

A seed morphometry study of selected species of *Bulbophyllum* and *Dendrobium* (Orchidaceae) in relation to their dispersals

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Abstract. Aprilianti P, Handini E, Puspitaningtyas DM. 2021. A seed morphometry study of selected species of *Bulbophyllum* and *Dendrobium* (Orchidaceae) in relation to their dispersals. *Biodiversitas* 22: 5564-5571. *Bulbophyllum* and *Dendrobium* are the two largest genera of Orchidaceae. The seed morphometry studies of *Bulbophyllum* and *Dendrobium*, mainly from Southeast Asia are still poorly reported. The research aimed to characterize seeds micromorphometry of selected species of *Bulbophyllum* and *Dendrobium*, namely *Bulbophyllum graveolens*, *B. macranthum*, *B. phalaenopsis*, *Dendrobium bicaudatum*, and *D. bifalce* in relation to their dispersal mechanisms. Twenty seeds of each species were studied using light and scanning electron microscope (SEM). Qualitative characters, such as seed shape, testa cell shape, wall structure, and its orientation and quantitative data, such as seed and embryo were analyzed. The results showed that the shape of the seeds of all the species was fusiform. The testa cell walls were smooth and covered by waxes. The anticlinal walls were raised and thickened. *Bulbophyllum* and *Dendrobium* can be differentiated in the orientation of testa cells, which is spirally oriented in *Bulbophyllum* and parallel to the long axis in the *Dendrobium*. There are no large variations in seed and embryo volume as well as seed air space (SAS) in different taxa. The SAS value can be used to predict the dispersal mechanism and distribution area of the orchid species. Unfortunately, the SAS values are not always positively correlated with the distribution area and other factors inhibit the spread of the species.

Keywords: *Bulbophyllum*, *Dendrobium*, distribution area, morphometry, orchid seed air space

Abbreviations: SAS: Seed air space, SEM: Scanning electron microscope

INTRODUCTION

Orchidaceae consists of 22.000 species in 880 genera (Barthlott et al. 2014). Orchid seeds are unique and vary in shape, color, size, embryo volume, and the structure of testa cells. Single fruit has numerous, light, and tiny seeds compared with other flowering plants (Ramudu and Khasim 2015). This large number of seeds have very specific germination requirements, such as mycotrophy (Arditti and Ghani 2000). These dust seeds are dispersed by the wind over a long-distance and exhibit broad morphological characters (Chaudhary et al. 2014; Ramudu et al. 2020).

The common shape of the orchid seed is fusiform and the color is whitish, brownish, greenish, yellowish, or black with the size ranging from 100 µm (*Oberonia similis* (Blume) Lindl.) to 6000 µm (*Epidendrum secundum* Jacq.) (Barthlott et al. 2014). Embryo volume varies between c. 0.0001 mm³ x 1000³ to 5.0 mm³ x 1000³ (Arditti and Ghani 2000). The testa wall that covers the embryo was smooth or reticulated with the secondary wall thickenings on periclinal and anticlinal cell walls as the most prominent pattern is (Swamy et al. 2004; Barthlott et al. 2014). There is also air space surrounding the embryo with various values based on the species (Yeung 2017; Diantina et al. 2020).

Scanning electron microscope (SEM) is commonly used to analyze the microstructures of orchid seeds. The

previous studies on microstructures have been conducted on some Turkish orchids (Güler 2016; Şeker et al. 2021), *Phalaenopsis*, *Paphiopedilum*, and *Dendrobium* native to Indonesia (Hariyanto 2019; Hariyanto et al. 2020), tropical and temperate orchid (Diantina et al. 2020), Western Himalayan orchids (Verma et al. 2014), Gulf of Guinean orchids (Gamarra et al. 2018), orchids of Manipur (Tongbram et al. 2012), *Paphiopedilum* and *Cypripedium* native to China (Zhang et al. 2015). These studies exhibit more understanding of the differences between species of orchid seed. Seed morphometry and morphology have given contributions to taxonomic, phylogenetic, and phytogeographic studies on orchids (Arditti and Ghani 2000; Barthlott et al. 2014; Koene et al. 2020). Seed morphology provides relevant information about evolution, ecological processes like dormancy, germination, and the dispersal adaptation of the orchid species to the specific habitat (Arditti and Ghani 2000; Gammara et al. 2018). It is also important to understand the reproduction biology process of the orchid species, which can be applied for its conservation and propagation study, including seed storage (Diantina et al. 2020).

