Comparison of Morphological Characters from Bornean Lowland *Nepenthes* Inflorescences: Case Study from Post-Mining Area of Sintang Regency West Kalimantan

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Abstract. The existence of inflorescence is an important aspect of *Nepenthes* conservation. However, it's usually abandoned because they are rarely found in nature. This research aimed to compare the morphological characters of the Bornean lowland *Nepenthes* inflorescences based on species and sexes. It was conducted in the post-mining area of Sintang Regency, West Kalimantan Province, Indonesia, from October 2022 to February 2023. Inflorescence samples were collected from males and females of *N. ampullaria*, *N. bicalcarata*, *N. gracillis*, *N. mirabilis*, *N. rafflesiana* var. typica. Inflorescence morphometry characters consist of inflorescence length, peduncle length, rachilla length, flower length, tepal length, tepal width, and number of pistillate/staminate per inflorescence. The results showed that morphological characters of Bornean lowland *Nepenthes* inflorescence differ between species and sexes. *N. ampullaria* is characterised by having the highest number of flowers of any *Nepenthes* species. In contrast, *N. bicalcarata* is characterised by inflorescence length and peduncle length. Males and females have different morphological characteristics. Males have significantly longer and wider tepals and more flowers per inflorescence than females of Bornean lowland *Nepenthes*.

1 Introduction

Nepenthes (Nepenthaceae) is a tropical pitcher plant with leaf modifications into jug-shaped organs called pitcher [1]. *Nepenthes* pitcher has a vital role in fulfilling nutritional needs. This plant lives in a habitat with poor macronutrients such as nitrogen and phosphorus [2].

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The nutritional gap is filled by pitchers, which trap and extract arthropod prey (carnivorous plants), leaf litter (detritivorous plants), and others for alternative nutrition sources [4, 5].

Nepenthes habitat is spread out on the palaeotropical area from Madagascar to New Caledonia, with the centre of its diversity on Southeast Asia [1]. To this day, *Nepenthes* species consist of more than 140 species in the world and are mostly found in the Philippines (40 species), Sumatera (35 species), Borneo (34 species), and Peninsular Malaysia (19 species) [5].

Altitudes are one of the important roles in *Nepenthes* categorisation [2]. If *Nepenthes* lives on a habitat altitude between 0-1100 m asl, it is categorised as lowland *Nepenthes*. Meanwhile, it will be categorised as highland *Nepenthes* if it lives on a habitat altitude of more than 1100 m asl [1]. Based on [5], the highland of Borneo (including Kalimantan, Sarawak, Sabah and Brunei Darussalam) is an important habitat for *Nepenthes* because of 21 species (62%) compared to 13 species (38%) in the lowland habitat. However, Cross et al. state that lowland habitat faces more anthropogenic threats than highland habitats, which threaten *Nepenthes*' existence in this area [6].

Nepenthes breeding occurs as a result of fertilisation of male and female flowers. *Nepenthes* is a dioecious species. Males and females are separated into different individuals [7]. This condition can be a conservation problem if the flowering time of *Nepenthes* is not in the same period. Post-mining area on Kalimantan Island is one of the suitable habitats for *Nepenthes* to live and breed [8]. Macro nutrients are scarce in post-mining areas. *Nepenthes* in this habitat adapt by increasing their breeding function through inflorescences.

Morphological description of *Nepenthes* inflorescence is usually being part of the new species description. Nevertheless, the inflorescence description of well-known species is hard to find and is based on qualitative data from field observation or herbarium specimens. Based on [1], comparing *Nepenthes* inflorescences is an important clue to species identification but is rarely used because of its periodicity. Some researchers interested in the *Nepenthes* flower usually describe the condition of the flower qualitatively [8, 10]. The research on morphometrics of lowland *Nepenthes* inflorescences is an interesting topic to be done. This research aims to compare the morphological characters of Bornean lowland *Nepenthes* inflorescences based on species and sexes. The results of this research can be a good addition for *Nepenthes* conservation strategy. It will give a different perspective on *Nepenthes* conservation act which usually focuses on pitchers rather than dioecious flowers.

