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Pollen morphology of the tribe *Hemimerideae*: possible evidence of ancestral pollen types and parallel evolution in the basalmost clade of *Scrophulariaceae* s.str.

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Abstract: Pollen morphology of nine species belonging to six genera of tribe *Hemimerideae* (representing the basalmost clade of *Scrophulariaceae* s.str.) was studied and illustrated with light microscopy (LM) and scanning electron microscopy (SEM). Pollen grains in *Hemimerideae* are 3- or 6–8-colpate or 3- or 5–8-colporate, prolate, spheroidal or oblate-spheroidal in shape; they are mainly medium-sized or occasionally small. The outline in polar view is 3-lobed, rounded-3-lobed or 5–8-lobed; the outline in equatorial view is elliptic or orbicular. Exine sculpture is rough, rough-foveolate, foveolate, microreticulate, rugulate, rugulate-foveolate or rugulate-microreticulate. Four major pollen types are recognized, based on original data: 3-colpate (*Alonsoa*), 6–8-colpate (*Diclis*, *Hemimeris*), 3-colporate (*Colpias*) and 5–8-colporate (*Diascia*, *Nemesia*). Within two of these pollen types, two subtypes can be further distinguished based on pollen size, exine sculpture, details of the colpi and endoapertures. Based on pollen morphological data presented here and in our previous studies combined with published molecular phylogenetic data and molecular clock estimates, we conclude that (1) the ancestral pollen type in *Scrophulariaceae* was 3-colporate with a rather “primitive” exine sculpture; (2) major trends and pathways of further morphological evolution of pollen among the all lineages of *Scrophulariaceae* were established at the early stages of diversification of the family, about the time of divergence of its main lineages; and (3) the signatures of parallel evolution of the main morphological pollen characters, combined with progressive diversification of exine sculpture patterns, are apparent in all major lineages of the family.

Key words: pollen evolution, pollen morphology, pollen type, exine sculpture, *Scrophulariaceae*, *Hemimerideae*

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Introduction

Current opinions on taxonomy and phylogeny of *Hemimerideae* and some other early-branching clades of *Scrophulariaceae* s.str.

According to recent studies, the tribe *Hemimerideae* Benth. is the earliest-branching phylogenetic lineage of *Scrophulariaceae* s.str. The tribe, as currently outlined, includes *Alonsoa* Ruiz & Pav. (c. 16 species, tropical America and S Africa), *Colpias* E. Mey. ex Benth. (one species, S Africa), *Diascia* Link & Otto (c. 38 species, S Africa; or c. 72 species, according to Steiner 2009), *Diclis* Benth. (c. ten species, tropical and S Africa and Madagas-

car), *Hemimeris* L. f. (four species, S Africa) and *Nemesia* Vent. (c. 65 species, tropical and S Africa) (Olmstead & al. 2001; Oxelman & al. 2005; Tank & al. 2006; Datson & al. 2008; Steiner 2009; Olmstead 2012). Olmstead (2012) also included *Schistanthe* Kunze (authorship erroneously cited as “Kuntze”) in *Hemimerideae*, with a question mark (probably better placed in *Alonsoa*: see Roux 1986).

The tribe was established in the new series of *Edwards’s Botanical Register* continued by Lindley (1835), who explicitly credited the authorship to Bentham: “For this valuable contribution we are indebted to George Bentham, Esq. the learned author of the *Genera and Species Labiatarum*.” Initially Bentham included in that

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tribe the following four genera: *Angelonia* Bonpl. (now placed in *Plantaginaceae* s.l. or *Veronicaceae* s.str.: see Olmstead & al. 2001; Tank & al. 2006; Olmstead & al. 2012), *Hemimeris* (incl. *Diascia*), *Nemesia* and “*Phylacanthus* Nees et Mart.” (a typographical error corrected in Bentham 1835: *Thylacantha* Nees & Mart., now considered a synonym of *Angelonia*). Further discussion followed in Bentham’s *Scrophularineae indicae* (Bentham 1835) and an article specifically on this tribe (Bentham 1836), in which he recognized six genera (with addition of *Diascia* and *Diclis*). Discussion continued later on in his monographic treatments of *Scrophulariaceae* (Bentham 1846, 1876), in which his vision of the tribe was somewhat changed, but not considerably.

Baillon (1888) proposed the name *Alonsoeae* and included in this tribe the genera *Alonsoa*, *Angelonia*, *Colpias*, *Diascia*, *Diclis*, *Hemimeris* and *Nemesia*. Since *Hemimeris* is the type of *Hemimerideae*, the tribal name coined by Baillon was nomenclaturally superfluous when published; however, it is not illegitimate because it is based on the stem of a legitimate generic name, *Alonsoa* (Art. 52.3 of ICN: McNeill & al. 2012). Consequently, the name *Alonsoeae* can be used if the tribe is accepted in a different circumscription, excluding *Hemimeris*. Barringer (1993) described *Alonsoeae* as a new tribe (attributed to Barringer) including only *Alonsoa* and *Schistanthe*; in fact, he re-circumscribed this tribe, and thus its authorship should be still attributed to Baillon (see Reveal 2012).

Steiner (1996), after considering morphological and karyological data, proposed to include *Colpias* in tribe *Bowkerieae* Barringer (now in *Stilbaceae*), which contradicts the recent molecular data, and included *Alonsoa* in *Hemimerideae*. Kornhall (2004) supported the placement of *Colpias* in *Hemimerideae*, based on molecular data and morphology of the oil-secreting trichomes, and placed *Charadrophila* Marloth in *Stilbaceae*. Fischer (2004) placed *Alonsoa* and *Charadrophila* in *Alonsoeae*, while leaving *Basistemon* Turcz., *Colpias*, *Diascia*, *Diclis*, *Hemimeris* and *Nemesia* in *Hemimerideae* (based mainly on morphological evidence). Takhtajan (1987, 1997, 2009) accepted *Hemimerideae* as a tribe of *Scrophulariaceae* in all recent versions of his system, but in somewhat differing circumscriptions. According to the latest version (Takhtajan 2009), the tribe contains *Basistemon* (now placed in *Plantaginaceae* s.l.: see Oxelman & al. 2005; Tank & al. 2006; Olmstead 2012), *Colpias*, *Diascia*, *Diclis*, *Hemimeris* and *Nemesia*. However, *Alonsoa* and the monotypic genus *Charadrophila* were placed by Takhtajan (2009) in a separate tribe, *Alonsoeae* (attributed by Takhtajan to Barringer). Molecular data (Olmstead & al. 2001) did not support the segregation of *Alonsoa* in a tribe separate from *Hemimerideae*. Doweld (2001) erected the separate family *Hemimeridaceae*; however, as far as we know, nobody followed that taxonomic concept. Reveal (2012) placed *Hemimerideae* in the newly established

subfamily *Hemimeridoideae* and subdivided the tribe into two subtribes, *Alonsoinae* and *Hemimeridinae*.

