UNTUK INDONESIA ADIL & BERADAB

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

3986369: Acknowledging Receipt

5 messages

Scientifica <rhea.lumbres@hindawi.com> Sun, Aug 11, 2019 at 10:01 PM
To: sucipto-h@fst.unair.ac.id
Cc: rhea.lumbres@hindawi.com, Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

The Research Article titled "Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw ," by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami has been received and assigned the number 3986369.

All authors will receive a copy of all the correspondences regarding this manuscript.

Thank you for submitting your work to Scientifica.

Best regards,

--
****
Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com
****

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Thu, Nov 14, 2019 at 7:35 PM
To: Scientifica <rhea.lumbres@hindawi.com>

Dear Prof. Rhea Lumbres
Editorial Office
Hindawi

I would like to ask about our Research Article titled " Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw ," by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami has been received and assigned the number 3986369.
This Article submitted in August 11 2019. Is it still in the review process?

Thank you for your attention and cooperation.

Best regards,
Sucipto Hariyanto
Biology Department, Faculty of Science and Technology, Airlangga University
[Quoted text hidden]

Rhealumbres <rhea.lumbres@hindawi.com> Fri, Nov 15, 2019 at 11:45 AM
To: sucipto-h@fst.unair.ac.id

Dear Dr. Hariyanto,

Thank you for your inquiry. The Editor who is overseeing the review process of your manuscript is currently assigning it to external reviewers. Once the reviewers have submitted their reports, the Editor will be able to make a decision.

We will notify you when the decision is finalized.

Best regards,

Rhea

--
****
Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com

[Quoted text hidden]

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sucipto hariyanto <sucipto-h@fst.unair.ac.id> Sun, Dec 1, 2019 at 6:41 PM
To: Rhealumbres <rhea.lumbres@hindawi.com>

Dear Prof. Rhea Lumbres

How about our article review process? Already enough for almost four months there no further information.
What should I do? Because according to Hindawi guidelines the 47 days review process.

I'm sorry I ask twice for you.
Best regards,
Sucipto Hariyanto

[Quoted text hidden]

Rhealumbres <rhea.lumbres@hindawi.com> Tue, Dec 3, 2019 at 11:55 AM
To: sucipto-h@fst.unair.ac.id

Dear Dr. Hariyanto,

Thank you for contacting us. As we rely on external Editors and reviewers to oversee the review process, some manuscripts may take longer than average to be reviewed. I am doing my best to expedite the review process of your manuscript.

Thank you for your patience and understanding.

Best regards,

Rhea

--
****
Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com

[Quoted text hidden]

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

3986369: Authors' Feedback Needed

3 messages

rhea.lumbres@hindawi.com <rhea.lumbres@hindawi.com> Thu, Aug 22, 2019 at 8:59 PM
To: sucipto-h@fst.unair.ac.id
Cc: Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

This is regarding manuscript 3986369 titled "Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw" submitted to Scientifica. While checking your manuscript, we have found that thedata availability statement is missing. Hindawi encourages all authors to share the data underlying the findings of their manuscripts. Data sharing allows researchers to verify the results of an article, replicate the analysis, and conduct secondary analyses. Accordingly, please update your manuscript's PDF to include a "Data Availability" statement for the data used in your manuscript and send the updated file to me by email. This statement should describe how readers can access the data supporting the conclusions of the study, and clearly outline the reasons why unavailable data cannot be released. Help with writing your statement can be found at https://www.hindawi.com/research.data/#statement.
The tables are not labeled correctly. Please email us an updated PDF file of the manuscript with the correct labels for the tables.

We look forward to hearing from you.

Best regards,

Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Fri, Aug 23, 2019 at 2:51 PM
To: rhea.lumbres@hindawi.com

Dear Prof. Lumbres
Editorial office Hindawi

We send back our manuscript that we revised according of your suggestion (the statement of data availability (page 17 in the manuscript) and labels of table 1 and table 2.
Thank you for your information about our manuscript and your attention.

Best regards,
Sucipto Hariyanto
[Quoted text hidden]

Article of Seed Morpho Dendro for SCIENTIFICA (1).pdf
1086K

Rhealumbres <rhea.lumbres@hindawi.com> Fri, Aug 23, 2019 at 3:04 PM
To: sucipto-h@fst.unair.ac.id

Dear Dr. Hariyanto,

Thank you for your feedback. I have updated your manuscript PDF file in the MTS.

Best regards,

Rhea

--
****

Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com

[Quoted text hidden]

request information about article number 3986369

3 messages

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Sat, Jan 18, 2020 at 9:29 AM
To: ltortiz@vet.ucm.es

To. Prof. Luis T. Ortiz
Editor Scientifica Journal Hindawi

Dear Prof. Ortiz
My name is Sucipto Hariyanto, lecturer in Biology Department Faculty of Science and Technology Airlangga University, Surabaya, Indonesia.
I submitted an article in the Scientifica Journal on August 11 2019, by title:"Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw ," by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami and at that time the Editor was Prof. Rhea Lumbres, but since December 2019 you are the editor.

For that, I want to know the clarity of the status of my article

I thank you for the information and deign as the editor of our article

Best regards,

Sucipto Hariyanto

Department of Biology

Faculty of Science and TechnologyAirlangga University

Campus C Unair, Jl. Soekarno, Surabaya, Indonesia 60115

LUIS TOMAS ORTIZ VERA <ltortiz@vet.ucm.es> Mon, Jan 20, 2020 at 6:53 PM
To: sucipto hariyanto <sucipto-h@fst.unair.ac.id>

Dear Dr. Sucipto:
I apologize for the delay in the decision on the acceptance of your paper, but at the moment we´re expecting a third opinion.
One of the reviewers recommend to resubmit the manuscript after major revisions while the other refuses acceptance of the paper.
I hope the answer comes in a few weeks.

Best regards
[Quoted text hidden]
--

Dr. Luis T. Ortiz
Facultad de Veterinaria
Universidad Complutense de Madrid
Spain

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Tue, Jan 21, 2020 at 6:35 AM

To: LUIS TOMAS ORTIZ VERA <ltortiz@vet.ucm.es>

Dear Prof. Ortiz

I say many thanks for the information about our article. I will wait for further information from you.

Best regards,

Sucipto Hariyanto

[Quoted text hidden]

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

3986369: Major Revision Required

2 messages

Luis T. Ortiz <scientifica@hindawi.com> Thu, Jan 30, 2020 at 8:06 PM
Reply-To: rhea.lumbres@hindawi.com
To: sucipto-h@fst.unair.ac.id
Cc: Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

Following the review of Research Article titled "Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw " by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami, I recommend that it should be revised taking into account the changes requested by the reviewer(s). Since the requested changes are major, the revised manuscript will undergo a second round of review by the same reviewer(s). Please login to the Manuscript Tracking System to read the submitted review report(s) and submit the revised version of your manuscript no later than Thursday, February 27, 2020.

To submit the revised version of your manuscript, please access "Author Activities" in your account and upload the PDF file of your revised manuscript. Also, please submit your replies to the comments of the reviewer(s) as an additional PDF file.

Best regards,

Luis T. Ortiz

---

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Fri, Jan 31, 2020 at 6:11 AM
To: Scientifica <rhea.lumbres@hindawi.com>

Dear Prof Ortiz

I would like to say many thanks for the information and you have been looking for additional reviews for our article.
I will revise according to the review recommendations as soon as possible.

Best regards
Sucipto Hariyanto
[Quoted text hidden]

UNTUK INDONESIA ADIL & BERADAB

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

3986369: Revised Version Received

1 message

Scientifica <rhea.lumbres@hindawi.com> Thu, Feb 27, 2020 at 8:04 PM
To: sucipto-h@fst.unair.ac.id
Cc: rhea.lumbres@hindawi.com, Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

The revised version of Research Article 3986369 titled "Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw " by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami has been received. The editor assigned to handle the review process of your manuscript will inform you as soon as a decision is reached.

Thank you for submitting your work to Scientifica.

Best regards,

--
****
Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com
****

3986369: Minor Revision Required

2 messages

Luis T. Ortiz <scientifica@hindawi.com> Mon, Mar 9, 2020 at 4:20 PM
Reply-To: rhea.lumbres@hindawi.com
To: sucipto-h@fst.unair.ac.id
Cc: Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

Following the review of your Research Article titled "Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw ," by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami, I recommend that it should be revised taking into account the changes requested by the reviewer(s). Please login to the Manuscript Tracking System to read the submitted review report(s) and submit the revised version of your manuscript not later than Monday, March 23, 2020.

To submit your revised manuscript, please access "Current Manuscripts" in your account and upload the PDF file of your revised manuscript. You are also asked to submit your replies to the reviewer(s) comments as an additional PDF file.

Best regards,

Luis T. Ortiz

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sucipto hariyanto <sucipto-h@fst.unair.ac.id> Mon, Mar 9, 2020 at 7:02 PM
To: Scientifica <rhea.lumbres@hindawi.com>

Dear Prof. Ortiz

Thanks for you for your information about our manuscript.
We will immediately revise our manuscript according to the reviewer(s) recommendations.
Best regards,
Sucipto Hariyanto

[Quoted text hidden]

3986369: Revised Version Received

1 message

Scientifica <rhea.lumbres@hindawi.com> Thu, Mar 12, 2020 at 7:03 PM
To: sucipto-h@fst.unair.ac.id
Cc: rhea.lumbres@hindawi.com, Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

The revised version of Research Article 3986369 titled "Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw " by Sucipto Hariyanto, Intan Ayu Pratiwi and Edy S.W. Utami has been received. The editor assigned to handle the review process of your manuscript will inform you as soon as a decision is reached.

Thank you for submitting your work to Scientifica.

Best regards,

--
****
Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com
****

3986369: Electronic Files

2 messages

Scientifica <rhea.lumbres@hindawi.com> Mon, Mar 16, 2020 at 3:56 PM
To: sucipto-h@fst.unair.ac.id
Cc: rhea.lumbres@hindawi.com, Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

This is regarding manuscript 3986369 that will be published shortly in Scientifica. None of the figures can be edited. We need to be able to edit lines, arrowheads, and fonts to match the journal's style. Each figure should be a separate ps, eps, fig, ai, Visio, wmf, emf, Word, Excel, PowerPoint, opj, or PDF file which can be edited. Please note that jpg, bmp, png, and tif files cannot be edited by default.

Please access the Manuscript Tracking System at
http://mts.hindawi.com/author/3986369/upload.files/ and upload the file(s) required.

Best regards,

--
****
Rhea Lumbres
Editorial Office
Hindawi
http://www.hindawi.com
****

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Mon, Mar 16, 2020 at 7:59 PM
To: Scientifica <rhea.lumbres@hindawi.com>

Dear Prof. Lumbres (Scientifica)

Thank you for your information about electronic file. That the figures must be editable.
I resubmit the electronic file.

Thank you again for your information and cooperation.

Best regards,
Sucipto Hariyanto

[Quoted text hidden]

3986369: Galley Proofs

1 message

Scientifica <production.b@hindawi.com> Fri, Apr 3, 2020 at 11:37 PM
To: sucipto-h@fst.unair.ac.id
Cc: Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

I am pleased to let you know that the first set of galley proofs of your Research Article 3986369 titled "Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium ," is ready. You can apply your corrections directly to the manuscript with the Online Proofing System (OPS).

Using the OPS, you can quickly and easily make corrections directly to your galley proofs and submit these corrections with a single click.

https://ops.hindawi.com/author/3986369/

If a new corresponding author is added, they must log into their manuscript tracking system account and add their ORCID ID. Any additional ORCID IDs added on during proofing will also need to be updated on that author's account. Delays can occur if this isn't done.

To expedite the publication of your manuscript, please send us your corrected galley proofs within three days.

Please ensure that you read the proofs thoroughly and make all necessary corrections at this stage. A second round of proofs may be requested only for checking essential changes or major revisions.

Best regards,

--
****
Hindawi Production Team
Hindawi
https://www.hindawi.com
****

3986369: Galley Proofs

2 messages

Scientifica <production.b@hindawi.com> Thu, Jun 18, 2020 at 9:53 AM
To: sucipto-h@fst.unair.ac.id
Cc: Intan.ayu.pratiwi@fst.unair.ac.id, edy-s-w-u@fst.unair.ac.id

Dear Dr. Hariyanto,

I am pleased to let you know that the second set of galley proofs of your Research Article 3986369 titled "Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium ," is ready. You can apply your corrections directly to the manuscript with the Online Proofing System (OPS).

Using the OPS, you can quickly and easily make corrections directly to your galley proofs and submit these corrections with a single click.

https://ops.hindawi.com/author/3986369/

If a new corresponding author is added, they must log into their manuscript tracking system account and add their ORCID ID. Any additional ORCID IDs added on during proofing will also need to be updated on that author's account. Delays can occur if this isn't done.

To expedite the publication of your manuscript, please send us your corrected galley proofs within three days.

Please ensure that you read the proofs thoroughly and make all necessary corrections at this stage. A second round of proofs may be requested only for checking essential changes or major revisions.

Best regards,

--
****
Hindawi Production Team
Hindawi
https://www.hindawi.com
****

sucipto hariyanto <sucipto-h@fst.unair.ac.id> Thu, Jun 18, 2020 at 10:29 AM
To: Scientifica <production.b@hindawi.com>

Hindawi Production Team

Dear Mrs./Mr.

I say many thanks for the completion of manuscript corrections.
After we double checked the manuscript (Research Article 3986369 titled "Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium ,"), the manuscript had no written error.

Thank you for your kindness and cooperation.

Best regards,
Sucipto Hariyanto
Biology Department
Faculty of Science & Technology
Universitas Airlangga
[Quoted text hidden]

FILE MESSAGE

Fri 26/06/2020 15:16

Rhea Lumbres <rhea.lumbres@hindawi.com>

3986369: Your article has been published

To sucipto-h@fst.unair.ac.id

Dear Dr. Hariyanto,

I am pleased to let you know that your article has been published in its final form in "Scientifica."

Sucipto Hariyanto, "Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium," Scientifica, vol. 2020, Article ID 3986369, 14 pages, 2020. https://doi.org/10.1155/2020/3986369

You can access this article from the Table of Contents of Volume 2020, which is located at the following link:

https://www.hindawi.com/journals/scientifica/contents/

Alternatively, you can access your article directly at the following location:

https://www.hindawi.com/journals/scientifica/2020/3986369/

"Scientifica" is an open access journal, meaning that the full-text of all published articles is made freely available on the journal's website with no subscription or registration barriers.

If you would like to order reprints of this article please click here, https://www.hindawi.com/journals/scientifica/2020/3986369/reprint/.

Best regards,

Rhea Lumbres
Scientifica
Hindawi
https://www.hindawi.com/

REVIEWER RECOMMENDATIONS AND COMMENTS & RESPONSES TO REVIEWER COMMENTS

Recommendation

Consider after major changes

Comments for the author

-The manuscript's findings not adequately presented. Is suggests that the Results section and Discussion section should be separated. A better Discussion section is missing for some morphological characters.

-On page 6: All the seeds of the orchids are small and have a seed air space. It is difficult to corroborate whether this air space contributes to a lesser or a more significant way during seed dispersion. How can authors verify this?
-The DOI of some papers should be a double check. Some are incorrect as:
[22] T. A. Akçin, Y. Ozdener, and A. Akçin, "Taxonomic Value of Seed Characters in Orchids from Turkey," Belg. J. Bot. v. 142, n. 2, p. 124-139, 2009. http://dx.doi.org/10.2307/41427182.
-The authors should check the spelling of some sentences.
-The data do not support the conclusions.
-Many modifications marked on the PDF.

Additional File

PDF file with further comments Detailed review report

Comments for the author

The ms “Study on Seed Morphometry of Native Indonesian Orchids in the Genus Dendrobium Sw” Show interesting data about the seed morphology of 10 Dendrobium species; however, the MS:
a) Lack of theoretical framework and hypotheses, it looks like a scholar report. I suggest authors to write a taxonomic context, Dendrobium is a species rich genus and the MS could be useful if the species studied belong to little studied infrageneric clades. Does the studied species represent a clade without studies of their seeds? How does the traits differ from those reported previously and why?
b) Too much information is duplicated between tables and text.
c) There are not statistical tests supporting differences between the species. Seeds coming from greenhouses, and from outcrossing could be larger than those coming from the wild. How this effect was controlled to truly represent the seed traits of the species.
d) Maybe the MS can be published as a short communication in Lankesteriana.

Minor comments
a) Keywords must be different from the title
a) Delete the word "Study" from the title

b) In the study area, add data about the type of vegetation and the phenology of the species
c) How do you know that the capsules were immature/mature?
d) Drop the disinfection protocol, is irrelevant for the purpose of the MS (seeds were not cultivated in vitro).

List of revision manuscript No. 3986369

Reviewer 1

Comments for the author 1

1. Does the studied species represent a clade without studies of their seeds? How does the traits differ from those reported previously and why?
2. To much information is duplicated between tables and text.
   I am agree, we have already tried to change it
3. There are not statistical test supporting differences between the species. Seeds coming from greenhouses, and from outcrossing could be larger than those coming from the wild. How the effect was controlled to truly represent the seed traits of the species.
   We have added statistical test supporting differences between species.

Minor comments

Number	Reviewer statement	Author(s) Answer
a.	Keyword must be different from the title	I am agree, I have change it
b.	Delete the word "Study" from the tittle	I am agree, I have deleted
c.	In the study area, add data about type of vegetation and the phenology of the species	We have added data type of vegetation and phenology of the species
d.	How do you know that the capsules were immature/mature?	The capsules were mature when they were yielding to the touch and had yellow, red or brown coloration.
e.	Drop the disinfection protocol, is irrelevant for purpose of the manuscript.	I am agree, I have drop the sentences the disinfection ….

