Pollen morphology of selected species of Ruellioideae (Acanthaceae) in Peninsular Malaysia

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Abstract: : The pollen morphology of eight species in subfamily Ruellioideae (Acanthaceae) from Peninsular Malaysia were investigated using light and scanning electron microscopes. Species studied are *Hemigraphis alternata* (Burm. f) T. Anderson, *Hemigraphis reptans* (G. Forst.) T. Anderson ex Hemsl., *Hygrophila pusilla* Blume ex Steud., *Ruellia repens* L., *Ruellia simplex* C. Wright, *Ruellia tuberosa,* L., *Sanchezia speciosa* Leonard and *Strobilanthes crispa* T. Anderson. The objective of this study is to assess the taxonomic value of pollen morphological characteristics. Findings in this study have shown some variations in the pollen morphology that can be used in species identification and classification. Pollen description includes pollen classes, shape, apertures, exine thickness and ornamentation. The results have demonstrated that pollen morphology characteristics have taxonomic significance and useful as additional data especially in species identification and classification in subfamily Ruellioideae.

Key words: pollen morphology, Ruellioideae, Acanthaceae

INTRODUCTION

The Acanthaceae is one the large pan-tropical families of herbs and shrubs. About 3,000 species in some 250 genera with centre of distribution in Indo-Malesia, Africa (including Madagascar), northern South America, Central America and Mexico, with 36 genera are native or naturalized in Peninsular Malaysia (Ridley 1923). Turner (1995) recorded 29 genera with 129 species in Peninsular Malaysia. Previous study by Scotland and Vollesen (2000) classified Acanthaceae into three subfamilies namely as Nelsonioideae, Acanthoideae and Thunbergioideae. However, McDade et al. (2008) consider this family to consist of four subfamilies, Acanthoideae, Nelsonioideae, Thunbergioideae and Andrographideae. But later, Vollesen (2008) elevated the Tribe Ruellieae to subfamily Ruellioideae. New findings by Schwarzbach and Mc Dade (2008) and Borg (2008) have suggested that *Avicennia* has a sister relationship with Acanthaceae but still questionable and unclear in Acanthaceae lineages.

The vast majority of genera and species belonging to Ruellioideae are characterized the presence of a unique fruits type with seeds borne on retinacula. Besides, the presence of cystolith cells can also be used in identification of species in subfamily Ruellioideae. This subfamily comprises mostly annuals to perennial herbs and shrubs. The leaves are opposite and usually entire without stipules. The inflorescences bracts are often well developed and showy. The flowers are gamopetalous and zygomorphic, usually with 2-lipped corolla and 2 or 4 stamens (Heywood 2007).

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Heywood (2007) stated that Acanthaceae is well known for the diversity of its pollen morphology characteristics especially in size, shapes, apertures, exine structures and ornamentation. Many genera can be classified by pollen types. The very first taxonomist who recognized the variability of pollen morphology in *Strobilanthes* and its potential in classification and identification of plant species was Radlkofer (1883). The pollen morphology of Acanthaceae has been widely studied in many countries such as in Yemen (Al-Hakimi and Latiff 2015), China (Wang and Blackmore 2003), India (Shendage and Yadav 2009), and Thailand (Ruengsawang et al. 2013) but yet still very limited especially in Peninsular Malaysia.

Therefore, the aim of this study is to explore and investigate the variation of pollen morphology characteristics in all Acanthaceae species as additional tools in species identification and classification especially in subfamily Ruellioideae (Acanthaceae).

MATERIALS AND METHODS

The pollen of eight species had been collected in various localities in Peninsular Malaysia (Table 1). The pollen were observed under scanning electron microscopy (SEM). The pollen samples were acetolysed and suspensed in ascending alcohol series and finally dehydrated by using critical point-drying method (CPD). The pollen samples were mounted on stubs and coated by gold. Pollen were observed under Scanning Electron Microscope Zeiss Supra 55VP with various magnification (2000x-15,000x). Images were analysed using SmartSEM Software. Five to ten pollen grains of each species were described and average of three measurements for the polar axis and equatorial diameter were documented. Terminology of pollen morphology follows Hesse et al. (2009). For light microscope, the pollen were mounted on the glycerine jelly and size measurement were taken based on 10 pollen grains; the value of P (polar axis length) and E (equatorial diameter) were measured and the P/E ratio was calculated. Exine thickeness were measured using Cell^B Software.

Table 1. List of specimens of Acanthaceae species studied

Species	Locality	Collector	Code and Date
Hemigraphis alternata (Burm. f.) T. Anderson	Selangor, Serdang	Nurul-Aini & Ruzi	CNA 93 (UKMB) 19.9.2012
Hemigraphis reptans (G. Forst.) T. Anderson ex Hemsl.	Selangor, Bangi	Nurul-Aini & Ruzi,	CNA 95 (UKMB) 30.5.2012
Hygrophila pusilla Blume ex Steud.	Perak, Tapah Kinjang Forest Reserves	Aiman & Ruzi	MAA 67 (UKMB) 8.3.2014
Ruellia repens L.	Pahang, Tasik Chini	Nurul-Aini, Aiman & Ruzi	CNA 115 (UKMB) 7.11.2012
Ruellia simplex C. Wright	Pahang, Tasik Chini	Nurul-Aini, Aiman & Ruzi	CNA 106-108 (UKMB) 7.11.2012
Ruellia tuberosa L.	Selangor, Sepang	Sani	SM 2266 (UKMB) 26.6.2012
Sanchezia speciosa Leonard	Pahang, Fraser Hill	Aiman & Ruzi	MAA 19 (UKMB) 22.5.13
Strobilanthes crispa T. Anderson	Terengganu, Besut, Lata Tembakah Forest Reserve	Nurul-Aini & Ruzi	CNA 84 (UKMB) 11.7.2012

RESULTS

General pollen descriptions

Previous research by Hesse et al. (2009) described generally the pollen type based on the characteristics of pollen class, aperture numbers, shape and exine ornamentations. Earlier Wang and Blackmore (2003) related the pollen morphology with the taxonomic implications in *Strobilanthes* (Acanthaceae). Based on this study, the variation of pollen morphology in selected species of Ruellioideae were described. The aim has been to discuss the characteristics of pollen morphology in each species studied and provide a key for identifications. Below are the description of each species:

Type 1

H. alternata (Burm. f.) T. Anderson (Figure 1A-F)

Pollen class: stephanoaperturate; shape subprolate; P/E ratio 1.19; apertures 3-colpi, > 6 pseudocolpi, 3- porate; exine ornamentation micro-reticulate; measurement sizes: equatorial diameter E (25.31 (29.78) 35.09µm), polar axis P (33.34 (35.29) 38.84 µm), exine thickness (1.76 (1.92) 2.21 µm), colpus length C (21.08 (27.72) 37.67 µm), diameter in polar axis D (30.67 µm).

