



sucipto hariyanto &lt;sucipto-h@fst.unair.ac.id&gt;

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**[biodiv] Submission Acknowledgement**

1 message

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**Ahmad Dwi Setyawan** <smujo.id@gmail.com>  
Reply-To: Ahmad Dwi Setyawan <editors@smujo.id>  
To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

Wed, Sep 18, 2019 at 6:47 AM

Sucipto Hariyanto:

Thank you for submitting the manuscript, "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum" to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: <https://smujo.id/biodiv/authorDashboard/submission/4466>  
Username: hariyanto

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

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**[biodiv] Editor Decision**

2 messages

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**Smujo Editors** <smujo.id@gmail.com>  
Reply-To: Smujo Editors <editors@smujo.id>  
To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

Fri, Oct 18, 2019 at 1:25 PM

Sucipto Hariyanto:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum".

Our decision is: Revisions Required

Smujo Editors  
[editors@smujo.id](mailto:editors@smujo.id)

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Reviewer C:  
Recommendation: Revisions Required

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Reviewer G:

1. Title - change to "Variations in seed micromorphology and morphometry of native Indonesian Phalaenopsis and Paphiopedilum orchid.

2. p48 - plant material change to "plant materials"

3. Figure 1 - It looks like no embryo in several seeds, including B, C, E, J.

4. Fig. 2-11 - Too many figures. Combine them into one panel.

5. There is a lack of significant difference between means in all data.

6. The authors claim that "the results of the study indicate that morphological and morphometric features can serve to identify live forms and distinguish between species and phylogeny", but how to use them to identify these species of orchids? Please specify at least one example in detail.

7. M&M - Please describe the maturity of capsules in detail, e.g. how long is the period from pollination to maturation, morphology and size, etc.

Recommendation: Revisions Required

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 **C-Edit\_4466-Article Text-11784-1-4-20190920 (1).doc**

3094K

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**sucipto hariyanto** <sucipto-h@fst.unair.ac.id>  
To: Smujo Editors <editors@smujo.id>

Mon, Nov 11, 2019 at 9:28 PM

Dear Smujo Editors,  
I apologize for the delay in completing the revision in accordance with the reviewer recommendations.

Following, I send an article that I have revised and a list of revisions / answers from the review (attached),

besides that I have also sent via the system (smujo).


Hopefully it is in accordance with the editor and review

Best regards,  
Sucipto Hariyanto

[Quoted text hidden]

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**2 attachments**

 **C-Edit\_4466-Article Text-11784-1-4-20191019 (1) (f).doc**  
13888K

 **List of Revisions 20191019.docx**  
22K



sucipto hariyanto <sucipto-h@fst.unair.ac.id>

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## [biodiv] New notification from Biodiversitas Journal of Biological Diversity

1 message

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**Smujo Editors** <smujo.id@gmail.com>

Thu, Nov 14, 2019 at 5:12 PM

Reply-To: Smujo Editors <editors@smujo.id>

To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Uncorrected Proof" regarding the submission "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum".

Link: <https://smujo.id/biodiv/authorDashboard/submission/4466>

Ahmad Dwi Setyawan

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sucipto hariyanto &lt;sucipto-h@fst.unair.ac.id&gt;

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**[biodiv] New notification from Biodiversitas Journal of Biological Diversity**2 messages

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**DEWI NUR PRATIWI** <smujo.id@gmail.com>  
Reply-To: DEWI NUR PRATIWI <biodiv07@gmail.com>  
To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

Fri, Nov 15, 2019 at 10:19 AM

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "BILLING" regarding the submission "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum".

Link: <https://smujo.id/biodiv/authorDashboard/submission/4466>

Ahmad Dwi Setyawan

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[Biodiversitas Journal of Biological Diversity](#)

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**Smujo Editors** <smujo.id@gmail.com>  
Reply-To: Smujo Editors <editors@smujo.id>  
To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

Fri, Nov 15, 2019 at 10:47 AM

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "Uncorrected Proof" regarding the submission "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum".

[Quoted text hidden]

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**[biodiv] New notification from Biodiversitas Journal of Biological Diversity**2 messages

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**Smujo Editors** <smujo.id@gmail.com>

Sat, Nov 16, 2019 at 8:17 AM

Reply-To: Smujo Editors &lt;editors@smujo.id&gt;

To: Sucipto Hariyanto &lt;sucipto-h@fst.unair.ac.id&gt;

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "Uncorrected Proof" regarding the submission "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum".

Link: <https://smujo.id/biodiv/authorDashboard/submission/4466>

Ahmad Dwi Setyawan

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**sucipto hariyanto** <sucipto-h@fst.unair.ac.id>

Sat, Nov 16, 2019 at 1:11 PM

To: Smujo Editors &lt;editors@smujo.id&gt;

Dear Setyawan,  
Thanks you for notice to me.

Best regards,  
Sucipto Hariyanto  
[Quoted text hidden]



sucipto hariyanto &lt;sucipto-h@fst.unair.ac.id&gt;

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**[biodiv] New notification from Biodiversitas Journal of Biological Diversity**

1 message

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**DEWI NUR PRATIWI** <smujo.id@gmail.com>  
Reply-To: DEWI NUR PRATIWI <biodiv07@gmail.com>  
To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

Sun, Nov 17, 2019 at 2:35 PM

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "BILLING" regarding the submission "Variations in seed micromorphology and morphometry of native Indonesian Orchids Phalaenopsis and Paphiopedilum".

Link: <https://smujo.id/biodiv/authorDashboard/submission/4466>

Ahmad Dwi Setyawan

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[Biodiversitas Journal of Biological Diversity](#)

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**[biodiv] Editor Decision**

2 messages

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**Smujo Editors** <smujo.id@gmail.com>  
Reply-To: Smujo Editors <editors@smujo.id>  
To: SUCIPTO HARIYANTO <sucipto-h@fst.unair.ac.id>

Mon, Nov 18, 2019 at 1:59 PM

SUCIPTO HARIYANTO :

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Variations in seed micromorphology and morphometry of native Indonesian Phalaenopsis and Paphiopedilum orchids".

Our decision is to: Accept Submission

Smujo Editors  
[editors@smujo.id](mailto:editors@smujo.id)

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[Biodiversitas Journal of Biological Diversity](#)

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**Smujo Editors** <smujo.id@gmail.com>  
Reply-To: Smujo Editors <editors@smujo.id>  
To: SUCIPTO HARIYANTO <sucipto-h@fst.unair.ac.id>

Mon, Nov 18, 2019 at 2:01 PM

SUCIPTO HARIYANTO :

The editing of your submission, "Variations in seed micromorphology and morphometry of native Indonesian Phalaenopsis and Paphiopedilum orchids," is complete. We are now sending it to production.

Submission URL: <https://smujo.id/biodiv/authorDashboard/submission/4466>

Smujo Editors  
[editors@smujo.id](mailto:editors@smujo.id)

[Quoted text hidden]



**REVIEWER RECOMMENDATIONS AND  
COMMENTS & RESPONSES TO  
REVIEWER COMMENTS**



sucipto hariyanto <sucipto-h@fst.unair.ac.id>

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**[biodiv] Editor Decision**

2 messages

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**Smujo Editors** <smujo.id@gmail.com>  
Reply-To: Smujo Editors <editors@smujo.id>  
To: Sucipto Hariyanto <sucipto-h@fst.unair.ac.id>

Fri, Oct 18, 2019 at 1:25 PM

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Reviewer G:


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Recommendation: Revisions Required

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[Biodiversitas Journal of Biological Diversity](#)

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 **C-Edit\_4466-Article Text-11784-1-4-20190920 (1).doc**  
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# Variations in seed micromorphology and morphometry of native Indonesian Orchids *Phalaenopsis* and *Paphiopedilum*

**Abstract.** Seeds of ten taxa of the genera *Phalaenopsis* and *Paphiopedilum* were studied using light and scanning electron microscopy (SEM). Quantitative characters (seed and embryo shape, seed and embryo colour, ornamentation of testa cell, testa cell wall shape) as well as quantitative data (seed and embryo length, seed and embryo width, seed and embryo volume, seed length/seed width, embryo length/embryo width, seed volume/embryo volume and air space) were analysed. Seeds of all studied taxa were fusiform in shape and had smooth testa surfaces. *Phalaenopsis* testa cells were elongated with cylindrical cell walls, while *Paphiopedilum* testa cells were polygonal with thin and flat rectangular cell walls. The shape of the embryos was generally ovoid in *Phalaenopsis* and prolate in *Paphiopedilum*. Seed colours ranged from brown to dark brown. Embryo colours varied from light yellow, yellowish brown, dark brown, black and white. Based on our investigation, there are variations in seed and embryo length, seed and embryo width, seed and embryo volume as well as the percentage of the air space, both in *Phalaenopsis* and *Paphiopedilum*. In general, the values of seed volume, embryo volume and air space in *Paphiopedilum* are higher than in *Phalaenopsis*. Together, the results of the study indicate that morphological and morphometric features can serve to identify live forms and distinguish between species and phylogeny.

**Keywords:** testa cell shapes, testa cell wall shapes, seed volume, seed air spaces, epiphytic versus terrestrial habitats

## INTRODUCTION

*Phalaenopsis* Blume and *Paphiopedilum* Pfitzer are orchids whose members are very popular and are commercialised as the most traded potted and cut flower plants in the world. Wild populations are under threat of extinction due to high rates of habitat modification, deforestation, forest fires, illegal harvesting and trade as a consequence of rapid economic development, high population growth and corrupt institutions (Sodhi et al. 2004). All *Paphiopedilum* species are listed by the Conservation on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Appendix I and *Phalaenopsis* are listed in Appendix II. This is in spite of the fact that, according to the CITES, all species from the genus *Paphiopedilum* are prohibited for trade.

Seeds occupy the first rank in the life history of plants. Orchid seeds disperse, germinate and grow into mature plants, and reproduce in a suitable place on certain parts of the tree or on the ground. In general, seeds are responsible for the regeneration and distribution of a species, even playing an important role in the conservation of orchids. Verma et al. (2014) and Tsutsumi et al. (2007) explain that seed dispersal mode and seed size are important factors in regulating the growth of new populations.

Seed morphology varies greatly in shape, colour, size, volume of the embryo and testa structure, and some of these characters have been used to establish the phylogeny of species in the genus (Gamarra et al. 2008, 2010, 2015; Cela et al. 2014; Guler, 2016). In addition, some seed characters can be related to germination and dispersal, especially in their ecological adaptation (Chaudhary et al. 2014; Zhang et al. 2015). According to research by Chaudhary et al. (2014) in the *Dendrobium* orchid, the volume of the embryo and the percentage of the air space are directly related to the climate and this is reflected in seed ultrastructure.

Previous studies on the morphology of orchid seeds in tropical areas have demonstrated the importance of the seed coat, related to the taxonomy and ecology (Chase and Pippen, 1988; Tsutsumi et al. 2007; Akçin et al. 2009; Verma et al. 2012), as demonstrated with scanning electron microscopy (SEM).

Not much is known about how certain seed characteristics may correlate with ecological adaptations. The aim of this study is to reveal the qualitative and quantitative characteristics of the seeds of several species of epiphytic and terrestrial orchids native to Indonesia from different genera, thus contributing to a better understanding of the differences and similarities in their adaptation strategies for seeds in the tropics.

### 48 Plant material and seed collection

49 Ten different orchid species were collected from Simanis and DD Orchids Nursery, East Java, Indonesia. The  
 50 collection for the present study included *Phal. amabilis*, *Phal. tetraspis*, *Phal. bellina*, *Phal. gigantea*, *Phal. amboinensis*,  
 51 *Phal. venosa*, *Paph. kolopakingii*, *Paph. liemianum*, *Paph. baccanum*, *Paph. Primulinum*; these plants were then hand  
 52 pollinated during their normal period of flowering. The seeds of mature capsules [WU3] were collected between 2016 to  
 53 2019 [WU4]. The capsules were washed using 10% sunlight detergent solution for 3 minutes to eliminate dust particles, then  
 54 rinsed 3 times with sterile distilled water. The surface of the capsules was sprayed with 70% alcohol, put on a Petri dish,  
 55 placed into a laminar flow cupboard and passed over a Bunsen flame; this was repeated 3 times. The capsule was cut into  
 56 four parts transversally and longitudinally using a sterile scalpel in a sterile Petri dish. The mature seeds were released  
 57 from the capsules and collected with the help of a sterile spatula. Fresh seeds were dried for at least 2 weeks [WU5] and  
 58 stored in small-cap vials at 4°C [WU6] in dry conditions. [...] [WU7]. All species within *Phalaenopsis* in this study are epiphytic  
 59 orchids while all species within *Paphiopedilum* are terrestrial orchids.

### 60 Morphological and statistical analyses

61 Seed samples were observed and photographed under a Light Microscope (LM) and Scanning Electron Microscope  
 62 (SEM). Our morphological parameters included seed shape (SS), seed colour (SC), seed length (SL), seed width (SW),  
 63 seed length/seed width (SL/SW), seed volume (SV). For embryos, the parameters included embryo shape (ES), embryo  
 64 colour (EC), embryo length (EL), embryo width (EW), embryo length/embryo width (EL/EW), embryo volume (EV), seed  
 65 volume/embryo volume (SV/EV), and air space (AS). The SV ( $\text{mm}^3 \times 10^{-3}$ ) was calculated using the formula  $2 \left[ \frac{L}{2} \right]$   
 66  $\left( \frac{W}{2} \right)^2 \left( \frac{\pi}{3} \right)$ , where L = length, W = width,  $\pi = \frac{22}{7}$  and embryo volume ( $\text{mm}^3 \times 10^{-3}$ ) with the formula  $\frac{4}{3} \pi \left( \frac{L}{2} \right) \left( \frac{W}{2} \right)^2$ ,  
 67 where L = length, W = width (adapted from Arditti et al. 1980). The SV/EV values were calculated following the  
 68 described method (Arditti et al. 1979). For calculating AS (%), the following formula was applied: (seed volume-embryo  
 69 volume)/seed volume) x 100% (adapted from Arditti and Ghani, 2000). To analyse each species statistically, we used the  
 70 mean for each quantitative character.

### 71 LM study

72 Seeds were spread on a slide with a drop of water and covered with a cover glass. Values for SL, SW, EL and EW (at  
 73 the longest and widest axis) were obtained from an average of thirty seeds and were observed for each species and  
 74 measurement using a light microscope (Olympus CH 20, Olympus Japan) and standardised ocular meter. Characteristics  
 75 such as SC, SS, EC and ES were observed under Tension stereomicroscope (Nikon SMZ-1, Japan). The SC and EC was  
 76 described in subjective terms.

### 77 SEM study

78 For SEM preparations, the samples (seeds) were mounted on SEM stubs. The samples were then sputter-coated with  
 79 palladium/gold (SEM coating system Q150R S mini sputter Coater). Detailed seed coat (testa cells) surface studies were  
 80 conducted by observing under a Hitachi TM3000, with a filament voltage of 15 kV. The parameters considered were seed  
 81 coat sculpturing and thickenings.

## 82 RESULTS AND DISCUSSION [WU8]

### 83 Qualitative character of seed and embryos

84 Qualitative characters were analysed by LM, and this showed the seeds [WU9] were generally uniform i.e. fusiform in  
 85 shape with colours from brown to dark brown. Brown transparent seeds were found in *Phal. amabilis*, *Phal. tetraspis*,  
 86 *Phal. bellina*, *Phal. gigantea*, *Phal. Amboinensis* and *Phal. venosa*. Dark brown seeds were recorded in *Paph. kolopakingii*  
 87 and *Paph. liemianum*. Brown seeds were observed in *Paph. baccanum* and *Paph. primulinum* (Table 1, Figure 1). The  
 88 shape of the embryos was generally ovoid in *Phalaenopsis* and prolate in *Paphiopedilum*, and embryos were distinct and  
 89 present in the centre. Embryo colour in the observed species varied from light yellow, yellowish brown to dark brown,  
 90 black and white. Light yellow embryos were found in *Phal. venosa*. Yellowish brown embryos were recorded in *Phal.*  
 91 *amabilis* and *Phal. tetraspis*. Brown embryos were common in *Phal. bellina*, *Phal. gigantea* and *Paph. kolopakingii*. Dark  
 92 brown embryos were found in *Paph. Baccanum* and yellow embryos were noticed in *Phal. amboinensis*. White embryos  
 93 characterised *Paph. liemianum* and black embryos were seen in *Paph. primulinum* (Table 1, Figure 1).

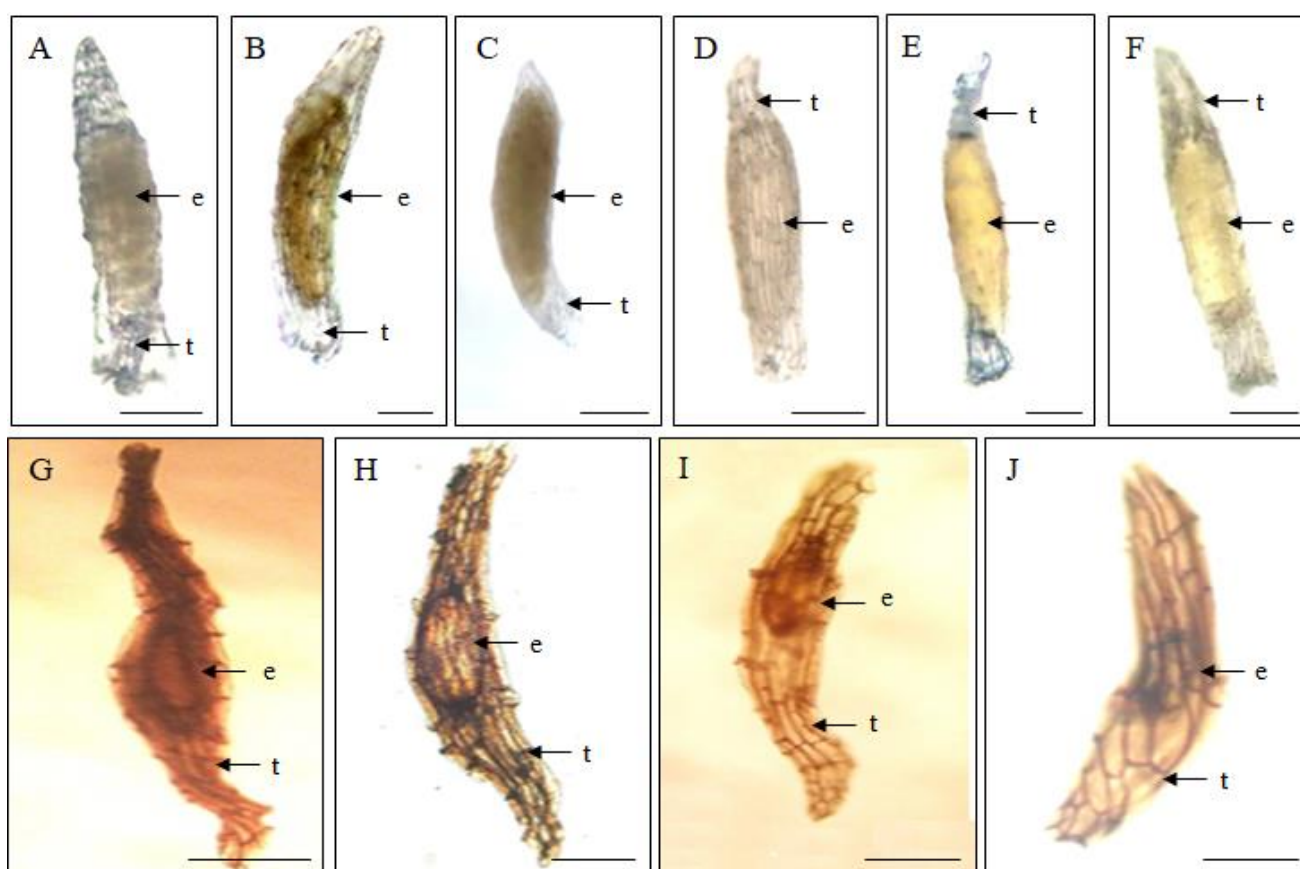
94 Molvray and Chase (1999) announced that features of the seed ornamentation can be based at higher taxonomic ranks.  
 95 In the present work, the testa surface of *P*[WU10]. *amabilis* did not have any ornamentation. The testa cells were elongated,  
 96 longitudinally oriented, parallel to the long seed axis and irregular. The testa cell walls were cylindrical, and exhibited  
 97 overlap (Figure 2B arrow), becoming round and rising at the end of the meeting between two testa cells [WU11] (Figure 2D  
 98 arrow). Certain parts of the testa formed deep furrows (Figure 2C arrow). There were no differences in the shape of the  
 99 testa cells at the two poles [WU12].

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**Table 1.** Qualitative data of the analysed species [WU13] of the genus *Phalaenopsis* and *Paphiopedilum*

Species name	Seed Shape	Seed Colour	Ornamentation of the periclinal walls	Testa cell shape	Testa cell walls shape	Embryo Shape	Embryo Colour
<i>Phal. amabilis</i> [WU14]	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish brown
<i>Phal. tetraspis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish brown
<i>Phal. bellina</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. gigantea</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. amboinensis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellow
<i>Phal. venosa</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Light yellow
<i>Paph. kolopakingii</i> [WU15]	Fusiform	Dark brown	Smooth	Polygonal	Flat rectangular	Prolate	Brown
<i>Paph. liemianum</i>	Fusiform	Dark brown	Smooth	Polygonal	Thin rectangular	Prolate	White
<i>Paph. baccanum</i>	Fusiform	Brown	Smooth	Polygonal	Thin rectangular	Prolate	Dark brown
<i>Paph. primulinum</i>	Fusiform	Brown	Smooth	Polygonal	Flat rectangular	Prolate	Black

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Figure 1. LM photographs of (A-F) *Phalaenopsis* - and (G-J) *Paphiopedilum*. A. *Phal. amabilis* (scale bar=72  $\mu$ m), B. *Phal. tetraspis* (scale bar=49  $\mu$ m), C. *Phal. bellina* (scale bar=65  $\mu$ m), D. *Phal. gigantea* (scale bar=51  $\mu$ m), E. *Phal. amboinensis* (scale bar=59  $\mu$ m), F. *Phal. venosa* (scale bar=55  $\mu$ m), G. *Paph. kolopakingii* (scale bar=206  $\mu$ m), H. *Paph. liemianum* (scale bar=162  $\mu$ m), I. *Paph. baccanum* (scale bar=185  $\mu$ m), J. *Paph. primulinum* (scale bar=161  $\mu$ m). e= embryo, t=testa.

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The testa surface of *P*[WU16]. *tetraspis* was smooth. The testa cells were elongated, longitudinally oriented, parallel to the long axis and irregular. The testa cell walls were cylindrical, and very close together [WU17](Figure 3). At the end of the meeting between two testa cells the cell walls became wider, rounder and rose up (Figure 3C arrow)[WU18]. There was a difference in the shape of the testa cells at the two poles. Cells at the basal pole were smaller than cells at the apical pole[WU19].

