands were observed in the five *Nepenthes* species from Bangka Belitung (Figure 6). These digestive glands are multicellular, either rounded (*N. gracilis*, *N. ampullaria*, and *N. mirabilis*) or oval (*N. reinwardtiana* and *N. rafflesiana*), consist of dense cells (Figure 6). The largest size ($55.3 \pm 2.8 \mu\text{m}$) and the highest density of digestive glands (96.3 per mm^2) are showed in *N. rafflesiana* (Table 5). This gland secretes digestive enzymes to degrade prey so that the nutrients can be absorbed [27].

Transverse structures

The structure of leaf transverse section composes of one layer of epidermal cells with a thin cuticle at the outer layer (Figure 7). The thickness of cuticle is diverse among the species of *Nepenthes* and is influenced by the environment [17]. The cuticle on *Nepenthes* is a waxy structure that has a function in reducing evaporation. There are 1 – 2 layers of hypodermis inside the epidermal layer (Figure 7G). This character is common in other *Nepenthes* species [12]. The hypodermal cells are larger than epidermal cells. The leaves of *Nepenthes* are bifacial, which is characterized by the mesophyll is differentiated into palisade parenchyma at the top layer and sponge parenchyma at the bottom layer. The thickness leaves of *Nepenthes* are varying among the species observed. It is probably affected by the environmental factor. Based on the field observation (Table 6), the species which is growing in higher elevation and drier habitat, for example *N. reinwardtiana*, have thicker leaves than those growing at the swamp habitat in lower elevation. The species *N. gracilis* have the intermediate thickness compare to *N. reinwardtiana* and *N. mirabilis*. This species is commonly found in many lowland habitats in

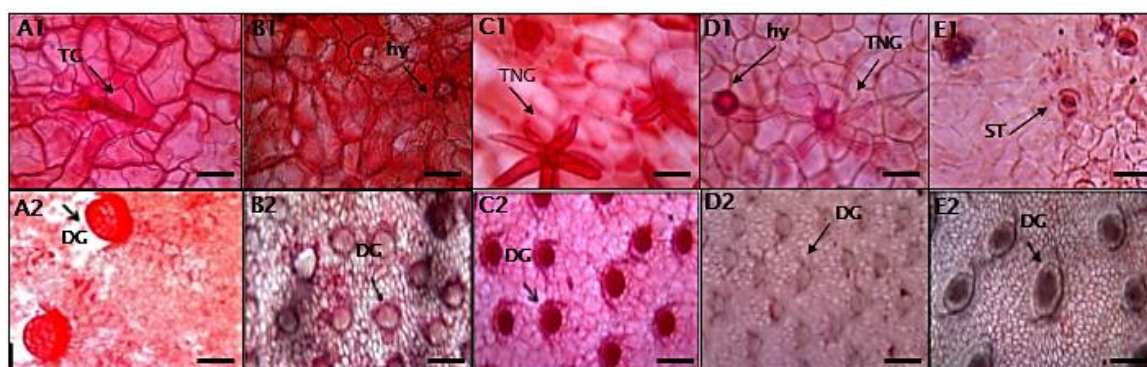


Figure 6. Paradermal section of outer (top rows) and inner (bottom rows) surfaces lower half pitcher of five *Nepenthes* species of Bangka Belitung. (Note: *N. gracilis* (A), *N. mirabilis* (B), *N. ampullaria* (C), *N. rafflesiana* (D), *N. reinwardtiana* (E) DG: digestive gland; hy: hydatoda; st: stoma; TG: uniselular trichome; TNG: non glandular trichome (Scale bar =50 μm))

