Comparison of leaf anatomy on some *Nepenthes* spp. (*Nepenthaceae*) from highland and lowland habitat in Indonesia

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Comparison of Leaf Anatomy on Some *Nepenthes* spp. (*Nepenthaceae*) from Highland and Lowland Habitat in Indonesia

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Abstract. *Nepenthes* (*Nepenthaceae*) is one of the unique plants with pitcher to absorb nutritional needs. This dicotyledonous plant is able to live in the lowland and highland. The difference of their habitat may influence its anatomical structures, such in leaves. This study aimed to compare the anatomy of lowland and highland *Nepenthes* leaves. We examined *Nepenthes rafflesiana* and *N. mirabilis* from the group of lowland *Nepenthes*. We also examined *Nepenthes aristolochioides* and *N. singalana* from the group of highland *Nepenthes*. Each species was represented by three adult leaves from 1-3 individual plants. Each leaf was made transverse section by using a hand mini microtome and the paradermal section was made by leaf screaping technique. Paradermal and the transverse section were dehydrated by using graded series of alcohol. Transverse section was stained with safranin 1 % and fastgreen 1 %, while the paradermal section with safranin 1 %. Microscopic observations were performed at Bioimaging Laboratory, Universitas Indonesia, Depok using a light microscope. The results showed there are differences in the anatomy structure between these two habitats. Highland *Nepenthes* has thicker and larger hypodermis than lowland *Nepenthes.* Cuticle layer in the highland *Nepenthes* was thicker than the lowland *Nepenthes*. Nectary gland on the highland *Nepenthes* was thicker and larger than the lowland *Nepenthes*. In addition, highland *Nepenthes* has bigger and fewer stomata density than the lowland *Nepenthes.*

Keywords: mesophyll, nectary gland, altitude

INTRODUCTION

The differences in the intensity of sunlight will affect to plant anatomy and morphology. The morphology and anatomy of plants that grow naturally in the wild can be influenced by environmental factors. Different habitat will show the different natural factors, such as altitude, temperature, air humidity, soil pH, and light intensity. Differences in habitat led to anatomical differences, especially on the leaves [1-3].

At the anatomical level, the leaves from plants that grow in the middle land with a high moisture content has palisade at adaxial and spongy tissue in abaxial leaf. The composition of the mesophyll called the bifacial or dorsiventral. The plant's leaves from dry areas (xerophytic) have arrangement of mesophyll unifacial or isobilateral (isolateral). The Palisade has specialized to increase the efficiency of photosynthesis process [4]. Buisson and Lee [5] showed that *Carica papaya* leaves that were placed in areas with high light intensity have more leaf mass, more total of cell layers, and thicker mesophyll than leaves that were under the shade.

Khawaja [2] observed five of Tribus Aveneae grass that was collected from the lowland (1100 m) and highland (2300 m). They were *Agrostis pilosa, Agrostis viridis, Polypogon monspellensis, Koeeria macrantha*, and *Koeleria crisnata*. Grass that live in highland has thicker leaves, thinner layer of epidermis abaxial, larger area of buliform

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cell, larger area of vascular bundle, smaller area of metaxylem, and thicker sclerenchyme than those living in the lowland [2].

In *Nepenthes*, Paluvi [6] has conducted research on *Nepenthes gracilis* that grow naturally in different intensity of light area between shaded areas and open areas. Morphologically, *N. gracilis* on open area colored more green and leaves size wider than that grown in the shade. While anatomically, *N. gracilis* in open area has a thicker epidermis layer [6].

Nepenthes is dicotyledonous plants with a unique pitcher that is able to trap and digest insects. *Nepenthes* needs insect to fulfill the nutritional needs, especially protein due to their habitat are arid and nutrient-poor [7]. The pitcher is a modification of midrib leaves extension which form the tendrils [8].

Nepenthes spread at southern China, Southeast Asia and northern Australia. There are 82 species of *Nepenthes* in the world with 64 species live in Indonesia. Borneo Island as center of *Nepenthes* distribution has 32 species, followed by Sumatra Island in the second place has 29 species. Other species found in Sulawesi, New Guinea, Maluku and Java [9].

Currently, research on anatomy of the lowland *Nepenthes* is sufficient. Paluvi *et al.* [6] observed the anatomical structure of leaf, pitcher, and tendrils *Nepenthes gracilis* [6]. Biati [5] observed the anatomy and structure of secretory plant *Nepenthes* (*Nepenthes* spp.). Biati examined *Nepenthes* hookeriana, N. gracilis, and N. Rafflesiana as her sample [5]. Yusmad [10] observed the anatomy of leaf, tendrils and pitcher on *N. reinwardtiana* [10]. Instead, the research is still lacking performed at highland *Nepenthes*. In addition, there are no studies that show comparative anatomy between highland *Nepenthes* and lowland *Nepenthes*, especially leaf organ. This study aimed to compare the anatomical differences between highland *Nepenthes* and lowland *Nepenthes*.

MATERIALS AND METHODS

Plant material used in this study came from Borneo and Kerinci Seblat National Park, Jambi, Sumatra. Each species was represented by three adults leaves from 1-3 individual plants. Each leaf was made transverse section by using a hand mini microtome and paradermal section was made by leaf screaping technique.