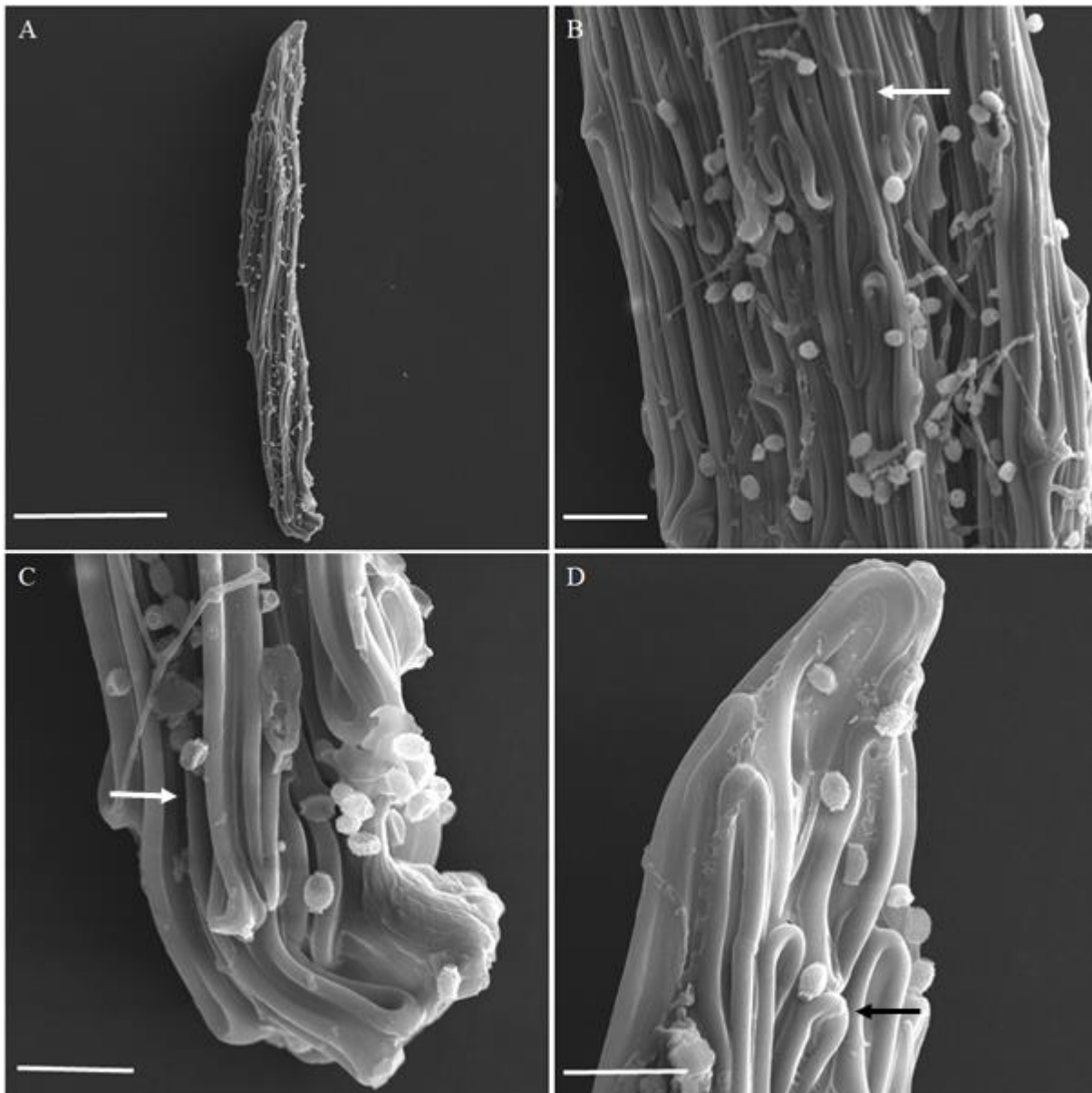
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*P*[WU20]. *bellina* did not have any ornamentation on the surface of its testa cells; the cells were elongated, longitudinally oriented, parallel to the long seed axis and irregular. The testa cell walls were cylindrical, and the distance between two cells formed a slit in a row (Figure 4B). The meeting point between the two ends of the testa cells was curved, thickened



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and raised to form a bulge (Figure 4B arrow). There was a difference in the shape of the testa cells at the two poles. Cells of the basal pole were bigger and rounder than cells of the apical pole [WU21](Figure 4C and D).



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128 **Figure 2**[WU22]. SEM photographs of *Phal. amabilis*. A. **Fusiform** seed shape, B. **Pattern of testa cells** [WU23]of the medial, C. **Cells of**  
129 **the basal pole**[WU24], D. **Cells of the apical pole**[WU25]. Scale bars: A=100 µm, B-D=10 µm

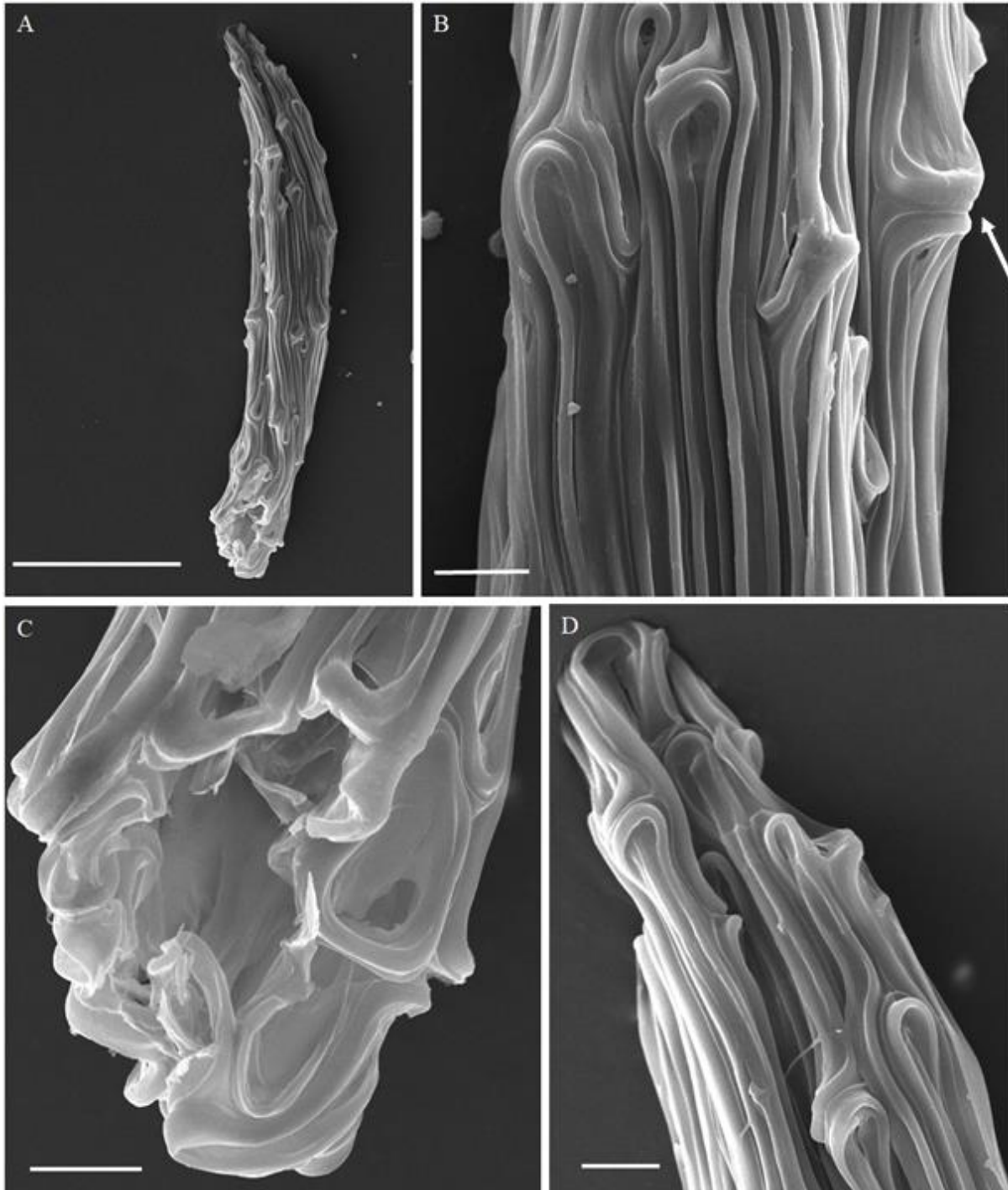
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132 In *P.*[WU26] *gigantea*, the testa surface was smooth. The testa cells were elongated, longitudinally oriented, parallel to  
133 the long seed axis and highly irregular. Ridges were elevated with a deep groove (Figure 5B black arrow). The testa cell  
134 walls were cylindrical. At the meeting point the two ends of the testa cells were curved and raised (Figure 5B white  
135 arrow). Cells of the basal pole were different to cells at the apical pole; cells at the basal pole had an appendage at the end  
136 and become pointed (Figure 5C arrow).[WU27]  
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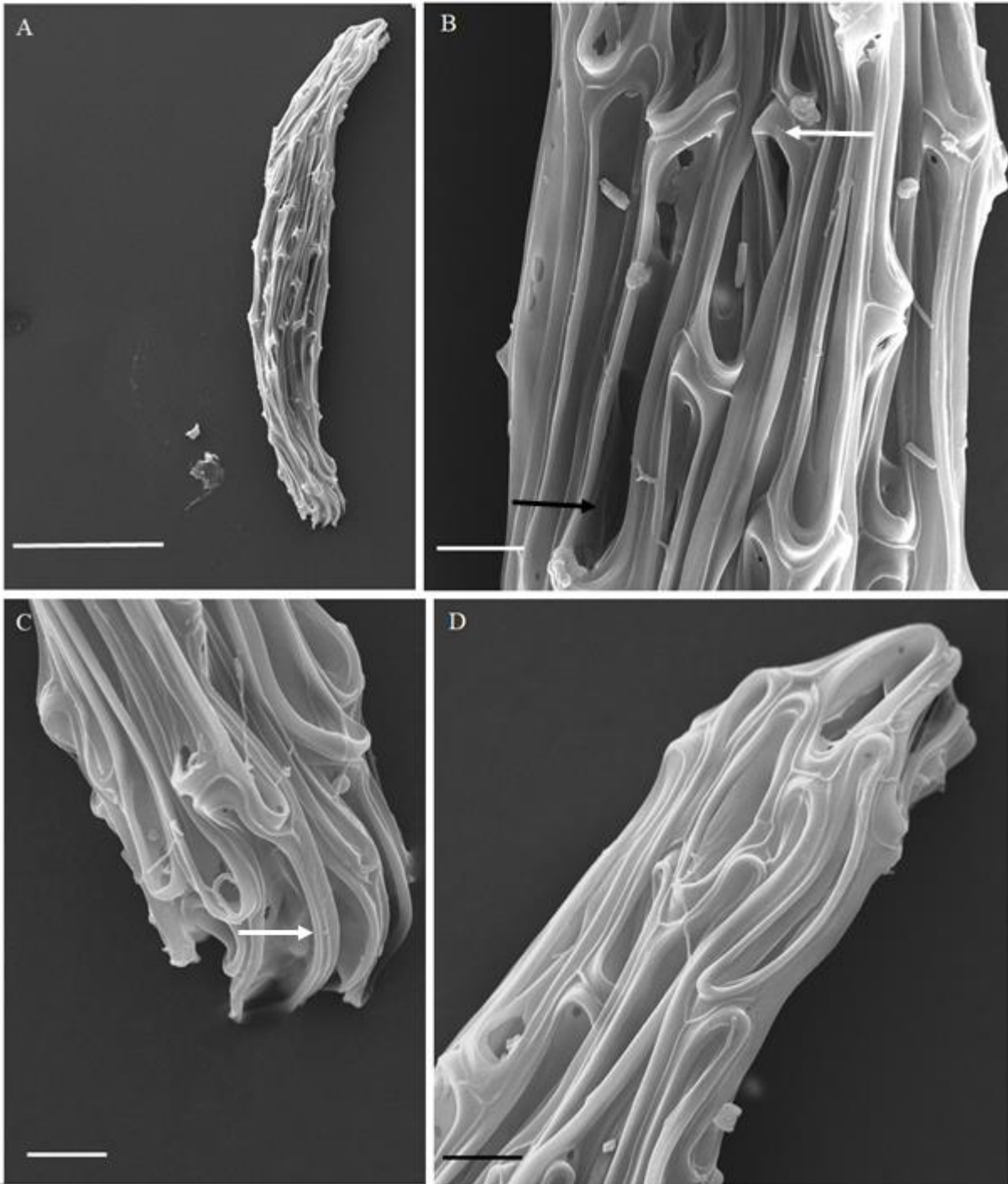
Figure 3[WU28]. SEM photographs of *Phal. tetraspis*. A. seed shape[WU29], B. Pattern of testa [WU30]cells of the medial, C. Cells of the basal pole[WU31], D. Cells of the apical pole[WU32]. Scale bars: A=100  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .



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Figure 4[wu33]. SEM photographs of *Phal. bellina*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .





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Figure 5. [WU34]SEM photographs of *Phal. gigantea*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .

The testa cells on the surface of *P. amboinensis* did not have any ornamentation. The testa cells were elongated, longitudinally oriented, parallel to long axis and irregular. The testa cells walls were cylindrical. Part of the meeting at the end of the two cells was rounded and slightly raised (Figure 6B arrow). The end of the basal pole was blunt (Figure 6C), while the end of the apical pole became pointed (Figure 6D).

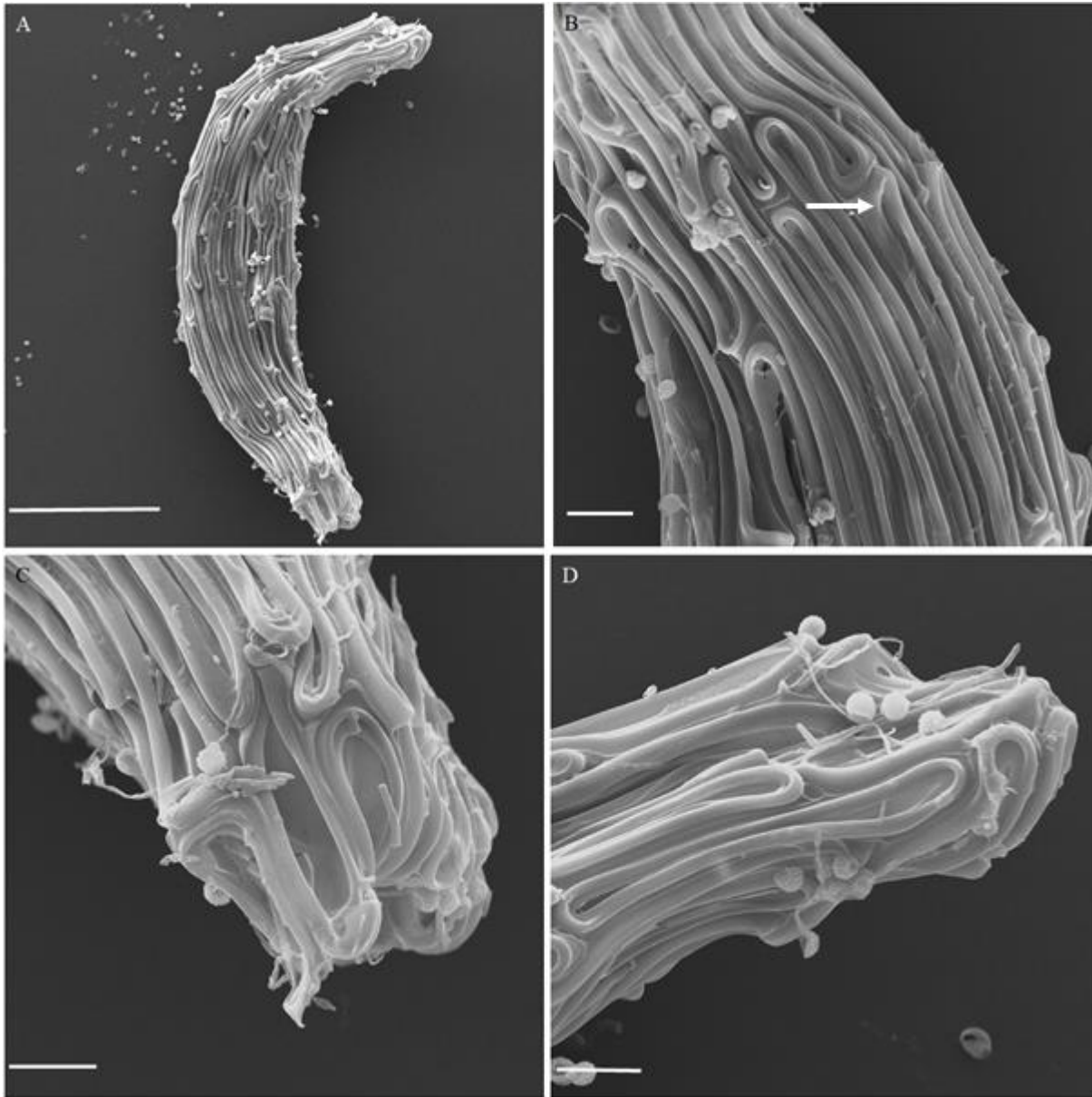


Figure 6. [WU35]SEM photographs of *Phal. amboinensis*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=80  $\mu$ m, B-D=10  $\mu$ m.

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In the case of *P. venosa*, the testa surface was smooth. The testa cells were elongated and longitudinally oriented, parallel to the long axis, irregular, and raised at the end of the meeting between two cells (Figure 7B arrow). The testa cell walls were cylindrical. Testa cells at the end of the basal pole were widened and rounded (Figure 7C arrow), but testa cells at the apical pole were more elongated (Figure 7D). The end of the basal pole was rounded (Figure 7C), while the end of the apical pole was pointed (Figure 7D).

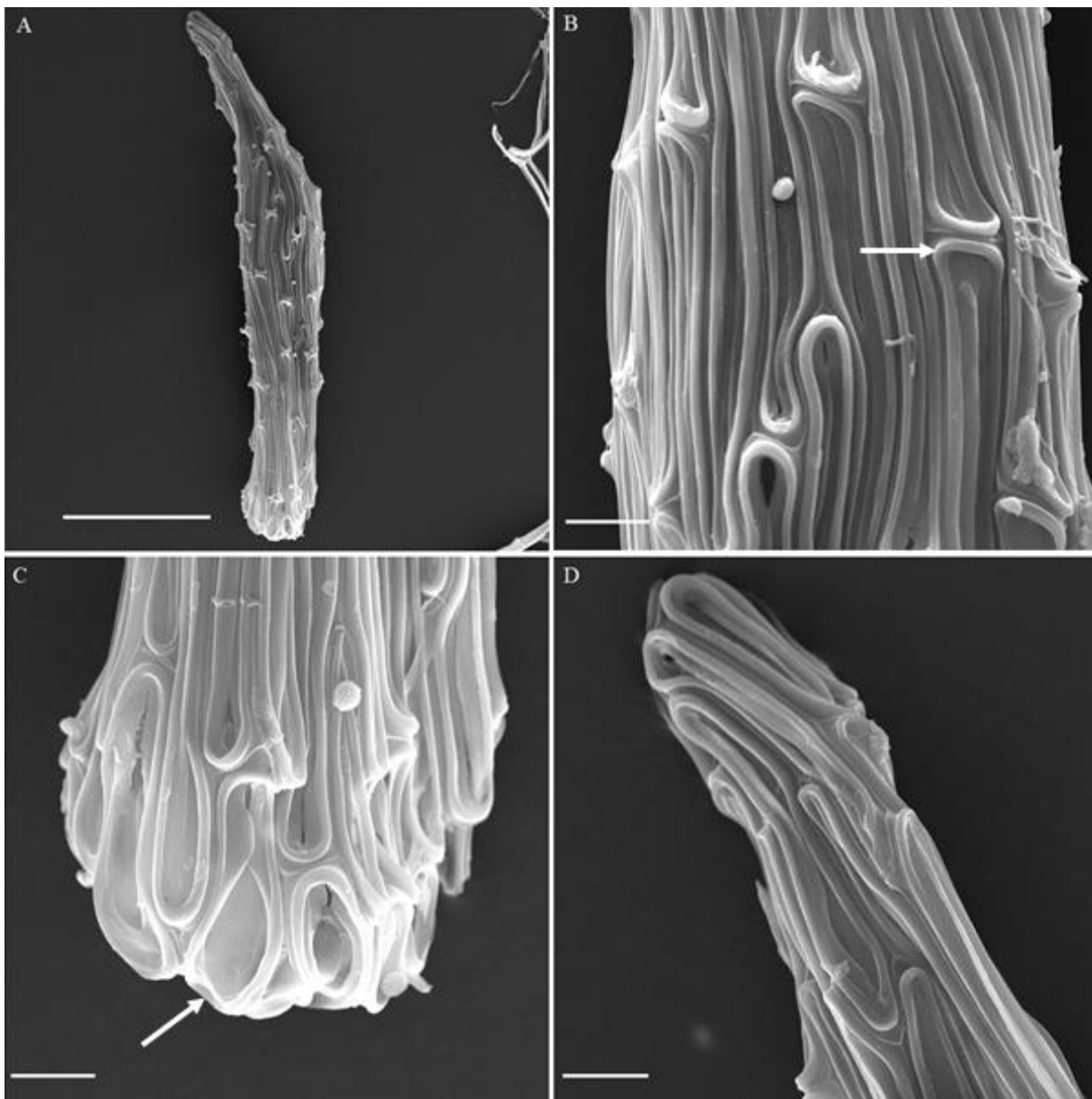
In the case of *Paph. kolopakingii*, testa cells were polygonal, longitudinally oriented with a deep groove, parallel to the long axis and irregular. The testa cell walls were flat and rectangular (Figure 8B arrow). The testa surfaces were smooth. The shape of the basal cell pole was larger and more dented than the cells of the apical pole (Figures 8C and D). The testa surfaces of *Paph. liemianum* were smooth. The testa cells were polygonal, longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 9). The testa cell walls were thin and rectangular (Figure 9B arrow). The medial region was wider than the two poles. The basal cells were short, polygonal and more numerous than the apical cells (Figure 9C arrow).

In the case of *Paph. baccanum*, the testa surface was smooth. Generally, the testa cells were polygonal, longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 10). The testa cell walls were thin and rectangular (Figure 10B arrow). Cells at the basal pole were longer compared to cells at the apical pole. The end of the basal pole became pointed, while the end of the apical pole became rounded (Figures 10C and D).

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*In Paph. primulinum*, the testa surface was smooth. Generally, testa cells were polygonal, longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 11). The testa cell walls were flat and rectangular (Figure 11B arrow). The end of the basal pole was blunt, while the end of the apical pole was suppressed (Figures 11C and D).

In this study, we did not observe any sculpturing on the cell walls or smooth in any species of the two different genera. This is probably because all of these species are equally alive and come from the tropics, despite their different living forms (epiphyte and terrestrial). This observation corresponds with the opinion of Chaudhary et al. (2014), who noted that seed ornamentations are directly related to the climatic preference of the species rather than its phylogeny. Similarly, according to Shimizu (2012), seed coat forms that are independent of plant habitat have a wider seed dispersal. The images we observed with SEM showed that different species in the genus *Phalaenopsis* had the same shape of testa cells and cell walls, namely elongated with cylindrical cell walls. Similarly, different species in the genus *Paphiopedilum* had testa cells and cell walls with the same shape, namely polygonal and with thin/flat rectangular cells walls. However, these results contradict with the results of previous research by Arditti et al. (1979, 1980) and Swamy et al. (2004), who showed that testa cells of different species can vary significantly in shape.



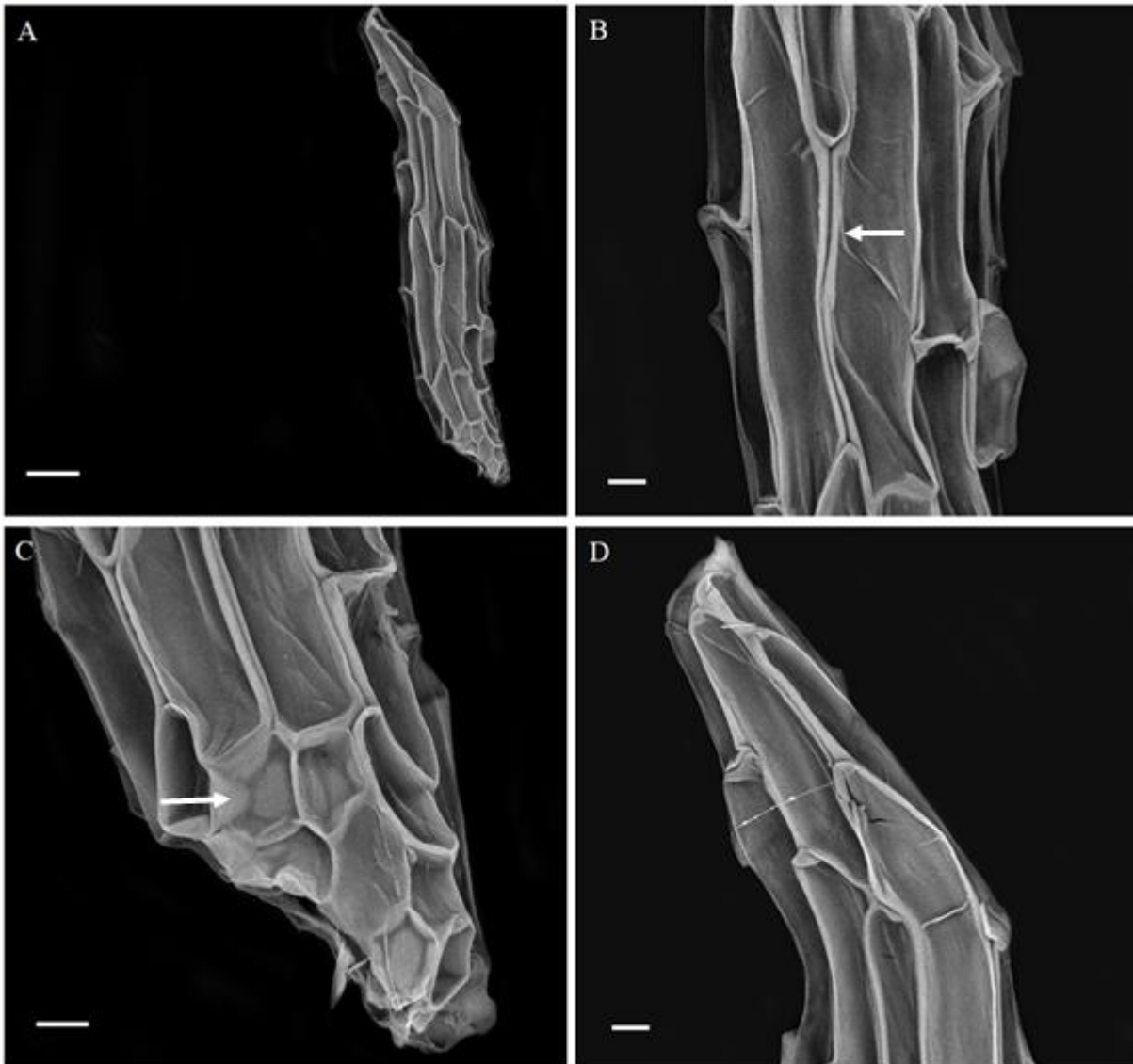
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Figure 7. [WU36]SEM photographs of *Phal. venosa*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=80  $\mu$ m, B-D=10  $\mu$ m.