H. reptans (G.Forst.) T. Anderson ex. Hemsl. (Figure 2A-E)

Pollen class: stephanoaperturate; shape prolate-spheroidal; P/E ratio 1.06; apertures: 3-colpi, > 6 pseudocolpi, 3-porate; exine ornamentation micro-reticulate; Measurement sizes: equatorial diameter E (30.88 (34.88) 39.49µm), polar axis P (31.66 (37.07) 42.75 µm), exine thickness (1.61 (1.98) 2.32 µm), colpus length C (22.11 (34.64) 37.42 µm), diameter in polar axis D (26.99 (24.59) 25.93 µm).

H. pusilla Blume ex Steud. (Figure 3A&B)

Pollen class: stephanoaperturate; Shape prolate-spheroidal; P/E ratio 1.00; Apertures: 3- colpi, > 6 pseudocolpi, 3-porate; exine ornamentation micro-reticulate; measurement sizes: Equatorial diameter E (19.84 (26.09) 29.88 µm), Polar axis P (19.75 (26.35) 30.00 µm), exine thickness (1.38 (1.55) 1.73 µm), colpus length C (15.62 µm), diameter in polar axis D (23.45 µm).

Type 2

R. repens L. (Figure 3C-E)

Pollen class: triporate; shape prolate-spheroidal; P/E ratio: 1.05; apertures 3-porate; exine ornamentation coarsely-reticulate; Measurement sizes: equatorial diameter E (40.60 (40.95) 41.29 µm), polar axis P (43.11 (43.18) 43.24 µm), exine thickness (1.56 (1.75) 1.90 µm.

R. simplex C. Wright (Figure 4A-D)

Pollen class: triporate; shape prolate-spheroidal; P/E ratio 1.04; apertures 3-porate; exine ornamentation coarsely-reticulate; measurement sizes: equatorial diameter E (53.18 (53.63) 54.08 µm), polar axis P (55.07 (55.65) 56.13 µm), exine thickness (1.32 (1.60) 2.00) µm.

R. tuberosa L. (Figure 5A-F)

Pollen class: triporate; shape prolate-spheroidal; P/E ratio: 1.02; Apertures 3- porate; exine ornamentation coarsely-reticulate; measurement sizes: equatorial diameter E (54.24 (57.13) 57.55 µm), polar axis P (54.27 (57.13) 58.90 µm), exine thickness (1.91 (2.08) 2.33) µm.

Type 3

S. speciosa Leonard (Figure 6A-E)

Pollen class: diporate; Shape: prolate; P/E ratio 1.37; Apertures 2-porate; Exine ornamentation striato-reticulate; Measurement sizes: equatorial diameter E (50.44 (53.54) 55.57 µm), polar axis P (66.65 (73.17) 78.42 µm), exine thickness (6.06 (5.94) 6.11) µm.

Type 4

S. crispa T. Anderson (Figure 7A-F)

Pollen class: stephanoaperturate; Shape: prolate; P/E ratio: 1.79; Apertures: 3-colpi, > 6 pseudocolpi, 3-porate; Exine ornamentation bireticulate; Measurement sizes: equatorial diameter E (18.99 (19.67) 20.04 µm), polar axis P (34.62 (35,.24) 36.04 µm), exine thickness (1.13 (1.26) 1.50) µm.

Key to pollen types

1 Aperture 3-colpi, > 6 pseudocolpi, 3-porate.....2
1 Apertures 2 or 3-porates.....3
2 Exine ornamentation microreticulate…….....Type 1
2 Exine ornamentation bireticulate…….....Type 4
3 Pollen shape prolate-spheroidal …….....Type 2
3 Pollen shape prolate……...…….....Type 3

DISCUSSION

Heywood et al. (2007) described Acanthaceae as a well-known plant family for the diversity of its pollen especially in size, shape, ornamentation and apertures. Besides, the characteristics of pollen type can also be used to differentiate many genera in Acanthaceae. The variability of pollen morphology in Acanthaceae and significant of this character in generic delimitation was firstly recognised by Lindau (1893, 1895). Carine and Scotland (1998) also stated a number of pollen characters that can be useful in species identification which are pollen shape, pollen class; number of aperture; distribution of apertures; ectoaperture and endoaperture shapes; cross-sectional structure of the exine and spine morphology.

In previous study, Perveen and Qaiser (2010) recorded the diversity in the pollen morphology in various characters such as in pollen size, shape, aperture and tectum. In Yemen, Al-Hakimi et al. (2015) has described the differences in pollen morphology in three genera of Acanthaceae that reflects the variations within this family. Al-Hakimi et al. (2015) stated that there are some pollen characteristics which can be used to differentiate the species in Yemen such as pollen apertures, class and shape. Results of this study are in agreement with Al-Hakimi et al. (2015) in which the pollen morphology is very uniform between *Ruellia* species and very similar in shape, class, apertures and exine ornamentation. Al-Hakimi et al. (2015) also reported that the details of pollen characteristics can be used to differentiate species in Yemen.

Findings of this study also have shown the significance of pollen characteristics that can be used to identify and differentiate species especially in subfamily Ruellioideae (Table 1). Four types of pollen are recognized in this study which are Type 1, Type 2, Type 3 and Type 4. The pollen types have been described based on the pollen characteristics such as pollen shape, class, apertures, exine ornamentation and exine thickness. Pollen shape varies from subprolate, prolate or prolate-spheroidal. Pollen class also varies which is either stephanoaperturate, triporate or diporate. While in exine ornamentation, four types of ornamentation has been recognised which are micro-reticulate, coarsely-reticulate, striato-reticulate and bireticulate.

