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# Pollen morphology of *Justicia* L. (Acanthaceae) from Thailand and its taxonomic value

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**Abstract**
The acetolysed pollen of 33 species of the genus *Justicia* in Thailand is investigated using both light and scanning electron microscopy. The pollen of the genus is characterised as being isopolar, bilaterally or radially symmetrical, with mostly prolate or prolate spheroidal shape. Based on characters with high factor loading in the cluster analysis and principal components analysis, the studied species are divided into two major groups; one with 3-colporate with six pseudocolpi and the other with 2-porate or 2–3-colporate with aperture areas. The pollen morphology of each group is described and illustrated. Pollen characters are useful for taxonomic delimitation and relationships among Thai species.

**Keywords:** *Acanthaceae, pollen morphology, taxonomy*

The genus *Justicia* L. belongs to Acanthaceae subtribe Justiciinae (Scotland & Vollesen, 2000), with seven and nine sections in the Old and the New Worlds, respectively (Graham, 1988). The genus is recognised by having simple spicate or compound inflorescences, rarely in solitary or sessile clusters, subtended by bracts and bracteoles, a tubular and bilabiate corolla, a fold or rugula along the centre of the upper lip and anthers with two completely superposed thecae. The circumscription of the genus here follows Graham (1988) and Hansen (1989) and includes species with a rising placenta in the capsule.

Numerous studies of pollen morphology within the Acanthaceae by various authors (e.g., Radlkofer, 1883; Lindau, 1895; Raj, 1961; Immelman, 1983; Graham, 1988; Hedrén, 1989; Furness, 1989, 1990, 1991, 1993, 1995, 1996; Ensermu, 1990; Hilsenbeck, 1990; Scotland, 1992, 1993; Furness & Grant, 1996; Daniel, 1998; Carine & Scotland, 1998; Wang & Blackmore, 2003) have revealed taxonomically useful characters. The early palynological observations of the family by Radlkofer (1883) were extended by Lindau (1895), who recognised 11 pollen types within the family, namely 'Daubenpollen', 'Glatter Pollen', 'Runder Pollen', 'Gürtelpollen', 'Knötchenpollen', 'Pollen von anderer Form', 'Rahmenpollen', 'Rippenpollen', 'Spangenpollen', 'Spaltenpollen', 'Stachelpollen' and 'Wabenpollen'. An understanding of pollen morphology derived from these previous studies has immense benefits for the understanding of relationships within Acanthaceae, in particular the pollen morphology of *Justicia*. Lindau (1895) classified the pollen of the genus as being 'Knötchenpollen', which is characterised by having two or three pores and an aperture area, with 1–3 rows of insulae on each side of the pore. Huang (1972), using data from Taiwanese *Justicia*, described the grains of *J. gendarussa*, *J. hayatai* var. *decumbens*, *J. procumbens* and *J. quadrifaria* as parasyncolpate (2–3-colporate). Additionally, Immelman (1983) studied the southern African species of *Justicia* and suggested that the number of colpi in the pollen was correlated with inflorescence type. An excellent account of the

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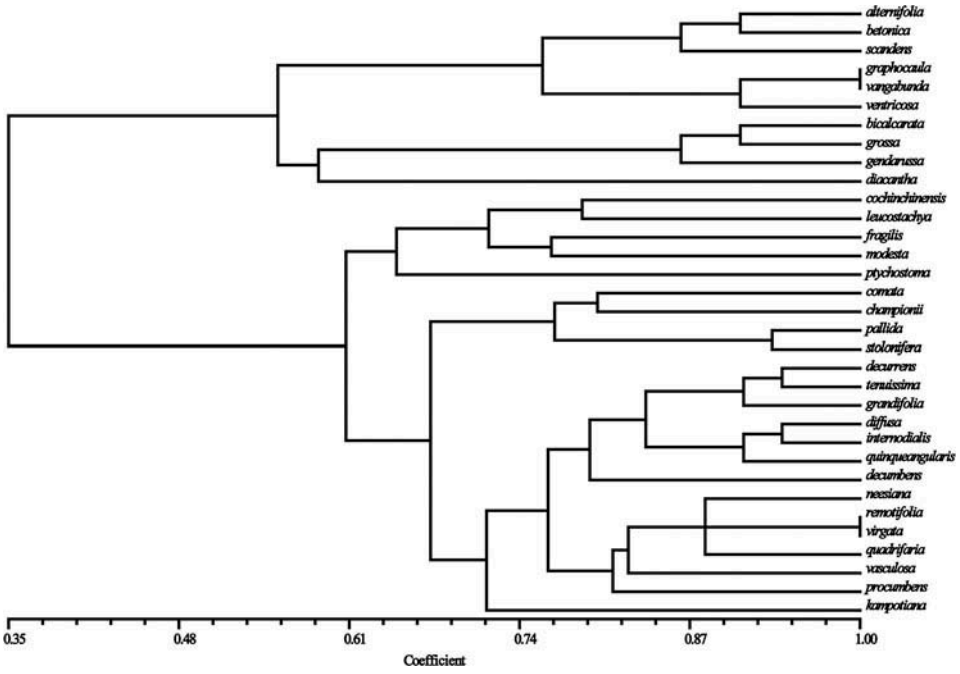


Figure 1. UPGMA phenogram resulting from cluster analysis of pollen morphology of Thai *Justicia* species. Major clusters 1 and 2 are indicated.

palynology of the genus was presented by Graham (1988). The author investigated the infrageneric classification of *Justicia* in both the Old and New Worlds and divided the pollen into ten types. Pollen of most species was characterised as 2–3-porate or colporate and subprolate to prolate (P/E 1.30–2.41), microreticulate in the aperture area and with 2–6 rows of insulae. Hedrén (1989) revised *Justicia* section *Harnieria* from tropical Africa, and showed that the pollen is characterised by two apertures and an aperture area with more than one row of insulae on each side of the pore. Ensermu (1990) studied the pollen of 12 taxa of *Justicia* section *Anellia* in Ethiopia, and described them as 2–4-porate, oval or triangular in outline in equatorial view and rectangular in polar view. One of the most important pollen studies of the genus was made by Hilsenbeck (1990). He divided the genus *Siphonoglossa s.l.* (=*Justicia*) from the New World into two pollen types: Type I, pollen is 3-colporate, with two pseudocolpi flanking each colpus ('Spangenpollen'), and type II, which is 2-porate ('Knötchenpollen').

Pollen morphology of *Justicia* in Thailand is still poorly understood. Therefore, this study aims to describe the pollen of Thai species and to evaluate the contribution of pollen data to the delimitation species on the basis of morphometric analyses.

## Material and methods

Pollen of 33 species of *Justicia* was collected in the field as well as from herbarium specimens in K and QBG (abbreviations follow Thiers, 2012). For each species, 1–2 individuals were sampled. Voucher specimens from the fieldwork were deposited in Khon Kaen University (KKU). The pollen was studied using light microscopy (LM) and scanning electron microscopy (SEM). Pollen samples were acetolysed following the technique of Erdtman (1960*a*), then mounted in silicone oil and sealed with paraffin. Observations and measurements of ten pollen grains were conducted using an Olympus BH2 light microscope. Polar axis, equatorial diameter and exine thickness at equator and pole were recorded. For the SEM study, droplets of pollen-ethanol suspension were placed on stubs with double-sided cellophane tape and dried, then sputter-coated with a gold-palladium mixture. Measurements of pseudocolpi length, insulae diameter, aperture area length and width, colpi length, pore diameter and mesocolpium width were observed with a LEO 1450 VP SEM. The terminology used follows Lindau (1895), Erdtman (1960*b*), Graham (1988) and Punt et al. (2007).

Morphometric studies of the pollen was based on 36 characters, 27 of which were quantitative, nine were qualitative (Table I). Multivariate analyses were carried out using NTSYS-PC version 2.1 (Rohlf, 1990). Two analyses were performed: cluster analysis (CA) and principal components analysis (PCA). CA was conducted using the unweighted pair-group method with arithmetic average (UPGMA) in SAHN. Ordination using PCA was also investigated. The first three eigenvectors were extracted from the correlation matrix.