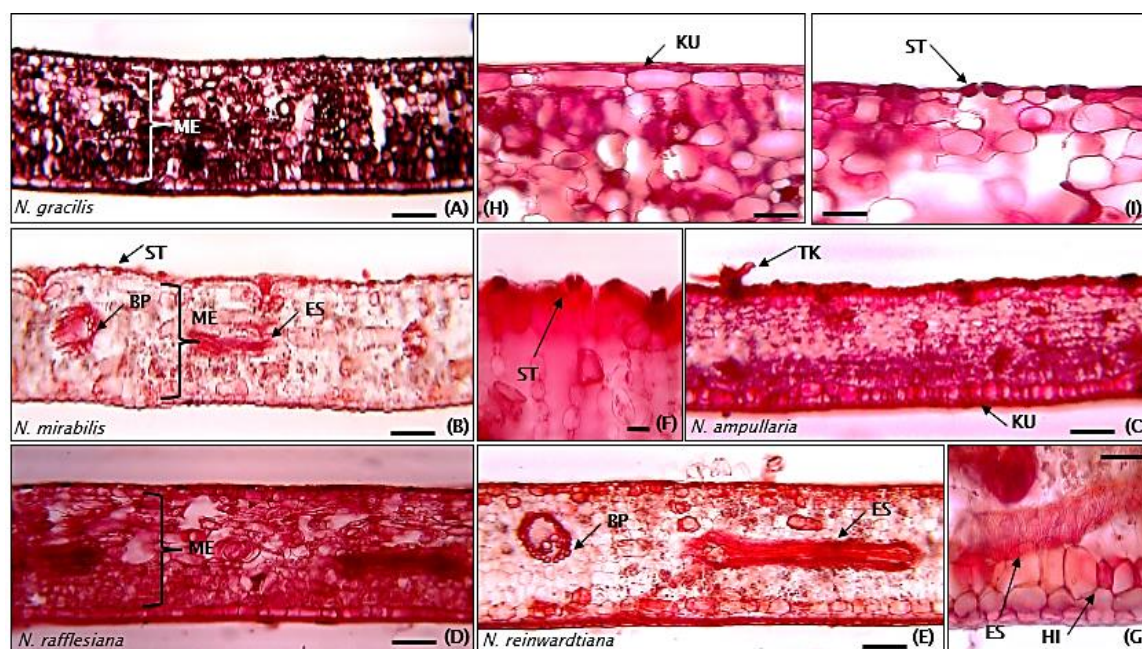


Figure 7. The leaf transverse sections of five *Nepenthes* species. (Note: *N. gracilis* (A, H, I); *N. mirabilis* (B, F); *N. ampullaria* (C); *N. rafflesiana* (D); *N. reinwardtiana* (E, G); *N. ampullaria* (F). BP: vascular bundle; ES: spiral element; HI: hydathode; KU: cuticle; ME: mesophyll; ST: stoma; TK: non-glandular trichome. Scale bar (A-E) = 100 μm; (F-I) = 30 μm))

Bangka Belitung such as heath forest and secondary forest.

The stomata observed at the leaf transverse section did not showed variation among the observed species (*N. gracilis*, *N. ampullaria*, *N. mirabilis*, *N. rafflesiana*, and *N. reinwardtiana*), the stomata are protruding above the epidermal cell layer (Figure 7F). Although the stomata that sunken below the surface of epidermis may occur among the *Nepenthes* [17], this type of stomata was not found in the five species of *Nepenthes* ob-

served.

The transverse sections of pitcher lids are different with those of leaves in term of the mesophyll are not differentiated into sponge and palisade tissue, and the presence of nectary glands. These glands were observed at the epidermal cell of abaxial surface of the three *Nepenthes* species of Bangka Belitung and absent at the two others (Figure 8). The nectary glands, which could be found at the pitchers and lids, are an extra-floral nectary derived from the development of epider-

