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*Floribunda* merupakan organ resmi Penggalang Taksonomi Tumbuhan Indonesia, diterbitkan dua kali setahun dan menerbitkan makalah dalam bahasa Indonesia dan Inggris mengenai pelbagai gatra sistematika keanekaragaman flora Malesia pada umumnya dan Indonesia pada khususnya yang berasal dari hasil penelitian, pengamatan lapangan, pengalaman pribadi, telaahan bergagasan, dan tinjauan kritis.

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##### **Penyunting**

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Makalah lengkap memuat hasil penelitian floristik, revisi, atau monografi unsur-unsur flora Malesia. Komunikasi pendek mencakup laporan kemajuan kegiatan penelitian, pengembangan dan rekayasa keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituari tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktual seputar kegiatan penelitian, pengembangan dan rekayasa tetumbuhan Indonesia, serta timbangan buku akan dimuat berdasarkan undangan.

#### **Rujukan pembakuan**

Pemakaian Bahasa Indonesia sepenuhnya mengikuti *Pedoman Umum Ejaan yang Disempurnakan*, *Pedoman Umum Pembentukan Istilah*, *Kamus Besar Bahasa Indonesia*, serta kamus-kamus istilah yang dikeluarkan Pusat Bahasa. Bahasa Inggris yang dipakai adalah the Queen English dengan berpedoman pada *Oxford Dictionary of*

*the English Language*. Ketentuan-ketentuan yang dimuat dalam *Pegangan Gaya Penulisan, Penyuntingan, dan Penerbitan Karya Ilmiah Indonesia*, serta *Scientific Style and Format: CBE Manuals for Author, Editor, and Publishers*, dan buku-buku pegangan pembakuan lain akan sangat diperhatikan. Kepatuhan penuh pada *International Code of Botanical Nomenclature* bersifat mutlak.

#### **Gaya penulisan**

Penulisan naskah yang akan diajukan supaya disesuaikan dengan gaya penulisan yang terdapat dalam nomor terakhir terbitan *Floribunda*.

Abstrak informatif supaya diberikan dalam bahasa Indonesia dan Inggris yang masing-masing tidak melebihi 200 kata. Sediakan sekitar 7 kata kunci untuk keperluan pengindeksan dan pemindaian.

Bilamana diperlukan ucapan terima kasih dan bentuk persantunan lain dapat dicantumkan sesudah tubuh teks tetapi sebelum daftar pustaka.

Pengacuan pada pustaka hendaklah dilakukan dengan sistem nama-tahun. Daftar pustaka supaya disusun berdasarkan alfabet nama pengarang dengan memakai sistem Harvard.

Gambar dan tabel merupakan pendukung teks sehingga perlu disusun secara logis dalam bentuk teks atau tabel atau sebagai gambar, tetapi tidak dalam bentuk ketiganya sekaligus. Siapkan gambar yang lebarnya dua kolom cetak.

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#### **Kantor penyunting**

Sidang penyunting *Floribunda*

Herbarium Bogoriense, Cibinong Science Center

Jalan Raya Bogor KM 46 Cibinong 16911

Telepon : (021) 8765066-67

Fax : (021) 8765059

E-mail : floribundaptti@gmail.com;

floribunda@ptti.or.id



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**LEAF ANATOMICAL COMPARISON BETWEEN NATURAL HYBRID *NEPENTHES AMPULLARIA* JACK × *NEPENTHES MIRABILIS* (LOUR.) DRUCE WITH THE PARENTAL SPECIES IN KERINCI, JAMBI**

**Dee Dee Al Farishy<sup>1,\*</sup>, Nisyawati<sup>2</sup>, Destario Metusala<sup>3</sup>**

<sup>1</sup>)Organisasi Mahasiswa Pecinta Tumbuhan (OMPT) Canopy, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Indonesia. Kampus UI Depok 16424, West Java, Indonesia.

<sup>2</sup>)Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

<sup>3</sup>)Purwodadi Botanical Garden, Indonesian Institute of Sciences (LIPI), Purwodadi 67163, Indonesia  
Email: deede.alfarishy@alumni.ui.ac.id. Phone: +62 838 9362 5971

Dee Dee Al Farishy, Nisyawati, Destario Metusala. 2020. Perbandingan Anatomi Hibrid Alam Daun *Nepenthes ampullaria* Jack x *Nepenthes mirabilis* (Lour.) Druce dengan Spesies Induk di Kerinci, Jambi. *Floribunda* 6(4): 141–153. — *Nepenthes* merupakan tanaman berumah dua yang dapat memproduksi persilangan alami, termasuk *N. ampullaria* dan *N. mirabilis*. Persilangan tersebut kurang lebih berbagi karakter atau peralihan antara kedua spesies parental. Objektif penelitian adalah untuk menganalisis kesamaan karakter taksa persilangan tersebut berdasarkan organ daun dasar. Sampel dikoleksi dari Danau Lingkat, Kerinci, Jambi. Data observasi diukur secara kualitatif, kuantitatif menggunakan SPSS 22 dengan tes parametrik, dan non-parametrik, serta Analisis Komponen Utama (AKU) dari 27 karakter. Secara kualitas, terdapat dua karakter persilangan yang berupa peralihan. Secara kuantitas, terdapat pula 14 karakter yang tidak berbeda signifikan, 1 karakter serupa *N. ampullaria*, 4 karakter serupa *N. mirabilis*, 1 karakter peralihan, dan 3 karakter berbeda dengan kedua parental.

Kata Kunci: *Nepenthes*, anatomi, persilangan alami, Kerinci, Sumatra.