Reviewer 2

Comment for the author 2

1. Is suggest that the results section and discussion section should be separated. A better Discussion is missing for some morphological characters.
   I am agree … We have separated results section and discussion section.
2. On page 6: All the seeds of the orchids are small and have a seed air space. It is difficult to corroborate whether this air space contributes to a lesser or mare significant way during seed dispersion. How can authors verify this?
   Fahn and Werker (1972) distinguished wind-dispersed (anomochores) seeds as flyers (meteoranomochores) and rollers (chamaechores), and orchid seeds, because of their small size and light weight, belong to the former category (flyers). The tiny nature of embryos makes them exceedingly air-filled, therefore helping them to float (fly). Across longer distances in air for wider dispersal (Arditti, 1992; Yam et al., 2002).

3. The DOI of some papers should be a double check. Some are incorrect as: [22] T.A Akin …
   Yes you are right. We were wrong, because we believe the DOI at article. From now we must be a double check. After we check there are 3 references wrong, i.e.
   [9] doi written 10.2307/23362707 correct doi 10.1600/036364412x656563
   [17] doi written 1058-5893/95/5604-0019$02.00 correct doi 10.1086/297279
   [22] Do not have doi

4. The authors should check the spelling of some sentences.
   I am agree
5. The data not support the conclusions.
   We have revised of conclusions

Page	Number	Reviewer Suggestions	The answer of Author(s)
1	2	… apical pole	micropylar pole
	3	..basal pole	chalazal pole
2	4	See Mattews and Levins 1986	I am agree, add …8 species in Portulaca (Portulacaceae)
	6	The author explain why these 10 species	These Dendrobium species are a collection off DD Orchids Nursery that are used as crosses.
	7	Sterilization or disinfection?	deleted
	8	The authority of each species should be included	
	10	*D. affine*, and *D. leporinum*	Ok
	12	, and east Java, Indonesia	OK
	14	Sunlight	Means brand name of disinfectant (deleted)
	16	Ethanol?	deleted
	18	dishes	deleted
3	1	5 ^{o}C	OK
	2	Under stereomicroscope, Light Microscope ..	OK
	4	Seed morphology and micromorphology	OK
	7	Tables	OK
	9	width	OK
	12	width	OK
	15	transparent	OK
	16	The SEM photographs are not appropriate to mention the color seed!	We agree Already we checked
	18	Figures 1A and 1B	OK
	19	In Figure 1A : translucent or transparent	transparent
	20	Oriented, while	OK
4	2	The apical pole means micropylar pole? The authors should use micropylar zone or micropylar pole	We agree, We have changed

	8	Big?, or bigger?, or biggest?	deleted
	9	With spheric epicuticular waxes?	yes
	12	I can see the substances on Figures 5D and 5E	OK
	14	Epicuticular waxes	OK
	17	I can see the substances on Figures 7C, D, and E	Ok
	20	The authors should use chalazal pole and micropylar pole on the manuscript	We agree, we have changed
	21	Cup wax??? What does it means? I can see the cup	… as cup
	25	I can see the cottony-white substances. The authors should not use the color on SEM pictures	We agree
5	1	I can see the “smooth waxes”	OK
	2	Check spelling	OK
	5	A discussion section is missing	We have separated results and discussion (as suggested reviewer)
	8	width	ok
	9	width	ok
	11	Check spelling	ok
	13	The properly figures should be use in each species	OK, We have changed
	17	The properly figures should be use in each species	OK, We have changed
	19	endosperm	dosperms …. endosperm
6	1	Observed, nine species	9 to nine
	3	2A, 3A, 4A ….10A should be mentioned in each species	OK, We have changed
	5	A better discussion should be mentioned	I am agree
11	1	The letter E (embryo) is on the testa	We have already correction
17	2	The results are not enough for the authors to mention this	

Study on Seed Morphometry of Native Indonesian Orchids in the Genus *Dendrobium* Sw

Sucipto Hariyanto, Intan Ayu Pratiwi, Edy Setiti Wida Utami

Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia

Corresponding Author: Sucipto Hariyanto, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Mulyorejo (Kampus C Unair). Surabaya, Post Code 60115, Indonesia. Tel: +6282139070704; E-mail: sucipto-h@fst.unair.ac.id

ABSRACT- In this study, seeds of 10 species of epiphytic orchids were examined using light and scanning electron microscope. Quantitative and qualitative characters were analyzed. All the presently investigated seeds showed are transparent with visible embryo and remarkable embryo color variations (such as pale yellow, light yellow, shiny yellow to yellow, orange and white). The species showed two group in seed shape (fusiform and filiform), prolate and ovaled embryo shape, positioned at the center of the long axis and near apical pole. Prolate embryo shaped and near apical pole position was only in *D. antennatum*. Based on our investigation, there are variations in seed and embryo volume as well as percentage air space in different taxa of orchids. The highest air space percentages were found in *D. leporinum*. According ornamentation testa cells, 3 types of seeds were discovered in this genus. Additionally, the clear variation in the testa ornamentation pattern include the species of *D. leporinum*, where the testa cells were in the medial regular rectangles, but in the apical and basal pole is irregular poygonal; testa cells of *D. antennatum* is poygonal and irregular oriented; and longitudinally oriented with regular rectangles in *D. purpureum*.

Key words: Indonesia, Scanning Electron Microscope, orchids, seed, morphometry.

1. Introduction

Indonesia has more than 5000 species of orchids spread in Islands of Sumatra, Kalimantan, Jawa, Sulawesi, Maluku, and Papua. Basically, *Dendrobium* is among the largest genera in orchid family, with 1509 currently described species [1], which mostly grow as epiphytes in tropical and subtropical Asia and Eastern Australia [2-4]. Seed morphology has long been perceived as an important aspect for taxonomic objectives and reflects the evolutionary history of plants [5]. The characteristic used to show morphological diversity in seed included size, shape and testa surface [6, 7]. These characteristic provide vital information at different taxonomic levels [5, 6]. While some studies support this hypothesis Gamarra et al. [8] and Celep et al. [9]; other investigations show the systematic and taxonomic value of seed micromorphology is limited [10-13]. Besides, seed morphology may affect important biological and ecological aspect such as seed dispersion mechanisms [14].

Variations in seeds morphology is an important source of systematic characters for establishing relationships between species within a genus [15-17]. These differences have served as taxonomic and/or phylogenetic markers on seeds of native California orchids and related species [18-21]. Several studies on the morphology of orchid seeds have been carried out including 19 orchids from Turkey [22], genus *Vanilla* [14], *Paphiopedilum* and *Cypripedium* [23], 13 species in tribe Chloraceeae [24], 95 species of 34 genera from the Gulf of Guinea [25] and ten *Dendrobium* species using 13 quantitative trait descriptors [26]. However, studies on native Indonesian orchid seeds covering morphometry and morphology, especially *Dendrobium*, have not been found. In this study therefore, a total 10 species from Indonesia were studied based on their seed morphology and morphometry. The purpose of the study furthermore was to investigate the range of variability regarding seed characteristics in native orchid species to establish their usefulness for future taxonomic works.

2. Material and Methods

2.1. Sterilization of mature capsules and seed collection. The seeds used were 10 species of the *Dendobium* genus including *D. antennatum*, *D. lineale*, *D. tonson*, *D. orodatum*, *D. discolor*, *D. mirbelianum*, *D. purpurium*, *D. nindii*, *D. affine*, *D. leporinum* collected from ripe capsules during 2015-2018 from DD Orchids Nursery, Batu, East Java, Indonesia. The capsules was washed using 10% sunlight detergent solution for 3 minutes to remove dust particles, and rinsed 3 times with sterile distilled water. Its surface was sprayed with 70% alcohol and passed over the Bunsen fire and repeated 3 times. The capsules was placed on sterile Petri dish in

laminar flow and cut transversally and longitudinally using a sterile scalpel into four parts. Afterward, the seeds were released from the capsule and collected using a sterile spatula. The fresh seeds were dried for at least 2 weeks and stored in tubes at 5 ^{0}C in dry conditions.

2.2. *Observation of seed morphology and micromorphology*. Seed samples were observed and photograph under stereomicroscope, Light Microscope (LM) and Scanning Electron Microscope (SEM). The morphological parameters included seed shape (SS), seed color (SC), seed length (SL), seed width (SW), seed length/ seed width (SL/SW) and seed volume (SV). On the basis of embryo, the parameters included embryo shape (ES), embryo color (EC), embryo length (EL), embryo width (EW), embryo length/ embryo width (EL/EW), embryo volume (EV), seed volume/embryo volume (SV/EV) and air space (AS) (Tables 1 and 2). Characteristics such as SS, ES, SC and EC were observed under Tension stereomicroscope, Nikon SMZ-1, Japan. The SC and EC was described in subjetive terms while SL, SW, EL and EW (at the longest and widest axis) using a light microscope (Olympus CH 20, Olympus Japan) and standardized ocular meter. The seed volume (mm^3 x 10^{-3}) was calculated using the formula 2 [(^{L}/2) (^{W}/2)^2 ($^{\pi}$/3)], where L = length, W = width, $\pi = {}^{22}/7$ and embryo volume (mm^3 x 10^{-3}) with the formula 4/3 π (L/2) (W/2)^2, where L = length, W = width, adapted from Arditti et al. [20]. The values for SL, SW, EL and EW were recorded from approximately 30 seeds per species. In calculating air space (%) the formula (seed volume-embryo volume)/seed volume) x 100%, adapted from Arditti and Ghani [27]. To analysis statistically each species, we used the mean for each quantitative character.

2.3. SEM study. For SEM preparations, the samples were mounted on SEM stubs, sputter-coated with paladium/gold (SEM coating system SC 7620 mini sputter Coater). Detailed seed coat (testa cells) surface were examined with Phenom Prox SEM generation five, using a filament voltage of 15 kV. The considered parameters were seed coat sculpturing and thickenings.

3. Results and Discussion

3.1. Seed shape and testa cells. According Vij et al. [28], Dressler [29], Molvray and Kores [30] the shape of orchid seeds vary and could be ellipsoid, oblongoid, ovoid, globose, fillamentous, spindle, irregular, fusiform or filiform. In the 10 species observed, the seeds were fusiform and filiform in shaped, and the majority central embryo in position. Verma et al. [31] also found filiform seed shaped in *Goodyera biflora* orchids. In the present work, seed of *D. antennatum* are transperent, fusiform shaped with small embryo located in the near apical pole and white colour in testa (Figures 1(A) and 1(B)). The testa cells are polygonal and irregular oriented while the surface is blunt. Testa cells walls were covered with smooth waxes (Figures

1(C), 1(D) and 1(E)). The seeds of *D. lineale* are transparent, fusiform-shaped with a big embryo at the center (Figures 2(A) and 2(B)). Testa cells are longitudinally oriented with regular rectangles (Figure 2(D)). The testa cells walls were covered with cottony-white substances, in any region of cells walls were covered with thickening white substances (Figures 2(C), 2(D) and 2(E) arrow). The seeds of *D. tonson* are transparent, fusiform-shaped with a visible embryo at the center and occupies a major part of the seed space (Figures 3(A) and 3(B)). Testa cells are longitudinally oriented with regular rectangles (Figures 3(C), 3(D) and 3(E)). The testa cells walls were covered with bead cottony substances which were more thick at the apical pole (Figure 3(E) arrow). In *D. odoratum*, the seeds are small and fusiform shaped (Figures 4(A) and 4(B)). Testa cells are longitudinally oriented with regular rectangles, but in the region near the apical pole is twisted (Figure 4(B) arrow). Besides, the testa cells walls were with small sphere waxes (Figures 4(C), 4(D) and 4(E)). In *D. discolor*, the seeds are big and fusiform shaped. The embryo distinct and were present in the center oriented long axis (Figure 5(A)). Testa cells are longitudinally oriented with regular rectangles and straight on all the seeds (Figure 5(B)). The testa cells walls were covered with cottony-white substances which were more visible at the center (Figures 5(C), 5(D) and 5(E)). In the case of *D. mirbelianum*, the seeds were fusiform shaped, embryo distinct and were present at the center (Figure 6(A)). Testa cells are longitudinally oriented with regular rectangles and straight (Figure 6(B)). Additionally, the cells walls have smooth wax which was thicker at the center (Figures 6(C), 6(D) and 6(E) arrow). In *D. purpureum*, the seeds are fusiform shaped, with big and distinct embryos, present in the center and occupies a major part of the seed space (Figure 7(A)). Testa cells are spiral oriented with regular rectangles, with twisted rope appearance and more twisted at the apical pole (Figures 7(B) and 7(E) arrow). Moreover, the testa cells walls were covered with smooth cotony-white substances (Figures 7(C), 7(D) and 7(E)). The seeds of *D. nindii* are transparent, fusiform shaped with the visible embryo, centrally located and occupies a major part of the seed space (Figure 8(A)). Testa cells are longitudinally oriented with regular rectangles (Figure 8(B)). Besides, the cells walls were covered with an adequate thickness of cottony white substances or cup wax (Figure 8(C) arrow), and thick wax in both poles (Figures 8(C) and 8(E)). The seeds of *D. affine* are transparent, fusiform shaped with the visible embryo, located in the center and occupies a major part of the seed space (Figure 9(A). Testa cells are longitudinally oriented with regular rectangles (Figure 9(B)), while the cells walls are covered with dispersed cottony-white substances (Figures 9(C), 9(D) and 9(E)). In case of *D. leporinum*, the seeds are transparent, filiform shaped, with the small embryo, distinct, present in the center (Figures 10(A) and 10(B)). Testa cells in the medial part are longitudinally oriented with regular

rectangles (Figure 10D), having anticlinal walls with a remakable deep thickening at the vertex. The testa cells were basal and the apical pole irregular polygonal (Figures 10(C) and 10(E)), and was covered with smooth waxes.

3.2. Seed size, seed volume, and ratio SL/SW. The table 1 illustrates the size of seeds from 10 species of the genus *Dendrobium*. Even though the seeds are microscopic, the result of the investigation shows high diversity in their size. The seeds range between 0.353±0.0019 mm in *D. purpureum* and 1.868±0.0128 mm in *D. leporinum* in length; 0.067±0.0018 mm in *D. purpureum* and 0.181±0.0078 mm in *D. leporinum* in width. Orchids seeds show an impressive variation in their length (0.351-1.881 mm) as well as width (0.065 – 0.19 mm). Such variations may be significant importance even within a genus or a species [27, 32].

In the present work, seed volume ranges from 0.001±0.000 mm^3x10^{-3} to 7.286±0.1569 mm^3x10^{-3} (Table 1). These differences are of taxonomic importance at the genus and/or species level [27]. The higher seed volume is noticed in *D. antennatum* (7.286±0.1569 mm^3x10^{-3}), followed by *D. tonson* (1.074±0.0816 mm^3x10^{-3}). Other species (*D. lineale, D. odoratum, D. discolor, D. mirbelianum, D. purpureum, D. nindii, D. affine and D. leporinum*) had seeds of lower volume (< 1.0) (Table 1). According to Arditti et al. [19] and Vij et al. [28] the relative degree of truncation of the orchid seeds is directly correlated with an increase in their length rather than their width, and is a good taxonomic character. In the present work, species with elongated seeds (SL/SW >6) was observed in *D. tonson, D. odoratum, D. nindii, D. affine and D. leporinum*. Other species (*D. antennatum, D. lineale, D. discolor, D. mirbelianum* and *D. purpureum*) produced truncated seeds (SL/SW <6) (Table 1). The maximum SL/SW ratio was noticed in *D. leporinum* (10.315±0.4152 mm) while the minimum was in *D. antennatum* (5.201±0.0740 mm).

3.3. Seed to embryo volume and free air space. Embryo color in the observed species varied, i.e shiny yellow, orange, yellow, pale yellow, light yellow, and white. Shiny yellow embryos characterized in *D. antennatum* (Figure 1(A)), orange embryos are recorded in *D. lineale, D. tonson* and *D. affine* (Figures 2(A), 3(A) and 9(A)). Yellow embryos are common in *D. odoratum* (Figure 4(A)). Pale yellow embryos show in *D. discolor* and *D. nindii* (Figures 5(A) and 8(A)). White embryos found in *D. purpureum* and *D. leporinum* (Figures 7(A) and 10(A)). Light yellow embryos characterized in *D. mirbelianum* (Figure 6(A)). According Arditti and Ghani [27] and Arditti and Ernst [33], orchid embryos are very small, simple, only composed of several cells, and most do not have endosperms, generally oval or spherical. In the majority

of investigated species, embryos were generally ovaled, noticed in *D. tonson, D. odoratum, D. nindii, D. affine, D. lineale, D. discolor, D. mirbelianum, D. purpureum* and *D. leporinum*, however embryos *D. antennatum* prolate. Similarly, the results of investigations on the position of the embryo, of the 10 species observed 9 species had a central embryo position, noticed in *D. tonson, D. odoratum, D. nindii, D. affine, D. lineale, D. discolor, D. mirbelianum, D. purpureum* and *D. leporinum* (Figures 2(A)-10(A)), but in *D. antennatum* the position of the embryo was near the apical pole (Figure 1(A)).

Variation has also been observed with reference to EL, EW, EL/EW, EV ratio, SV/EV ratio, and AS. EL/EW ratio was observed highest in *D. odoratum* (4.208±0.114 mm^3x10^{-3}). On the other hand, the EL/EW ratio was observed in its lowest in *D. antennatum* (1.299±0.024 mm^3x10^{-3}) (Table 2). According Healey et al. [21] the size of orchid embryos in the same genus tends to be uniform, but in this study the size of the embryo varies, smallest (0.335±0.017 mm^3x10^{-3}) in *D. purpureum* and biggest (0.927±0.036 mm^3x10^{-3}) found in *D. antennatum* and (0.922±0.125 mm^3x10^{-3}) in *D. leporinum*.

