Morphological Studies on *Nepenthes* Species from Mount Pulai, Johor

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ABSTRACT

Nepenthes ampullaria, N. gracilis, N. rafflesiana and N. hookeriana were recorded in the study plots of Mount Pulai, Johore. Morphological studied showed that N. gracilis significantly different than the other species in morphological characters such as stem, leaves, leaf base, shape of upper pitcher, digestive glands on the inner pitcher wall of upper pitcher and on the inner wall of lower pitcher, type of digestive glands on the inner wall of upper and lower pitcher and peristome. Meanwhile N. ampullaria showed different characters in size of pitcher's lid, honey glands on the lower lid surface, inflorescence type, pedicel and pedicel extended by bract.

Keywords: *Nepenthes, Mount Pulai, morphological characters*

Introduction

Pitcher plants is the most extraordinary plants kingdom, for the handsome hanging pitcher which appear to drip forth on long stems from the end of leaves, are remarkable and most unleaf-like structure, and it is not surprising that the uninformed almost invariably confuse them with flowers (Slack, 1979). Nepenthes has been described in the literature as the scandent herb, undershrubs, scrambling shrubs, prostrate evergreen undershrubs, climbers, climbing vines, clambering vines, lianas to epiphytes (Adam, 1990; Clarke, 2002; Kurata, 1976, Green, 1967; Phillipps and Lamb, 1988 and 1996; Rohana Md. Som, 1988; Shivas, 1984: Symthies, 1965). Nepenthes is a terrestrial plants but some species may grow epiphytically on trees. One of the main characters of the genus is the presence of pitchers variously differ from ojne another in their leaves (whether stalked or not, shape, size, hairiness and vein), in their pitcher and in their flowers (Holttum, 1969).

Materials and Methods

Four plots each with a size of 10m x 100m were set along the slope from the foothill to near submit of Gunung Pulai. Samples of the species found had been taken for herbarium specimens. The entire morphological characteristics were being noted. Scanning Electron Microscope (SEM) were used for identification of the surface features of the lower and upper pitcher of all the species.

Results and Discussion

There are four species of Nepenthes being found in the study area. The species are *Nepenthes rafflesiana, N. ampullaria, N. gracilis* and *N. hookeriana*. Detailed morphological characteristics of all the species found recorded in Table 1. Morphological characters for all the species found were significantly different. Identification for all the species are using the key to the Nepenthes in Peninsular Malaysia by Shivas (1984) and Rohana Md. Som (1988). All the morphological characters of species found are similar and support all the description of the species reported by past researchers (Adam, 2002a; Jebb & Cheek, 1997; Kurata, 1976; Phillipps & Lamb, 1996; Rohana, 1988; Shivas, 1984).

Based on Rohana (1988), leaves attachment to the stem, wing of the petiole and peristome are the main characteristic to determine the Nepenthes species, however Adam (1990), describe that lid and glands also play an important role in the identification process. Leaves attachment to the stem are categorized into three; sessile, where the lamina is directly attached to the stem without petiole; petiole, the leaves are attached to the stem and intermediate between the above two conditions.

N. gracilis collected from the study area showed several distinctive and diagnostic characters thus differentiate itself from the other species. These diagnostic attributes includes the stem were angular to triangular in shape, leaves were distinctly sessile or without stalk, leaves bases were decurrent into to wings, the pitcher lips or peristome were very narrow <1 mm in thickness; lower lid surface scarcely covered with nectar or honey glands (Figure 1), pitchers of lower and upper pitchers were ventricose in shape on the lower half and tubulose on the upper half, inner wall surface was covered with waxy layer on the upper tubulose part and digestive glands on the lower ventricose part. This type of pitchers were called wholly glandular pitcher (Adam et al., 2005; Danser, 1928; Kurata, 1976). Scanning Electron Microscope photograph showed the waxy layer with numerous deformed stomata (Figure 2) and digestive glands (Figure 3). The digestive glands in *N. gracilis* is called by Danser (1928) and Adam et al. (2005) as exposed glands in which the epidermal flap forming the roof of the digestive glands are less developed thus exposed most part of the glands (Figure 4.3). The digestive glands in other three species have very well developed roof epidermal flap almost concealed the digestive glands (Figure 3). The honey glands were found in shallow pouch between the peristome teeth in *N. gracilis* (Figure 4 and 5) but in the shallow pouch on the peristome surface in the other three species.