Dee Dee Al Farishy, Nisyawati, Destario Metusala. 2020. Leaf Anatomical Comparison Between Natural Hybrid *Nepenthes ampullaria* Jack × *Nepenthes mirabilis* (Lour.) Druce with the Parental Species in Kerinci, Jambi. *Floribunda* 6(4): 141–153. — *Nepenthes* are dioecious plant that could produce natural hybrid, including *N. ampullaria* and *N. mirabilis*. The hybrid more or less have intermediate or sharing character between two parent species. The objective of the research were to analyze character similarity according to basic leaves organ. Sample collected from Lingkat Lake, Kerinci, Jambi. Observation data were measured qualitatively, quantitatively using SPSS 22 with parametric test, non-parametric test, and Principal Component Analysis (PCA) from 27 characters. Qualitatively, there are two hybrid characters that intermediate. Quantitatively, there are 14 characters are not significantly different, 1 character similar to *N. ampullaria*, 4 characters similar to *N. mirabilis*, 1 character intermediate, and 3 characters are different with two parent.

Keywords: *Nepenthes*, anatomy, natural hybrid, Kerinci, Sumatra.

*Nepenthes* are known to produce natural hybrid (Damayanti *et al.* 2011; Gronemeyer *et al.* 2016). This is common when two or more populations of *Nepenthes* species live in the same habitat and not isolated reproducibly (Heon & Clarke 2015). Morphologically, natural hybrid of *Nepenthes* could be distinguish in by similiar or

sharing characteristics between the two parental species (Clarke 1997).

More than 280 names of natural hybrid *Nepenthes* have been published with the nomenclature added of noto- (×) ephitet between two parental. These natural hybrid names include *N. alata* × *mirabilis*, *N. fusca* × *rajah*, and *N. gracilis*

× *reinwardtiana* (Kurata & Toyoshima 1972; Cheek & Jebb 1997; Mansur 2007; van der Ent *et al.* 2015). There are also hybrid that use new name marked by placing epitet notho-before the name of the taxa, such as *N. × alisaputraiana*, *N. × hookeriana*, *N. × kinabaluensis*, and *N. × trichocarpa* (Adam *et al.* 1992; Clarke 1997; Cheek & Jebb 2001).

One of natural hybrid of *Nepenthes* that have been found is *N. ampullaria* Jack × *mirabilis* (Lour.) Druce (Clarke 1997; Clarke 2001). In morphology, pitcher part of the taxa is not much different from the *N. mirabilis*. The characters include in the size of the pitcher are more elongated pitcher's body, wider pitcher's lid, and frequently produces upper pitcher. Without pitcher, added by same typical fimbriate leaf shape, the natural hybrid is very difficult to distinguish from parent *N. mirabilis* (Clarke 1997; Clarke 2001).

Taxa of *N. ampullaria* × *mirabilis* could be found in the Lingkat Lake area, Lempur Village, at the edge of Kerinci Seblat National Park (Susanti *et al.* 2014; Al Farishy *et al.* 2017). The population coexists with both parental species by the Lingkat Lake on freshwater swamp. There are no ecological boundaries of the two parental, so the cross-breeding path very likely to occur sustainably.

Basic research on anatomy of *Nepenthes* had been done since Metcalfe & Chalk (1950). Furthermore, identification of anatomical characteristics continues in both wild type and cultivated species (Toma *et al.* 2002; Pavlovic *et al.* 2007; Paluvi *et al.* 2015; Al Farishy *et al.* 2017). However, there have no studies outlining the tendency of *Nepenthes* anatomical similarities among natural hybrid with the parent, especially from natural habitat.

The aim of this research was to analyze the anatomical structure on the leaves of natural hybrid between *N. ampullaria* × *mirabilis* compared with the parental species grown in their natural habitat. Leaf blade was chosen because it shown to have an important role in *Nepenthes* adaptation although morphologically similar (Pavlovic *et al.* 2007). Pitchers organ are not included in this research as a limited range between the basic organ and the modification.

## MATERIAL AND METHODS

All the *Nepenthes* taxa used in this research were collected from Lake Lingkat, Desa Lempur, on the border of the Kerinci Seblat National Park, in March 2016. Three individuals have been collected for each taxa: *N. ampullaria* and *N. mira-*

*bilis* and natural hybrid *N. ampullaria* × *mirabilis*. Perfectly developed leaf blades as samples were selected and collected from each individual, with criteria that do not had curled sheets, dry parts, nor damaged and torn. Environmental notes were also recorded as additional data.

Each leaf sample were sliced transversally using mini-microtome with liquid preservation method (Metusala 2017), while the paradermals have been collected using leaf scraping method. Transversal sections were done on the midrib near the leaf base, middle, and near the tip part. Paradermal sections were done on the adaxial and abaxial side of the area other than the midrib. Ten repetitions of slices were performed from each individual.

The sections were stained using one to two drops of 1% safranin and 0.1% fast green in a modified solution of 70% alcohol and glycerin by comparison 3:1. Then, the sections were preserved with liquid preservation technique inside the microtube 1,5 mL. In this stage, sections can be stored for years (Metusala 2017).

Anatomical characteristics that observed include stomata, sessile glands, trichomes, cuticles, epidermis, hypodermis, mesophyll, and other features if it present. The sections was observed under the LEICA DM500 light microscope in the Department of Biology, Universitas Indonesia. Observation data were calculated qualitatively, quantitatively using SPSS 22 with parametric test One-Way ANOVA with Duncan's Multiple Range test for normal data, non-parametric test Kruskal-Wallis with Mann-Whitney test for abnormal data, and Principal Component Analysis (PCA).