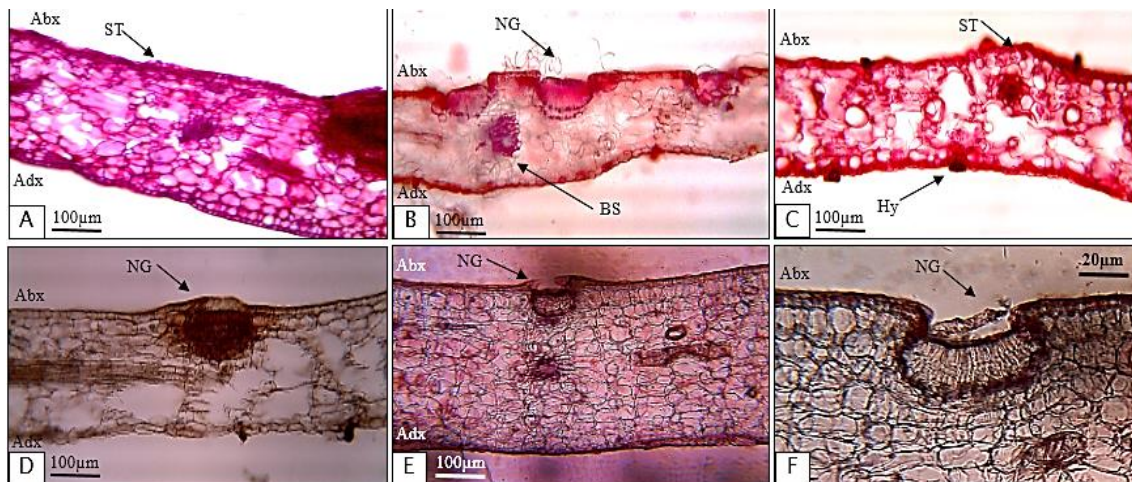


Figure 8. The transverse sections of pitcher lid from five species *Nepenthes* of Bangka Belitung. (Note: *N. gracilis* (A); *N. mirabilis* (B); *N. ampullaria* (C); *N. rafflesiana* (D); *N. reinwardtiana* (E); Nectary gland structure in *N. reinwardtiana* (F). Abx: Abaxial surface; Adx: Adaxial surface; BS: vascular bundle; HY: hydathode; NG: nectary gland; ST: stoma))