According Arditti et al. [20], Augustine et al. [34], and Swamy et al. [35] emphasizes the importance of observing the air space in orchid seeds. The existence of air space in orchid seeds is a reflection of the condition the seeds are shows the main mode of seed dispersal. In the present investigation, higher percentage of air space of *D. leporinum*, *D. antennatum*, and *D. odoratum* shown more than 50% i.e. (94.245±0.874%); (87.273±0.621%) and (55.269±3.688%). The ratio of SV/EV shown more than two also noticed in *D. leporinum* (17.758±2.630 mm^3x10^{-3}), *D. antennatum* (7.875±0.374 mm^3x10^{-3}), and *D. odoratum* (2.250±0.187 mm^3x10^{-3}). In fact, seeds with a greater percentage of air space as in *D. leporinum, D. antennatum*, and *D. odoratum* indicate which make the seed light, float, so that it is easily carried away by the wind, so may get dispersed over wide geographical areas. The other seven species namely *D. lineale*, *D. tonson*, *D. discolor*, *D. mirbelianum*, *D. purpureum*, *D. nindii* and *D. affine* have seeds with air spaces below 40% (Table 2). With the result that the seven species may get convined to a few narrow distributed in nature, which will potentially be endemic. If these species become endangered, conservational measures are more difficult.

TABLE 1. Shape and measurement data of seeds (Genus *Dendrobium* Sw).

No	Species	Shape	SL (mm)	SW (mm)	SL/SW (mm)	Volume (mm^3x10^{-3})
1	*D. antennatum*	Fusiform	0,910 ± 0,0071	0,175 ± 0,0019	5.201 ± 0.0740	7.286±0.1569
2	*D. lineale*	Fusiform	0.480 ± 0.0061	0.081 ± 0.0003	5.894 ± 0.0782	0.001 ± 0.0000
3	*D. tonson*	Fusiform	0.564 ± 0.0034	0.085 ± 0.0032	6.624 ± 0.2465	1.074 ± 0.0816
4	*D. orodatum*	Fusiform	0.551 ± 0.0037	0.073 ± 0.0028	7.513 ± 0.2696	0.779 ± 0.0614
5	*D. discolor*	Fusiform	0.405 ± 0.0044	0.078 ± 0.0030	5.220 ± 0.1965	0.642 ± 0.0509
6	*D. mirbelianum*	Fusiform	0.439 ± 0.0086	0.082 ± 0.0045	5.367 ± 0.2971	0.774 ± 0.0864
7	*D. purpurium*	Fusiform	0.353 ± 0.0019	0.067 ± 0.0018	5.307 ± 0.1573	0.410 ± 0.0222
8	*D. nindii*	Fusiform	0.486 ± 0.0019	0.075 ± 0.0015	6.462 ± 0.1356	0.719 ± 0.0269
9	*D. affine*	Fusiform	0.518 ± 0.0046	0.085 ± 0.0015	6.110 ± 0.0805	0.975 ± 0.0411
10	*D. leporinum*	Filiform	1.868 ± 0.0128	0.181 ± 0.0078	10.315 ± 0.4152	0.016 ± 0.0014

± Standard deviation
SL: Seed length; SW: Seed width

TABLE 2. Shape, colour and measurement data of embryos (Genus *Dendrobium* Sw).

No	Species	Shape	Colour	EL (mm)	EW (mm)	EL/EW (mm)	Volume (mm^3x10^{-3})	SV/EV (mm^3x10^{-3})	AS (%)
1	*D. antennatum*	Prolate	Shiny yellow	0.144±0.002	0.111±0.002	1.299±0.024	0.927±0.036	7.875±0.374	87.273±0.621
2	*D. lineale*	Ovaled	Orange	0.215±0.003	0.069±0.001	3.104±0.070	0.542±0.023	1.541±0.065	34.978±2.868
3	*D. tonson*	Ovaled	Orange	0.226±0.006	0.076±0.002	2.970±0.107	0.685±0.049	1.572±0.132	35.962±5.297
4	*D. orodatum*	Ovaled	Yellow	0.227±0.003	0.054±0.001	4.208±0.114	0.347±0.013	2.250±0.187	55.269±3.668
5	*D. discolor*	Ovaled	Pale yellow	0.165±0.011	0.077±0.003	2.130±0.159	0.524±0.057	1.233±0.117	18.414±5.493
6	*D. mirbelianum*	Ovaled	Light yellow	0.185±0.004	0.082±0.004	2.264±0.138	0.652±0.070	1.187±0.036	15.679±2.571
7	*D. purpurium*	Ovaled	White	0.145±0.003	0.067±0.002	2.173±0.088	0.335±0.017	1.223±0.028	18.161±1.800
8	*D. nindii*	Ovaled	Pale yellow	0.186±0.001	0.070±0.003	2.674±0.121	0.473±0.043	1.534±0.147	34.237±6.206
9	*D. affine*	Ovaled	Orange	0.084±0.002	0.034±0.002	2.496±0.133	0.750±0.038	1.304±0.091	22.979±5.292
10	*D. leporinum*	Ovaled	White	0.210±0.009	0.091±0.005	2.304±0.146	0.922±0.125	17.758±2.630	94.245±0.874

± Standard deviation; EL: Embryo length; EW: Embryo width; SV: Seed volume; EV: Embryo volume; AS: Air space; Percentage air space was calculated as [(seed volume-embryo volume)/(seed volume)] x 100. $N = 30$.

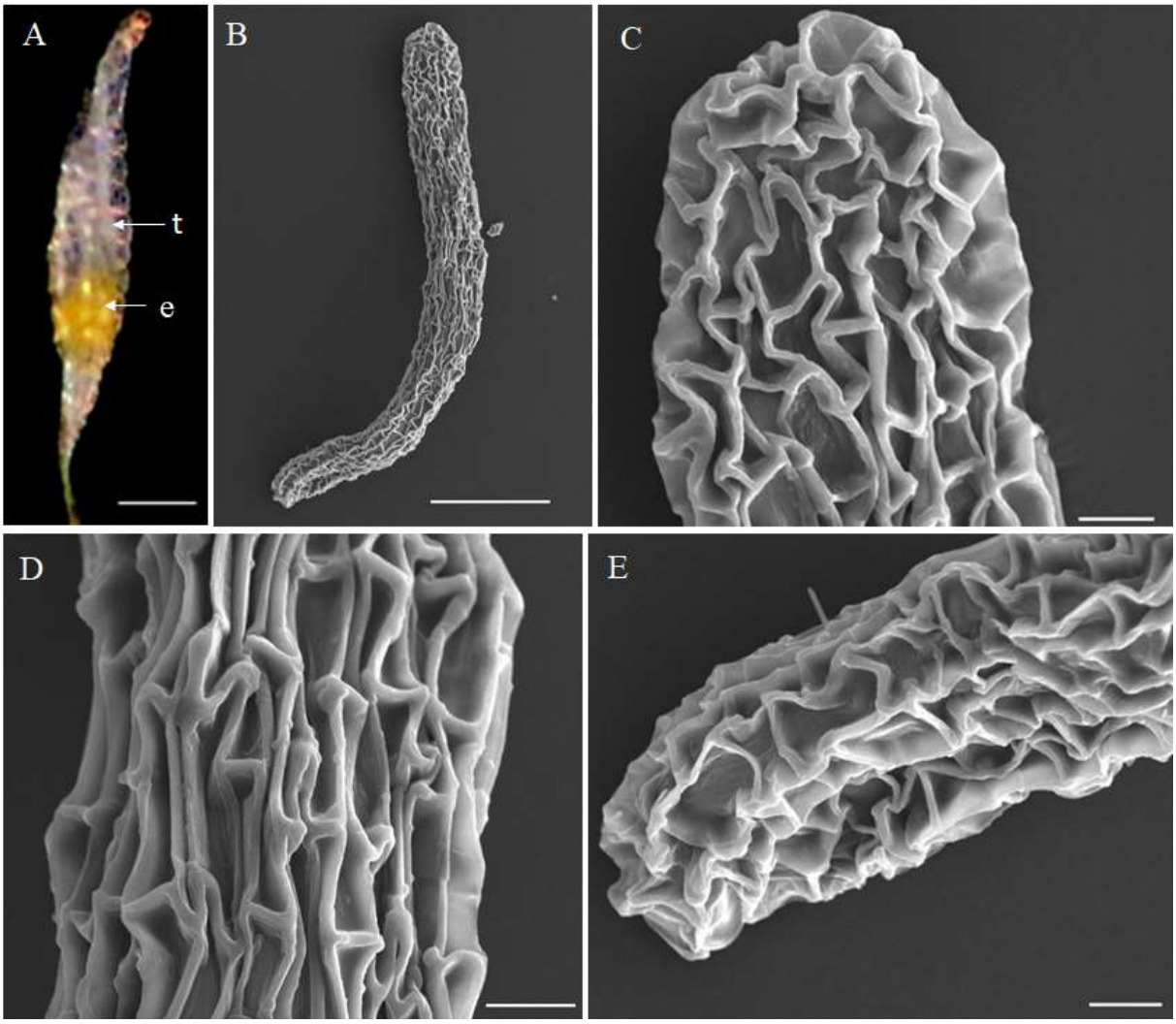

Figure 1. LM and SEM photographs of *D. antennantum.* A. A seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e=embryo, t=testa. Scale bars: (A) 175 µm, (B) 100 µm, (C-E) 10 µm.

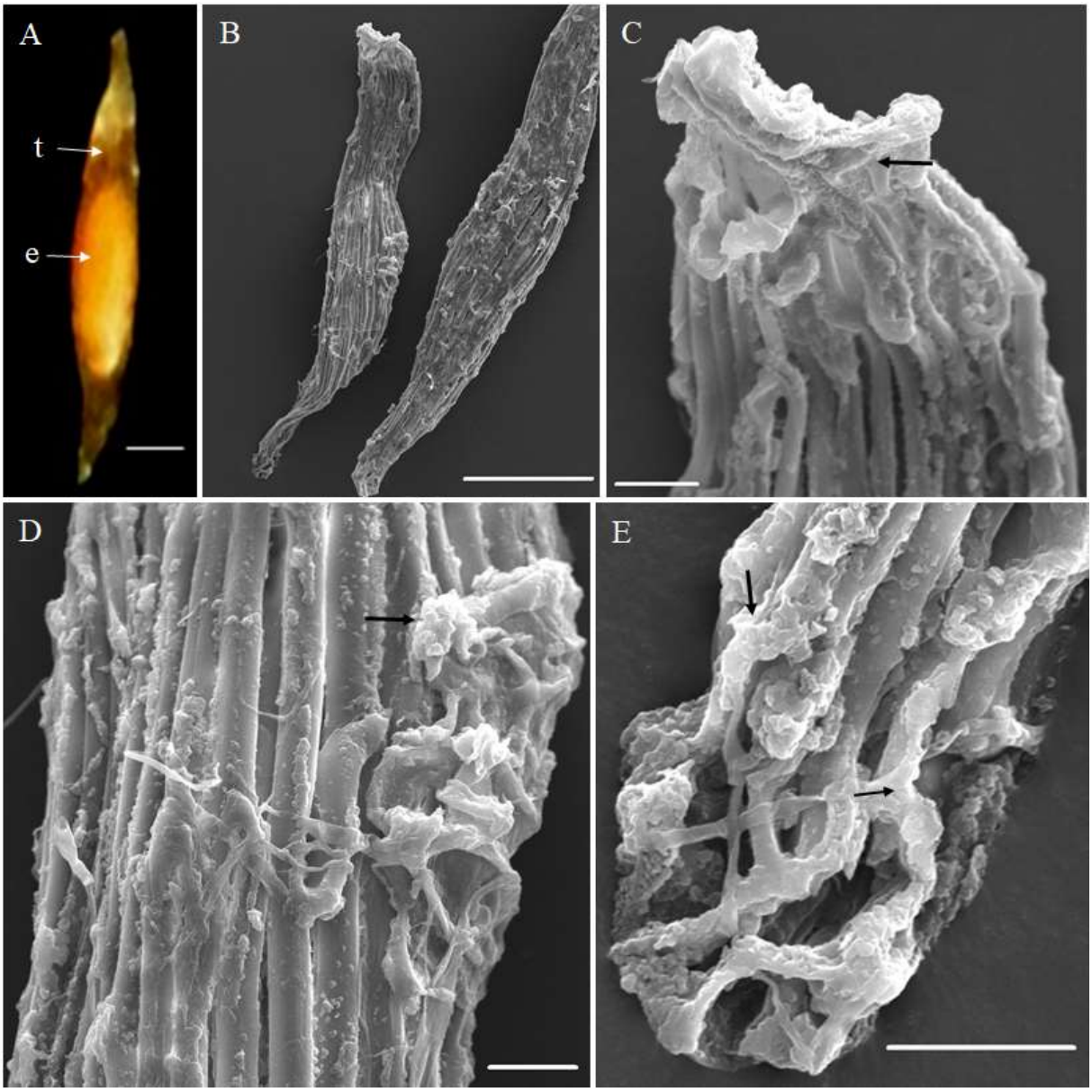

Figure 2. LM and SEM photographs of *D. lineale*. A. A seed under LM; B. Seeds under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e=embryo, t=testa. Scale bars: (A) 81µm, (B) 100 µm, (C-E) 10 µm.

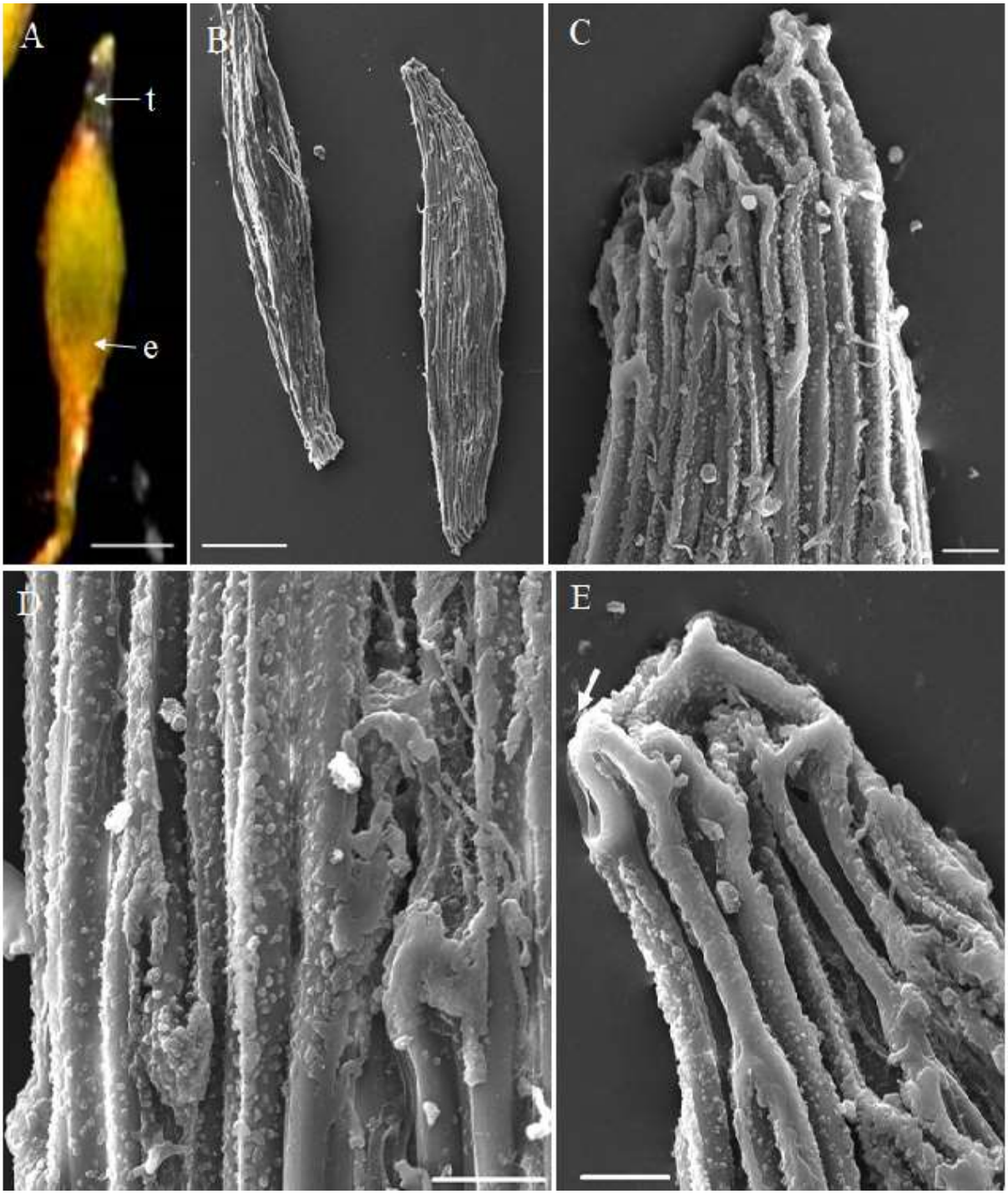

Figure 3. LM and SEM photographs of *D. tonson.* A. A seed under LM; B. Seeds under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 85 µm, (B) 100 µm, (C-E) 10 µm.