## RESULTS AND DISCUSSION

### Habitat

Three *Nepenthes* taxa which found lives in conditions with almost the same temperature and humidity (Table 1). The three taxa also live at altitude of 1019 m above sea level (asl) on the lake's peat swamp. In addition, environmental data obtained can show that *N. ampullaria*, *N. mirabilis*, and *N. ampullaria* × *mirabilis* were able to live in full sunlight with less to intermediate shade, warm temperatures from 24 to near 27 °C, and mid-high humidity about 80%. These environmental conditions are at the threshold for *Nepenthes* growing requirement by minimum humidity of 70%, maksimum temperature of 30 °C, and of sufficient sunlight (Lloyd 1942; Heathcote 1985; Clarke 2001; Mansur 2006).

Table 1. Environmental conditions

Conditions	<i>N. ampullaria</i>	<i>N. mirabilis</i>	<i>N. ampullaria</i> × <i>mirabilis</i>
Light intensity (Klux)	1,21–15,65	10,34–19,2	6,55–7,00
Temperature (°C)	24–25,6	26,5–26,8	24,5–25,5
Air humidity (%)	86–87	79–82	86–88

### Qualitative Analysis

Result shows that there are some character similarities in anatomical leaves of *N. ampullaria* × *mirabilis* compared with the parental species. These similarities are the presence of adaxial cuticle layer, adaxial epidermis, adaxial hypodermis, palisade parenchyma, mesophyll, abaxial hypodermis, abaxial epidermis, kidney shaped stomatal guard cells, anomocytic type of subsidiary cells, presence of sessile gland, and circular arrangement of sclerenchyma in the midrib surrounding vascular bundles. The whole characters said to be general in *Nepenthesaceae* family (Metcalf & Chalk 1950; Osunkoya & Muntassir 2017). Other than that, Owen Jr. & Lennon (1999) and

Toma *et al.* (2002) found that idioblasts occur in mesophyll tissue in pitcher bodies before. In this study, idioblast observed extend to leaf blade mesophyll tissue. In addition, there are variations that can be measured from these basic characters (Figures 1, 2, and 3).

Result of the qualitative analysis shows that there are differences on the stellate trichomes and the abaxial sessile gland between the three taxa (Table 2). Species of *N. ampullaria* show a greater number of abaxial trichomes. On the other hand, *N. mirabilis* and *N. ampullaria* × *mirabilis* have trichomes that only present on the midrib and evenly scattered respectively.

Table 2. Leaf anatomical qualitative characters in differences

No.	Characters	<i>N. ampullaria</i>	<i>N. mirabilis</i>	<i>N. ampullaria</i> × <i>mirabilis</i>
1	Presence of abaxial trichome	Abundant	Very rare	Abundant only on midrib area
2	Shape of head sessile gland abaxial	orbicular from above	plus-shaped from above	plus-shaped from above

Variation in shape and distribution of trichomes within the three *Nepenthes* taxa that can be used as a distinguish character. Based on this study, there is a note that trichomes in *N. ampullaria* × *mirabilis* were only observed in transversal sections in the midrib area so that could not be measured quantitatively. However, the presence of trichomes on natural that hybrid taxa shows that there are sharing character appear on the leaf organ.

Shape and distribution of non-glandular trichomes on leaf blade varies greatly in each plant taxa. In other genera, non-glandular trichomes can be differentiated in some members of *Melolobium*, *Quercus*, *Capsicum*, and *Marubium* (Moteetee *et*

*al.* 2002; Vázquez 2006; Kim *et al.* 2012; Olaniran & Olamide 2014; Ahvazi *et al.* 2016). Therefore, the presence of a trichome can be a distinguish character on the lower taxa level.

Different shape of sessile glands was also shown on *N. ampullaria* by rounded head shape from the upper view, while both *N. mirabilis* and *N. ampullaria* × *mirabilis* share similar plus-shaped head. The difference is quite striking even though the number of head cells are 8 in each taxa. Metcalfe & Chalk (1950) suggested that glandular head in *Nepenthes* generally has eight cells. However, it is assumed that there is a potential variation in cell number and shape on the other species within family.