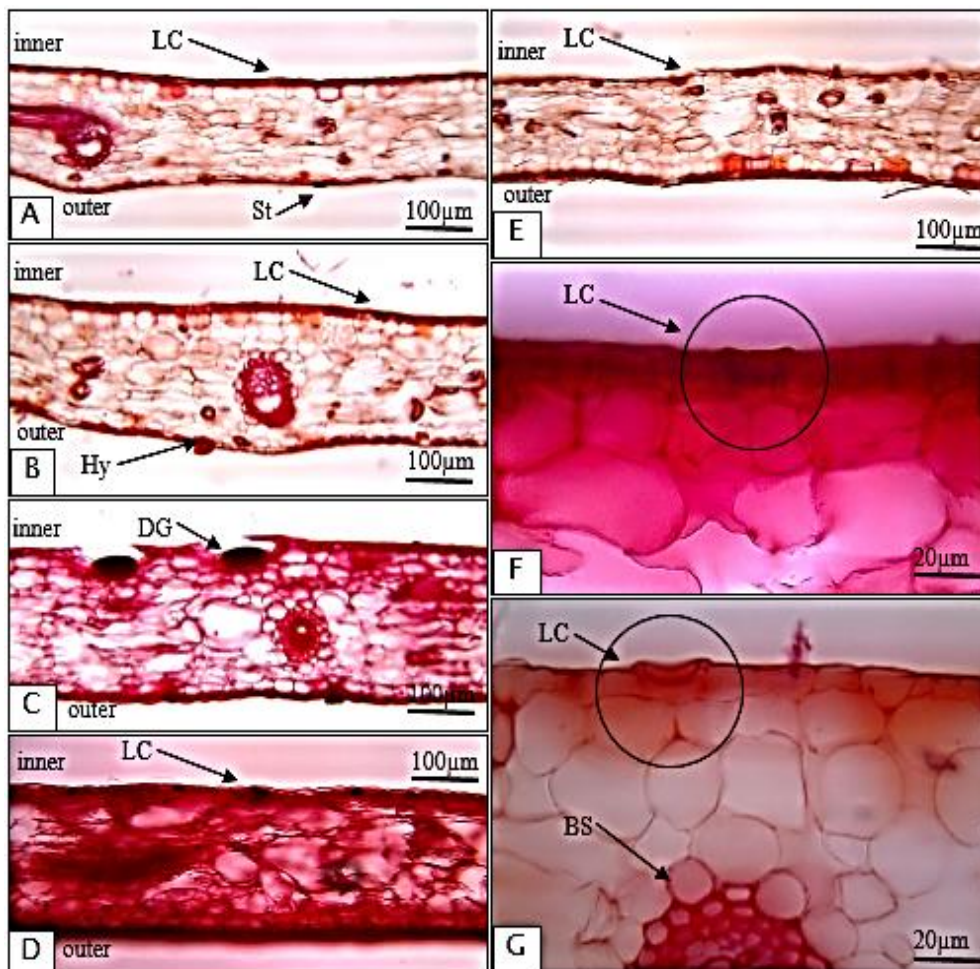


Figure 9. The transverse section of upper half pitcher (waxy zone) of five species *Nepenthes* of Bangka Belitung. (Note: *N. gracilis* (A); *N. mirabilis* (B); *N. ampullaria* (C); *N. rafflesiana* (D, F); *N. reinwardtiana* (E); Lunate cell as it is appeared in *N. rafflesiana* (F, G). BS: vascular bundle; DG: digestive gland; HY: hydathode; LC: lunate cell; ST: stoma))

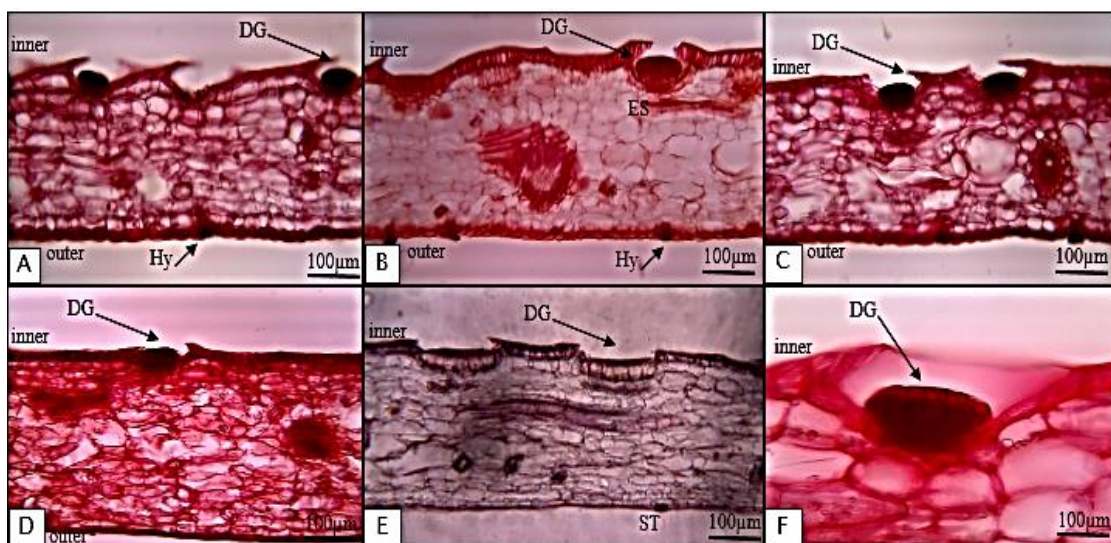


Figure 10. The transverse sections of lower half (digestive zone) pitcher of five species *Nepenthes* species of Bangka Belitung. (Note: *N. gracilis* (A); *N. mirabilis* (B); *N. ampullaria* (C); *N. rafflesiana* (D); *N. reinwardtiana* (E); *N. ampullaria* (F). DG: digestive glands; HY: hydatode; ST: stomata)