Pollen Type 1 differs from other types in apertures with 3–colpi, > 6 pseudocolpi, 3–porate, pollen class stephanoaperturate and exine ornamentation micro-reticulate. This type is exemplified by *Hemigraphis* and *Hygrophila*. However, pollen shape can be used to differentiate species in *Hemigraphis*. *Hemigraphis alternata* can be recognised by its subprolate pollen shape while prolate-spheroidal is observed in *H. reptan.* For *Hygrophila*, the pollen shape is similar to that of *Hemigraphis reptans* which is prolate-spheroidal as in *Hygrophila pusilla*. Perveen and Qaiser (2010) also reported the similar characters for pollen class and shape in *Hygrophila polysperma.*

Pollen Type 2 that has been recognized is present only in *Ruellia* and characterized by triporate pollen with three porate apertures and coarsely-reticulate exine ornamentation. Al-Hakimi and Latiff (2015) also reported similar pollen morphology in *R. prostrata, R. patula, R. grandiflora, R. insignis* and *R. dioscoridis*. This study is also supported by previous study on Acanthaceae in Pakistan that also demonstrated the similar characteristics in pollen class and exine ornamentation as in *R. tuberosa, R. patula, R. brittoniana, R. prostrata* and *R. linearibracteolata* (Perveen and Qaiser 2010). Besides, for the pollentype 3 that has been described in *Sanchezia* is easily recognised and delimited by having diporate pollen class and striato-reticulate for exine ornamentation which is not recorded in other species studied.

In the pollen Type 4 that has been described for *Strobilanthes* it is very similar to pollen Type 1 in *Hemigraphis* and *Hygrophila* with pollen class stephanoaperturate and apertures 3–colpi, > 6 pseudocolpi, 3–porate but differ in the variation of exine ornamentation and in pollen shape. Pollen shape Type 1 is either subprolate or prolate-spheroidal but prolate in Type 4 whereas exine ornamentation is micro-reticulate that has been recorded in Type 1 and bireticulate in Type 4. According to Carine and Scotland (1998), *Strobilanthes* contributed great variations and can be divided into 22 pollen types based on pollen shape, apertural types and exine ornamentation. The earliest study by Bremekamp (1944) on selected species of *Strobilanthes* can be used to split this genus into segregate genera using pollen morphology as one of the main characteristics but yet still problematic (Carine and Scotland 1998).

Therefore in conclusion, the present pollen morphological study in subfamily Ruellioideae demonstrates great variation that can give high impact in taxonomic implication of Acanthaceae especially in Peninsular Malaysia. It can be used as supportive data especially in identification and classification at the genus and species level.

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REFERENCES

Al-Hakimi, A. S. and Latiff, A. 2015. Pollen and seed morphology of *Ruellia* L., Phaulopsis Willd. and *Dyschoriste* Nees (Acanthaceae: Ruellioideae: Ruellieae) of Yemen. *Plant Systematic and Evolutions* 301(1): 1-13.

Al-Hakimi, A. S., Haja Maideen and Latiff, A. 2015. Pollen and seed morphology of Rhinacanthus Nees and Hypoestes Sol. ex R. Br. (Acanthaceae) of Yemen. Sains Malaysiana 44(1): 7-15.

Borg, A. J. 2008. *Molecular phylogenetics and morphological evolution of Thunbergioideae* (Acanthaceae). Taxon 57(3): 811-822.

Carine, M. A. and Scotland, R. W. 1998. Pollen morphology of Strobilanthes Blume (Acanthaceae) from Southern India and Sri Lanka. *Review of Palaeobotany and Palynology* 103: 143-165.

Hesse, M., Halbritter, H., Weber, M., Buchner, R., Frosch-Radivo, A. and Ulrich, S. 2009. Pollen Terminology. An Illustrated Handbook. Berlin: Springer Verlag.

Heywood, V. H., Brummitt, R. K., Culham, A. and Seberg, O. 2007. *Flowering Plants Families of The World*, pp. 23-24. Canada: Firefly Books.

Lindau G. 1893. Beiträge zur Systematik der Acanthaceen. *Botanische Jahrbüchen*. 18: 36-64.

Lindau, G. 1895. Acanthaceae. Dlm. Engler, A. and Prantl, K. (pnyt..) *Die natürlichen Pflanzenfamilien* 4(3b), pp. 274–353. Leipzig: Verlag von Wilhem Engelmann.

McDade, L. A., Daniel, T. F. and Kiel, C. A. 2008. Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae S.L. (Lamiales). *American Journal of Botany* 95(9): 1136-1152.

Perveen, A. and Qaiser, M. 2010. Pollen flora of Pakistan—LXVII : Acanthaceae. *Pakistan Journal Botany* (Special Issues) 42:175–191.

Ridley, H. N. 1923. *The Flora of the Malay Peninsula*, pp. 554-610. London: L. Reeve and Co.

Ruengsawang, K., Chantaranothai, P. and Simpson, D. A. 2013. Pollen morphology of *Justicia* L. (Acanthaceae) from Thailand and its taxonomic value. Grana 52(4) 275-288.

Schwarzbach, A. E. and McDade, L. A. 2002. Phylogenetic relationships of the mangrove family Avicenniaceae based on chloroplast and nuclear ribosomal DNA sequences. *Systematic Botany* 27:84–98

Scotland, R. W. and Vollesen. K. 2000. Classification of Acanthaceae. *Kew Bull*. 5: 513- 580.

Shendage, S. M. and Yadav, R. V. 2009. Pollen morphology of Barleria L. (Acanthaceae) from India. *Phytomorphology* 59(3/4): 121-126

Turner, I. M. 1995. A catalogue of the vascular plants of Malaya. *Gards' Bull*. Sing. 47(2): 486-489.

Vollesen, K. 2008. *Flora of Tropical East Africa: Acanthaceae*, pp. 1-285. Kew: Royal Botanic Gardens.

Wang, H. and Blackmore, S. 2003. Pollen morphology of *Strobilanthes* Blume (Acanthaceae) in China and its taxonomic implications. *Grana* 42: 82-87.

Table 2. Morphological characteristic of pollen grains of *Hemigraphis, Ruellia, Sanchezia* and *Strobilanthes*.