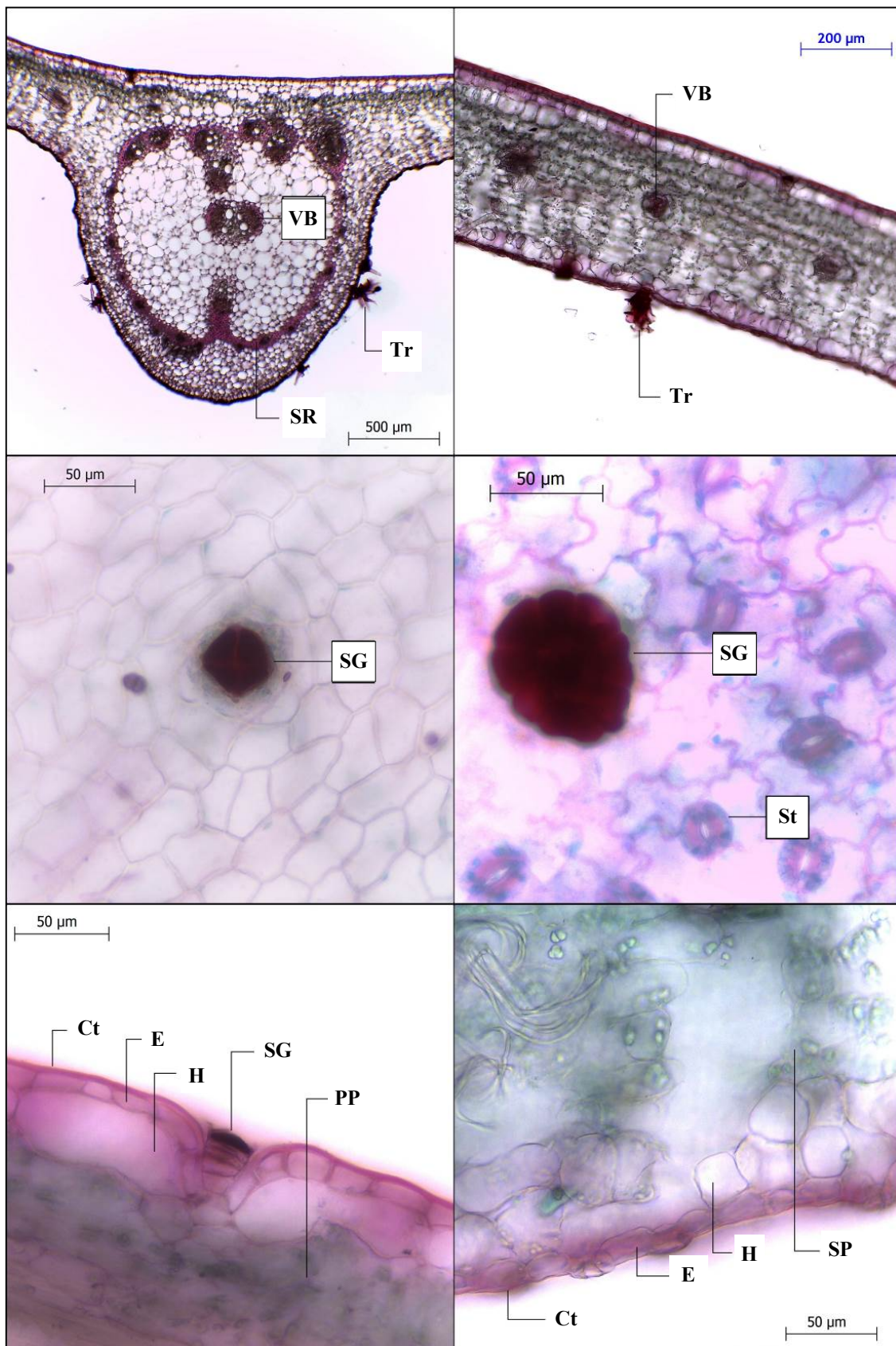


Figure 1. *N. ampullaria* Jack Ct: Cuticle layer; E: Epidermal layer; H: Hypodermal layer; PP: Palisade Parenchyma; SG: Sessile Gland; SP: Spongy Parenchyma; SR: Schlerenchyma Ring; St: Stomata; Tr: Trichome; VB: Vascular Bundle.