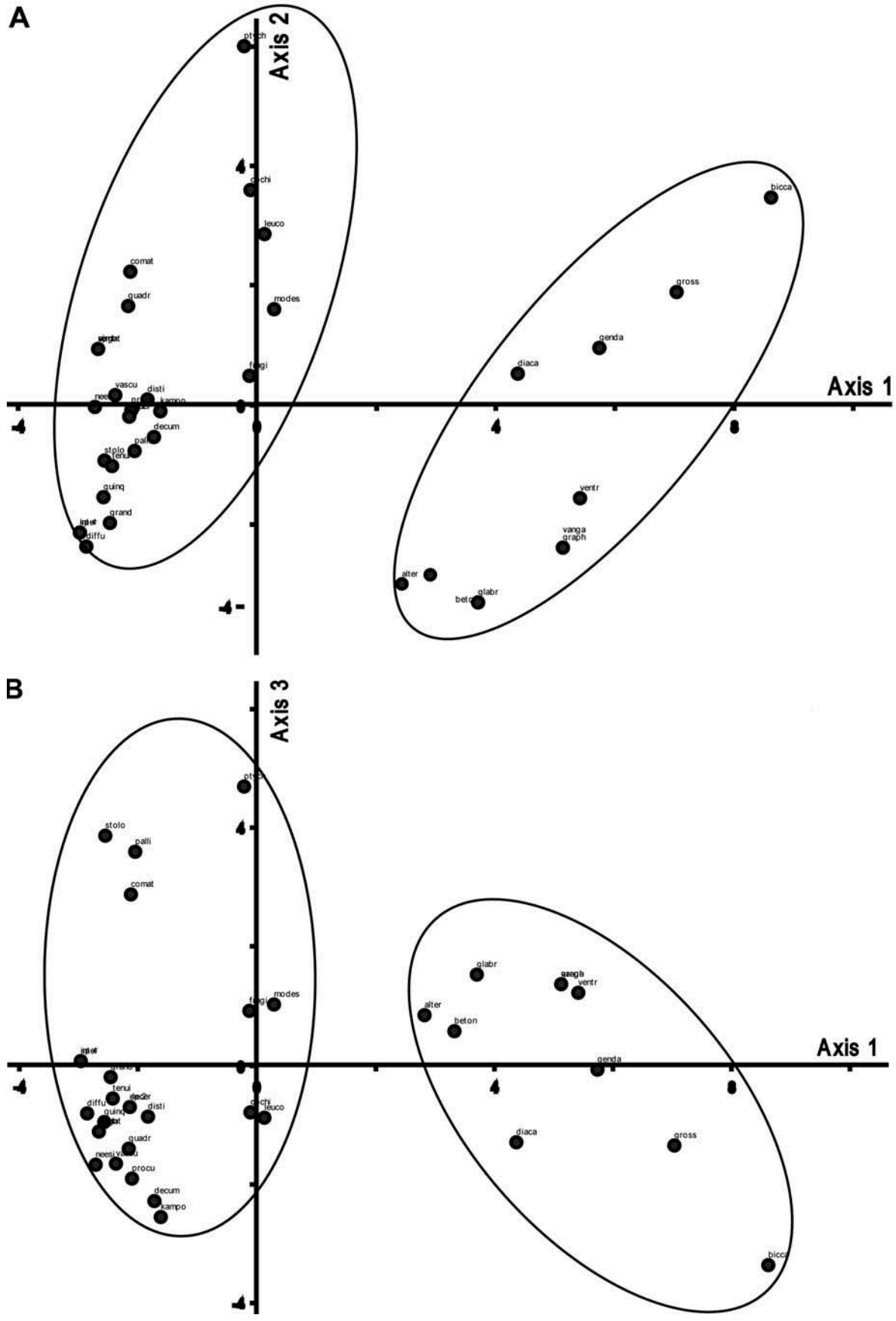


Figure 2. PCA of the pollen morphology. **A.** Plot of the PCA axis I against axis II accounts for 43.62% of total variance. **B.** Plot of the PCA axis I against axis III accounts for 40.56% of total variance.

## Results

### *General pollen morphology*

Pollen morphology is summarised in Table II. Pollen is isopolar, bilaterally or radially symmetrical, 2-porate, 2–3-colporate with aperture areas or 3-colporate with six pseudocolpi. The aperture area surrounding the pore has 2–6 rows of 3–33 insulae. The pseudocolpi are long and flank each colpus in 3-colporate pollen. The size of the pollen varies from small to medium ($P = 13.8$–$55.2\,\mu m$, $E = 9.8$–$41.3\,\mu m$). The shape is mostly prolate ($P/E = 1.32$–$1.66\,\mu m$) or prolate spheroidal ($P/E = 1.08$–$1.11\,\mu m$), circular to elliptic or triangular in polar view, elliptic in equatorial view. The ectocolpi show lolongate or lalongate pores, 1.5–6.1 μm in diameter. The exine thickness is 0.9–3.9 μm at equator, 1.2–4.7 μm at the poles, and is usually thicker at the poles than at the equator. Ornamentation in the aperture area and ectocolpus membrane is granulate or microreticulate, and at the mesocolpium is microreticulate or reticulate with scattered granules in muri.

### *Morphometric analyses*

The phenogram obtained from the CA (Figure 1) has a cophenetic correlation of 0.88, and therefore a high reliability. Two major groups are recognised. The first group consists of ten species and the second one is the larger group with 23 species.

The PCA of 36 pollen characters is visualised by the first three principal components, their eigenvalues and equivalent percentage of the variance accounted (Table III). The first three axes show 53.1% of the total variation, each one contributing 31%, 12.5% and 9.5%, respectively. Plots of the scores for each species in relation to the first three components are illustrated in Figure 2. As shown in Table I, axis I consists of the strongest

Table I. Loading on the first three principal components of 36 characters. The important characters show high factor loading > ±0.6.

| | Pollen characters | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|---|
| 1. | 3-colporate | 0.8882 | –0.2117 | 0.0864 |
| 2. | 2-colporate | –0.6971 | –0.0311 | –0.6207 |
| 3. | 2-porate | –0.2001 | 0.3353 | 0.7865 |
| 4. | pseudocolpi length (0 μm) | 0.9334 | –0.2831 | 0.0274 |
| 5. | pseudocolpi length (1–25 μm) | 0.6168 | –0.5258 | 0.2094 |
| 6. | pseudocolpi length (26–35 μm) | 0.6335 | 0.2938 | –0.2559 |
| 7. | polar axis length (1–30 μm) | –0.5932 | –0.2743 | 0.3218 |
| 8. | polar axis length (31–40 μm) | 0.5932 | 0.2743 | –0.3218 |
| 9. | equatorial axis length (1–30 μm) | –0.4105 | –0.0844 | 0.1224 |
| 10. | equatorial axis length (31–40 μm) | 0.4105 | 0.0844 | –0.1224 |
| 11. | exine thickness at equator (1–2 μm) | –0.6778 | –0.3664 | –0.0299 |
| 12. | exine thickness at equator (2.1–3 μm) | 0.7267 | 0.3726 | 0.0582 |
| 13. | shape (*P*/*E*) (1.08–1.11 μm) | –0.4297 | –0.2720 | 0.3033 |
| 14. | shape (*P*/*E*) (1.32–1.66 μm) | 0.4297 | 0.2720 | –0.3033 |
| 15. | row of insulae (0) | –0.9334 | 0.2831 | –0.0274 |
| 16. | row of insulae (1–2) | –0.8244 | 0.3011 | 0.0136 |
| 17. | row of insulae (3–6) | –0.0803 | –0.0079 | –0.2295 |
| 18. | number of insulae (2–5) | –0.0109 | 0.4703 | 0.4236 |
| 19. | number of insulae (6–18) | –0.7322 | 0.0390 | –0.0371 |
| 20. | number of insulae (19–35) | –0.1194 | –0.0358 | –0.3124 |
| 21. | insulae diameter (1–2 μm) | –0.4027 | –0.3715 | 0.1325 |
| 22. | insulae diameter (2.1–3 μm) | –0.4281 | 0.5726 | –0.1720 |
| 23. | aperture area length (0 μm) | –0.9334 | 0.2831 | –0.0274 |
| 24. | aperture area length (10–25 μm) | –0.7718 | –0.2244 | –0.2272 |
| 25. | aperture area length (26–35 μm) | –0.0762 | 0.6065 | 0.2580 |
| 26. | aperture area width (5–10 μm) | –0.5698 | –0.2256 | 0.1270 |
| 27. | aperture area width (11–15 μm) | –0.2893 | 0.5196 | –0.1642 |
| 28. | aperture area granulate | –0.5647 | 0.2499 | 0.3136 |
| 29. | aperture area reticulate | –0.3212 | 0.0019 | –0.4103 |
| 30. | colpi length (0 μm) | 0.2001 | –0.3353 | –0.7865 |
| 31. | colpi length (1–20 μm) | –0.5449 | –0.2650 | –0.5798 |
| 32. | colpi length (21–41 μm) | 0.7754 | 0.0324 | 0.0287 |
| 33. | pore diameter (1–3 μm) | –0.0877 | –0.7248 | 0.2278 |
| 34. | pore diameter (3.1–4 μm) | 0.0877 | 0.7248 | –0.0838 |
| 35. | mesocolpium width (1–10 μm) | –0.2321 | –0.4227 | 0.0626 |
| 36. | mesocolpium width (11–20 μm) | 0.2321 | 0.4227 | –0.0626 |