2 Materials and method

This research was done on the post-mining area in Sintang Regency West Kalimantan Province Indonesia (Coordinate 0°2'28,482" N and 9°28'57,396 E) (Fig. 1). [8] state that the post-mining area is ideal for lowland *Nepenthes* in the Sintang Regency. This area is a former heath forest (*kerangas*) converted to illegal gold mining operated by the local community. After the gold mine was not operating, it was then used as a sand and stone mining area for local community construction. Some areas that were not carried out sand and stone mining operations were then revegetated by several types of plants including *Nepenthes* species. The gold mine operated from the 1990s to the early 2000s. Then the sand and stone mining area is carried out since the gold mine has not been operating until now (in 2023).

This research was conducted from October 2022 to February 2023; it is a rainy season in the Sintang Regency area. [5] state that wet conditions (in rainy season or humid areas) can help *Nepenthes* to maintain the evaporation from its pitcher and help wet the peristome for prey capturing mechanism, which leads to *Nepenthes* flourishing after fulfilling the macronutrients.

The exploration method with purposive sampling was used to observe *Nepenthes* on flowering conditions. For the location of *Nepenthes* inflorescences used as research stations,

the researchers applied three plots of 10 m x 10 m on three stations which separated 100 m of minimum distance to observe *Nepenthes* (Fig. 1). This plot size (10 m x 10 m) is an optimal minimum area on *Nepenthes* observation [8]. The researchers used 122 males and 105 females on the morphometric data from five lowland *Nepenthes* species. We used a guidance book from [1] and other relevant sources to identify *Nepenthes* species found. Based on Handayani [9], males are more commonly found on *N. mirabilis* than females and can be a reference for other lowland *Nepenthes* which share the same habitat with this species. All the *Nepenthes* inflorescence used in this research are safe (not harmful), except one of each *Nepenthes* flower used as the photograph sampled (one flower per species).

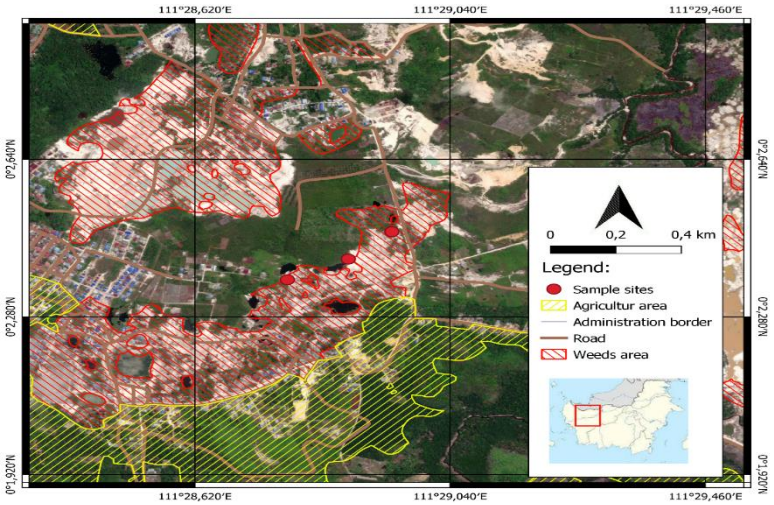

Fig. 1. Map of study location in Sintang District, West Kalimantan Province, Indonesia

The samples used in this research consist of males and females of *N. ampullaria*; *N. bicalcarata*; *N. gracilis*; *N. mirabilis*, and *N. rafflesiana* var. typica, which can be seen in Fig. 2. We used 16 males and two females of *N. ampullaria*, seven males and four females of *N. bicalcarata*, 34 males and 34 females of *N. gracilis*, 32 males and 32 females of *N. mirabilis*, and 33 males and 33 females of *N. rafflesiana* var. typica. The number of flowers is varied on each species depending on its existence in nature.

