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Pollen Morphology of Six Indonesian *Begonia* (*Begoniaceae*) Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author HMS designed the study, collected plant materials and wrote the first draft of the manuscript. Author Sudarmono wrote the methods and made interpretation of the results. Authors JRW and HW managed the study, helped in the interpretation of the results and tidy up the draft according to journal template. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: This study aims to determine pollen morphological differences in six Indonesian *Begonia* species.

Study Design: All fresh pollen grain of six Indonesian *Begonia* species were collected using a standard method and observed at SEM (Scanning Electron Microscope) Laboratory.

Place and Duration of Study: The pollen is collected from Bogor Botanic Gardens, then observed using an SEM in The Museum Zoologicum Laboratory, Research Center for Biology, Indonesian Institute of Sciences (LIPI), on August 2018.

Methodology: The results of SEM pollen ultrastructure observations were analyzed descriptively by describing images of size, P/E index, aperture, endoaperture, and ornamentation of pollen. The size of pollen morphology is calculated based on the length of the polar axis and the equatorial axis on a micrometer scale. Determination of the shape of pollen can be determined by comparing the length of the polar axis with the equatorial axis.

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Results: All species examined share common pollen grain characters, i.e. isopolar and three zonocolporate. The longest polar axis (P) and equatorial diameter (E) presented by *B. puspitae*, whereas the shortest of P is shown by *B. kudoensis* and the shortest of E by *B. sudjanae*. Only *B. hooveriana* has prolate – perprolate aperture, whereas other species perprolate. Endoaperture types of *B. puspitae*, *B. sudjanae*, and *B. hooveriana* is lalongate, while the other species is lolongate. The coarsely striate ornamentation forms are presented by *B. holosericeoides* and *B. natunaensis*, whereas other species has fine striate ornamentation.

Conclusion: The size, P/E index, aperture, endoaperture, and ornamentation of pollen are not useful for section classification of six Indonesian *Begonia* species. Pollen morphology should be incorporated to other characters, such as morphological, cytological, and molecular characters for making delimitation of *Begonia* species.

Keywords: *Begonia*; pollen; SEM; Bogor Botanic Gardens; Indonesia.

1. INTRODUCTION

Begonia L. (*Begoniaceae*) is a pantropical genus as known as the sixth-largest genus of flowering plants. It consists of 1891 accepted species, belonged to 70 sections, and distributed throughout tropics and subtropics to central China [1,2]. *Begonia* species are common to secondary and primary forests, along rivers or creeks, mostly in humid areas and around waterfalls [3]. Many species of *Begonia* use as ornamental plants, either as species or hybrids [4], spices or vegetables [5], and traditional medicine [6].

The Malesian region is one of the centers of *Begonia* diversity with an estimated number of 579 species [5,7,8,9]. The island of Borneo is harboring the highest *Begonia* diversity and endemism among the major islands with 194 species [7]. *Begonia* is easily recognizable by diagnostic characters such as asymmetrical leaves, unisexual monoecious flowers, twisted-, papillose stigmas, and dry-, and three-winged capsules [10]. *Begonia* is a complicated genus, since it consists of huge species with a wide range of distribution, and paraphyletic. The evidence at hand in terms of phylogeny, biogeography, morphology including pollen characters is necessary to establish a stable infrageneric classification in the genus.

Pollen grains are known to be useful characters in a study at all levels of the taxonomic hierarchy from the genus, species, and intra-species levels, and can often be helpful in suggesting a relationship [11]. Some studies have proved that pollen morphological characteristics play a major role in solving taxonomic problems in some genera within family Dipsacaceae [12], Orchidaceae [13], Arecaceae [14], *Centaurea* (Asteraceae) [15], and *Arundina graminifolia*

(Orchidaceae) [16]. Whereas in *Begonia*, some pollen studies have been reported in Africa [17], Kuching-Malaysia [18], Nepal [19], and China [20].

Study on pollen morphology of *Begonia* in Indonesia has not been studied and reported. This current study aimed to determine pollen morphological differences in six Indonesian *Begonia* species.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant materials of six species of *Begonia* were collected from living collections cultivated in the *Begonia* Green House of Bogor Botanic Gardens on August 2018. All *Begonia* species studied are native to Indonesia, namely *B. kudoensis* Girm., *B. puspitae* Ardi, *B. sudjanae* C.-A.Jansson from Sumatra, *B. natunaensis* C.W.Lin & C.I Peng from Natuna Isl. (near Borneo), *B. hooveriana* Wiriad. from Sulawesi, and *B. holosericeoides* Ardi & DC Thomas from Maluku. According to Moonlight et al. [1], *B. kudoensis*, *B. puspitae*, *B. sudjanae*, and *B. natunaensis* belong to section Petermannia, whereas *B. hooveriana* and *B. holosericeoides* belong to section Jackia.