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Figure 8[wU37]. SEM photographs of *Paph. kolopakingii*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu\text{m}$ , B-D=20  $\mu\text{m}$ .



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Figure 9. [WU38]SEM photographs of *Paph. liemianum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.



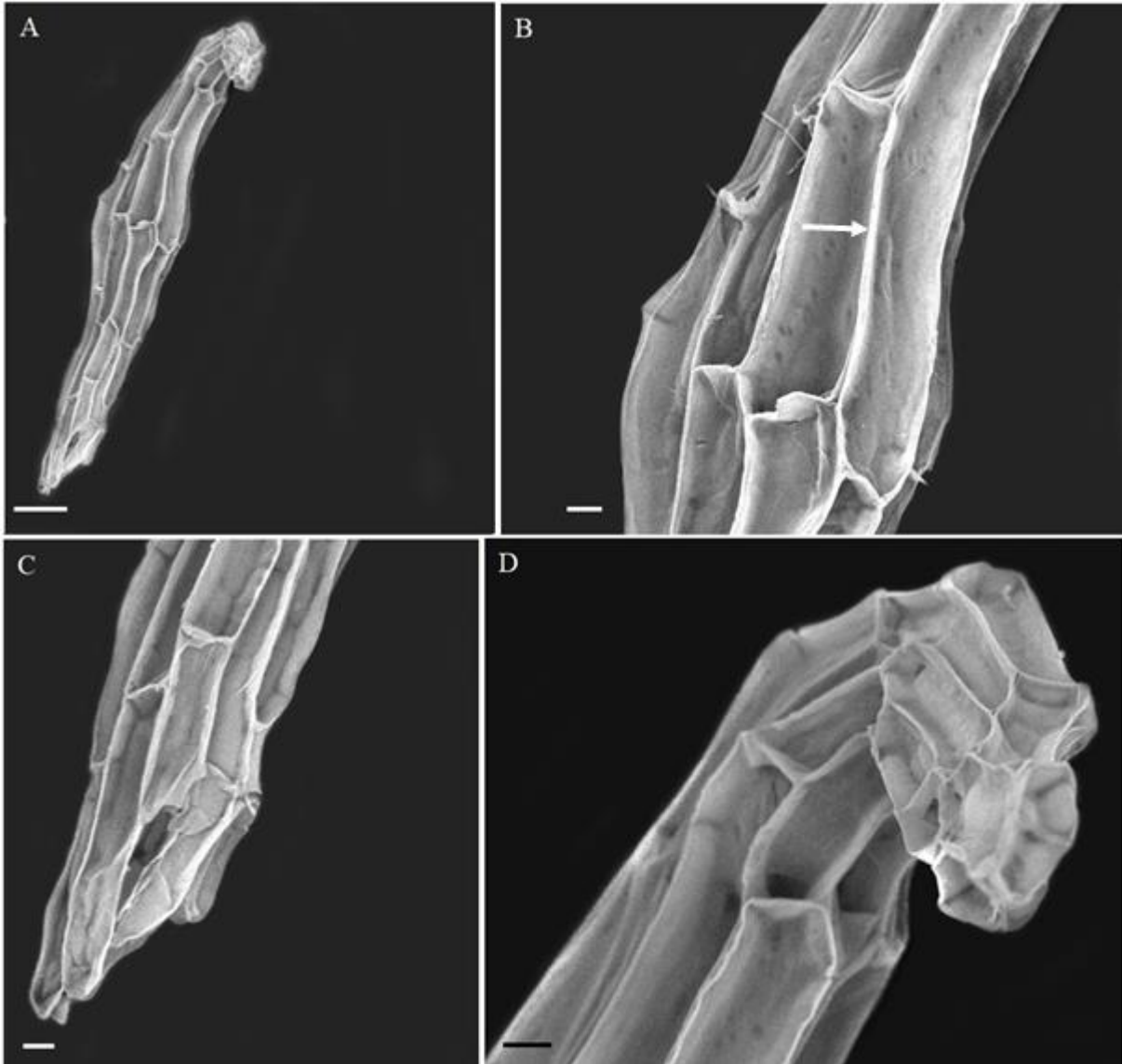


Figure 10[wU39]. SEM photographs of *Paph. baccanum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.

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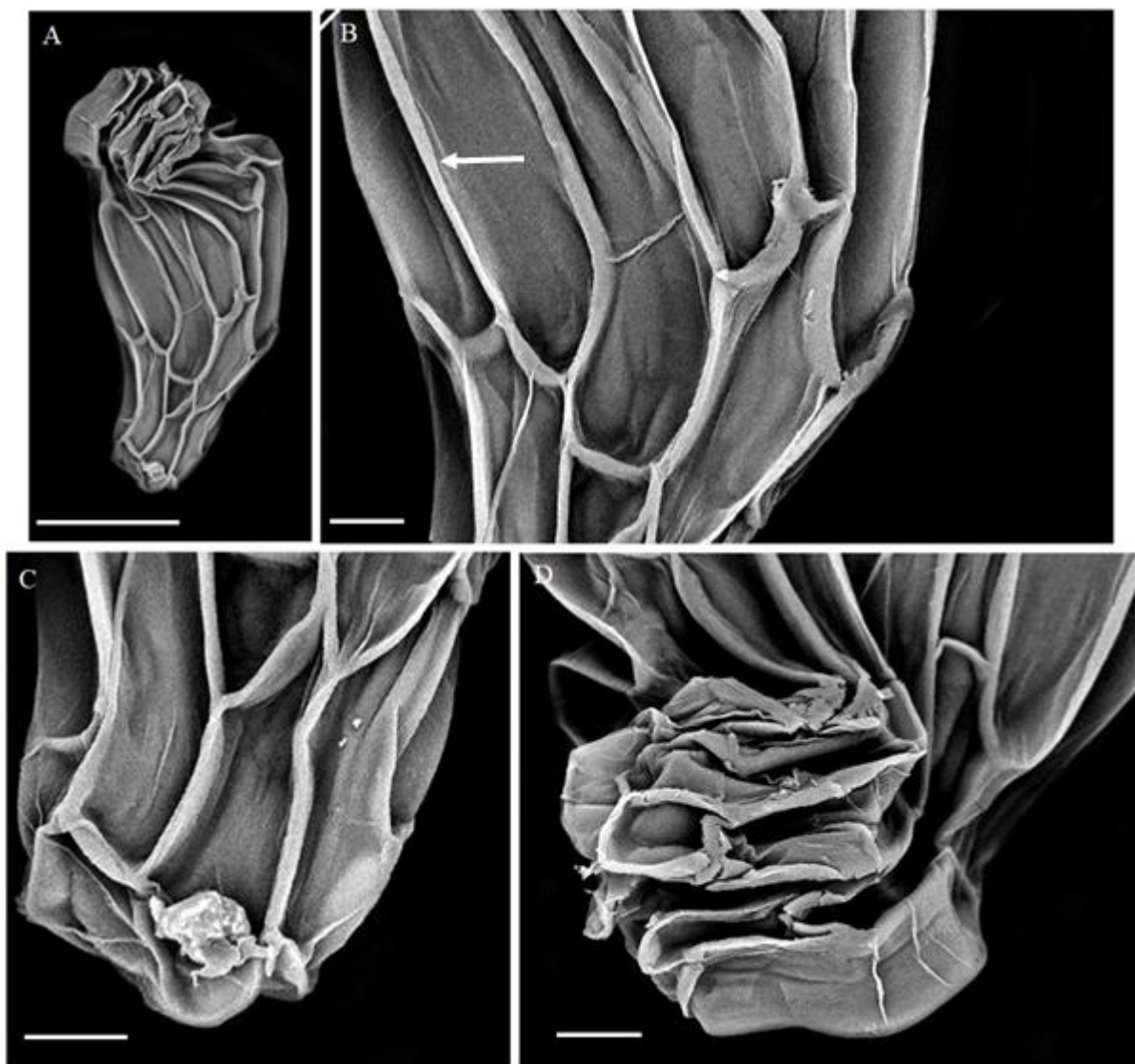


Figure 11 [WU40]. SEM photographs of *Paph. primulinum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu\text{m}$ , B-D=20  $\mu\text{m}$ .

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### Seeds size, ratio SL/SW and seed volume

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The table 2 exhibits the size of seeds from ten species of the genus *Phalaenopsis* and *Paphiopedilum*. In spite of the fact that the seeds are microscopic, the result of the investigation showed a great diversity in their size. Seed length ranged from  $0.302 \pm 0.0079$  mm (*Phal. bellina*) and  $0.983 \pm 0.0035$  mm (*Paph. liemianum*), and width ranged from  $0.049 \pm 0.0039$  mm (*Phal. tetraspis*) to  $0.206 \pm 0.0016$  mm (*Paph. kolopakingii*). According to Verma et al. (2014), the SL/SW ratio provides information on the degree of seed truncation and is a good taxonomic character (Arditti et al. 1979 and Vij et al. 1992). In the present work, the species with seeds that were truncated (SL/SW < 6) were *Phal. bellina*, *Phal. amboinensis*, *Paph. kolopakingii*, *Paph. baccanum* and *Paph. primulinum*. The following species had elongated seeds (SL/SW > 6): *Phal. amabilis*, *Phal. tetraspis*, *Phal. gigantea*, *Phal. venosa* and *Paph. liemianum* (Table 2). The most truncated seeds (SL/SW =  $4.254 \pm 0.0494$  mm) were recorded in *Paph. kolopakingii* and the most elongated seeds (SL/SW =  $7.472 \pm 0.6574$  mm) in *Phal. tetraspis*, both of which are members of different genus. The seed volume showed significant variations in both *Phalaenopsis* and *Paphiopedilum* orchids. On average, low seed volume was found in *Phal. gigantea* ( $0.240 \pm 0.0327$   $\text{mm}^3 \times 10^{-3}$ ), *Phal. tetraspis* ( $0.242 \pm 0.0363$   $\text{mm}^3 \times 10^{-3}$ ), *Phal. venosa* ( $0.272 \pm 0.0316$   $\text{mm}^3 \times 10^{-3}$ ), *Phal. amboinensis* ( $0.318 \pm 0.0196$   $\text{mm}^3 \times 10^{-3}$ ), *Phal. bellina* ( $0.333 \pm 0.0268$   $\text{mm}^3 \times 10^{-3}$ ) and *Phal. amabilis* ( $0.589 \pm 0.0497$   $\text{mm}^3 \times 10^{-3}$ ). When compared with epiphytic species, the seed volume was found to be higher in the terrestrial species [ $9.668 \pm 0.1768$   $\text{mm}^3 \times 10^{-3}$  (the highest) in *Paph. kolopakingii*,  $7.324 \pm 0.1283$   $\text{mm}^3 \times 10^{-3}$  in *Paph. baccanum*,  $6.755 \pm 0.2672$   $\text{mm}^3 \times 10^{-3}$  in *Paph. liemianum* and  $5.532 \pm 0.6396$   $\text{mm}^3 \times 10^{-3}$  in *Paph. primulinum*]. Clifford and Smith (1969), Rasmussen (1995), Swamy et al. (2004) and Verma et al. (2012) stated that seed size shows a direct correlation with plant habitat, and

253 terrestrial orchids generally possess larger seeds as compared to epiphytic orchids. Yoder et al. (2010) also stated that the  
 254 seeds of terrestrial orchids are bigger as compared to epiphytic orchids.  
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256 Table 2. Micro-morphometric data of seeds related characters from species of the genus *Phalaenopsis* and *Paphiopedilum*  
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Species name	SL[WU41] (mm)	SW (mm)	SL/SW (mm)	SV (mm <sup>3</sup> x10 <sup>-3</sup> )
<i>Phalaenopsis</i>				
<i>Phal. amabilis</i>	0.433 ± 0.0084[WU42]	0.072 ± 0.0030	6.029 ± 0.2851	0.589 ± 0.0497
<i>Phal. tetraspis</i>	0.370 ± 0.0086	0.049 ± 0.0039	7.472 ± 0.6574	0.242 ± 0.0363
<i>Phal. bellina</i>	0.302 ± 0.0079	0.065 ± 0.0024	4.646 ± 0.1999	0.333 ± 0.0268
<i>Phal. gigantea</i>	0.348 ± 0.0095	0.051 ± 0.0034	6.823 ± 0.4870	0.240 ± 0.0327
<i>Phal. ambonensis</i>	0.349 ± 0.0062	0.059 ± 0.0018	5.915 ± 0.2214	0.318 ± 0.0196
<i>Phal. venosa</i>	0.345 ± 0.0069	0.055 ± 0.0032	6.305 ± 0.3947	0.272 ± 0.0316
<i>Paphiopedilum</i>				
<i>Paph. kolopakingii</i>	0.874 ± 0.0077	0.206 ± 0.0016	4.254 ± 0.0494	9.668 ± 0.1768
<i>Paph. liemianum</i>	0.983 ± 0.0035	0.162 ± 0.0033	6.074 ± 0.1291	6.755 ± 0.2672
<i>Paph. baccanum</i>	0.815 ± 0.0022	0.185 ± 0.0017	4.401 ± 0.0429	7.324 ± 0.1283
<i>Paph. primulinum</i>	0.815 ± 0.0435	0.161 ± 0.0088	5.083 ± 0.3878	5.532 ± 0.6396

258 .....[WU43]

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 260 Table 3. Micro-morphometric data of embryos related characters from species of the genus *Phalaenopsis* and *Paphiopedilum*  
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Species name	EL [WU44] (mm)	EW (mm)	EL/EW (mm)	EV (mm <sup>3</sup> x10 <sup>-3</sup> )	SV/EV (mm <sup>3</sup> x10 <sup>-3</sup> )	AS (%)
<i>Phal. amabilis</i> [WU45]	0.187 ± 0.0044	0.072 ± 0.0030	2.607 ± 0.1322	0.509 ± 0.0433	1.158 ± 0.0333	13.554 ± 2.4693
<i>Phal tetraspis</i>	0.171 ± 0.0081	0.046 ± 0.0034	3.739 ± 0.3049	0.191 ± 0.0301	1.273 ± 0.0821	21.128 ± 5.0900
<i>Phal bellina</i>	0.211 ± 0.0132	0.051 ± 0.0022	4.149 ± 0.3611	0.286 ± 0.0244	1.168 ± 0.0305	14.294 ± 2.2379
<i>Phal gigantea</i>	0.192 ± 0.0074	0.043 ± 0.3022	4.496 ± 0.3022	0.184 ± 0.0241	1.308 ± 0.0745	23.291 ± 4.3589
<i>Phal ambonensis</i>	0.203 ± 0.0046	0.051 ± 0.0019	3.993 ± 0.1897	0.274 ± 0.0200	1.165 ± 0.0529	14.245 ± 3.6410
<i>Phal venosa</i>	0.183 ± 0.0029	0.047 ± 0.0018	3.929 ± 0.1922	0.209 ± 0.0141	1.309 ± 0.1718	22.338 ± 10.0278
<i>Paph. kolopakingii</i> [WU46]	0.270 ± 0.0032	0.157 ± 0.0038	1.726 ± 0.0416	3.466 ± 0.1863	2.797 ± 0.1499	64.150 ± 1.9360
<i>Paph. liemianum</i>	0.271 ± 0.0044	0.083 ± 0.0045	3.267 ± 0.2025	0.797 ± 0.1031	6.974 ± 0.7653	85.501 ± 1.5279
<i>Paph. baccanum</i>	0.223 ± 0.0030	0.115 ± 0.0018	1.933 ± 0.0367	1.557 ± 0.0539	4.709 ± 0.2083	78.725 ± 0.9214
<i>Paph. primulinum</i>	0.274 ± 0.0111	0.105 ± 0.0090	2.636 ± 0.2165	1.590 ± 0.3090	3.608 ± 0.8454	70.898 ± 6.3436

262 .....[WU47]

264 **Seed to embryo volume and free air space**

265 According to Verma et al. (2014), EV is an important character as it directly affects the percentage of the procurable air  
 266 space inside the seed. Like their EL/EV ratios, EV were observed in various *Phalaenopsis* species; it was lowest in *Phal.*  
 267 *gigantea* (0.184±0.0241 mm<sup>3</sup> x 10<sup>-3</sup>) and highest in *Phal. amabilis* (0.509±0.0433 mm<sup>3</sup> x 10<sup>-3</sup>). In *Paphiopedilum*, EV the  
 268 ranged between 0.797±0.1031 mm<sup>3</sup> x 10<sup>-3</sup> (*Paph. liemianum*) and 3.466±0.1863 mm<sup>3</sup> x 10<sup>-3</sup> (*Paph. kolopakingii*).  
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270 Our data showed that variation was observed in SV/EV. In the species of *Phalaenopsis*, the SV/EV ratio never  
 271 exceeded two (Table 3), but in the species of *Paphiopedilum* measurements peaked at 6.974±0.7653 mm<sup>3</sup> x 10<sup>-3</sup> in *Paph.*  
 272 *liemianum*. This was followed by *Paph. baccanum* (4.709±0.2083 mm<sup>3</sup> x 10<sup>-3</sup>), *Paph. primulinum* (3.608±0.8454 mm<sup>3</sup> x  
 273 10<sup>-3</sup>) and *Paph. kolopakingii* (2.797±0.1499 mm<sup>3</sup> x 10<sup>-3</sup>). Burgeff (1936) experimentally demonstrated the relationship  
 274 between SV/EV ratio and seed buoyancy. Seeds with a high SV/EV ratio are more buoyant because they possess a greater  
 275 air space.

276 Swamy et al. (2004, 2007) stated that AS is an important character because the seeds of most orchids are wind  
 277 dispersed, implying that seeds with a greater AS percentage will float in the air for a longer time and thus spread to more  
 278 distant places. We observed that terrestrial species possessed a comparatively greater percentage of air space in their seeds  
 279 as compared with epiphytic species. A greater percentage of air space for *Paph. kolopakingii*, *Paph. liemianum*,  
 280 *Paph. baccanum* and *Paph. primulinum* was shown more than 60% of seed i.e. (64.150±1.9360), (85.501±1.5279),  
 281 (78.725±0.9214) and (70.898±6.3436). Zhang et al. (2015) informed that higher AS values in seeds of terrestrial species  
 282 can help spread them further along the forest floor where wind speeds are lower. Seeds with a greater percentage of air  
 283 space in *Paphiopedilum* are expected to be more widely distributed and, in fact, we found it difficult to collect their seeds.

284 .....[WU48]

285 The results of this study can be concluded that species within *Paphiopedilum* produce larger seeds with smaller  
 embryos and a bigger percentage of air space (AS) than those of *Phalaenopsis* species. Likewise, the volume of seeds and



286 seed volume/embryo volume in *Paphiopedilum* are larger than in *Phalaenopsis*. Large air spaces will increase seed  
287 buoyancy and seeds can be dispersed further. Testa cells are elongated in shape, with cylindrical cells walls, in  
288 *Phalaenopsis* but they are polygonal, with thin or flat rectangular cell walls, in *Paphiopedilum*. There are some similarities  
289 in character that might be related to adaptation to the tropical area, because in all species studied, both epiphytic and  
290 terrestrial orchids have the same form of fusiform seeds and a smooth testa surface. The general results of this study  
291 inform us that the morphological and morphometric properties of orchid seeds could be utilised as a means of  
292 distinguishing between life forms and habitat similarity and aid in identification.

.....[WU49]

## 294 REFERENCES [WU50]

- 295 Akçin TA, Ozdener Y, Akçin A. 2009. Taxonomic Value of Seed Characters in Orchids from Turkey. *Belg. J. Bot* [WU51].  
296 142 (2):124-139. <http://dx.doi.org/10.2307/41427182>.
- 297 Arditti J, Ghani AKA. 2000. Numerical and physical properties of orchid seeds and their biological implications. *New*  
298 *Phytol.* 145:367-421. DOI: 10.1046/j.1469-8137.2000.00587.x.
- 299 Arditti J, Michaud JD, Healey PL. 1979. Morphometry of orchid seeds. I. *Paphiopedilum* and native Californian and  
300 related species of *Cypripedium*. *American Journal of Botany* [WU52]. 66:1128-1137. <http://dx.doi.org/10.2307/2442211>.
- 301 Arditti J, Michaud JD, Healey PL. 1980. Morphometry of Orchid Seeds. II. Native California and Related Species of  
302 *Calypso*, *Cephalanthera*, *Corallorhiza* and *Epipactis*. *Amer. J. Bot.* 67 (3):347-360. <http://dx.doi.org/10.2307/2442345>.
- 303 Burgeff H. 1936. *Samenkeimung der Orchideen und Entwicklung ihrer Keimpflanzen*, Jena: Gustav Fischerr Verlag.
- 304 Cela PG, Seligrat I, Ortúñez E, Gamarra R, Vivar A, Scrugli A. 2014. A study of seed micromorphology in the genus  
305 *Ophrys* (Orchidaceae). *Anales del Jardín Botánico de Madrid.* 71 (2):1-6. <http://dx.doi.org/10.3989/ajbm.2370>.
- 306 Chase MW, Pippen JS. 1988. Seed morphology in the Oncidiinae and related subtribes orchidaceae. *Systematic Botany.* 13  
307 (3):313-323.
- 308 Chaudhary B, Chattopadhyay P, Banerjee N. 2014. Modulations in seed micromorphology reveal signature of adaptive  
309 species-diversification in *Dendrobium* (Orchidaceae). *Open Journal of Ecology.* 4 (2):33-42.  
310 <http://dx.doi.org/10.4236/oje.2014.42005>.
- 311 Clifford HT, Smith WK. 1969. Seed morphology and classification of Orchidaceae. *Phytomorphology.* 19:133-139.
- 312 Gamarra R, Galán P, Herrera I, Ortúñez E. 2008. Seed micromorphology supports the splitting of *Limnorchis* from  
313 *Platanthera* (Orchidaceae). *Nordic Journal of Botany.* 26:61-65. DOI: 10.1111/j.1756-1051.2008.00135.x
- 314 Gamarra R, Galan P, Pedersen HE, Ortunez E, Sanz E. 2015. Seed micromorphology in *Dactylorhiza Necker ex Nevski*  
315 (Orchidaceae) and allied genera. *Turkish Journal of Botany.* 39:298-309. DOI: 10.3906/bot-1401-66.
- 316 Gamarra R, Ortúñez E, Sanz E, Esparza I, Galán P. 2010. Seeds in subtribe Orchidinae (Orchidaceae): the best  
317 morphological tool to support molecular analyses. In: Nimis P. L., R. Vignes-Lebbe (eds.). *Tools for Identifying*  
318 *Biodiversity: Progress and Problems.* 323-326. ISBN 978-88-8303-295-0. EUT.
- 319 Guler N. 2016. Seed micromorphology of *Orchis Tourn. Ex L.* (Orchidaceae) and allied genera growing in Edirne  
320 province, Turkey. *Phyto Keys.* 69:9-25. <http://dx.doi.org/10.3897/phytokeys.68.8746>.
- 321 Molvray M, Chase M. 1999. Seed morphology. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds.). *Genera*  
322 *Orchidacearum, General Introduction, Apostasioideae, Cypripedioideae.* Oxford University Press, Oxford, 59-66.
- 323 Rasmussen HN. 1995. *Terrestrial orchids from seed to mycotrophic plant.* Cambridge, UK: Cambridge University Press.
- 324 Shimizu N. 2012. Adaptation and evolution of seed shape on bleeding area in Japanese orchids. *International Journal of*  
325 *Biology.* 4: 47-53.
- 326 Sodhi NS, Koh LP, Brook BW, Ng PKL. 2004. Southeast Asian biodiversity: An impending disaster. *Trends in Ecology &*  
327 *Evolution.* 19 (12):654-660. DOI: 10.1016/j.tree.2004.09.006
- 328 Swamy KK, Kumar HNK, Ramakrishna TM, Ramaswamy SN. 2004. Studies on Seed Morphometry of Epiphytic Orchids  
329 from Western Ghats of Karnataka. *Taiwania.* 49 (2):124-140. DOI: 10.6165/tai.2004.49 (2).124.
- 330 Swamy KK, Kumar HNK, Ramaswamy SN. 2007. Studies on seed morphometry of *Dendrobium* species.  
331 *Phytomorphology.* 57:33-43.
- 332 Tsutsumi C, Yukawa T, Lee NS, Lee CS, Kato M. 2007. Phylogeny and comparative seed morphology of epiphytic and  
333 terrestrial species of *Liparis* (Orchidaceae) in Japan. *Journal of Plant Research.* 120 (3):405-412. DOI:  
334 10.1007/s10265-007-0077-0
- 335 Verma J, Kusum K, Thakur K, Sembi JK, Vij SP. 2012. Study on seed morphometry of seven threatened Himalayan  
336 orchids exhibiting varied life modes. *Acta Botanica Gallica: Botany Letters.* 159 (4):443-449. DOI:  
337 10.1080/12538078.2012.750582.
- 338 Verma J, Sharma K, Thakur K, Sembi JK, Vij SP. 2014. Study on Seed Morphometry of Some Threatened Western  
339 Himalayan Orchids. *Turkish Journal of Botany.* 38:234-251. DOI: 10.3906/bot-1307-14.
- 340 Vij SP, Kaur P, Kaur S, Kaushal PS. 1992. The Orchid Seeds: Taxonomic, Evolutionary and Functional Aspects. *J. Orchid*  
341 *Soc.* 6:91-107.