Fig. 2. Bornean lowland *Nepenthes* Inflorescence. A1&A2 are males and females of *N. ampullaria*, B1&B2 are males and females of *N. bicalcarata*, C1&C2 are males and females of *N. gracilis*,

D1&D2 are males and females of *N. mirabilis*, E1&E2 are males and females of *N. rafflesiana* var. typica.

Morphological characteristics of *Nepenthes* inflorescences consist of length, peduncle length, rachilla length, flower length, tepal length, tepal width, and number of flowers per inflorescence. All morphological characters are illustrated in Fig. 3.

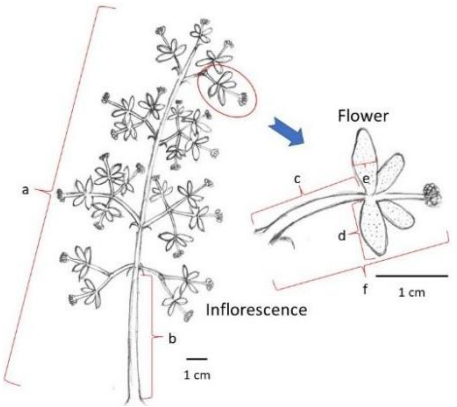

Fig. 3. Illustration of *Nepenthes* inflorescences morphological characters (panicle-like). a) Inflorescence length; b) peduncle length; c) rachilla length; d) tepal length; e) tepal width; f) flower length

Qualitative characters of *Nepenthes* inflorescences, such as colour, odour, and hairiness, were also recorded. For all these data, a binary quantitative score was applied (0/1) [10]. The binary code for colour was 0 for green and 1 for another colour, then green. Meanwhile, the binary code was defined as 0 for no odour and 1 for any odour that could be smelled from inflorescences. The binary code for hairiness was 0 for no hair found and 1 for any hair in *Nepenthes* inflorescences.

The differences in inflorescence morphometry data on each *Nepenthes* were analysed using Brown Forsythe and followed by the Games Howell test at the 0.05 level using SPSS 16.00. Morphometry data and qualitative data on flower morphology were analysed using PCA (PAST software version 4.03) to determine the comparison between inflorescences.

3 Results and discussions

3.1 Results

The morphological characters of Bornean lowland *Nepenthes* inflorescences show differences in the character of each *Nepenthes* species. Morphological characters differ significantly between species, and parameters are distinguished by different notations (Fig. 4).

Inflorescence length data showed that all *Nepenthes* species have a significantly different average length (Fig. 4A). *Nepenthes* with the longest inflorescences are males of *N. bicalcarata* with an average of 72.46 cm, while the shortest are females of *N. gracilis* with 13.54 cm long. There are significant differences between sexes in *N. ampullaria*, *N. gracilis,* and *N. mirabilis* inflorescences, while there were no significant differences in *N. bicalcarata* and *N. rafflesiana* (Fig. 4A).

Tepal length characters show significant differences between *Nepenthes* species (Fig. 4E). The longest tepal length average is in males of *N. rafflesiana*; it is 9.77 mm. It is

compared to females of *N. gracilis* which only have a 2.83 mm tepal length average. There are no significant differences in mean tepal length between the sexes in *N. bicalcarata*, while all other species have significant differences. In *N. ampullaria*, *N. gracilis*, *N. mirabilis,* and *N. rafflesiana*, the average tepal length of male flowers is longer than that of female flowers (Fig. 4E).