Table II. Pollen morphology and measurements of Thai *Justicia* species investigated.

| Taxa | Ap | *P* | *E* | *P*/*E* | Eq | Ep | Ri | Ni | Tl | Tw | Mw |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Group I* | | | | | | | | | | | |
| *J. alternifolia* | 3-colporate-6 pseudocolpi | 19.2 ± 1.7 (18.0–20.4) | 11.5 ± 1.5 (10.4–12.5) | 1.66 | 1.4 ± 0.0 (1.3–1.4) | — | — | — | — | — | 5.7 ± 0.6 (5.1–6.4) |
| *J. betonica* | 3-colporate-6 pseudocolpi | 38.1 ± 8.9 (25.4–55.2) | 23.1 ± 5.1 (15.5–33.4) | 1.64 | 2.0 ± 0.5 (1.0–3.0) | — | — | — | — | — | 4.7 ± 0.6 (4.2–5.1) |
| *J. bicalcarata* | 3-colporate-6 pseudocolpi | 43.6 ± 1.6 (40.7–46.6) | 39.2 ± 1.5 (36.9–41.3) | 1.11 | 2.2 ± 0.5 (1.6–3.1) | — | — | — | — | — | 14.2 ± 1.1 (12.9–15.0) |
| *J. diacantha* | 3-colporate-6 pseudocolpi | 36.9 ± 3.6 (28.4–41.6) | 33.0 ± 3.0 (25.9–36.5) | 1.11 | 2.3 ± 0.4 (1.5–3.0) | — | — | — | — | — | 9.5 ± 3.0 (6.1–11.6) |
| *J. gendarussa* | 3-colporate-6- pseudocolpi | 40.5 ± 5.6 (28.6–52.5) | 26.5 ± 2.7 (22.4–30.9) | 1.52 | 2.8 ± 0.5 (1.5–3.9) | — | — | — | — | — | 9.0 ± 3.4 (6.6–11.5) |
| *J. graphocaula* | 3-colporate-6 pseudocolpi | 39.1 ± 3.2 (30.6–42.7) | 29.5 ± 2.7 (23.2–34.5) | 1.32 | 2.6 ± 0.4 (2.0–3.6) | — | — | — | — | — | 7.4 ± 1.5 (6.4–8.6) |
| *J. grossa* | 3-colporate-6- pseudocolpi | 36.5 ± 1.3 (35.4–37.3) | 33.6 ± 1.4 (32.2–34.3) | 1.08 | 2.3 ± 1.2 (1.5–3.2) | — | — | — | — | — | 17.1 ± 2.9 (15.7–19.9) |
| *J. scandens* | 3-colporate-6- pseudocolpi | 27.6 ± 3.9 (21.4–32.7) | 18.6 ± 2.4 (13.0–22.0) | 1.48 | 1.8 ± 0.4 (1.2–2.8) | — | — | — | — | — | 7.2 ± 0.7 (6.7–8.1) |
| *J. vagabunda* | 3-colporate-6 pseudocolpi | 34.9 ± 4.9 (28.8–46.4) | 23.7 ± 4.4 (17.8–30.7) | 1.47 | 2.2 ± 0.4 (1.2–2.8) | — | — | — | — | — | 8.4 ± 1.0 (7.3–9.4) |
| *J. ventricosa* | 3-colporate-6 pseudocolpi | 40.8 ± 4.9 (30.6–47.4) | 28.4 ± 3.3 (22.5–32.4) | 1.43 | 2.6 ± 0.5 (1.6–3.5) | — | — | — | — | — | 15.5 ± 1.7 (14.3–16.8) |
| *Group II: subgroup 1* | | | | | | | | | | | |
| *J. championii* | 2-porate | 32.7 ± 3.9 (26.8–40.7) | 23.0 ± 2.4 (19.2–25.9) | 1.42 | 1.9 ± 0.3 (1.3–2.3) | 2.6 ± 0.5 (1.6–3.3) | 2 | 14–16 | 26.0 ± 3.1 (22.4–26.8) | 8.9 ± 1.2 (8.0–9.8) | 9.0 ± 2.5 (7.2–10.8) |
| *J. cochinchinensis* | 2-colporate | 42.2 ± 5.4 (33.9–51.5) | 26.6 ± 3.4 (20.4–35.8) | 1.58 | 2.4 ± 0.6 (1.5–3.6) | 3.3 ± 0.8 (1.8–4.7) | 2 | 12–14 | 28.9 ± 2.2 (27.3–30.5) | 11.2 ± 1.6 (10.0–12.4) | 16.2 ± 1.6 (15.0–17.4) |
| *J. comata* | 2-porate | 22.9 ± 1.8 (20.8–24.4) | 15.8 ± 3.4 (11.8–17.8) | 1.44 | 1.4 ± 0.3 (1.3–1.8) | 1.9 ± 0.1 (1.8–2.1) | 2 | 10 | 22.6 ± 2.5 (20.3–25.3) | 9.9 ± 3.0 (6.5–12.5) | 11.5 ± 3.6 (9.0–14.2) |
| *J. decumbens* | 2-colporate | 36.1 ± 3.5 (31.1–41.7) | 22.8 ± 1.7 (20.5–24.6) | 1.58 | 1.7 ± 0.3 (1.2–2.3) | 2.6 ± 0.6 (2.1–3.6) | 2 | 11–13 | 22.2 ± 0.6 (21.6–22.8) | 11.3 ± 1.6 (10.4–12.3) | 18.0 ± 5.0 (13.4–22.7) |
| *J. decurrens* | 2-colporate | 38.5 ± 5.4 (30.9–48.2) | 23.7 ± 3.8 (18.3–28.7) | 1.62 | 1.8 ± 0.3 (1.2–2.5) | 2.7 ± 0.5 (2.0–3.5) | 2 | 12–14 | 21.7 ± 0.1 (21.6–21.8) | 7.5 ± 2.9 (9.6–11.7) | 16.1 ± 7.0 (11.2–21.1) |
| *J. diffusa* | 2-colporate | 21.4 ± 2.5 (17.2–24.6) | 15.7 ± 1.8 (13.4–18.1) | 1.36 | 1.5 ± 0.2 (1.2–2.0) | 2.6 ± 0.4 (1.8–3.4) | 2 | 12 | 12.0 ± 0.0 (12.1–12.2) | 5.0 ± 0.9 (4.1–5.5) | 8.7 ± 0.14 (8.6–8.8) |
| *J. grandifolia* | 2-colporate | 33.5 ± 3.6 (28.2–39.0) | 20.7 ± 2.1 (17.2–25.0) | 1.61 | 1.9 ± 0.3 (1.2–2.4) | 2.8 ± 0.5 (2.0–3.7) | 2 | 8–10 | 18.2 ± 5.0 (14.7–21.8) | 5.8 ± 1.1 (5.0–6.6) | 9.2 ± 2.8 (7.2–11.2) |
| *J. internodialis* | 2-colporate | 28.5 ± 3.3 (22.4–32.5) | 20.4 ± 1.9 (16.3–22.4) | 1.39 | 1.6 ± 0.4 (1.0–2.2) | 2.2 ± 0.4 (1.2–3.0) | 2 | 9–10 | 15.5 ± 2.7 (12.3–17.4) | 7.3 ± 1.6 (5.6–8.8) | 6.0 ± 2.1 (4.5–7.6) |

(*Continued*)

Table II. (*Continued*).

