

# Prey composition and correlation between morphometry and prey biomass weight of the endemic *Nepenthes bicalcarata* in Kalimantan, Indonesia

HENDRA SETIAWAN<sup>1,2,\*</sup>, LUCHMAN HAKIM<sup>3</sup>, ADJI ACHMAD RINALDO FERNANDES<sup>4</sup>,  
CATUR RETNANINGDYAH<sup>3,✉</sup>

<sup>1</sup>Departement of Biology Education, Faculty of Teaching Training and Education, Universitas Kapuas. Jl. Y.C. Oevang Oeray No. 92, Sintang 78612, West Kalimantan, Indonesia. Tel.: +62-565-2020034, ✉email: hendra\_setiawan09@yahoo.com

<sup>2</sup>Doctoral Program in Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

<sup>3</sup>Departement of Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia. Tel.: +62-341-554403, ✉email: catur@ub.ac.id

<sup>4</sup>Departement of Statistics, Faculty of Mathematics and Natural Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

Manuscript received: 16 August 2022. Revision accepted: 23 October 2022.

**Abstract.** Setiawan H, Hakim L, Fernandes AAR, Retnaningdyah C. 2022. Prey composition and correlation between morphometry and prey biomass weight of the endemic *Nepenthes bicalcarata* in Kalimantan, Indonesia. *Biodiversitas* 23: 5453-5460. *Nepenthes bicalcarata* Hook.f. is an endemic plant of the north-western part of Borneo, which is threatened by the conversion of its habitat. The understanding of the ecology of *N. bicalcarata* in regard to its prey is limited. This research aims to analyze the prey composition of *N. bicalcarata* and to investigate the correlation between pitcher morphometry and prey biomass weight. This research was done from November 2021 to March 2022 in a peat swamp primary forest in Sintang District, West Kalimantan Province, Indonesia. We used a quantitative descriptive approach and exploration method to gather data from *N. bicalcarata* in-situ habitat. The morphometry data of *N. bicalcarata* pitchers were collected in the field while the biomass trapped in *N. bicalcarata* pitcher liquid was sampled with a plastic bag and preserved with 70% of alcohol. Seven upper pitchers and 39 lower pitchers were recorded in this research. The composition of *N. bicalcarata* preys was dominated by Formicidae family including *C. schmitzi*, *Polyrhachis* sp. and *Crematogaster* sp.. Besides that, there were also mosquito larvae, ladybugs larvae, Ceratopogonidae larvae and some unidentified flying arthropods as the prey of *N. bicalcarata*. We found the plant's parts in the lower pitchers of *N. bicalcarata* in the peat swamp forest, suggesting a possible mechanism of this plant to extract plant's parts as alternative nutrient sources, yet this needs further research and intensive field observation. The correlation between pitcher volume and prey biomass weight was significant with  $P\text{-value} = 0.00 (<0.01)$  and regression is formulated as  $Y$  (prey weight) =  $-0.1727 + 0.0039X$  (pitcher volume). This means that any increase in *N. bicalcarata*'s pitcher volume will increase the weight of the biomass linearly.

**Keywords:** Alternative syndrome, Kalimantan endemic plant, prey composition, tropical pitcher plant

## INTRODUCTIONS

*Nepenthes bicalcarata* Hook.f. is an endemic tropical pitcher plant with geographical distribution in the north-western part of Borneo Island (Bazile et al. 2012). *N. bicalcarata* is a threatened species and has been listed as Vulnerable based on IUCN Red List since 2000 (IUCN 2022). This species grows in habitats with poor nutrients, such as peat-swamp forests and primary heath forests at an altitude of 0-950 m above sea level (Setiawan et al. 2018; Sunardi and Mansur 2021). *N. bicalcarata* plants are usually found with other *Nepenthes* species, namely *N. ampullaria*, *N. gracilis*, *N. mirabilis* and *N. rafflesiana*, in which in a particular condition it will produce natural hybridization (Setiawan et al. 2018).

The natural habitat of *N. bicalcarata* in Kalimantan (Indonesian Borneo) is threatened by conversion to agricultural lands, settlements and mining areas (Setiawan et al. 2018; Cross et al. 2020). In particular, forest conversion into oil palm plantations (both conducted by large-scale corporations and small-scale farmers) is the

major driver of the habitat loss of *N. bicalcarata* in Kalimantan (Meijaard et al. 2020). Almost all forested lands outside the state-owned conservation areas have been surrounded by oil palm plantations, rubber, pepper, and other agricultural plantations (Gaveau et al. 2016). As a consequence, such forest loss affects the existence of endemic species like *N. bicalcarata*. The loss of habitat makes the holistic conservation of *N. bicalcarata* problematic since there is limited knowledge regarding the ecology, population and physiology of this species. Based on IUCN, the population of *N. bicalcarata* in the wild is still unknown (IUCN, 2022). Nonetheless, a pre-observation in Sintang District, West Kalimantan Province, indicated that the population of *N. bicalcarata* in its natural habitat was decreasing because of habitat degradation (Setiawan et al. 2018).

