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Analysis Of Natural Hybrid Between Nepenthes Gymnamphora Reinw. Ex Nees With Nepenthes Inermis Danser Based On Geometric Morphometrics

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Abstract – Recently, based on preliminary observations in the field of *Nepenthes* populations found in Mount Kunyit, Kerinci Regency, Sumatra, several *Nepenthes* species were found to live sympatrically, namely *N. gymnamphora* with *N. inermis*. On the other hand, a population of *Nepenthes* that is assumed to be a natural hybrid of the two species was also found. This unidentified taxon is very different from other *Nepenthes* in the habitat because it has *intermediate* pitcher characters with its parents. However, it is not yet known which taxon is the parent. In this study, the approach used was geometric morphometric analysis. The images were photographed from two different views, i.e., top view (lid) and front view (body with peristome). A total of 17 images (5 images from *N. gymnamphora*, 6 images from *N. inermis* and 6 images from its natural hybrid. All analyses used *Momocs Package* in *R Statistical Software*. The results of this study are variations in the shape of the pitcher of putative natural hybrids between *N. gymnamphora* and *N. inermis* species in the shape of the pitcher in front view and the size of the pitcher lid in lid view. *Nepenthes* individuals that have *intermediate* shape characters with both parents (*N. gymnamphora* and *N. inermis*) are natural hybrid individuals.

Keywords – Geometric morphometrics, intermediate, Natural hybrid, *Nepenthes*, PCA.

I. INTRODUCTION

Nepenthes L. or pitcher plants is the only genus of the family Nepentheaceae and the largest carnivorous plant family of the order Caryophyllales. Currently, there are 168 species of *Nepenthes* in the world [1]. The pitcher character is the most important part to distinguish the native species of *Nepenthes* and its natural hybrids due to flowers and fruits do not show distinctive character differences [2]. The taxonomy of *Nepenthes* is complicated due to the high number of species and hybrids [3].

During a survey in 2021 of a Mount Kunyit, Kerinci Seblat National Park, Kerinci Regency, two species *Nepenthes* founded, namely *N. gymnamphora* Reinw. ex Nees and *N. inermis* Danser. Based on preliminary observations in the field, these two species *Nepenthes* live sympatric in the area and are founded to flower and fruit almost at all time. Sympatrically population and their ability to flower and fruit almost all the time provide greater possibilities for out crossing natural hybrid formation in the area [4]. In this habitat, *Nepenthes* assumed to be a crossbreed of the two species was found. These unidentified the taxon is very different from the parent in the habitat due to it have a mix pitchers characters between the parent both. Thus, it can be assumed that the group that has combined characters is probably a natural hybrid, and in this case it is very necessary to prove and further study.

In the case, the correct solution to prove the shape variation of the pitcher character and its natural hybrids can be explained more objectively and accurately through quantitative analysis, namely by geometric morphometric analysis methods. By using geometric morphometric methods, the resulting shape and size quantification is more accurate and specific [5]. On the other hand, this method has been successfully used to explain the variation in pitcher shape among several species of *Nepenthes* in Kalimantan [6].

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Thus, this research is very important to conduct with the assumption that geometric morphometric analysis can be used to determine the existence of natural hybrids of *Nepenthes* in Sumatra morphologically. This research is expected to be able to get information about the differences or similarities of the characters of these natural hybrid individuals with their parents, so as not to cause confusion in the recognition of *Nepenthes* in the future.

II. Research Methods

Study area

This study was conducted in Mount Kunyit, Kerinci Seblat National Park, Talang Kemuning Village Gunung Raya sub-district, Kerinci district, Jambi Province, Sumatra Mount Kunyit is located at coordinates 02°16'34.6" S and 101°29'07.8" E, as depicted in Figure 4. Further analysis was carried out at the Herbarium of Andalas University (ANDA), Department of Biology, Padang.

III. Methods

Image of pitcher *Nepenthes* from Mount Kunyit were collected in September to Desember 2021. The images were photographed from two different views, i.e., top view (lid) and front view (body with peristome). A total of 18 images (6 images from *N. gymnamphora*, 6 images from *N. inermis* and 6 images from its natural hybrid). Before conducting the outline analysis, the original images will be transformed into black silhouettes. On the other hand, outlines must be aligned to remove differences in rotation, translation, or size. We used two landmarks to align the outlines using a full Procrustes superimposition [7]. One landmark were defined at the bottom of the image as a starting point of the coordinates, and the another landmark on the outline vertically above the first landmark. The silhouettes were then converted into (x; y) coordinates. All the procedures of statistical analyses were conducted using the Momocs package version 1.2.3 [5] in the R version 3.2.4 12 environment [8].