| Taxa | Ap | *P* | *E* | *P*/*E* | Eq | Ep | Ri | Ni | Tl | Tw | Mw |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *J. kampotiana* | 2-colporate | 32.0 ± 1.5 (29.7–33.4) | 20.2 ± 1.1 (18.1–22.4) | 1.58 | 1.4 ± 0.3 (0.9–2.0) | 2.1 ± 0.3 (1.6–2.6) | 5–6 | 26–33 | 17.0 ± 5.2 (13.2–20.6) | 10.0 ± 2.1 (9.1–12.1) | 6.9 ± 1.5 (6.3–8.2) |
| *J. leucostachya* | 2-colporate | 34.6 ± 2.0 (31.1–37.8) | 23.3 ± 1.9 (20.8–26.9) | 1.48 | 2.1 ± 0.4 (1.3–2.7) | 3.9 ± 0.3 (2.6–4.1) | 2 | 13–15 | 28.0 ± 0.0 (28.6–28.7) | 14.3 ± 1.6 (13.2–15.5) | 10.7 ± 0.2 (10.6–10.9) |
| *J. neesiana* | 2-colporate | 26.0 ± 2.3 (23.4–28.3) | 17.4 ± 1.8 (14.7–19.5) | 1.49 | 1.4 ± 0.1 (1.3–1.6) | 2.3 ± 0.4 (1.7–2.9) | 2 | 10–14 | 24.0 ± 1.1 (23.0–25.2) | 8.4 ± 0.2 (8.2–8.6) | 10.0 ± 0.2 (9.8–10.2) |
| *J. pallida* | 2-porate | 30.3 ± 2.7 (23.0–34.3) | 18.2 ± 1.8 (12.9–20.4) | 1.66 | 1.3 ± 0.2 (1.0–1.5) | 1.8 ± 0.2 (1.5–2.2) | 2 | 10–13 | 15.0 ± 6.2 (9.7–22.1) | 8.0 ± 4.1 (5.5–12.9) | 10.0 ± 0.7 (9.6–10.0) |
| *J. procumbens* | 2-colporate | 32.8 ± 5.7 (23.1–40.2) | 21.3 ± 2.8 (16.2–28.3) | 1.53 | 1.7 ± 0.4 (1.2–2.6) | 2.8 ± 1.0 (1.5–4.2) | 2 | 11–14 | 25.0 ± 0.0 (24.9–25.0) | 10.2 ± 0.8 (9.4–11.1) | 10.6 ± 1.4 (9.6–11.6) |
| *J. ptychostoma* | 2-porate | 27.9 ± 2.4 (24.2–32.6) | 18.6 ± 1.5 (15.8–20.6) | 1.50 | 2.1 ± 0.5 (1.2–3.2) | 3.0 ± 0.4 (2.3–3.5) | 2 | 3–4 | 26.5 ± 1.3 (25.4–28.1) | 12.3 ± 0.4 (12.0–12.0) | 10.0 ± 0.9 (9.6–11) |
| *J. quadrifaria* | 2-colporate | 28.0 ± 2.0 (23.5–30.5) | 19.2 ± 2.4 (15.7–23.2) | 1.45 | 1.7 ± 0.3 (1.0–2.5) | 2.7 ± 0.4 (1.8–3.2) | 2 | 9–10 | 26.2 ± 0.8 (25.6–26.8) | 12.0 ± 1.0 (11.0–13.0) | 10.9 ± 1.0 (10.2–11.7) |
| *J. quinqueangularis* | 2-colporate | 27.2 ± 1.3 (25.3–28.4) | 19.7 ± 1.3 (17.4–21.2) | 1.38 | 1.6 ± 0.2 (1.3–1.8) | 2.4 ± 0.6 (1.8–3.3) | 2 | 10–12 | 17.0 ± 2.1 (15.9–19.0) | 6.7 ± 0.9 (6.1–7.4) | 11.9 ± 1.2 (11.0–12.8) |
| *J. remotifolia* | 2-colporate | 28.4 ± 1.5 (25.7–29.7) | 19.9 ± 1.3 (18.5–21.9) | 1.42 | 1.4 ± 0.4 (1.0–2.0) | – | 2 | 8–12 | 22.6 ± 8.6 (14.7–31.9) | 13.9 ± 4.7 (8.8–18.1) | 7.9 ± 0.0 (7.8–8.0) |
| *J. stolonifera* | 2-porate | 22.2 ± 5.9 (13.8–27.3) | 15.0 ± 3.6 (9.8–17.7) | 1.48 | 1.6 ± 0.4 (1.0–2.1) | 2.5 ± 0.4 (2.1–3.2) | 2 | 8–10 | 14.0 ± 1.5 (12.1–14.3) | 8.5 ± 0.7 (8.0–9.1) | 6.9 ± 0.4 (6.6–7.3) |
| *J. tenuissima* | 2-colporate | 34.8 ± 4.9 (32.0–43.5) | 22.1 ± 4.7 (16.6–29.4) | 1.57 | 1.9 ± 0.3 (1.3–2.3) | 2.8 ± 0.2 (2.4–3.1) | 2 | 10–12 | 20.0 ± 1.6 (17.3–20.6) | 7.4 ± 0.4 (7.7–8.1) | 9.3 ± 1.3 (7.7–10.2) |
| *J. vasculosa* | 2-colporate | 29.5 ± 3.9 (25.2–35.8) | 21.9 ± 2.4 (18.9–27.2) | 1.34 | 1.6 ± 0.4 (1.1–2.3) | 2.1 ± 0.3 (1.6–2.5) | 2 | 12–14 | 23.0 ± 1.5 (21.5–23.7) | 11.5 ± 2.6 (9.7–13.4) | 11.0 ± 0.7 (10.0–12.0) |
| *J. virgata* | 2-colporate | 23.0 ± 1.8 (19.5–27.1) | 15.5 ± 1.6 (11.5–17.6) | 1.48 | 1.4 ± 0.1 (1.2–1.7) | 2.1 ± 0.4 (1.3–2.9) | 2 | 10–11 | 22.0 ± 2.5 (18.9–22.5) | 12.2 ± 0.9 (11.5–12.9) | 7.6 ± 0.8 (7.0–8.2) |
| *Group II: subgroup 2* | | | | | | | | | | | |
| *J. fragilis* | 3-colporate | 39.3 ± 3.7 (34.6–46.1) | 26.2 ± 2.6 (20.4–30.0) | 1.50 | 2.3 ± 0.3 (2.0–2.8) | — | 2 | 8–10 | 34.0 ± 2 (32.0–36.0) | 10.7 ± 1.5 (9.6–11.8) | 10.1 ± 3.9 (7.4–13.0) |
| *J. modesta* | 3-colporate | 40.2 ± 3.8 (34.2–49.9) | 25.6 ± 3.4 (19.6–29.4) | 1.57 | 2.0 ± 0.4 (1.3–2.5) | — | 2 | 10–12 | 37.0 ± 3.7 (35.2–43.0) | 11.8 ± 1.7 (10.3–13.8) | 11.6 ± 2.2 (10.0–13.2) |

Notes: Measurements represent mean, low and high values. Ap = aperture type; *P* = length of polar axis; *E* = equatorial diameter; *P*/*E* = ratio of polar axis and equatorial axis; Ep = exine thickness at pole; Eq = exine thickness at equator; Ri = number of row of insulae; Ni = number of insulae; Tl = length of aperture area; Tw = width of aperture area; Mw = width of mesocolpium; — = no data. All units in micrometres.