N. gracilis differs from the other three taxa by it sessile leaves, leaves base being decurrent, the stem triangular in shape, inconspicuous peristome teeth, very thin peristome (=1 mm) and inner surface of pitcher wall covered with exposed digestive glands. Hooker (1859) as cited in Lloyd (1942), divided the inside of the pitcher into zones on the basis of structure as related to function. The 'attractive zone' (upper part of the pitcher including lid and peristome) of *N. gracilis* posses numerous of nectar glands. The peristome nectar glands are located so that insects alighting on the peristome often fall into the pitcher while attempting to reach the nectar (Figure 4 and 5).

Table 1. Differences of morphological characters between *N. ampullaria, N. hookeriana, N. gracilis* and *N. rafflesiana*

	Morphological character	*N. rafflesiana*	*N. ampullaria*	*N. gracilis*	*N. hookeriana*
1	Stem	Cylindrical	Cylindrical	Triangular	Cylindrical
2	Leaf base	Petiolate	Petiolate	Sessile	Petiolate
3	Leaves	Semi-amplexicaul	Semi-amplexicaul	Decurrent	Semi-amplexicaul
4	Shape of upper pitcher	Infundibulate	Absent	Tubulose-ventricose	Infundibulate
5	Digestive glands (DG) on the inner pitcher wall of upper pitcher	Wholly glandular	No upper pitcher	Partly glandular	Wholly glandular
6	Shape of lower pitcher	Ellipsoidal	Urceolate	Tubulose-ventricose	Ellipsoidal
7	DG on the inner wall of lower pitcher	Wholly glandular	Wholly glandular	Only covering the lower ventricose part	Wholly glandular
8	Type of DG on the inner wall of upper and lower pitcher	Gland overarched with well developed external flap	Gland overarched with well developed external flap	Exposed type and without extended epidermal flap	Overarched
9	Peristome	Thick (>1 mm)	Thick (>1 mm)	Very thin (1 mm)	Thick (>1 mm)
10	Size of pitcher's lid	The same size of the pitcher mouth	Distinctly smaller than the pitcher mouth	The same size of the pitcher mouth	The same size of the pitcher mouth
11	Honey glands on the lower lid surface	Sparsely in the middle and becoming dense towards the margin	Glandless	Sparsely distributed	Sparsely distributed
12	Inflorescence type	Raceme	Panicle	Raceme	Raceme
13	Pedicel	Simple	Branching into 2-4	Simple	Simple
14	Pedicel extended by bract	Ebracteolate	Bracteolate	Ebracteolate	Ebracteolate

Entrapment of insects is facilitated by features of the 'conductive zones (middle part of pitcher). Insects are unable to maintain traction in this zone as its surface is composed of a powdery ablative wax which is extremely slippery, and the unique stomata found in this area, in which the lower guard cell is overlapped by the upper, may deny the insects the ledge-like foothold that would otherwise be provided. (Lyold, 1942) which is confirm in the same Figure of *N. rafflesiana* by Adams & Smith (1977) and based on Kaul (1982), *Nepenthes* in general, it is believed that the nectar glands of the lid and peristome attract foraging insect such as ants and small dipterans some of which in their effort to obtain nectar, slip or fall into the pitcher. The slippery lining, the overarched stomata and in most species, the peristome prevent escape the prey eventually drowning in the pitcher's copious fluid containing acids and enzymes.