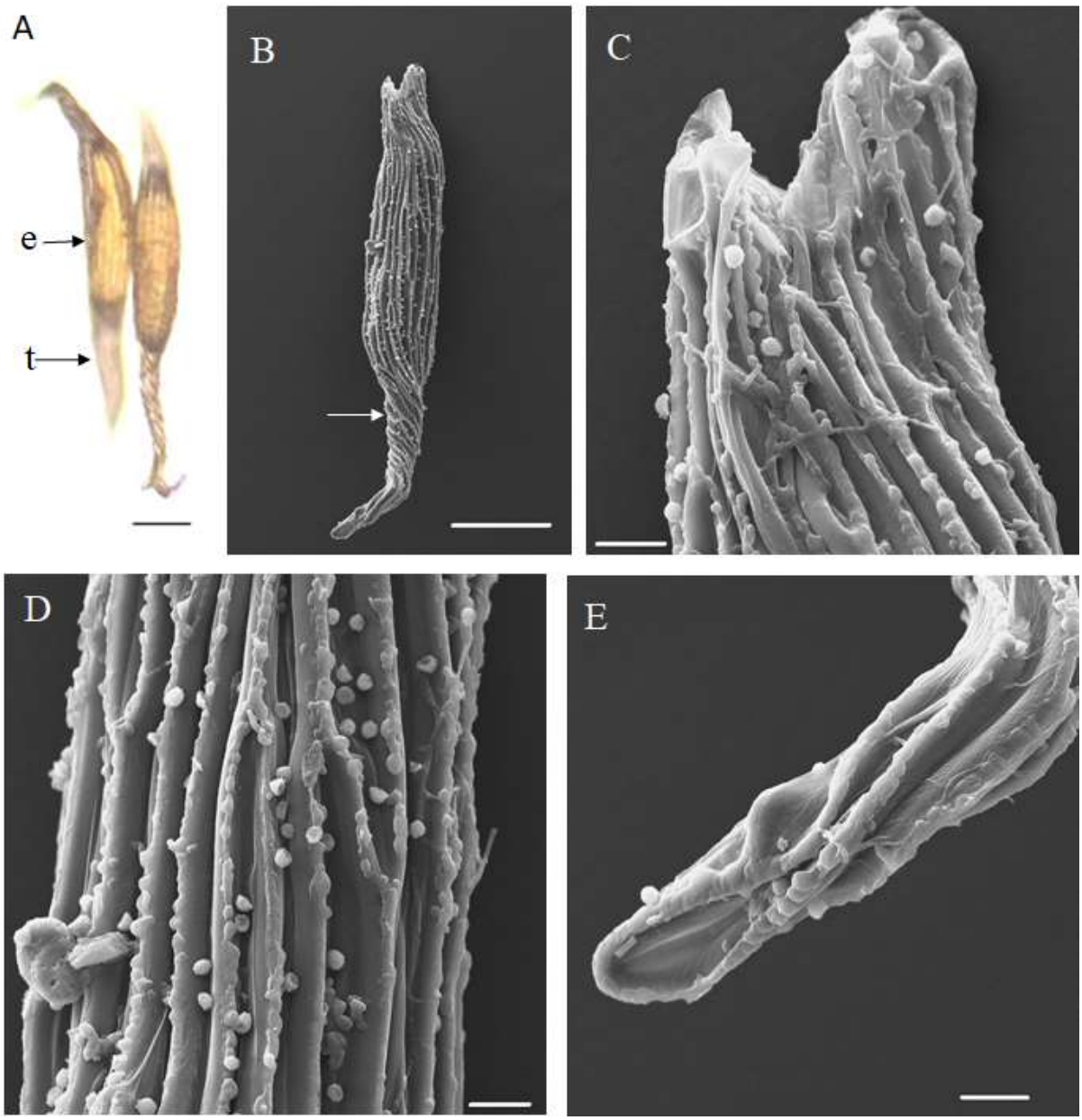

Figure 4. LM and SEM photographs of *Dendrobium odoratum.* A. A seed under LM; B. A seed shape under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 73 µm, (B) 100 µm, (C-E) 10 µm.

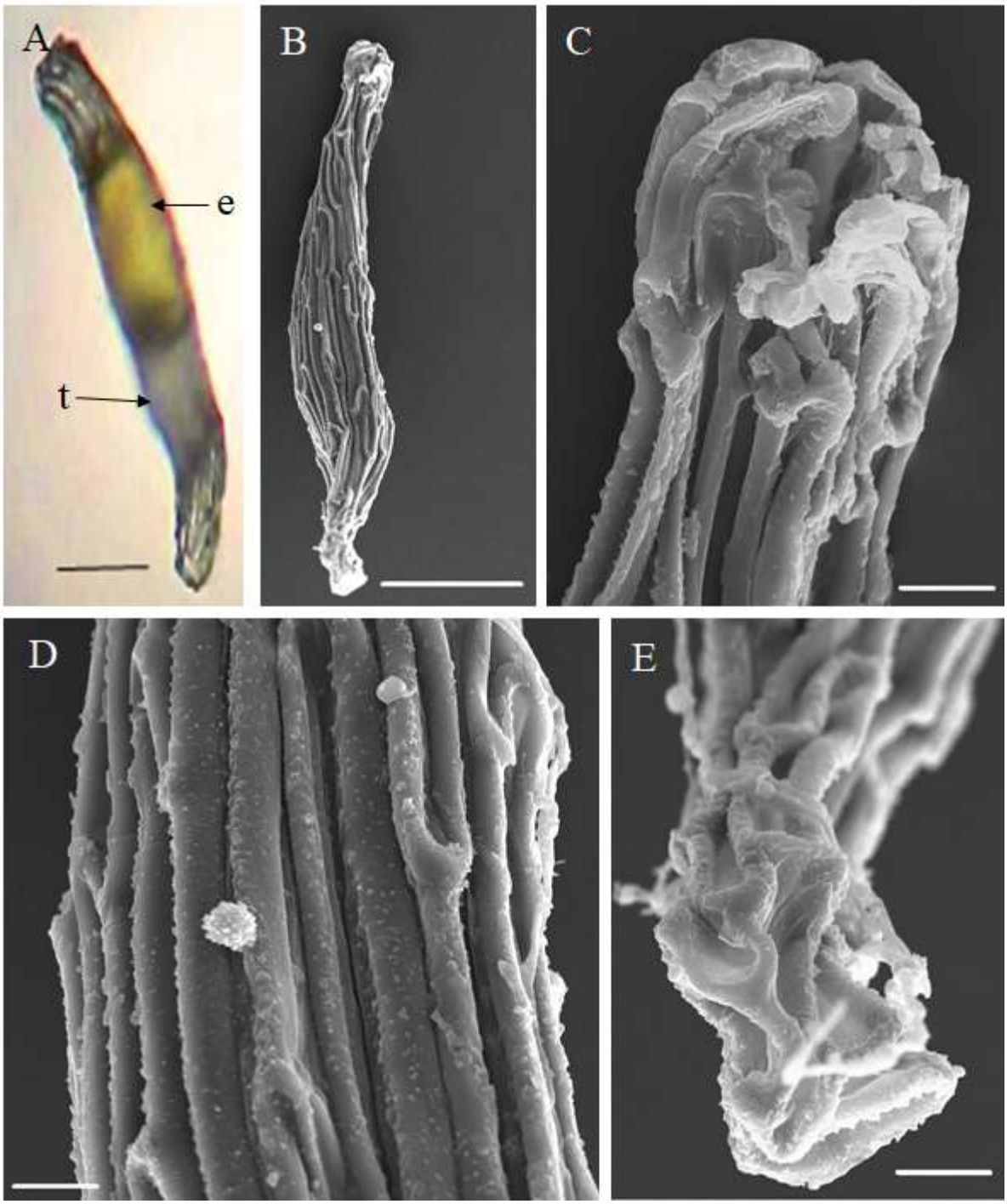

Figure 5. LM and SEM photographs of *D. discolor*. A. A seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 78 µm, (B)100 µm, (C-E)10 µm.

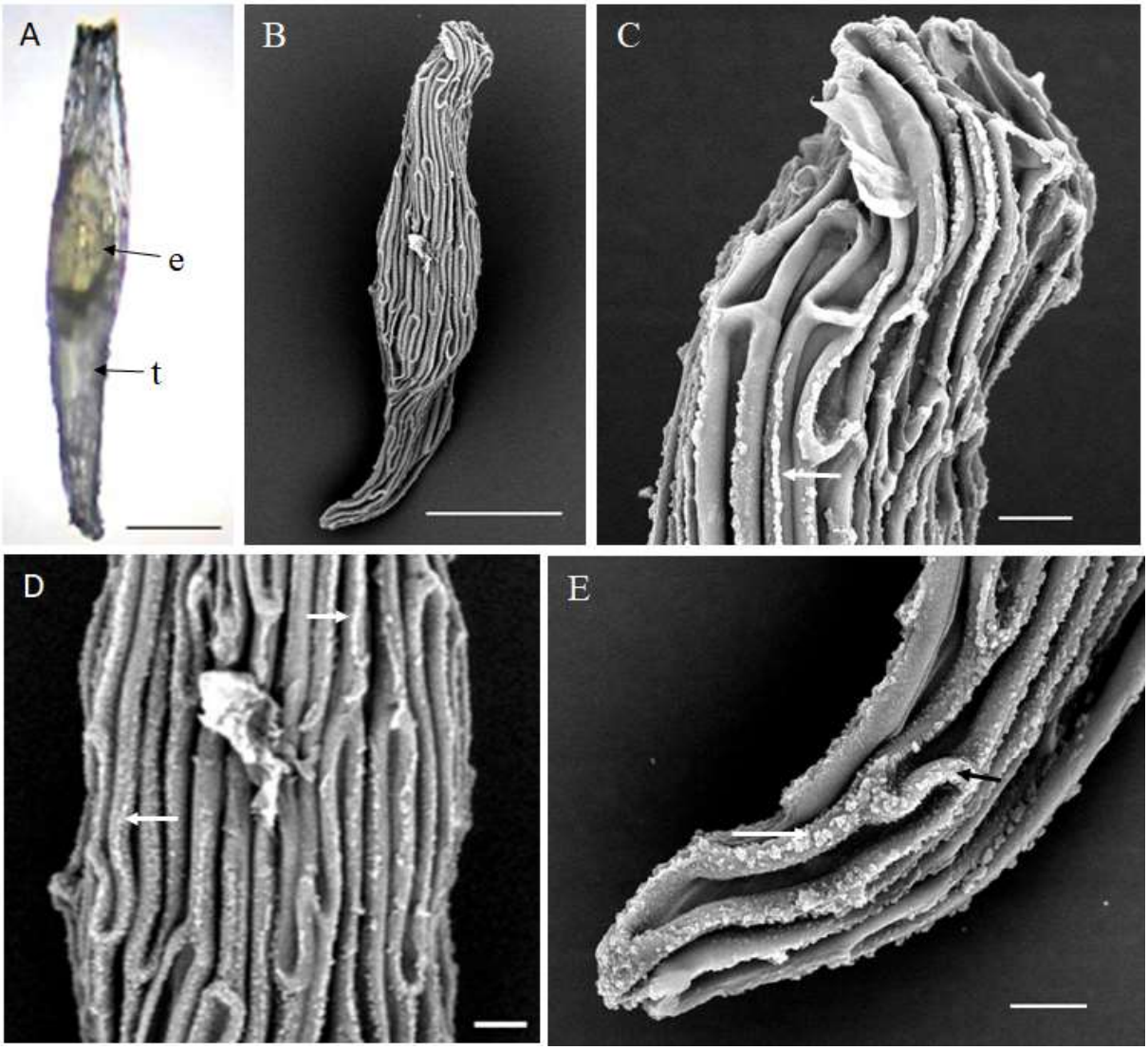

Figure 6. LM and SEM photographs of *D. mirbelianum.* A. seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 82µm, (B) 100µm, (C-E) 10µm.

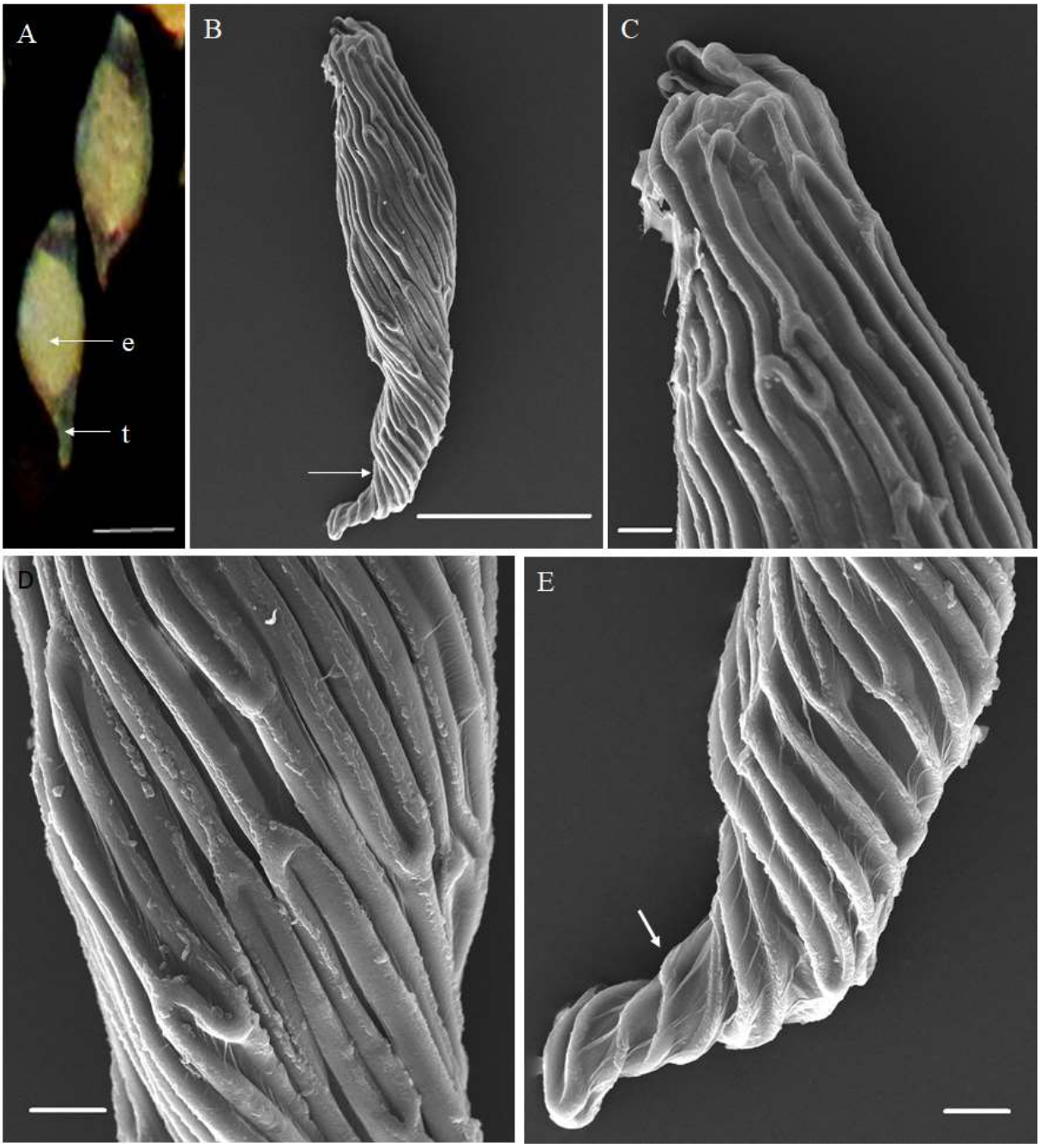

Figure 7. LM and SEM photographs of *D. purpureum.* A. seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 67 µm, (B) 100 µm, (C-E) 10 µm.

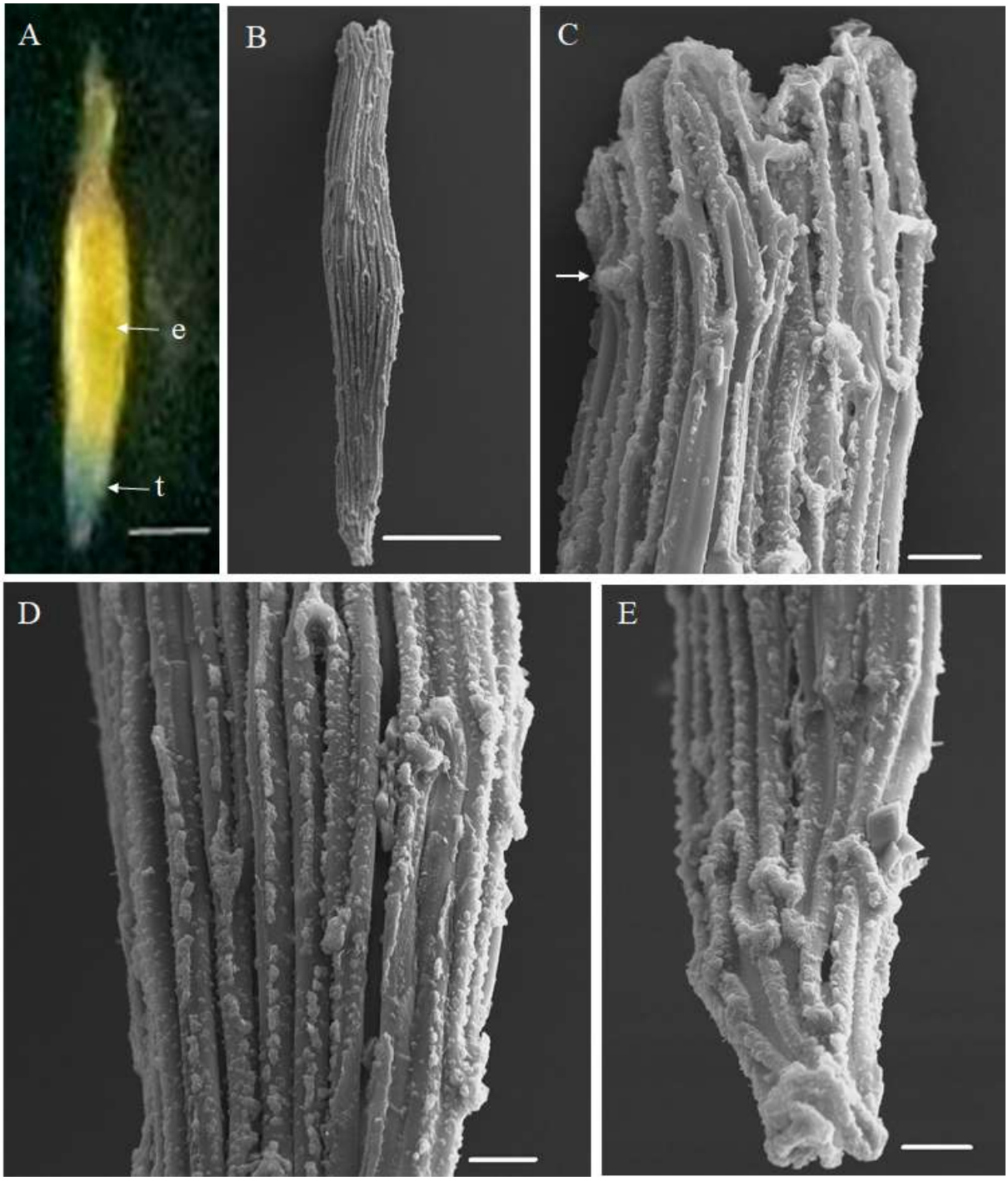

Figure 8. LM and SEM photographs of *D. nindii*. A. Seeds under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A)75 µm, (B) 100 µm, (C-E) 10 µm.