Orchid significance as the ornamental plants made them overexploited. The loss and destruction of their habitat are getting severe due to human activities and the effect of climate change (Barman and Devadas 2013; Zhang et al. 2015). *Bulbophyllum* and *Dendrobium* are the two largest

genera of Orchidaceae. *Bulbophyllum* consists of more than 2200 that are distributed widely in tropical and subtropical regions and Asia was considered as the major diversity center of *Bulbophyllum* (Comber 2001; Vermeulen et al. 2015). *Dendrobium* is also diverse genera with approximately 1580 species (Wood 2013).

The studies of seed micromorphometry of *Bulbophyllum* and *Dendrobium* species from Southeast Asia are still poorly reported. More studies are needed to learn about their dispersal mechanisms compared with seed characters. So far, there have been approximately eight species of *Bulbophyllum* (Arditti and Ghani 2000; Swamy et al. 2004) and 20 species of *Dendrobium* in the tropical zone ($\pm 20\%$ of *Dendrobium* in Asia mainland) (Arditti and Ghani 2000; Chaudhary 2014; Diantina et al. 2020; Hariyanto et al. 2020) from Asia that have been studied for their seed micromorphometry. The present study was conducted on *Bulbophyllum* and *Dendrobium* which were collected in Bogor Botanic Gardens. This study aimed to characterize the seeds' micromorphometry of the selected species of *Bulbophyllum* and *Dendrobium* in relation to their dispersal mechanisms.

MATERIALS AND METHODS

Plant materials

The research was conducted from January to April 2021. Seeds of five orchid species of Bogor Botanic Gardens' Indonesia collections were harvested from mature and naturally dehiscent capsules. For obtaining the fruits, the flowers were hand-pollinated in the Orchid Glass House. There were three species of *Bulbophyllum*, namely *B. graveolens* (F.M. Bailey) J. J. Sm., *B. macranthum* Lindl., and *B. phalaenopsis* J. J. Sm. and two species of

Dendrobium, namely *D. bicaudatum* Reinw. ex Lindl. and *D. bifalce* Lindl. (Figure 1). The mature seeds were released from the capsule and dried for 7 days in the desiccator.

Observation of seed micromorphology

Seed samples were sterilized with 5% chlorox solution for 5 minutes and rinsed with sterile distilled water. Twenty seed samples were then observed and photographed under Light Microscope (LM) Olympus U-TV0.5XC-3 with a magnification 10 or 40 times. The morphological parameters used LM, including seed shape (SS), seed length (SL), seed width (SW), seed length/seed width (SL/SW), and seed volume (SV). For embryos, we also measured embryo length (EL), embryo width (EW), embryo length/embryo width (EL/EW), and embryo volume (EV).

Data analysis

Seed Volume (SV), Embryo Volume (EV), and Seed Air Space (SAS) were calculated with the formula:

$$SV = 2[(SL/2) (SW/2)^2 (\pi/3)]$$

$$EV = 4/3 \pi (EL/2) (EW/2)^2$$

$$SAS = ((SV - EV)/SV) \times 100\%$$

(Arditti and Ghani 2000).

The qualitative parameters were observed with Scanning Electron Microscope (SEM), such as seed shape, anticlinal and periclinal wall structure, cell shape in chalazal, micropylar, and median region (Şeker et al. 2021). The seed samples were sputter-coated with gold and observed with SEM JSM-IT 200. The terminology of qualitative and quantitative characters was based on Arditti and Ghani (2000) and Barthlott et al. (2014).



Figure 1. Orchid species were used in the study. A. *Bulbophyllum graveolens*, B. *B. macranthum*, C. *B. phalaenopsis*, D. *Dendrobium bicaudatum*, E. *D. bifalce*

RESULTS AND DISCUSSION

Morphological character of seeds

Bulbophyllum and *Dendrobium* are the genera that are included in subfamily Epidendroideae subtribe Dendrobiinae (Barthlott et al. 2014). Based on the observation, *Bulbophyllum* and *Dendrobium* had no difference in seed shape. The seeds of all investigated species were fusiform and did not exhibit great variations (Figures 2A-6A). This seed shape is also known as scobiform elongate seeds and is common to *Bulbophyllum* and *Dendrobium* (Barthlott et al. 2014). Most studies showed that seed shape could not be applied for the delimitation of different genera, because fusiform seeds are mostly found in all the subfamilies (Verma et al. 2014).

There was no difference in the location of the embryo inside the seed between *Bulbophyllum* and *Dendrobium* observed. All embryos were located in the center of the long axis (Table 1; Figures 2A, 3A, 4A, and 6A), except for *D. bicaudatum* that had a small embryo directed to the micropylar pole (Figure 5A). *Dendrobium bifalce* had a large embryo occupying a major part of the seed and located at the center of the long axis (Figure 6A). It was also similar to *D. tosons*, *D. purpureum*, *D. nindii*, and *D. affine* that is native to Indonesia (Hariyanto et al. 2020). *Bulbophyllum graveolens* and *B. phalaenopsis* had a wide opening of suspensor of the seed (Figures 2C and 4C, brown arrow), while suspensor opening in *B. macranthum* was small (Figure 3C, yellow arrow). This opening suspensor is used for allowing the entry of water and fungal hyphae that are required for germination (Arditti and Ghani 2000).