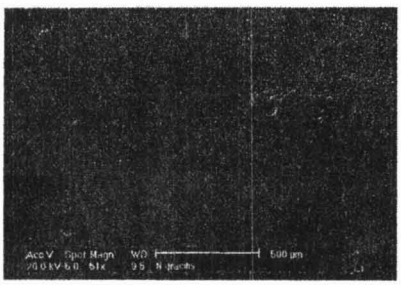

Figure 1. Honey glands on the lower lid surface on *N. gracilis*

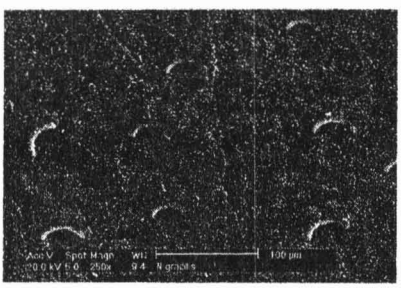

Figure 2. Deframed stomata the waxy zones of inner pitcher wall of *N. gracilis*

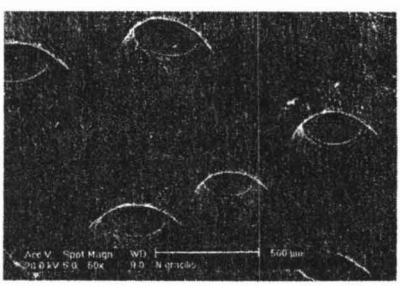

Figure 3. Exposed digestive glands with slight extension of epidermal on inner pitcher wall of *N. grancilis*

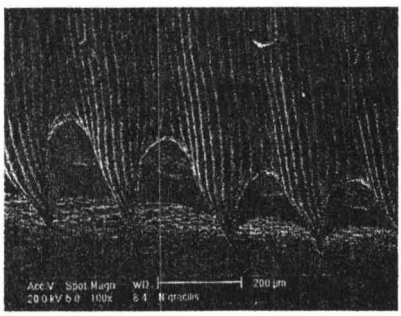

Figure 4. Marginal glands in between the peristome teeth of the pitcher of *N. gracilis*

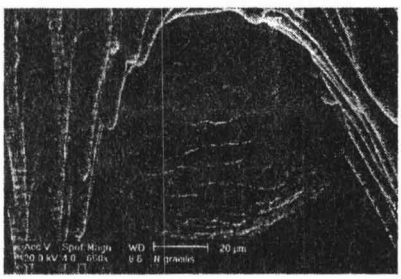

Figure 5. Close up marginal glands in between the peristome teeth of *N. gracilis*

Nepenthes ampullaria had being identified as a climber. The species also show that if the main stem or lateral branches fail to reach a support, it may creep along the ground or clamber over bushes. The roots of the species abundantly at the nodes, and there it produces short lateral branches with dense clusters of leaves with reduced lamina but large and often richly colored pitchers. The stem of the species sometimes found prostrating on the ground.

Nepenthes ampullaria is the only species in this study and the genus possessing only lower pitchers in the form of rosettes. The pitcher color of *N. ampullaria* is uniform green colors and sometimes blotches with red (Slack, 1979). *N. ampullaria*, revealed by Rohana (1988) consist of two variants based on the color of the pitchers, one having uniform green pitchers while the other having pitchers that are light green with red. Based on (Kurata, 1976; Phillipps & Lamb, 1988; Shivas, 1984; Tan & Wong, 1996), *N. ampullaria* seldom produces upper pitcher. The shape of upper and lower pitcher is tubulose-ventricose.

This species can easily be identified from the three other species in the research plots by possessing lower pitchers which were urceolate in shape and with horizontal mouth, very narrow cuneate lids of the pitchers which is deflexed to the front in very closely related species of *N. hookeriana*, lower lid surface of this species was not covered with honey glands, the lower surface of the leaves and on the stems were densely covered with ferruginous and star shaped trichomes or hairs.