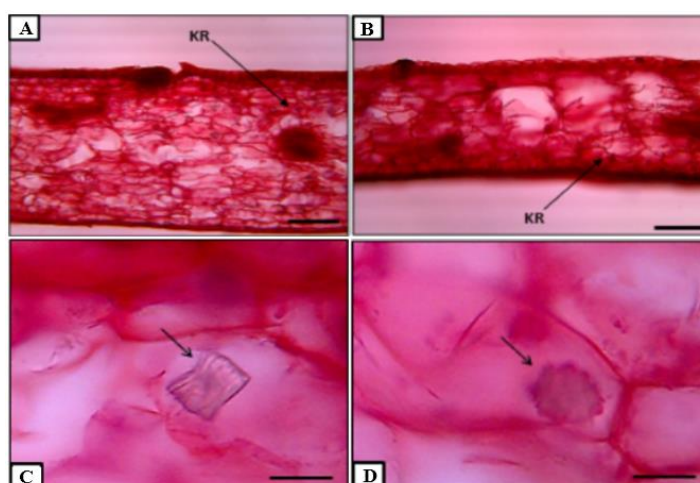


Figure 11. Transverse sections of the pitcher of *N. rafflesiana* showing crystal structures. (Note: Digestive zone (scale bar = 100 µm) (A); waxy zone (scale bar = 100 µm) (B); a prism crystal (scale bar = 30 µm) (C); a druse crystal (scale bar = 30 µm) (D). KR: Crystal)

mal cells [20]. The nectary glands often abundantly occur at peristome, which is a collar-shaped structure surrounding and overhanging at the mouth of the pitchers [20].

The structure of pitcher transverse section is cuticle layer at the inner and outer surface, inner and outer epidermis layer and undifferentiated mesophyll layer (Figure 9 and Figure 10). This mesophyll is lacking of air spaces. The inner surface of the upper half pitcher lined with either secretory or digestive glands (Figure 9). The secretory gland showed at transverse section of upper half pitcher are lunate cells (a stomata-like struc-

ture with a single guard cell) (Figure 9). The lunate cells are coated by a thick layer of epicuticular wax forming loose scales [9]. The digestive glands are noticeable as an ovoid-spherical structure of three cell layers protected by hooded ridges. This hooded ridge comprises a modified epidermal cell extending over at least part of the depression [28]. The first layer is called the glandular head, consist of multicellular masses of tissue with the outermost layer of cells are columnar [14, 9]. The second and the third layers are thinner than the first one and have various shape. The digestive glands that immersed in cavity were distributed on the

lower half pitcher of all the observed species (Figure 10).

Spiral elements were observed at the transverse section of leaves, pitchers, and pitcher's lids (Figure 7, 8, 9, 10). This structure is common for *Nepenthes* [14]. It was noticed that the spiral elements were frequently found between digestive glands and seem to connect them.

Prism and druse crystal structures scattered in the hypodermic layer, mesophyll tissue and vascular bundle were found at the pitcher transverse sections of *N. rafflesiana* (Figure 11). Such a crystal structure is absent at the other four *Nepenthes* species. However, stellate crystals were reported found throughout the pitcher of *N. alata* [9]. Presumably, the crystal found in *Nepenthes* is calcium oxalate. The crystals of plants are commonly found in idioblast cells, but can also be stored in the epidermal cell, palisade, and vascular bundle [29]. It was reported that the presence and shape of crystal are determined by genetic factors, this character constant for a species [30] and is a valuable taxonomical character [31]. This character has been reported to be used to distinguish groups of monocots at the family level [32].

The character used in separating the *Nepenthes* species of Bangka Belitung

Five species of *Nepenthes* from Bangka Belitung islands could be identified based on the anatomical characters of leaf epidermal cell, the presence of nectary glands at the abaxial surface of pitcher lid, the presence of crystal at the parenchymal tissue of pitcher transverse section, and the length of lunate cells (compared with the epidermal cells) at the inner surface of the upper half pitchers. These first three anatomical character is a good character since it is fixed and genetically inherited. However, the length of lunate cell is less valuable because it is probably various and affected by environment or growth stages.

The identification key for five species *Nepenthes* from Bangka Belitung Islands based on anatomical characters:

1. a. Nectar glands are absent in the abaxial of pitcher lid 2
- b. Nectar glands are present in the abaxial of pitcher lid 3
2. a. The anticlinal wall of leaf epidermal cell is sinuous *N. gracilis*

- b. The anticlinal wall of leaf epidermal cells is straight *N. ampullaria*
3. a. Prism and druse crystals occur in the cells distribute at parenchyma tissue of pitcher transverse section *N. rafflesiana*
- b. Crystals are not found at the pitcher transverse section 4
4. a. The lunate cells (observed at paradermal section) are shorter than the epidermal cells *N. mirabilis*
- b. The lunate cells are longer than the epidermal cells *N. reinwardtiana*

