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

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

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 Madagascar), 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...
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 “Phyla-
canthus Nees et Mart.” (a typographic error corrected in Bentham 1835: Thylectanthera Nees & Mart., now con-
sidered a synonym of Angelonia). Further discussion fol-
lowed in Bentham’s Scrophulariaeae indicae (Bentham 1835) and an article specifically on this tribe (Bentham 1836), in which he recognized six genera (with addi-
tion of Diascia and Dichis). Discussion continued later on in his monographic treatments of Scrophulariaeae (Bentham 1846, 1876), in which his vision of the tribe was somewhat changed, but not considerably.

Baillon (1888) proposed the name Alonsoaee and included in this tribe the genera Alonsoa, Angelonia, Colpia, Diascia, Dichis, Hemimeris and Nemesia. Since Hemimeris is the type of Hemimerideae, the trib-
al name coined by Baillon was nomenclaturally super-
fluous 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 Alonsoaee can be used if the tribe is accepted in a different circumscription, exclud-
ing Hemimeris. Barringer (1993) described Alonsoaee 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 attrib-
uted to Baillon (see Reveal 2012).

Steiner (1996) proposed a name Alonsoaee and included in this tribe the genera Alonsoa, Ang
elonia, Colpia, Diascia, Dichis, 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 Alonsoaee can be used if the tribe is accepted in a different circumscription, excluding Hemimeris. Barringer (1993) described Alonsoaee 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 Colpia in tribe Bowkerieae Barringer (now in Stilbaceae), which con-
tricts the recent molecular data, and included Alonsoa in Hemimerideae. Kornhall (2004) supported the placement of Colpia 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 Alonso-
seeae, while leaving Basistemum Turcz., Colpia, Dias-
cia, Dichis, Hemimeris and Nemesia in Hemimerideae (based mainly on morphological evidence). Takhtajan (1987, 1997, 2009) accepted Hemimerideae as a tribe of Scrophulariaeae in all recent versions of his sys-
tem, but in somewhat differing circumscriptions. Ac-
cording to the latest version (Takhtajan 2009), the tribe contains Basistemum (now placed in Plantaginaceae s.l.: see Oxelman & al. 2005; Tank & al. 2006; Olm-
stead 2012), Colpia, Diascia, Dichis, Hemimeris and Nemesia. However, Alonsoa and the monotypic genus Charadrophila were placed by Takhtajan (2009) in a separate tribe, Alonsoaee (attributed by Takhtajan to Barringer). Molecular data (Olmstead & al. 2001) did not support the segregation of Alonsoa in a tribe separ-
ate from Hemimerideae. Doweld (2001) erected the separate family Hemimeridaeae; 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 (bas-
al) lineage of Scrophulariaeae 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 Scrophulariaeae 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 (Scrophulariaeae 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 ear-
lier (Datson & al. 2008).

Importance of Hemimerideae for understanding pol-
en 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; Snij-
man 2013).

However, published data on pollen morphology of representatives of Hemimerideae are very scarce. Erdt-
man (1952), using light microscopy only, studied pollen grains of Alonsoa acidofilha Ruiz & Pav., Dichis 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 com-
parative 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 Scrophulariaeae makes this group very interesting and promising for revealing the pat-
terns and trends of morphological pollen evolution in the family, especially in comparison with pollen morphology data obtained for other early-branching lineages of Scrophulariaeae 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 & Tsymbalyuk 2015a, 2015b) and briefly reported here.

For LM (Biolar, × 700), the pollen was acetylised 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 ½–¾ as thick as or nearly equaling 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, Nemesis 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* (L.f.) Steud.

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, microreticulate. Colpi membrane smooth and granulate. Exine 2–5.3 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. ½ 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

*Diclis* 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 ± distinct, strict margins, rounded at ends; aperture membrane smooth. Endoapertures indistinct. Exine 1.3–2.4 µm thick. Tectum nearly equaling 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** Link & Otto

**Hemimerideae**

**Alonsoa** Ruiz & Pav.

*Alonsoa unilabia*ta (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 equaling 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°16.01'3S, 18°25.249'E, 165 m, 4 Sep 2001, K. Steiner 3726 (MO).

**Colpis** E. Mey, ex Benth.

*Colpis 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. ½ 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: 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 sphe-
roidal and oblate-spheroidal, in polar view 6-lobed, in equatorial view elliptic or orbicular. P=17.3 – 21.3 µm, E=14.6 – 21.3 µm. Colpi 0.7 – 1.1 µm wide, with ± distinct, uneven margins and acute ends; colpus membrane smooth. Endoapertures indistinct. Exine 1.1 – 1.3 µm thick. Tectum nearly equalling infratectum. Columellae indistinct. Exine sculpture indistinct, foveolate.

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 µm; B, C, E, F, H, I, K, L, N, O = 1 µm.

*SEM* — Sculpture foveolate and microreticulate. Luminaria of reticulum rounded or elongated; muri wide. Colpus membrane smooth.
**Hemimeris racemosa** L. f.