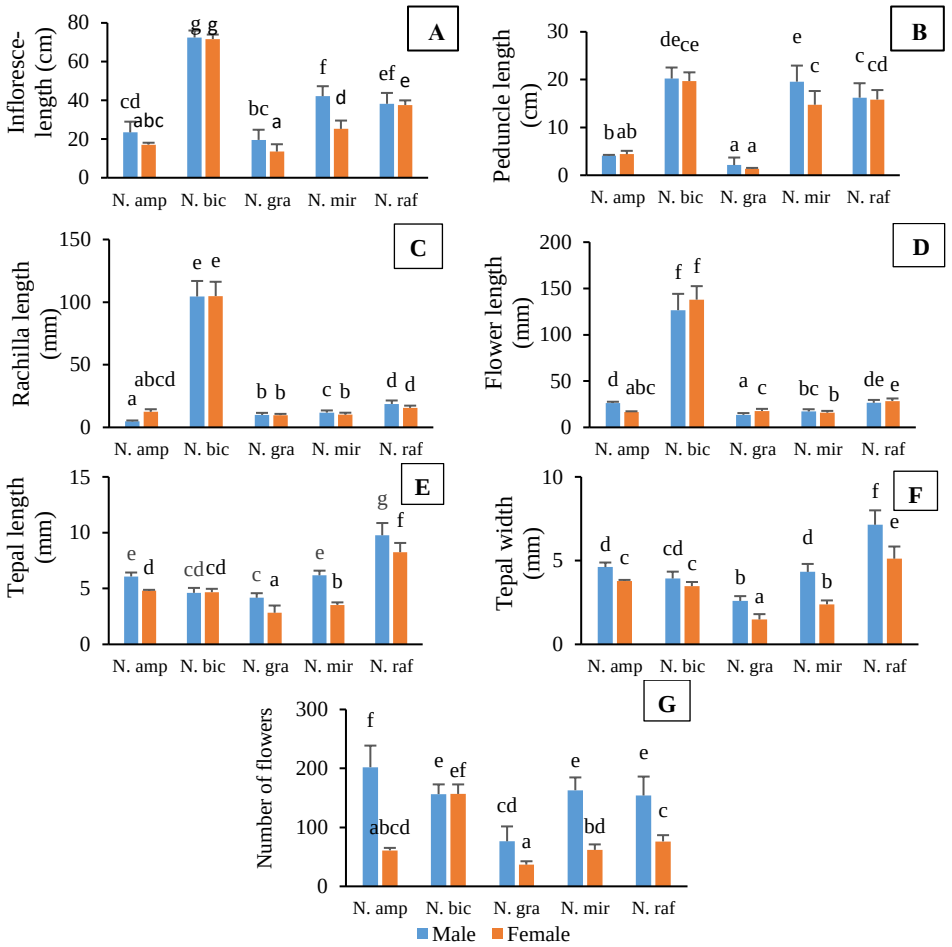

Fig. 4. Comparisons of Bornean *Nepenthes* lowland inflorescence characters. The same notation on the same parameter indicates no significant difference based on the Brown Forsythe test followed by Games Howell α= 0.05. N. amp = *N. ampullaria*, N. bic = *N. bicalcarata,* N. gra = *N. gracilis,* N. mir = *N. mirabilis*, N. raf = *N. rafflesiana*.

Table 1. Qualitative morphological data on Bornean Lowland *Nepenthes* flowers

Parameter	Sexes	N. amp	N. bic	N. gra	N. mir	N. raf
Colour	Male	1	1	1	1	1
	Female	1	1	1	1	1
Odor	Male	1	1	1	1	1
	Female	1	1	1	1	1
Hairiness	Male	1	0	0	0	1
	Female	1	0.	0	0	1

Note: Color 0= green color, 1 =other color than green; Odor 0 = odorless, 1=odors; Hairiness 0 =no hair, 1=hairy. N. amp = *N. ampullaria*, N. bic = *N. bicalcarata,* N. gra = *N. gracilis,* N. mir = *N. mirabilis*, N. raf = *N. rafflesiana*

Qualitative data of *Nepenthes* inflorescences consist of a variety of colors, the presence of odor and hair. The qualitative data of *Nepenthes* inflorescences can be seen in Table 1. All Bornean lowland *Nepenthes* flowers have a variety of colors (not only green). For example, *N. ampullaria* has yellowish-green flowers ranging from rachillas to tepals. *N. bicalcarata* flowers have light green on rachillas, while tepals are brownish-red. *N. gracilis* flowers have dark green on the rachilla, while tepals are brownish-red. *N. mirabilis* flowers are light green on the rachilla, while tepals are white to brownish red. *N. rafflesiana* is dark green on rachilla, while tepals are white to brownish-red (see Fig. 2).