All recent molecular phylogenetic studies (Olmstead & al. 2001; Kornhall 2004; Oxelman & al. 2005; Tank & al. 2006; Schäferhoff & al. 2010, etc.) have revealed the clade of *Hemimerideae* (in the circumscription discussed above; see Olmstead 2012) as the earliest-branching (basal) lineage of *Scrophulariaceae* s.str., which is sister to the large clade containing all other representatives of the re-circumscribed family. Molecular clock estimates of the initial evolutionary radiation of *Scrophulariaceae* s.str. and the divergence time of its basal groups remain controversial (Bremer & al. 2004; Wikström & al. 2001; see an overview and new assessments in Datson & al. 2008). The *Hemimerideae* clade, in any case, represents an ancient lineage within the family (*Scrophulariaceae* s.str.), the most recent common ancestor (MRCA) of which existed not later than 42–47.5 million years ago (the Eocene or earlier epochs) or most probably even earlier (Datson & al. 2008).

Importance of *Hemimerideae* for understanding pollen evolution, and objectives of the present study

Representatives of *Hemimerideae* are also interesting from the viewpoint of their life forms and chromosome evolution (Steiner 1996; Datson & al. 2008), pollination syndromes (Kampny 1995; Renner & Schaefer 2010) and some other aspects (Steiner 2006, 2009). In particular, some species of that group are pollinated by specialized oil-collecting bees (Buchmann 1987; Steiner 1990; Steiner & Whitehead 1990, 1991, 2002; Renner & Schaefer 2010) and (in *Hemimeris*) possess a very peculiar form of heterostyly (Pauw 2005). Members of this tribe are also biogeographically important and morphologically diverse, especially in the Cape flora of S Africa (Manning & Goldblatt 2012; Snijman 2013).

However, published data on pollen morphology of representatives of *Hemimerideae* are very scarce. Erdtman (1952), using light microscopy only, studied pollen grains of *Alonsoa acutifolia* Ruiz & Pav., *Diclis petiolaris* Benth., *D. reptans* Benth. and *Nemesia affinis* Benth. Roux (1986) provided scanning electron photomicrographs of pollen grains of *Alonsoa peduncularis* (Kunze) Wettst. (*Schistanthe peduncularis* Kunze) without descriptions and discussion. Because of that, the palynomorphological data provided by Roux (1986) cannot be used in our comparative analysis below. We are not aware of any other specialized studies of pollen morphology of the tribe.

The basalmost (earliest-branching) phylogenetic position of *Hemimerideae* within *Scrophulariaceae* makes this group very interesting and promising for revealing the patterns and trends of morphological pollen evolution in the family, especially in comparison with pollen morphology data obtained for other early-branching lineages of *Scrophulariaceae* as summarized in Tsymbalyuk & Mosyakin (2013) and Mosyakin & Tsymbalyuk (2015a, 2015b).

Because of that, our main objectives for the present publication were to study pollen morphology patterns of selected species of *Hemimerideae* representing all genera of the tribe (at least those now definitely placed here), to compare the obtained data with the data available on other taxa of *Scrophulariaceae* and to try to reconstruct the main trends and some general patterns of pollen evolution in the early-branching lineages of the family.

Material and methods

Pollen from nine species, belonging to all six genera of *Hemimerideae*, was sampled in the herbarium of the Missouri Botanical Garden, St. Louis, Missouri, U.S.A. (MO). Pollen from one species of *Nemesia* was sampled in the herbarium of the M. G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Kyiv (Kiev), Ukraine (KW; herbarium codes according to Thiers 2016+). The investigated specimens are cited according to the label information, sometimes with changes in the order of data, for consistency.

Pollen morphology was studied using light microscopy (LM) and scanning electron microscopy (SEM), following the techniques described in our previous articles (Mosyakin & Tsybalyuk 2015a, 2015b) and briefly reported here.

For LM (Biolar, $\times 700$), the pollen was acetolysed and mounted on slides following Erdtman (1952). For size determinations, 20 measurements were taken along the polar (P) and equatorial (E) axes for each species.

For SEM (JSM-6060LA), pollen grains were treated with 96% ethanol, then sputter-coated with gold at the Center of Electron Microscopy of the M. G. Kholodny Institute of Botany. The obtained micrographs were minimally edited with Adobe Photoshop 6.0 to enhance the images, with no alteration of the images themselves.

Abbreviation of taxon author names follows Brummitt & Powell (1992), with corrections and additions available from IPNI (2016+).

Terminology used in our descriptions of pollen grains mainly follows the glossaries by Punt & al. (1994) and Tokarev (2002), with some minor adjustments.

Results

General description of pollen grains

Hemimerideae

Pollen grains in monads, radially symmetrical, isopolar, 3- or 6–8-colpate or 3- or 5–8-colporate, prolate, spheroidal or oblate-spheroidal, mainly medium-sized, occasionally in some taxa small: P=15.9–45.2 μm , E=14.6–34.6 μm . Outline in polar view 3-lobed, rounded-3-lobed or 5–8-lobed, in equatorial view elliptic or orbicular. Colpi 0.7–9.3 μm wide, with distinct or indistinct, strict margins and blunt, rounded, acute or

indistinct ends. Endoapertures mainly indistinct, rarely covered by margins of colpi, or elliptic when distinct.

Exine 1.1–2.7 μm thick, in some species thickened at apocolpia to 3.7 μm . Tectum $\frac{1}{2}$ – $\frac{2}{3}$ as thick as or nearly equalling infratectum. Columellae mainly distinct or sometimes indistinct, thick or rarely thin, arranged regularly. Exine sculpture rough, rough-foveolate, foveolate, microreticulate, rugulate, rugulate-foveolate and/or rugulate-microreticulate. Colpus membrane smooth, granulate or rugulate.

Pollen types and subtypes

Pollen grains in the studied taxa can be subdivided into four basic types, based on their aperture types; two types can be additionally subdivided into two subtypes each. The subtypes are distinguished mainly according to the pollen size, exine sculpture and details of colpi and endoapertures.

Type I: 3-colpate.

This type includes *Alonsoa unilabiata*.

P=30.6–45.2 μm , E=22.6–34.6 μm . Exine sculpture rugulate-microreticulate. Colpi 5.3–9.3 μm wide, with blunt ends. Colpus membrane granulate. Exine 2–2.7 μm thick.

Type II: 6–8-colpate.

This type includes *Diclis ovata*, *Hemimeris racemosa* and *H. sabulosa*.

Subtype IIa: P=17.3–21.3 μm , E=14.6–21.3 μm . Exine sculpture foveolate and microreticulate. Colpi 0.7–1.1 μm wide, with acute ends. Colpus membrane smooth. Exine 1.1–1.3 μm thick. *Diclis ovata*.

Subtype IIb: P=21.3–35.9 μm , E=21.3–29.3 (–31.9) μm . Exine sculpture rugulate-microreticulate and microreticulate. Species of *Hemimeris*.

1. Colpi 0.7–1.6 μm wide, with acute or rounded ends. Colpus membranes smooth and rugulate. Exine 2–2.4 (–3.7) μm thick. *Hemimeris sabulosa*.

2. Colpi 2–2.7 μm wide, with acute ends. Colpus membrane smooth and granulate. Exine 2.4–2.7 (–3.3) μm thick. *Hemimeris racemosa*.