Species	E length (µm)	P diameter (µm)	P/E	P diameter (µm)	E length (µm)	Pollen class	Exine ornamentations	Exine thickness	Apertures
Hemigraphis alternata	25.31 (29.78) 35.09 µm	33.34 (35.29) 38.84 µm	1.19	33.34 (35.29) 38.84 µm	25.31 (29.78) 35.09 µm	Stephanoaperturate	Micro-reticulate	1.76 (1.92) 2.21 µm	3- colpi, > 6 pseudocolpi, 3- porate
Hemigraphis reptans	30.88 (34.88) 39.49 µm	31.66 (37.07) 42.75 µm	1.06	31.66 (37.07) 42.75 µm	30.88 (34.88) 39.49 µm	Stephanoaperturate	Micro-reticulate	1.61 (1.98) 2.32 µm	3- colpi, > 6 pseudocolpi, 3- porate
Hygrophila pusilla	19.84 (26.09) 29.88 µm	19.75 (26.35) 30.00 µm	1.00	19.75 (26.35) 30.00 µm	19.84 (26.09) 29.88 µm	Stephanoaperturate	Micro-reticulate	1.38 (1.55) 1.73 µm	3- colpi, > 6 pseudocolpi, 3- porate
Ruellia repens	40.60 (40.95) 41.29 µm	43.11 (43.18) 43.24 µm	1.05	43.11 (43.18) 43.24 µm	40.60 (40.95) 41.29 µm	triporate	Coarsely-reticulate	1.56 (1.75) 1.90 µm	3-porate
Ruellia simplex	53.18 (53.63) 54.08 µm	55.07 (55.65) 56.13 µm	1.04	55.07 (55.65) 56.13 µm	53.18 (53.63) 54.08 µm	triporate	Coarsely-reticulate	1.32 (1.60) 2.00 µm	3-porate
Ruellia tuberosa	54.24 (57.13) 57.55 µm	54.27 (57.13) 58.90 µm	1.02	54.27 (57.13) 58.90 µm	54.24 (57.13) 57.55 µm	triporate	Coarsely-reticulate	1.91 (2.08) 2.33 µm	3-porate
Sanchezia speciosa	50.44 (53.54) 55.57 µm	66.65 (73.17) 78.42 µm	1.37	66.65 (73.17) 78.42 µm	50.44 (53.54) 55.57 µm	Diporate	Striato-reticulate	6.06 (5.94) 6.11 µm	3-porate
Strobilanthes crispa	18.99 (19.67) 20.04 µm	34.62 (35.24) 36.04 µm	1.79	34.62 (35.24) 36.04 µm	18.99 (19.67) 20.04 µm	Stephanoaperturate	Bireticulate	1.13 (1.26) 1.50 µm	3- colpi, > 6 pseudocolpi, 3- porate

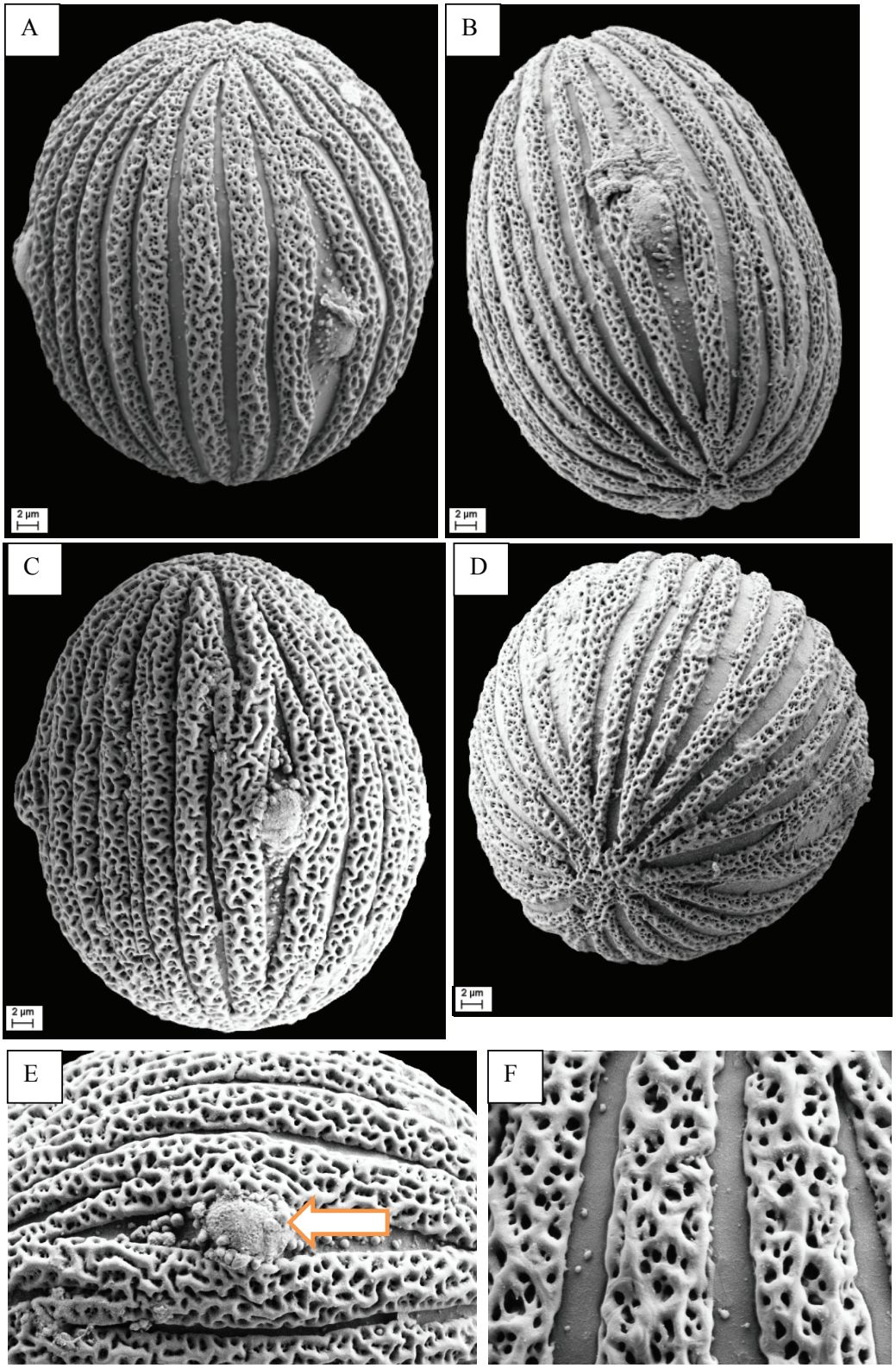

Figure 1. Pollen grains of *Hemigraphis alternata*: A-C) Equatorial view, D) Polar view, E) Aperture (arrow). F) Exine ornamentation. Scale: A, C, D, E = 2 µm, B =10 µm, F =1 µm.