Transverse and paradermal section was made at Perkembangan Tumbuhan Laboratory, Biology Department, Universitas Indonesia. Leaf transverse section was taken from center of leaf include midrib in 1x2 cm. Leaf paradermal section was taken from piece of middle lamina exclude midrib in 1x1 cm. The abaxial side was scraped to get the adaxial side, vice versa. This technique was named leaf screaping technique.

The section was inputted in the microtube with alcohol 70 %. Three days later, alcohol 70 % was substituted by alcohol 96 % to dissolve the chlorophyll. Then, alcohol 96 % was substituted by preservation solution. A day later, the section was put on the object glass and covered by cover glass.

Observation was done using microscope LEICA DM500 at Bioimaging Laboratory, Department Biology, Universitas Indonesia. Sections were observed qualitatively. Qualitative data included epidermal cell, hypodermic cell, stomata, and nectary gland.

RESULTS AND DISCUSSION

High fluctuations in average temperature and mean annual rainfall along the altitudinal gradient impact to significant variations in soil physic-chemical attributes. High elevations have less Na^+ ionic concentration and soil pH. Otherwise, it increase soil organic matter and all other ionic content, moisture content, and soil ECe. Sometimes, magnesium concentration increase because of decrease in mean temperature, soil pH, and sodium content [11].

The increase of soil organic matter due to high rainfall and decrease of soil pH and temperature affect high altitude has good decomposition mineralization of soil organic matter. Low altitude has less total nitrogen and organic matter content [3, 12]. The survival of the species in any stressful conditions may depend on intensity of sclerification, which is a prominent feature for minimizing water loss [13, 14]. In addition, sclerification can minimize root damage as one of protective features in response to environmental stresses [15].

Highland *Nepenthes* have thicker and larger hypodermis than lowland *Nepenthes*. Highland *Nepenthes* have 2--4 layers of hypodermis, while lowland *Nepenthes* have 1--2 layers of hypodermis. A form of hypodermis in lowland *Nepenthes* more cubical than highland *Nepenthes*. Cuticle in highland *Nepenthes* thicker than lowland *Nepenthes* (Fig. 1). Tanner and Kapos [16] said that altitudinal gradient may increase development of hypodermis, epidermal thickness, and cuticle deposition. Generally, leaves in highland increase their leaf thickness but decrease their

length, width, and area [17, 18]. Increase of altitude affect alteration of leaf morphological such as biomass, leaf thickness, leaf area, and density of stomata [19, 20].

Leaf blade thickness is an important feature encountering different stresses, especially due to increased storage parenchyma, and therefore, enables plants to survive for longer periods of a stressful environment. A number of studies have suggested that leaf thickness increases with altitude, while frequency of leaf and size of leaf decreases [21, 22, 23]. Fibrous and thin leaves are more capable of rolling because of intensive sclerification [24] and it protecting their stomata from directs exposure to external environments [25].

Highland and lowland Nepenthes have different form nectary gland. Nectary gland on highland Nepenthes thicker and larger than lowland Nepenthes. It looks like flower in highland Nepenthes and plus sign or circle in lowland Nepenthes (Fig. 2). The epidermal cell of highland Nepenthes has fewer angle than lowland Nepenthes. Highland Nepenthes have bigger and fewer stomata than lowland Nepenthes (Fig. 3). Sari and Susilo [26] observing

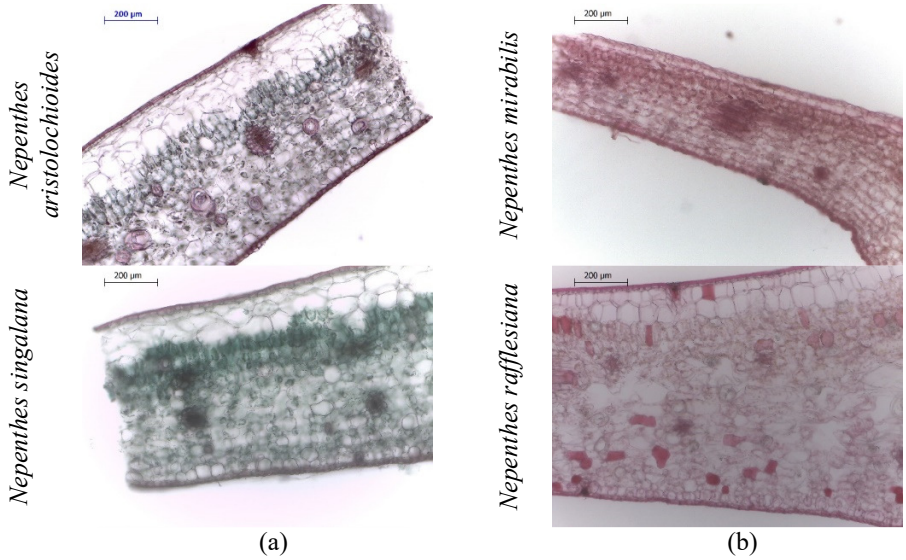

FIGURE 1. Laminal cross section (a) *Nepenthes aristolochioides* and *N. singalana* as highland *Nepenthes* and (b) *N. mirabilis* and *N. rafflesiana* as lowland *Nepenthes*