Type III: 3-colporate.

This type includes *Colpias mollis*.

P=29.3–34.6 μm , E=(25.3–)27.9–31.9 μm . Sculpture rough, rough-foveolate and foveolate. Colpi 2.4–4 μm wide, with acute ends. Colpus membrane smooth. Endoapertures indistinct, covered by margins of colpi. Exine 1.3–2 (–2.7) μm thick.

Type IV: 5–8-colporate.

This type includes *Diascia capsularis*, *D. elongata*, *Nemesia cheiranthus* and *N. strumosa*.

Subtype IVa: P=19.9–27.9 μm , E=18.6–25.3 μm . Colpi 0.7–1.3 μm wide, with acute or rounded ends. En-

doapertures indistinct. Exine sculpture rugulate-foveolate or rugulate-microreticulate. Species of *Diascia*.

1. Colpi 0.7–1.3 μm wide, with rounded ends. Colpus membrane smooth and granulate. Endoapertures indistinct. Exine 1.3–2.4 μm thick. *Diascia capsularis*.

2. Colpi 0.7–1.1(–1.3) μm wide, with acute ends. Colpus membrane smooth and rugulate. Endoapertures indistinct. Exine 2–2.4(–2.7) μm thick. *Diascia elongata*.

Subtype IVb: P=15.9–25.3 μm , E=15.9–26.6 μm . Colpi 1.1–2.7 μm wide, with indistinct ends. Endoapertures indistinct or distinct. Exine sculpture rugulate, rugulate-foveolate, rugulate-microreticulate or microreticulate. Characters mainly overlapping with those of the previous subtype, but colpi with indistinct ends and exine usually thinner. Species of *Nemesia*.

1. P=19.9–25.3 μm , E=21.3–26.6 μm . Exine sculpture rugulate. Colpi 2–2.7 μm wide, with indistinct ends. Colpus membrane smooth and rugulate. Endoapertures indistinct. Exine 1.3–2 μm thick. *Nemesia strumosa*.

2. P=15.9–22.6 μm , E=15.9–21.3 μm . Sculpture rugulate-foveolate, rugulate-microreticulate, microreticulate. Colpi 1.1–1.3 μm wide, with indistinct ends. Colpus membrane smooth and rugulate. Endoapertures distinct, elliptic, 6.6–9.3 μm long, 2.7–3.3 μm wide. Exine 1.3–1.6 μm thick. *Nemesia cheiranthus*.

Descriptions of pollen grains

Genera and species within genera are listed alphabetically. Main pollen morphology characters are additionally summarized in Table 1.

Hemimerideae

Alonsoa Ruiz & Pav.

Alonsoa unilabiata (L. f.) Steud. (Fig. 1A–C; 3A–D).

LM — Pollen grains 3-colpate, prolate, rarely spheroidal, in polar view 3-lobed, in equatorial view elliptic. P=30.6–45.2 μm , E=22.6–34.6 μm . Colpi 5.3–9.3 μm wide, with uneven margins, slightly tapering to blunt ends; colpus membrane smooth and granulate. Exine 2–2.7 μm thick, thickened at apocolpia. Tectum nearly equalling infratectum. Columellae distinct, thick, arranged regularly. Exine sculpture distinct, reticulate.

SEM — Sculpture rugulate-microreticulate. Colpus membrane granulate, granules large.

Specimen investigated — SOUTH AFRICA: Western Cape, grid ref. 3218 AD Clanwilliam, farm Skerpheuwel, road to Elands Bay, 6.5 km S of R 365, 32°16.013'S, 18°25.249'E, 165 m, 4 Sep 2001, K. Steiner 3726 (MO).

Colpias E. Mey. ex Benth.

Colpias mollis E. Mey. ex Benth. (Fig. 1D–F; 3E–H).

LM — Pollen grains 3-colporate, prolate, rarely oblate-spheroidal, in polar view 3-lobed or rounded-3-lobed, in equatorial view elliptic or rarely orbicular. P=29.3–34.6 μm , E=(25.3–)27.9–31.9 μm . Colpi

2.4–4 μm wide, with distinct, strict margins, slightly tapering to acute ends; aperture membrane smooth. Endoapertures indistinct, covered by margins of colpi. Exine 1.3–2 μm thick, at apocolpia thickened to 2.4–2.7 μm . Tectum c. $\frac{2}{3}$ as thick as infratectum. Columellae indistinct, thin, arranged regularly. Exine sculpture distinct, foveolate and microreticulate.

SEM — Sculpture rough, rough-foveolate and foveolate. Colpus membrane smooth.

Specimen investigated — SOUTH AFRICA: Cape, grid ref. 2917 DD Springbok, 5.7 km NW of N7 at Buffelsrivier on road to farm Koringhuis, E-facing slope above road, 510 m, 23 Aug 1990, K. Steiner 2176 (MO).

Diascia Link & Otto

Diascia capsularis Benth. (Fig. 1G–I; 3I–L).

LM — Pollen grains 6- or 8-colporate, prolate, rarely spheroidal and oblate-spheroidal, in polar view 6–8-lobed, in equatorial view elliptic or orbicular. P=22.6–27.9 μm , E=19.9–25.3 μm . Colpi 0.7–1.3 μm wide, with \pm distinct, strict margins, rounded at ends; aperture membrane smooth. Endoapertures indistinct. Exine 1.3–2.4 μm thick. Tectum nearly equalling infratectum. Columellae distinct, thick, arranged regularly. Exine sculpture distinct, reticulate.

SEM — Sculpture rugulate-foveolate and rugulate-microreticulate. Colpus membrane smooth and granulate.

Specimen investigated — SOUTH AFRICA: Eastern Cape, grid ref. 3225 AA, S Mountain Zebra National Park, farm Elandsfontein, 32°11'25"S, 25°08'58"E, 1626 m, 13 Dec 2005, S. P. Bester 6363 (MO).

Diascia elongata Benth. (Fig. 1J–L; 3M–P).

LM — Pollen grains 6- or 7-colporate, prolate, rarely spheroidal and oblate-spheroidal, in polar view 6- or 7-lobed, in equatorial view elliptic or orbicular. P=19.9–27.9 μm , E=18.6–23.9 μm . Colpi 0.7–1.1(–1.3) μm wide, with distinct, uneven margins, slightly tapering to acute ends; colpus membranes smooth. Endoapertures indistinct. Exine 2–2.4 μm thick, at apocolpia thickened to 2.7 μm . Tectum c. $\frac{1}{2}$ as thick as infratectum. Columellae distinct, thick, arranged regularly. Exine sculpture distinct, reticulate.

SEM — Sculpture rugulate-foveolate and rugulate-microreticulate. Colpus membrane smooth and rugulate.

Specimen investigated — SOUTH AFRICA: Northern [?Western] Cape, grid. ref. 3118 DD Vanrhynsdorp, top of Gifberg, 13 Aug 1998, P. Goldblatt 10981 & J. C. Manning (MO).

Diclis Benth.

Diclis ovata Benth. (Fig. 1M–O; 3Q–T).