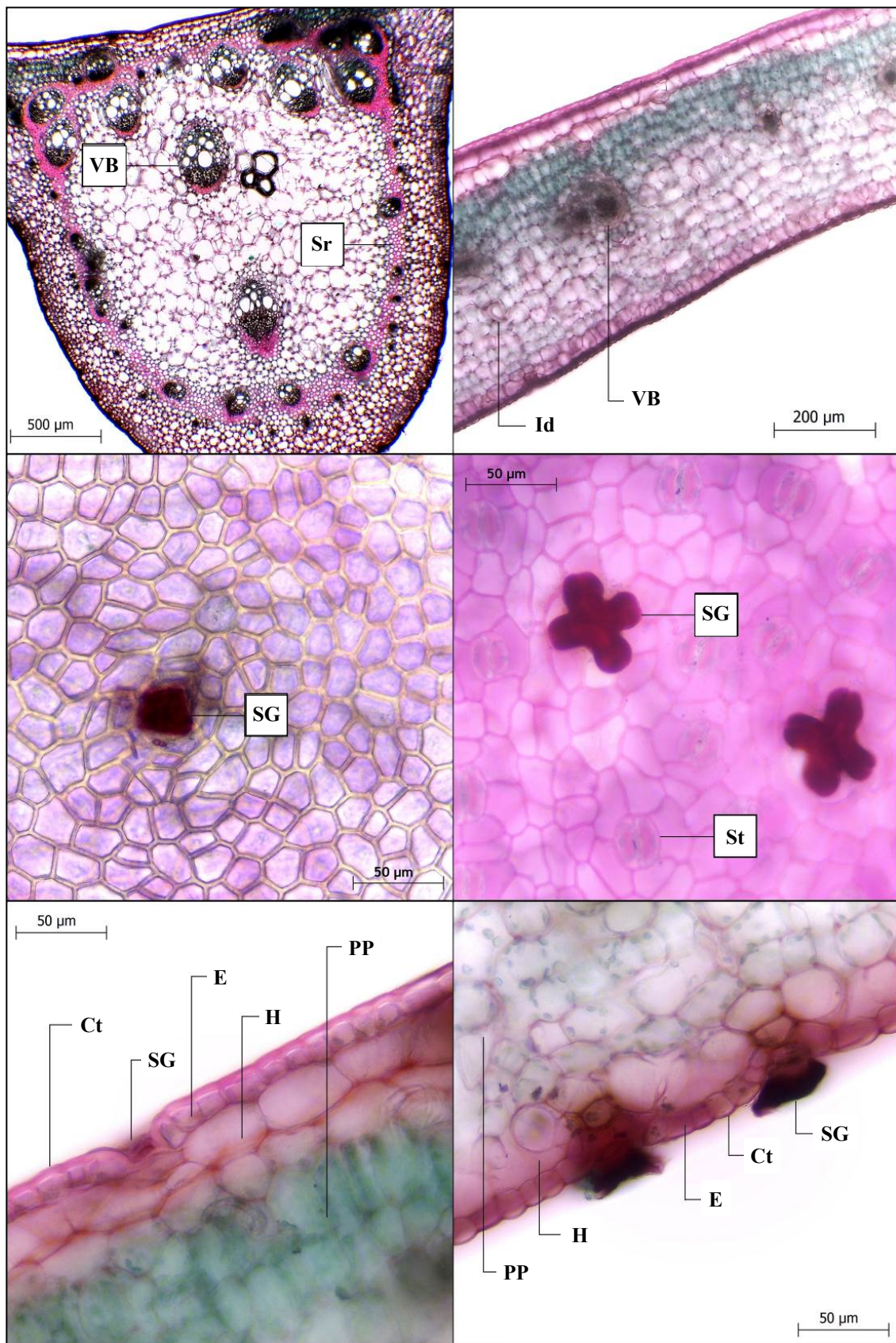


Figure 2. *N. mirabilis* (Lour.) Druce Ct: Cuticle layer; E: Epidermal layer; H: Hypodermal layer; PP: Palisade Parenchyma; SG: Sessile Gland; SP: Spongy Parenchyma; SR: Schlerenchyma Ring; St: Stomata; Tr: Trichome; VB: Vascular Bundle; Id: Idioblast.

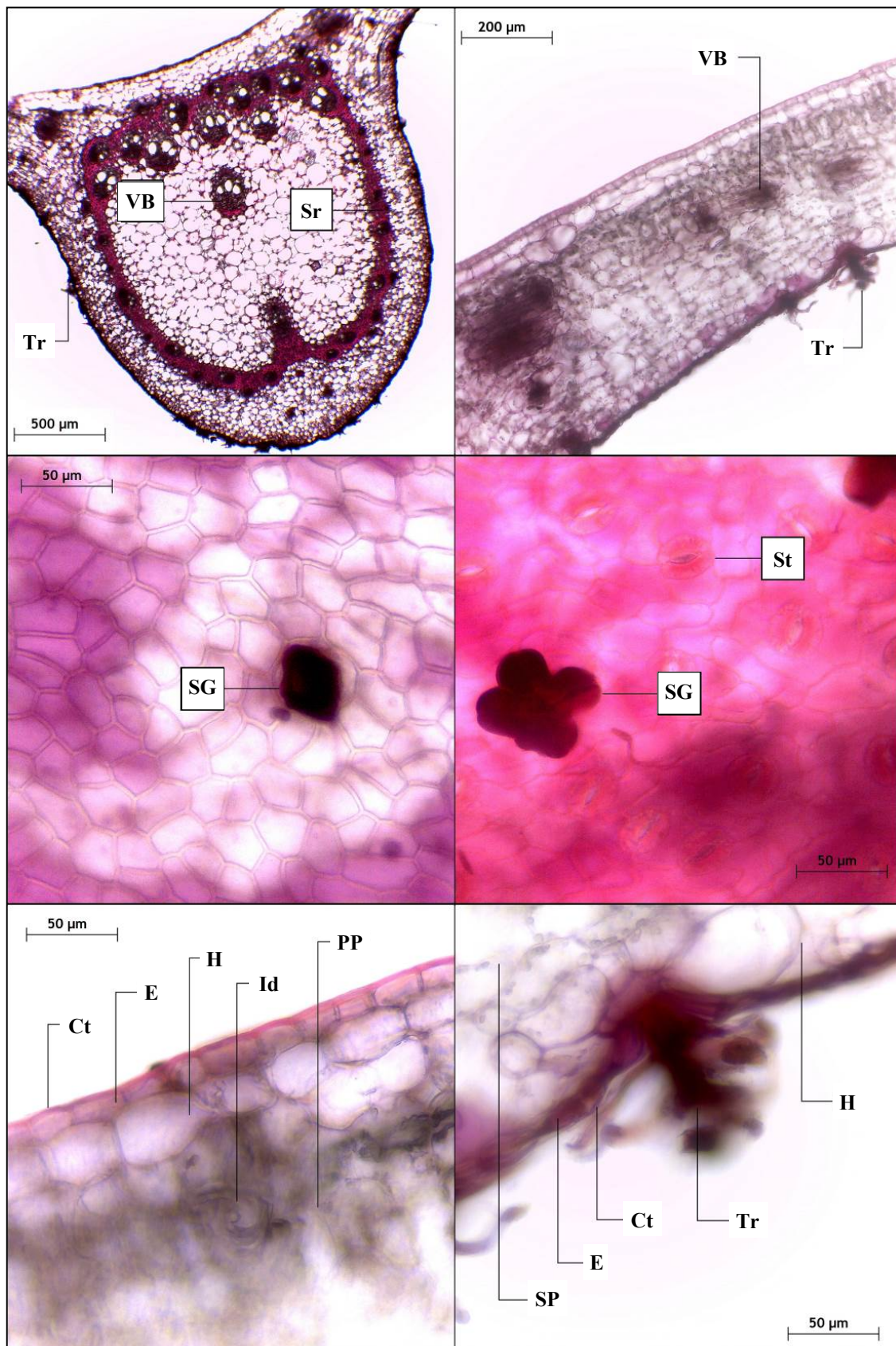


Figure 3. *N. ampullaria* Jack  $\times$  *mirabilis* (Lour.) Druce Ct: Cuticle layer; E: Epidermal layer; H: Hypodermal layer; PP: Palisade Parenchyma; SG: Sessile Gland; SP: Spongy Parenchyma; SR: Schlerenchyma Ring; St: Stomata; Tr: Trichome; VB: Vascular Bundle; Id: Idioblast.



Shape of the sessile glands in *Nepenthes* genera look similar to one of glandular trichomes in members of the *Bignoniaceae* family. Although both families has no close relationship in the taxa, the glands can also be distinguished based on variations by the head cells shape, attachment position to the epidermal surface, and number of stalk cells (Noguiera et al. 2013; Fróes et al. 2015). Through this study, specific characters in the glandular trichomes can be potential differentiating character by using morpho-anatomical approach.