- 342 Yoder JA, Imfeld SM, Heydinger DJ, Hart CE, Collier MH, Gribbins KM, Zettler LW. 2010. Comparative water balance  
343 profiles of Orchidaceae seeds for epiphytic and terrestrial taxa endemic to North America. *Plant Ecol.* 211:7-17. DOI:  
344 10.1007/s11258-010-9765-2.
- 345 Zhang FP, Zhang JJ, Yan N, Hu H, Zhang SB. 2015. Variations in seed micromorphology of *Paphiopedilum* and  
346 *Cypripedium* (Cypripedioideae, Orchidaceae). *Seed Science Research.* 25:395-401. DOI:  
347 10.1017/S0960258515000276.  
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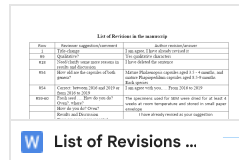
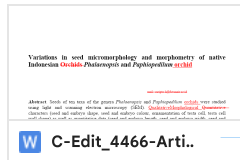


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# Variations in seed micromorphology and morphometry of native Indonesian *Phalaenopsis* and *Paphiopedilum* orchid

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**Abstract.** Seeds of ten taxa of the genera *Phalaenopsis* and *Paphiopedilum* orchids were studied using light and scanning electron microscopy (SEM). Qualitative Morphological Quantitative [WU1] characters (seed and embryo shape, seed and embryo colour, ornamentation of testa cell, testa cell wall shape) as well as quantitative data (seed and embryo length, seed and embryo width, seed and embryo volume, seed length/seed width, embryo length/embryo width, seed volume/embryo volume and air space) were analysed. Seeds of all studied taxa were fusiform in shape and had smooth testa surfaces. *Phalaenopsis* testa cells were elongated with cylindrical cell walls, while *Paphiopedilum* testa cells were polygonal with thin and flat rectangular cell walls. The shape of the embryos was generally ovoid in *Phalaenopsis* and prolate in *Paphiopedilum*. Seed colours ranged from brown to dark brown. Embryo colours varied from light yellow, yellowish brown, dark brown, black and white. Based on our investigation, there are variations in seed and embryo length, seed and embryo width, seed and embryo volume as well as the percentage of the air space, both in *Phalaenopsis* and *Paphiopedilum*. In general, the values of seed volume, embryo volume and air space in *Paphiopedilum* are higher than in *Phalaenopsis*. Together, the results of the study indicate that morphological and morphometric features can serve to identify live forms and distinguish between species. Together, the results of the study indicate that morphological and morphometric features can serve to identify live forms and distinguish between species, and phylogeny [WU2].

**Keywords:** testa cell shapes, testa cell wall shapes, seed volume, seed air spaces, epiphytic versus terrestrial habitats

## INTRODUCTION

*Phalaenopsis* Blume and *Paphiopedilum* Pfitzer are orchids whose members are very popular and are commercialised as the most traded potted and cut flower plants in the world. Wild populations are under threat of extinction due to high rates of habitat modification, deforestation, forest fires, illegal harvesting and trade as a consequence of rapid economic development, high population growth and corrupt institutions (Sodhi et al. 2004). All *Paphiopedilum* species are listed by the Conservation on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Appendix I and *Phalaenopsis* are listed in Appendix II. This is in spite of the fact that, according to the CITES, all species from the genus *Paphiopedilum* are prohibited for trade.

Seeds occupy the first rank in the life history of plants. Orchid seeds disperse, germinate and grow into mature plants, and reproduce in a suitable place on certain parts of the tree or on the ground. In general, seeds are responsible for the regeneration and distribution of a species, even playing an important role in the conservation of orchids. Verma et al. (2014) and Tsutsumi et al. (2007) explain that seed dispersal mode and seed size are important factors in regulating the growth of new populations.

Seed morphology varies greatly in shape, colour, size, volume of the embryo and testa structure, and some of these characters have been used to establish the phylogeny of species in the genus

44 (Gamarra et al. 2008, 2010, 2015; Cela et al. 2014; Guler, 2016). In addition, some seed characters  
45 can be related to germination and dispersal, especially in their ecological adaptation (Chaudhary et al.  
46 2014; Zhang et al. 2015). According to research by Chaudhary et al. (2014) in the *Dendrobium* orchid,  
47 the volume of the embryo and the percentage of the air space are directly related to the climate and  
48 this is reflected in seed ultrastructure.

49 Previous studies on the morphology of orchid seeds in tropical areas have demonstrated the  
50 importance of the seed coat, related to the taxonomy and ecology (Chase and Pippen, 1988; Tsutsumi  
51 et al. 2007; Akçin et al. 2009; Verma et al. 2012; Molvray and Chase, 1999), as demonstrated with  
52 scanning electron microscopy (SEM).

53 Not much is known about how certain seed characteristics may correlate with ecological  
54 adaptations. The aim of this study is to reveal the qualitative and quantitative characteristics of the  
55 seeds of several species of epiphytic and terrestrial orchids native to Indonesia from different genera,  
56 thus contributing to a better understanding of the differences and similarities in their adaptation  
57 strategies for seeds in the tropics.  
58

## 59 MATERIALS AND METHODS

### 60 Plant materials and seed collection

61 Ten different orchid species were collected from Simanis and DD Orchids Nursery, East Java,  
62 Indonesia. The collection for the present study included *Phal. amabilis*, *Phal. amboinensis*, *Phal.*  
63 *tetraspis*, *Phal. bellina*, *Phal. gigantea*, *Phal. amboinensis*, *Phal. tetraspis*, *Phal. venosa*, *Paph.*  
64 *baccanum*, *Paph. kolopakingii*, *Paph. liemianum*, *Paph. baccanum*, *Paph. pPrimulinum*; these plants  
65 were then hand pollinated during their normal period of flowering. The seeds of mature capsules were  
66 2-3 mature capsules each species [WU3] were collected from between 2016 to 2019 [WU4]. The seed  
67 samples were are taken randomly from -1-2 capsule(s) for each species. The matured capsules that are  
68 Age of mature Phalaenopsis capsules is 3.5-4 months old for Phalaenopsis and dan a mature  
69 Phapiopedilum capsules is 8.5-9 months for Phapiopedilum after hand self pollination were used infor  
70 this study. The capsules period from pollination to maturation of Phalaenopsis, which are between 3.5  
71 —4 moths and Phapiopedilum between 8.5-9 months after self pollination .— The capsules were  
72 washed using 10% sunlight detergent solution for 3 minutes to eliminate dust particles, then rinsed 3  
73 times with sterile distilled water. The surface of the capsules was sprayed with 70% alcohol, put on a  
74 Petri dish, placed into a laminar flow cupboard and passed over a Bunsen flame; this was repeated 3  
75 times. The capsule was cut into four parts transversally and longitudinally using a sterile scalpel in a  
76 sterile Petri dish. The mature seeds were released from the capsules and collected with the help of a  
77 sterile spatula. The specimens used for SEM were dried for at least 4 weeks [WU5] at room  
78 temperature and stored in small paper envelope. The seeds obtained from mature and opened capsules  
79 were used morphology investigations. The seeds specimens used for SEM Fresh seeds were dried for at  
80 least 42 weeks [WU6] at room temperature and stored in small paper envelopes. Al cap vials at 4°C  
81 [WU7] in dry conditions. ... [WU8] All species within *Phalaenopsis* in this study are epiphytic orchids  
82 while all species within *Paphiopedilum* are terrestrial orchids.

### 83 Morphological and statistical analyses

84 Seed samples were observed and photographed under a Light Microscope (LM) and Scanning  
85 Electron Microscope (SEM). Our morphological parameters included seed shape (SS), seed colour  
86 (SC), seed length (SL), seed width (SW), seed length/seed width (SL/SW), seed volume (SV). For  
87 embryos, the parameters included embryo shape (ES), embryo colour (EC), embryo length (EL),  
88 embryo width (EW), embryo length/embryo width (EL/EW), embryo volume (EV), seed  
89 volume/embryo volume (SV/EV), and air space (AS). The SV ( $\text{mm}^3 \times 10^{-3}$ ) was calculated using the  
90 formula  $2 \left[ \frac{L}{2} \left( \frac{W}{2} \right)^2 \left( \frac{\pi}{3} \right) \right]$ , where L = length, W = width,  $\pi = \frac{22}{7}$  and embryo volume ( $\text{mm}^3 \times 10^{-3}$ )

91 <sup>3</sup>) with the formula  $4/3 \pi (L/2) (W/2)^2$ , where L = length, W = width (adapted from Arditti et al. 1980).  
92 The SV/EV values were calculated following the described method (Arditti et al. 1979). For  
93 calculating AS (%), the following formula was applied: (seed volume-embryo volume)/seed volume))  
94 x 100% (adapted from Arditti and Ghani, 2000). To analyse each species statistically, we used the  
95 mean for each quantitative character.

## 96 LM study

97 Seeds were spread on a slide with a drop of water and covered with a cover glass. Values for SL,  
98 SW, EL and EW (at the longest and widest axis) were obtained from an average of thirty seeds and  
99 were observed for each species and measurement using a light microscope (Olympus CH 20, Olympus  
100 Japan) and standardised ocular meter. Characteristics such as SC, SS, EC and ES were observed under  
101 Tension stereomicroscope (Nikon SMZ-1, Japan). The SC and EC was described in subjective terms.

## 102 SEM study

103 For SEM preparations, the samples (seeds) were mounted on SEM stubs. The samples were then  
104 sputter-coated with palladium/gold (SEM coating system Q150R S mini sputter Coater). Detailed seed  
105 coat (testa cells) surface studies were conducted by observing under a Hitachi TM3000, with a  
106 filament voltage of 15 kV. The parameters considered were seed coat sculpturing and thickenings.

# 107 RESULTS AND DISCUSSION [WU9]

## 108 Qualitative character of seed and embryos

109 ~~Qualitative characters were analysed by LM, and this showed the characteristics of *Phalaenopsis*~~  
110 ~~seeds [WU10] that can be distinguished from *Paphiopedilum* seeds are elongated testa cell shape,~~  
111 ~~cylindrical testa cell wall shape, and ovoid embryo shape and, and brown transparent seeds colour~~  
112 ~~are showed in (Table 1) seeds of two genera~~

113 ~~*Phal. amabilis*, *Phal. amboinensis*, *Phal. bellina*, *Phal. gigantea*, *Phal. tetraspis* and *Phal. venosa*~~  
114 ~~show similar character i.e fusiform seed shape, brown and transparent seed colour, smooth~~  
115 ~~ornamentation of the periclinal walls, elongated testa cell shape, cylindrical testa cell walls shape and~~  
116 ~~ovoid embryo shape (Table 1).~~

117  
118 ~~*Phal. amabilis*, *Phal. amboinensis*, *Phal. bellina*, *Phal. gigantea*, *Phal. tetraspis* and *Phal.*~~  
119 ~~*venosa* show similar character i.e fusiform seed shape, brown and transparent seed colour, smooth~~  
120 ~~ornamentation of the periclinal walls, elongated testa cell shape, cylindrical testa cell walls shape and~~  
121 ~~ovoid embryo shape (Table 1). The seeds were generally uniform i.e. fusiform in shape with colours~~  
122 ~~from brown to dark brown. Brown transparent seeds were found in *Phal. amabilis*, *Phal. tetraspis*,~~  
123 ~~*Phal. amboinensis*, *Phal. bellina*, *Phal. gigantea*, *Phal. Amboinensis* *Phal. tetraspis* and *Phal. venosa*.~~  
124 ~~Dark brown seeds were recorded in *Paph. kolopakingii* and *Paph. liemianum*. Brown seeds were~~  
125 ~~observed in *Paph. baccanum* and *Paph. primulinum* (Table 1, Figure 1). The shape of the embryos~~  
126 ~~was generally ovoid in *Phalaenopsis* and prolate in *Paphiopedilum*, and embryos were distinct and~~  
127 ~~present in the centre. Embryo colour in the observed species varied from light yellow, yellowish~~  
128 ~~brown to dark brown, black and white. Light yellow embryos were found in *Phal. venosa*. Yellowish~~  
129 ~~brown embryos were recorded in *Phal. amabilis* and *Phal. tetraspis*. Brown embryos were common in~~  
130 ~~*Phal. bellina* and, *Phal. giganteantea* and *Paph. kolopakingii*. Dark brown embryos were found in~~  
131 ~~*Paph. bBaccanum* and yellow embryos were noticed in *Phal. amboinensis*. White embryos~~  
132 ~~eharacterised *Paph. liemianum* and black embryos were seen in *Paph. primulinum* (Table 1, Figure 1).~~

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134 ~~Molvray and Chase (1999) announced that features of the seed ornamentation can be based at~~  
135 ~~higher taxonomic ranks. In the present work, the embryo colour testa surface of P[WU11] *hal.*~~  
136 ~~*amabilis* was yellowish brown did not have any ornamentation. The testa cells were elongated,~~



137 longitudinally oriented, parallel to the long seed axis and irregular. The testa cell walls were  
 138 cylindrical shape and cylindrical, and exhibited overlap (Figure 2.1B arrow), becoming circle round  
 139 and rising at the end of the meeting between two testa cells [WU12](Figure 2.1D arrow). Certain  
 140 parts of the testa formed deep furrows (Figure 2.1C arrow).

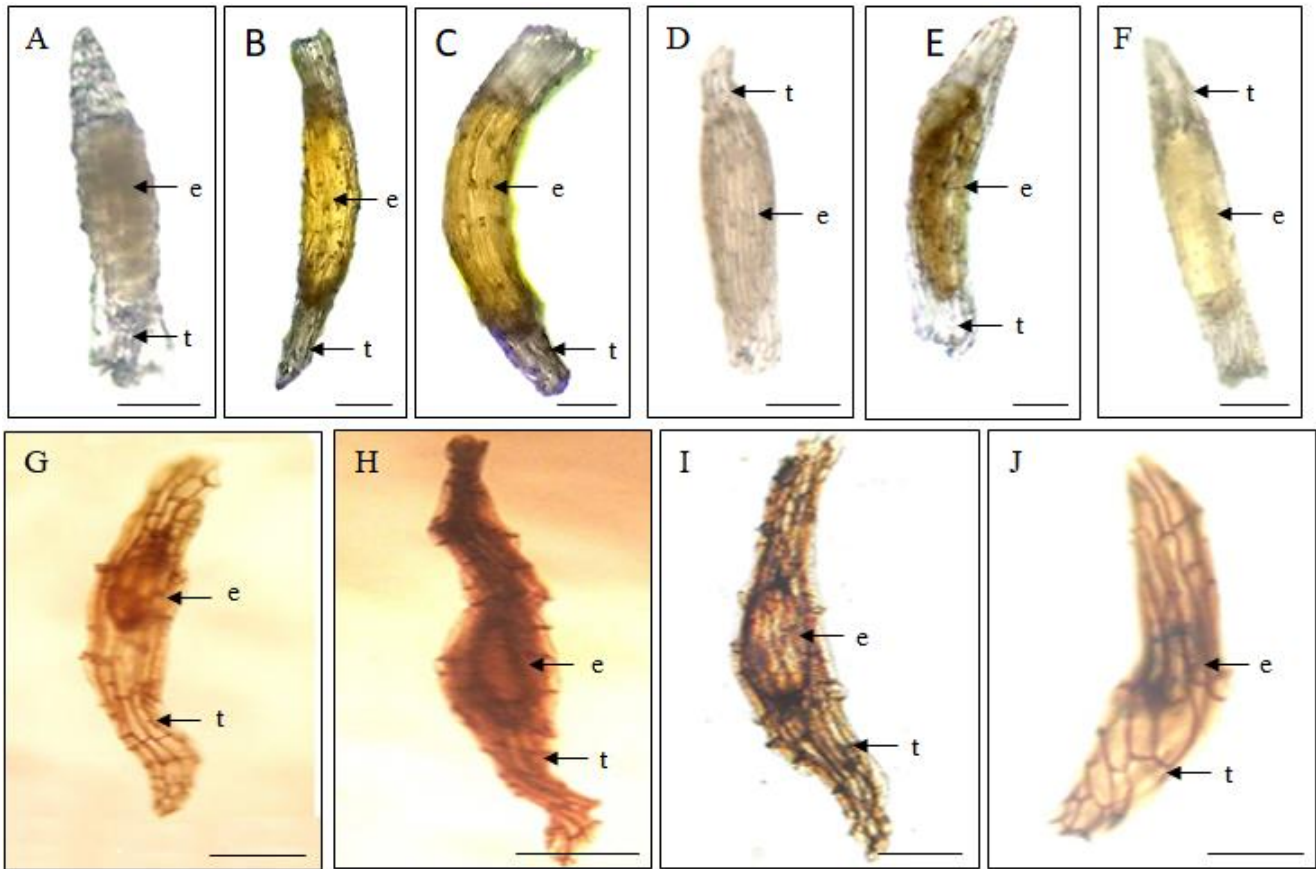
141  
 142 There were no differences in the shape of the testa cells at the two poles [WU13].  
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145 **Table 1.** Morphological characteristics Qualitative data of the analysed species [WU14] of the genus  
 146 *Phalaenopsis* and *Paphiopedilum*  
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<u>Species name</u>	<u>Seed Shape</u>	<u>Seed Colour</u>	<u>Ornamentation of the periclinal walls</u>	<u>Testa cell shape</u>	<u>Testa cell walls shape</u>	<u>Embryo Shape</u>	<u>Embryo Colour</u>
<i>Phalaenopsis</i>							
<i>Phal. amabilis</i> [WU15]	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish brown
<i>Phal. amboinensis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellow
<i>Phal. bellina</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. gigantea</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. tetraspis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish brown
<i>Phal. venosa</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Light yellow
<i>Paphiopedilum</i>							
<i>Paph. baccanum</i>	Fusiform	Brown	Smooth	Polygonal	Thin rectangular	Prolate	Dark brown
<i>Paph. kolopakingii</i> [WU16]	Fusiform	Dark brown	Smooth	Polygonal	Flat rectangular	Prolate	Brown
<i>Paph. liemianum</i>	Fusiform	Dark brown	Smooth	Polygonal	Thin rectangular	Prolate	White
<i>Paph. primulinum</i>	Fusiform	Brown	Smooth	Polygonal	Flat rectangular	Prolate	Black

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 149 In the present work, the embryo colour of *P* [WU17] *hal. amabilis* was yellowish brown. The testa  
 150 cells were elongated, longitudinally oriented, parallel to the long seed axis and irregular. The testa cell  
 151 walls were cylindrical shape and exhibited overlap (Figure 2.1B arrow), becoming circle and rising at  
 152 the end of the meeting between two testa cells [WU18](Figure 2.1D arrow). Certain parts of the testa  
 153 formed deep furrows (Figure 2.1C arrow).

154 The embryo colour of *Phal. amboinensis* was yellow. The testa cells were elongated, longitudinally  
 155 oriented and parallel to long axis and irregular. The testa cells walls were cylindrical. Part of the  
 156 meeting at the end of the two cells was rounded and slightly raised (Figure 2.2B arrow). The end of  
 157 the basal pole was blunt (Figure 2.2C), while the end of the apical pole became pointed (Figure 2.2D).  
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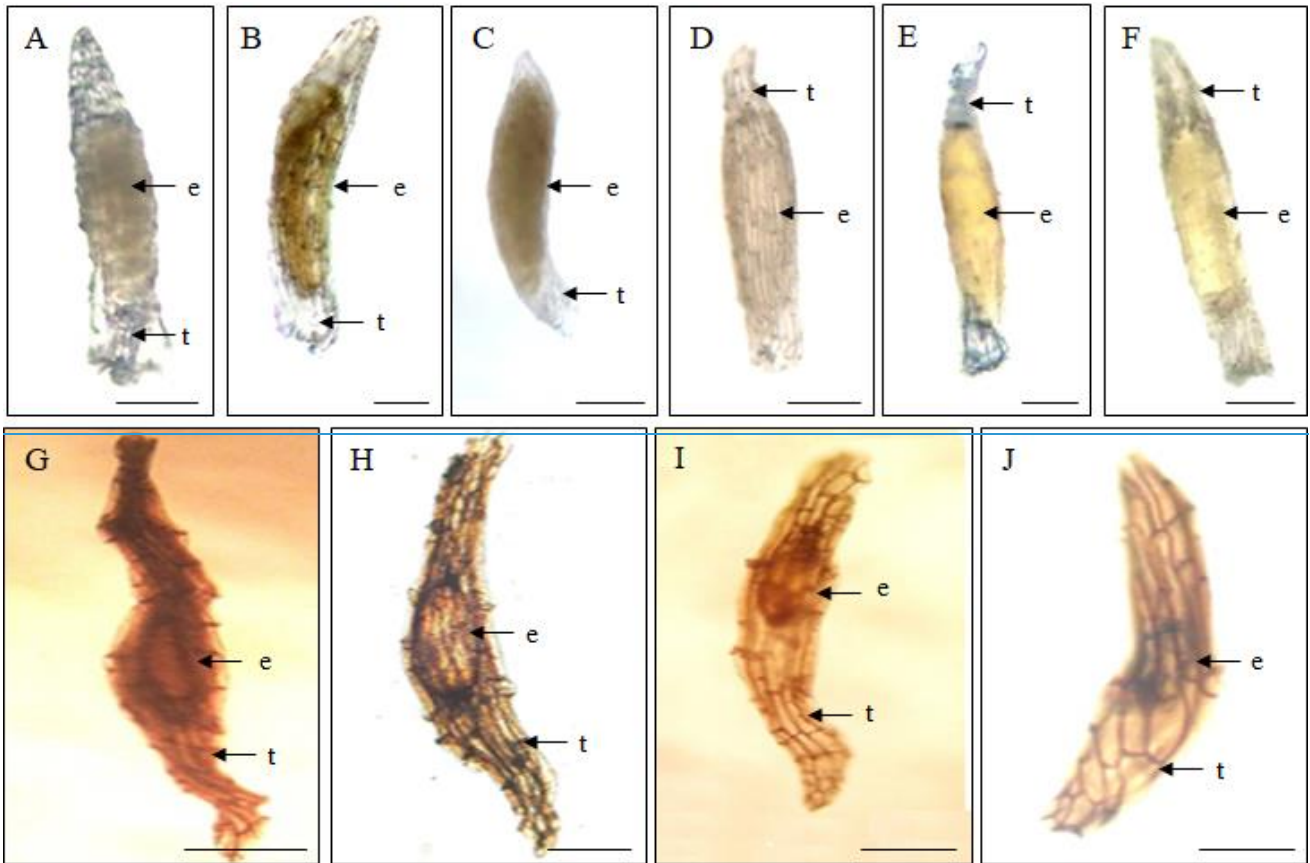


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Species-name	Seed-Shape	Seed-Colour	Ornamentation of the periclinal-walls	Testa-cell shape	Testa-cell-walls shape	Embryo Shape	Embryo Colour
<i>Phalaenopsis</i>							
<i>Phal. amabilis</i> [WU19]	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish brown
<i>Phal. tetraspis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish brown
<i>Phal. bellina</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. gigantea</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. amboinensis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellow
<i>Phal. venosa</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Light yellow
<i>Paphiopedilum</i>							
<i>Paph. kolopakingii</i> [WU20]	Fusiform	Dark brown	Smooth	Polygonal	Flat-rectangular	Prolate	Brown
<i>Paph. lieinianum</i>	Fusiform	Dark brown	Smooth	Polygonal	Thin-rectangular	Prolate	White
<i>Paph. baecanum</i>	Fusiform	Brown	Smooth	Polygonal	Thin-rectangular	Prolate	Dark brown
<i>Paph. primulinum</i>	Fusiform	Brown	Smooth	Polygonal	Flat-rectangular	Prolate	Black

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167 Figure 1. LM photographs of (A-F) *Phalaenopsis* and (G-J) *Paphiopedilum*. A. *Phal. amabilis* (scale  
168 bar=72  $\mu\text{m}$ ), B. *Phal. amboinensis* (scale bar=59  $\mu\text{m}$ ), C. *Phal. tetraspis* (scale bar=49  $\mu\text{m}$ ), C. *Phal.*  
169 *bellina* (scale bar=65  $\mu\text{m}$ ), D. *Phal. gigantea* (scale bar=51  $\mu\text{m}$ ), E. *Phal. tetraspis* (scale bar=49  $\mu\text{m}$ ),  
170 *Phal. amboinensis* (scale bar=59  $\mu\text{m}$ ), F. *Phal. venosa* (scale bar=55  $\mu\text{m}$ ), G. *Paph. baccanum* (scale  
171 bar=185  $\mu\text{m}$ ), H. *Paph. kolopakingii* (scale bar=206  $\mu\text{m}$ ), I. *Paph. liemianum* (scale bar=162  $\mu\text{m}$ ), J.  
172 *Paph. baccanum* (scale bar=185  $\mu\text{m}$ ), J. *Paph. primulinum* (scale bar=161  $\mu\text{m}$ ). e= embryo, t=testa.