Every Borneo lowland *Nepenthes* inflorescence has a distinctive odour. However, it generally can be observed in the morning, when the smell decreases during the day. *N. ampullaria* and *N. rafflesiana* have delicate hair on their rachilla, tepals, male column and female ovary. Meanwhile, *N. bicalcarata*, *N. gracilis* and *N. mirabilis* have no hairs on their flower. Furthermore, only hairiness data can be continued with PCA analysis because no colour and odour data variation exists (Fig. 5). This PCA results can explain 80% from all variables used.

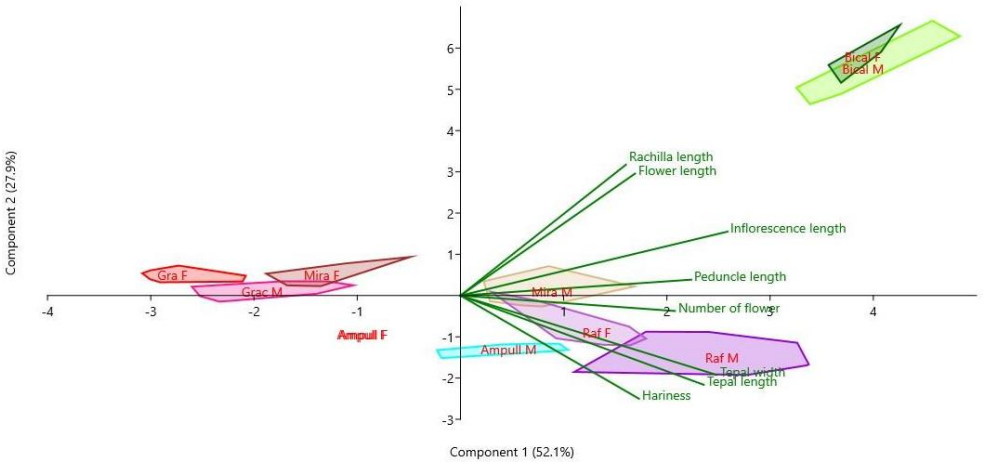

Fig. 5. Biplot for *Nepenthes* inflorescence characters. F = females; M = Males; Ampull = *N. ampullaria*; Bical = *N. bicalcarata*; Gra = *N. gracilis*; Mira = *N. mirabilis*; Raf = *N. rafflesiana*.

Based on PCA analysis, the morphological characteristic of males and females are different; it can be seen from the non-overlap grouping of male and female data. *N. bicalcarata* generally has a higher inflorescence, rachilla, and flower length than other species. Males and females of *N. rafflesiana* and males of *N. mirabilis* are characterised by higher peduncle length, number of flowers, petal length, and petal width. Meanwhile, males and females of *N. gracilis*, females of *N. ampullaria,* and females of *N. mirabilis* have opposite characters with lower values of all parameters.

3.2 Discussions

Bornean lowland *Nepenthes* with the most extended average inflorescences length are *N. bicalcarata*, followed by *N. mirabilis*, *N. rafflesiana*, *N. ampullaria*, and *N. gracilis*. *N. bicalcarata* is known to have an alternative nutrition source such as leaf litter and other sources which lead this species to adapt more with their habitat including the length of

inflorescences [4]. Inflorescence and peduncle length are suggested to have correlations to avoiding conflicts between prey and pollinators. This indication based on research by El-Sayed et al. [11] mention that the distance between the inflorescence and the nearest traps affects pollination's success in the genus Drosera's carnivorous plants. The farther the distance between the inflorescence and trap, can minimise the possibility of pollinating insects being caught as prey.