From the morphological perspective, *N. bicalcarata* has two distinctive pitchers, namely upper and lower pitchers. Either the lower and upper pitcher of *N. bicalcarata* is used to catch a different type of prey which increases the efficiency to attract and catch more diverse prey (Chin et

al. 2014; Gaume et al. 2016). The volume (morphometry) of the *N. bicalcarata* pitcher is relatively large compared to other lowland *Nepenthes* pitchers, although it is not the largest one in this genus. The large size of the pitcher in *N. bicalcarata* is presumed to be able to catch more prey. However, it is not yet known exactly whether with larger pitchers, *N. bicalcarata* is able to catch more prey. Previous research by Bazile et al. (2012) in Brunei Darussalam revealed that there was a linear correlation between pitcher volume and prey biomass on the lower pitcher of *N. bicalcarata*. Nevertheless, other researchers argued that different habitats might affect the prey composition of *Nepenthes* species (Rembold et al. 2010; Tarigan et al. 2021).

Prey availability is one of the main aspects of *N. bicalcarata* sustainability in natural habitat. The sufficient availability of prey in terms of quantity (i.e., number of individual) and quality (i.e., prey diversity) might provide the required nutrients for *N. bicalcarata* to grow which cannot be obtained from the soils. A study in Rampa-Sitahuis Hill of North Sumatra Province, Indonesia found that the main prey of *Nepenthes* consisted of families Culicidae, Formicidae, Araneidae, Calliphoridae, Rhyarochromidae, Salticidae, Curculionidae, Blattellidae, Coccinellidae and Tridactylidae (Tarigan et al. 2021). Gaume et al. (2016) showed that besides arthropods, some *Nepenthes* species extracts another source of nitrogen from leaf litter, treeshrew feces, mouse, and other big animals. The relationship between *N. bicalcarata* and the ant species *Componatus schmitzi* also gives the opportunity to discover an alternative source of *Nepenthes* prey (Bazile et al. 2012).

While the existing studies as mentioned above provide a good foundation for understanding the ecology of *N. bicalcarata*, further research is required to reveal the composition of prey and its biomass weight. The aim of the research was to analyze the prey composition digested by *N. bicalcarata* in West Kalimantan and to investigate the correlation between the volume of pitchers and the prey composition and biomass weight of the prey. We hypothesized that the bigger pitcher would catch prey with heavier biomass.

## MATERIALS AND METHODS

### Study site

This research was conducted in Kantuk customary forest (*Hutan Adat Kantuk*), Tanjung Perada Village, Tempunak Sub-district, Sintang District, West Kalimantan Province, Indonesia on November 2021 - March 2022. Kantuk customary forest, which is located at geographical coordinates of 0.0217101 N and 111.3098015 E (Figure 1) is a primary peat swamp forest with an extent of  $\pm 320$  Ha and dominated by *Shorea* spp. The forest floor is usually logged with water during the rainy season (September-February) and muddy in the dry season (March - August). The average air temperature is 23-32°C and humidity can reach 100%.

### Procedure

This research used a quantitative descriptive approach by providing a description of the findings (results) of direct observation in the field. The exploration method was used to investigate the existence of *N. bicalcarata* in its natural habitat. To explore the existence of *N. bicalcarata*, we followed the traditional route used by local people who usually collected the rattan from the forest in Kantuk customary forest. Every *N. bicalcarata* species found in the forest was used as the sample of this research.

Pitcher morphometry data was determined by measuring the height and diameter of the pitcher to obtain the volume of the pitcher. The upper pitcher is assumed to have a cylindrical shape and the lower pitcher is assumed to have a sphere shape. The formula to calculate the volume of the pitcher are as follows:

$$V_{UP} = h \times \pi \times r^2 \text{ (Gaume et al. 2016)}$$

$$V_{LP} = 4 \times \pi \times (h/2)^3/3 \text{ (Gaume et al. 2016)}$$

Where:

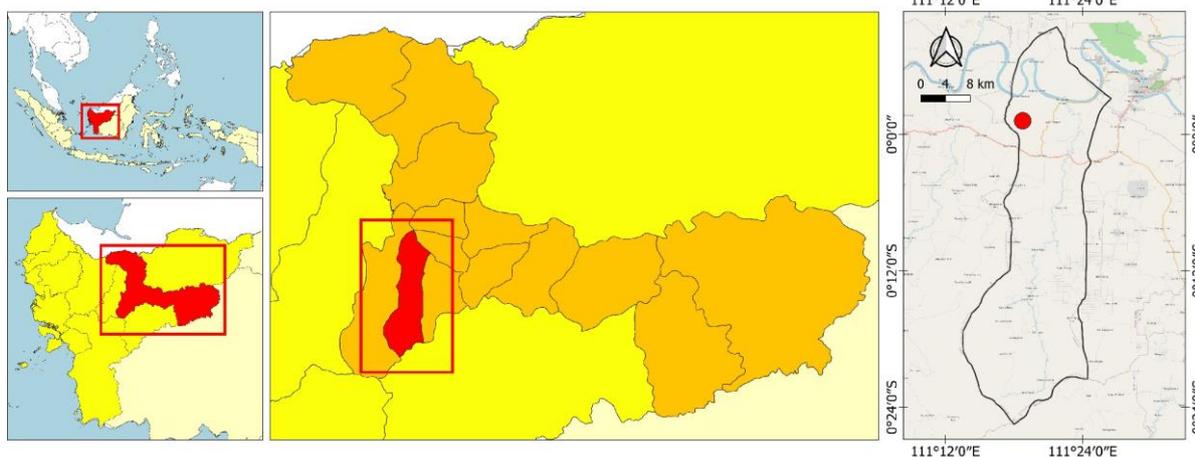
$V_{UP}$  = Upper pitcher volume

$V_{LP}$  = Lower pitcher volume

h = pitcher height

r = 1/2 of pitcher diameter

$\pi$  = 3.14



**Figure 1.** Map of the study area in Kantuk customary forest, Sintang District, West Kalimantan Province, Indonesia

The height of the lower and upper pitcher of the *N. bicalcarata* was measured from the bottom of the peristome to the base of the pitcher before curving into tendrils. The diameter of the upper and lower pitcher of *N. bicalcarata* was measured at the wider part of each one. The liquid and whole biomass content in the pitcher were sampled in a plastic bag, then added with 70% of alcohol to preserve them and brought to the Laboratory of Biology Universitas Kapuas Sintang for further analysis.