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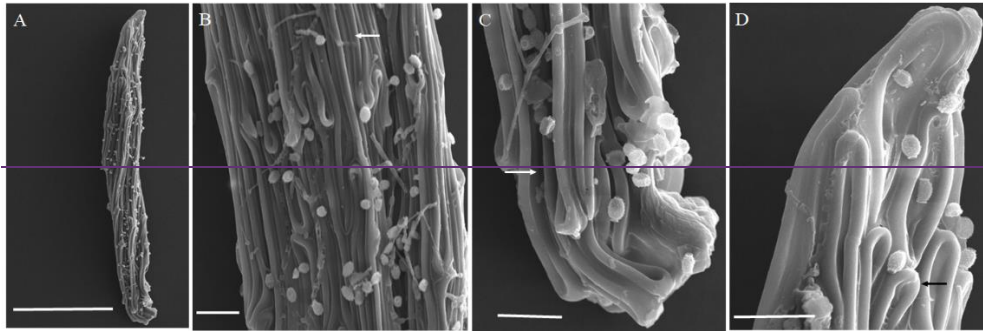
176 The embryo colour testa cells on the surface of *PhalP. amboinensis* was yellow. did not have  
177 any ornamentation. The testa cells were elongated elongated, longitudinally oriented and, parallel to  
178 long axis and irregular. The testa cells walls were cylindrical. The testa cells walls were cylindrical.  
179 Part of the meeting at the end of the two cells was rounded and slightly raised (Figure 2.2 3B arrow).  
180 The end of the basal pole was blunt (Figure 2.2 3C), while the end of the apical pole became pointed  
181 (Figure 2.23D).

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183 The testa surface of *Phal*[WU21]. *tetraspis* was smooth. The testa cells were  
184 elongated, longitudinally oriented, parallel to the long axis and irregular. The testa cell  
185 walls were cylindrical, and very close together [WU22](Figure 3). At the end of the  
186 meeting between two testa cells the cell walls became wider, rounder and rose up  
187 (Figure 3C arrow)[WU23]. There was a difference in the shape of the testa cells at the  
188 two poles. Cells at the basal pole were smaller than cells at the apical pole[WU24].

189 *P*[WU25]*hal. bellina* did not have any ornamentation on the surface of its testa  
190 cells; the cells were elongated, longitudinally oriented, parallel to the long seed axis  
191 and irregular. The testa cell walls were cylindrical, and the distance between two cells

192 formed a slit in a row (Figure 4B). The meeting point between the two ends of the testa  
193 cells was curved, thickened and raised to form a bulge (Figure 4B arrow). There was a  
194 difference in the shape of the testa cells at the two poles. Cells of the basal pole were  
195 bigger and rounder than cells of the apical pole [WU26](Figure 4C and D).  
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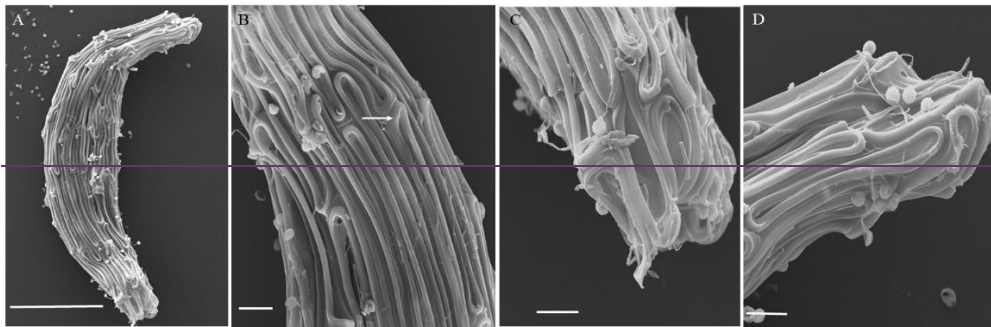
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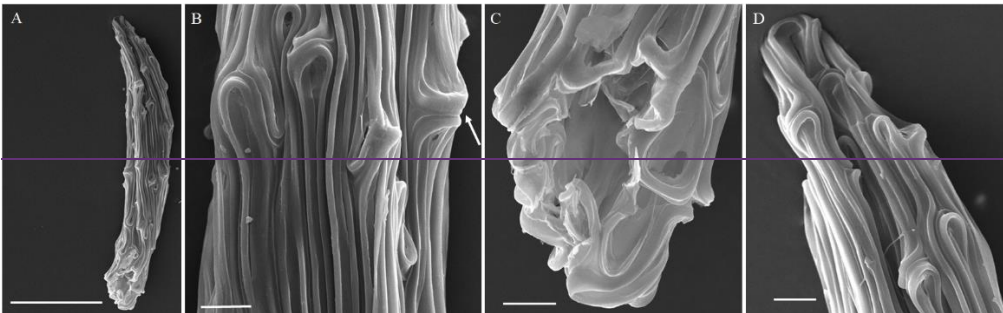
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Figure 2 [WU27]. SEM photographs of *Phal. amabilis*. A. Fusiform seed shape, B. Pattern of testa cells [WU28] of the medial, C. Cells of the basal pole [WU29], D. Cells of the apical pole [WU30]. Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.



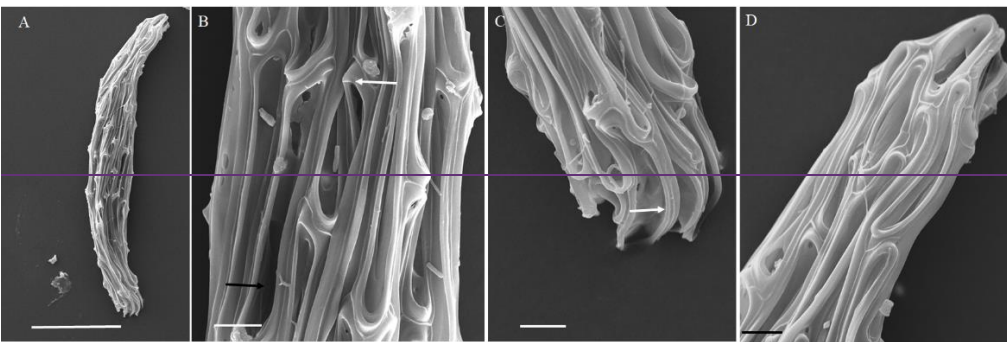
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Figure 36 [WU31] SEM photographs of *Phal. amboinensis*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=80  $\mu$ m, B-D=10  $\mu$ m.



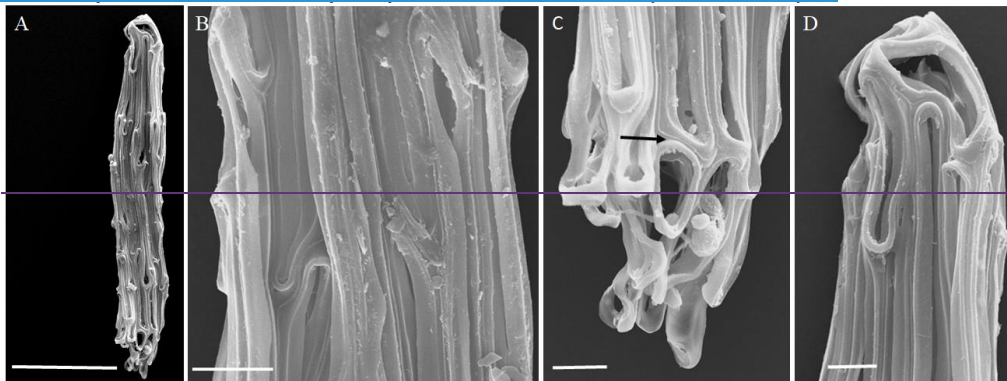
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Figure 4 [WU32]. SEM photographs of *Phal. bellina*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.



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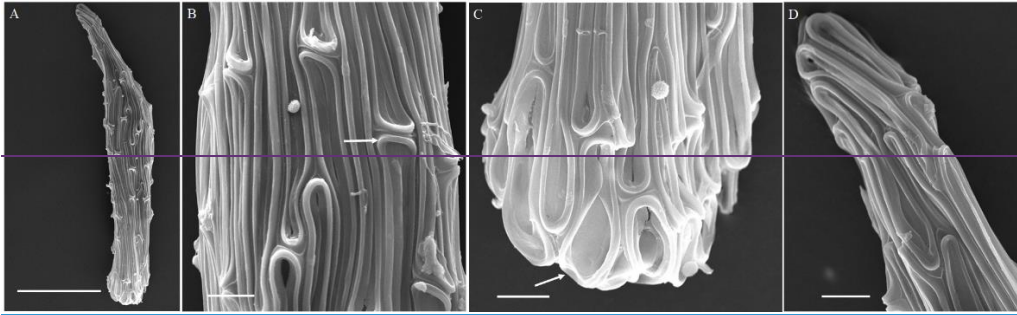
Figure 5 [WU33] SEM photographs of *Phal. gigantea*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.



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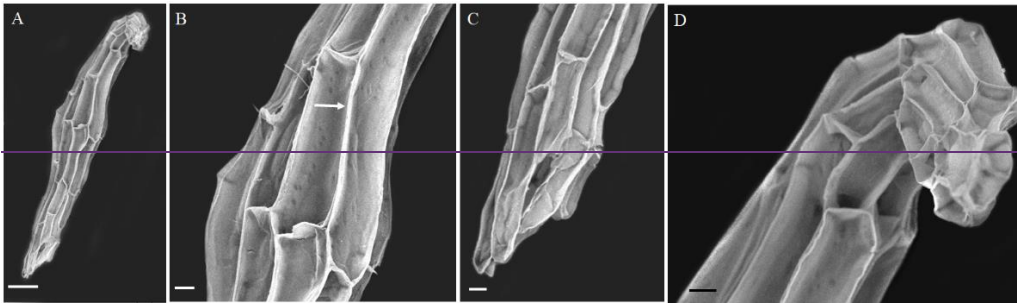
Figure 3 [WU34]. SEM photographs of *Phal. tetraspis*. A. seed shape [WU35], B. Pattern of testa [WU36] cells of the medial, C. Cells of the basal pole [WU37], D. Cells of the apical pole [WU38]. Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.





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Figure 7. [wU39] SEM photographs of *Phal. venosa*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=80  $\mu$ m, B-D=10  $\mu$ m.



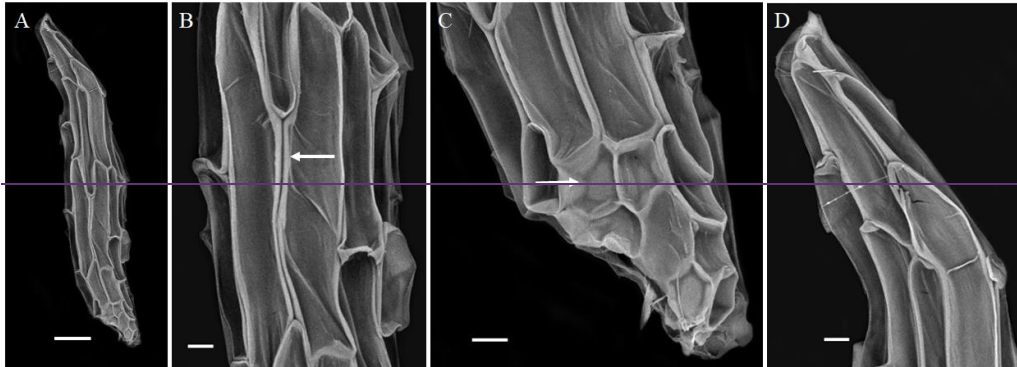
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Figure 8 [wU40]. SEM photographs of *Paph. baccanum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.



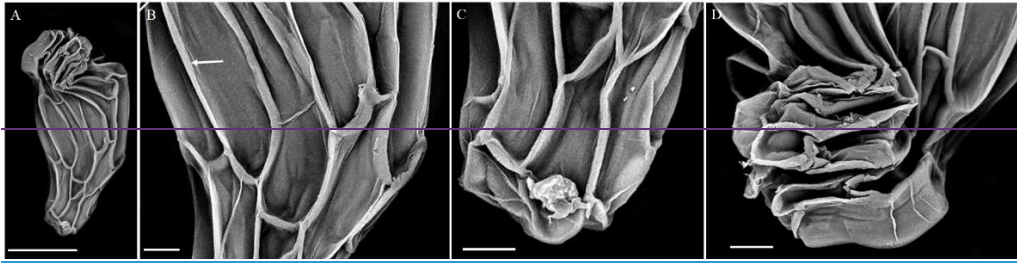
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Figure 8 [wU41]. SEM photographs of *Paph. kolopakingii*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.



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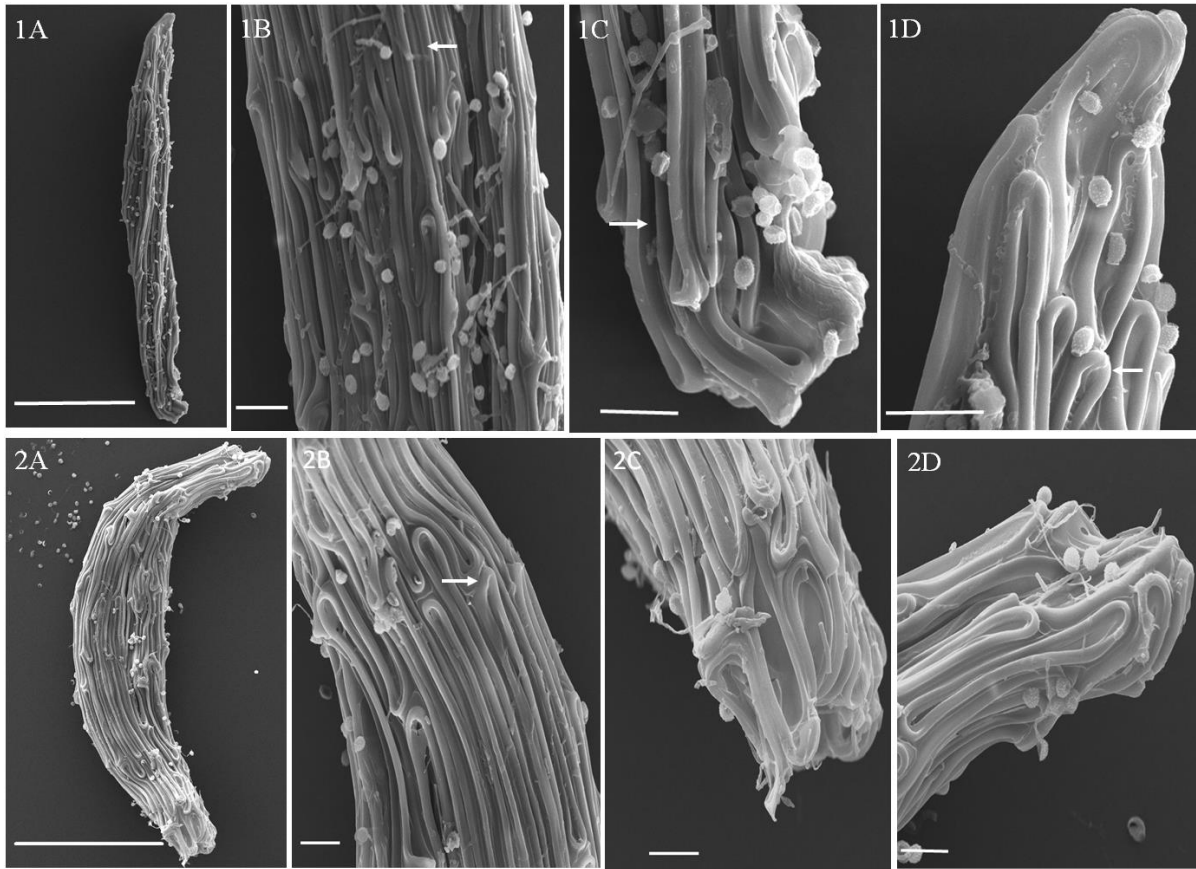
Figure 109. [wU42] SEM photographs of *Paph. liemianum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.



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237 Figure 11 [WU43]. SEM photographs of *Paph. primulinum*. A. seed shape, B. Pattern of testa cells of the medial, C.  
238 Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.  
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240 *P.* [WU44] *hal. bellina* have brown embryo colour. The testa cells were elongated, did not have  
241 any ornamentation on the surface of its testa cells; the cells were elongated, longitudinally oriented,  
242 parallel to the long seed axis and irregular. The testa cell walls were cylindrical, and the testa cells  
243 walls were cylindrical and the distance between two cells formed a slit in a row (Figure 3.14B). The  
244 meeting point between the two ends of the Ttesta cells was curved, thickened and raised to form a  
245 bulge at the meeting point between the two ends (Figure 3. 14B arrow). There was a difference in the  
246 shape of the testa-cells at the two poles. Cells of the basal pole were bigger and rounder than cells of  
247 the apical pole [WU45](Figure 3.1 4C and D).  
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249 In *Phal.* [WU46] *gigantea*, the embryo colour was brown testa surface was smooth. The testa cells  
250 were elongated, longitudinally oriented, parallel to the long seed axis and highly irregular. Ridges  
251 were elevated with a deep groove (Figure 3. 25B black black arrow). The testa cell walls were  
252 cylindrical. At the meeting point the Ttwo ends of the testa cells were curved and raised at the  
253 meeting point (Figure 3.25B white white arrow). Cells of the basal pole were different to cells at the  
254 apical pole; cells at the basal pole had an appendage at the end and become pointed (Figure 3. 25C  
255 arrow). [WU47]  
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**Figure 2**[WU48]. SEM photographs. 1. *Phal. amabilis*, 2. *Phal. amboinensis*, A. Seed shape, B. Testa cells [WU49] of the medial, (1B. arrow indicate the testa cell overlap, 2B. arrow indicate the end of two cells was rounded and slightly raised) C. Basal pole [WU50], (1C. arrow indicate the testa formed deep furrow) -D. Apical pole [WU51] (1D. arrow indicate the end of the meeting between two testa cells becoming round and rising). Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.

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265 *Phal. bellina* have brown embryo colour. The testa cells were elongated, longitudinally oriented, parallel to the long seed axis and irregular. The testa cells walls were cylindrical and the distance between two cells formed a slit in a row (Figure 3.1B). Testa cells was curved, thickened and raised to form a bulge at the meeting point between the two ends (Figure 3. 1B arrow). There was a difference in the shape of the testa cells at the two poles. Cells of the basal pole were bigger and rounder than cells of the apical pole [WU53] (Figure 3.1C and D).

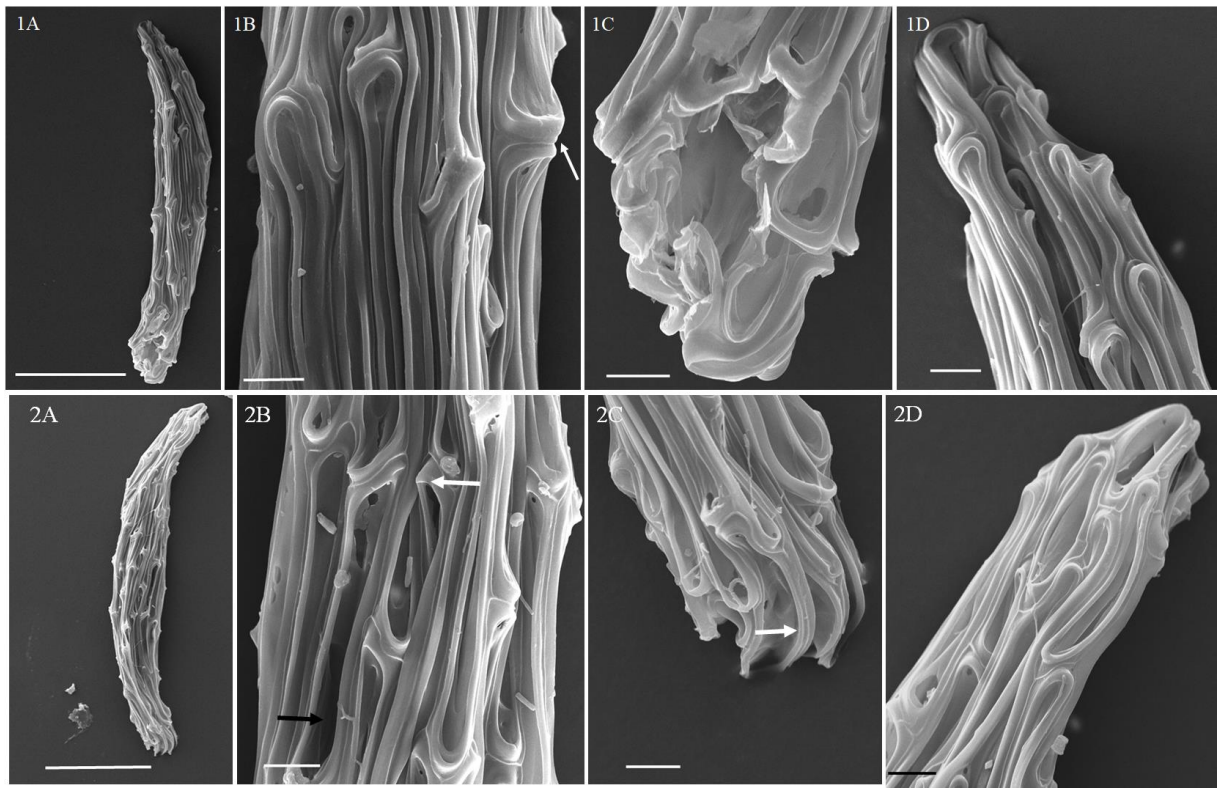
271 In *Phal.* [WU54] *gigantea*, the embryo colour was brown. The testa cells were longitudinally oriented, parallel to the long seed axis and highly irregular. Ridges were elevated with a deep groove (Figure 3. 2B black arrow). Two ends of the testa cells were curved and raised at the meeting point (Figure 3.2B white arrow). Cells of the basal pole were different to cells at the apical pole; cells at the basal pole had an appendage at the end and become pointed (Figure 3. 2C arrow). [WU55]

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277 The embryo colour of *Phal.* [WU56] *tetraspis* was yellowish brown. The testa cells were elongated, longitudinally oriented and parallel to the long axis and irregular. The testa cell walls were cylindrical and close proximity [WU57] (Figure 4.1). At the end of the meeting between two testa cells the cell walls became wider, rounder and rose up (Figure 4. 1C arrow) [WU58]. There was a difference in the shape of the testa cells at the two poles. Cells at the basal pole were smaller than cells at the apical pole [WU59].