2.2 Pollen Collecting and Observation

The pollen is collected between 10:30 to 11:30 when the flowers are in full blooming and the anthesis is then cleaned of the stamen. Pollen is stored into small-sized tubes (1.5 - 10 ml) and placed in a freezer temperature of -5 - (-80)^oC in the Treub Laboratory of the Bogor Botanic Gardens, then observed using an SEM (Scanning Electron Microscope) in The Museum Zoologicum Laboratory, Research Center for Biology, Indonesian Institute of Sciences (LIPI).

The pollen walls were cleaned so that they were easily observed, then the specimen installation glued to the stub specimen. Furthermore, coating the specimen with aurum using the ion coater (ion sputtering) JEOL IB2 for 15 minutes. The holder is taken, then mounted on an SEM JEOL JSM 5310 LV scanner. The best pollen is taken and photographed for morphological analysis [16].

2.3 Data Analysis

The results of SEM pollen ultrastructure observations were analyzed descriptively by describing images of size, P/E index, aperture, endoaperture, and ornamentation of pollen. The size of pollen morphology is calculated based on the length of the polar axis and the equatorial axis on a micrometer scale. Determination of the shape of pollen can be determined by comparing the length of the polar axis with the equatorial axis. The size of the pollen is determined followed van den Berg [21] and Rajbhandary et al. [19].

3. RESULTS AND DISCUSSION

In general, pollen morphology of *Begonia* by equatorial view present polar axis, equatorial diameter, striate ornamentation, and colpus are shown in Fig. 1.

The comparison in polar axis (P) and equatorial diameter (E) of pollen, P/E index, aperture, endoaperture, and ornamentation of six Indonesian *Begonia* species is shown in Table 1. Pistillate flower and pollen observation results including pollen morphology, striate ornamentation, and colpus of six Indonesian *Begonia* species were presented in Fig. 2. Based on our observation, all pollen grain of six *Begonia* species showed that the proximal and distal faces of the axine are alike (isopolar) and has three compound apertures (three zonocolporate).

The longest polar axis (P) and equatorial diameter (E) presented by *B. puspitae* of 21.85-26.10 μm and 10.25-11.35 μm , respectively, whereas the shortest of P is shown by *B. kudoensis* of 15.50-19.45 μm and the shortest of E is shown by *B. sudjanae* of 5.05-6.65 μm (Table 1). The P and E size of six Indonesian *Begonia* species are ranged of *Begonia* from Nepal, with the longest P and E presented by *B. flagellaris* (section *Diploclinium*) of 24.3-30.6 \times 11.4-12.0 μm , respectively and the shortest P and E size shown by *B. roxburghii* (section

Sphenanthera) of 11.2-12.8 \times 6.4-7.0 μm , respectively [19].

The shape of pollen can be determined by a comparison of the length of P and the E, herein after referred to as the P/E index. The highest P/E index is presented by *B. sudjanae* of 3.11-3.24, followed by *B. natunaensis* of 2.72-3.22, *B. kudoensis* of 2.66-2.81, *B. holosericeoides* of 2.50-2.75, *B. puspitae* of 2.13-2.29, and *B. hooveriana* of 1.95-2.47. According to Punt et al. [22], the classification of P/E index as follows: P/E index < 1 is spheroidal, P/E index 1-1.14 is prolate spheroidal, P/E index 1.14-1.33 is subprolate, P/E index 1.33-2.00 is prolate, and P/E index > 2.00 perprolate. Since the lowest range P/E index of *B. hooveriana* is 1.95, the aperture of the species is categorized as prolate, whereas the aperture of other species with P/E index > 2.00 is categorized as perprolate (Table 1). It means that aperture of six Indonesian *Begonia* species is categorized as prolate-perprolate type. Pollen shapes are mainly exhibited as perprolate (P/E index > 2.00) [22]. However prolate aperture with P/E index of 1.33 – 2.00 was presented in *B. edulis*, *B. fangii*, and *B. lanternaria* [20].