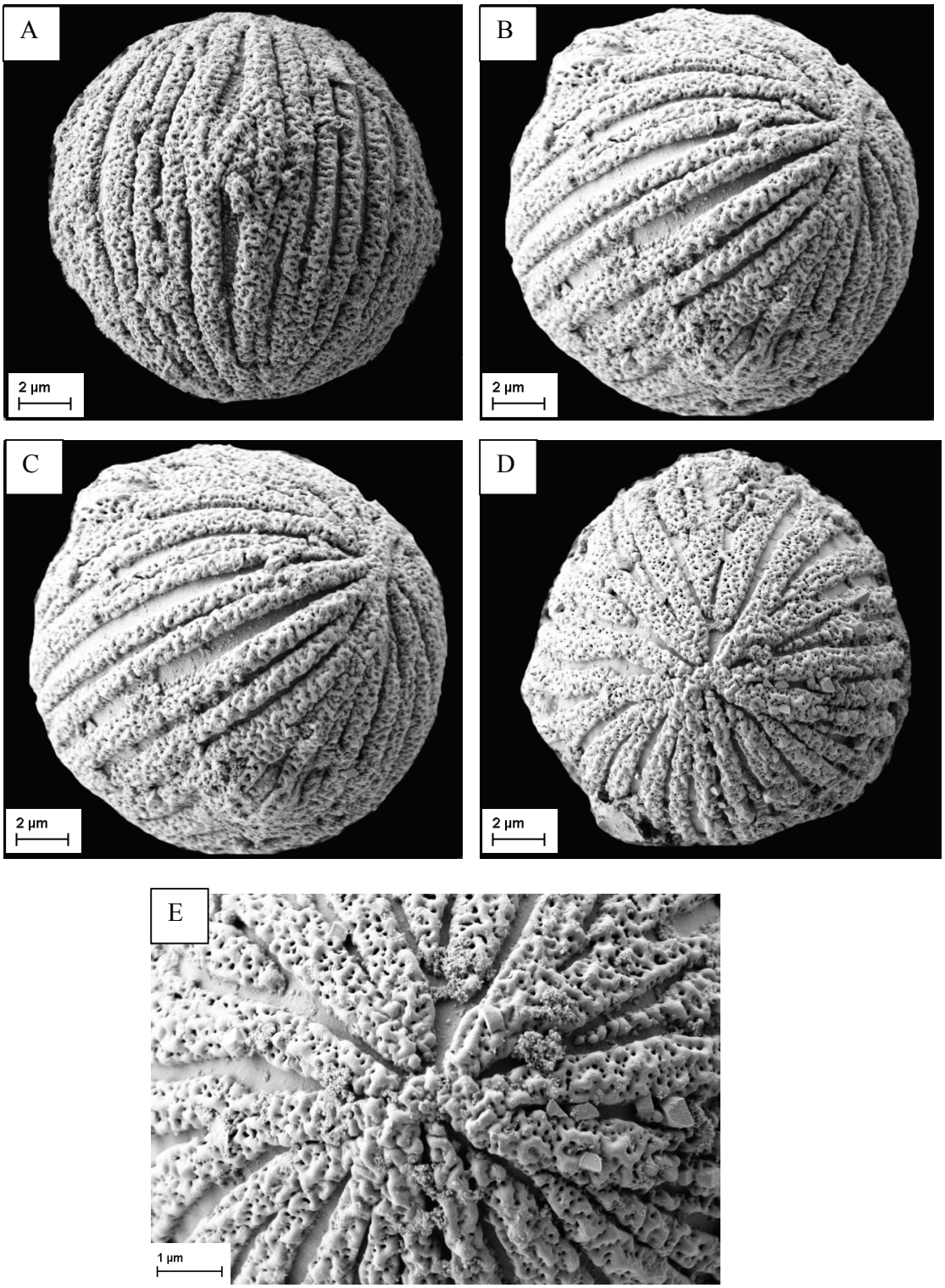

Figure 2. Pollen grains of *Hemigraphis reptans*: A) Equatorial view, B-D) Polar view, E) Exine ornamentation. Scale: A – D = 2 µm. E = 1 µm.