All species observed had smooth testa cell walls and the periclinal walls had no ridges (Table 1). According to

Hariyanto et al. (2020), some species of *Dendrobium* native to Indonesia presented similar results that testa cell walls of all *Dendrobium* examined were smooth. In this study, the testa cell walls of all species were covered with smooth and thin cottony-white substances, resulting in a dull velvety appearance of the seeds. This verrucosity is much common in the subfamily Epidendroideae, predominantly in the subtribe Dendrobiinae (Barthlott et al. 2014). In addition, *B. phalaenopsis* has filament cottony-white substances at the center part (Figure 4D, red arrow). Meanwhile, in *D. bifalce*, there were prominent ridges of the anticlinal walls (Figure 6D, red arrow). The verrucosity shows the presence of waxes (Table 1) in epiphytic taxa, related to their adaptation as epiphytes, and also creates a hydrophobic surface as embryo protection (Gamarra et al. 2018).

Bulbophyllum seeds can be differentiated from seeds of *Dendrobium* in terms of the orientation of testa cells. All *Bulbophyllum* observed was spirally oriented. Meanwhile, all *Dendrobium* was longitudinally oriented and parallel to the long axis (Table 1). Twisted medial cells in *Bulbophyllum* were revealed by Swamy et al. (2004) and Gamarra et al. (2018) that *B. falcatum* and *B. mysorens* also had this type of testa cells. Furthermore, the observation made on *B. graveolens* showed that medial cells apart from being elongated also had a deep groove (Figure 2D, blue arrow) and rounded at the ends (Figure 2D, red arrow). Twisted cells of *B. macranthum* looked like a rope and more twisted at the micropylar pole (Figures 3B, C, E) and *B. phalaenopsis* was strongly twisted with the irregular shape of testa cells at the micropylar pole (Figures 4B, C, E).

Table 1. Main micromorphological characters in *Bulbophyllum* and *Dendrobium* seeds

Species	Seed shape	Location of embryo	Ornamentation of the periclinal wall	Waxes	Orientation testa cells	Testa cell shape differences*	Testa cell shape of medial part	Anticlinal wall
<i>Bulbophyllum</i>								
<i>B. graveolens</i>	Fusiform	Center of the long axis	Smooth	Present	Spirally oriented	Absent	Elongated	Raised and thickened
<i>B. macranthum</i>	Fusiform	Center of the long axis	Smooth	Present	Spirally oriented	Absent	Elongated	Elevated and arch-like
<i>B. phalaenopsis</i>	Fusiform	Center of the long axis	Smooth	Present	Spirally oriented	Absent	Elongated	Raised and thickened
<i>Dendrobium</i>								
<i>D. bicaudatum</i>	Fusiform	Directed to micropylar pole	Smooth	Present	Parallel	Present	Rectangular	Raised and thickened
<i>D. bifalce</i>	Fusiform	Center of the long axis	Smooth	Present	Parallel	Absent	Elongated	Raised and thickened

Note: *Testa cell shape differences between chalazal, micropylar, and medial part

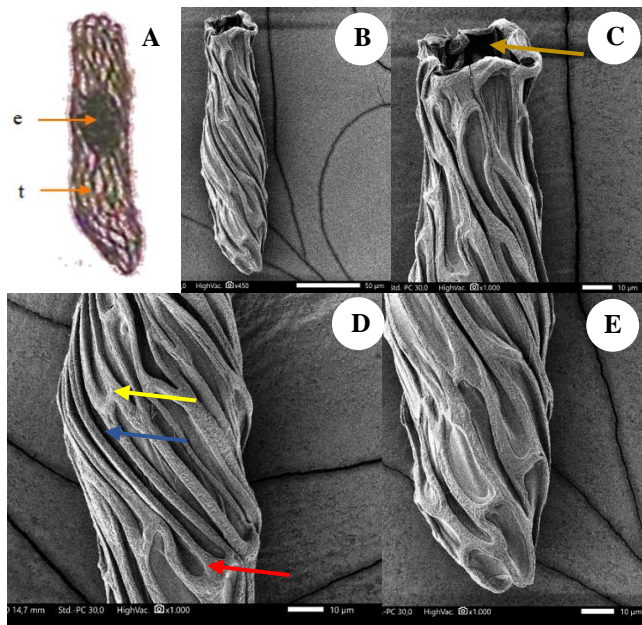


Figure 2. LM and SEM photographs of *Bulbophyllum graveolens*. A. a seed under LM (e: embryo, t: testa), B. a seed under SEM, C. cells of the chalazal pole (brown arrow indicates the opening of suspensor), D. pattern of testa cells of the medial, (blue arrow indicates deep groove of periclinal testa cell walls; red arrow shows rounded testa cell at the ends; yellow arrow indicates testa cell corners is raised and thickened), E. cells of the micropylar pole. Scale bars: B: 50 µm and C-E: 10 µm