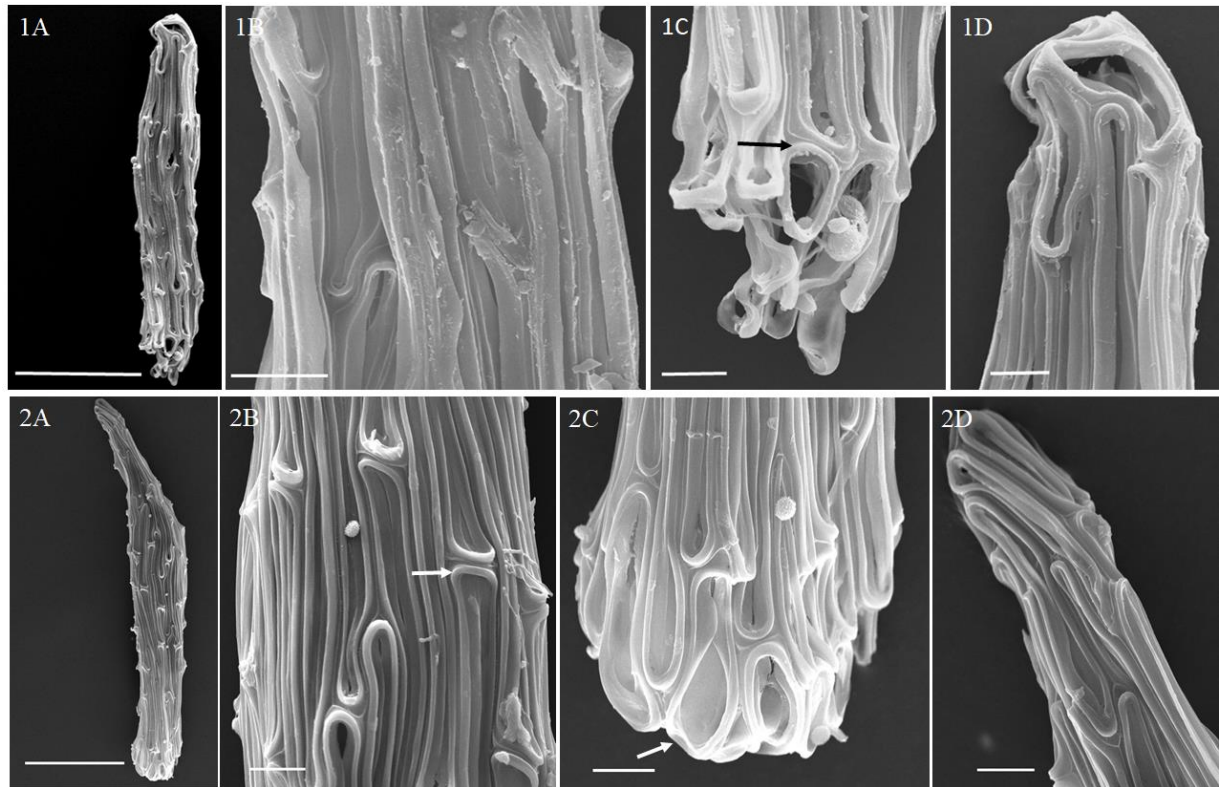
283 In the case of *Phal. venosa*, the embryo colour was light yellow. The testa cells were longitudinally oriented, parallel to the long axis, irregular, and raised at the end of the meeting between two cells (Figure 4. 2B arrow). Testa cells at the end of the basal pole were widened and rounded (Figure 4. 2C arrow), but testa cells at the apical pole were more elongated and pointed (Figure 4. 2D).

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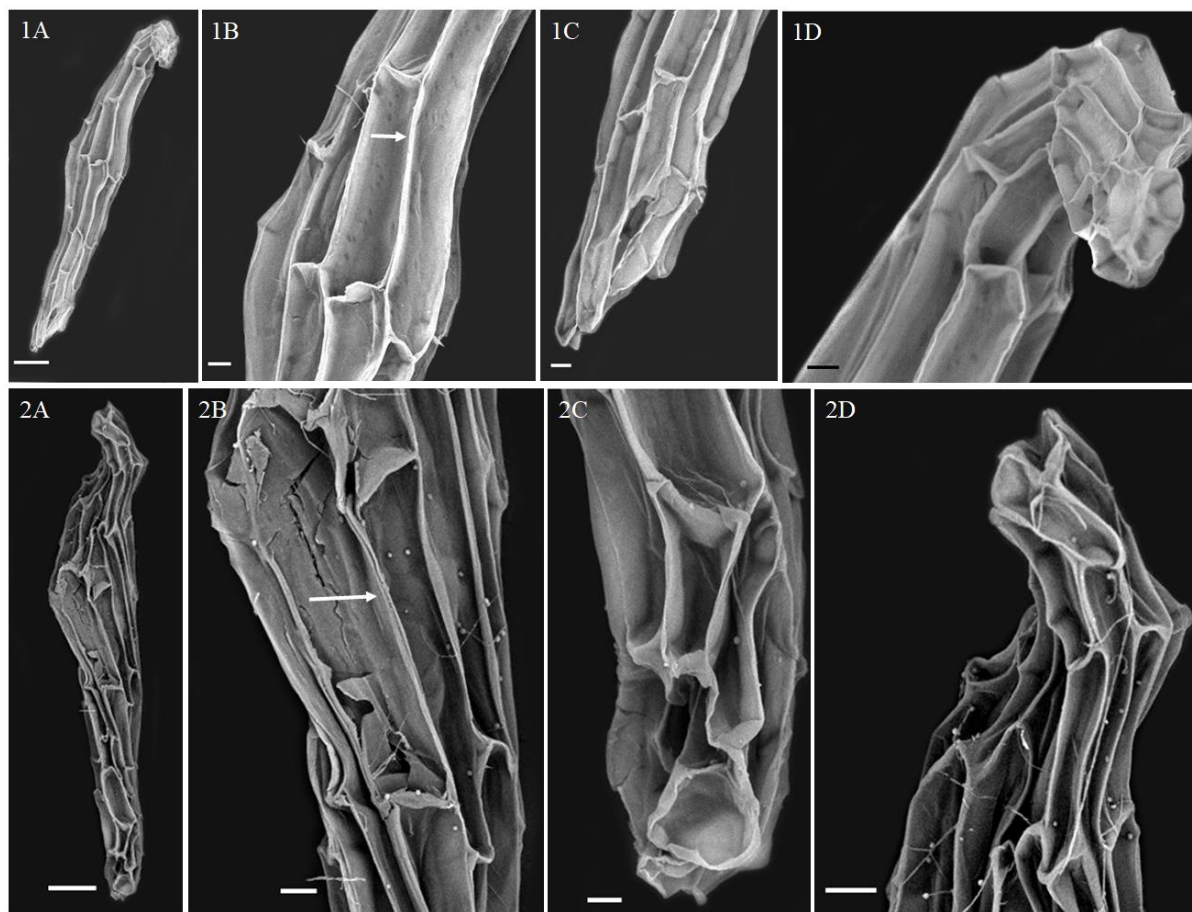


**Figure 3.** SEM photographs. 1. *Phal. bellina*, 2. *Phal. gigantea*, A. Seed shape, B. Testa cells of the medial (1B. arrow indicate cells are rounded and slightly raised, 2B white arrow indicate the testa cell are curved and raised at the end of two testa cells, black arrow indicate ridge with deep groove), C. Basal pole (2C. arrow indicate the end testa cells at the basal pole had appendage with pointed of the end), D. Apical pole. Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.



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Figure 4. [WU63]SEM photographs. 1. *Phal. tetraspis*. 2. *Phal. venosa*. A. seed shape, B. Testa cells of the medial (2B. arrow indicate the testa cell walls are raised at the end of the meeting between two cells), C. Basal pole (1C. black arrow indicate the testa cell become wider, rounder, and rose up. 2C. arrow indicate the testa cells at the end of basal pole were widened and rounded), D. Apical pole. Scale bars: A=100  $\mu$ m; B-D = 10  $\mu$ m.



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Figure 5. [WU64]SEM photographs. 1. *Paph. baccanum*. 2. *Paph. kolopakingii*. A. Fusiform seed shape, B. Testa cells of the medial (1B. arrow indicate the testa cell walls are thin, 2B. arrow indicate the cell walls were flat), C. Basal pole, D. Apical pole. Scale bars: A=80  $\mu$ m, B-D = 20  $\mu$ m.

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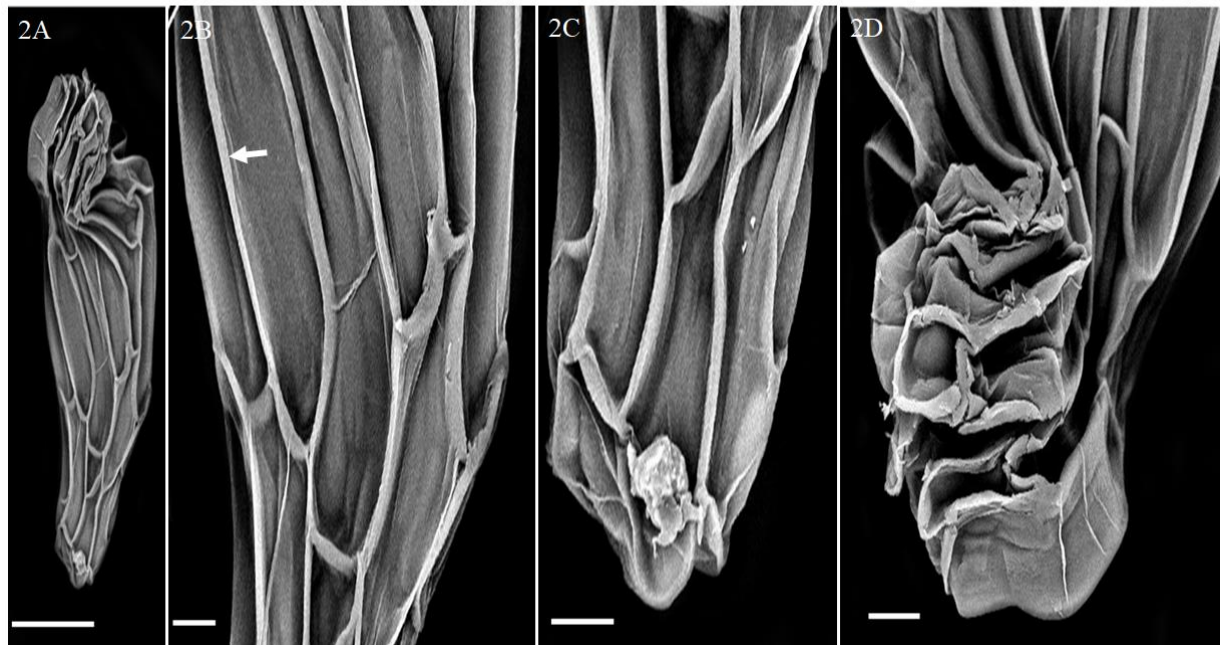
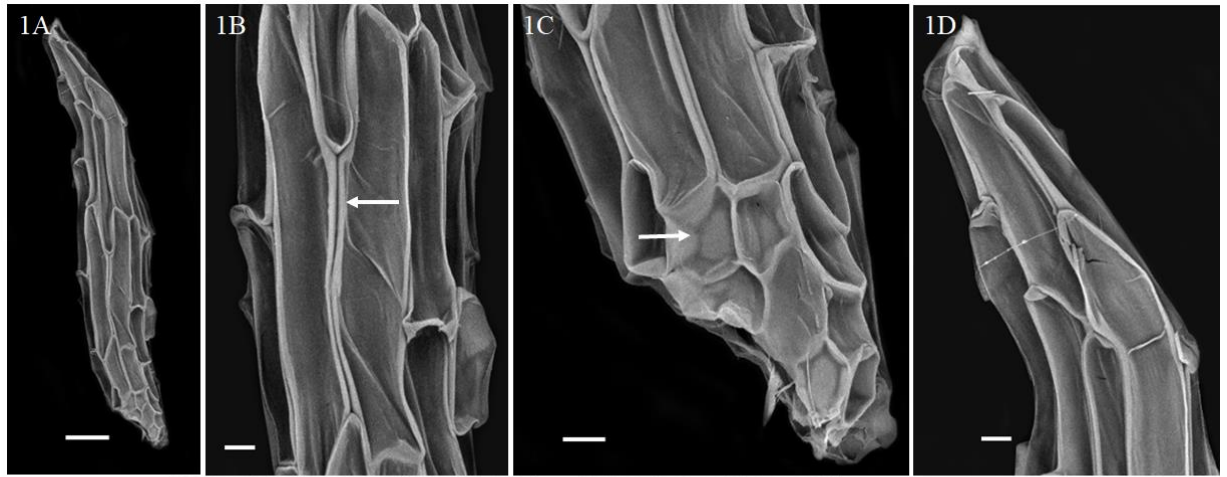
*Paphiopedilum* seeds can be differentiated with seeds of *Phalaenopsis* in terms of polygonal testa cell shape, rectangular testa cell walls and prolate embryo shape. *Paph. baccanum*, *Paph. kolopakingii*, *Paph. liemianum* and *Paph. primulinum* have same character i.e fusiform seed shape, smooth ornamentation of the periclinal walls, polygonal testa cell shape, rectangular testa cell walls shape and prolate embryo shape (Table 1).

In the case of *Paph. baccanum*, the seed colour was brown and embryo colour was dark brown. Generally, the testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 5.1). The testa cell walls were thin (Figure 5.1B arrow). Cells at the basal pole were longer compared to cells at the apical pole. The end of the basal pole became pointed, while the end of the apical pole became rounded (Figures 5. 1C and D).

In *Paph. kolopakingii*, the seed colour was dark brown and embryo colour was brown. Testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular. The testa cell walls were flat (Figure 5. 2B arrow).. The basal cell pole was larger and more dented than the cells of the apical pole (Figures 5.2C and D).

The seed colour of *Paph. liemianum* were dark brown and white embryo. The testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 6.1). The testa cell walls were thin (Figure 6.1B arrow). The medial region was wider than the two poles. The basal cells were short, polygonal and more numerous than the apical cells (Figure 6.1C arrow).





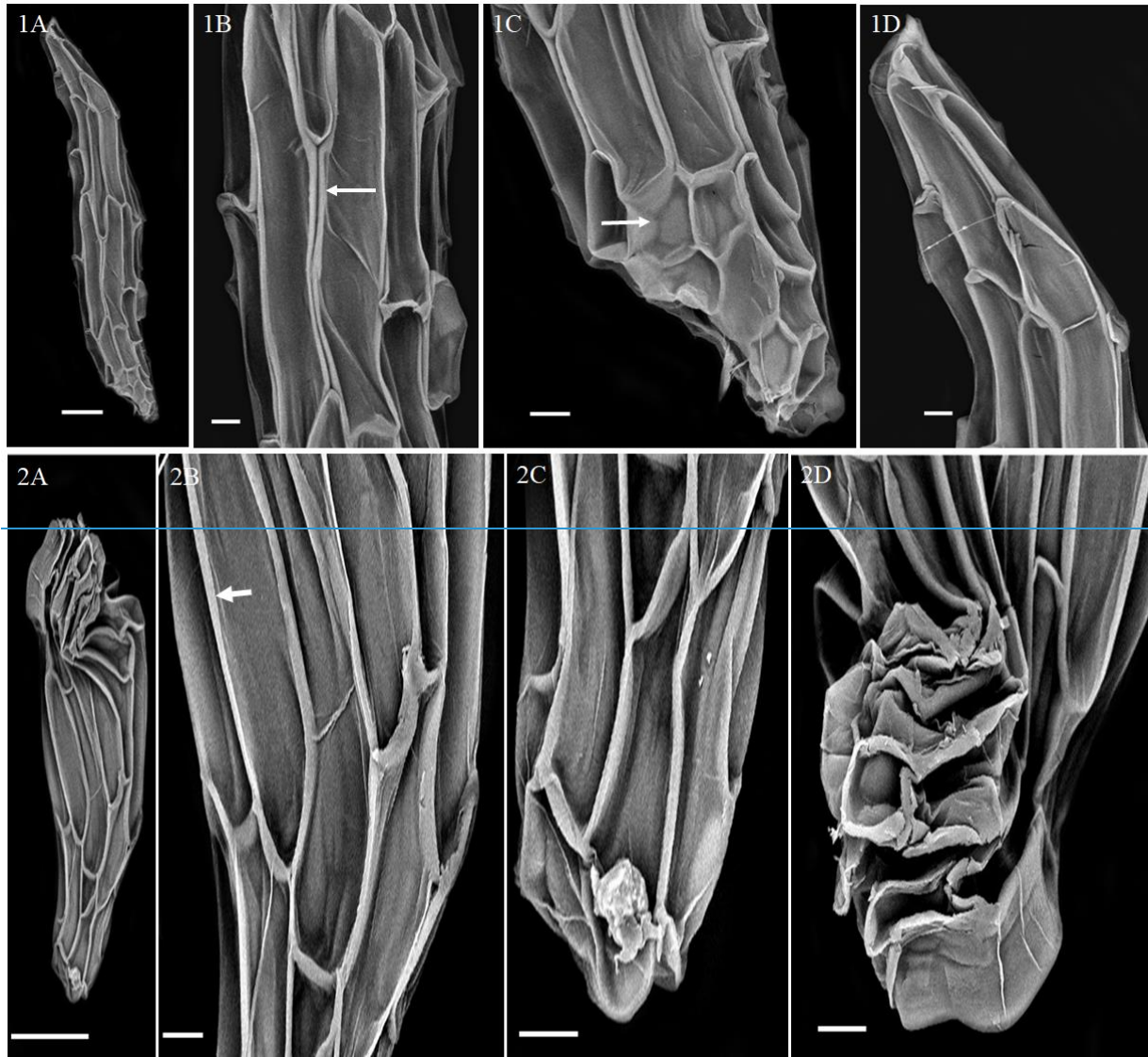
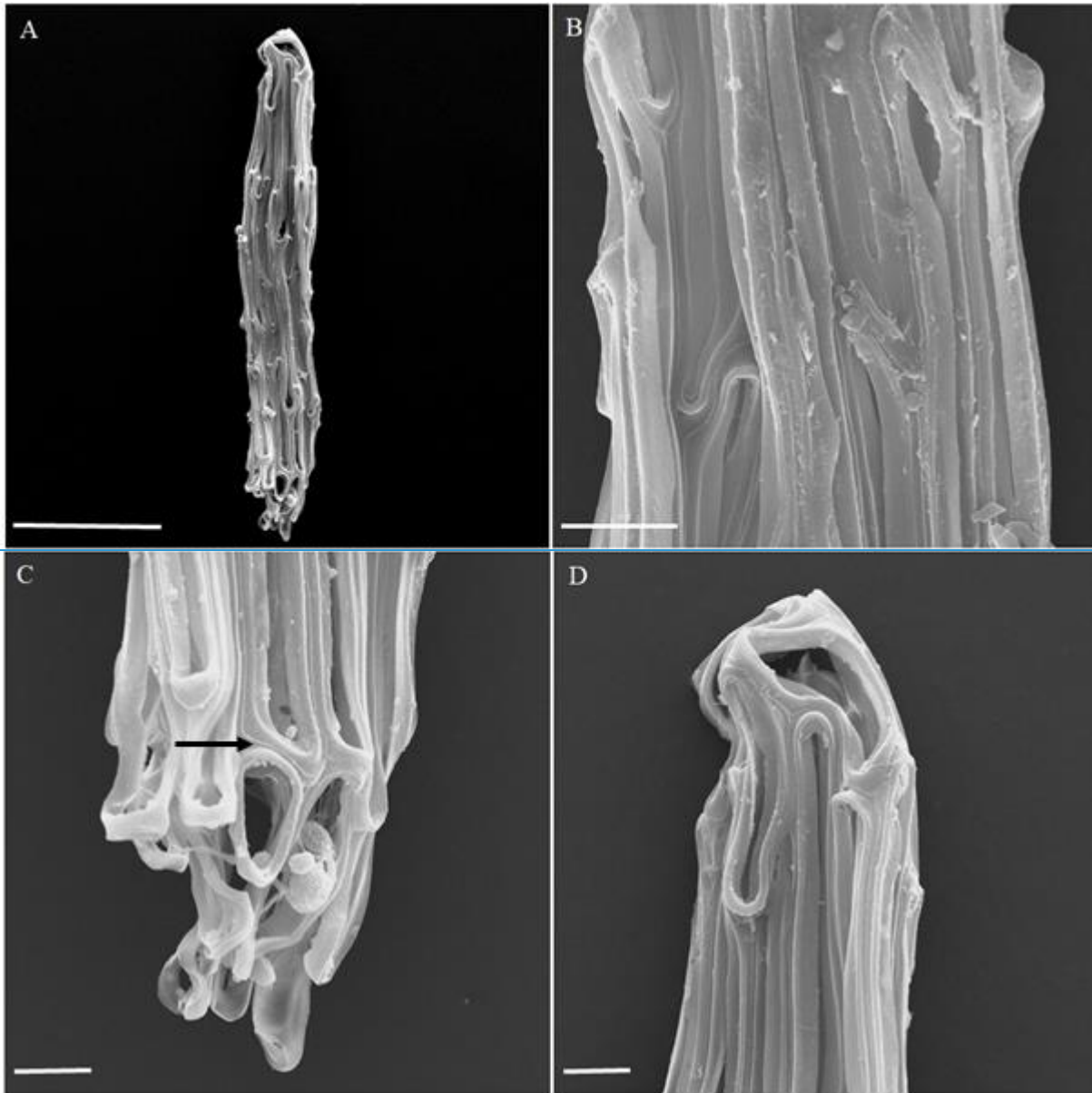


Figure 6. [WU65]SEM photographs. 1. *Paph. liemianum*. 2. *Paph. primulinum*. A. seed shape, B. Testa cells of the medial (1B. arrow indicate the testa cell walls are thin, 2B. arrow indicate the testa cell walls were flat), C. Basal pole (1C arrow indicate the shape of testa cells at basal pole are polygonal), D. Apical pole. Scale bars: A=80  $\mu\text{m}$ , B-D = 20  $\mu\text{m}$ .

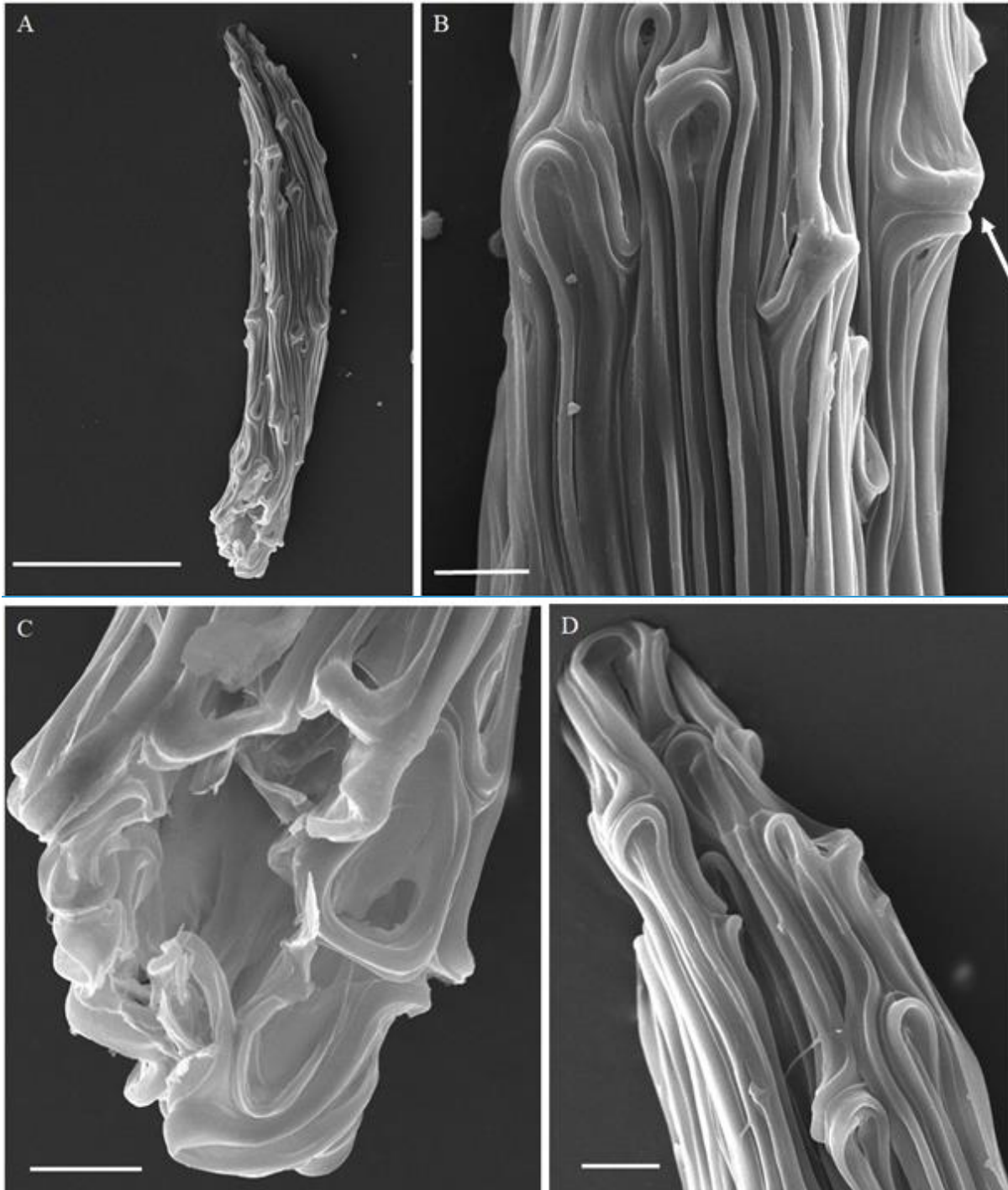
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The embryo colour testa surface of *Phal. tetraspis* was yellowish brown smooth. The testa cells were elongated, elongated, longitudinally oriented and, parallel to the long axis and irregular. The testa cell walls were cylindrical and cylindrical, and very close proximity together [WU67] (Figure 4.16). [At the end of the meeting between two testa cells the cell walls became wider, rounder and rose up (Figure 4. 16C arrow)] [WU68]. There was a difference in the shape of the testa cells at the two poles. Cells at the basal pole were smaller than cells at the apical pole [WU69]. RW