LM — Pollen grains 6-colpate, prolate, rarely spheroidal,

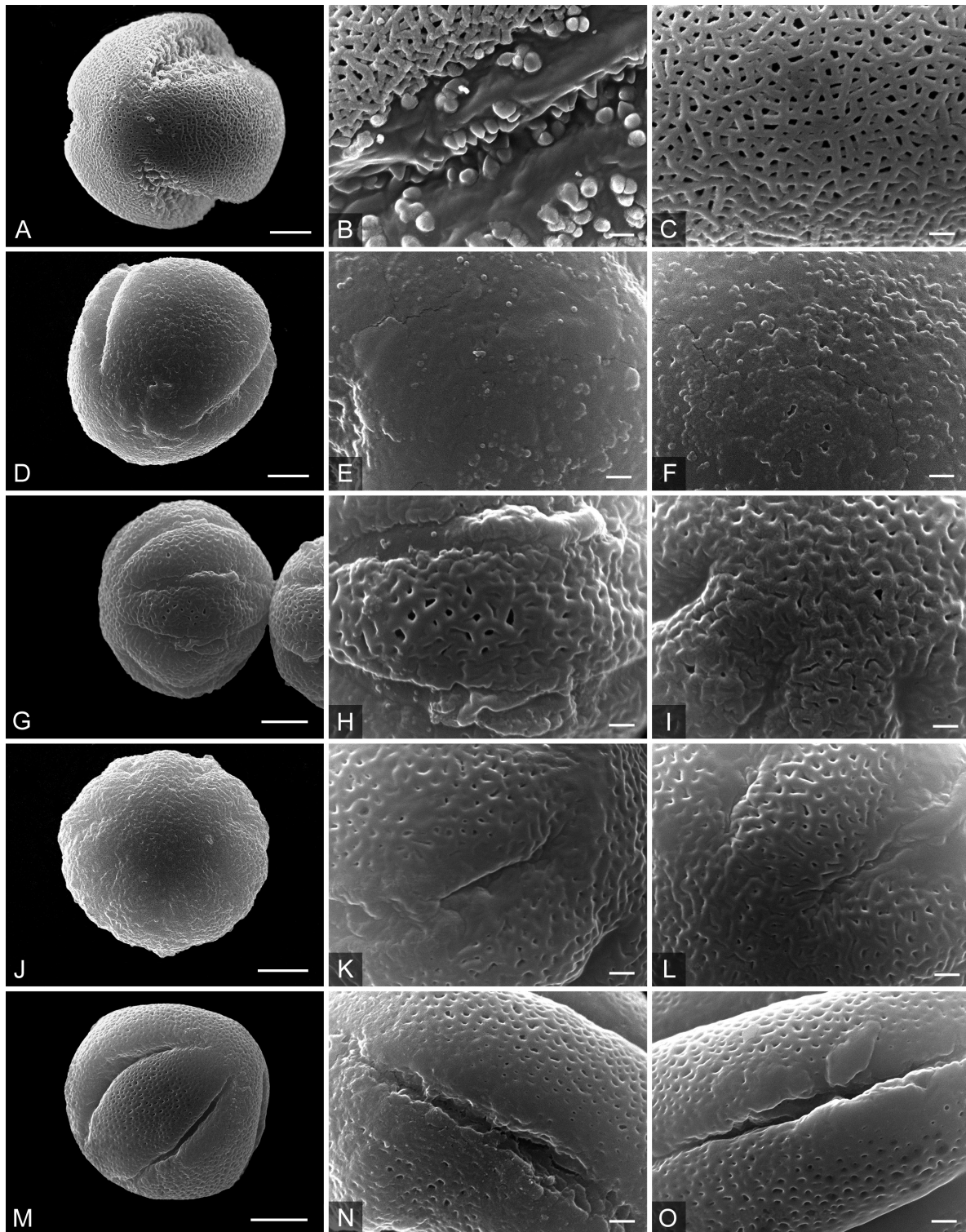


Fig. 1. Pollen grains of *Alonsoa*, *Colpias*, *Diascia* and *Diclis* (SEM). – A–C: *Alonsoa unilabiata*; D–F: *Colpias mollis*; G–I: *Diascia capsularis*; J–L: *Diascia elongata*; M–O: *Diclis ovata*. – A, J: polar view; D, G, M: equatorial view. – Exine sculpture: C, H, I, K, L: rugulate-microreticulate; E: rough; F: rough-foveolate; N, O: microreticulate; B: granulate aperture membranes. – Scale bars: A, D, G, J, M = 5 μ m; B, C, E, F, H, I, K, L, N, O = 1 μ m.

roidal and oblate-spheroidal, in polar view 6-lobed, in equatorial view elliptic or orbicular. P=17.3–21.3 μ m, E=14.6–21.3 μ m. Colpi 0.7–1.1 μ m wide, with \pm distinct, uneven margins and acute ends; colpus membrane smooth. Endoapertures indistinct. Exine 1.1–1.3 μ m

thick. Tectum nearly equalling infratectum. Columellae indistinct. Exine sculpture indistinct, foveolate.

SEM — Sculpture foveolate and microreticulate. Lumina of reticulum rounded or elongated; muri wide. Colpus membrane smooth.

Specimen investigated — MALAWI: South Region, Bvumbwe, 1150 m, 1 Sep 1985, *I. F. La Croix* 3322 (MO).

Hemimeris L. f.

Hemimeris racemosa (Houtt.) Merr. (Fig. 2A–C; 4A–D).

LM — Pollen grains 6- or 7-colpate, prolate, spheroidal, rarely oblate-spheroidal, in polar view 6- or 7-lobed, in equatorial view elliptic or orbicular. $P=21.3\text{--}29.3\ \mu\text{m}$, $E=21.3\text{--}29.3\text{--}(31.9)\ \mu\text{m}$. Colpi 2–2.7 μm wide, with indistinct, uneven margins, slightly tapering to acute ends; colpus membranes smooth, sometimes granulate. Exine 2.4–2.7 μm thick, at apocolpia thickened to 3.3 μm . Tectum c. $\frac{1}{2}$ as thick as infratectum. Columellae distinct, thick, arranged regularly. Exine sculpture distinct, macroreticulate, near colpi almost microreticulate (with smaller lumina).

SEM — Sculpture rugulate-microreticulate and microreticulate. Lumina of reticulum small, rounded, elongated or rounded-angular; muri thick. Colpus membrane smooth and granulate.

Specimen investigated — SOUTH AFRICA: Western Cape Province, Pakhuis Pass area E of Clanwilliam on road to Pakhuis, 2200–2700', 8 Oct 2001, *W. Elisens* 946 (MO).

Hemimeris sabulosa L. f. (Fig. 2D–F; 4E–H).

LM — Pollen grains 6–8-colpate, prolate, rarely spheroidal, in polar view 6- or 7-lobed, in equatorial view elliptic or rarely orbicular. $P=26.6\text{--}35.9\ \mu\text{m}$, $E=23.9\text{--}27.9\ \mu\text{m}$. Colpi 0.7–1.6 μm wide, with \pm distinct, uneven margins, slightly tapering to acute or rounded ends; colpus membrane smooth. Exine 2–2.4 μm thick, at apocolpia thickened to 3.7 μm . Tectum nearly equalling infratectum. Columellae distinct, thick, arranged regularly. Exine sculpture distinct, reticulate.

