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Size doesn't matter shape does: A morphological study of pitcher plant in distinct forest canopy structures

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Abstract. Light exposure from the sun is the most crucial variable for producing optimal pitcher size and colour variation in *Nepenthes*. This study aimed to observe the effect of forest canopy structure on morphological character of *Nepenthes ampullaria*'s pitcher parts (longitudinal, front, and peristome) both on size and shape using Geometric morphometric (GM) approach and its prey diversity. We classified the forest canopy structure into two categories: inside the canopy and open space area (gap). We used Unmanned Aerial Vehicle (UAV) images to build Canopy Height Model (CHM). Then, ForestGapR R package used to analyse and generated the forest gaps area. The prey specimens and photograph samples comprised from 9 individuals with three lower pitchers in open space area with high light exposure as well as shaded area inside the canopy. Total of 54 images were marked by point and curve to generated a landmark analysis using GeoMorph R package. Based on GM analysis, we observed that the forest canopy structure could affect *Nepenthes* pitcher shape but not in pitcher size. Our field result revealed *Nepenthes ampullaria* likely dominated by darker colour with a red spot in the shaded area and bright green colour in the open. However, based on our study the pitcher in shaded area inside the canopy have a larger number of prey species than the open area.

Keywords: canopy structure, habitat, photogrammetry, pitcher plant.

1. Introduction

Nepenthes is the largest genus of pitcher plants which the Sumatra species is one of the richest in the world. A total of 36 *Nepenthes* species were described in Sumatra, followed by Borneo with 34 species [1,2]. *Nepenthes* species are commonly found between 1.500 to 2.500 m. *Nepenthes* species are found in areas with lack of nutrients soil and acidic environmental conditions where the canopy is sparse or thin [3]. The genus *Nepenthes* capture and digest animal prey using a modified-leaf pitfall trap [4,5].



The digestion of prey is a source of a nitrogen [6,7]. This result comes from the adaptation of plants to their environment.

One of the most remarkable species is *Nepenthes ampullaria*. The species is widespread being found throughout South East Asia. *N. ampullaria* developed strategies for obtaining sources of nitrogen besides from prey animal but also from litterfall, including twigs, dead leaves and flowers, and scat from above tree canopy [8,9]. Moran *et al.* [6] estimated that *N. ampullaria* growing under the shade of tree canopy could derive 35.7% ($\pm 0.1\%$) of their foliar nitrogen from leaf litter. Light is the most crucial variable for producing optimal pitcher size and colour variation. The plants are grown under shade cloth, a light density level from 50% up to about 80% has good results [9].

The gap opening in the forest is correlated with abiotic factors such light availability, soil and air temperature, air vapor pressure deficit, soil nutrient and water content [10]. There is evidence that variation of colour and size of the pitcher could be affected by light availability [3,11]. The *Nepenthes* variation, even though with a single species, could be explained by geometric morphometric (GM) method [12,13]. The GM method has been successfully used to identify *Nepenthes* species [14] and revealed the shapes variations of the peristome and pitcher body of *Nepenthes saranganiensis* [15]. Based on that reason, this study used a geometric morphometric approach to investigate *Nepenthes* variation based on high and low forest canopy gaps. In addition, we recorded the prey animal on *Nepenthes* in such conditions.

2. Materials and Methods

2.1 Survey and occurrence recording

The study was conducted in Biological Education and Research Forest (HPPB) Andalas University (Figure 1). We surveyed alongside the “resam” vegetation (*Gleichenia linearis*) to record the *Nepenthes ampullaria* occurrences. We selected two different habitats of *Nepenthes ampullaria* based on the low and high vegetation gaps. We photographed the pitcher with scale and 30 cm range. In this study, we sampled three lower pitchers from 9 different individuals in each habitat. We photographed the longitudinal part, front and peristome (Figure 1).



Figure 1. The photograph of pitcher plant body parts, (A) longitudinal look (B) front (C) peristome.

2.2 Prey sorting and identification

Prey specimens were sorted from a pitcher into the microtube and identified to the lowest taxonomic level. We sampled the prey specimens from 27 pitcher from the open area as well as the shaded area. We followed [16-19] for ants, beetle, and spider description. To group the prey composition into pitcher habitats we used quantitative analysis and visualize with alluvial plot in ggplot2 R package [20].

2.3 Geometric morphometric analysis

We used tpsUtil [21] & tpsDig [22] software to build and landmark from the photograph. The output exported as .tps contained a curve, point, and scale of the images. In this study used a total number of landmark (LM) as follow: front (34), longitudinal (37), and peristome (115). We used an R package “Geomorph” [23] for landmark analysis. Analysis of Variance (procD.lm) function (procrustes analysis) used with 1000 permutations in Geomorph R package. All of the statistical analyses of this study were performed by RStudio [24].