### Quantitative Analysis

Comparison of the third *Nepenthes* taxa was obtained through parametric and non-parametric

tests with each confidence value of 95% (Table 3). By the 25 quantitative characters observed in *N. ampullaria* × *mirabilis*, there were 14 characters that were not significantly different with both parental taxa, 1 character that is similar only to *N. ampullaria*, 4 characters that are similar only to *N. mirabilis*, 1 intermediate character between two parental taxa, and 3 spesific characters that are different from both parental taxa. In addition, there were also 2 other characters that cannot be measured because not all taxa are observed to has exact same features in position, that is area and density of trichome.

Table 3. Leaf anatomical quantitative measurement

No.	Species	<i>N. ampullaria</i>	<i>N. mirabilis</i>	<i>N. ampullaria</i> × <i>mirabilis</i>	Value
1	Abaxial stomatal length (µm)	30,28 ± 0,41 a	29,79 ± 0,38 a	30,55 ± 0,54 a	NS
2	Abaxial stomatal width (µm)	23,48 ± 0,44 a	24,61 ± 0,40 ab	25,49 ± 0,42 b	**
3	Abaxial stomatal area (µm <sup>2</sup> )	544,54 ± 17,15 a	598,80 ± 15,19 b	605,21 ± 23,28 b	*
4	Abaxial stomatal density (/mm <sup>2</sup> )	270 ± 24,17 a	240 ± 13,38 a	210 ± 17,95 a	NS
5	Stomatal Index	0,13 ± 0,01 a	0,11 ± 0,01 a	0,11 ± 0,01 a	NS
6	Abaxial sessile glands area (µm <sup>2</sup> )	2051,26 ± 111,65 b	2045,63 ± 46,54 b	1741,74 ± 64,73 a	**
7	Abaxial sessile glands density (/mm <sup>2</sup> )	15,00 ± 2,12 a	11,50 ± 0,08 a	13,00 ± 0,30 a	NS
8	Abaxial trichome area (µm <sup>2</sup> )	10836,37 ± 2097,70	Very rare	Only on midrib area	-
9	Abaxial trichome density (/mm <sup>2</sup> )	7,50 ± 1,76	Very rare	Only on midrib area	-
10	Abaxial epidermal cell number (/mm <sup>2</sup> )	1001,50 ± 24,79 a	1646,00 ± 84,90 b	1335,00 ± 23,30 a	*
11	Adaxial sessile glands area (µm <sup>2</sup> )	802,70 ± 51,96 a	724,75 ± 45,74 a	1245,32 ± 82,12 b	*
12	Adaxial sessile glands density (/mm <sup>2</sup> )	8,50 ± 0,11 a	9,50 ± 1,53 a	8,00 ± 2,00 a	NS
13	Adaxial epidermal cell number (/mm <sup>2</sup> )	1676,00 ± 63,70 a	1969,00 ± 179,50 a	1836,00 ± 24,41 a	NS
14	Total leaf thickness (µm)	389,27 ± 16,21 a	394,69 ± 13,59 a	409,74 ± 7,70 a	NS
15	Adaxial cuticle thickness (µm)	4,68 ± 0,30 a	2,87 ± 0,43 b	4,07 ± 0,18 ab	*
16	Abaxial cuticle thickness (µm)	1,74 ± 0,18 a	1,64 ± 0,18 a	2,14 ± 0,25 a	NS

Table 3. Leaf anatomical quantitative measurement (*continue*)

No.	Species	<i>N. ampullaria</i>	<i>N. mirabilis</i>	<i>N. ampullaria</i> × <i>mirabilis</i>	Value
17	Adaxial epidermal thickness (µm)	12,87 ± 1,18 a	11,69 ± 0,89 a	10,99 ± 0,61 a	NS
18	Abaxial epidermal thickness (µm)	9,76 ± 0,83 a	10,40 ± 0,92 a	10,84 ± 1,07 a	NS
19	Adaxial hipodermal layer number (µm)	1,15 ± 0,08 a	1,40 ± 0,11 a	1,50 ± 0,17 a	NS
20	Adaxial hipodermal layer thickness (µm)	31,98 ± 2,56 a	39,34 ± 4,20 a	54,22 ± 3,97 b	*
21	Abaxial hipodermal layer number (µm)	1,05 ± 0,05 a	1,10 ± 0,07 a	1,30 ± 0,15 a	NS
22	Abaxial hipodermal layer thickness (µm)	32,50 ± 2,69 a	29,57 ± 1,63 a	32,91 ± 1,85 a	NS
23	Total esophyll thickness (µm)	262,26 ± 12,20 a	287,34 ± 13,54 a	297,24 ± 6,09 a	NS
24	Palisade parenchyma thickness (µm)	114,51 ± 5,44 b	94,32 ± 5,35 a	88,78 ± 6,79 a	**
25	Spongy parenchyma thickness (µm)	144,97 ± 8,33 a	197,21 ± 8,72 b	207,18 ± 7,46 b	**

\* : level of confident 95% at non-parametric test

\*\* : level of confident 95% at parametric test

NS : Not significant

- : Not calculate

The natural hybrid *N. ampullaria* × *mirabilis* has shared similar leaf anatomical characters with *N. mirabilis* including: thinner palisade parenchyma, thicker spongy parenchyma, and broader and wider stomata compared to *N. ampullaria*. Whole mentioned tissue characters has similar function related to photosynthesis. That is supported by physiological measurement by Mansur (2017) through the rate of CO<sub>2</sub> absorption, transpiration, and amount of chlorophyll content. Those three result of the measurement shows the overlapping data of *N. ampullaria* × *mirabilis* with *N. mirabilis* while significantly different from *N. ampullaria* which has the lowest measurement. Therefore, natural hybrid *N. ampullaria* × *mirabilis* has adapted to high photosynthesis rate and may inherit only from *N. mirabilis* parental taxa.