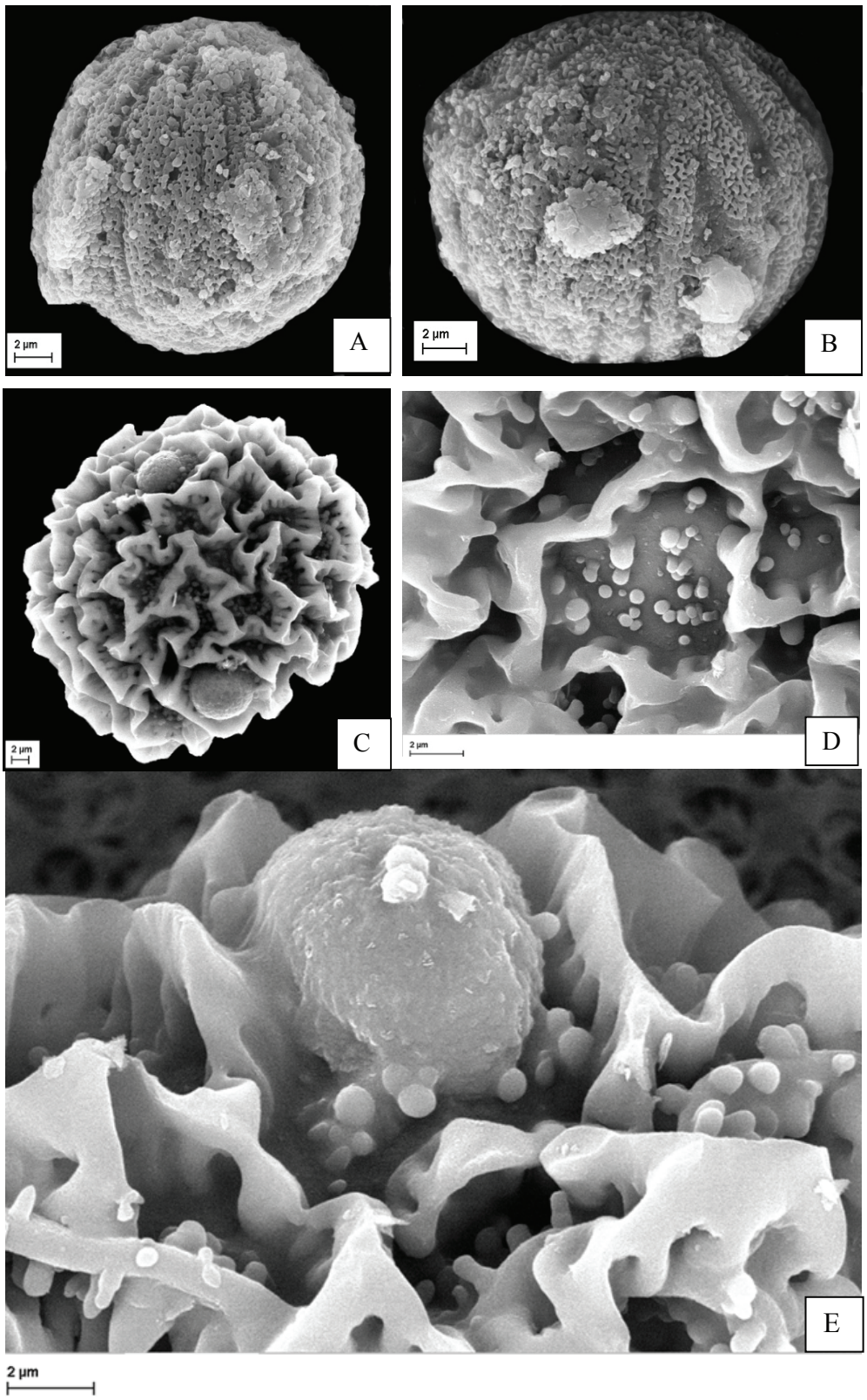

Figure 3. Pollen grains of *Hygrophila pusilla*: A&B) Equatorial view. Scale: A & B = 2 µm. Pollen grains of *Ruellia repens*: C) Polar view, D) Exine ornamentation, E) Porate. Scale: A – C = 2 µm.

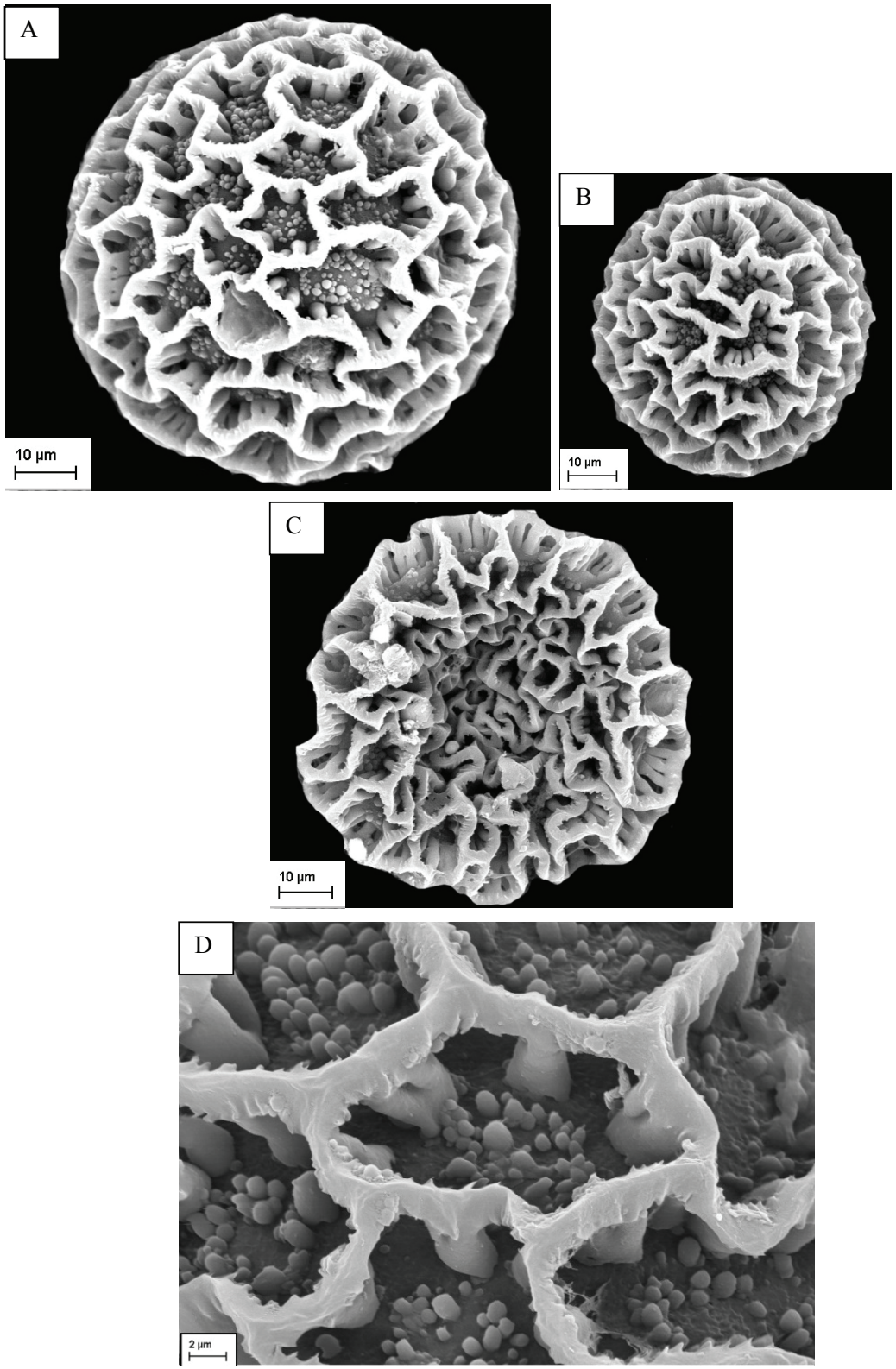

Figure 4. Pollen grains of *Ruellia simplex:* A-C) Polar view, D) Exine ornamentation. Scale: A – C = 10 µm, D = 2 µm. .