The inner wall surface of the pitcher of this species is wholly covered with overarched digestive glands (Figure 6 and 7). This species have a panicle inflorescence, pedicels subtended by branched and bracteolate pedicels. This inflorescence type is not shared by the other three species in the research plots. The other species which is known to share the same inflorescence

type was *N. bicalcarata* but this species have its distribution confined to the island of Borneo. Marginal glands of *N. ampullaria* were hidden in the pouches on the peristome (Figure 8).

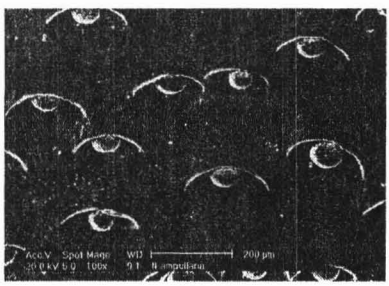

Figure 6. Overarched digestive glands on the inner pitcher wall of *N. ampullaria*

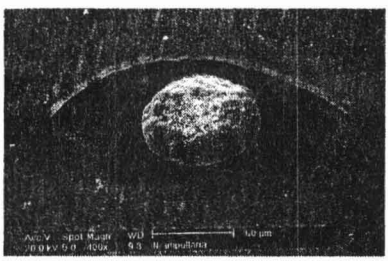

Figure 7. Close up overarched digestive glands on the inner pitcher wall of *N. ampullaria*

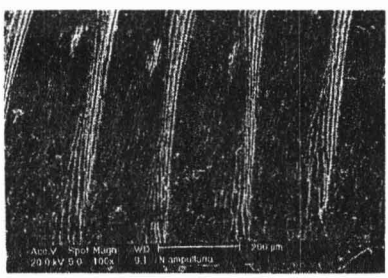

Figure 8. Marginal glands hidden in the pouches on the peristome of *N. ampullaria*

Nepenthes hookeriana was reported a natural hybrid resulting from natural crossing of the parental species of *N. ampullaria* and *N. rafflesiana.* This hybrid was found to have strong resemblance to *N. rafflesiana* by possessing ellipsoidal lower pitcher and funnel shape upper pitcher but differed from the species by its lower lid surface which was sparsely covered with honey glands. *N. hookeriana* differs from *N. ampullaria* in having ellipsoidal shape of lower pitcher, with very broad and reflex pitcher lids and its lower lid surface covered with honey glands. *N. hookeriana* rosette shown in Appendix B. Rosette of pitchers of *N.hookeriana* is produced around the base of the plants, but these are very loose (Clarke, 1997). Based on Rohana (1988), the hybrid shows a much stronger affinity towards *N. ampullaria* as compared to *N. rafflesiana* and generally they have intermediate characters between the two parents. Characters for *N. hookeriana* determination had been summarized in Table 2.

Nepenthes hookeriana has cylindrical stem with petiolate leaves, which is same characteristic shown by its parents. Overarched digestive glands on inner surface pitcher wall of the species also were shown in Figure 9 and 10. The overarched digestive glands of this species are quite same with digestive glands of *N. campulata* and *N. gracilis* which is discovered by Adam (1990). From the present study, marginal glands of the species were hidden in the pouches on the peristome (Figure 11). Meanwhile, SEM also discovered stellate trichomes on the external pitcher wall of *N. hookeriana* (Figure 12 and 13) which is illustrated by Rohana (1988) as four armed top view, stellate trichomes (Adam, 1990) and contain peltate hair.

N. hookeriana species exhibits some characteristic of each of the suspected parent species (Shiva, 1984). N. hookeriana differs from *N. ampullaria* by the present of both upper and lower pitchers in the former species and the absence of upper pitcher in the later species. *N. hookeriana* differs from *N. gracilis* by its cylindrical upper stem, infundibulate upper pitcher and inner pitcher cavity of both upper and lower pitchers covered with digestive glands.