Endoaperture is the inner part of a compound aperture [23]. Endoaperture of six Indonesian *Begonia* species is classified as lalongate and lolongate types. Lalongate type is identified by the shape of a transversely elongated endoaperture, whereas lolongate type is identified by the shape of a longitudinally elongated endoaperture [22]. Three species have identified as lalongate type, namely *B. puspitae*, *B. sudjanae* (section *Petermannia*), and *B. hooveriana* (section *Jackia*), while three species *B. holosericeoides* (section *Jackia*), *B. kudoensis* and *B. natunaensis* (section *Petermannia*) as lolongate type. In Burseraceae, there are four types of endoaperture, i.e. lolongate, lalongate, circular, and subcircular [24]. However, endoaperture of *Begonia* has not been reported so far.

The striated pattern shown by *Begonia* pollen can be defined as a regular pattern around a parallel jury. More specifically in striated grains or ridges of exineous material, referred to as lirae and grooves between ridges as striate [21]. Depending on the width of the lirae and striae, the striate pattern can be designated as coarsely striate, fine striate, and fine faint striate. The ornamentation of six Indonesian *Begonia* species is classified as coarsely striate and fine striate.

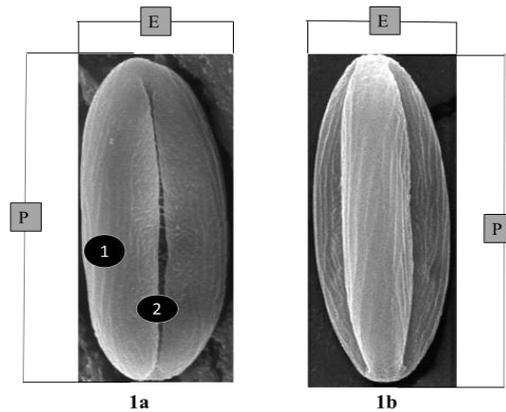


Fig. 1. Pollen morphology of *Begonia* by equatorial view; (1a) pollen with colpus, (1b) pollen without colpus. P=polar axis, E=equatorial diameter, 1=striate ornamentation, 2=colpus