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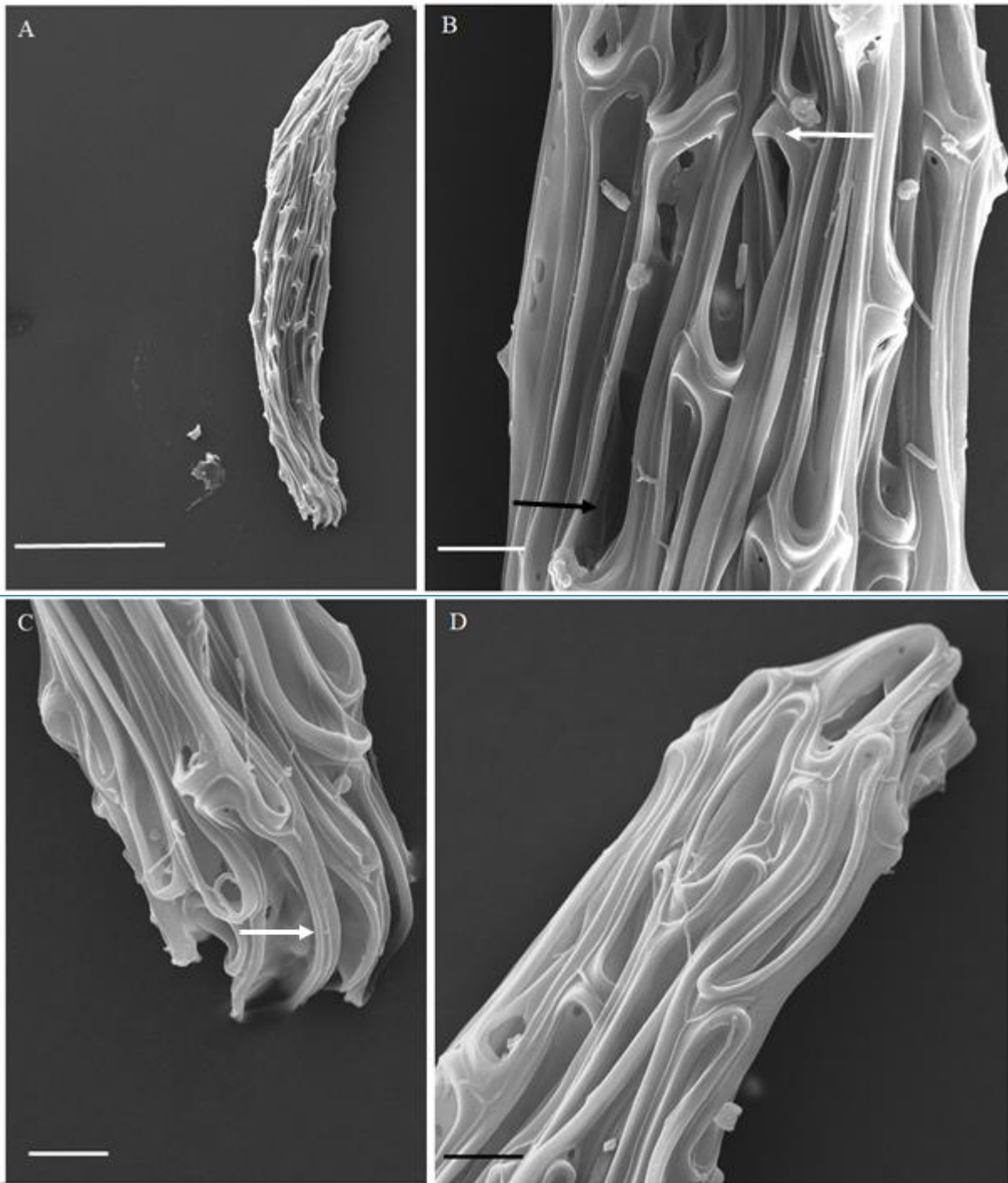
Figure 3 [WU70]. SEM photographs of *Phal. tetraspis*. A. seed shape [WU71], B. Pattern of testa [WU72] cells of the medial, C. Cells of the basal pole [WU73], D. Cells of the apical pole [WU74]. Scale bars: A=100  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .



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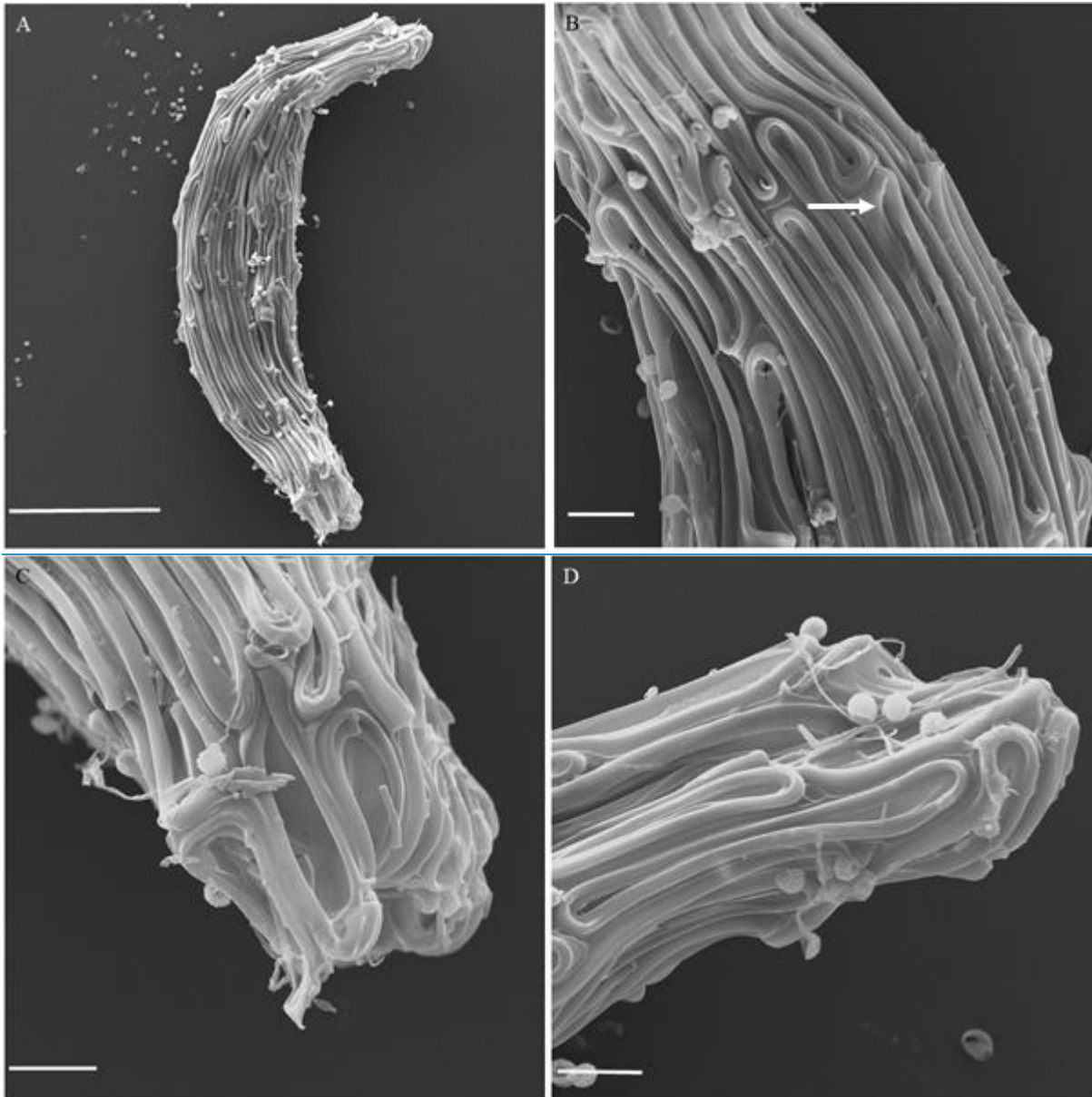
Figure 4 [WU75]. SEM photographs of *Phal. bellina*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=10  $\mu$ m.





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 363 **Figure 5.** [WU76] SEM photographs of *Phal. gigantea*. A. seed shape, B. Pattern of testa cells of the  
 364 medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .  
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366 The testa cells on the surface of *P. amboinensis* did not have any ornamentation. The testa cells  
 367 were elongated, longitudinally oriented, parallel to long axis and irregular. The testa cells walls were  
 368 cylindrical. Part of the meeting at the end of the two cells was rounded and slightly raised (Figure 6B  
 369 arrow). The end of the basal pole was blunt (Figure 6C), while the end of the apical pole became  
 370 pointed (Figure 6D).  
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374 **Figure 6.** [WU77] SEM photographs of *Phal. amboinensis*. A. seed shape, B. Pattern of testa cells of  
375 the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=80  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .  
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378 In the case of *Phal. venosa*, the embryo colour testa surface was light yellow smooth. The testa cells  
379 were elongated and longitudinally oriented, parallel to the long axis, irregular, and raised at the end of  
380 the meeting between two cells (Figure 4. 27B arrow). The testa cells walls were cylindrical. Testa  
381 cells at the end of the basal pole were widened and rounded (Figure 4. 27C arrow), but testa cells at  
382 the apical pole were more elongated and pointed (Figure 4. 27D). The end of the basal pole was  
383 rounded (Figure 7C), while the end of the apical pole was pointed (Figure 7D).  
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385 In *Paphiopedilum* seeds can be differentiated with seeds of *Phalaenopsis* in terms of have  
386 polygonal testa cell shape, rectangular testa cell walls, and prolate embryo shape. *Paph. baccanum*,  
387 *Paph. kolopakinggii*, *Paph. liemianum* and *Paph. primulinum* have same character i.e fusiform seed  
388 shape, smooth ornamentation of the periclinal walls, polygonal testa cell shape, rectangular testa cell  
389 walls shape and prolate embryo shape (Table 1).



390 ~~The seeds were generally uniform i.e. fusiform in shape with colours from brown to dark brown.~~  
391 ~~Dark brown seeds were recorded in *Paph. kolopakingii* and *Paph. liemianum*. Brown seeds were~~  
392 ~~observed in *Paph. baccanum* and *Paph. primulinum*, embryos were distinct and present in the centre.~~  
393 ~~Dark brown embryos were found in *Paph. baccanum*. White embryos characterised *Paph. Liemianum*~~  
394 ~~and black embryos were seen in *Paph. Primulinum*~~

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396 ~~In the case of *Paph. baccanum*, the seed colour testa surface was brown and embryo colour was~~  
397 ~~dark brown smooth. Generally, the testa cells were polygonal, longitudinally oriented with a deep~~  
398 ~~groove, parallel to the long axis and irregular (Figure 5.18). The testa cell walls were thin and~~  
399 ~~rectangular (Figure 5.18B arrow). Cells at the basal pole were longer compared to cells at the apical~~  
400 ~~pole. The end of the basal pole became pointed, while the end of the apical pole became rounded~~  
401 ~~(Figures 5.18C and D).~~

402  
403 ~~In the case of *Paph. kolopakingii*, the seed colour was dark brown and embryo colour was brown.~~  
404 ~~Ttesta cells were polygonal, longitudinally oriented with a deep groove, parallel to the long axis and~~  
405 ~~irregular. The testa cell walls were flat and rectangular (Figure 5.298B arrow). The testa surfaces~~  
406 ~~were smooth. The shape of the basal cell pole was larger and more dented than the cells of the apical~~  
407 ~~pole (Figures 5.298C and D).~~

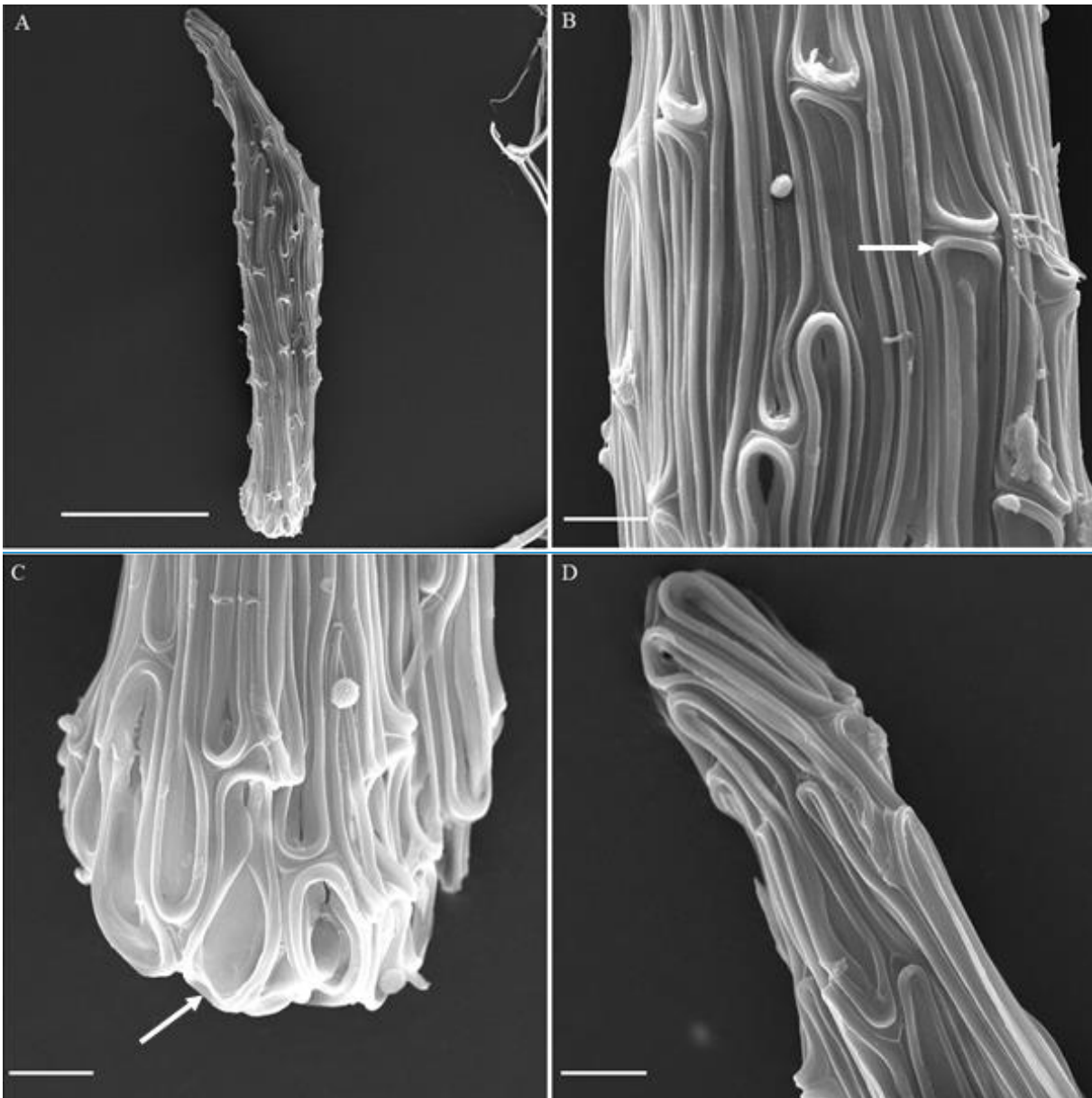
408 ~~The seed colour testa surfaces of *Paph. liemianum* were dark brown and white embryos smooth. The~~  
409 ~~testa cells were were polygonal, longitudinally oriented with a deep groove, parallel to the long axis~~  
410 ~~and irregular (Figure 96.110). The testa cell walls were thin and rectangular (Figure 9B 6.110B~~  
411 ~~arrow). The medial region was wider than the two poles. The basal cells were short, polygonal and~~  
412 ~~more numerous than the apical cells (Figure 9C 6.110C arrow).~~

413 ~~In the case of *Paph. baccanum*, the testa surface was smooth. Generally, the testa cells were~~  
414 ~~polygonal, longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure~~  
415 ~~10). The testa cell walls were thin and rectangular (Figure 10B arrow). Cells at the basal pole were~~  
416 ~~longer compared to cells at the apical pole. The end of the basal pole became pointed, while the end of~~  
417 ~~the apical pole became rounded (Figures 10C and D).~~

418 ~~In *Paph. primulinum* had, the brown seed colour and black embryo colour. smooth testa surfacee~~  
419 ~~was smooth. Generally, testa cells weree polygonal, longitudinally oriented with a deep groove,~~  
420 ~~parallel to the long axis and irregular (Figure 6.244). The testa cell walls were flat and rectangular~~  
421 ~~(Figure 6.244B arrow). The end of the basal pole was blunt, while the end of the apical pole was~~  
422 ~~suppressed (Figures 6.244C and D).~~

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424 In this study, we did not observe any sculpturing on the cell walls or smooth in any species of the  
425 two different genera. This is probably because all of these species are equally alive and come from the  
426 tropics, despite their different living forms (epiphyte and terrestrial). This observation corresponds  
427 with the opinion of Chaudhary et al. (2014), who noted that seed ornamentations are directly related to  
428 the climatic preference of the species rather than its phylogeny. Similarly, according to Shimizu  
429 (2012), seed coat forms that are independent of plant habitat have a wider seed dispersal. The images  
430 we observed with SEM showed that different species in the genus *Phalaenopsis* had the same shape of  
431 testa cells and cell walls, namely elongated with cylindrical cell walls. Similarly, different species in  
432 the genus *Paphiopedilum* had testa cells and cell walls with the same shape, namely polygonal and  
433 with thin/flat rectangular cells walls. However, these results contradict with the results of previous  
434 research by Arditti et al. (1979, 1980) and Swamy et al. (2004), who showed that testa cells of  
435 different species can vary significantly in shape.

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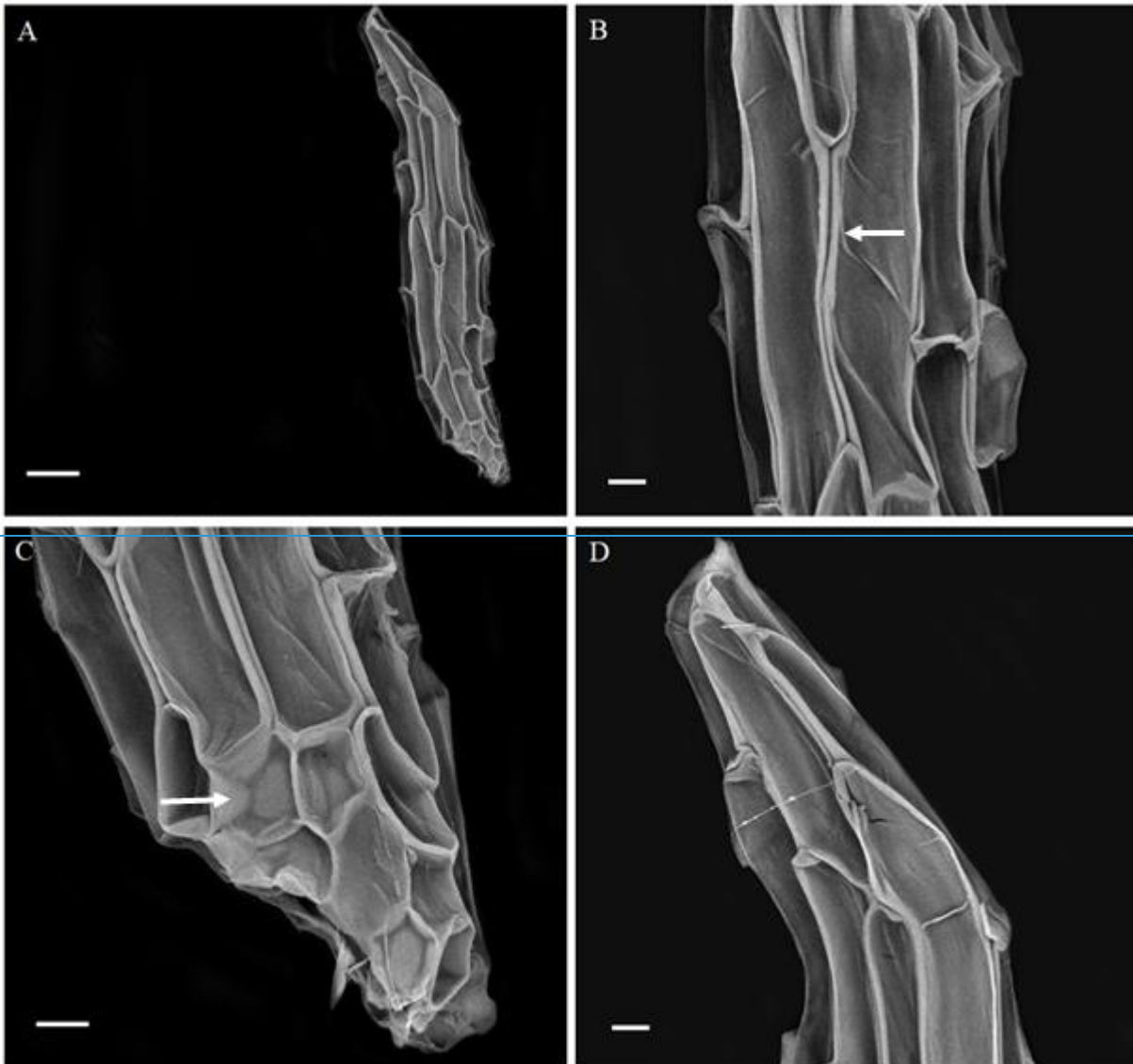
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Figure 7. [WU78] SEM photographs of *Phal. venosa*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=80  $\mu\text{m}$ , B-D=10  $\mu\text{m}$ .



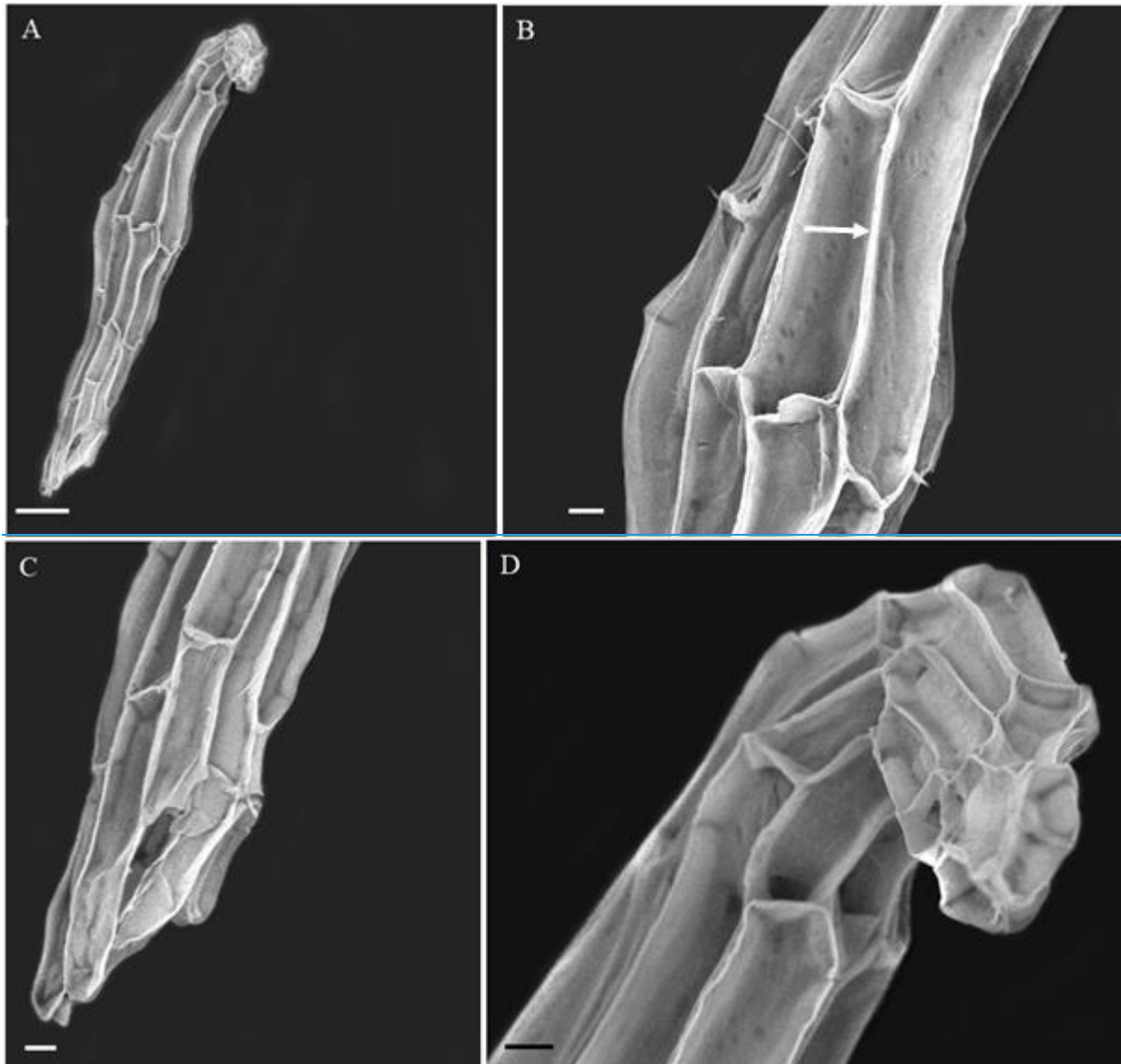
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Figure 8 [WU79]. SEM photographs of *Paph. kolopakingii*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu\text{m}$ , B-D=20  $\mu\text{m}$ .