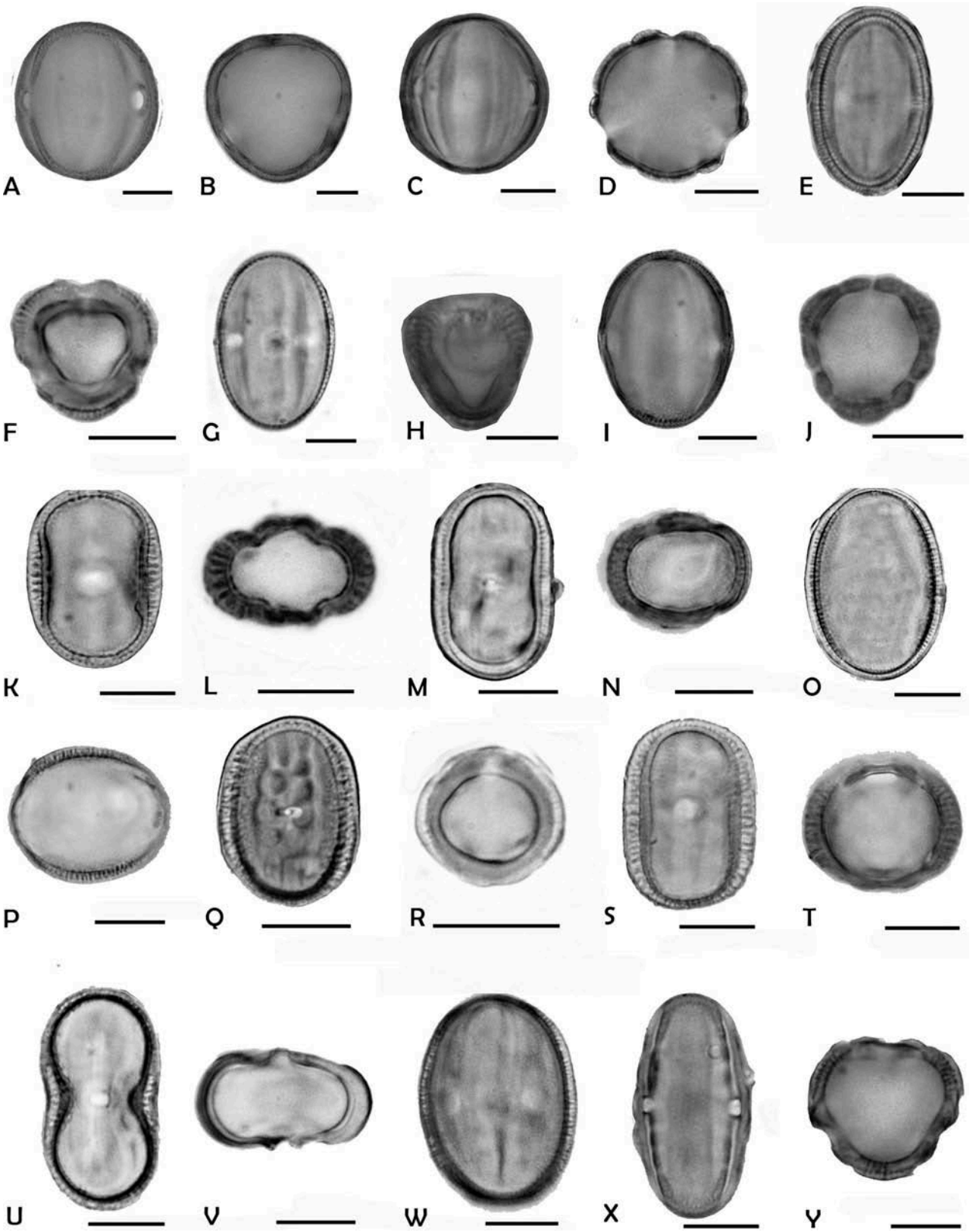


Figure 3. **A–J.** LM micrographs of pollen group I. **A, B.** *Justicia bicalcarata*; **C, D.** *J. diacantha*; **E, F.** *J. gendarussa*; **G, H.** *J. vagabunda*; **I, J.** *J. ventricosa*. **K–Y.** Pollen group II: **K, L.** *J. championii*; **M, N.** *J. decurrens*; **O, P.** *J. decumbens*; **Q, R.** *J. internodialis*; **S, T.** *J. leucostachya*; **U, V.** *J. ptychostoma*; **W.** *J. fragilis*; **X, Y.** *J. modesta*. Whole grain in equatorial view (A, C, E, G, I, K, M, O, Q, S, U, W, X) and in polar view (B, D, F, H, J, L, N, P, R, T, V, Y). Scale bars – 30 μm.

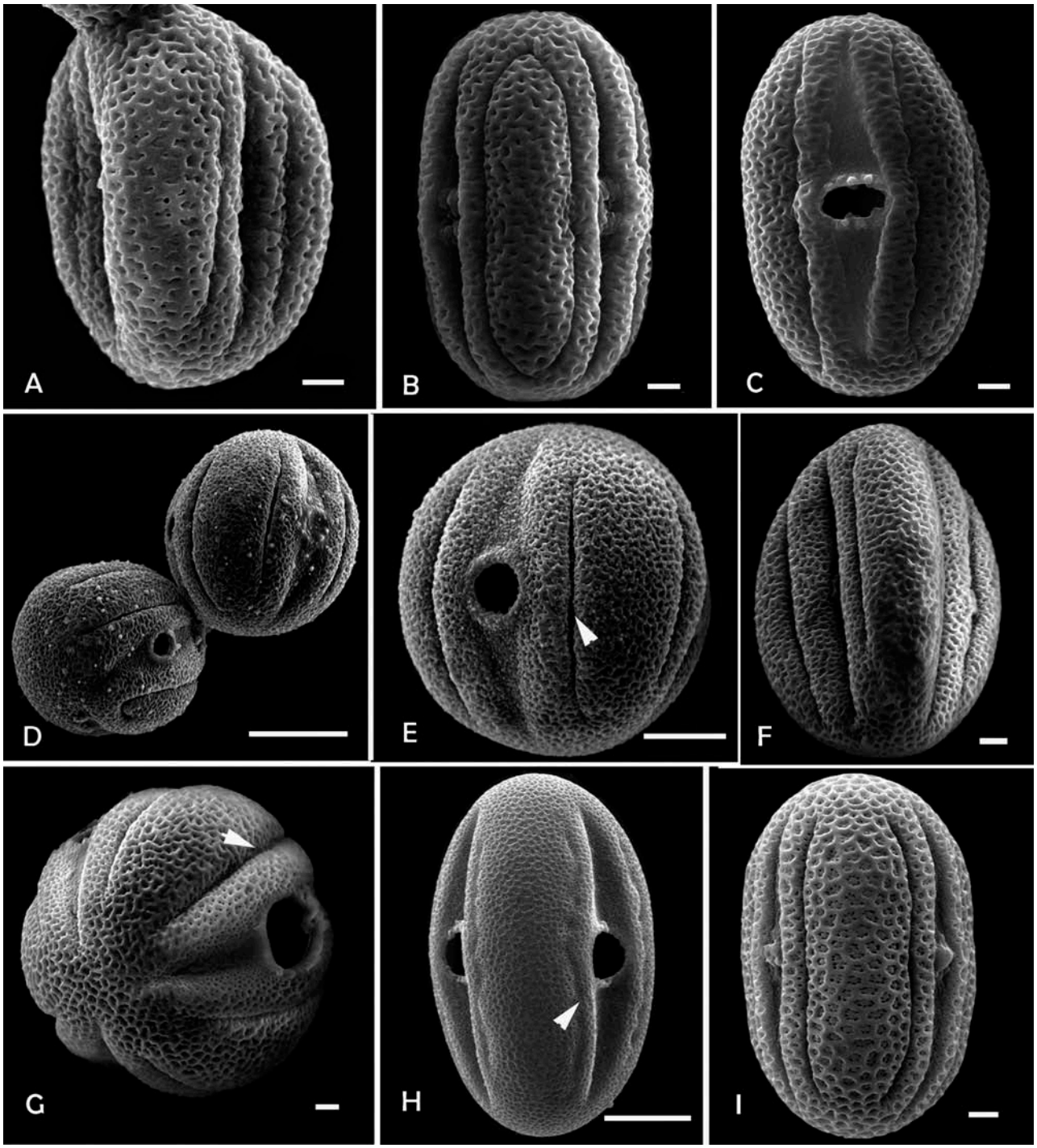


Figure 4. **A–I.** SEM micrographs of pollen group I: **A.** *Justicia alternifolia*; **B, C.** *J. betonica*; **D, E.** *J. bicalcarata*; **F, G.** *J. diacantha*; **H.** *J. gendarussa*; **I.** *J. scandens*. Arrows indicate pseudocolpi. Scale bars –10 μm (D, E, H), 2 μm (A, B, C, F, G, I).

characters, which include presence/absence of pseudocolpi, aperture area, insulae, row of insulae (1–2), 3-colporate, pseudocolpi length, number of insulae (6–8), aperture area length (10–25 μm), 2-colporate, colpi length (21–41 μm) and exine thickness at equator, which separated the second cluster from the other groups. However, the pore diameter (1–4 μm) and length of aperture area (26–35 μm) are important characters on axis II. The lack of colpi, the number of apertures and the type of apertures (2-porate) are important characters on axis III (characters with a high factor loading $> \pm 0.6$). Therefore, the first and second axes strongly divide the studied species into two groups.