2.4 Vegetation gap generation based on photogrammetry

We used DJI Phantom 4 Pro drone to map the study area at 50 m altitude. We used Pix4D software trial to generate a Digital Surface Model (DSM) & Digital Terrain Model (DTM) from drone images. After that, we performed a Canopy Height Model (CHM) by DSM-DTM in Raster R package [25]. The canopy gaps were generated with R package GapForestR [26] based on our CHM.

3. Results and Discussion

The samples were collected in two forest canopy structures, open space areas and inside tree canopy. The open space area is classified as a red colour and indicated as an open area with lack shaded from canopy tree (Figure 2). We discovered a bright green-yellowish colour in the open space area. However, the pitcher inside the tree canopy tends to be darker and have a red spot in the body (Inset picture in Figure 2). [9][27] mentioned that the light intensity strongly affected the colour in *Nepenthes*. The bright colours in low canopy gaps are related to prey capture and insect visual sensitivity maxima, especially flying insects.



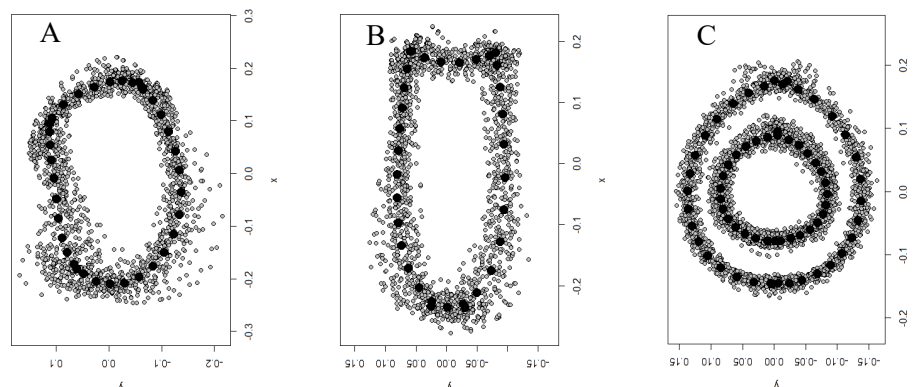
Figure 2. The map forest canopy gaps in study area.

In this study, we analysed variance on size and shape from points and landmarks on pitcher plants (Figure 3). Based on P-Value (0.006), we observed a forest canopy gap affected only the longitudinal shape and peristome of the pitcher plant (Table 1). The plants that grow in opened habitats with full light intensity have larger pitcher besides waist pitchers in shaded habitat [28]. In contrast with *N. saranganiensis*, when the canopy is constantly shading parts of pitcher, it would produce leaves and pitchers that are wider and larger than those exposed to sunlight [15].

Table 1. The p Value for each part of the Pitcher body, *significant.

Pitcher parts	P value (significant < 0.05)	
	Shape	Size
Front body	0.288	0.163
Longitudinal body	0.006*	0.82
Peristome	0.029*	0.58

The study on *N. saranganiensis* showed that the size and structure of the pitcher could be analysed using the geometric morphometric method. It was revealed that variations in the lateral and basal peristome and entire *N. saranganiensis* pitcher were formed. [15] [29] also conducted a study using geometric morphometric analysis of *Nepenthes rafflesiana* pitcher development stages. This study suggests that day/night-light hours might be one of the most significant contributing factors in pitcher development. However, in this study, we did not find the size variation between these canopy categories.

**Figure 3.** The landmark coordinate used in this study (A) longitudinal, (B) front, and (C) peristome.

We found a large number of prey in pitchers trapped inside canopy tree than an open space area (Figure 4). We identified six families (Agelenidae, Cnetidae, Formicidae, Linyphiidae, Oxyopidae, and Scarabaeidae) from open area pitcher and six families (Acanthosomatidae, Charinidae, Culicidae, Formicidae, Meloidae, and Saltidae) from shaded canopy area.

We have updated the [30] study while they only found four families (Formicidae, Ichneumonidae, Termitidae, Ixodidae) and an order (Araneae) of *N. ampullaria* in the same study site. [31] and [5] noted that several species have been recorded from the pitchers of *N. ampullaria*. The prey was composed largely from crawling invertebrate taxa such the ant (Formicidae), crab-spider *Misumenops nepenthicola* (Araneae), and large bug (*Lisarda* spp. and *Metochus* sp.). We suggest the difference of prey between pitcher in open area and inside canopy caused by insect preference habitat. [32] study revealed even the small-scale canopy structure could greatly affect distribution of insect.