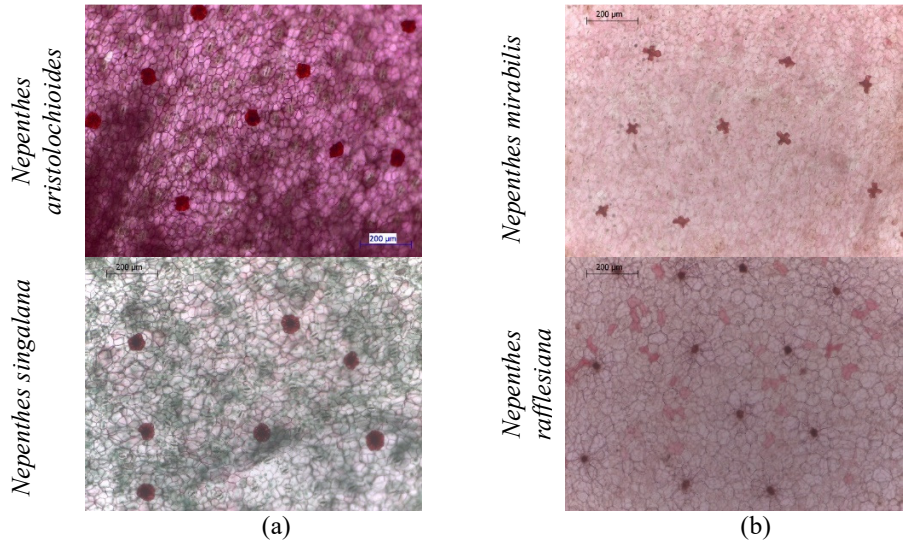

FIGURE 2. Nectary gland on abaxial leaf of (a) *Nepenthes aristolochioides* and *N. singalana* as highland *Nepenthes*and (b) *N. mirabilis* and *N. rafflesiana* as lowland *Nepenthes*

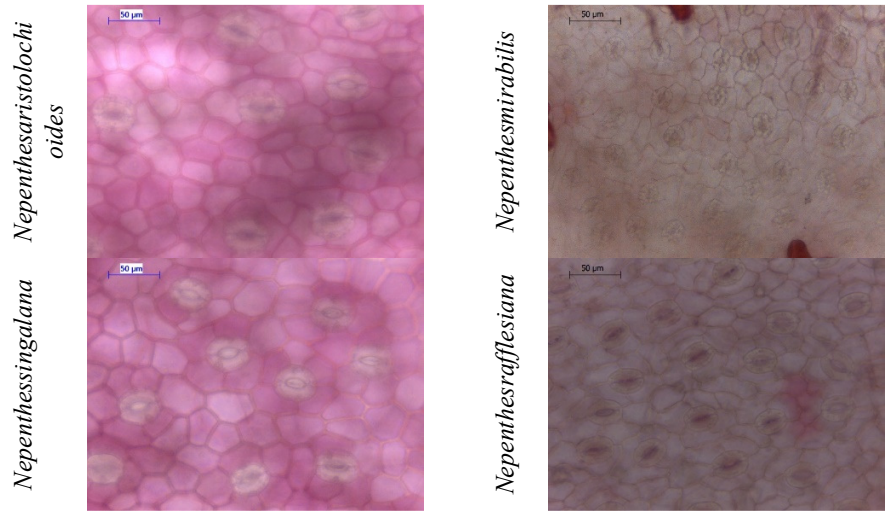

FIGURE 3. Stomata on abaxial leaf of (a) *Nepenthes aristolochioides* and *N. singalana* as highland *Nepenthes*and (b) *N. mirabilis* and *N. rafflesiana* as lowland *Nepenthes*

Theobroma cacao L. leaves in lowland area had greater amount stomata, while in highland had greater diameter and opening width of stomata [27]. Stomata have the important role to adapt to their environment. Fewer stomata could reduce the water loss from plant or evaporation. Otherwise, higher stomata could increase transpiration rate [27].

A comprehensive analysis of altitudinal effect on plants would include fall in temperature and change in radiation, pattern of rainfall, and other meteorological conditions responsible for the ecotype variability on the Borneo and Sumatera. Growth rates may decline with altitude because of reduced supply of nutrients, reduced air and soil temperatures, exposure to high wind velocity, and shorter growing seasons. Apart from the above, physiology of the plant, their response and edaphic environment can be count in important factors to diverse anatomy.

CONCLUSIONS

There were several differences on the leaves anatomy between lowland and highland *Nepenthes*. Highland *Nepenthes* showed thicker and larger hypodermis than lowland *Nepenthes.* Cuticle layer in the highland *Nepenthes* was thicker than the lowland *Nepenthes*. Nectary gland on the highland *Nepenthes* was thicker and larger than the lowland *Nepenthes*. In addition, highland *Nepenthes* has bigger and fewer stomata density than the lowland *Nepenthes.*

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