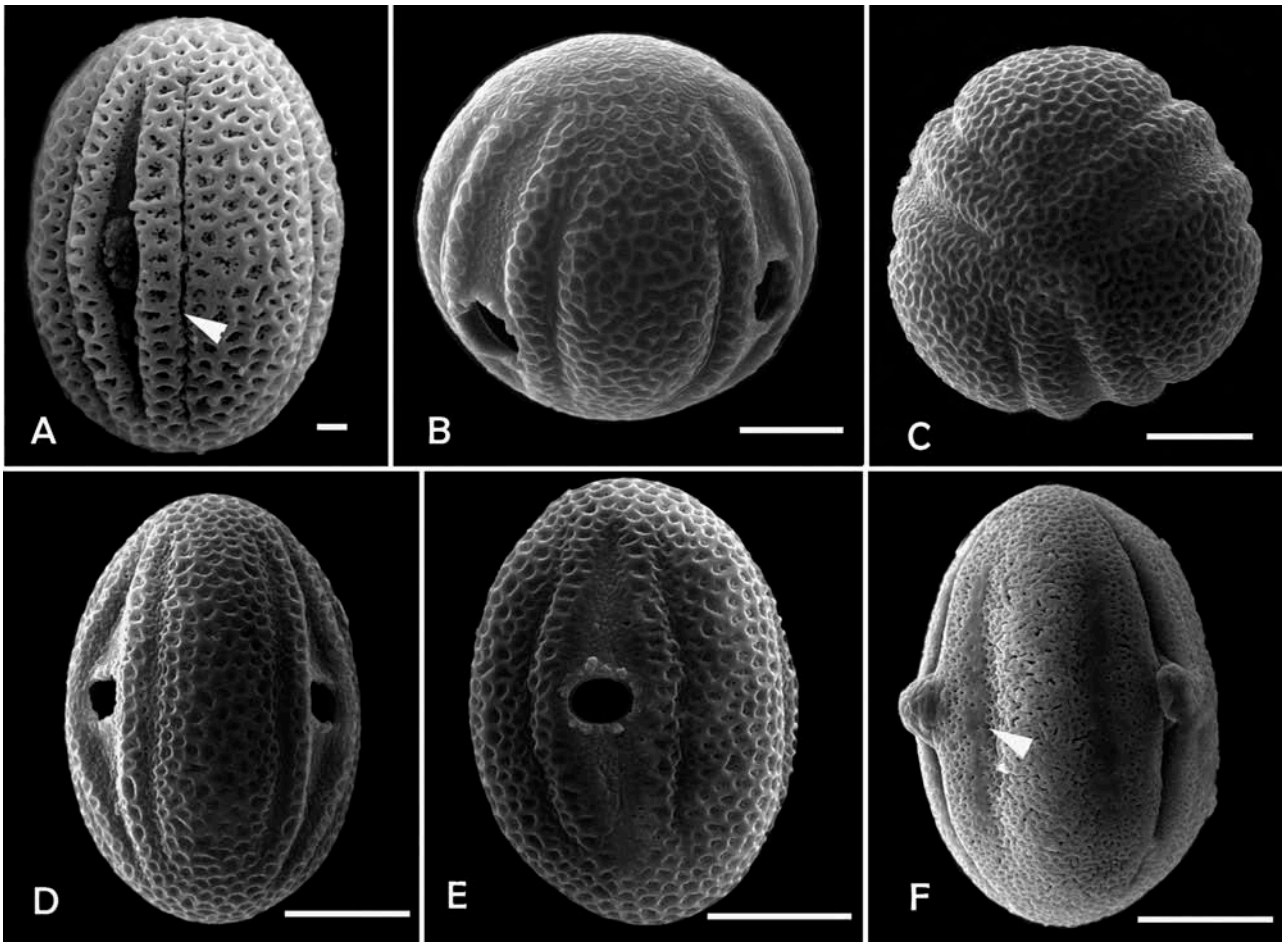


Figure 5. **A–F.** SEM micrographs of pollen group I: **A.** *Justicia graphocaula*; **B, C.** *J. grossa*; **D, E.** *J. vagabunda*; **F.** *J. ventricosa*. Arrows indicate pseudocolpi. Scale bars – 10 μm (B, C, D, E, F), 2 μm (A).

*Key to pollen groups*

(1) Pollen with pseudocolpi Group I
(2) Pollen without pseudocolpi Group II

*Group I.* — Pollen is 3-colporate with six pseudocolpi, $P = 18.0–55.2$ μm, $E = 10.4–41.3$ μm. The shape is mostly prolate ($P/E = 1.32–1.66$ μm) or rarely prolate spheroidal in *Justicia bicalcarata*, *J. diacantha* and *J. grossa* ($P/E = 1.08–1.11$ μm), circular or triangular in polar view, elliptic in equatorial view. The exine thickness at the pole ranges 1–3.9 μm. Ornamentation at ectocolpus membrane is granulate, at the mesocolpium is microreticulate or reticulate with scattered granules in muri (Figures 3A–J, 4, 5).

Species included: *Justicia alternifolia*, *J. betonica*, *J. bicalcarata*, *J. diacantha*, *J. gendarussa*, *J. graphocaula*, *J. grossa*, *J. scandens*, *J. vagabunda* and *J. ventricosa*.

*Group II.* — Pollen is 2-porate or 2–3-colporate, $P = 13.8–51.5$ μm, $E = 9.8–35.8$ μm. The shape is prolate ($P/E = 1.33–1.66$ μm), circular to elliptic or rarely triangular in polar view, elliptic in equatorial view. The aperture area comprises 2–6 rows of 3–33 insulae. The exine thickness ranges from 0.9–3.6 μm at the equator to 1.2–4.7 μm at the poles, usually thicker at the poles than at the equator. Ornamentation of the insulae resembles that found at the mesocolpium. Ornamentation at the aperture area and ectocolpus membrane is granulate or microreticulate and at the mesocolpium is microreticulate or reticulate with scattered granules in muri. This group is divided into two subgroups (Figures 3K–Y, 6, 7) as follows.

*Subgroup 1.* — Pollen is 2-porate or 2-colporate, bilaterally symmetrical. The shape in polar view is circular to elliptic, elliptic in equatorial view. The aperture area provides 2–6 rows of 3–33 insulae (Figures 3K–V, 6, 7A–G).

Species included: *Justicia championii*, *J. cochinchinensis*, *J. comata*, *J. decurrens*, *J. decumbens*, *J. diffusa*, *J. grandifolia*, *J. internodialis*, *J. kampotiana*, *J.*