Conclusion

The anatomical characters of leaf, pitcher, and pitchers' lid could be used for differentiating the five species of *Nepenthes* from Bangka Belitung Islands. The presence of crystal at the parenchymal tissue of pitcher differentiate the species *N. rafflesiana* from the four others. The absence of nectary gland at the abaxial surface of pitcher lids of the species *N. gracilis* and *N. ampullaria* separate them from the others species. The leaf epidermis of these two species have different cell wall. The length of lunate cells at the inner surface of upper half pitchers are comparable for identifying the pitchers of *N. mirabilis* from those of *N. reinwardtiana*. For the conclusion, in identifying the *Nepenthes*, the anatomical characters could be used for supporting the morphological character.

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References

1. Mansur M (2013) Tinjauan tentang *Nepenthes* (Nepenthaceae) di Indonesia. *Berita Biologi* 12 (1): 1 – 7.
2. CITES (2017) Convention on international trade in endangered species of wild fauna and flora: *Nepenthes*. <https://www.cites.org/eng/search/site/nepenthes>. Accessed date: 1 October 2017.

3. Hidayat S, Hidayat J, Hamzah et al. (2003) Vegetation analysis of two insectivorous plants in Padang Pinang Anyang, Belitung Island. *Biodiversitas* 4 (2): 93 – 96. doi: 10.13057/biodiv/d040205.
4. Latifah S (2003) Kegiatan reklamasi lahan pada lahan bekas tambang. <http://library.usu.ac.id/>. Accessed date: September 2018.
5. Cheek M, Jebb M (2013) *Nepenthes ultra* (Nepenthaceae), a new species from Luzon, Phillipines. *Blumea* 58 (3): 241 – 244. doi: 10.3767/000651913X675124.
6. Gronemeyer T, Coritico F, Wistuba A et al. (2014) Four New Species of *Nepenthes* L. (Nepenthaceae) from the Central Mountains of Minadano, Phillipines. *Plants* 3 (2): 284 – 303. doi: 10.3390/plants3020284.
7. Gronemeyer T, Suarez W, Nuytemans H et al. (2016) Two New *Nepenthes* species from the Philippines and an amended description of *Nepenthes ramos*. *Plants* 5 (2): 23 – 37.
8. Gaume L, Gorb S, Rowe N (2002) Function of epidermal surfaces in the trapping efficiency of *Nepenthes alata* pitchers. *The New Phytologist* 156 (3): 479 – 489. doi: 10.1046/j.1469-8137.2002.00530.x.
9. Owen PT, Lennon KA (1999) Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (Nepenthaceae). *American Journal of Botany* 86 (10): 1382 – 1390.
10. Arimy NQ, Nisyawati, Metusala D (2017) Comparison of leaf anatomy on some *Nepenthes* spp. (Nepenthaceae) from highland and lowland habitat in Indonesia. In AIP Conference Proceedings 1862 of International Symposium on Current Progress in Mathematics and Sciences: 1- 2 November 2016; Depok. Edited by: Mart T, Triyono D, Sugeng KA. doi: 10.1063/1.4991215.
11. Gaume L, Di Giusto B (2009) Adaptive significance and ontogenetic variability of the waxy zone in *Nepenthes rafflesiana*. *Annals of Botany* 104 (7): 1281 – 1291. doi: 10.1093/aob/mcp238.
12. Osunkoya OO, Muntassir NA (2017) Comparative anatomy of the assimilatory organs of *Nepenthes* species. *Australian Journal of Botany* 65 (1): 67 – 79. doi: 10.1071/BT16157.
13. Sass JE (1951) *Botanical microtechnique* 2nd edition. Iowa, Iowa State College Press.
14. Metcalfe CR, Chalk L (1950) *Anatomy of Dicotyledons: Leaves, stem, and wood in relation to taxonomy with notes on economic uses (Nepenthaceae) Volume III*. London, Oxford at the Clarendon Press.