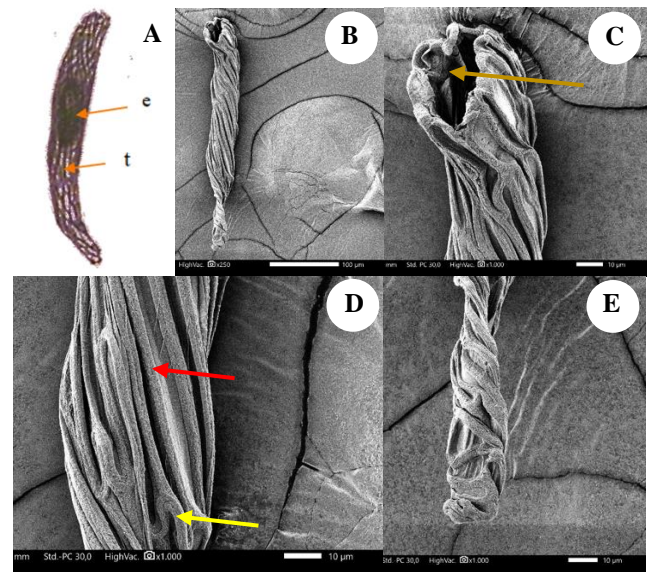


Figure 4. LM and SEM Photographs of *Bulbophyllum phalaenopsis*. A. a seed under LM (e: embryo, t: testa), B. a seed under SEM, C. cells of the chalazal pole (brown arrow indicates the opening of suspensor), D. pattern of testa cells of the medial (red arrow indicates filament cottony white substance; yellow arrow indicates anticlinal cell walls raised to form a bulge at meeting points between two ends), E. cells of the micropylar pole. Scale bars: B: 100 µm and C-E: 10 µm

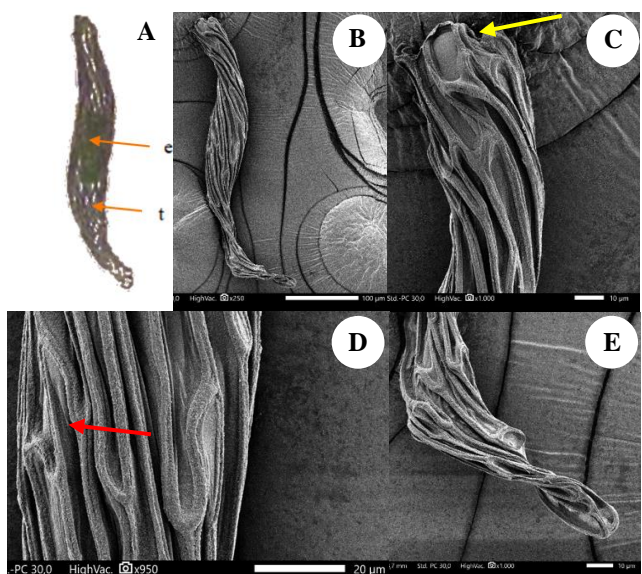


Figure 3. LM and SEM Photographs of *Bulbophyllum macranthum*. A. a seed under LM (e: embryo, t: testa), B. a seed under SEM, C. cells of the chalazal pole (yellow arrow indicates the opening of suspensor), D. pattern of testa cells of the medial (red arrow indicates the elevated and arch-like anticlinal walls), E. cells of the micropylar pole. Scale bars: B: 100 µm, C and E: 10 µm, D: 20 µm