The types of biomasses contained in *N. bicalcarata* pitcher fluids were analyzed based on their types, such as insects (arthropods) and other small animals, leaf litter, and other sources. The arthropods and animal contents were identified until the closest taxa. All biomass contents (arthropods, plant parts, etc.) in each pitcher were then dried in the oven at 70°C for 22 hours. The dried biomass was then measured using an analytical balance of two decimals. All data were gathered from every dried pitcher biomass, which found in field observation. Identification of arthropod species was carried out up to the family level or more if available, while for leaf litter, the identification process was through the type of vegetation found in the habitat.

#### Data analysis

Prey composition data were analyzed descriptively and displayed in the form of descriptions, tables and images. The difference in morphometrics (volume) and biomass of the upper and lower pitcher was tested separately using an independent sample t-test with an  $\alpha$  of 0.05. The correlation between morphometry and biomass was determined through a simple regression test using R Studio. The results are then processed using Microsoft Excel and displayed in the form of tables and diagrams.

## RESULTS AND DISCUSSION

### *Nepenthes bicalcarata* in Kantuk customary forest

There were 7 upper pitchers and 39 lower pitchers of *N. bicalcarata* found in Kantuk customary forest, Sintang District, West Kalimantan Province. All these pitchers were used as the sample in this research. The unequal number of samples of the upper and lower pitcher was obtained from 15 individuals of *N. bicalcarata*, which referring to Sunardi and Mansur (2021) the plants had not reached an adult phase yet. The lower pitcher is usually formed earlier than the upper pitcher and when the upper pitcher is scrimping, it indicates that the plant is not mature enough. Based on field observation, all *N. bicalcarata* found in the studied area did not grow well and only reached 2-3 meters long in the forest. This is likely caused by the competition among vegetation to obtain nutrition and light since *N. bicalcarata* is usually found in open canopy areas with a high level of light intensity. Some *N. bicalcarata* plants were used by other small liana species to climb and assess the sunlight and support their lives.

*Nepenthes bicalcarata* plants in Kantuk customary forest varied in size and color (Figure 2). Usually, the lower pitcher of *N. bicalcarata* had a bigger size (in height

and diameter) than the upper one. The lower pitchers were commonly found to lay on the ground, but some lower pitchers were also found hanging like typically the upper pitcher. The position of *N. bicalcarata* lower pitcher provided an opportunity to obtain diverse biomass composition. During the rainy season, the lower pitcher which lay on the forest ground, was usually drowned in water, while during the dry season, it left the pitcher with some litter which can serve as a nitrogen source for *Nepenthes*. This mechanism is possible since *N. bicalcarata* has the same habitat as *N. ampullaria* which also uses the leaf litter as an alternative nitrogen source (Pavlovič 2012).

### Composition and biomass of *N. bicalcarata* preys

The preys found in *N. bicalcarata* pitchers were either the whole part or only a small part of arthropods. This result is in line with the studies by Gaume et al. (2016) and Tarigan et al. (2021) which found that the composition of prey is usually damaged, thus making it difficult for further identification. In this research, preys that can be identified were dominated by ant species from Formicidae taxa (i.e. *Camponotus schmitzi*, *Polyrhachis pruinosa*, *Crematogaster* sp.). The other preys included Culicidae (mosquito larvae in some larvae life stages), Coccinellidae (ladybugs larvae), and Ceratopogonidae larvae (Table 1).