The thickness size of palisade and spongy parenchyma of *N. ampullaria* similar with *N. gracilis*, *N. bicalcarata*, *N. rafflesiana*, and *N. hemsleyana* hat measured by Osunkoya & Muntassir (2017). The study showed that palisade parenchymal tissue tended to be thicker rather than spongy parenchyma. There is no detail number that mentions specific size of the mesophyll of each

species, so that it cannot be compared one by one. However, that measured thickness could be suspected precisely influenced by habitats. It said that the location of samples collected occur at the edges of the kerangas secondary forest and low-land peat swamp, so that tend to be drought and hot climate. Therefore, those *Nepenthes* which live in that habitat has adapted to control the photosynthesis and transpiration rate in order to keep available water content.

Palisade and spongy parenchyma as a whole have different densities in typical xerophyte, mesophyte, and hydrophyte (Fahn 1990; Cutler *et al.* 2008). Ivanova & P'yankov (2002) also stated that the thickness of spongy parenchyma could increase in line within shade conditions related to optimization of light reflection and CO<sub>2</sub> flow, vice versa. It is estimated that there are basic high photosynthesis rates on *N. mirabilis* and *N. ampullaria* × *mirabilis* against related environmental conditions. Therefore, the thickness of palisade and spongy parenchyma must be maintained in such a way.

Natural hybrid *N. ampullaria* × *mirabilis* has shared similar leaf anatomical characters with *N.*

*ampullaria* in the number of abaxial epidermal cells. They tend to have significantly lesser epidermal cells per area compared to *N. mirabilis*, which means larger epidermal cells. The character has a function related to lower layer tissues protection, where number of cell is commonly affected by light intensity (Dunn *et al.* 2015). Such as in *N. gracilis*, the epidermal cell density and thickness are affected by light intensity and shade (Paluvi *et al.* 2015). More obvious evidence collected in *Coffea arabica* and *Allium sativum* where epidermal cells are increasingly tenuous and larger in shaded area, make leaf surface are also wider (Rahim & Fordham 1991; Pompelli *et al.* 2010).

Epidermal density, size, and cell number are generally influenced by internal and external factors. Internal factors come from genetic formation and leaf growth duration (Melaragno *et al.* 1993; Guimil & Dunand 2007; Tisné *et al.* 2008; Hara *et al.* 2009). In the external factors, exposure to sunlight and ultraviolet radiation could also cause the epiderm develop tighter and more rigid to avoid high damage to mesophyll tissue (Wagner *et al.* 2000; Kolb *et al.* 2001). Therefore, the natural hybrid *N. ampullaria* × *mirabilis* and *N. ampullaria* are expected to have better adaptation to shaded habitat rather than *N. mirabilis* which more adapted to bright and exposed habitat.

Further more, *N. ampullaria* × *mirabilis* also has showed similar adaxial cuticle thickness with *N. ampullaria*, but significantly thicker than *N. mirabilis*. Cuticles are product from oxidation and condensation of fat that secreted by the epiderm (Lee & Priestley 1924; Javelle *et al.* 2011; Yeats & Rose 2013). However, the result of cuticle thickness is also influenced by the intensity of light and temperature. Cuticle deposits on epiderm become larger as the first defensive mechanism of extreme heat or cold through risk control of water loss (Cutler *et al.* 2008; Lee *et al.* 2015). Therefore, the natural hybrid has developed intermediate size of cuticle thickness due to accomodate large range of environmental condition between *N. ampullaria* to *N. mirabilis*.

There are three anatomical characters *N. ampullaria* × *mirabilis* that are significantly different from the two parental taxa: wider abaxial sessile glands, smaller adaxial sessile glands area, and thicker adaxial hypodermis. These three characters have similar function to regulate water content (Metcalf & Chalk 1950; Cheek & Jebb 2001). Wider size of the sessile glands on the abaxial position compared to adaxial strengthen the indication that abaxial side has better adaptation to the

transpiration rate control. This is similar to the effect of denser and wider stomatal area towards water control (Jones & Rotenberg 2001; Lawson & Blatt 2014; Buckley *et al.* 2017). It means that the natural hybrid has developed adaptation ability to the transpiration rate. The ability to maintain water content is expected to be better from two parental taxa.

The natural hybrid data *N. ampullaria* × *mirabilis* also shared 14 similar leaf anatomical characters compared with *N. ampullaria* and *N. mirabilis* as its parental taxa. There is no aberrant characters occurred. Therefore, it could be noted that more than half of the observed characters were not differ significantly between those three *Nepenthes* taxa.

### Principal Component Analysis (PCA).

There are total 50 repetitions of measurement from the three taxa *N. ampullaria*, *N. mirabilis*, and *N. ampullaria* × *mirabilis*. Those fifty repetitions included in the quantitative character analysis measured through the PCA. The analysis includes 25 quantitative characters and 2 qualitative characters that are numerated.

The PCA result shows there are 2 components of the highest variation. The first component was dominated by the presence of trichomes and the shape of the abaxial sessile gland, while the second component was dominated by the total leaf thickness and total mesophyll thickness. The two components of variation then selected in sequence as the y axis and x in the scatter diagram.