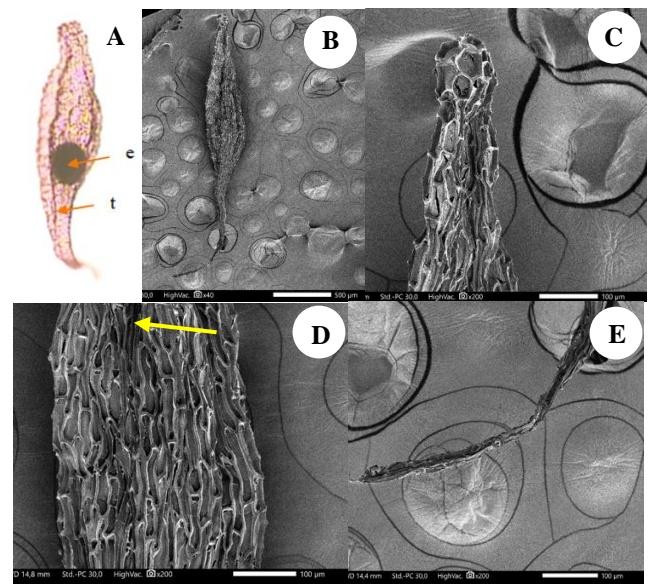


Figure 5. LM and SEM Photographs of *Dendrobium bicaudatum*. A. a seed under LM (e = embryo, t = testa), B. a seed under SEM, C. cells of the chalazal pole, D. pattern of testa cells of the medial (yellow arrow indicates longitudinally deep groove), E. cells of the micropylar pole. Scale bars: B: 500 µm and C-E: 100 µm.

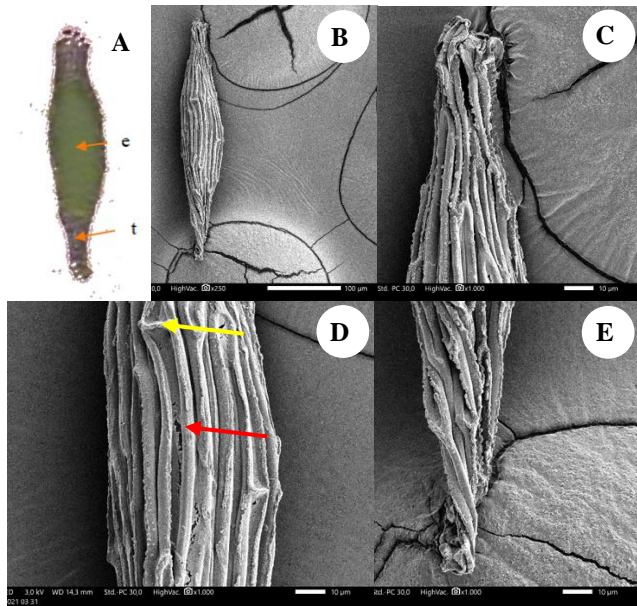


Figure 6. LM and SEM Photographs of *Dendrobium bifalce*. A. a seed under LM (e: embryo, t: testa), B. a seed under SEM, C. cells of the chalazal pole, D. pattern of testa cells of the medial (yellow arrow indicates anticlinal cell walls raised to form a bulge at the meeting points between two ends; red arrow indicates prominent ridges of the anticlinal walls), E. cells of the micropylar pole. Scale bars: B: 100 µm and C-E: 10 µm

Testa cell shape differences between chalazal, micropylar, and medial parts existed only in *D. bicaudatum* (Table 1). Our examination results showed that the testa cell shape in the medial part was elongated in all species, except for *D. bicaudatum* that had irregular shapes with a smaller size than other species. This species also had a polygonal shape of testa cells in the chalazal pole (Figure 5C) and from the medial part to the chalazal pole, there was a deep groove along the axis (Figure 5D, yellow arrow). Cells at the micropylar pole had an appendage at the end with irregular forms (Figure 5E). A similar result was obtained by Hariyanto et al. (2020) that *D. leporinum* also had this polygonal shape of testa walls in the chalazal pole.

The anticlinal walls of testa cells of *Bulbophyllum* and *Dendrobium* were raised and thickened (Table 1). This result is similar to the research conducted by Chaudhary et al. (2014) on *Dendrobium* spp. which was collected from eastern North India. All of the seeds showed thickening of testa cell walls and thus giving important information that anticlinal cell walls were well developed and strongly raised in *Dendrobium*. In *B. phalaenopsis* and *D. bifalce*, these walls were raised to form a bulge at the meeting points between two ends (Figures 4D and 6D, yellow arrow). *Bulbophyllum macranthum* had anticlinal walls that elevated and arch-like (Figure 3D, red arrow). Barthlott et al. (2014) has also mentioned that many species within subfamily Epidendroideae have this character. The seeds that have these elevated anticline walls will fall more slowly, so they can better attach to the substrate (Barthlott et al. 2014; Gamarra et al. 2018). Furthermore, Vij et al. (1992) described that the thickening testa wall provided rigidity to the seed coat and protected the embryo, and the

hygroscopic nature of seed provided metabolic activities during germination.