SEM — Sculpture rugulate-microreticulate and microreticulate. Lumina of reticulum small, rounded, elongated or rounded-angular; muri wide. Colpus membrane smooth and rugulate.

Specimen investigated — SOUTH AFRICA: Western Cape, grid. ref. 3118 DA Vanrhynsdorp, farm Aties Extension, R27 3.2 km NE of junction with R362, 30 m, 21 Aug 1995, *K. E. Steiner* 3004 (MO).

Nemesia Vent.

Nemesia cheiranthus E. Mey. ex Benth. (Fig. 2G–I; 4I–L).

LM — Pollen grains 6- or 7-colporate, prolate, rarely oblate-spheroidal and spheroidal, in polar view 6- or 7-lobed, in equatorial view elliptic or orbicular. $P=15.9\text{--}22.6\ \mu\text{m}$, $E=15.9\text{--}21.3\ \mu\text{m}$. Colpi 1.1–1.3 μm wide, with indistinct margins and ends; colpus membrane smooth. Endoapertures with indistinct margins, elliptic, 6.6–9.3 μm long, 2.7–3.3 μm wide. Exine 1.3–1.6 μm thick. Tectum thinner than infratectum. Columellae distinct. Exine sculpture distinct, reticulate.

SEM — Sculpture rugulate-foveolate, rugulate-microreticulate and microreticulate. Muri indistinct. Colpus membrane smooth and rugulate.

Specimen investigated — SOUTH AFRICA: [Western Cape Province], near Table Mountain, “mont. tabul. septentr.”, Aug, *U. J. [C. F.] Ecklon* (KW Turczaninow memorial collection).

Note: The letters U. J. on the printed label of this historical specimen are not initials of the collector (as sometimes erroneously cited in other botanical databases); abbreviations U. J. (or sometimes U. I. on other labels) indicate that this collection made by C. F. Ecklon was distributed by *Unio Itineraria*, a botanical exchange club founded and managed by C. F. Hochstetter and E. G. Steudel (see Gunn & Codd 1981: 388).

Nemesia strumosa Benth. (Fig. 2J–L; 4M–P).

LM — Pollen grains 5- or 6-colporate, oblate-spheroidal and spheroidal, in polar view 5- or 6-lobed, in equatorial view orbicular. $P=19.9\text{--}25.3\ \mu\text{m}$, $E=21.3\text{--}26.6\ \mu\text{m}$. Colpi 2–2.7 μm wide, with indistinct margins, slightly tapering to indistinct ends; aperture membrane smooth. Endoapertures with indistinct margins. Exine 1.3–2 μm thick. Tectum nearly equalling infratectum. Columellae \pm distinct. Exine sculpture distinct, microreticulate.

SEM — Sculpture rugulate. Muri \pm distinct. Colpus membrane smooth and rugulate.

Specimen investigated — SOUTH AFRICA: Western Cape, grid ref. 3219 AC Clanwilliam, Sandveld between Sauer and Velddrif, 15 Sep 1999, *P. Goldblatt* 11144 & *J. C. Manning* (MO).

Discussion

Comparative pollen morphology of genera of *Hemimerideae*

Pollen grains in *Alonsoa*, in particular in *A. unilabata*, are 3-colpate with a rugulate-microreticulate exine sculpture. They have the widest colpi among pollen grains of the studied species of the tribe, and a distinctly granulate colpus membrane (a unique feature in *Hemimerideae*).

Pollen grains of *Diclis ovata*, the only species of *Diclis* studied by us, are small-sized, 6-colpate, with foveolate, microreticulate exine sculpture. Erdtman (1952) described pollen grains of *D. petiolaris* as 4- or 5-colpate and those of *D. reptans* as 6- or 7-colpate, small in both species. Judging from these data, species of the genus differ in the number of colpi, or at least demonstrate a considerable variation of that character. Additional palynomorphological studies in this genus are needed.

The two studied species of *Hemimeris* are similar in their type of apertures (6- or 7-colpate in *H. racemosa*