In addition to inflorescence length, flower color also affects the possibility of inflorescence being observed visually [9]. All Bornean lowland *Nepenthes* flowers have various colors (other than green), contrasting them with the background (Table 1). Flower color is one of the characteristics that attract pollinators to visit and spread pollen to various types of flowers; insects carry out this pollination.[12] stated that the more flower color contrasts with the background, makes it easier to be found by pollinators.

Based on Kato [13], non-flying insects are very rarely able to help pollination because of the antibiotic substances' presence produced by metapleural glands that harm pollen. The flower length of Bornean lowland *Nepenthes* shows that *N. bicalcarata* has the longest flowers among other species. The flower length of *N. bicalcarata* aligns with the body length of *N. bicalcarata,* which is known as the largest among other lowland species [1]. Moreover, the association between *N. bicalcarata* and the ant *Camponotus schmitzi* can be the reason for the length of the flowers compared to other species. *C. szhmitzi* may interfered *N. bicalcarata* pollen production to some degree.

Tepals are nectar producers in *Nepenthes* flowers. Tepal size indicates the amount of nectar produced by *Nepenthes* flowers [9]. The larger the tepal of *Nepenthes* flowers, the greater the number of nectars produced. This nectar production is an attraction for pollinator insects so that they can spread the pollen. The largest tepal size (length and width) in Bornean Lowland *Nepenthes* is owned by *N. rafflesiana*. Males of *N. rafflesiana* have an average tepal length of 9.77 mm and width of 7.14 mm, while females have an average length of 8.26 mm and width of 5.12 mm. It is inversely proportional to the size of *N. gracilis* tepals, with the smallest size among other species. The average length of males of *N. gracilis* is 4.17 mm, and the width is 2.59 mm. While for females' average length is 2.83 mm, and the width is 1.49 mm.

Interestingly, all males' tepal sizes (length and width) of Bornean lowland *Nepenthes* are larger than females (Fig. 2, Fig. 4 E&F). The tepal size of males of *N. ampullaria*, *N. gracilis*, *N. mirabilis,* and *N. rafflesiana* is significantly larger than females. Based on [14] males can attract more visitors (pollinator, prey or herbivore) than females due to the larger tepal size, estimated nectar production, and other complex interactions of dioecious flowers in *Eurya japonica*. It is also supported by [13] who states that the amount of nectar produced by male tepals of *N. gracilis* is more than females, with a total of 85 µl compared to 26 µl each. However, this statement needs to be confirmed by further research. Besides, there has been no research that explains the preferences of visitors to *Nepenthes'* male and female flowers, so that it will be interesting for future research.

Nepenthes species are known to produce flowers in large numbers in support of the breeding process [1]. The lowland *Nepenthes* that produced the most flowers were male flowers of *N. ampullaria,* with an average of 202.06 flowers per inflorescence, while females of *N. gracilis* owned the fewest with an average of 76.41 flowers per inflorescence. The results showed that males of all Bornean lowland *Nepenthes* were more abundant than females. It is due to the need for male flowers to spread pollen to be able to fertilise female flowers.

4 Conclusion

Morphological characters of Bornean lowland *Nepenthes* inflorescences differ between species and sexes. *N. ampullaria* has the highest number of male flowers than others. *N. bicalcarata* is characterised by inflorescences and rachilla length. *N. gracilis* was characterised by the lowest measurement values for all characters compared to other species. Males and females have different morphological characteristics. Males have significantly longer and wider tepals and more flowers per inflorescence than females of Bornean lowland *Nepenthes*. Inflorescences are an important part of Bornean lowland *Nepenthes* conservation. When *Nepenthes* are able to produce more numbers and bigger sizes of males compared to females and females succeed in becoming fruit then the conservation act of *Nepenthes* can be categorised as successful.

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