Seed size, seed volume, and seed air space

The seeds size of the *Bulbophyllum* and *Dendrobium* studied varies greatly and there were no differences between seed length (SL) and seed width (SW). The SL ranged between 0.28 ± 0.035 mm to 1.1 ± 0.082 mm and SW ranged between 0.067 ± 0.009 to 0.337 ± 0.048 mm (Table 2). Barthlott et al. (2014) used the length of the seed for grouping the seed size and there are five categories of seeds, i.e. very small (0.1-0.2 mm), small (0.2-0.5 mm), medium (0.5-0.9 mm), large (0.9-2.0 mm), and very large (2.0-6.0 mm). Based on this classification, the seeds of *D. bicaudatum* were classified as large seeds (1.100 ± 0.082 mm) and the others as small seeds. *Bulbophyllum mysorensense* from India had a shorter SL (0.174 ± 0.014 mm) than all of the *Bulbophyllum* taxa observed in this research but had the same average of SW (0.073 ± 0.009 mm) (Swamy et al. 2004). *Dendrobium bifalce* in this research has a similar length with *D. purpureum* (0.353 ± 0.0019 mm) (Hariyanto et al. 2020).

The maximum SL/SW ratio of the seeds was observed in *B. phalaenopsis* (6.375 ± 0.797), while *D. bicaudatum* had the minimum value (3.294 ± 0.389) (Table 2). This SL/SW ratio provides the data of the relative degree of truncation of the orchid seeds (Verma et al. 2014). The value of SL/SW between *Bulbophyllum* and *Dendrobium* was not different significantly, except for *B. macranthum* and the species that had truncated seed ($SL/SW < 6$) were *B. graveolens*, *B. macranthum*, *D. bicaudatum*, and *D. bifalce*. Meanwhile, only *B. phalaenopsis* had elongated seeds ($SL/SW > 6$). Previous studies showed that higher seed volume was the result of an increase in SL and SW of the seeds and embryos (Vij et al. 1992; Arditti and Ghani 2000; Swamy et al. 2004; Verma et al. 2014). This statement is congruent with the result of this study, there was a positive correlation between the size of the seed and SV, and *D. bicaudatum* has the biggest value of seed volume (SV) (33.400 ± 10.0 mm³ $\times 10^{-3}$).

The length of the embryo (EL) of the investigated species varies between 0.067 ± 0.005 mm (*B. graveolens*) and 0.250 ± 0.00 mm (*D. bicaudatum*) and widths of the embryos (EW) between 0.052 ± 0.009 (*B. phalaenopsis*) and 0.200 ± 0.00 (*D. bicaudatum*) (Table 3). Based on the results, EL and EW of *Dendrobium* species are bigger than *Bulbophyllum* (Table 3). Larger embryos give more benefit to epiphytic species since they will increase the success of germination and seedling establishment because they can deposit more nutrients and are beneficial to adapt in poor environments (Chaudhary et al. 2014). The large embryo have a bigger chance of being survived and establishment success (Zhang et al. 2015; Calevo et al. 2017). The shape of embryos of *B. graveolens*, *B. macranthum* and *D. bicaudatum* were not different significantly and seemed to be spherical with a low EL/EW value (1.083 ± 0.096 to 1.756 ± 0.218) (Table 3). *Bulbophyllum phalaenopsis* and *D. bifalce* had high EL/EW values, representing that the embryos are more elliptical rather than spherical (Güler 2016).

Table 2. Micromorphometric data of *Bulbophyllum* and *Dendrobium* seeds

Species	SL (mm)	SW (mm)	SL/SW (mm)	SV (mm ³ x10 ⁻³)
<i>Bulbophyllum</i>				
<i>B. graveolens</i>	0.280±0.035	0.072±0.005	3.888±0.66	0.383±0.044
<i>B. macranthum</i>	0.395±0.288	0.070±0.014	5.827±1.31	0.517±0.209
<i>B. phalaenopsis</i>	0.425±0.019	0.067±0.009	6.375±0.797	0.516±0.16
<i>Dendrobium</i>				
<i>D. bicaudatum</i>	1.100±0.082	0.337±0.048	3.294±0.389	33.400±10.0
<i>D. bifalce</i>	0.337±0.033	0.075±0.006	4.522±0.608	0.498±0.083