Table 2. Characters for the determination of the hybrid species for *N. hookeriana*

Characters	N. ampullaria	N. hookeriana	N. rafflesiana
Pitcher shape	Urceolate	Intermediate	Ellipsoidal
Leaf shape	Cuneate	Ovate-oblong, elliptic	Caudate-orbiculate
Glands on lid	None	Few	Numerous
Peristome neck	None	Short	Long
Peristome ribs	Fine	Intermediate	Coarse
Peristome teeth	Short	Intermediate	Long

Source: Rohana Md. Som (1998)

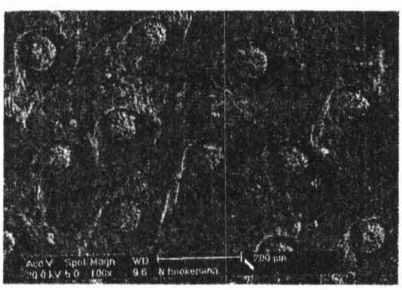

Figure 9. Overarched digestive glands on inner surface wall of *N. hookeriana*

Figure 10. Digestive gland on inner pitcher wall of *N. hookeriana*

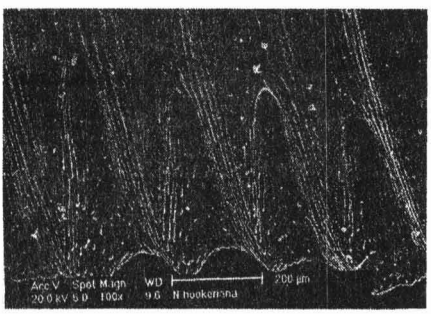

Figure 11. Marginal glands hidden in the pouches on the peristome of *N. hookeriana*

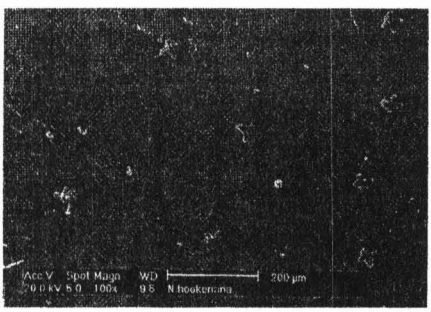

Figure 12. Stellate trichomes on the external pitcher wall of *N. hookeriana*

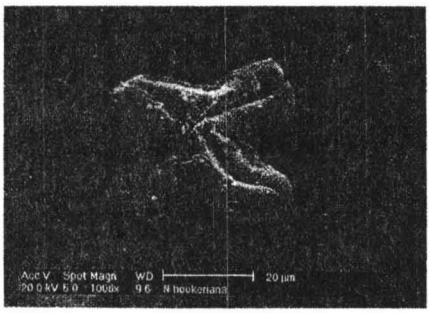

Figure 13. Close up stellate trichomes on the external pitcher wall of *N. hookeriana*

Nepenthes rafflesiana is a very distinctive pitchers in which the mouth is horizontal in front and strongly elevated in to a vert long neck towards the pitcher lids. The other distinctive features of the species includes the trapezoid shape of the peristome at the front, inner peristome teeth are long and distinct, lower lid surface is densely covered with honey glands on the outer half portion and glandless on the inner portion.

Nepenthes rafflesiana stem is cylindrical. It is covered with tricomes and dense on the young shoots. The colour of the stems are light green but sometimes tinged with purplish brown. The leaves of the species are attached to the stems by ends of distinct petiole. Its petiole usually winged. The upper pitcher found on *N. rafflesiana* is unfundibulate which is an open funnel, narrowest at the base and expanded at the top and the lower pitcher of the plant is also different, where it has ellipsoidal shape.

The lower pitcher is light green with blotches and the upper pitcher is whitish green at the basal region and light green with or without red blotches in the upper region. Based on Green (1967), the shape of upper pitcher lessens wind resistance besides the lesser load of pitcher fluid they have to carry. The peristome of the species is thick, narrower in front but it is much broader or expanded at the sides and especially towards the lid.