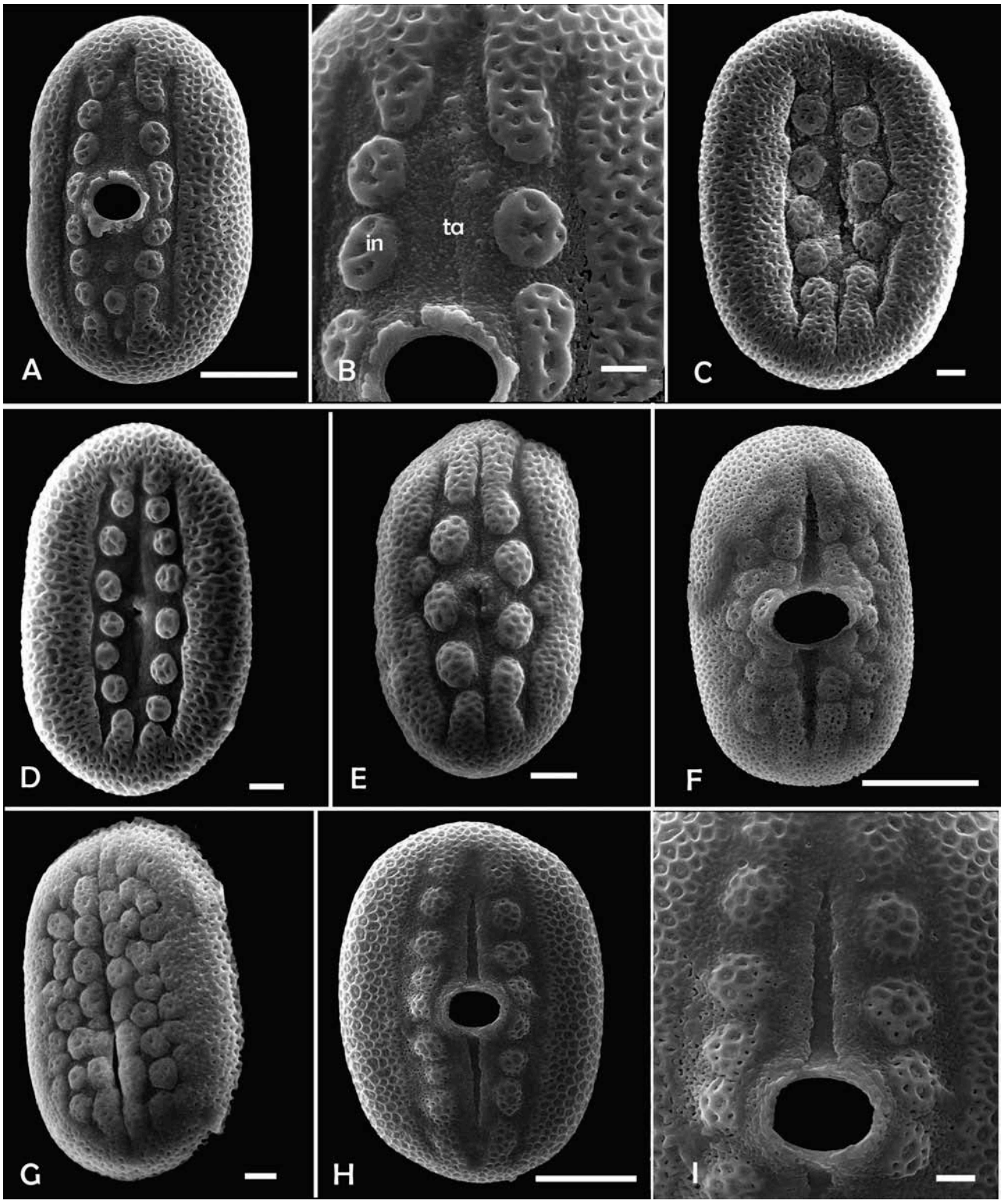


Figure 6. **A–I.** SEM micrographs of pollen group II: **A, B.** *Justicia cochinchinensis*; **C.** *J. decumbens*; **D.** *J. grandifolia*; **E.** *J. internodialis*; **F, G.** *J. kampotiana*; **H, I.** *J. leucostachya*. Abbreviations: in, insulae; ta, aperture area. Scale bars – 10 μm (A, F, H), 3 μm (D, E, G), 2 μm (B, C, I).

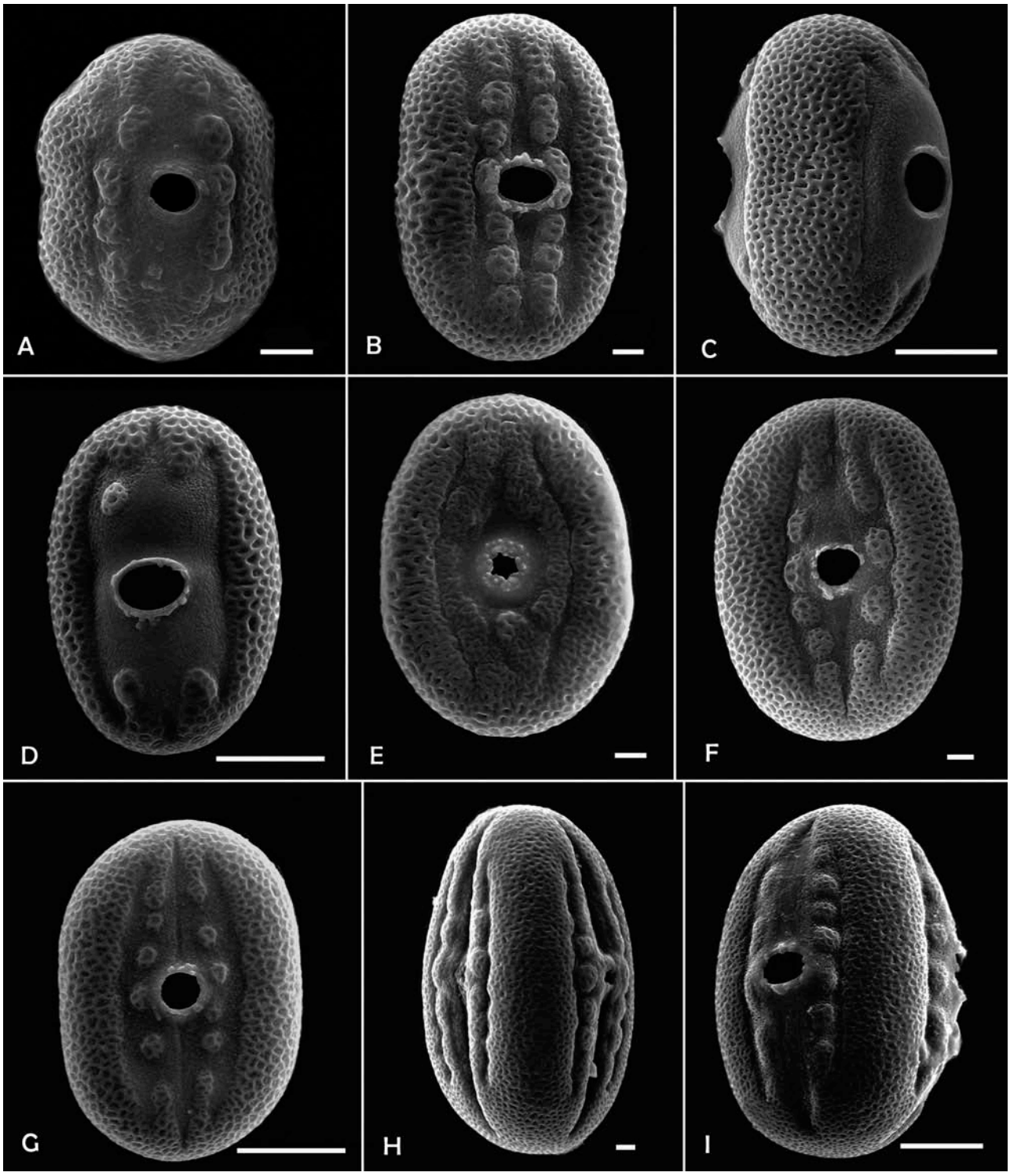


Figure 7. **A–I.** SEM micrographs of pollen group II: **A.** *Justicia pallida*; **B.** *J. procumbens*; **C, D.** *J. ptychostoma*; **E.** *J. stolonifera*; **F.** *J. tenuissima*; **G.** *J. virgata*; **H, I.** *J. modesta*. Scale bars – 10 μm (C, D, G, I), 3 μm (A), 2 μm (B, E, F, H).

*leucostachya*, *J. neesiana*, *J. pallida*, *J. procumbens*, *J. ptychostoma*, *J. quadrifaria*, *J. quinqueangularis*, *J. remotifolia*, *J. stolonifera*, *J. tenuissima*, *J. vasculosa* and *J. virgata*.

*Subgroup 2.* — Pollen is 3-colporate. The aperture area provides two rows of 8–12 insulae (Figures 3W–Y, 7H–I).

Species included: *Justicia fragilis* and *J. modesta*.

## Discussion

Pollen morphology of the Thai species of *Justicia* has a typical morphology for the genus with the pollen being isopolar, bilaterally or radially symmetrical, 2-porate or 2–3-colporate. This study revealed the pollen to be comparatively homogeneous in terms of the features noted in the previous sentence (e.g., Lindau, 1895; Raj, 1961; Huang, 1972; Immelman, 1983; Graham, 1988; Ensermu, 1990; Hilsenbeck, 1990). The size, shape and ornamentation of the pollen grains show a great range of variation, but there is insufficient variation to recognise the differences in these characteristics taxonomically. However, distinct differences in the number of apertures among Thai species have been revealed for the first time. The differences in number of apertures is essential for taxonomic discrimination at the infrageneric level and correlates very well with the results found by Graham (1988). Another distinguishing character is the presence or absence of insulae on each side of the aperture on the aperture area. The insulae on each side of the aperture can only be seen in equatorial view and are restricted to pollen group II. For example, in *J. kampotiana*, there are 26–33 insulae on the aperture area, while only two insulae are present in *J. ptychostoma*.

The morphometric analyses of pollen morphological characters were performed to assess relationships that exist among several taxonomic groups (e.g. Olvera et al., 2006; Castro et al., 2009). The findings of the current study show that two different pollen morphological groups correlate with the recent taxonomic delimitation by Hilsenbeck (1990). Pollen group I is distinguished by being 3-colporate with six pseudocolpi. This character was described by Lindau (1895), Raj (1961) and Bremekamp (1965) as 'Spangenpollen'. This group includes sections *Rhaphidospora* (seven species), *Grossa* (two species) and *Betonica* (one species). Clarke (1885) placed *Justicia gendarussa* and *J. ventricosa* into sections *Gendarussa* and *Betonica*, respectively. Graham (1988) placed these species into section *Rhaphidospora* on the basis of their inflorescences comprising a compound spike although most species in this section have a dichasial inflorescence. However, based on the current studies, there are no significant differences in pollen morphology between the sections. Thus, pollen morphology does not appear to support Clarke's or Graham's classifications.

Immelman (1983) indicated that 3-colporate pollen is found in those species with a terminal spike or spike-like inflorescence. The same correlation was described with regard to *Justicia* in both the Old and New Worlds by Graham (1988). In contrast, the pollen morphology of Thai *Justicia* species shows that most species in group I have a dichasial inflorescence, probably because a dichasial inflorescence can be spicate inflorescence in nature, with all peduncles and pedicels absent. Furthermore, the presence of pseudocolpi is a distinctive feature of the pollen in this group, a fact not mentioned by previous workers.