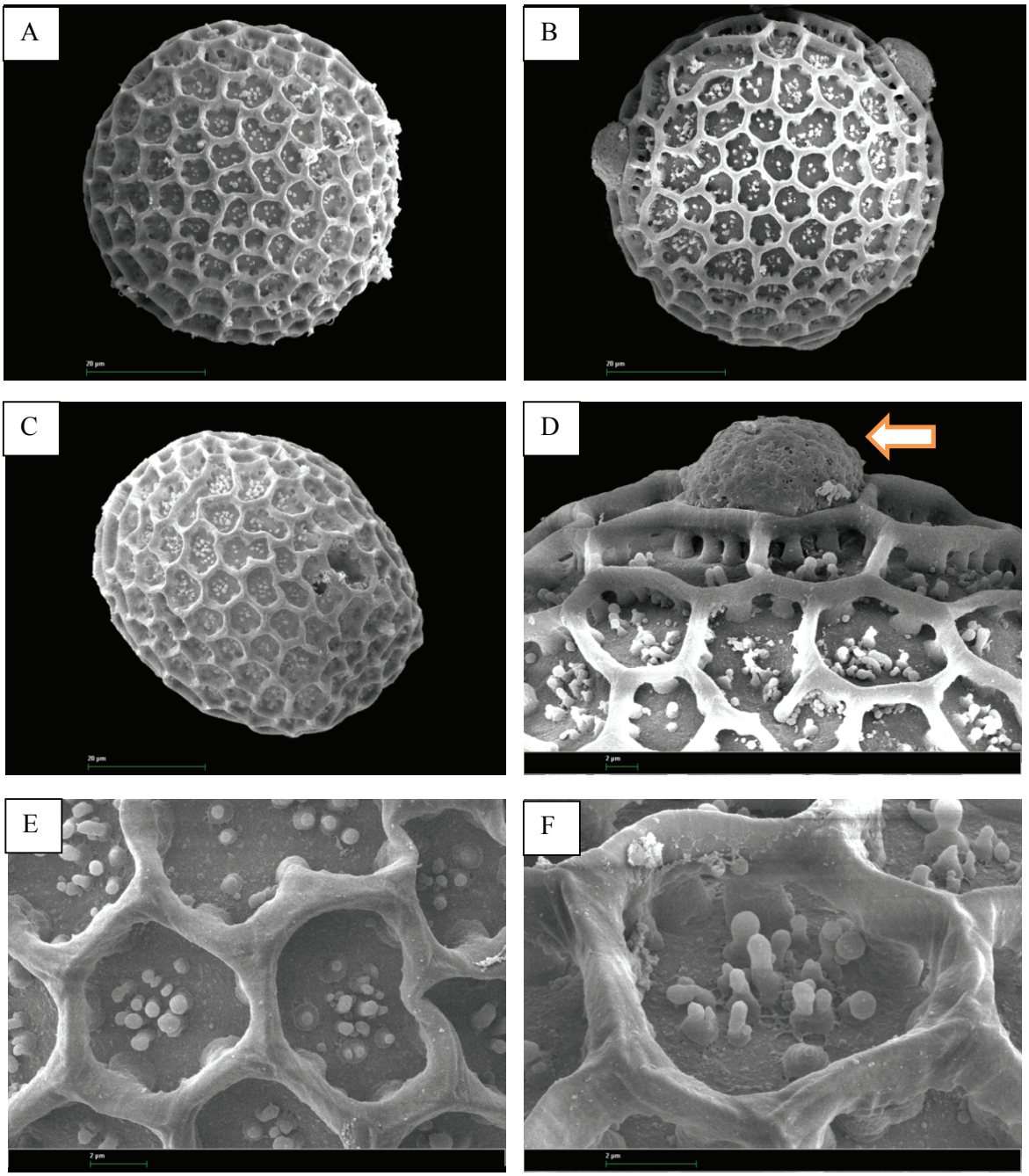

Figure 5. Pollen grains of *Ruellia tuberosa*: A - C) Polar view, D) Porate, E & F) Exine ornamentation. Scale: A & B = 20 µm, C =10 µm, D & F = 2 µm.