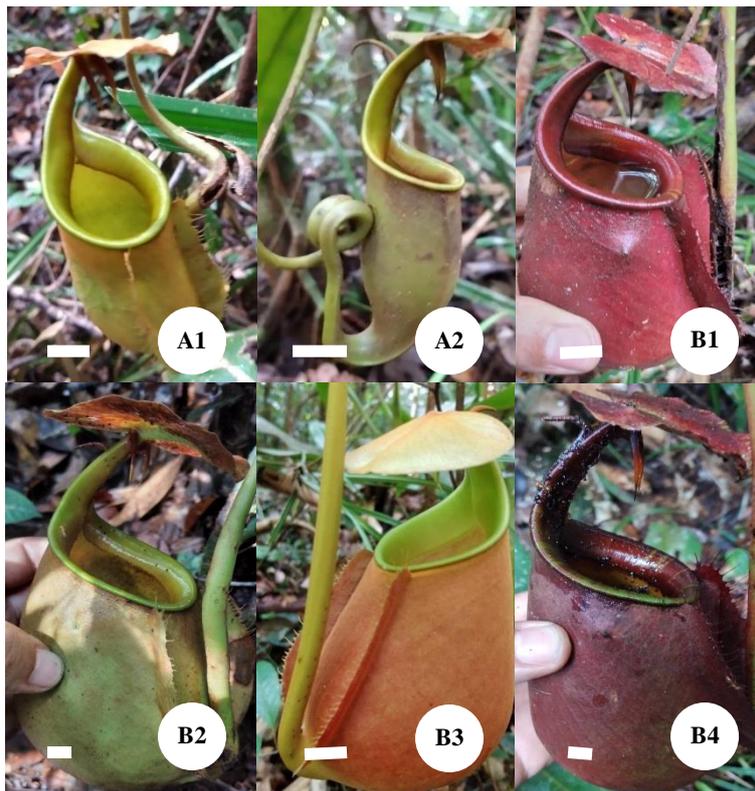
The preys of *N. bicalcarata* in Kantuk customary forest have a similar composition to the results of Bonhomme et al. (2011), but their result had a more diverse composition namely Hymenoptera order, Formicidae family (Formicinae, Myrmicinae, Dolichoderinae subfamily and unidentified ants), Lepidoptera (on larvae stage), Diptera, Isoptera (Termitidae family), Coleoptera and Araneae. The more diverse composition in the Bonhomme et al. (2011) research was because the research was done in a peat swamp and heath forest in Brunei Darussalam, suggesting that the sampling locations were from two kinds of habitat. Gaume et al. (2016) who conducted research in heath forest also found that the main prey of *N. bicalcarata* lower pitcher was from Hymenoptera order (ants species) followed by non-ants Hymenoptera, Diptera and Coleoptera, however in the upper pitcher was dominated by Isoptera and less than 10% of Hymenoptera (ants). On the other hand, compared to the study by Tarigan et al. (2021) in lowland Sumatra, the prey composition is different since it consisted of Gryllidae, Formicidae, Culicidae, Calliophoridae, Curculionidae, Coccinellidae, Rhyparochromidae, Blattellidae, Salticidae, and Araneidae. Such differences imply that environmental conditions of the habitat affect the prey composition trapped on the pitchers.

*Camponotus schmitzi* is an ant species that is well known to have a mutualism relation with *N. bicalcarata*. Based on Bonhomme et al. (2011) and Bazile et al. (2012), this ant species helps plants to catch, retain and digest the prey captured on its pitcher. The absent of this species will decrease the effectiveness of *N. bicalcarata* in acquiring nutrients from prey. However, based on our observation, *C. schmitzi* was also the prey of *N. Bicalcarata*. This is presumably *C. schmitzi* found in *N. bicalcarata* pitcher

liquid was a dead body or dying old one that cannot swim perfectly. It is also part of the mutualism relation between *N. bicalcarata* and *C. schmitzi* where ants provide food for plants and *N. bicalcarata* become host plants and gives some extra floral nectary on the pitcher organ (Scharmann et al. 2013). Based on Bazile et al. (2012), *C. schmitzi* also serves as a “regulator” for prey composition on the pitcher. This ant will swim to the pitcher liquid to dispose of the undigested prey.

This research found that there were differences in prey composition between the upper and lower pitchers of *N.*

*bicalcarata* (Table 1 and Figure 3). We cannot find flying arthropods (only found part of wings) in the lower pitcher while there were many larvae found in the lower pitcher. This finding suggests that there are different functions between both pitchers. Bauer et al. (2012) stated that the morphological characters of pitchers affect the function of pitcher in trapping prey. The upper pitcher of *N. bicalcarata* has a cylindrical shape and is slimmer than the lower one (Figure 2).



**Figure 2.** Variation of size and color of *Nepenthes bicalcarata* pitchers in Kantuk customary forest, Sintang District, West Kalimantan Province, Indonesia. Notes: A1-A2 are upper pitchers, B1-B4 are lower pitchers

**Table 1.** The composition of preys of *Nepenthes bicalcarata* in Kantuk customary forest, Sintang District, West Kalimantan Province, Indonesia

Arthropods	UP/LP*	Descriptions
<b>Formicidae</b>		
<i>Camponotus schmitzi</i>	UP, LP	<i>C. schmitzi</i> was found to be life inside the tendril and has mutualism with <i>N. bicalcarata</i> , but when it died then it is used as <i>N. bicalcarata</i> prey
<i>Polyrhachis</i> sp.	UP, LP	Commonly found in peat swamp forest and usually came to <i>N. bicalcarata</i> pitcher for nectar
<i>Crematogaster</i> sp.	UP, LP	Usually life as colony in less canopy forest and came to <i>N. bicalcarata</i> pitcher for nectar
<b>Culicidae</b>		
Mosquito larvae	UP, LP	Mosquito larvae is infauna of <i>N. bicalcarata</i> pitcher liquid but if it died it also became prey for the host plant
<b>Coccinellidae</b>		
Ladybugs larvae	LP	Only found in the lower pitcher and only in one stage of larvae
Ceratopogonidae larvae	LP	This larva was abundant inside of <i>N. bicalcarata</i> liquid but only found in the lower pitcher
Unidentified flying arthropod	UP	We only found the wings of this arthropods on upper pitcher

Note: \*UP = upper pitcher, LP = lower pitcher

The lower pitcher supports the upper pitcher in the climbing forest canopy and competes for light and flying arthropods in a forest (Sunardi and Mansur 2021). However, the lower pitcher was found to be more bulbous and bigger in volume (Figure 2). It is because the function of the lower pitcher is to catch non-flying arthropods which usually only lay on the forest floor (Lam et al. 2018). Based on our findings, there were a lot of arthropod larvae in the pitcher liquid namely mosquito larvae, ladybug larvae, and Ceratopogonidae larvae. Lam et al. (2017) state that this Dipterans larva helps *N. gracilis* to extract important nutrients from the prey. But in *N. bicalcarata*, this infauna can extract the nutrient in pitcher liquid but *C. schmitzi* prevents nutrition loss by this infauna (Scharmann et al. 2013).