Based on the Scanning Electron Microscope Figure, *N. rafflesiana* differ from the other species found based on its digestive glands (DG) on the inner pitcher wall of upper pitcher and lower pitcher where both of it are wholly glandular. Overarched digestive glands on the inner pitcher wall of upper pitcher of *N. rafflesiana* were shown in Figure 14 and Figure 15.

Based on Toekes et al. (1974), numerous glands secrete liquid containing proteolytic enzymes by which the insects are digested. It is suggested that the hood covering each glands may prevent insect from using the glands as footholds and protect the secretory cells from abrasion by an insects's grappling (Lloyd, 1942).

Its honey glands on the lower lid surface are sparsely in the middle and becoming dense towards the margin. The inflorescence type of *N. rafflesiana* is raceme, which simple pedicel and the pedicel extended by bract is ebracteolate.

The stem so the species is cylindrical and covered with tricomes and especially dense on the young shoots. Based on Slack (1979), the ground level rosettes contains small leaves which are white to pink. Many sprounting from underground rhizomes around a single climbing stem, and each is surrounded by several terrestrial pitchers springing from the ground which is equally unique features of *N. ampullaria.*

The leaves are attached to the stem by means of a distinct petiolate. The leaves of *N. ampullaria* is differentiated from *N. rafflesiana* by the fact that the wings of the former are directed horizontally, whereas those of *N. rafflesiana* are directed vertically upwards or oblique

so as to give a canaliculate appearance (Rohana, 1988). *N. ampullaria* differs from the other species by possessing the paniculate inflorescence, bracteolate flowers, having only lower pitchers, the lids sizes are distinctly smaller the mouth of the pitcher, lower lid surface glandless and trifid spurs. All the morphology of the spur in all species found being summarized in Table 3.

Table 3. Morphology of the spur of *Nepenthes* species

Species	Shape	Branched or unbranched	Point of attachment
N. ampullaria	Flattened	Trifid	On the lid base
N. gracilis	Flattened	Not branched	On the lid base
N. hookeriana	Flattened	Not branched	3 mm below the lid base
N. rafflesiana	Cylindrical	Not branched	On the lid base

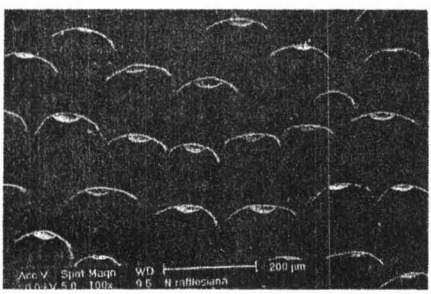

Figure 14. Overarched digestive glands on the inner pitcher wall of upper pitcher of *N. rafflesiana*

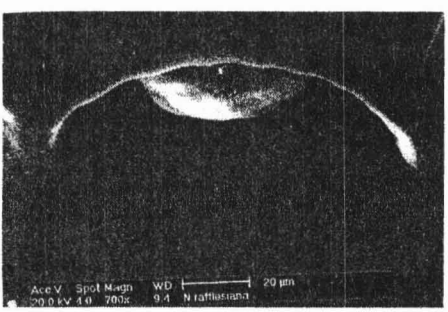

Figure 15. Close up overarched digestive glands on the inner pitcher wall of upper pitcher of *N. rafflesiana*

Conclusion

From the study, morophological characteristic of the species found are differ. Based on the 14 characteristic studied, *N. gracilis* significantly different than the other species in stem, leaves, leaf base, shape of upper pitcher, digestive glands on the inner pitcher wall of upper pitcher and on the inner wall of lower pitcher, type of digestive glands on the inner wall of upper and lower pitcher and peristome and *N. ampullaria* showed different characters in size of pitcher's lid, honey glands on the lower lid surface, inflorescence type, pedicel and pedicel extended by bract.

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