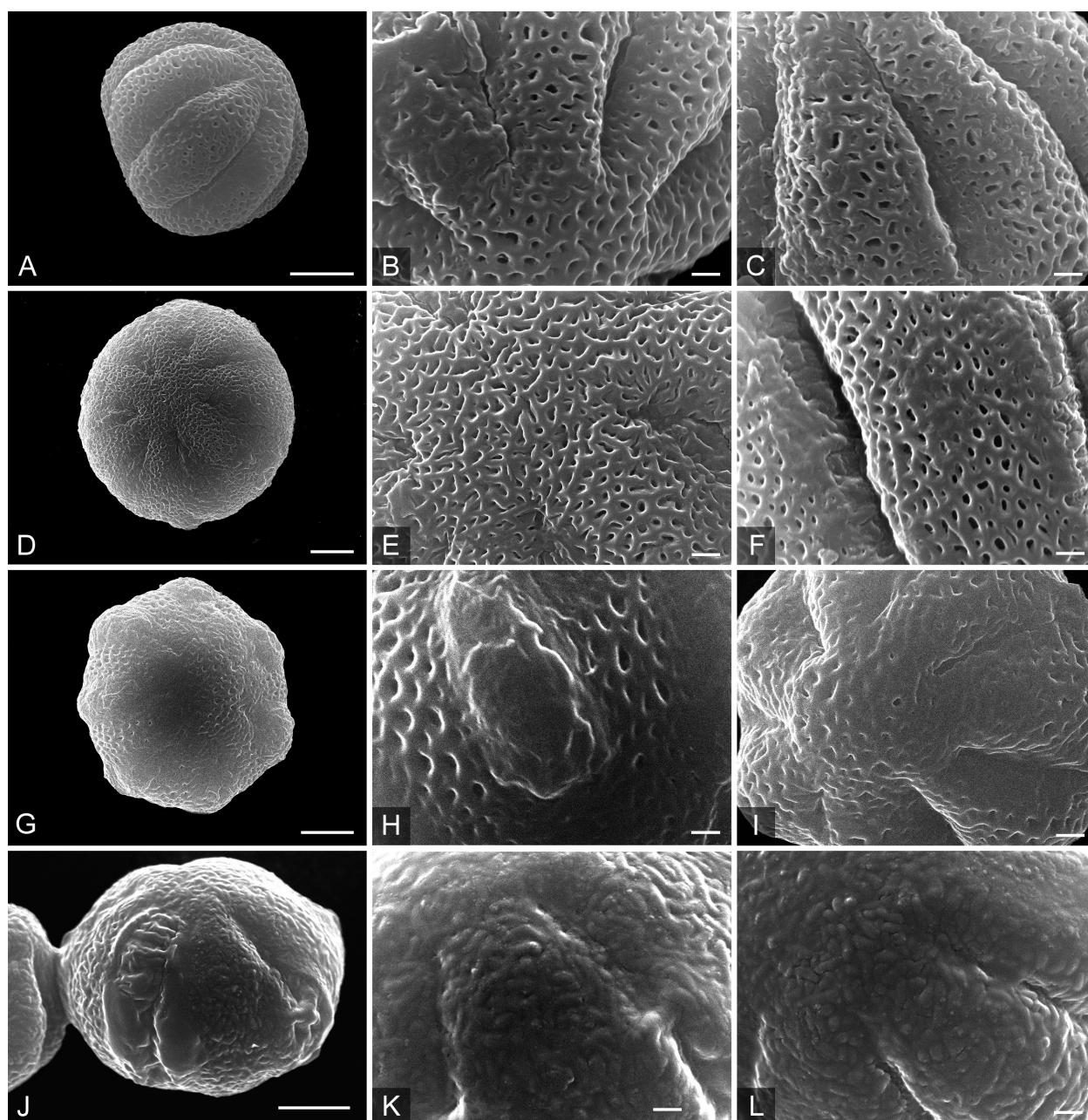


Fig. 2. Pollen grains of *Hemimeris* and *Nemesia* (SEM). – A–C: *Hemimeris racemosa*; D–F: *H. sabulosa*; G–I: *Nemesia cheiranthus*; J–L: *N. strumosa*. – A, J: equatorial view; D, G: polar view. – Exine sculpture: B, C, F, H, I: microreticulate; E: rugulate-microreticulate; K, L: rugulate. – Scale bars: A, D, G, J = 5 μ m; B, C, E, F, H, I, K, L = 1 μ m.

and 6–8-colpate in *H. sabulosa*), pollen size and exine sculpture; they differ, however, by their colpi structure: in pollen grains of *H. racemosa* colpi are wider (2–2.7 μ m) with acute ends, whereas in *H. sabulosa* colpi are narrower (0.7–1.6 μ m) with acute and rounded edges. Further studies are needed to reveal any other distinctive characters of species of the genus.

In general, pollen grains of *Diclis* and *Hemimeris* are similar in their aperture types, but they differ somewhat in their size, thickness of exine, distinct versus indistinct columellae of the infratectum, and characters of their exine sculpture.

Pollen grains in *Colpias* are 3-colporate, with rough

and foveolate exine sculpture, clearly differing by these characters from those of other genera of the tribe.

The two studied species of *Diascia* are similar in having the 6–8-colporate (6- or 8-colporate in *D. capsularis* and 6- or 7-colporate in *D. elongata*) aperture type, rugulate-foveolate and rugulate-microreticulate exine sculpture and rather wide colpi; they differ, however, by some elements of their colpi structure: in *D. capsularis* colpi ends are rounded, whereas in *D. elongata* colpi ends are acute and pointed. It should be noted that the two species that we sampled represent the two currently recognized sections within the genus, *D. sect. Diascia* (*D. elongata*) and *D. sect. Racemosae* (*D. capsularis*) (Hilliard & Burt 1984).

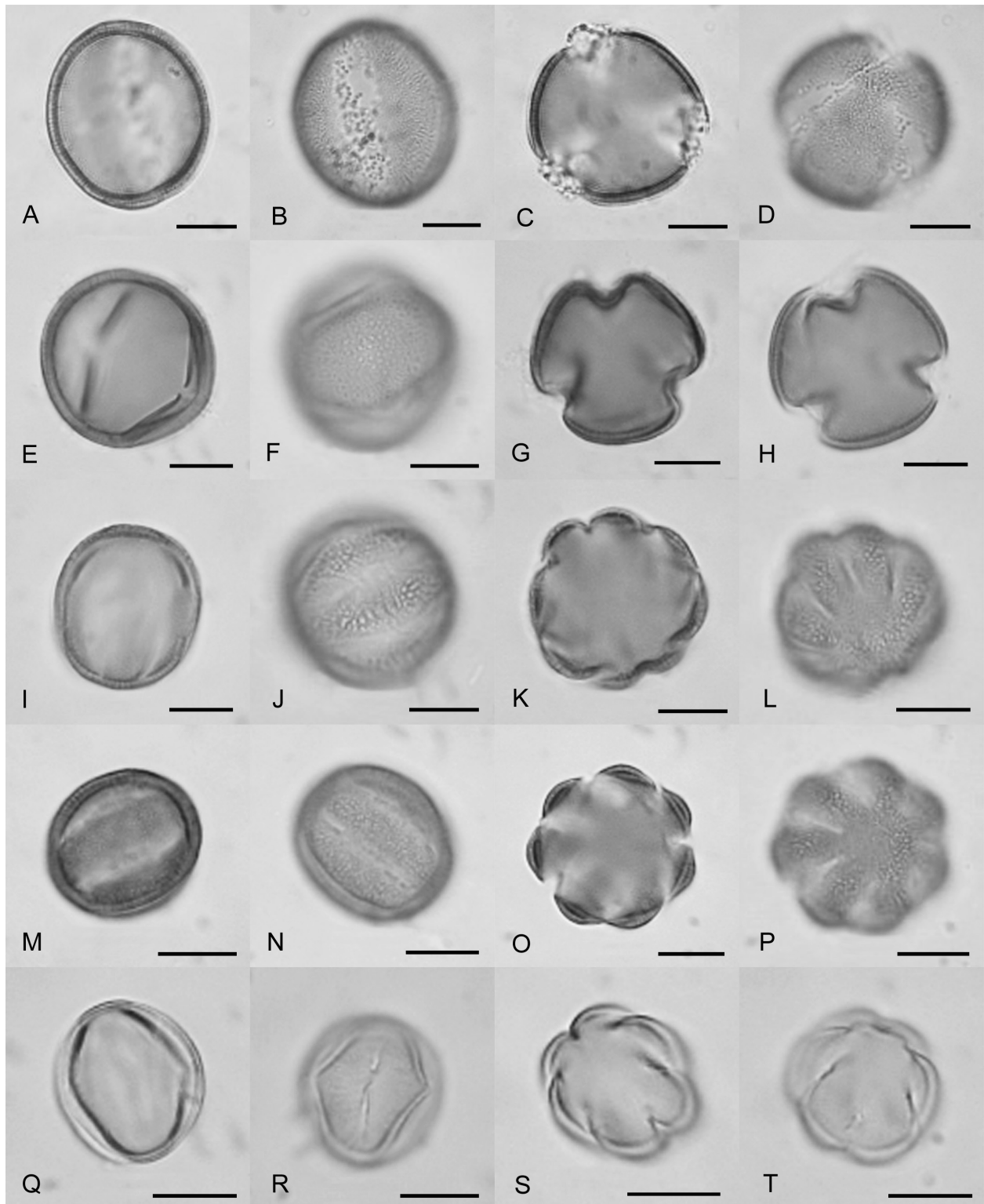


Fig. 3. Pollen grains of *Alonsoa*, *Colpias*, *Diascia* and *Diclis* (LM). – A–D: *Alonsoa unilabiata*; E–H: *Colpias mollis*; I–L: *Diascia capsularis*; M–P: *Diascia elongata*; Q–T: *Diclis ovata*. – A, B, E, F, I, J, M, N, Q, R: equatorial view; C, D, G, H, K, L, O, P, S, T: polar view. – Scale bars: A–T = 10 μ m.