The lower pitcher can be categorized into two distinct types based on its position on the forest floor: (i) the lower pitcher which hangs with its tendrils and does not lay on the forest floor (called the aerial lower pitcher); and (ii) the lower pitcher which lays on the forest floor (a lower pitcher). The prey composition in the aerial lower pitcher was more similar with the upper pitcher but with fewer

flying arthropods. Based on observation (Figure 4), the aerial lower pitcher caught more ant species (Formicidae) and was cleaner than the lower pitcher because no acidic water from the forest floor filled inside. Nevertheless, the lower pitcher which lay on the forest floor caught more ants. Its liquid had darker color affected by flood water in the rainy season since the leaf debris and another part of plants come inside of the pitcher (Figure 4). This affects the composition of biomass on the lower pitcher. Theoretically, when a solid component comes to pitcher liquid, it will affect the physical and chemical component in it namely color, turbidity, pH, etc (Gaume et al. 2016). However, based on our observation, there was no effect on the lower pitcher, which lay on the forest floor and seemed even more fit to the habitat. We suspect that there were some other aspects that affect the biochemical components which already fit this habitat that was not observed in this research. It can be a question for the next research to compare biochemical components in *N. bicalcarata* pitcher between pitchers which down in peat swamp forest flood and those which not.



**Figure 3.** The prey of *Nepenthes bicalcarata* in Kantuk customary fores: A-B. Mosquito larvae; C. ant's *Camponotus schmitzi*, D. *Polyrhachis* sp.; E. *Crematogaster* sp.; F. Ladybugs larvae; G. Ceratopogonidae larvae; H-I. unidentified species. Bar = 5 mm



**Figure 4.** Differences in biomass composition between the aerial lower pitcher (A) and the lower pitcher which laid on the forest floor (B)

**Table 2.** Plants and plant parts found in *Nepenthes bicalcarata* pitcher

Plants name	Family	Form	Local name
<i>Palaquium</i> sp.	Sapotaceae	Tree	<i>Nyatu' burung</i>
Unidentified sp 1	-		
Unidentified sp 2	-		

Several parts of the plant (i.e., leaf debris, stem, fruit, flower, root, etc.) were found in the lower pitcher of *N. bicalcarata*. These plant parts were usually found in a damaged condition which affected the identification process (Figure 5). Among the plant parts found in the lower pitcher, we only can properly identify one species namely *Palaquium* sp. The identification of this species was based on the part of the fruit, flower, and leaf bud found in the lower pitcher of *N. bicalcarata* (Table 2). Based on Kalima and Denny (2019) *Palaquium* sp. is the species with the highest Important Value Index (IVI) at the tree and the pole stages in peat swamp forest at Sebangau National Park, Central Kalimantan, Indonesia. This mean that *Palaquium* sp. is one of the most dominant and abundant species in peat swamp forest, making it reasonable if part of this species were found in the lower pitcher of *N. bicalcarata* in the rainy season.

The plant parts found in the lower pitcher of *N. bicalcarata* liquid are an interesting finding because *Nepenthes* is well known to be a carnivorous plant that generally only uses insects as prey. The possible explanation is the plant parts found most likely entered the pitcher when flooding occurred in the forest. Some of the plant parts were likely to have undergone a digestive process (Figure 5C). There are two possibilities about the digested leaf in the lower pitcher of *N. bicalcarata*. Firstly, the plant part had undergone a digestive process by microorganisms on the forest floor then due to flooding, it entered the lower pitcher. Secondly, the leaves entered the pitcher during when rainy season and were slowly digested by the *N. bicalcarata* pitcher as an alternative source of nitrogen. Nevertheless, this premise requires further research to ascertain whether it is true that *N. bicalcarata* digests plant parts as an alternative source of nitrogen.