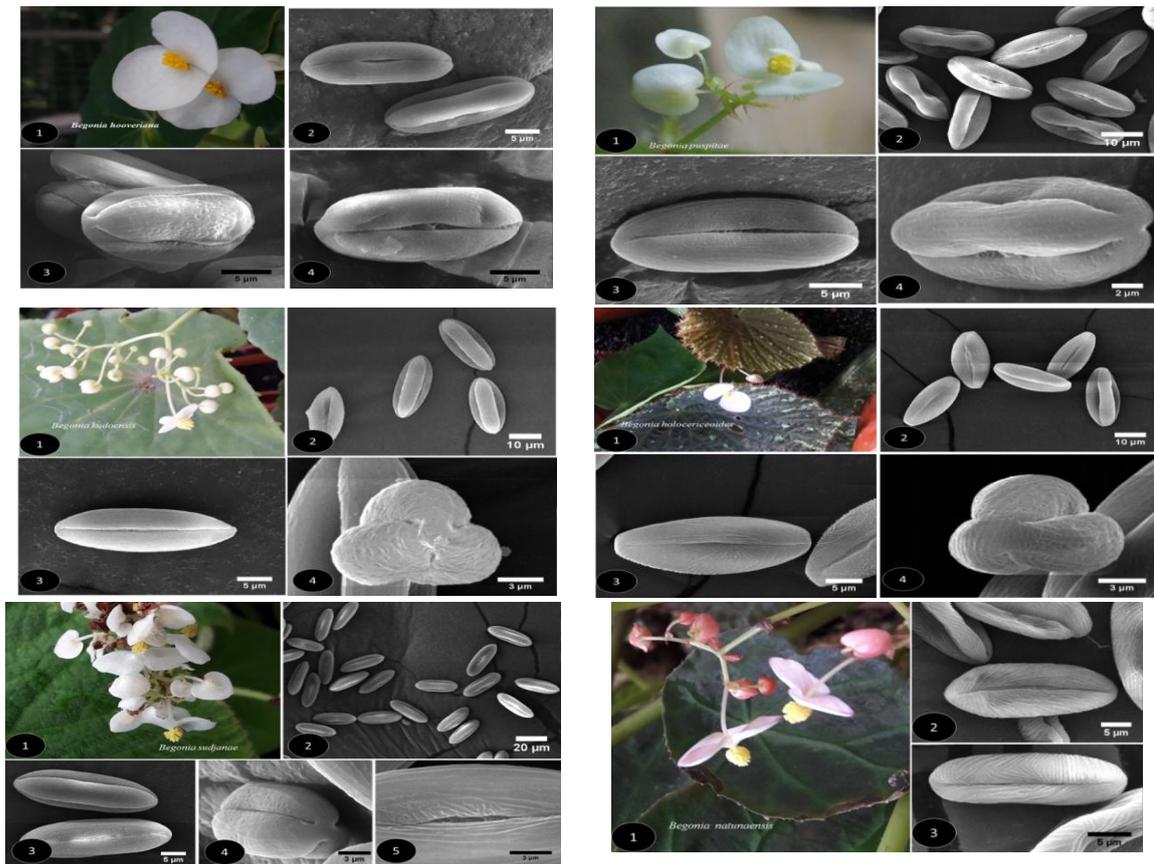


Fig. 2. Pistillate flower and pollen morphology of six Indonesian *Begonia* species. A. *B. hooveriana*: (1) pistillate flower, (2) pollen morphology by polar view, (3) fine striate ornamentation by equatorial view, (4) colpus; B. *B. puspitae*: (1) pistillate flower, (2) pollen morphology, (3) fine striate ornamentation by polar view, (4) pollen by an equatorial view; C. *B. kudoensis*: (1) pistillate flower, (2) pollen morphology, (3) fine striate ornamentation by polar view, (4) pollen by an equatorial view; D. *B. holosericeoides*: (1) pistillate flower, (2) pollen morphology, (3) striate ornamentation by polar view, (4) pollen by an equatorial view; E. *B. sudjanae*: (1) pistillate flower, (2) pollen morphology, (3) fine striate ornamentation by a polar view, (4) pollen by an equatorial view, (5) colpus; F. *B. natunaensis*: (1) pistillate flower, (2) pollen morphology, (2) striate ornamentation

Table 1. Comparison in pollen size, P/E index, aperture, endoaperture, and ornamentation of six Indonesian *Begonia* species

No	Species	Section	Pollen size (μm)		P/E Index	Aperture	Endoaperture	Ornamentation
			P = Polar axis	E = equatorial diameter				
1	<i>B. hooveriana</i> Wiriad.	Jackia	16.95–17.70	6.85–9.10	1.95–2.47	prolate -perprolate	lalongate	fine striate
2	<i>B. holosericeoides</i> Ardi & DC Thomas	Jackia	16.25–19.45	6.50–7.10	2.50–2.75	perprolate	lolongate	coarsely striate
3	<i>B. kudoensis</i> Girm.	Petermannia	15.50–19.45	5.50–7.30	2.66–2.81	perprolate	lolongate	fine striate
4	<i>B. natunaensis</i> C.W. Lin & C.I Peng	Petermannia	17.40–21.40	6.40–6.65	2.72–3.22	perprolate	lolongate	coarsely striate
5	<i>B. puspitae</i> Ardi	Petermannia	21.85–26.10	10.25–11.35	2.13–2.29	perprolate	lalongate	fine striate
6	<i>B. sudjanae</i> C.- A.Jansson	Petermannia	16.35–20.70	5.05–6.65	3.11–3.24	perprolate	lalongate	fine striate

The coarsely striate ornamentation forms are presented by *B. holosericeoides* and *B. natunaensis*, whereas fine striate ornamentation is shown in *B. puspitae*, *B. sudjanae*, *B. kudoensis*, and *B. hooveriana* (Table 1). According to Rajbhandary et al. [19], three types of ornamentation of *Begonia* in Nepal has identified, i.e. coarsely striate, fine striate and fine faint striate. However, striate ornamentation could not be used to separate *Begonia* in section Diploclinium, Platycentrum, Sphenanthera, and Monopteron in Nepal.

4. CONCLUSION

The pollen of six Indonesian *Begonia* species is isopolar, three zonocolporate, and prolate to perprolate aperture. The longest P and E presented by *B. puspitae*, whereas the shortest of P is shown by *B. kudoensis* and the shortest of E by *B. sudjanae*. The coarsely striate ornamentation forms are presented by *B. holosericeoides* and *B. natunaensis*, whereas fine striate ornamentation is shown in *B. puspitae*, *B. sudjanae*, *B. kudoensis*, and *B. hooveriana*. P/E index, aperture, and ornamentation of pollen are not useful to classify six Indonesian *Begonia* species. Pollen morphology should be incorporated to other characters, such as morphological, cytological, and molecular characters for making delimitation of *Begonia* species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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