**Hemimeris I. F. La Croix 3322**

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

**Hemimeris sabulosa** L. f. (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 = 21.3–29.3 µm, E = 21.3–29.3(–31.9) µm. 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. 1/2 as thick as infratectum. Columellae distinct, thick, arranged regularly. Exine sculpture distinct, macroteicticulate, near colpi almost microtecticulate (with smaller lumina).

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

Specimen investigated — **SOUTH AFRICA**: Western Cape Province, Pahkuis Pass area E of Clanwilliam on road to Pahkuis, 2200–2700’, 8 Oct 2001, W. Elsens 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 = 26.6–35.9 µm, E = 23.9–27.9 µm. Colpi 0.7–1.6 µm wide, with ± 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-microtecticulate and microtecticulate. 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-colpate, prolate, rarely oblate-spheroidal and spheroidal, in polar view 6- or 7-lobed, in equatorial view elliptic or orbicular. P = 15.9–22.6 µm, E = 15.9–21.3 µm. 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-microtecticulate and microtecticulate. 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 Turczanianow 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 & Cod 1981: 388).

**Nemesia strunosa** Bentham. (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 = 19.9–25.3 µm, E = 21.3–26.6 µm. 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 ± distinct. Exine sculpture distinct, microtecticulate.

**SEM** — Sculpture rugulate. Muri ± 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-microtecticulate 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, microtecticulate 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*...
and 6–8-colporate 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 μm) with acute ends, whereas in *H. sabulosa* colpi are narrower (0.7–1.6 μm) 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 *Colpia* 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 & Burtt 1984).
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. 3. Pollen grains of *Alonsoa, Colpia, Diascia* and *Diclis* (LM). – A–D: *Alonsoa unilabiata*; E–H: *Colpia 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 µm.
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 suggestion and demonstrate that 3-colporate pollen grains of *Colpias* are more similar to (but also distinguishable from) 3-colpate pollen of *Alonsoa*, but easily distinguished from 4–8-colpate 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-colpate pollen. *Alonsoa* is probably sister to the *Diclis* + *Hemimeris* clade; however, it differs in having 3-colpate 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 colpate pollen, as opposed to colporate

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**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 µm.
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 |
|------------------------|--------------|------------|---------------|------------------------|-------------------|------------------|-------------------------------|----------------------|---------------|
| Alonsoa acutifolia     | 27           | 23         | 3-colpate     | —                      | —                 | —                | microreticulate               | —                    | —             |
| (Erdtman 1952)         |              |            |               |                        |                   |                  |                               |                      |               |
| Alonsoa unilabiata     | 30.6–45.2    | 22.6–34.6  | 3-colpate     | wide, ends blunt       | 5.3–9.3           | granulate        | rugulate-microreticulate       | 2–2.7                | absent        |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Colpia mollis          | 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 |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Diascia capsularis     | 22.6–27.9    | 19.9–25.3  | 6- or 8-colpate| narrow, ends rounded  | 0.7–1.3           | smooth and granulate | rugulate-foveolate, rugulate-microreticulate | 1.3–2.4              | indistinct     |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Diascia elongata       | 19.9–27.9    | 18.6–23.9  | 6- or 7-colpate| narrow, ends acute     | 0.7–1.1(–1.3)     | smooth and rugulate | rugulate-foveolate, rugulate-microreticulate | 2–2.4                | indistinct     |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Diclis ovata           | 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        |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Diclis petiolaris      | 16           | 13.5       | 4- or 5-colpate| —                      | —                 | —                | —                            | —                    | —             |
| (Erdtman 1952)         |              |            |               |                        |                   |                  |                               |                      |               |
| Diclis reptans         | 20           | 15         | 6- or 7-colpate| —                      | —                 | —                | —                            | —                    | —             |
| (Erdtman 1952)         |              |            |               |                        |                   |                  |                               |                      |               |
| Hemimeris racemosa     | 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        |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Hemimeris sabulosa     | 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        |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Nemesia affinis        | 20.5         | 16         | 6- or 7-colpate| —                      | —                 | —                | reticulate                    | —                    | —             |
| (Erdtman 1952)         |              |            |               |                        |                   |                  |                               |                      |               |
| Nemesia cheiranthus    | 15.9–22.6    | 15.9–21.3  | 6- or 7-colpate| 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 |
|                        |              |            |               |                        |                   |                  |                               |                      |               |
| Nemesia strumosa       | 19.9–25.3    | 21.3–26.6  | 5- or 6-colpate| 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 colporate and colporate 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 Colpis) 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-colpate 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 morphological transition (or a trend) toward more advanced types observed in next-branching clades (Aptosineae, 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 Buddlejaceae (Mosyakin & Tsymbalyuk 2015a, 2015b). Pollen grains of Androya H. Perrier also have 3-colporate pollen (Mosyakin & Tsymbalyuk 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 & Tsymbalyuk 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 (Colpis in Hemimerideae, Androya in the Leucophylleae / Myoporeae / Androya clade, Buddleja L. in Buddlejaceae 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 Limoselieae) we also often observe 3-colpate pollen, but usually with more advanced sculpture.

That peculiar pattern, in our opinion, supports our hypothesis of the ancestral pollen type in Scrophulariaceae (Mosyakin & Tsymbalyuk 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-colpate 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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