15. Stone BC (1977) The Morphology and systematics of Pandanus today (Pandanaeae). *Gardens Bulletin* 29 (4): 137 – 142.
16. Nurit-Silva K, Costa-Silva R, Basilio IJL D, de Fatima Agra M (2012) Leaf epidermal characters of Brazilian species of *Solanum* section *Torya* as taxonomic evidence. *Botany* 90 (9): 806 – 814. doi: 10.1139/b2012-046.
17. Damayanti F, Roostika I, Mansur M (2015) Kajian morfologi, sitologi dan struktur anatomi daun *Nepenthes* spp. asal Kalimantan Barat. *Bioedukasi: Jurnal Pendidikan Biologi* 8 (2): 5 – 11. doi: 10.20961/bioedukasi-uns.v8i2.3862.
18. Chen CC, Chen YR (2005) Study on laminar hydrotodes of *Ficus formosana* (Moraceae) II. *Botanical Bulletin – Academia Sinica Taipei* 46: 205 – 215.
19. Benz MJ, Gorb EV, Gorb SN (2012) Diversity of the slippery zone microstructure in pitchers of nine carnivorous *Nepenthes* taxa. *Arthropod-Plant Interactions* 6 (1): 147 – 158. doi: 10.1007/s11829-011-9171-2.
20. Moran JA, Clarke CM (2010) The carnivorous syndrome in *Nepenthes* pitcher plants: Current state of knowledge and potential future directions. *Plant signaling and Behavior* 5 (6): 644 – 648. doi: 10.4161/psb.5.6.11238.
21. Sutriani Y (1992) *Pengantar anatomi tumbuh-tumbuhan*. Jakarta, Rineka Cipta.
22. Moran JA, Clarke CM, Hawkins BJ (2003) From carnivore to detritivore? Isotopic evidence for leaf litter utilization by the tropical pitcher plant *Nepenthes ampullaria*. *International Journal of Plant Science* 164 (4): 635 – 639. doi: 10.1086/375422.
23. Adamec L (1997) Mineral nutrition of carnivorous plants: A review. *Botanical Review* 63 (3): 273 – 299. doi: 10.1007/2FBF02857953.
24. Wang LX, Zhou Q (2011) Friction force of locust *Locusta migratoria manilensis* (orthoptera, Locustidae) on slippery zones surface of pitchers from four *Nepenthes* species. *Tribology Letters* 44: 345 – 353. doi: 10.1007/2Fs11249-011-9858-7.
25. Riedel M, Eichner A, Meimberg H, Jetter R (2007) Chemical composition of epicuticular wax crystals on the slippery zones in pitcher of five *Nepenthes* and hybrids. *Planta* 225 (6): 1517 – 1534. doi: 10.1007/2Fs00425-006-0437-3.
26. Haberlant G (1914) *Physiological plant anatomy*. Macmillan and Company, London.
27. Fahh A (1979) *Secretory tissues in plants*. New York, Academic Press.
28. Gorb E, Kastner V, Peressadko A et al. (2004) Structure and properties of the glandular surface in the digestive zone of the pitcher in the carnivorous plant *Nepenthes ventrata* and its role in insect trapping and retention. *The Journal of Experimental Biology* 207: 2947 – 2963. doi: 10.1242/jeb.01128.
29. Faheed F, Mazen A, Abd Elmohsen S (2013) Physiological and ultrastructural studies on calcium oxalate crystal formation in some plants. *Turkish Journal of Botany* 37: 139 – 152. doi: 10.3906/bot-1112-19.
30. Fritsch FE (1903) The use of anatomical characters for systematic purpose. *New Phytologist Journal* 2 (8): 177 – 184. doi: 10.1111/j.1469-8137.1903.tb04972.x.
31. Prychid CJ, Rudall PJ (1999) Calcium oxalate crystals in Monocotyledons: A review of their structure and systematics. *Annals of Botany* 84 (6): 725 – 739. doi: 10.1006/anbo.1999.0975.
32. Wang L, Zhou Q (2014) *Nepenthes* pitchers: surface structure,

physical property, anti-attachment function and potential application in mechanical controlling plaque locust. Chinese Science

Bulletin 59 (21): 2513 – 2523. doi: 10.1007/2Fs11434-014-0383-6.