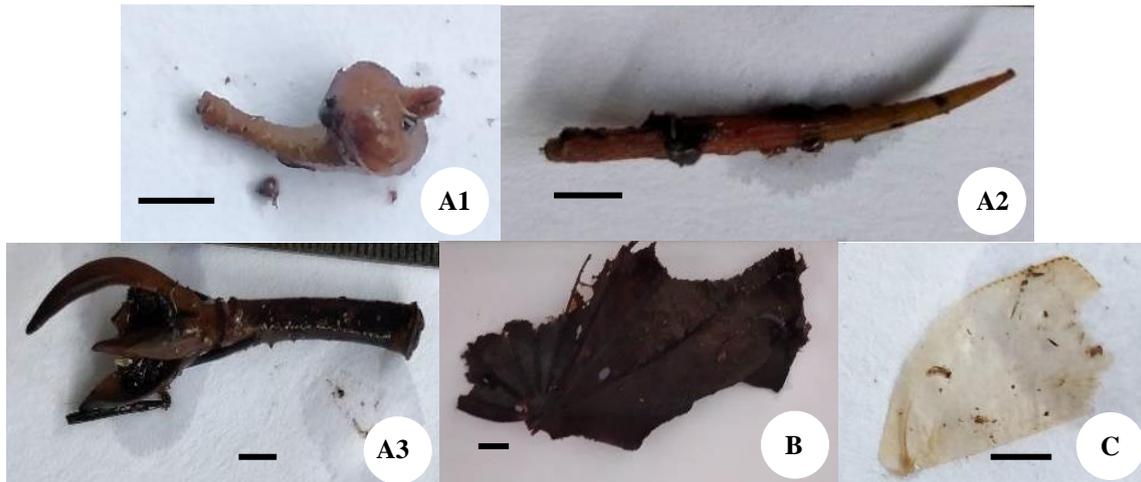
The fact that *N. bicalcarata* shared the same habitat with *N. ampullaria* in peat swamp forest might strengthen our hypothesis (Chin et al. 2014). *N. ampullaria* is known to be able to digest leaf debris for extra nitrogen (Pavlovič 2012; Gaume et al. 2016). *N. ampullaria* has the perfect morphological organ to support leaf debris extraction, such as a broad peristome, small rear lid, and small waxy zone inside the pitcher (Pavlovič 2012). On the other hand, *N. bicalcarata* does not have morphological organs that support a similar mechanism, but the fact that it shared the same habitat with *N. ampullaria* and had a mutualism relation with *C. schmitzi* led us to the hypothesis that *N. bicalcarata* uses leaf debris as an alternative nutrition source. Pavlovič (2012) state that *N. ampullaria* has detritivore syndrome since it has a relatively long functionally live pitcher (> 6 months), compared to *N. mirabilis* in which its pitchers can only last for less than 1 month thus this species is known as a pure carnivorous plant. The pitcher of *N. bicalcarata* is reported to have a

longer functionally live pitcher of more than 9 months, which is longer than that of *N. ampullaria* (> 6 months) (Gaume et al. 2016). This is an indication of a slow but steady trickle of nutrients, which also became the main reason for the assumption that *N. bicalcarata* has the same syndrome with *N. ampullaria*. Bazile et al. (2012) reported that *C. schmitzi* is consumed by pitcher plants in the form of a dead body drowning in pitcher liquid than hauled up to the underside of the peristome. It will leave a small part of prey for *N. bicalcarata* and make it easier to digest by the plant. The same case can happen with leaf debris because *C. schmitzi* has a variety of diets from carnivory to omnivore by eating the nectary in *N. bicalcarata* pitchers (Gaume et al. 2016). Besides that, Bazile et al. (2012) and Thornham et al. (2012) stated that *C. schmitzi* retrieves big prey from *N. bicalcarata* liquid and leaves a small part of prey for its host. It is a very useful habit that prevents the pitcher from putrefaction affected by overloaded unused biomass. All of these reasons lead us to the conclusion that *N. bicalcarata* can possibly use leaf litter and other plant debris as an alternative nutrition source.

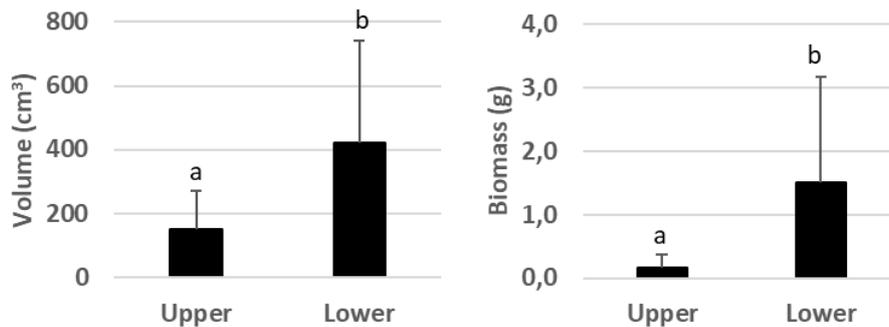
#### Correlation between pitcher morphometry and biomass caught in the pitcher

*Nepenthes* pitcher morphometry is represented by pitcher volume following the formula proposed by Gaume et al. (2016), while biomass caught in the pitcher was calculated as an average weight of all biomass on *N. bicalcarata* pitcher liquid. The independent sample T-test was used to find out the difference between the volume and weight of biomass between the upper and lower pitcher. The difference in the average pitcher volume was significant, with a P-value of 0.01 (<0.05), meaning that there was a significant difference between the volume of the upper and lower pitchers. Meanwhile, the comparison of the average biomass weight between the upper and the lower pitchers resulted in a significant difference with P-value = 0.00 (<0.01), meaning that there was a highly significant difference between the biomass weight in the upper and lower pitchers of *N. bicalcarata* (Figure 6).