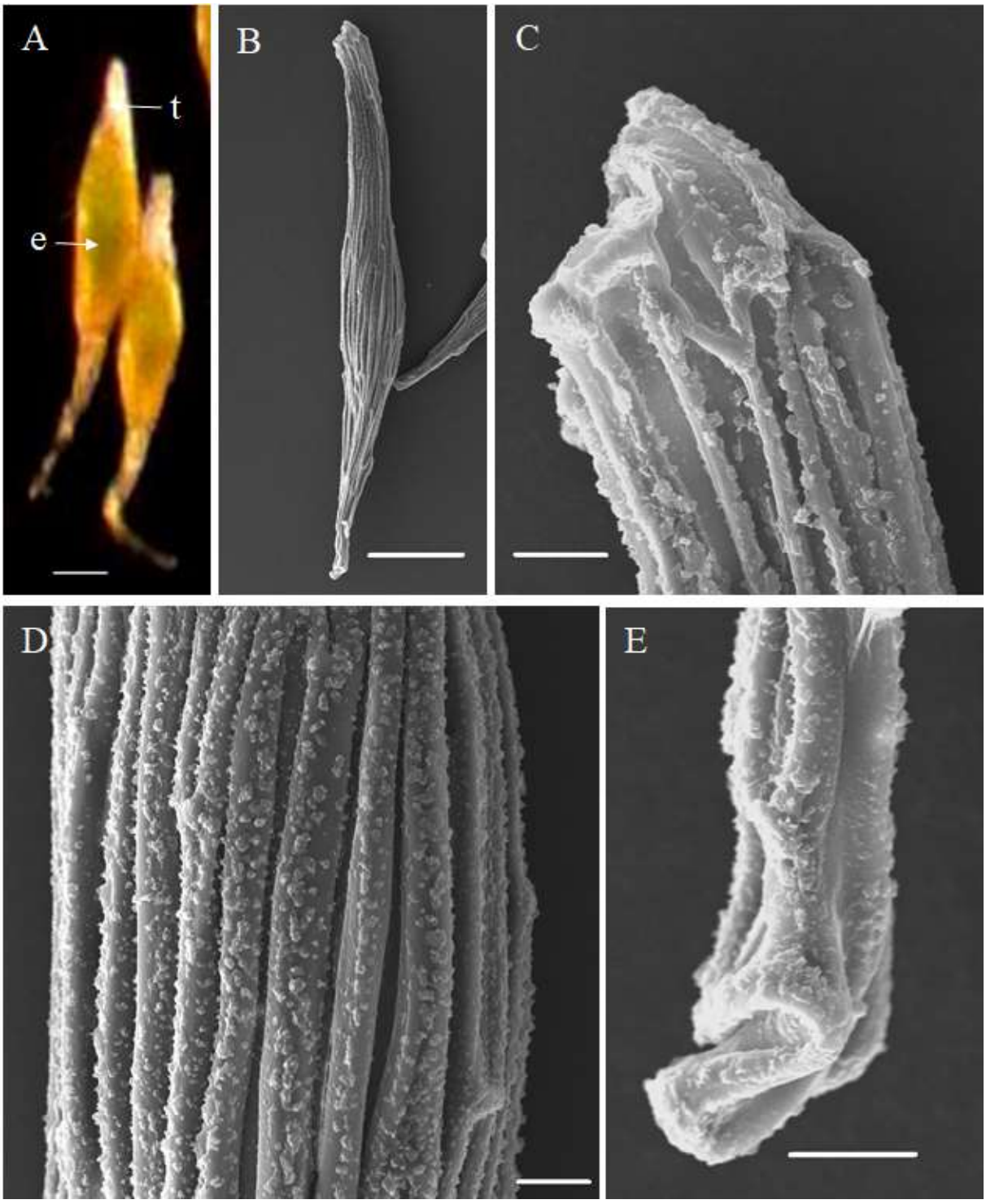

Figure 9. LM and SEM photographs of *D. affine*. A. Seeds under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 85 µm, (B) 100 µm, (C-E) 10 µm.

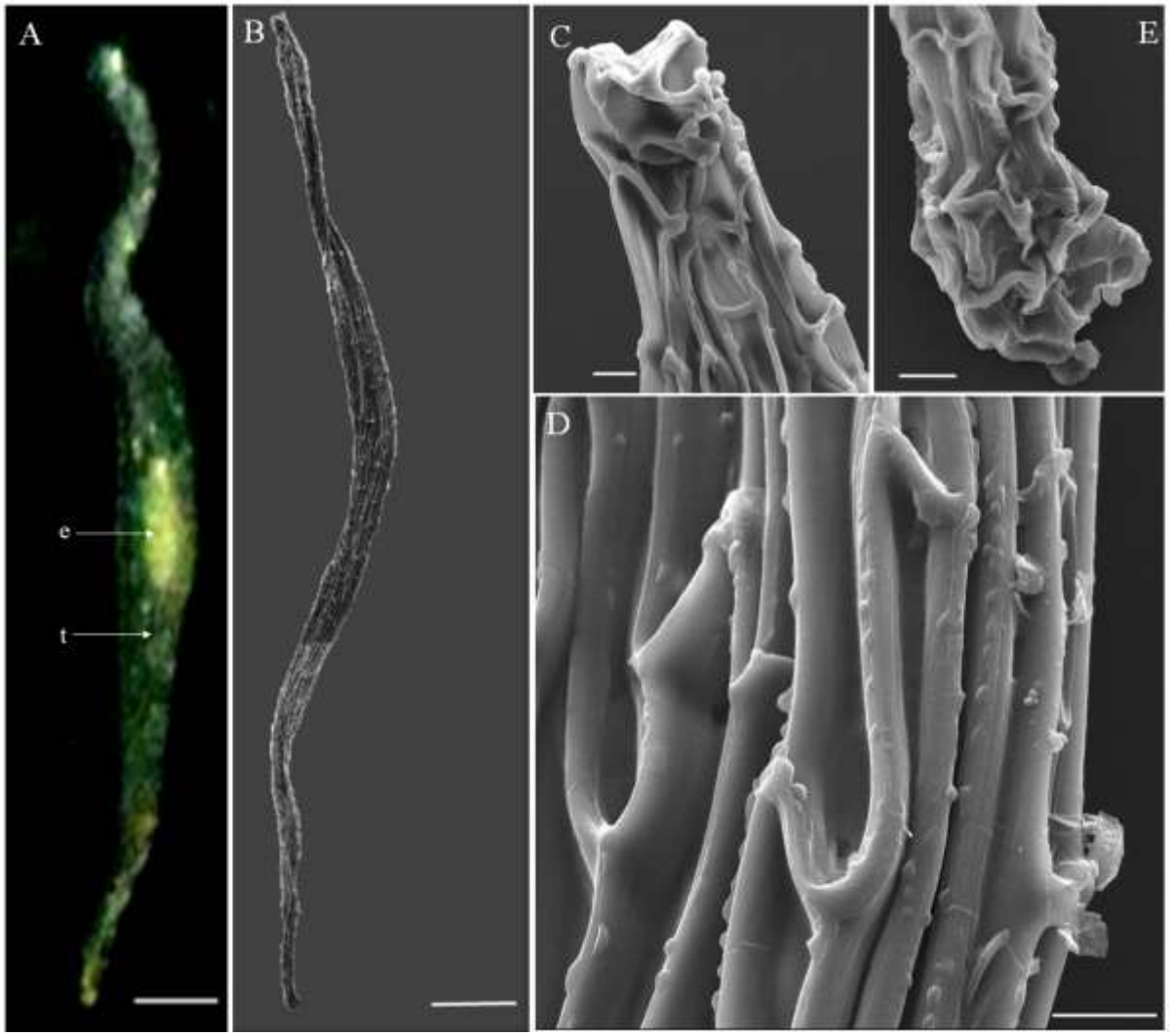

Figure 10. LM and SEM photographs of *D. leporinum.* A. A seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A)181 µm, (B)100 µm, (C-E) 10 µm.

4. Conclusion

The results of this study show orchid seeds vary in micromorphology, size, ultrastructure features and finer detail. Importanly, the characteristics of seeds are used in explaining taxonomic, phylogenetic, and phytogeographic relationships between different orchid taxa. The seeds with percentage of air space below 49% were found in *D. lineale*, *D. tonson*, *D. discolor*, *D. mirbelianum*, *D. purpureum*, *D. nindii* and *D. affine*. This imlpies the seeds are limited in distribution, hence potentially endemic species. In case the habitat of these 7 species are not properly maintained, their existence is threatened.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Number	line	Reviewer Suggestions	The answer of Author(s)
2	2	The authority of the genus should be removed from the title and move to the first time it is mentioned in the introduction section (line 2 of Introduction	Ok, I'm agree ….. I have moved
A	6	On line 6 of Materials and Methods: 5 0C	5°C
B		Remove the point (.) from all subtitles	I'm agree ….. I have removed
C		In section 3.3. Embryo size, embryo volume and free air space are missing double parenthesis () Double-check in all RESULTS	I'm agree ….. I've added parentheses (red)…. Line 2 page 6 (*D, discolour* (Figure 5 (A)), *D. mirbelianum* (Figure 6 (A)), *D. purpureum* (Figure 7 (A)), *D. nindii* (Figure 8 (A)) …. I have checked all the results

Study on Seed Morphometry of Native Indonesian Orchids in the Genus *Dendrobium* Sw

Sucipto Hariyanto, Intan Ayu Pratiwi, Edy Setiti Wida Utami

Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia

Corresponding Author: Sucipto Hariyanto, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Mulyorejo (Kampus C Unair). Surabaya, Post Code 60115, Indonesia. Tel: +6282139070704; E-mail: sucipto-h@fst.unair.ac.id

ABSRACT- In this study, seeds of 10 species of epiphytic orchids were examined using light and scanning electron microscope. Quantitative and qualitative characters were analyzed. All the presently investigated seeds showed are transparent with visible embryo and remarkable embryo color variations (such as pale yellow, light yellow, shiny yellow to yellow, orange and white). The species showed two group in seed shape (fusiform and filiform), prolate and ovaled embryo shape, positioned at the center of the long axis and near apical pole. Prolate embryo shaped and near apical pole position was only in *D. antennatum*. Based on our investigation, there are variations in seed and embryo volume as well as percentage air space in different taxa of orchids. The highest air space percentages were found in *D. leporinum*. According ornamentation testa cells, 3 types of seeds were discovered in this genus. Additionally, the clear variation in the testa ornamentation pattern include the species of *D. leporinum*, where the testa cells were in the medial regular rectangles, but in the apical and basal pole is irregular poygonal; testa cells of *D. antennatum* is poygonal and irregular oriented; and longitudinally oriented with regular rectangles in *D. purpureum*.

Key words: Indonesia, Scanning Electron Microscope, orchids, seed, morphometry.

1. Introduction

Indonesia has more than 5000 species of orchids spread in Islands of Sumatra, Kalimantan, Jawa, Sulawesi, Maluku, and Papua. Basically, *Dendrobium* is among the largest genera in orchid family, with 1509 currently described species [1], which mostly grow as epiphytes in tropical and subtropical Asia and Eastern Australia [2-4]. Seed morphology has long been perceived as an important aspect for taxonomic objectives and reflects the evolutionary history of plants [5]. The characteristic used to show morphological diversity in seed included size, shape and testa surface [6, 7]. These characteristic provide vital information at different taxonomic levels [5, 6]. While some studies support this hypothesis Gamarra et al. [8] and Celep et al. [9]; other investigations show the systematic and taxonomic value of seed micromorphology is limited [10-13]. Besides, seed morphology may affect important biological and ecological aspect such as seed dispersion mechanisms [14].

Variations in seeds morphology is an important source of systematic characters for establishing relationships between species within a genus [15-17]. These differences have served as taxonomic and/or phylogenetic markers on seeds of native California orchids and related species [18-21]. Several studies on the morphology of orchid seeds have been carried out including 19 orchids from Turkey [22], genus *Vanilla* [14], *Paphiopedilum* and *Cypripedium* [23], 13 species in tribe Chloraceeae [24], 95 species of 34 genera from the Gulf of Guinea [25] and ten *Dendrobium* species using 13 quantitative trait descriptors [26]. However, studies on native Indonesian orchid seeds covering morphometry and morphology, especially *Dendrobium*, have not been found. In this study therefore, a total 10 species from Indonesia were studied based on their seed morphology and morphometry. The purpose of the study furthermore was to investigate the range of variability regarding seed characteristics in native orchid species to establish their usefulness for future taxonomic works.

2. Material and Methods

2.1. Sterilization of mature capsules and seed collection. The seeds used were 10 species of the *Dendobium* genus including *D. antennatum*, *D. lineale*, *D. tonson*, *D. orodatum*, *D. discolor*, *D. mirbelianum*, *D. purpurium*, *D. nindii*, *D. affine*, *D. leporinum* collected from ripe capsules during 2015-2018 from DD Orchids Nursery, Batu, East Java, Indonesia. The capsules was washed using 10% sunlight detergent solution for 3 minutes to remove dust particles, and rinsed 3 times with sterile distilled water. Its surface was sprayed with 70% alcohol and passed over the Bunsen fire and repeated 3 times. The capsules was placed on sterile Petri dish in

laminar flow and cut transversally and longitudinally using a sterile scalpel into four parts. Afterward, the seeds were released from the capsule and collected using a sterile spatula. The fresh seeds were dried for at least 2 weeks and stored in tubes at 5^0C in dry conditions.

2.2. *Observation of seed morphology*. Seed samples were observed and photograph under a Light Microscope (LM) and Scanning Electron Microscope (SEM). The morphological parameters included seed shape (SS), seed color (SC), seed length (SL), seed width (SW), seed length/ seed width (SL/SW) and seed volume (SV). On the basis of embryo, the parameters included embryo shape (ES), embryo color (EC), embryo length (EL), embryo width (EW), embryo length/ embryo width (EL/EW), embryo volume (EV), seed volume/embryo volume (SV/EV) and air space (AS) (Table 1 and 2). Characteristics such as SS, ES, SC and EC were observed under Tension stereomicroscope, Nikon SMZ-1, Japan. The SC and EC was described in subjetive terms while SL, SW, EL and EW (at the longest and widest axis) using a light microscope (Olympus CH 20, Olympus Japan) and standardized ocular meter. The seed volume (mm^3 x 10^{-3}) was calculated using the formula 2 [$(^L/2)$ $(^W/2)^2$ $(^\pi/3)$], where L = length, W = widh, $\pi = {}^{22}/7$ and embryo volume (mm^3 x 10^{-3}) with the formula $4/3\,\pi$ (L/2) $(W/2)^2$, where L = length, W = widht, adapted from Arditti et al. [20]. The values for SL, SW, EL and EW were recorded from approximately 30 seeds per species. In calculating air space (%) the formula (seed volume-embryo volume)/seed volume) x 100%, adapted from Arditti and Ghani [27]. To analyze statistically each species, we used the mean for each quantitative character.

2.3. SEM study. For SEM preparations, the samples were mounted on SEM stubs, sputter-coated with paladium/gold (SEM coating system SC 7620 mini sputter Coater). Detailed seed coat (testa cells) surface were examined with Phenom Prox SEM generation five, using a filament voltage of 15 kV. The considered parameters were seed coat sculpturing and thickenings.