The insects trapped into the pitcher is strongly influenced by the peristome condition. The prey is attracted to the peristome by the nectar-secreting gland. The wettable peristome could cause the prey to slip easily into the pitcher. Such conditions would create the *Nepenthes* trapping strategies more efficient and caused higher incoming insects [33,34]. In the open area, we assumed that the nectar fluid will evaporate shortly and the peristome would become drier than the area shaded by canopy cover. We suggest this is one of the reasons why the shaded area has a higher number of contained prey in the pitcher than the open area.

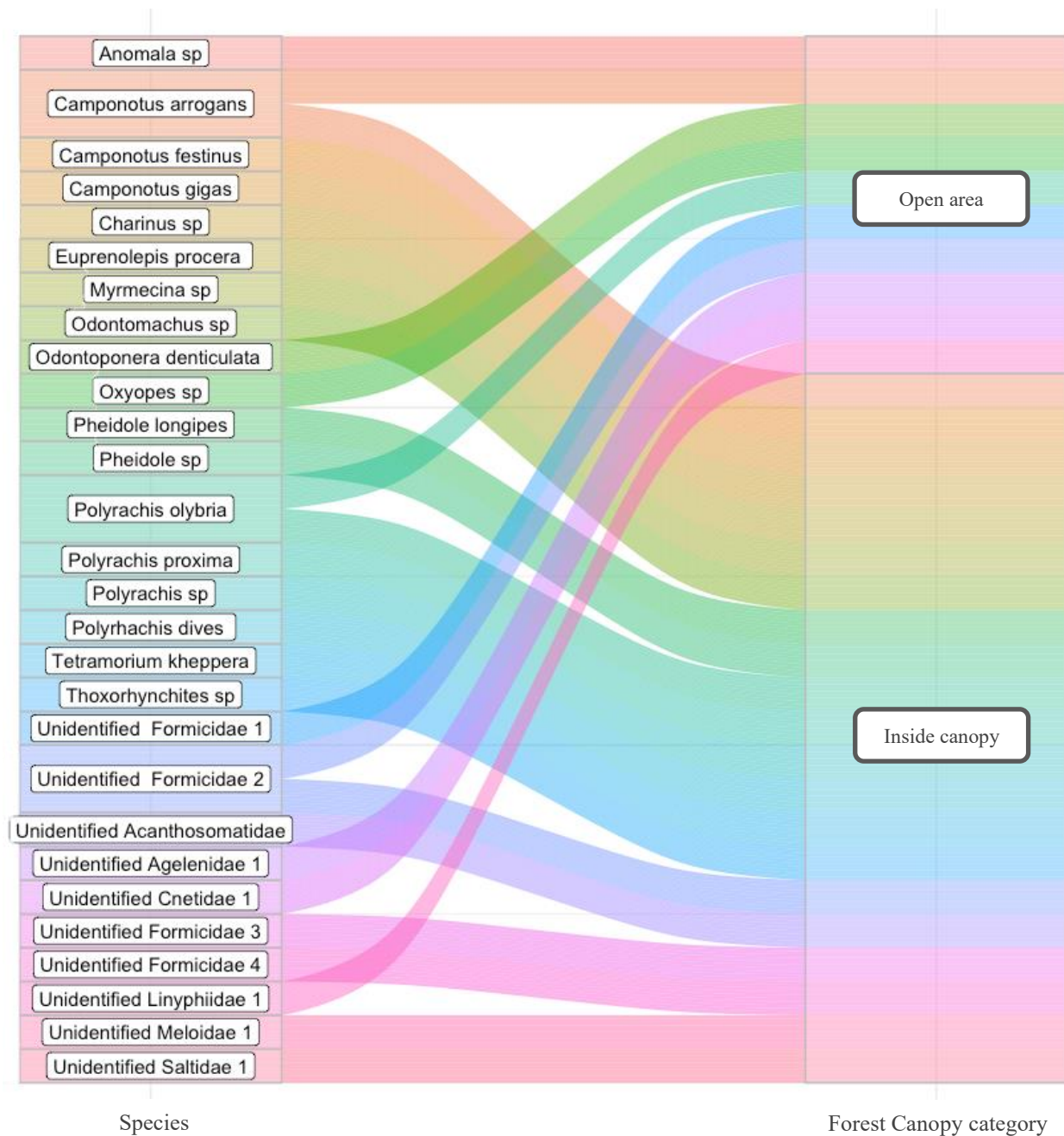


Figure 4. The number of species of two different forest canopy categories: open space area and inside the tree canopy.

4. Conclusion

The forest gaps could influence the shape, colour and number of prey on *Nepenthes ampularia*. The bright green colouration and low number of preys indicated *Nepenthes* grow in high forest canopy gaps while darker with red spot colour in the pitcher and a high number of preys is characters for *Nepenthes* that occurs in shaded area.

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