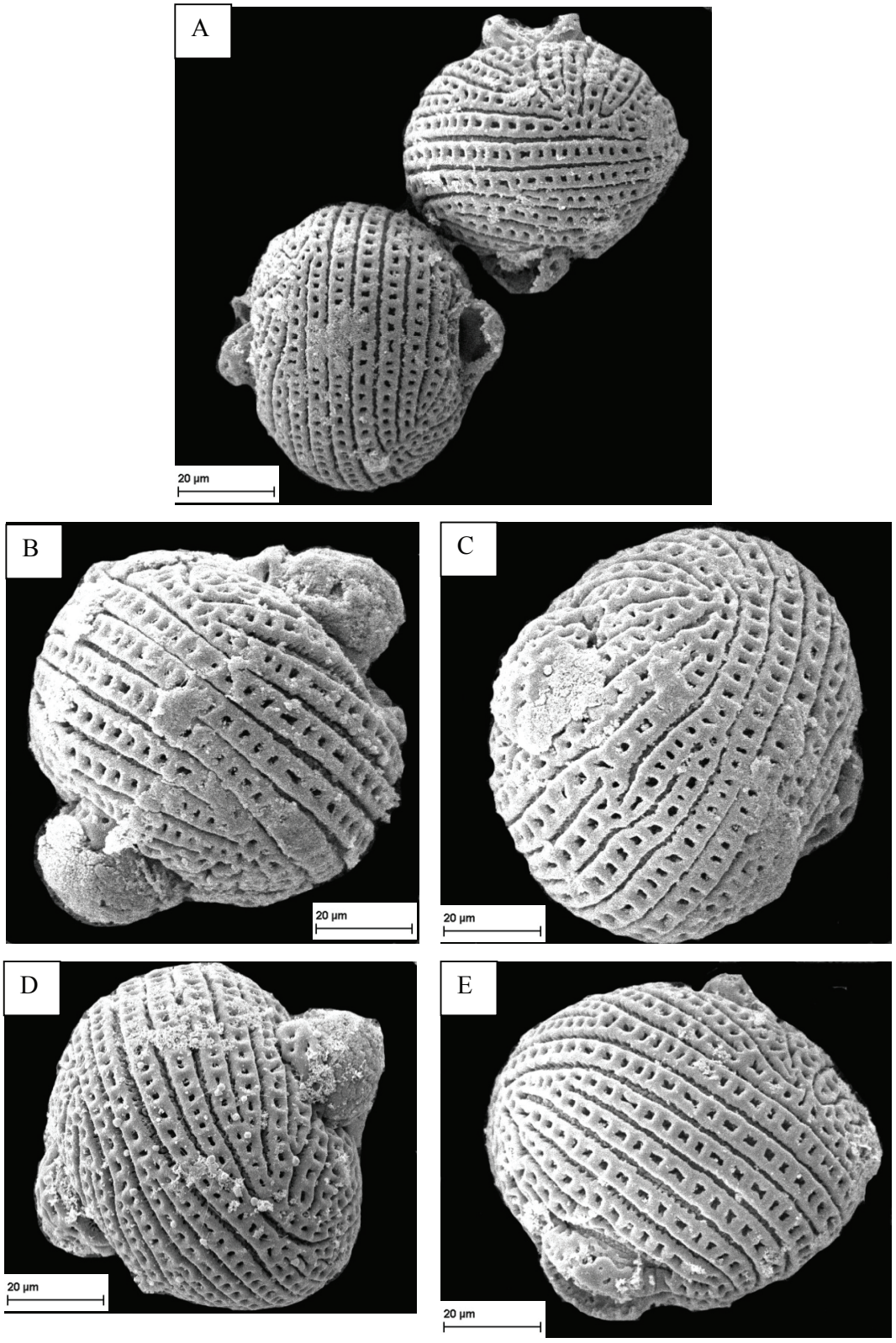

Figure 6. Pollen grains of *Sanchezia speciosa*: A) Equatorial view. Scale: A –E = 20 µm.

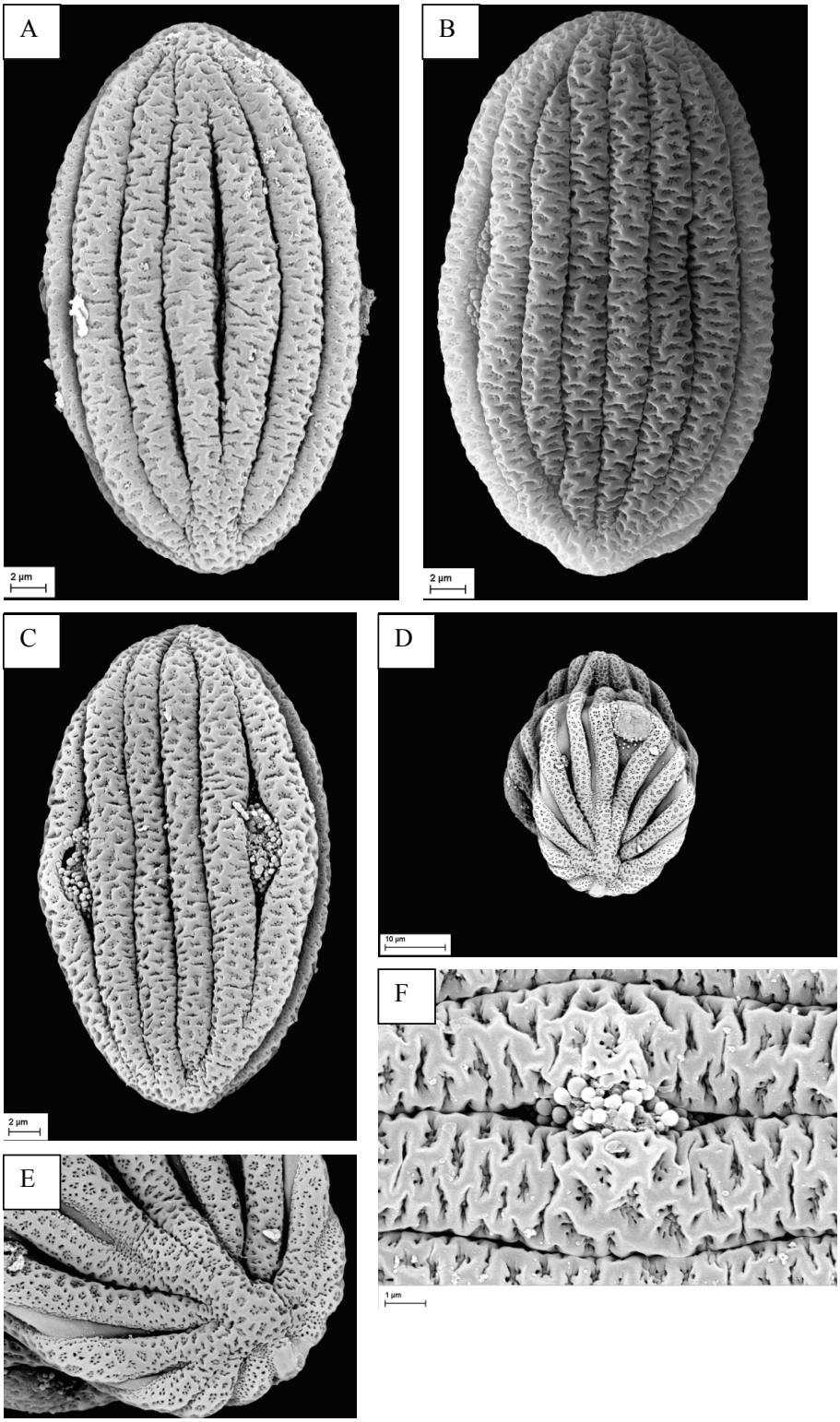

Figure 7. Pollen grains of *Strobilanthes crispa*: A-C) Equatorial view, D & E. Polar view, F) Exine ornamentation. Scale: A, B,C & E= 2 µm, D = 10 µm, F = 1 µm.