The diagram (Figure 4) shows that the anatomical characters of *N. ampullaria* × *mirabilis* leaves occurred in transition between *N. mirabilis* and *N. ampullaria*. However, there is a tendency that variations in the character of the natural hybrid to be closer to *N. mirabilis*. Therefore, the natural hybrid *N. ampullaria* × *mirabilis* can be expected has more resemblance in adaptation characters to *N. mirabilis* rather than *N. ampullaria*.

Shared similarities in the morphological or anatomical characteristics of natural hybrid compared to its parental taxa have been investigated in various plant groups. Previous study showed that *Passiflora* × *rosea* have similarity towards parental *P. pinnatistipula* based on the corona development, while trichomal shape of leaves are more similar to *P. tripartita* var. *mollissima* (Jørgensen & Vásquez 2009). Pliszko & Kostrakiewicz-Gieralt (2018) also showed that *Erigeron* × *huelsenii* has 12 intermediate characters between parental *E. acris* and *E. canadaensis* morphologically. *Pyrus* × *mylos-*

*lavensis* even shares the characteristics of both parent *P. communis* and *P. salicifolia* down to the

micromorphological character of the seeds (Antkowiak *et al.* 2016).

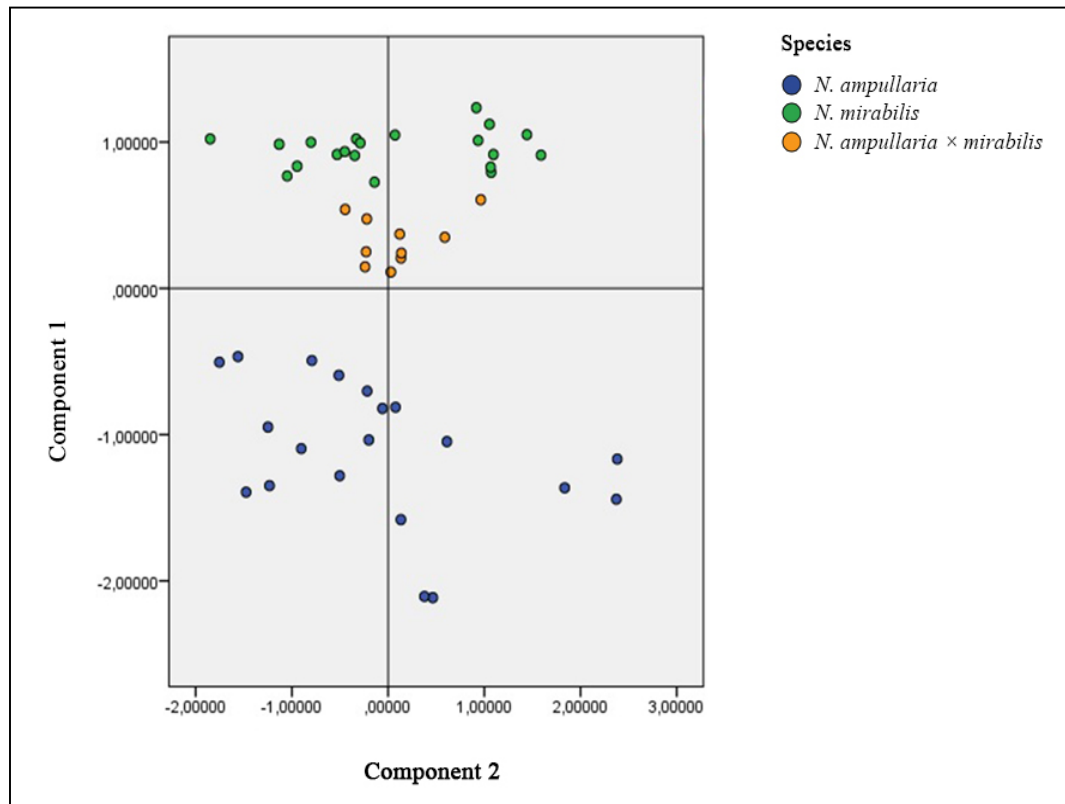


Figure 4. PCA Diagram.

The diagram (Figure 4) shows that the anatomical characters of *N. ampullaria* × *mirabilis* leaves occurred in transition between *N. mirabilis* and *N. ampullaria*. However, there is a tendency that variations in the character of the natural hybrid to be closer to *N. mirabilis*. Therefore, the natural hybrid *N. ampullaria* × *mirabilis* can be expected has more resemblance in adaptation characters to *N. mirabilis* rather than *N. ampullaria*.

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(Antkowiak *et al.* 2016).

The comparison study between natural hybrid and the relation with parental taxa through anatomical approach is still limited. Most of the data analysis has done through morphological, intracell, and molecular approaches (King & Irvine 2010; López-Caamal & Tovar-Sánchez 2014; Santos *et al.* 2014). Therefore, this research could be an evidence that anatomical study is an effective approach to provide a deeper understanding in the relationship between natural hybrid and its parental taxa. That also could be done for relation analysis as well as the basic information related to the habitat preferences.

## CONCLUSION

There are 27 anatomical characters in leaf strands of *N. ampullaria* × *mirabilis* compared to the two parent. Two characters are different qualitatively. Quantitatively, 14 characters are not significantly different, 1 character similar to *N. ampullaria*, 4 characters similar to *N. mirabilis*, 1 character intermediate, and 3 characters are different. The results of the PCA showed that there

anatomical leaf sheet *N. ampullaria* × *mirabilis* similar to *N. mirabilis*. Although quantitative character raises more differences, qualitative characters play a more important role in determining the direction of the similarity.

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d.a. “Herbarium Bogoriense” Bidang Botani, Puslit Biologi, CSC-LIPI  
Jl. Raya Jakarta Bogor, Km. 46. Cibinong, Bogor. 16911. Indonesia