However, the pollen diversity within the genus could be much greater than revealed in the present study.

Pollen grains of the studied species of *Nemesia* (*N. cheiranthus* and *N. strumosa*) are distinguished by their size, wide colpi, clarity and structure of endoapertures,

and exine sculpture. Pollen grains of *Nemesia* show similarities to those of *Diascia* by their aperture types, pollen grain size, and sculpture of aperture membranes, but differ in the structure of apertures and exine sculpture characters (see Table 1).

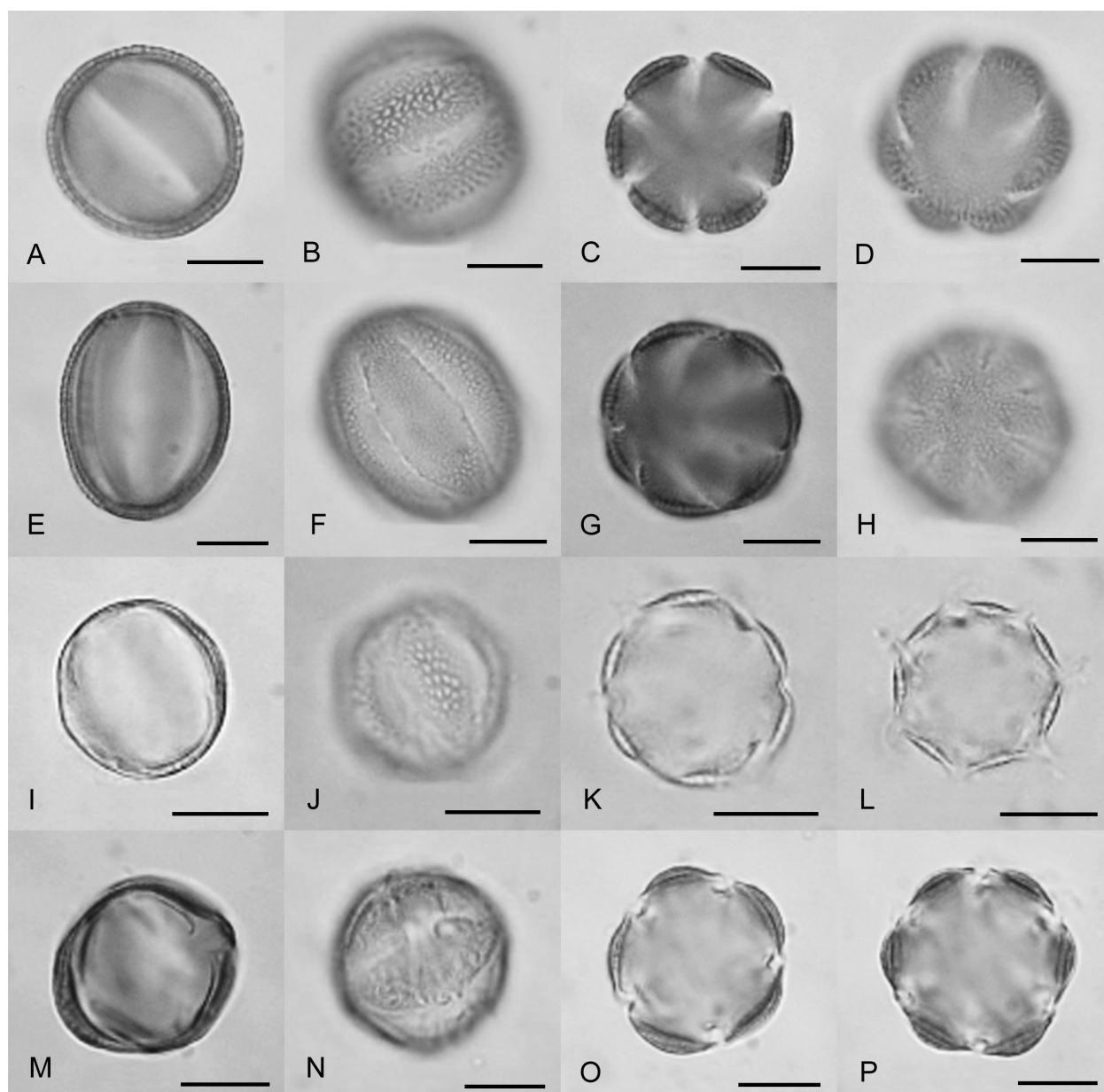


Fig. 4. Pollen grains of *Hemimeris* and *Nemesia* (LM). – A–D: *Hemimeris racemosa*; E–H: *H. sabulosa*; I–L: *Nemesia cheiranthus*; M–P: *N. strumosa*. – A, B, E, F, I, J, M, N: equatorial view; C, D, G, H, K, L, O, P: polar view. – Scale bars: A–P = 10 μ m.

Comparison of palynomorphological and molecular phylogenetic evidence

Judging from molecular phylogenetic data (Oxelman & al. 2005), *Diascia* and *Nemesia* are included in one clade, which is weakly supported as sister to all other members of *Hemimerideae*. The close links of these two genera are also confirmed by palynomorphological characters: the same pollen type IV, characterized by 5–8-colporate pollen, and similar exine sculpture patterns (rugulate, rugulate-foveolate, rugulate-microreticulate and/or microreticulate).

In turn, *Colpias* may be sister to the clade that includes the genera *Alonsoa* + (*Diclis* + *Hemimeris*) (Oxelman & al. 2005). Our data are consistent with this sug-

gestion and demonstrate that 3-colporate pollen grains of *Colpias* are more similar to (but also distinguishable from) 3-colporate pollen of *Alonsoa*, but easily distinguished from 4–8-colporate pollen observed in *Diclis* and *Hemimeris*.

According to molecular phylogenetic data (Oxelman & al. 2005), *Diclis* and *Hemimeris* are sister members of the same terminal clade of the tribe. Palynomorphological data also suggest a close affinity of *Diclis* and *Hemimeris* because they share 4–8-colporate pollen. *Alonsoa* is probably sister to the *Diclis* + *Hemimeris* clade; however, it differs in having 3-colporate pollen, at least in the two species studied here and by Erdtman (1952). In general, the clade that includes *Alonsoa*, *Diclis* and *Hemimeris* is characterized by colporate pollen, as opposed to colporate

Table 1. Summary of main pollen morphological characters. Original data, except four species studied by Erdtman (1952). “—” = no data reported.