IV. Result And Discussion

Outline analysis and Variation in Putative Pitcher Shape of Natural Hybrids between *N. gymnamphora* and *N. inermis*.

The right number of harmonics was decided based on the reconstruction of the pitcher outline using harmonic power. In the study, eight harmonics for the side view, eight harmonics for the front view, eleven harmonics for the lid view, where enough to gather at least 99% of the total harmonic power. The power is proportional to the harmonic amplitude and can be considered as a measure of pitcher shape information.

Figure 1 show the image recronstruction of the front view of Hybrid putative. In this view using harmonics same with side view number of harmonics (h). 8 harmonics were enough to recronstruct the original image with gathered 99% of the harmonic power. The number of harmonics depends on the complexity of the shape [5]. Higher harmonics add detail to the outline. Harmonics are trigonometric sine and cosine waves, the first of which describes a simple ellipse.

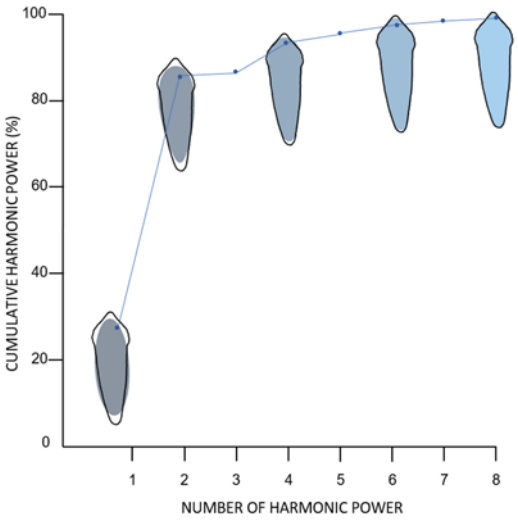

Fig. 1. The outline reconstruction of the original image of the front view. 8 harmonics were enough to reconstruct the original image which gathered 99% of the harmonic power.

Proving Natural Hybrids of *N. gymnamphora* and *N. inermis* with *Principal Component Analysis* (PCA).

Nepenthes species produces a natural hybrid in the wild. Hybridization in this genus produces a pitcher which characters that are intermediate in appearance between its parents [9]. This study proving the natural hybrid of *N. gymnamphora* with *N. inermis* using principal component analysis (PCA) or scatter diagram. In PCA, it proves that individuals with intermediate traits are natural hybrid individuals. [10] reported that principal component analysis achieved a high classification accuracy of 99.5% in a computation time of 0.4 seconds for each query image data.

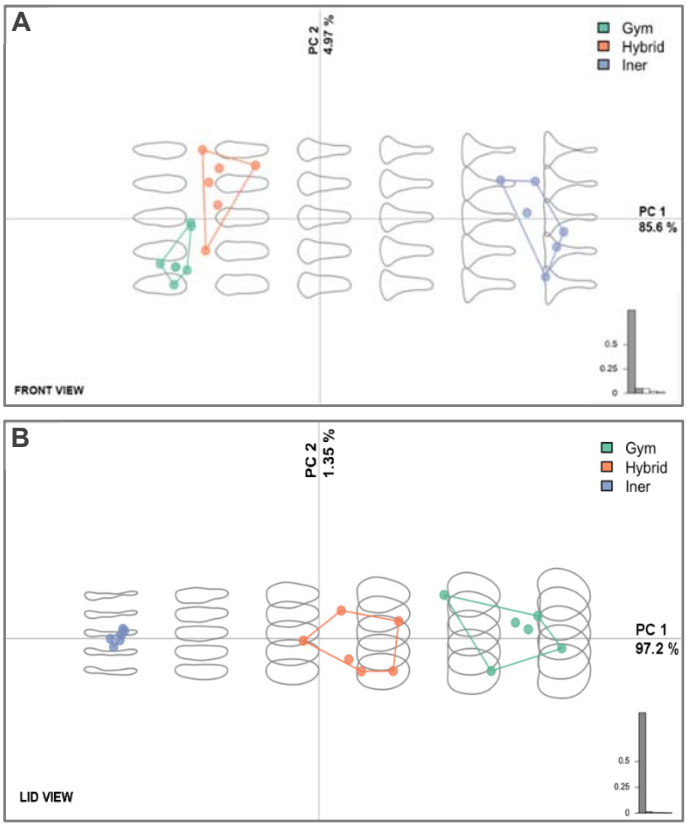

Figure 2. Principal component analysis (PCA) results of the matrix of the Fourier coefficient for putative hybrids *N. gymnamphora* x *N. inermis* with its parents (*N. gymnamphora* and *N. inermis*). (A) The scatterplot of the scores on the first two PCs show the variation in the shapes of pitcher on the front view. (B) The scatterplot of the scores on the first two PCs show the variation in the shapes of pitcher on the lid view. The morphological space were shown in the background to clarify the differences of pitcher shapes.