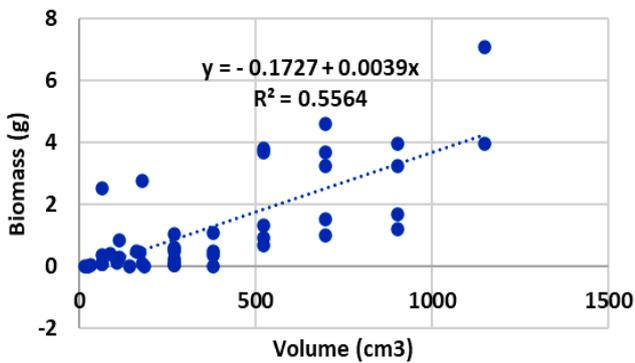
The biomass weight of *N. bicalcarata* upper pitcher and lower pitchers had a significant difference with p-value 0.00 <0.01. It is because the lower pitchers which laid on the forest floor, trapped the leaf litter and other debris which came with the flood in peat swamp forest during the rainy season. The bigger volume of *Nepenthes* pitcher made more litter can be trapped in. In other research Moran et al. (2013) explained that season affected the composition of the prey in *Nepenthes* species. When humidity is high in the rainy season, the prey composition is more abundant and more diverse compared to period with less humid condition especially in *Nepenthes* with broad peristome like *N. bicalcarata*. In contrast, Rembold et al. (2010) stated that pitcher volume has no correlation with prey composition in the pitcher fluid of *N. madagascariensis*. Nevertheless, that results only considered the *Nepenthes* prey from arthropods and others animals. *N. bicalcarata* which suggested to have a same syndrome with *N. ampullaria* that used leaf litter and other plants debris as alternative nutrition source can be excluded from the general perception of *Nepenthes* as carnivorous plants only.



**Figure 5.** Plants parts found in the lower pitcher of *N. bicalcarata*: A1. fruit of *Palaquium* sp., A2. leaf bud of *Palaquium* sp. A3. Flower stalk of *Palaquium* sp., B. leaf of unidentified species 1, C. leaf of unidentified species 2. Bar = 5 mm



**Figure 6.** The difference in morphometry and biomass weight between the upper and lower pitchers of *N. bicalcarata*. Notes: charts with similar letters indicate that there was no significant difference based on the independent sample t-test  $\alpha$  0.05 (n=7 for the upper and 39 for lower pitchers)



**Figure 7.** Linear regression showing the relationship between pitcher volume and weight of biomass trapped in the pitcher

The relationship between morphometry and biomass was determined through a simple regression test. The overall test results of this regression model can be seen

from the results of the Anova test which showed a significant relationship with a P-value 0.00 ( $<0.01$ ) so that the alternative hypothesis was accepted and can be concluded that there is a linear relationship between pitcher volume (X) and biomass weight (Y) in *N. bicalcarata* (Figure 7).

The relationship between volume and biomass is strong referring to the high value of the correlation coefficient (R) of 0.746. The  $R^2$  (R square), which is the coefficient of determination, showed the percentage of pitcher volume affecting biomass which was 0.556 or it can be said that pitcher biomass was influenced by volume by 55.6%, while the rest 44.4% was influenced by other factors (e.g. the availability of prey, type of prey or other environmental factors) (Rembold et al. 2010; Clarke and Moran 2015; Gaume et al. 2016).

The explanation for the result regarding the high correlation between volume and biomass weight on *N. bicalcarata* is that the plants occurred in peat swamp forests which drowned by swampy water in the rainy season and the vegetation debris is captured and digested

(even slowly) by the pitcher. This is because other research which studies the composition of *N. bicalcarata* prey never considered the vegetation debris as an alternative nutrition source and that study is taken in heath forests that usually dry throughout the year (Gaume et al. 2016).

In conclusion, the composition of *N. bicalcarata* prey in Kantuk customary forest was dominated by Formicidae family including *C. schmitzi*, *Polyrhachis* sp. and *Crematogaster* sp. Besides that, there were also mosquito larvae, ladybugs larvae, Ceratopogonidae larvae and some unidentified flying arthropods were *N. bicalcarata* preys. We found plant parts in the lower pitcher of *N. bicalcarata* in a peat swamp forest, suggesting a possible mechanism of this plant to extract plants part as an alternative nutrition source, yet this needs further research and intensive field observation. The correlation between pitcher volume and prey biomass weight was significant with  $P\text{-value} = 0.00$  ( $<0.01$ ) and regression is formulated as  $Y$  (prey weight) =  $-0.1727 + 0.0039X$  (pitcher volume). This means that any increase in *N. bicalcarata*'s pitcher volume will increase the weight of the biomass linearly. The results of this research might serve as the basic information for further research and field observation to understand the possibility of *N. bicalcarata* acting as detritivorous like its close relative of *N. ampullaria*.

#### ACKNOWLEDGEMENTS

This research was funded by the Centre for Education Financial Services (*Pusat Layanan Pembiayaan Pendidikan/ Puslapdik*), the Ministry of Education, Culture and the Higher Education Republic of Indonesia. We also thank Internal Research Grant of Kapuas University 2022. Our high gratitude to the reviewers who provided excellent input and suggestions