Note: SL: Seed length, SW: Seed width, SV: Seed volume

Table 3. Micromorphometric data of *Bulbophyllum* and *Dendrobium* embryos

Species	EL (mm)	EW (mm)	EL/EW (mm)	EV (mm ³ x10 ⁻³)	SV/EV (mm ³ x10 ⁻³)	SAS (%)
<i>Bulbophyllum</i>						
<i>B. graveolens</i>	0.067±0.005	0.062±0.005	1.083±0.096	0.139±0.0283	2.879±0.802	62.64±12.79
<i>B. macranthum</i>	0.117±0.012	0.067±0.009	1.756±0.218	0.287±0.0979	1.808±0.304	43.33±10.82
<i>B. phalaenopsis</i>	0.140±0.011	0.052±0.009	2.712±0.361	0.211±0.086	2.602±0.645	59.81±9.70
<i>Dendrobium</i>						
<i>D. bicaudatum</i>	0.250±0.00	0.200±0.00	1.250±0.00b	5.233±0.00	6.40±2.03	83.15±5.18
<i>D. bifalce</i>	0.187±0.043	0.075±0.005	2.522±0.701	0.549±0.114	0.901±0.125	10.12±26.88

Note: EL: Embryo length, EW: Embryo width, EV: Embryo volume, SAS: Seed air space

Dangat and Gurav (2016) stated that the increase of embryo volume (EV) results in increasing seed weight, thus improving the ability of the seed to float on air and the distribution of the species (Arditti and Ghani 2000). In the end, EV will directly affect the percentage of available air space inside the seed (Verma et al. 2014). In addition to having the largest SV, *D. bicaudatum* also had the largest volume of EV (5.233 mm³x10⁻³). This value shows that the species has a bigger seed weight. Seeds with a higher ratio of SV/EV are expected to be more buoyant because they have high air space inside the seed (SAS) (Dangat and Gurav 2016). This statement is congruent with our finding that *D. bicaudatum* has the highest SV/EV ratio (6.40±2.03 mm³x10⁻³) among others and indeed it has the largest SAS (Table 3).

Seed air space in relation to dispersal area of the species

The seed air space (SAS) plays a key role in the distribution of orchid species and the large SAS is beneficial in increasing the dispersal area (Arditti and Ghani 2000). Our study result showed that *D. bicaudatum* had the largest value of SAS (83.15±5.18%) and *D. bifalce* had the smallest value of SAS (10.12±26.88%). Among the taxa observed, the SAS value of *Bulbophyllum* ranged from 43.33±10.82% to 62.64±12.79% (Table 3). According to O'Byrne (2001), *D. bicaudatum* had a limited area of distribution (Moluccas, Sulawesi, and the Philippines) (Figure 7, white line). This finding contradicts the statement that the species with higher SAS will have a wide geographic area because the seeds will float in the air longer (Swamy et al. 2004; Hariyanto et al. 2020). The

SAS value above 50% indicates the seed of the species is light and buoyant, so it can be easily dispersed by wind and spread to a larger occupied area (Hariyanto et al. 2020). The study conducted by Tongbram et al. (2012) on some orchids such as (*Aerides odorata* Lour., *Ascocentrum himalaicum* (Deb, Sengupta & Malick) Aver., *Cymbidium iridioides* D. Don, *Dendrobium crepidatum* Lindl. & Paxton, *Hygrochilus parishii* (Rchb.f.) Pfitzer, *Paphiopedilum hirsutissimum* (Lindl. ex Hook.) Stein, *Papilionanthe vandarum* (Rchb.f.), *Rhynchostylis retusa* (L.) Blume, *Taeniophyllum glandulosum* Blume, and *Vanda coerulea* Griff. ex Lindl.) from Manipur, India also gave the same result. Seed air space of *C. iridioides* was the largest among the investigated taxa (90.5%), but the species has a limited distribution area than the others with lower air space. This situation is related to the condition during dispersion that the seeds of both species (*C. irioides* and *D. bicaudatum*) did not get the suitable environment to germinate or, in other words, these species have the narrow amplitude of environmental conditions and a highly specific habitat (Tongbram et al. 2012; Verma et al. 2014). Moreover, the existence of orchid seeds depends on the availability of mycorrhizal fungi to obtain the nutrients to germinate naturally. Also, larger air spaces in the orchid seed would affect the turbulence because the seed will float longer in the air. Thus, increased air space may increase floatation time during dispersal event and reduce the average travel distance of the seed. This is reasonable, since the seeds have limited of viability. Most seeds perhaps have lost their viabilities just before reaching a suitable environment (Arditti and Ghani 2000).