The scatter plot PCA shows that the shapes are separated and grouped based on species (Fig.1. A). On the front view, the natural hybrids are grouping close to *N. gymnamphora*. On the other wise, the pitchers of *Nepenthes* on the lid view, showed a clear separation between the two *Nepenthes* parents and their natural hybrids (Fig.2.B). The position of natural hybrids is between *N. gymnamphora* and *N. inermis* on the scatterplot of PCA with a morphological space in the background. [3] states that, the shape of hybrid is somewhat a mixture between the putative parental species. The first principal component (PC1), accounting for 85.6% of the total variance, highlighted the shape changes in the pitchers as a whole, as well as separated *N. gymnamphora*, *N. inermis* and their natural hybrids. Shapes presenting the lowest scores on this PC belonged to *N. gymnamphora* with a pitcher shape such as ovate. On the other hand, shapes presenting the highest values on this component belonged to *N. inermis* species with a pitcher shape as strikingly infundibular. Meanwhile, the natural hybrid was on the PC axis between the two parents, but adjacent to *N. gymnamphora*. natural hybrids have a pitcher shape like obovate and show a negative value. The second principal component (PC2), explaining 4.97% of the variability, highlighted a change in the pitcher shape from obovate to oblanceolate appearance. Shapes presenting the lowest values on this component belonged to natural

hibrid species with a pitcher shape such as obovate. On the contrary, shape presenting the highest values on this component belonged to natural hibrid species with a pitcher shape such as oblanceolate.

Finally, on the lid view, showed a clear separation between the two *Nepenthes* parents and their natural hybrids. The natural hybrids group between *N. gymnamphora* and *N. inermis* on the scatter plot. The first principal component (PC1), explaining 97.2% of the variability, represents a change in the lid shape and size. Shapes presenting the lowest values on this component belonged to *N. gymnamphora* species with a lid shape such as orbicular, shapes presenting the highest values on this component belonged to *N. inermis* species with a lid shape such as linear and narrow. The second principal component (PC2), describing 1.35% of total variability, represent a change in the lid apex from rounded to acute. Shapes presenting the lowest and highest values on this component belonged to natural hibrid species with a lid shape such as ovate. On the other hand, based on the front and lid view, the multivariate analysis of variance (MANOVA) shows that the shapes of *N. gymnamphora*, *N. inermis* and the natural hybrid are distinct, with P-value ($p<0.05$) (Table 1 and 2).

Table 1. The multivariate analysis of variance (MANOVA) on the front view

	gym	gym × iner	iner
N. gymnamphora		***	***
gym × iner			***

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '-' 1; ' ;

Table 2. The multivariate analysis of variance (MANOVA) on the lid view

	gym	gym × iner	iner
N. gymnamphora		***	***
gym × iner			***

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '-' 1; ' ;

Classification of Pitcher Shapes and Model Training

The machine learning algorithms used contributed to successful identification based on the pitcher outlines. The accuracy of the identification from two different views is shown in Table 3. In this table, *N. inermis* reach impeccable identification (100%) for the front and lid view. On the other hand, *N. gymnamphora* have an identification accuracy value of 80% for the front view, and 100% for the lid view. In addition, natural hybrids have an identification accuracy value of 83% for the front view, and 100% for the lid view.

Table 4 The accuracy of identification from LOOCV

Spesies	Front view (%)	Lid view (%)	N
N. gymnamphora	80		5
N. inermis	100		6
N. gym × *N. iner*	83		6

N= Total of Samples

V. CONCLUSIONS

Variations in the shape of the pitcher of putative natural hybrids between *N. gymnamphora* and *N. inermis* species in the shape of the pitcher in front view and the size of the pitcher lid in lid view. *Nepenthes* individuals that have intermediate shape characters with both parents (*N. gymnamphora* and *N. inermis*) are natural hybrid individuals. MANOVA values of the shape characters of *Nepenthes* elders and natural hybrids in the two views had significant p-values (<0.05).

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