3. Results and Discussion

3.1. Seed shape and testa cells. According Vij et al. [28], Dressler [29], Molvray and Kores [30] the shape of orchid seeds vary and could be ellipsoid, oblongoid, ovoid, globose, fillamentous, spindle, irregular, fusiform or filiform. In the 10 species observed, the seeds were fusiform and filiform in shaped, and the majority central embryo in position. Verma et al. [31] also found filiform seed shaped *Goodyera biflora* orchids. In the present work, seed of *D. antennatum* are transperent, fusiform shaped with small embryo located in the near apical pole and white colour in testa (Figures 1(A) and 1(B)). The testa cells are polygonal and irregular oriented while the surface is blunt. Testa cells walls were covered with smooth waxes (Figures

1(C), 1(D) and 1(E)). The seeds of *D. lineale* are transparent, fusiform-shaped with a big embryo at the center (Figures 2(A) and 2(B)). Testa cells are longitudinally oriented with regular rectangles (Figure 2(D)). The testa cells walls were covered with cottony-white substances, in any region of cells walls were covered with thickening white substances (Figures 2(C), 2(D) and 2(E) arrow). The seeds of *D. tonson* are transparent, fusiform-shaped with a visible embryo at the center and occupies a major part of the seed space (Figures 3(A) and 3(B)). Testa cells are longitudinally oriented with regular rectangles (Figures 3(C), 3(D) and 3(E)). The testa cells walls were covered with bead cottony substances which were more thick at the apical pole (Figure 3(E) arrow). In *D. odoratum*, the seeds are small and fusiform shaped (Figures 4(A) and 4(B)). Testa cells are longitudinally oriented with regular rectangles, but in the region near the apical pole is twisted (Figure 4(B) arrow). Besides, the testa cells walls were with small sphere waxes (Figures 4(C), 4(D) and 4(E)). In *D. discolor*, the seeds are big and fusiform shaped. The embryo distinct and were present in the center oriented long axis (Figure 5(A)). Testa cells are longitudinally oriented with regular rectangles and straight on all the seeds (Figure 5(B)). The testa cells walls were covered with cottony-white substances which were more visible at the center (Figures 5(C), 5(D) and 5(E)). In the case of *D. mirbelianum*, the seeds were fusiform shaped, embryo distinct and were present at the center (Figure 6(A)). Testa cells are longitudinally oriented with regular rectangles and straight (Figure 6(B)). Additionally, the cells walls have smooth wax which was thicker at the center (Figures 6(C), 6(D) and 6(E) arrow). In *D. purpureum*, the seeds are fusiform shaped, with big and distinct embryos, present in the center and occupies a major part of the seed space (Figure 7(A)). Testa cells are spiral oriented with regular rectangles, with twisted rope appearance and more twisted at the apical pole (Figures 7(B) and 7(E) arrow). Moreover, the testa cells walls were covered with smooth cotony-white substances (Figures 7(C), 7(D) and 7(E)). The seeds of *D. nindii* are transparent, fusiform shaped with the visible embryo, centrally located and occupies a major part of the seed space (Figure 8(A)). Testa cells are longitudinally oriented with regular rectangles (Figure 8(B)). Besides, the cells walls were covered with an adequate thickness of cottony white substances or cup wax (Figure 8(C) arrow), and thick wax in both poles (Figures 8(C) and 8(E)). The seeds of *D. affine* are transparent, fusiform shaped with the visible embryo, located in the center and occupies a major part of the seed space (Figure 9(A). Testa cells are longitudinally oriented with regular rectangles (Figure 9(B)), while the cells walls are covered with dispersed cottony-white substances (Figures 9(C), 9(D) and 9(E)). In case of *D. leporinum*, the seeds are transparent, filiform shaped, with the small embryo, distinct, present in the center (Figures 10(A) and 10(B)). Testa cells in the medial part are longitudinally oriented with regular

rectangles (Figure 10D), having anticlinal walls with a remakable deep thickening at the vertex. The testa cells were basal and the apical pole irregular polygonal (Figures 10(C) and 10(E)), and was covered with smooth waxes.

3.2. Seed size, seed volume, and ratio SL/SW. The table 1 illustrates the size of seeds from 10 species of the genus *Dendrobium*. Even though the seeds are microscopic, the result of the investigation shows high diversity in their size. The seeds range between 0.353±0.0019 mm in *D. purpureum* and 1.868±0.0128 mm in *D. leporinum* in length; 0.067±0.0018 mm in *D. purpureum* and 0.181±0.0078 mm in *D. leporinum* in width. Orchids seeds show an impressive variation in their length (0.351-1.881 mm) as well as width (0.065 – 0.19 mm). Such variations may be significant importance even within a genus or a species [27, 32].

In the present work, seed volume ranges from 0.001±0.000 mm^3x10^{-3} to 7.286±0.1569 mm^3x10^{-3} (Table 1). These differences are of taxonomic importance at the genus and/or species level [27]. The higher seed volume is noticed in *D. antennatum* (7.286±0.1569 mm^3x10^{-3}), followed by *D. tonson* (1.074±0.0816 mm^3x10^{-3}). Other species (*D. lineale, D. odoratum, D. discolor, D. mirbelianum, D. purpureum, D. nindii, D. affine and D. leporinum*) had seeds of lower volume (< 1.0) (Table 1). According to Arditti et al. [19] and Vij et al. [28] the relative degree of truncation of the orchid seeds is directly correlated with an increase in their length rather than their width, and is a good taxonomic character. In the present work, species with elongated seeds (SL/SW >6) was observed in *D. tonson, D. odoratum, D. nindii, D. affine and D. leporinum*. Other species (*D. antennatum, D. lineale, D. discolor, D. mirbelianum* and *D. purpureum*) produced truncated seeds (SL/SW <6) (Table 1). The maximum SL/SW ratio was noticed in *D. leporinum* (10.315±0.4152 mm) while the minimum was in *D. antennatum* (5.201±0.0740 mm).

3.3. Seed to embryo volume and free air space. Embryo color in the observed species varied, i.e shiny yellow, orange, yellow, pale yellow, light yellow, and white. Shiny yellow embryos characterized in *D. antennatum* (Figure 1(A)), orange embryos are recorded in *D. lineale, D. tonson* and *D. affine* (Figures 2(A), 3(A) and 9(A)). Yellow embryos are common in *D. odoratum* (Figure 4(A)). Pale yellow embryos show in *D. discolor* and *D. nindii* (Figures 5(A) and 8(A)). White embryos found in *D. purpureum* and *D. leporinum* (Figures 7(A) and 10(A)). Light yellow embryos characterized in *D. mirbelianum* (Figure 6(A)). According Arditti and Ghani [27] and Arditti and Ernst [33], orchid embryos are very small, simple, only composed of several cells, and most do not have endosperms, generally oval or spherical. In the majority

of investigated species, embryos were generally ovaled, noticed in *D. tonson, D. odoratum, D. nindii, D. affine, D. lineale, D. discolor, D. mirbelianum, D. purpureum* and *D. leporinum*, however embryos *D. antennatum* prolate. Similarly, the results of investigations on the position of the embryo, of the 10 species observed 9 species had a central embryo position, noticed in *D. tonson, D. odoratum, D. nindii, D. affine, D. lineale, D. discolor, D. mirbelianum, D. purpureum* and *D. leporinum* (Figures 2(A)-10(A)), but in *D. antennatum* the position of the embryo was near the apical pole (Figure 1(A)).

Variation has also been observed with reference to EL, EW, EL/EW, EV ratio, SV/EV ratio, and AS. EL/EW ratio was observed highest in *D. odoratum* (4.208±0.114 mm^3x10^{-3}). On the other hand, the EL/EW ratio was observed in its lowest in *D. antennatum* (1.299±0.024 mm^3x10^{-3}) (Table 2). According Healey et al. [21] the size of orchid embryos in the same genus tends to be uniform, but in this study the size of the embryo varies, smallest (0.335±0.017 mm^3x10^{-3}) in *D. purpureum* and biggest (0.927±0.036 mm^3x10^{-3}) found in *D. antennatum* and (0.922±0.125 mm^3x10^{-3}) in *D. leporinum*.

According Arditti et al. [20], Augustine et al. [34], and Swamy et al. [35] emphasizes the importance of observing the air space in orchid seeds. The existence of air space in orchid seeds is a reflection of the condition the seeds are shows the main mode of seed dispersal. In the present investigation, higher percentage of air space of *D. leporinum*, *D. antennatum*, and *D. odoratum* shown more than 50% i.e. (94.245±0.874%); (87.273±0.621%) and (55.269±3.688%). The ratio of SV/EV shown more than two also noticed in *D. leporinum* (17.758±2.630 mm^3x10^{-3}), *D. antennatum* (7.875±0.374 mm^3x10^{-3}), and *D. odoratum* (2.250±0.187 mm^3x10^{-3}). In fact, seeds with a greater percentage of air space as in *D. leporinum, D. antennatum*, and *D. odoratum* indicate which make the seed light, float, so that it is easily carried away by the wind, so may get dispersed over wide geographical areas. The other seven species namely *D. lineale*, *D. tonson*, *D. discolor*, *D. mirbelianum*, *D. purpureum*, *D. nindii* and *D. affine* have seeds with air spaces below 40% (Table 2). With the result that the seven species may get convined to a few narrow distributed in nature, which will potentially be endemic. If these species become endangered, conservational measures are more difficult.

TABLE 1. Shape and measurement data of seeds (Genus *Dendrobium* Sw).

No	Species	Shape	SL (mm)	SW (mm)	SL/SW (mm)	Volume (mm^3x10^{-3})
1	*D. antennatum*	Fusiform	0,910 ± 0,0071	0,175 ± 0,0019	5.201 ± 0.0740	7.286±0.1569
2	*D. lineale*	Fusiform	0.480 ± 0.0061	0.081 ± 0.0003	5.894 ± 0.0782	0.001 ± 0.0000
3	*D. tonson*	Fusiform	0.564 ± 0.0034	0.085 ± 0.0032	6.624 ± 0.2465	1.074 ± 0.0816
4	*D. orodatum*	Fusiform	0.551 ± 0.0037	0.073 ± 0.0028	7.513 ± 0.2696	0.779 ± 0.0614
5	*D. discolor*	Fusiform	0.405 ± 0.0044	0.078 ± 0.0030	5.220 ± 0.1965	0.642 ± 0.0509
6	*D. mirbelianum*	Fusiform	0.439 ± 0.0086	0.082 ± 0.0045	5.367 ± 0.2971	0.774 ± 0.0864
7	*D. purpurium*	Fusiform	0.353 ± 0.0019	0.067 ± 0.0018	5.307 ± 0.1573	0.410 ± 0.0222
8	*D. nindii*	Fusiform	0.486 ± 0.0019	0.075 ± 0.0015	6.462 ± 0.1356	0.719 ± 0.0269
9	*D. affine*	Fusiform	0.518 ± 0.0046	0.085 ± 0.0015	6.110 ± 0.0805	0.975 ± 0.0411
10	*D. leporinum*	Filiform	1.868 ± 0.0128	0.181 ± 0.0078	10.315 ± 0.4152	0.016 ± 0.0014

± Standard deviation
SL: Seed length; SW: Seed width

TABLE 2. Shape, colour and measurement data of embryos (Genus *Dendrobium* Sw).

No	Species	Shape	Colour	EL (mm)	EW (mm)	EL/EW (mm)	Volume (mm^3x10^{-3})	SV/EV (mm^3x10^{-3})	AS (%)
1	*D. antennatum*	Prolate	Shiny yellow	0.144±0.002	0.111±0.002	1.299±0.024	0.927±0.036	7.875±0.374	87.273±0.621
2	*D. lineale*	Ovaled	Orange	0.215±0.003	0.069±0.001	3.104±0.070	0.542±0.023	1.541±0.065	34.978±2.868
3	*D. tonson*	Ovaled	Orange	0.226±0.006	0.076±0.002	2.970±0.107	0.685±0.049	1.572±0.132	35.962±5.297
4	*D. orodatum*	Ovaled	Yellow	0.227±0.003	0.054±0.001	4.208±0.114	0.347±0.013	2.250±0.187	55.269±3.668
5	*D. discolor*	Ovaled	Pale yellow	0.165±0.011	0.077±0.003	2.130±0.159	0.524±0.057	1.233±0.117	18.414±5.493
6	*D. mirbelianum*	Ovaled	Light yellow	0.185±0.004	0.082±0.004	2.264±0.138	0.652±0.070	1.187±0.036	15.679±2.571
7	*D. purpurium*	Ovaled	White	0.145±0.003	0.067±0.002	2.173±0.088	0.335±0.017	1.223±0.028	18.161±1.800
8	*D. nindii*	Ovaled	Pale yellow	0.186±0.001	0.070±0.003	2.674±0.121	0.473±0.043	1.534±0.147	34.237±6.206
9	*D. affine*	Ovaled	Orange	0.084±0.002	0.034±0.002	2.496±0.133	0.750±0.038	1.304±0.091	22.979±5.292
10	*D. leporinum*	Ovaled	White	0.210±0.009	0.091±0.005	2.304±0.146	0.922±0.125	17.758±2.630	94.245±0.874

± Standard deviation; EL: Embryo length; EW: Embryo width; SV: Seed volume; EV: Embryo volume; AS: Air space; Percentage air space was calculated as [(seed volume-embryo volume)/(seed volume)] x 100. $N = 30$.

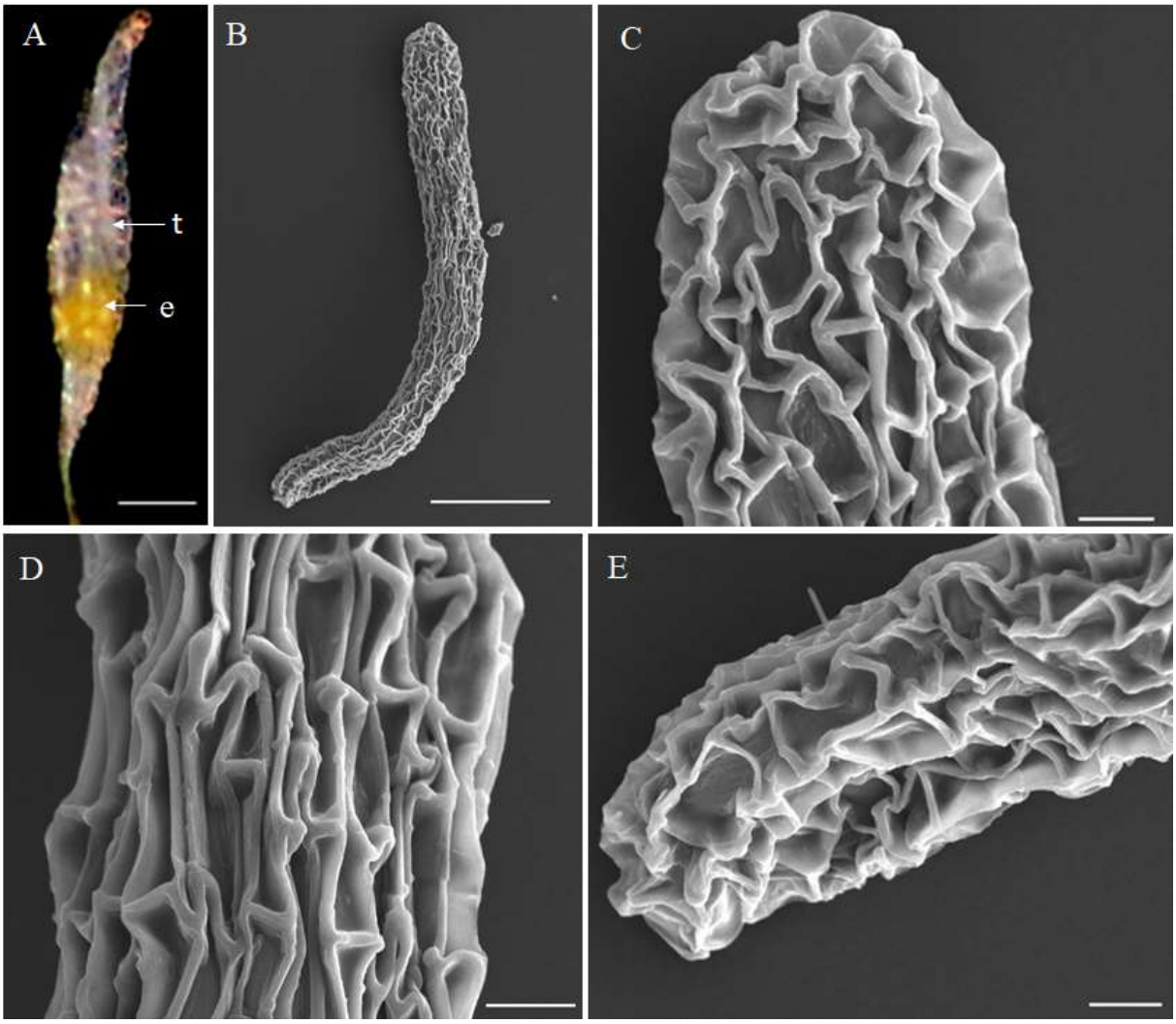

Figure 1. LM and SEM photographs of *D. antennantum.* A. A seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e=embryo, t=testa. Scale bars: (A) 175 µm, (B) 100 µm, (C-E) 10 µm.