Imlay (1938) described *Justicia diacantha* as distinct from *J. grossa* by its flower size and leaf shape. In contrast, Hansen (1987) reduced *J. diacantha* to the synonymy of *J. grossa* because of its spike-like inflorescence, lanceolate bract, white to pale green corolla and densely pubescent capsule. The phenogram revealed that the pollen morphology of *J. diacantha* differs from *J. grossa* by the ratio of the polar and equatorial axes, thus supporting Imlay's classification.

Pollen group II is the larger group comprising 23 species. Lindau (1895) referred to the pollen of species in this group as 'Knötchenpollen'. This group includes the sections *Betonica*, *Harnieria*, *Orthotactus*, *Plagiacanthus*, *Rhaphidospora*, *Rostellaria* and *Sarotheca*. It is congruent with the distribution of pollen types in the sections *Harnieria*, *Orthotactus*, *Plagiacanthus* and *Rostellaria* of Graham (1988). The present study indicates that the aperture area has a wide range of variation in terms of size and cannot be used in pollen morphological analyses. The number of rows of insulae can be useful for the delimitation of species, but for most of the species we studied there is only one discernible row of insulae, except in *Justicia kampotiana*, which has 2–3 rows of insulae on each side. However, this is contrary to the findings recorded by Bremekamp (1948). He characterised Asian *Justicia* as having one row of insulae one each side and two rows in the African taxa. The insulae differ greatly in size and shape. For example, the shape of the insulae in *J. stolonifera* gives the impression of a continuous band (Figure 7E).

The PCA analysis supports *Justicia fragilis* and *J. modesta* as belonging to group II by the presence of insulae or a band on each side of the aperture at the aperture area. However, both species

Table III. Eigenvalues, percentage of total variance explained by each axis and cumulative percentage along the first three axes obtained.

| Axis | Eigenvalues | Percentage of variance explained | Cumulative percentage of variance explained |
|---|---|---|---|
| 1 | 11.183 | 31.064 | 31.064 |
| 2 | 4.521 | 12.557 | 43.622 |
| 3 | 3.422 | 9.505 | 53.127 |

are 3-colporate but group II otherwise comprises 2-porate or 2-colporate pollen taxa. Therefore, the pollen of both species is sufficiently distinct to be placed in a subgroup of group II.

## Conclusion

The present results are congruent with previous studies indicating that the number of apertures, insulae or bands on each side of the aperture at the aperture area and the characteristics of the pseudocolpi are of considerable taxonomic importance in *Justicia* species. Pollen group II is the most prevalent in Thai *Justicia* and most of the species are placed in this group. However, pollen alone does not give much useful information for species relationships and the data need to be combined with other morphological and molecular characters for a full understanding of relationships in *Justicia*.

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## Specimens investigated

*Justicia alternifolia* C.B. Clarke. Thailand: Ranong, Khlong Nakha Wildelife Sanctuary. K. Rueangsawang 243 (KKU).

*Justicia betonica* L. Thailand: Mae Hong Son, Muang. K. Rueangsawang 224 (KKU).

*Justicia bicalcarata* Craib. Thailand: Phrae, Muang. A. F. G. Kerr 993 (K).

*Justicia championii* T. Anderson ex Benth. Thailand: Phetchaburi, Nam Nao National Park. K. Rueangsawang 130 (KKU).

*Justicia cochinchinensis* Benoist. Thailand: Phetchaburi, Kaeng Krachan National Park. K. Rueangsawang 171 (KKU); Surat Thani, Tai Rom Yen waterfall. K. Rueangsawang 259 (KKU).

*Justicia comata* (L.) Lam. Thailand: Chumphon, Sawi, Horticultural Research Centre. K. Rueangsawang 236(KKU); Phangnga, Khura Buri. K. Rueangsawang 248 (KKU).

*Justicia decumbens* Craib. Thailand: Chiang Mai, Doi Chiang Dao Wildlife Sanctuary. K. Rueangsawang 168 (KKU).

*Justicia decurrens* J.B. Imlay. Thailand: Chiang Rai. *P. Srisanga* 1268 (QBG).

*Justicia diacantha* J.B. Imlay. Thailand: Phetchaburi, Kaeng Krachan National Park. K. Rueangsawang 175 (KKU); Prachuap Khiri Khan, Sam Roi Yot. K. Rueangsawang 207 (KKU).

*Justicia diffusa* Willd. Thailand: Prachuap Khiri Khan, Klong Wan. K. Rueangsawang 147 (KKU); Phitsanulok, Thung Salaeng Luang National Park. K. Rueangsawang 241 (KKU).

*Justicia fragilis* Wall. Thailand: Kanchanaburi. M.C. Lakshnakara 1354 (K).

*Justicia gendarussa* Burm. f. Thailand: Kamphaeng Phet, Khlog Wang Chao National Park. K. Rueangsawang 273 (KKU).

*Justicia grandifolia* T. Anderson. Thailand: Phetchaburi, Kaeng Krachan National Park. K. Rueangsawang 181 (KKU).

*Justicia graphocaula* J.B. Imlay. Thailand: Surat Thani, Khao Sok National Park. D. J. Middleton et al. 4052 (K).

*Justicia grossa* C.B. Clarke. Thailand: Prachuap Khiri Khan, Huai Yang waterfall. K. Rueangsawang 274 (KKU).

*Justicia internodialis* (Bremek.) B. Hansen. Thailand: Phetchaburi, Kaeng Krachan National Park. K. Rueangsawang 180 (KKU).

*Justicia kampotiana* Benoist. Thailand: Ubon Ratchathani, Phu Jong Nayoi National Park. K. Rueangsawang 210 (KKU).

*Justicia leucostachya* (Bremek.) V.A.W. Graham. Thailand: Tak, Doi Hua Mot. K. Rueangsawang 156 (KKU); Kanchanaburi, Si Sawat. K. Rueangsawang 229 (KKU).

*Justicia modesta* (Bremek.) V.A.W. Graham. Thailand: Saraburi, Sam Lan. K. Rueangsawang 120 (KKU).

*Justicia neesiana* (Nees) T. Anderson. Thailand: Nakhon Si Thammarat, Tung Song, Yong waterfall. K. Rueangsawang 246 (KKU).

*Justicia pallida* J.B. Imlay. Thailand: Loei. K. Rueangsawang 209 (KKU).

*Justicia procumbens* L. Thailand: Chiang Mai, Doi Inthanon. K. Rueangsawang 161 (KKU).

*Justicia ptychostoma* Nees in Wall. Thailand: Nakhon Si Thammarat, Khao Luang, Karom waterfall. K. Rueangsawang 249 (KKU).

*Justicia quadrifaria* (Nees) T. Anderson. Thailand: Bueng Kan, Phu Wua Wildlife Sanctuary. K. Rueangsawang 159; Chanthaburi, Soi Dao. K. Rueangsawang 202 (KKU).

*Justicia quinqueangularis* K.D. Koenig ex Roxb. Thailand: Chiang Rai, Chiang Saen. K. Rueangsawang 280 (KKU).

*Justicia remotifolia* Ridl. Thailand: Chaiyaphum, Ban Nam Phrom. K. Rueangsawang 260 (KKU).

*Justicia scandens* Vahl. Thailand: Prachuap Khiri Khan, Huai Yang waterfall. K. Rueangsawang 146 (KKU); Phetchaburi, Kaeng Krachan National Park. K. Rueangsawang 174 (KKU).

*Justicia stolonifera* (C.B. Clarke) B. Hansen. Thailand: Loei, Phu Luang. K. Rueangsawang 245 (KKU).

*Justicia tenuissima* J.B. Imlay. Thailand: Surat Thani, Khao Sok National Park. K. Rueangsawang 247 (KKU).

*Justicia vagabunda* Benoist. Thailand: Chiang Rai, Khonkon waterfall. K. Rueangsawang 266 (KKU).

*Justicia vasculosa* (Wall. ex Nees) T. Anderson. Thailand: Phayao, Doi Luang National Park. K. Rueangsawang 270 (KKU).

*Justicia ventricosa* Wall. ex Hook.f. Thailand: Khon Kaen, Phu Pha Man National Park. K. Rueangsawang 214 (KKU).

*Justicia virgata* (Nees) T. Anderson. Thailand: Mae Hong Son, Tham Pla. K. Rueangsawang 228 (KKU).

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