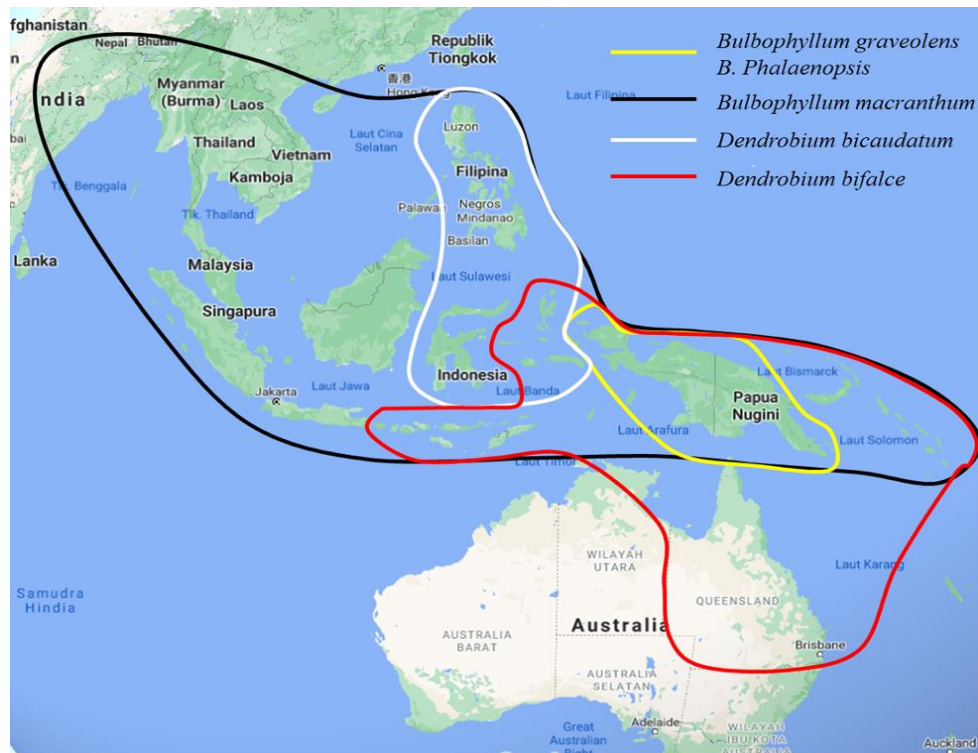


Figure 7. The distribution area of investigated taxa

Bulbophyllum macranthum has a smaller SAS value ($43.33 \pm 10.82\%$) than the other taxa, then it can be predicted that the species are distributed in a limited area. In fact, *B. macranthum* was dispersed to a larger area than other species. Based on Vermeulen (1991), it is distributed from Assam, Burma, Vietnam, Thailand, Malaysia, Sumatra, Java, Borneo, Sulawesi, Papua New Guinea, Philippines, reaching Solomon Island (Figure 7, black line). Howe and Smallwood (1982) assumed that the dispersal mechanism for the species with small SAS like *B. macranthum* is by water (hydrochory) along the coast. Moreover, Diantina et al. (2020) stated that the testa covered by waxes could be a very useful character for seed distribution along the coastal stream. This hypothesis also applies to *D. bifalce* that has the smallest air space (10.12 ± 26.88) (Table 3) and distributes in the Lesser Sunda Islands, Moluccas, New Guinea, and Queensland to Solomon Islands (Figure 7, red line) (O'Byrne 1994).

The SAS values between *B. graveolens* and *B. phalaenopsis* were not different significantly. Both of them have SAS ranging from $59.81 \pm 9.70\%$ to $62.64 \pm 12.79\%$. Those values were bigger than *D. bifalce*, then it can be analyzed that the distribution area is larger. On the contrary, based on O'Byrne (1994), *B. graveolens* and *B. phalaenopsis* are native to Papua New Guinea (Figure 7, yellow line). *Bulbophyllum graveolens* was reported found in scattered locations. This species is uncommon and usually found only in isolated stands, growing on tree trunks of the lower and middle layers of the forest (O'Byrne 1994). The reason for this condition is that they have a very specific environment (habitat and substrate)

and mycorrhiza requirements for seed germination (Arditti and Ghani 2000; Tongbram et al. 2012; Ramudu et al. 2020). Zhang et al. (2018) stated that orchids display various levels of specificity to their fungal hosts, then it can be seen clearly that the environment with low fungal diversity would restrict the orchids to only certain habitats where these specific fungi appear. There would be heavy mortality at any stage of seedling development and very few seed seedlings will grow successfully and give a rise to mature plants (Ramudu et al. 2020).

In conclusion, all seeds of the orchid species examined have the same shape and similar testa walls condition with verrucosity as one of the key characteristics of epiphytes. The difference between *Bulbophyllum* and *Dendrobium* observed is in the orientation of testa walls. There are no large variations in the quantitative characters of those two genera, including the SAS value. The dispersal mechanism and distribution area of the orchid species can be predicted by their SAS values. However, the values are not always positively correlated with the distribution area of the species. Even SAS value is large enough. Other factors inhibit the spread of the species, such as the absence of specific mycorrhiza. The seed with small SAS tends to be dispersed by water.

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