#### REFERENCES

- Bauer U, Clemente CJ, Renner T, Federle W. 2012. Form follows function: Morphological diversification and alternative trapping strategies in carnivorous *Nepenthes* pitcher plants. *J Evol Biol* 25 (1): 90-102. DOI: 10.1111/j.1420-9101.2011.02406.x.
- Bazile V, Moran JA, Moguedec GL, Marshall DJ, Gaume L. 2012. A carnivorous plant fed by its ant symbiont: a unique multi-faceted nutritional mutualism. *PLoS ONE* 7 (5): e36179. DOI: 10.1371/journal.pone.0036179.
- Bonhomme V, Gounand I, Alaux C, Jusselin E, Barthélémy D, Gaume L. 2011. The plant-ant *Camponotus schmitzi* helps its carnivorous host-plant *Nepenthes bicalcarata* to catch its prey. *J Trop Ecol* 27 (1): 15-24. DOI: 10.1017/S0266467410000532.
- Chin L, Chung AYC, Clarke C. 2014. Interspecific variation in prey capture behavior by co-occurring nepenthes pitcher plants: Evidence for resource partitioning or sampling-scheme artifacts? *Plant Signaling Behav* 9 (1): 27930. DOI: 10.4161/psb.27930.
- Clarke C, Moran JA. 2015. Climate, soils and vicariance - their roles in shaping the diversity and distribution of *Nepenthes* in Southeast Asia. *Plant Soil* 403 (1): 37-51. DOI: 10.1007/s11104-015-2696-x.
- Cross AT, Krueger TA, Gonella PM, Robinson AS, Fleischmann AS. 2020. Conservation of carnivorous plants in the age of extinction. *Global Ecol Conserv* 24, e01272. DOI: 10.1016/j.gecco.2020.e01272.
- Gaume L, Bazile V, Huguin M, Bonhomme V. 2016. Different pitcher shapes and trapping syndromes explain resource partitioning in *Nepenthes* species. *Ecol Evol* 6 (5): 1378-1392. DOI: 10.1002/ece3.1920.
- Gaveau DLA, Sheil D, Husnayaen, Salim MA, Arjasakusuma S, Anrenaz M, Pacheco P, Meijaard E. 2016. Rapid conversions and avoided deforestation: Examining four decades of industrial plantation expansion in Borneo. *Sci Rep* 6, 1-13. DOI: 10.1038/srep32017.
- IUCN. 2022. IUCN red list. IUCN Red List. <https://www.iucnredlist.org/search?query=nepenthes&searchType=species>.
- Kalima T, Denny D. 2019. Komposisi Jenis Dan Struktur Hutan Rawa Gambut Taman Nasional Sebangau, Kalimantan Tengah." *Jurnal Penelitian Hutan dan Konservasi Alam* 16 (1): 51-72. DOI: 10.20886/jphka.2019.16.1.51-72. [Indonesian]
- Lam WN, Chong KY, Anand GS, Wah Tan HT. 2017. Dipteran larvae and microbes facilitate nutrient sequestration in the *Nepenthes gracilis* pitcher plant host. *Biol Lett* 13 (3). DOI: 10.1098/rsbl.2016.0928.
- Lam WN, Lai HR, Lee CC, Tan HTW. 2018. Evidence for pitcher trait-mediated coexistence between sympatric *Nepenthes* pitcher plant species across geographical scales. *Plant Ecol Divers* 11 (3): 283-294. DOI: 10.1080/17550874.2018.1517831.
- Meijaard E, Brooks TM, Carlson KM, Slade EM, Garcia-Ulloa J, Gaveau DLA, Lee JSH., Santika T, Juffe-Bignoli D, Struebig MJ, Wich SA, Anrenaz M, Koh LP, Zamira N, Abrams JF, Prins HHT, Sendashonga CN, Murdiyarsa D, Furumo PR, Sheil D. 2020. The environmental impacts of palm oil in context. *Nat Plants* 6 (12): 1418-1426. DOI: 10.1038/s41477-020-00813-w.
- Moran JA, Gray LK, Clarke C, Chin L. 2013. Capture mechanism in Palaeotropical pitcher plants (*Nepenthes*) is constrained by climate. *Ann Bot* 112, 1279-1291. DOI: 10.1093/aob/mct195.
- Pavlovič A. 2012. Sequestration strategies in the carnivorous plants of the genus *Nepenthes*. *Plant Signaling Behav* 7, 295-297. DOI: 10.4161/psb.18842.
- Rembold K, Fischer E, Wetzel MA, Barthlott W. 2010. Prey composition of the pitcher plant *Nepenthes madagascariensis*. *J Trop Ecol* 26, 365-372. DOI: 10.1017/S026646741000012X.
- Scharmann M, Thornham DG, Grafe TU, Federle W. 2013. A novel type of nutritional ant - Plant interaction: ant partners of carnivorous pitcher plants prevent nutrient export by dipteran pitcher infauna. *PLoS ONE* 8 (5): e63556. DOI: 10.1371/journal.pone.0063556.
- Setiawan H, Wardhani HAK, Kamaludin K, Hutagaol RR, Afriani R. 2018. The diversity of *Nepenthes* at the post-mining area in Sintang District, West Kalimantan, Indonesia. *Biodiversitas* 19 (5): 1820-1827. DOI: 10.13057/biodiv/d190532.
- Sunardi S, Mansur M. 2021. Kelimpahan, asosiasi dan ancaman habitat *Nepenthes bicalcarata* Hook.f. di Cagar Alam Mandor, Kalimantan Barat. *Buletin Kebun Raya* 24 (2): 66-75. DOI: 10.14203/bkr.v24i2.734. [Indonesian]
- Tarigan MRM, Corebima AD, Zubaidah S, Rohman F. 2021. Arthropods discovered in lower and upper pitchers of *Nepenthes* at rampa-sitahuis hill, north sumatra, indonesia. *Biodiversitas* 22 (12): 5358-5366. DOI: 10.13057/biodiv/d221217.
- Thornham DG, Smith JM, Grafe TU, Federle W. 2012. Setting the trap cleaning behaviour of *Camponotus schmitzi* ants increases long-term.pdf. *Funct Ecol* 26, 11-19. DOI: 10.1111/j.1365-2435.2011.01937.x.