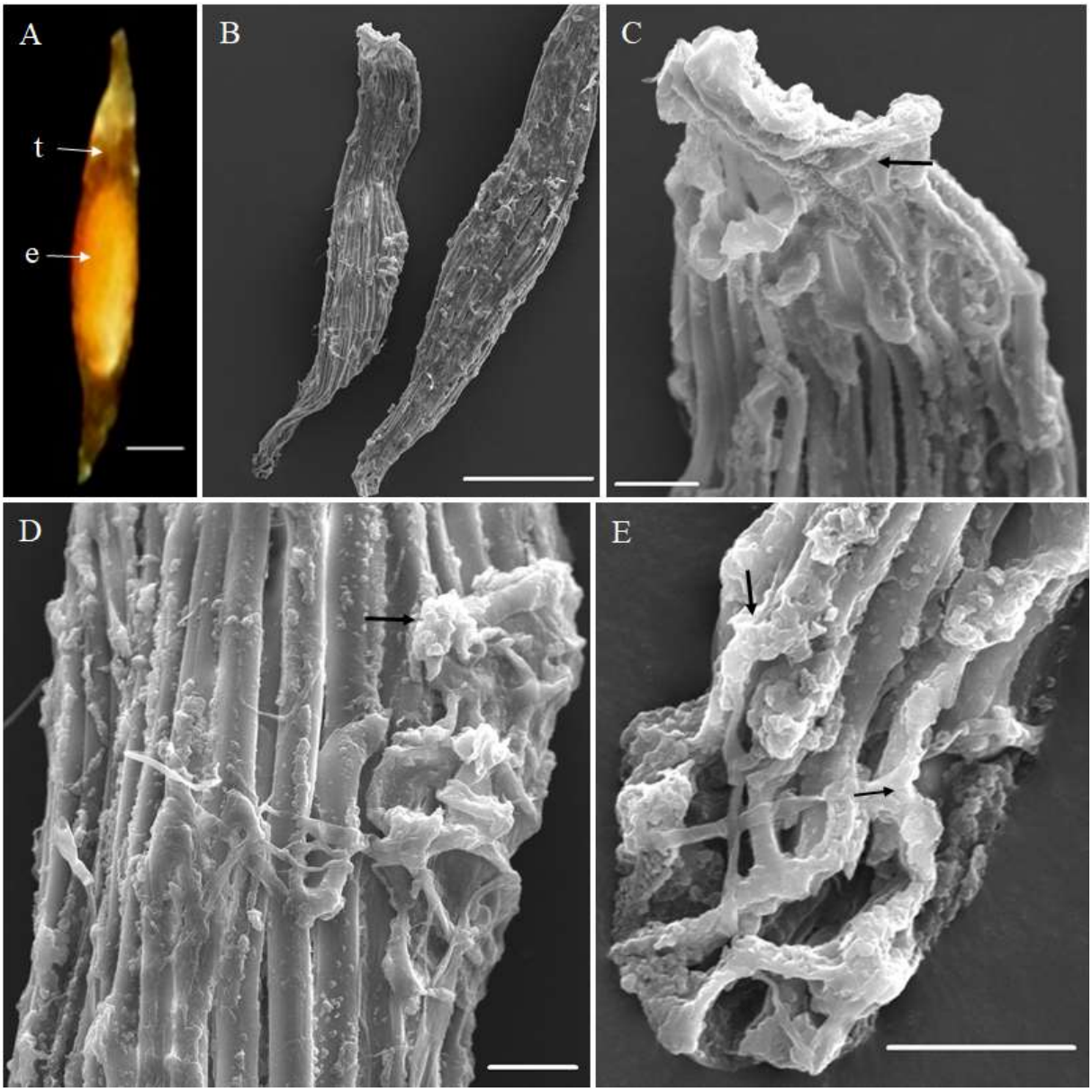

Figure 2. LM and SEM photographs of *D. lineale*. A. A seed under LM; B. Seeds under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e=embryo, t=testa. Scale bars: (A) 81µm, (B) 100 µm, (C-E) 10 µm.

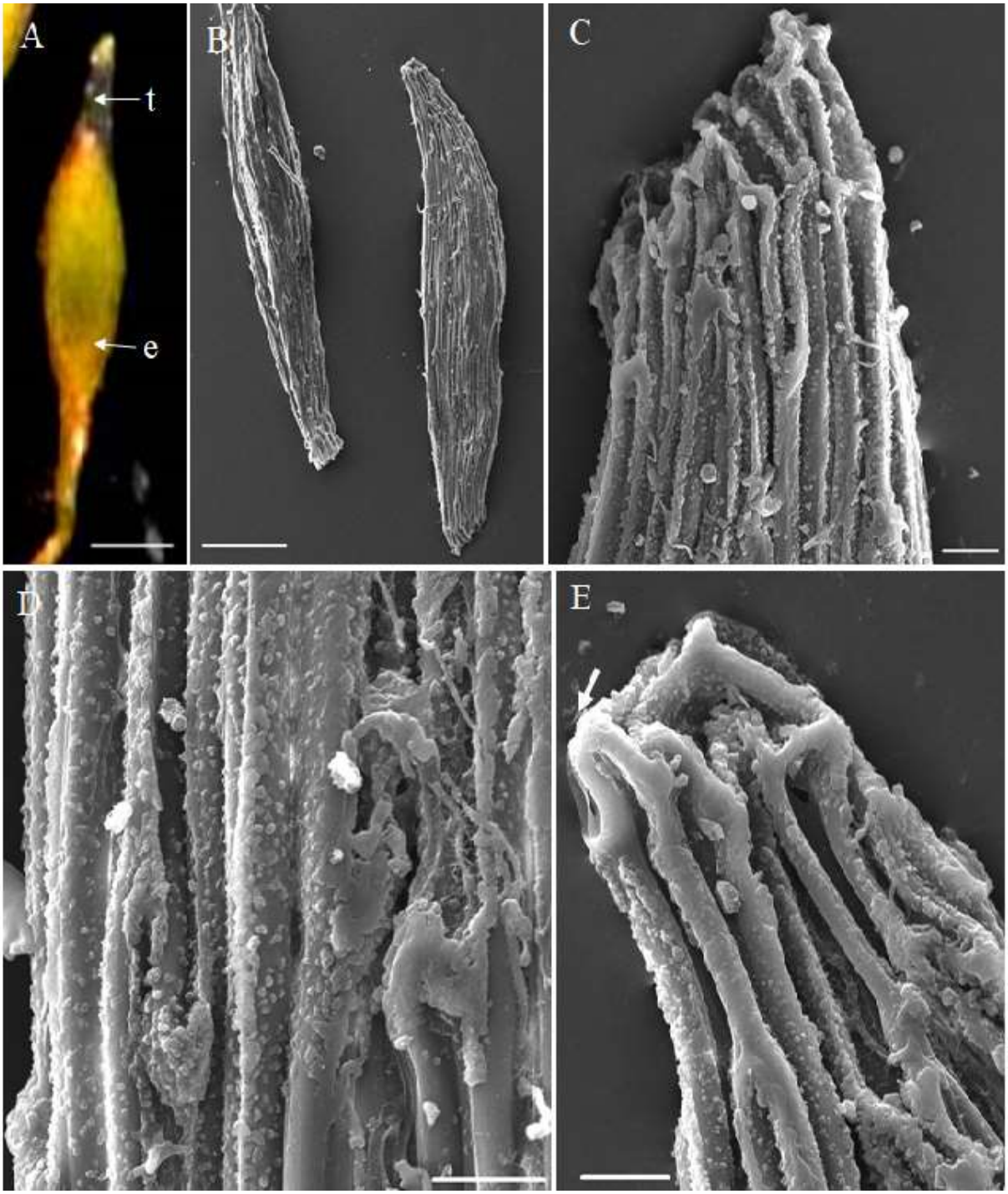

Figure 3. LM and SEM photographs of *D. tonson.* A. A seed under LM; B. Seeds under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 85 µm, (B) 100 µm, (C-E) 10 µm.

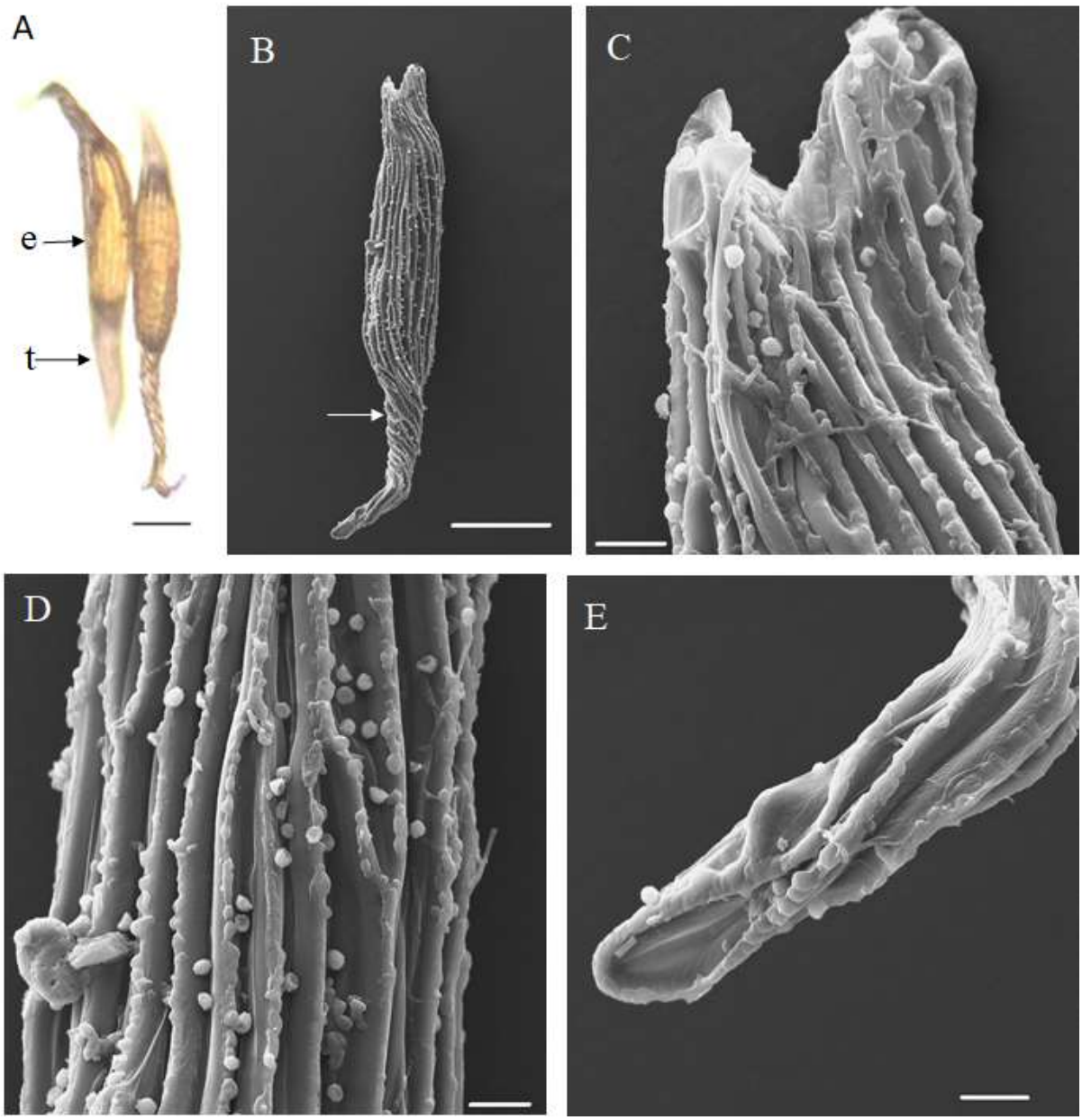

Figure 4. LM and SEM photographs of *Dendrobium odoratum.* A. A seed under LM; B. A seed shape under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 73 µm, (B) 100 µm, (C-E) 10 µm.

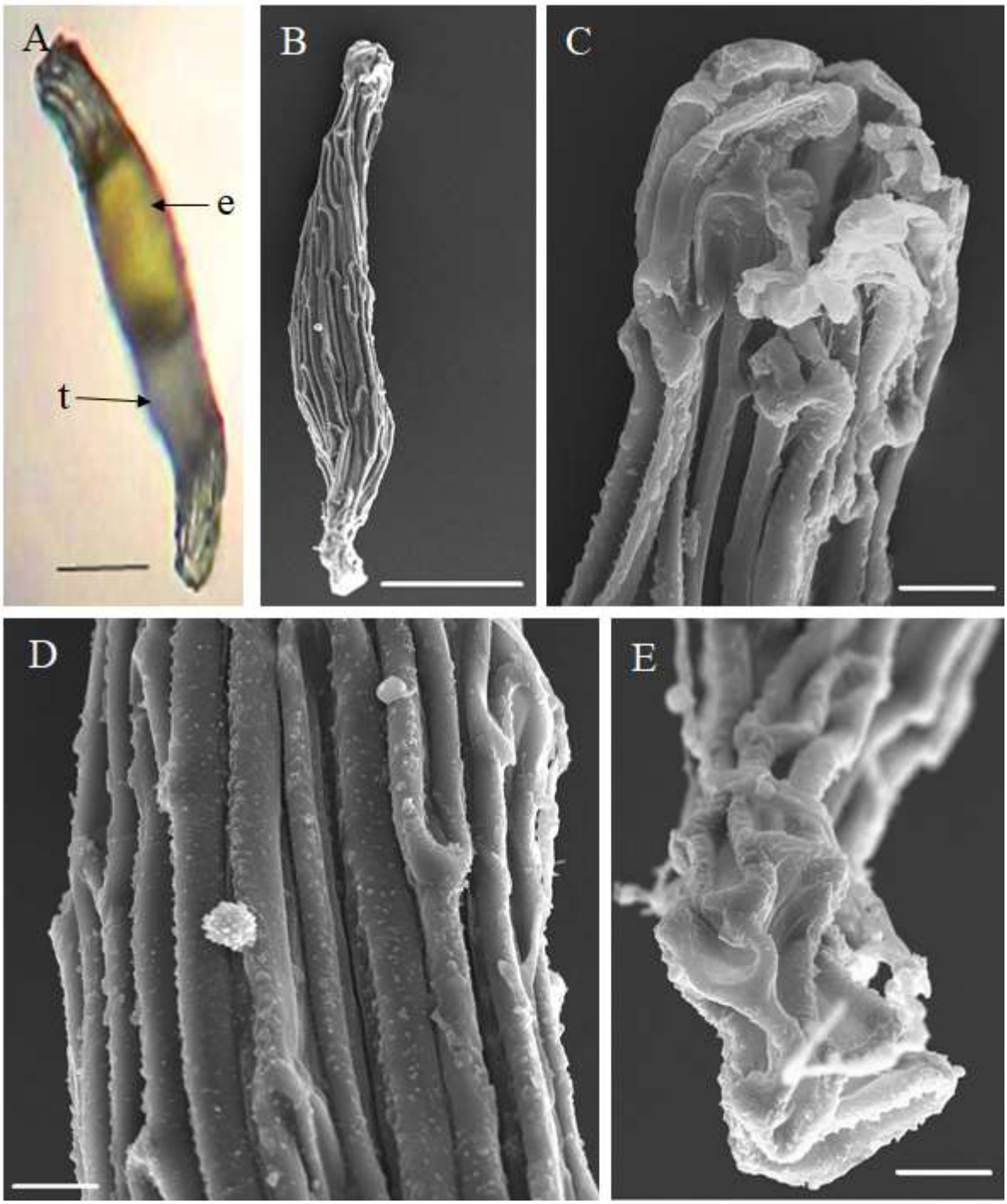

Figure 5. LM and SEM photographs of *D. discolor*. A. A seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 78 µm, (B)100 µm, (C-E)10 µm.

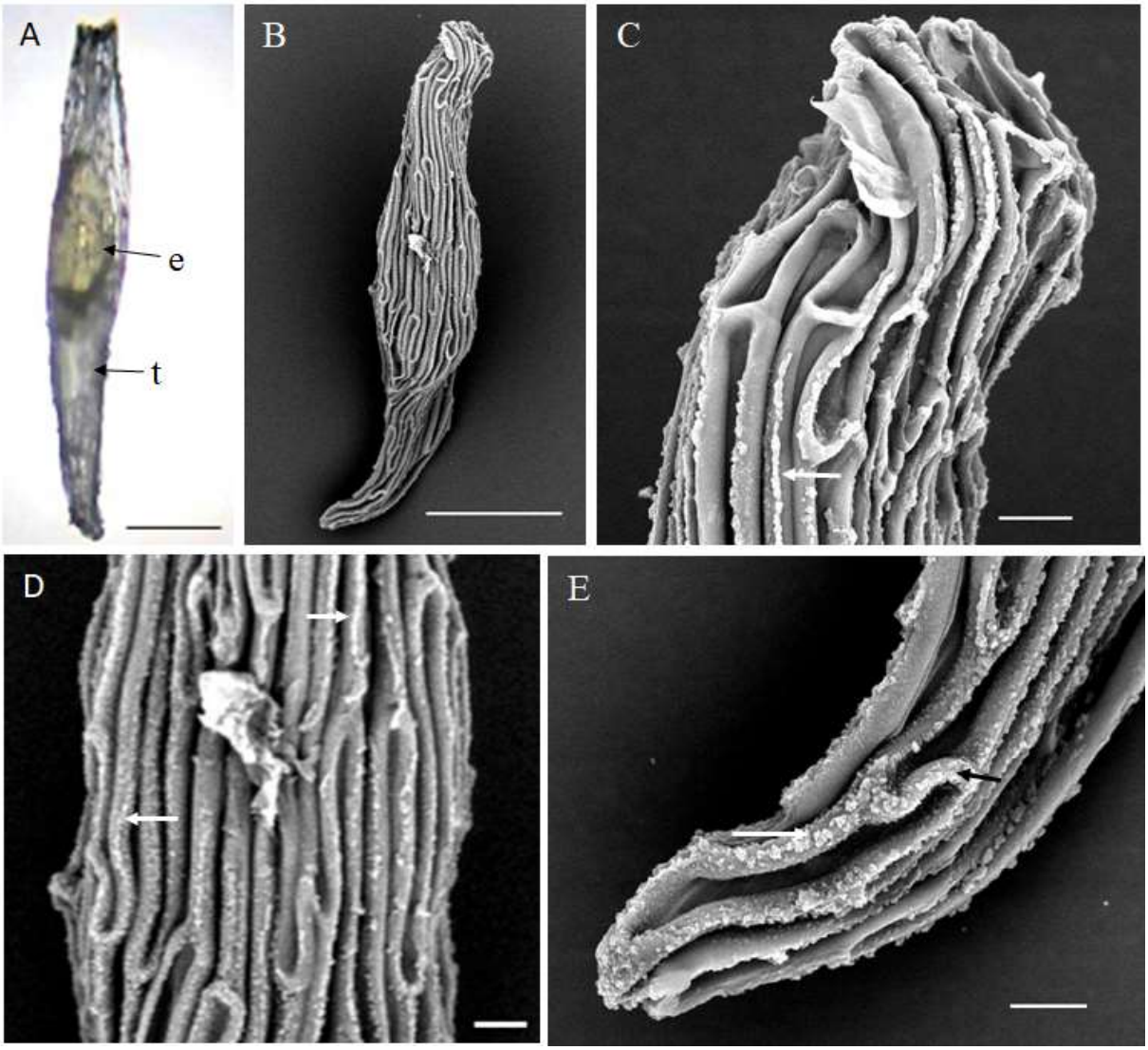

Figure 6. LM and SEM photographs of *D. mirbelianum.* A. seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 82µm, (B) 100µm, (C-E) 10µm.