Taxon	P (µm)	E (µm)	Apertures	Colpi	Colpus width (µm)	Colpus membrane	Exine sculpture	Exine thickness (µm)	Endoapertures
<i>Alonsoa acutifolia</i> (Erdtman 1952)	27	23	3-colpate	—	—	—	microreticulate	—	—
<i>Alonsoa unilabiata</i>	30.6–45.2	22.6–34.6	3-colpate	wide, ends blunt	5.3–9.3	granulate	rugulate-microreticulate	2–2.7	absent
<i>Colpasia mollis</i>	29.3–34.6	(25.3–)27.9–31.9	3-colporate	medium width, ends acute	2.4–4	smooth	rough, rough-foveolate, foveolate	1.3–2(–2.7)	indistinct, covered by margins of colpi
<i>Diascia capsularis</i>	22.6–27.9	19.9–25.3	6- or 8-colporate	narrow, ends rounded	0.7–1.3	smooth and granulate	rugulate-foveolate, rugulate-microreticulate	1.3–2.4	indistinct
<i>Diascia elongata</i>	19.9–27.9	18.6–23.9	6- or 7-colporate	narrow, ends acute	0.7–1.1(–1.3)	smooth and rugulate	rugulate-foveolate, rugulate-microreticulate	2–2.4	indistinct
<i>Diclis ovata</i>	17.3–21.3	14.6–21.3	6-colpate	narrow, ends acute	0.7–1.1	smooth	foveolate, microreticulate	1.1–1.3	absent
<i>Diclis petiolaris</i> (Erdtman 1952)	16	13.5	4- or 5-colpate	—	—	—	—	—	—
<i>Diclis reptans</i> (Erdtman 1952)	20	15	6- or 7-colpate	—	—	—	—	—	—
<i>Hemimeris racemosa</i>	21.3–29.3	21.3–29.3(–31.9)	6- or 7-colpate	medium width, ends acute	2–2.7	smooth and granulate	rugulate-microreticulate, microreticulate	2.4–2.7(–3.3)	absent
<i>Hemimeris sabulosa</i>	26.6–35.9	23.9–27.9	6–8-colpate	narrow, ends acute or rounded	0.7–1.6	smooth and rugulate	rugulate-microreticulate, microreticulate	2–2.4(–3.7)	absent
<i>Nemesia affinis</i> (Erdtman 1952)	20.5	16	6- or 7-colporate	—	—	—	reticulate	—	—
<i>Nemesia cheiranthus</i>	15.9–22.6	15.9–21.3	6- or 7-colporate	narrow, ends indistinct	1.1–1.3	smooth and rugulate	rugulate-foveolate, rugulate-microreticulate, microreticulate	1.3–1.6	elliptic, 6.6–9.3 µm long, 2.7–3.3 µm wide
<i>Nemesia strumosa</i>	19.9–25.3	21.3–26.6	5- or 6-colporate	medium width, ends indistinct	2–2.7	smooth and rugulate	rugulate	1.3–2	indistinct

pollen observed in all other studied members of *Hemimerideae*.

Main trends of pollen evolution in early-branching *Scrophulariaceae*

Furness & Rudall (2004) indicated that there is a general trend, both in angiosperms in general and in their main clades, toward an increase in pollen aperture number, suggesting that aperture number is under strong selection pressure. A similar pattern is observed in *Scrophulariaceae* and it is best manifested in taxa with advanced pollination syndromes (specialized entomophily), which demonstrate a trend toward developing more apertures than less specialized taxa. However, morphological patterns in early-branching clades do not always indicate ancestral character states (see Crisp & Cook 2005) and ancestral character state recognition often needs a very careful approach (Cunningham 1999; Cunningham & al. 1998). Nevertheless, when recurrent patterns are observed in various early-branching clades of a particular group (or a phylogenetic clade), one can assume that these patterns may indicate possible ancestral character states. The recent attempts at reconstruction of the early evolution of pollen grains of angiosperms (Wortley & al. 2015) confirm these predictions. Thus, reconstructions of ancient pollen patterns in *Scrophulariaceae* are in agreement with those ideas.

Our data demonstrate that pollen grains of the studied species of *Hemimerideae* are characterized by colpate and colpiate aperture types. We can assume that pollen with the 3-colporate type of apertures and rough and foveolate exine sculpture (similar to the characters observed in *Colpasia*) was ancestral in *Hemimerideae* and probably in *Scrophulariaceae* in general. Further evolutionary trends manifested in the tribe are the increase in the number of colpi leading to the formation of 5–8-colporate pollen, such as revealed in *Diascia* and *Nemesia*. Pollen grains of representatives of these genera have rugulate, rugulate-foveolate, rugulate-microreticulate and microreticulate exine sculpture patterns (the last two with indistinct muri). This can be seen as an evolutionary bridge to more advanced and more typical rugulate-microreticulate and microreticulate sculpture with well-developed muri, as observed in *Alonsoa* and many members of crown clades of *Scrophulariaceae*. Further reduction of endoapertures (ora) leads to development of 4–8-colpate pollen grains (apertures are represented by colpi only), like those in *Diclis* and *Hemimeris*. Further evolutionary changes through reduction in the number of colpi may result in formation of the 3-colpate type, as that seen in *Alonsoa*. However, the type of pollen observed in *Alonsoa* may also develop from the 3-colporate type by reduction of ora.

Ancestral types of exine sculpture are probably rough and foveolate ones, whereas rugulate, rugulate-foveolate and rugulate-microreticulate types can be seen as a mor-

phological transition (or a trend) toward more advanced types observed in next-branching clades (*Aptosimeae*, *Leucophylleae* / *Myoporeae* and core *Scrophulariaceae*). The 3- and 4(or 5)-colporate types of apertures with rough, foveolate and microreticulate exine sculpture (similar to the patterns seen in *Hemimerideae*) also occur in some representatives of the tribes *Teedieae* and *Buddlejeae* (Mosyakin & Tsybalyuk 2015a, 2015b). Pollen grains of *Androya* H. Perrier also have 3-colporate pollen (Mosyakin & Tsybalyuk 2015a, 2015b).

Conclusions

The palynomorphological data obtained for members of *Hemimerideae* agree with the results of molecular phylogenetic studies. In particular, close relationships of the two sister groups, *Diascia* + *Nemesia* and *Diclis* + *Hemimeris*, are supported by our palynomorphological studies.

As we have already demonstrated (Mosyakin & Tsybalyuk 2015a, 2015b and in the present article), in all main basal clades of *Scrophulariaceae* we see a recurrent pollen morphological pattern, namely representatives of early-branching lineages within these clades (*Colpasia* in *Hemimerideae*, *Androya* in the *Leucophylleae* / *Myoporeae* / *Androya* clade, *Buddleja* L. in *Buddlejeae* and *Freylinia* Colla in *Teedieae*) usually have 3-colporate pollen with rather “primitive” (smooth, rough and/or foveolate) exine sculpture. In the core *Scrophulariaceae* clades (*Scrophularieae* and *Limoselleae*) we also often observe 3-colporate pollen, but usually with more advanced sculpture.

That peculiar pattern, in our opinion, supports our hypothesis of the ancestral pollen type in *Scrophulariaceae* (Mosyakin & Tsybalyuk 2015a, 2015b). Judging from pollen morphological evidence compared with molecular phylogenetic data and molecular clock estimates mentioned above, we may conclude that (1) the ancestral pollen type within the *Scrophulariaceae* was 3-colporate with a rather “primitive” exine sculpture; (2) main trends and pathways of further morphological evolution of pollen in all lineages of *Scrophulariaceae* had already formed at the early stages of diversification of the family, about the time of divergence of its main lineages; (3) because of that we observe now in all main lineages of the family the signatures of parallel evolution of the major morphological pollen characters, combined with progressive diversification of exine sculpture patterns.

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