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Figure 9. [WU80]SEM photographs of *Paph. liemianum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.



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Figure 10 [WU81]. SEM photographs of *Paph. baccanum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.





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Figure 11 [WU82]. SEM photographs of *Paph. primulinum*. A. seed shape, B. Pattern of testa cells of the medial, C. Cells of the basal pole, D. Cells of the apical pole. Scale bars: A=100  $\mu$ m, B-D=20  $\mu$ m.

### 473 Seeds size, ratio SL/SW and seed volume

474 The table 2 exhibits the size of seeds from ten species of the genus *Phalaenopsis* and  
475 *Paphiopedilum*. In spite of the fact that the seeds are microscopic, the result of the investigation  
476 showed a great diversity in their size. Seed length ranged between  $0.302 \pm 0.00879$  mm (*Phal. bellina*)  
477 and  $0.983 \pm 0.0035$  mm (*Paph. liemianum*), and width ranged from  $0.049 \pm 0.00439$  mm (*Phal.*  
478 *tetraspis*) to  $0.206 \pm 0.00246$  mm (*Paph. kolopakingii*). According to Verma et al. (2014), the SL/SW  
479 ratio provides information on the degree of seed truncation and is a good taxonomic character (Arditti  
480 et al. 1979 and Vij et al. 1992). In the present work, the species with seeds that were truncated  
481 (SL/SW < 6) were *Phal. amboinensis*, *Phal. bellina*, *Paph. baccanum*, *Phal. amboinensis*, *Paph.*  
482 *kolopakingii*, *Paph. baccanum* and *Paph. primulinum*. The following species had elongated seeds  
483 (SL/SW > 6): *Phal. amabilis*, *Phal. tetraspis*, *Phal. gigantea*, *Phal. tetraspis*, *Phal. venosa* and *Paph.*  
484 *liemianum* (Table 2). The most truncated seeds (SL/SW =  $4.254 \pm 0.0494$ ) were recorded in *Paph.*  
485 *kolopakingii* and the most elongated seeds (SL/SW =  $7.472 \pm 0.6574$ ) in *Phal. tetraspis*, both of  
486 which are members of different genus. The seed volume showed significant variations in both  
487 *Phalaenopsis* and *Paphiopedilum* orchids. On average, low seed volume was found in *Phal. gigantea*



488 (0.240±0.0327 mm<sup>3</sup>×10<sup>-3</sup>), *Phal. tetraspis* (0.242±0.0363 mm<sup>3</sup>×10<sup>-3</sup>), *Phal. venosa* (0.272±0.03216  
 489 mm<sup>3</sup>×10<sup>-3</sup>), *Phal. amboinensis* (0.318±0.020196 mm<sup>3</sup>×10<sup>-3</sup>), *Phal. bellina* (0.333±0.02768 mm<sup>3</sup>×10<sup>-3</sup>)  
 490 and *Phal. amabilis* (0.589±0.050497 mm<sup>3</sup>×10<sup>-3</sup>). When compared with epiphytic species, the seed  
 491 volume was found to be higher in the terrestrial species (9.668±0.17768 mm<sup>3</sup>×10<sup>-3</sup> (the highest) in  
 492 *Paph. kolopakingii*, 7.324± 0.1283 mm<sup>3</sup>×10<sup>-3</sup> in *Paph. baccanum*, 6.755±0.2672 mm<sup>3</sup>×10<sup>-3</sup> in *Paph.*  
 493 *liemianum* and 5.532±0.640396 mm<sup>3</sup>×10<sup>-3</sup> in *Paph. primulinum*). Clifford and Smith (1969),  
 494 Rasmussen (1995), Swamy et al. (2004) and Verma et al. (2012) stated that seed size shows a direct  
 495 correlation with plant habitat, and terrestrial orchids generally possess larger seeds as compared to  
 496 epiphytic orchids. Yoder et al. (2010) also stated that the seeds of terrestrial orchids are bigger as  
 497 compared to epiphytic orchids.

500 Table 2. Micro-morphometric data of seeds related characters (mean ± SD) from species of the genus  
 501 *Phalaenopsis* and *Paphiopedilum*

502

Species name	SL [WU83] (mm)	SW (mm)	SL/SW (mm)	SV (mm <sup>3</sup> ×10 <sup>-3</sup> )
<i>Phalaenopsis</i>				
<i>Phal. amabilis</i>	0.433 ± 0.0084 [WU84]	0.072 ± 0.0030	6.029 ± 0.2854	0.589 ± 0.054097
<i>Phal. ambonensis</i>	0.349 ± 0.006	0.059 ± 0.002	5.915 ± 0.221	0.318 ± 0.020
<i>Phal. tetraspis</i>	0.370 ± 0.0080096	0.049 ± 0.0030049	7.472 ± 0.6574	0.242 ± 0.0363
<i>Phal. bellina</i>	0.302 ± 0.0070089	0.065 ± 0.0024	4.646 ± 0.2004999	0.333 ± 0.02768
<i>Phal. gigantea</i>	0.348 ± 0.0095	0.051 ± 0.0034	6.823 ± 0.4870	0.240 ± 0.03327
<i>Phal. tetraspis</i> <i>Phal. ambonensis</i>	0.370 ± 0.0090.349 ± 0.0062	0.049 ± 0.0040.059 ± 0.0010028	7.472 ± 0.6575.915 ± 0.2214	0.242 ± 0.0360.318 ± 0.020196
<i>Phal. venosa</i>	0.345 ± 0.0060079	0.055 ± 0.0032	6.305 ± 0.3943957	0.272 ± 0.03216
<i>Paphiopedilum</i>				
<i>Paph. baccanum</i> <i>Paph. kolopakingii</i>	0.815 ± 0.0020.874 ± 0.0070087	0.185 ± 0.0020.206 ± 0.0010026	4.401 ± 0.0434.254 ± 0.0494	7.324 ± 0.1289.668 ± 0.17768
<i>Paph. kolopakingii</i>	0.874 ± 0.008	0.206 ± 0.002	4.254 ± 0.049	9.668 ± 0.177
<i>Paph. liemianum</i>	0.983 ± 0.0035	0.162 ± 0.0033	6.074 ± 0.1294	6.755 ± 0.2672
<i>Paph. baccanum</i>	0.815 ± 0.0022	0.185 ± 0.0017002	4.401 ± 0.04329	7.324 ± 0.1283
<i>Paph. primulinum</i>	0.815 ± 0.0435	0.161 ± 0.0088009	5.083 ± 0.38878	5.532 ± 0.643096

503 Abbreviations: SL → Seed length; SW → Seed width; SL/SW → Seed length/Seed width; SV → Seed volume

504 [WU85]

505  
 506 Table 3. Micro-morphometric data of embryos related characters (mean ± SD) from species of the  
 507 genus *Phalaenopsis* and *Paphiopedilum*

508

Species name	EL [WU86] (mm)	EW (mm)	EL/EW (mm)	EV (mm <sup>3</sup> ×10 <sup>-3</sup> )	SV/EV (mm <sup>3</sup> ×10 <sup>-3</sup> )	AS (%)
<i>Phalaenopsis</i>						
<i>Phal. amabilis</i> [WU87]	0.187 ± 0.0044	0.072 ± 0.0030	2.607 ± 0.1322	0.509 ± 0.0433	1.158 ± 0.0333	13.554 ± 2.4693
<i>Phal. ambonensis</i> <i>Phal. tetraspis</i>	0.203 ± 0.0050.171 ± 0.0081	0.051 ± 0.0020.046 ± 0.0034	3.993 ± 0.1903.739 ± 0.30549	0.274 ± 0.0200.191 ± 0.0301	1.165 ± 0.0531.273 ± 0.0821	14.245 ± 3.64121.128 ± 5.0900
<i>Phal. bellina</i>	0.211 ± 0.0132	0.051 ± 0.0022	4.149 ± 0.3614	0.286 ± 0.0244	1.168 ± 0.0305	14.294 ± 2.23879
<i>Phal. gigantea</i>	0.192 ± 0.0074	0.043 ± 0.3022	4.496 ± 0.3022	0.184 ± 0.0244	1.308 ± 0.0745	23.291 ± 4.35289
<i>Phal. ambonensis</i>	0.203 ± 0.00546	0.051 ± 0.00219	3.993 ± 0.190897	0.274 ± 0.0200	1.165 ± 0.05329	14.245 ± 3.6410
<i>Phal. tetraspis</i>	0.171 ± 0.008	0.046 ± 0.003	3.739 ± 0.305	0.191 ± 0.030	1.273 ± 0.082	21.128 ± 5.090
<i>Phal. venosa</i>	0.183 ± 0.00329	0.047 ± 0.00218	3.929 ± 0.1922	0.209 ± 0.0144	1.309 ± 0.17248	22.338 ± 10.02878
<i>Paphiopedilum</i>						
<i>Paph. kolopakingii</i> [WU88]	0.270 ± 0.0032	0.157 ± 0.00438	1.726 ± 0.04216	3.466 ± 0.1863	2.797 ± 0.150499	64.150 ± 1.9360
<i>Paph. baccanum</i>	0.223 ± 0.003	0.115 ± 0.002	1.933 ± 0.037	1.557 ± 0.054	4.709 ± 0.208	78.725 ± 0.921
<i>Paph. kolopakingii</i>	0.270 ± 0.003	0.157 ± 0.004	1.726 ± 0.042	3.466 ± 0.186	2.797 ± 0.150	64.150 ± 1.936

[WU89]

<i>Paph. liemianum</i>	0.271 ± 0.0044	0.083 ± 0.0045	3.267 ± 0.2025	0.797 ± 0.1034	6.974 ± 0.7653	85.501 ± 1.52879
<i>Paph. baccanum</i>	0.223 ± 0.0030	0.115 ± 0.00218	1.933 ± 0.03767	1.557 ± 0.05439	4.709 ± 0.2083	78.725 ± 0.9214
<i>Paph. primulinum</i>	0.274 ± 0.0114	0.105 ± 0.0090	2.636 ± 0.2165	1.590 ± 0.3090	3.608 ± 0.8454	70.898 ± 6.34436

Abbreviations: EL- :-Embryo length, :-EW- :-Embryo width, weight, :-EL/EW: Embryo length/Embryo width, EV- :- Embryo volume, A:- :-Air space, SV/EV: Seed volume/Embryo volume, AS-S: Air space.

## Seed to embryo volume and free air space

According to Verma et al. (2014), EV is an important character as it directly affects the percentage of the procurable air space inside the seed. Like their EL/EV ratios, EV were observed in various *Phalaenopsis* species; it was lowest in *Phal. gigantea* ( $0.184 \pm 0.024 \text{ mm}^3 \times 10^{-3}$ ) and highest in *Phal. amabilis* ( $0.509 \pm 0.043 \text{ mm}^3 \times 10^{-3}$ ). In *Paphiopedilum*, EV the ranged between  $0.797 \pm 0.103 \text{ mm}^3 \times 10^{-3}$  (*Paph. liemianum*) and  $3.466 \pm 0.186 \text{ mm}^3 \times 10^{-3}$  (*Paph. kolopakingii*).

Our data showed that variation was observed in SV/EV. In the species of *Phalaenopsis*, the SV/EV ratio never exceeded two (Table 3), but in the species of *Paphiopedilum* measurements peaked at  $6.974 \pm 0.765 \text{ mm}^3 \times 10^{-3}$  in *Paph. liemianum*. This was followed by *Paph. baccanum* ( $4.709 \pm 0.208 \text{ mm}^3 \times 10^{-3}$ ), *Paph. primulinum* ( $3.608 \pm 0.845 \text{ mm}^3 \times 10^{-3}$ ) and *Paph. kolopakingii* ( $2.797 \pm 0.150 \text{ mm}^3 \times 10^{-3}$ ). Burgeff (1936) experimentally demonstrated the relationship between SV/EV ratio and seed buoyancy. Seeds with a high SV/EV ratio are more buoyant because they possess a greater air space.

Swamy et al. (2004, 2007) stated that AS is an important character because the seeds of most orchids are wind dispersed, implying that seeds with a greater AS percentage will float in the air for a longer time and thus spread to more distant places. We observed that terrestrial species possessed a comparatively greater percentage of air space in their seeds as compared with epiphytic species. A greater percentage of air space for *Paph. kolopakingii*, *Paph. liemianum*, *Paph. baccanum* and *Paph. primulinum* was shown more than 60% of seed i.e. ( $64.150 \pm 1.936$ ), ( $85.501 \pm 1.528$ ), ( $78.725 \pm 0.921$ ) and ( $70.898 \pm 6.344$ ). Zhang et al. (2015) informed that higher AS values in seeds of terrestrial species can help spread them further along the forest floor where wind speeds are lower. Seeds with a greater percentage of air space in *Paphiopedilum* are expected to be more widely distributed and, in fact, we found it difficult to collect their seeds.

## Conclusion

The results of this study can be concluded that species within *Paphiopedilum* produce larger seeds with smaller embryos and a bigger percentage of air space (AS) than those of *Phalaenopsis* species. Likewise, the volume of seeds and seed volume/embryo volume in *Paphiopedilum* are larger than in *Phalaenopsis*. Large air spaces will increase seed buoyancy and seeds can be dispersed further. Testa cells are elongated in shape, with cylindrical cells walls, in *Phalaenopsis* but they are polygonal, with thin or flat rectangular cell walls, in *Paphiopedilum*. There are some similarities in character that might be related to adaptation to the tropical area, because in all species studied, both epiphytic and terrestrial orchids have the same form of fusiform seeds and a smooth testa surface. The general results of this study inform us that the morphological and morphometric properties of orchid seeds could be utilised as a means of distinguishing between life forms and habitat similarity and aid in identification.

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## REFERENCES[WU93]

- 554 Akçin TA, Ozdener Y, Akçin A. 2009. Taxonomic Value of Seed Characters in Orchids from Turkey.  
 555 *Belgian Journal of Botany*[WU94] 142 (2):124-139. <http://dx.doi.org/10.2307/41427182>.
- 556 Arditti J, Ghani AKA. 2000. Numerical and physical properties of orchid seeds and their biological  
 557 implications. *New Phytol.* 145:367-421. DOI: 10.1046/j.1469-8137.2000.00587.x.
- 558 Arditti J, Michaud JD, Healey PL. 1979. Morphometry of orchid seeds. I. *Paphiopedilum* and native  
 559 Californian and related species of *Cypripedium*. *American Journal of Botany*[WU95]. 66:1128-  
 560 1137. <http://dx.doi.org/10.2307/2442211>.
- 561 Arditti J, Michaud JD, Healey PL. 1980. Morphometry of Orchid Seeds. II. Native California and  
 562 Related Species of *Calypso*, *Cephalanthera*, *Corallorhiza* and *Epipactis*. *American Journal of*  
 563 *Botany*. 67 (3):347-360.  
 564 <http://dx.doi.org/10.2307/2442345>.
- 565 Burgeff H. 1936. *Samenkeimung der Orchideen und Entwicklung ihrer Keimpflanzen*, Jena: Gustav  
 566 Fischerr Verlag.
- 567 Cela PG, Seligrat I, Ortúñez E, Gamarra R, Vivar A, Scrugli A. 2014. A study of seed  
 568 micromorphology in the genus *Ophrys* (Orchidaceae). *Anales del Jardín Botánico de Madrid.* 71  
 569 (2):1-6. <http://dx.doi.org/10.3989/ajbm.2370>.
- 570 Chase MW, Phippen JS. 1988. Seed morphology in the Oncidiinae and related subtribes orchidaceae.  
 571 *Systematic Botany.* 13 (3):313-323.
- 572 Chaudhary B, Chattopadhyay P, Banerjee N. 2014. Modulations in seed micromorphology reveal  
 573 signature of adaptive species-diversification in *Dendrobium* (Orchidaceae). *Open Journal of*  
 574 *Ecology.* 4 (2):33-42. <http://dx.doi.org/10.4236/oje.2014.42005>.
- 575 Clifford HT, Smith WK. 1969. Seed morphology and classification of Orchidaceae.  
 576 *Phytomorphology.* 19:133-139.
- 577 Gamarra R, Galán P, Herrera I, Ortúñez E. 2008. Seed micromorphology supports the splitting of  
 578 *Limnorchis* from *Platanthera* (Orchidaceae). *Nordic Journal of Botany.* 26:61-65. DOI:  
 579 10.1111/j.1756-1051.2008.00135.x
- 580 Gamarra R, Galan P, Pedersen HE, Ortunez E, Sanz E. 2015. Seed micromorphology in *Dactylorhiza*  
 581 *Necker ex Nevski* (Orchidaceae) and allied genera. *Turkish Journal of Botany.* 39:298-309. DOI:  
 582 10.3906/bot-1401-66.
- 583 Gamarra R, Ortúñez E, Sanz E, Esparza I, Galán P. 2010. Seeds in subtribe Orchidinae (Orchidaceae):  
 584 the best morphological tool to support molecular analyses. In: Nimis P. L., R. Vignes-Lebbe (eds.).  
 585 *Tools for Identifying Biodiversity: Progress and Problems.* 323-326. ISBN 978-88-8303-295-0.  
 586 EUT.
- 587 Guler N. 2016. Seed micromorphology of *Orchis Tourn. Ex L.* (Orchidaceae) and allied genera  
 588 growing in Edirne province, Turkey. *Phyto Keys.* 69:9-25. <http://dx.doi.10.3897/phyto>  
 589 [keys.68.8746](http://dx.doi.10.3897/phyto).
- 590 Molvray M, Chase M. 1999. Seed morphology. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen  
 591 FN (eds.). *Genera Orchidacearum, General Introduction, Apostasioideae, Cypripedioideae.* Oxford  
 592 University Press, Oxford, 59-66.
- 593 Rasmussen HN. 1995. *Terrestrial orchids from seed to mycotrophic plant.* Cambridge, UK:  
 594 Cambridge University Press.
- 595 Shimizu N. 2012. Adaptation and evolution of seed shape on bleeding area in Japanese orchids.  
 596 *International Journal of Biology.* 4: 47-53.
- 597 Sodhi NS, Koh LP, Brook BW, Ng PKL. 2004. Southeast Asian biodiversity: An impending disaster.  
 598 *Trends in Ecology & Evolution.* 19 (12):654-660. DOI: 10.1016/j.tree.2004.09.006
- 599 Swamy KK, Kumar HNK, Ramakrishna TM, Ramaswamy SN. 2004. Studies on Seed Morphometry  
 600 of Epiphytic Orchids from Western Ghats of Karnataka. *Taiwania.* 49 (2):124-140. DOI:  
 601 10.6165/tai.2004.49 (2).124.

- 602 Swamy KK, Kumar HNK, Ramaswamy SN. 2007. Studies on seed morphometry of *Dendrobium*  
603 species. *Phytomorphology*. 57:33-43.
- 604 Tsutsumi C, Yukawa T, Lee NS, Lee CS, Kato M. 2007. Phylogeny and comparative seed  
605 morphology of epiphytic and terrestrial species of *Liparis* (Orchidaceae) in Japan. *Journal of Plant*  
606 *Research*. 120 (3):405-412. DOI: 10.1007/s10265-007-0077-0
- 607 Verma J, Kusum K, Thakur K, Sembi JK, Vij SP. 2012. Study on seed morphometry of seven  
608 threatened Himalayan orchids exhibiting varied life modes. *Acta Botanica Gallica: Botany Letters*.  
609 159 (4):443-449. DOI: 10.1080/12538078.2012.750582.
- 610 Verma J, Sharma K, Thakur K, Sembi JK, Vij SP. 2014. Study on Seed Morphometry of Some  
611 Threatened Western Himalayan Orchids. *Turkish Journal of Botany*. 38:234-251. DOI:  
612 10.3906/bot-1307-14.
- 613 Vij SP, Kaur P, Kaur S, Kaushal PS. 1992. The Orchid Seeds: Taxonomic, Evolutionary and  
614 Functional Aspects. *Journal- Orchid Society*,- 6:91-107.
- 615 Yoder JA, Imfeld SM, Heydinger DJ, Hart CE, Collier MH, Gribbins KM, Zettler LW. 2010.  
616 Comparative water balance profiles of Orchidaceae seeds for epiphytic and terrestrial taxa endemic  
617 to North America. *Plant Ecology*,- 211:7-17. DOI: 10.1007/s11258-010-9765-2.
- 618 Zhang FP, Zhang JJ, Yan N, Hu H, Zhang SB. 2015. Variations in seed micromorphology of  
619 *Paphiopedilum* and *Cypripedium* (Cypripedioideae, Orchidaceae). *Seed Science Research*. 25:395-  
620 401. DOI: 10.1017/S0960258515000276.
- 621

### List of Revisions in the manuscript

Row	Reviewer suggestion/comment	Author revision/answer
1	Title-change	I am agree, I have already revised it
R9	Qualitative?	Yes qualitative characters
R18	Need/clarify some more reasons in results and discussion	I have deleted the sentence
R54	How old are the capsules of both genera?	Mature Phalaenopsis capsules aged 3.5 - 4 months, and mature Paphiopedilum capsules aged 8.5-9 months Each species
R54	Correct: between 2016 and 2019 or from 2016 to 2019	I am agree with you,.... From 2016 to 2019
R59-60	Fresh seed .... How do you do? Oven?, where?	The specimens used for SEM were dried for at least 4 weeks at room temperature and stored in small paper envelope
	How do you do? Oven?	
	Results and Discussion There are some more repeated sentences. (please try to reduce those sentences) It is possible, if the writing can be improved and corrected. For example, 1. (I) Describe the important characteristics of each genus , followed by in each species. Phal.....(characteristics of genus)..... Species.....(characteristics of each species) Paph..... Species..... 2. (II) Characteristics related adaptation/evolution/ others	I have already revised as your suggestion
R-80	, and this showed the seed ... Of 2 genera?	Yes, of 2 genera
R-95	<i>P. amabilis</i>	<i>Phal. amabilis</i>
R-96-97	Rewrite (rw)	Yes, I rewrite it already
R-99	.... two poles .... and how	I have rewrite
R-101 (WU13)	Change topic: For example Morphological characteristic	Ok, I am agree with you, I have changed
(WU14) (WU15)	Add genus name and rearranged in alphabetical	Ok, I am agree with you. I have changed
R.107	Font not italic	Ok .... I have corrected
R.114	<i>P. tetraspis</i>	<i>Phal. tetraspis</i>
R.118	<i>P. bellina</i>	<i>Phal. bellina</i>
R. 119-120	<i>RW</i>	Ok I have rewrite
R-128	Try to insert the important characteristics (in all figure)	I have inserted the important characteristics
	Indicating arrow	I have added of information at all figures
R283	Write a ... Conclusion	I am agree, already I have write a conclusion

No	Recommendation of reviewer	Author revision/answer
1	Title – change to "Variations in seed micromorphology and morphometry of native Indonesian Phalaenopsis and Paphiopedilum orchid.	I am agree, I have changed it

2	p48 – plant material change to “plant materials”	I am agree, I have changed it
3	Figure 1 – It looks like no embryo in several seeds, including B, C, E, J.	I have changed it, in my opinion can already distinguished between embryos and testa
4	Fig. 2-11 – Too many figures. Combine them into one panel.	I have made three pages, if combine them into one panel, there are no visible characteristics of surface morphology of the seeds
5	There is a lack of significant difference between means in all data.	The word: significant, is deleted, I did not analyse statistically
6	The authors claim that “the results of the study indicate that morphological and morphometric features can serve to identify live forms and distinguish between species and phylogeny”, but how to use them to identify these species of orchids? Please specify at least one example in detail.	Qualitative and quantitative characters can distinguished between two genus and Interspecies. Qualitative characters analysed by EM can distinguished live form
7	M&M - Please describe the maturaty of capsules in detail, e.g. how long is the period from pollination to maturation, morphology and size, etc.	The period from pollination to maturation of <i>Phalaenopsis</i> is 3.5-4 months, and of <i>Phapiopedilum</i> is 8.5-9 months. I did not measured of capsules. I have some photos collection of capsules