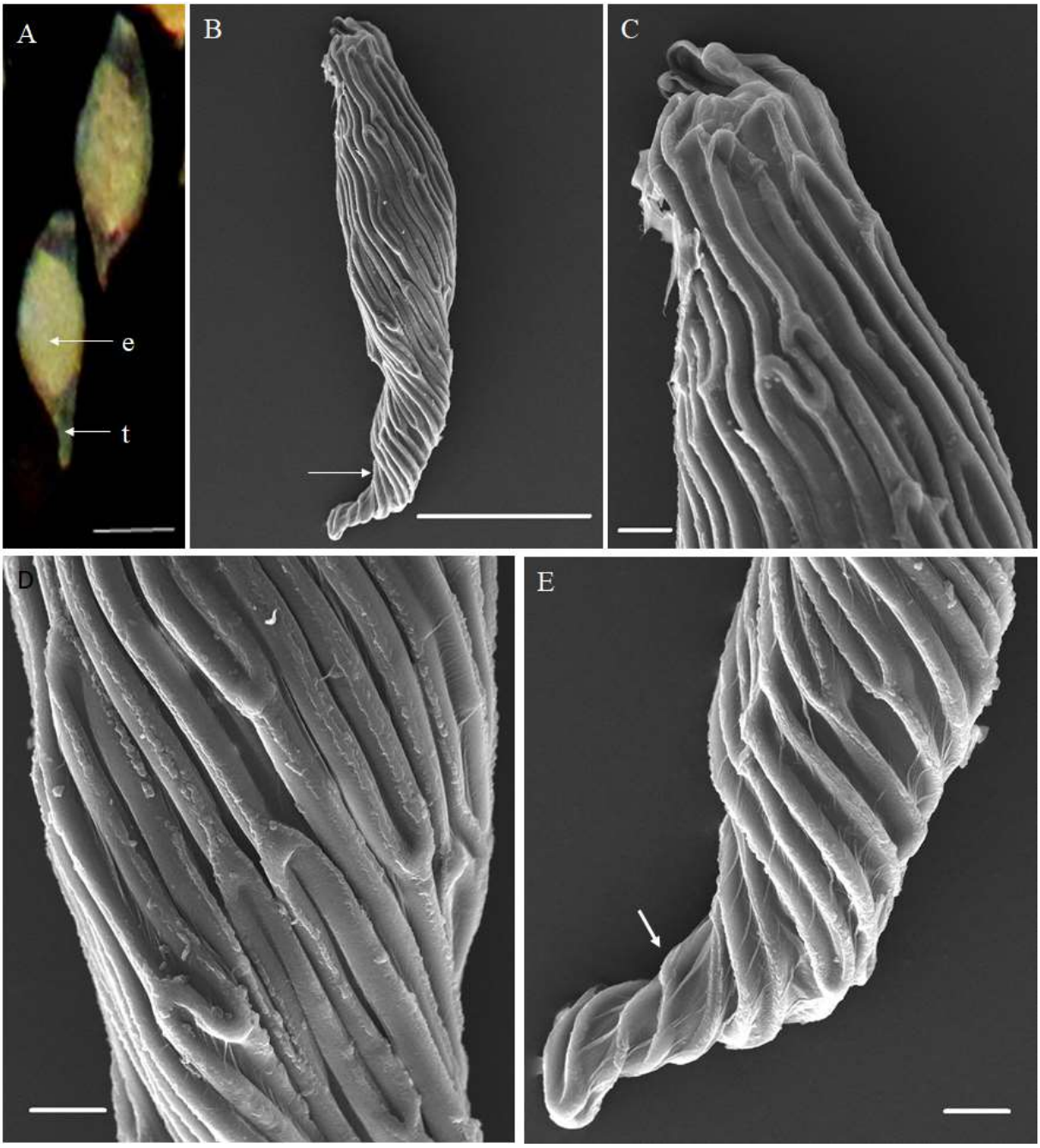

Figure 7. LM and SEM photographs of *D. purpureum.* A. seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 67 µm, (B) 100 µm, (C-E) 10 µm.

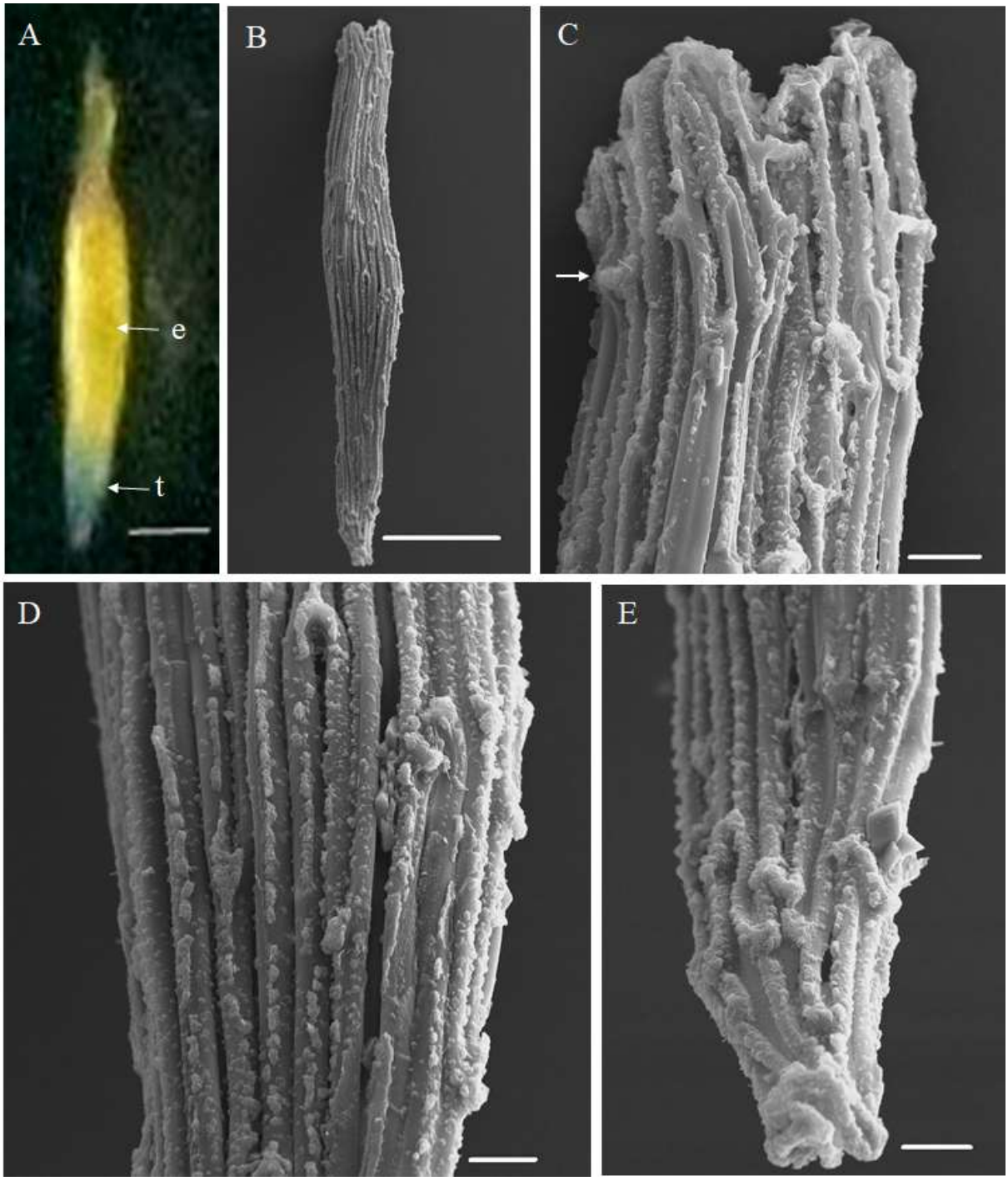

Figure 8. LM and SEM photographs of *D. nindii*. A. Seeds under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A)75 µm, (B) 100 µm, (C-E) 10 µm.

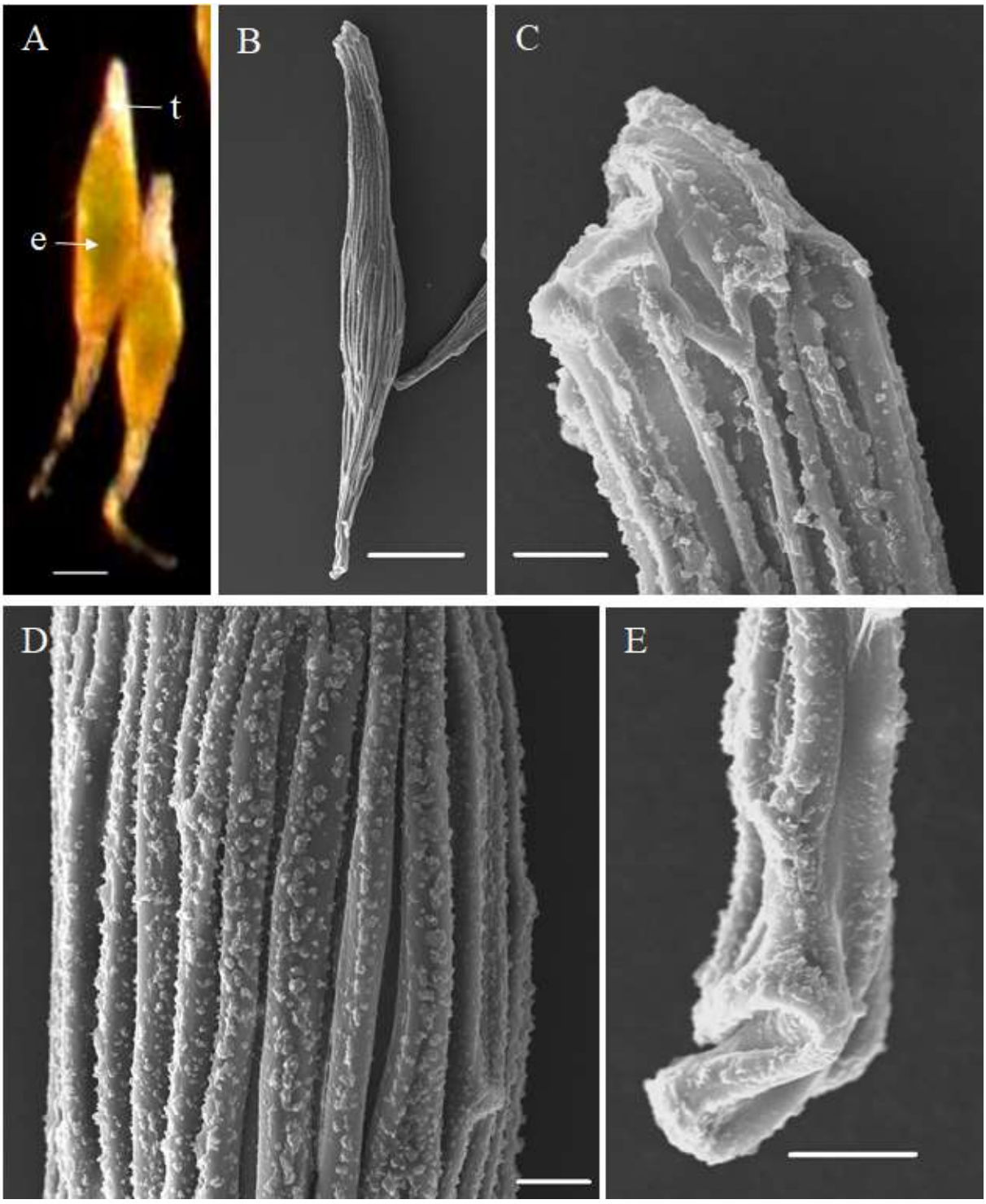

Figure 9. LM and SEM photographs of *D. affine*. A. Seeds under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A) 85 µm, (B) 100 µm, (C-E) 10 µm.

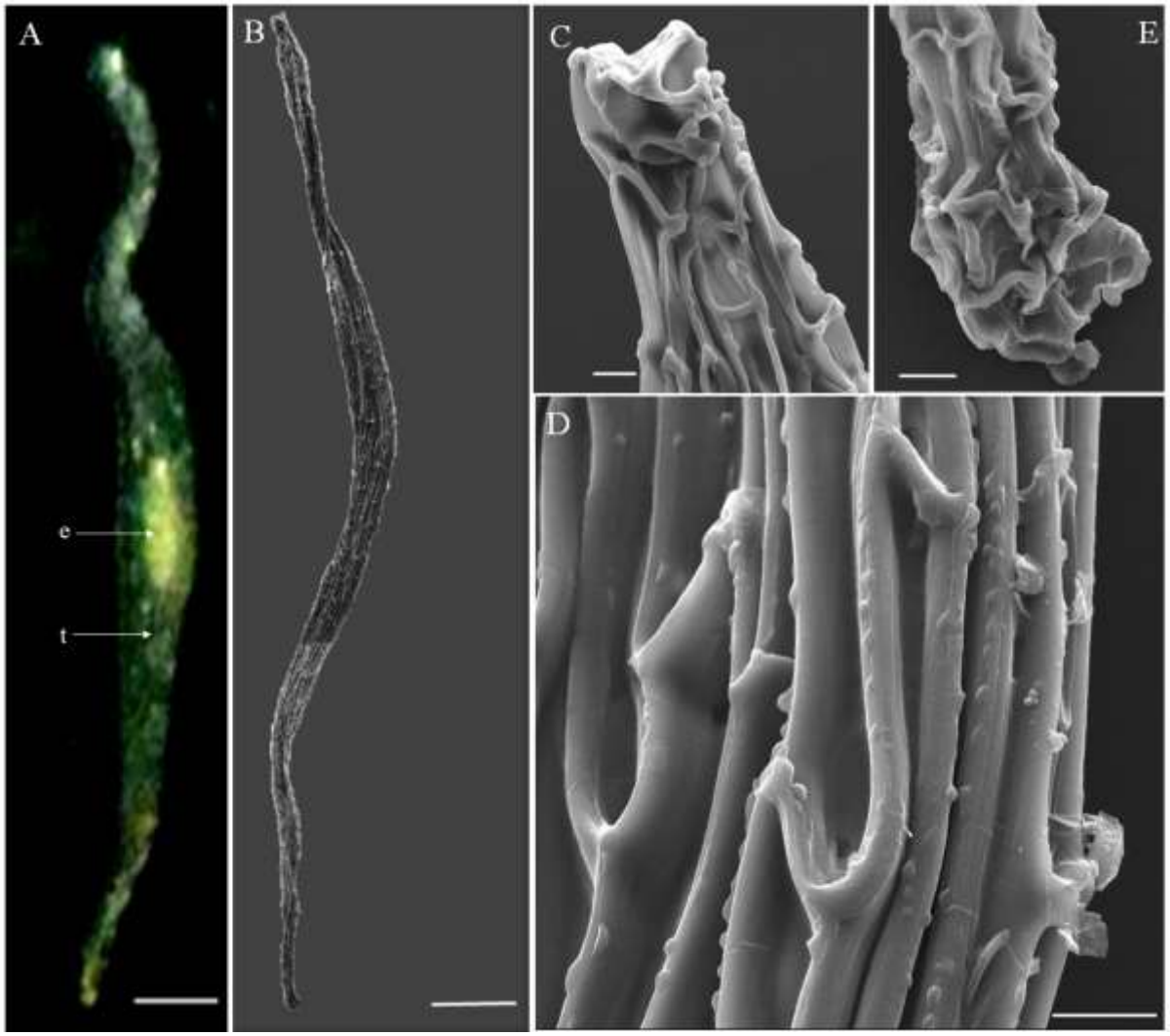

Figure 10. LM and SEM photographs of *D. leporinum.* A. A seed under LM; B. A seed under SEM; C. Cells of the basal pole; D. Pattern of testa cells of the medial; E. Cells of the apical pole. e:embryo, t:testa. Scale bars: (A)181 µm, (B)100 µm, (C-E) 10 µm.

4. Conclusion

The results of this study show orchid seeds vary in micromorphology, size, ultrastructure features and finer detail. Importanly, the characteristics of seeds are used in explaining taxonomic, phylogenetic, and phytogeographic relationships between different orchid taxa. The seeds with percentage of air space below 49% were found in *D. lineale*, *D. tonson*, *D. discolor*, *D. mirbelianum*, *D. purpureum*, *D. nindii* and *D. affine*. This imlpies the seeds are limited in distribution, hence potentially endemic species. In case the habitat of these 7 species are not properly maintained, their existence is threatened.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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