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Pollen morphology of tribes *Aptosimeae* and *Myoporeae* supports the phylogenetic pattern in early-branching *Scrophulariaceae* revealed by molecular studies

Abstract

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Pollen morphology of six species representing three genera of tribe *Aptosimeae* and six species representing two genera of tribe *Myoporeae* (*Scrophulariaceae*) is described and illustrated using light microscopy (LM) and scanning electron microscopy (SEM). Pollen grains in *Aptosimeae* are 3-syncolporate, prolate, sometimes oblate-spheroidal in shape; mainly medium-sized, occasionally in some taxa small; exine sculpture striate, rarely with microperforations and small granules. Pollen grains in *Myoporeae* are 3-colpate-diorate, spheroidal, rarely oblate-spheroidal or prolate in shape; mainly medium-sized, occasionally in some taxa small; exine sculpture foveolate, microreticulate, rugulate-microperforate, rugulate, sometimes with small granules. Two major pollen types, 3-syncolporate (*Aptosimeae*) and 3-colpate-diorate (*Myoporeae*) are recognized by aperture types. Within these pollen types, eight subtypes are distinguished (three in *Aptosimeae*, five in *Myoporeae*) based on pollen size, exine sculpture and details of colpi and endoapertures. Our analysis of palynomorphological data (both published earlier and newly reported here) outlines the same main clades as those revealed by molecular phylogenetic studies, corresponding to *Aptosimeae*, *Leucophylleae*+*Myoporeae* and *Androya*, which differ by their pollen aperture types: 3-syncolporate, 3-colpate-diorate, and 3-colporate, respectively. There are also some differences in exine sculpture patterns. Thus, palynomorphological data are mainly consistent with recent results of molecular phylogenetic studies. Considerations on ancestral pollen character states in early-branching *Scrophulariaceae* are provided. It is assumed that the colporate type was probably ancestral in *Scrophulariaceae*; however, the ancestral status of the colpate type cannot be excluded as well.

Additional key words: exine sculpture, systematics, phylogeny, palynomorphology

Introduction

Current opinions on taxonomy and phylogeny of early-branching clades of *Scrophulariaceae*

Judging from the phylogenetic pattern revealed by recent molecular phylogenetic studies (Olmsted & al. 2001; Oxelman & al. 2005; Tank & al. 2006), the first early-branching lineage of the re-circumscribed *Scrophulariaceae* Juss. (s.str.) is represented by the tribe *Hemimerideae*, which is sister to the clade containing all other members of *Scrophulariaceae*. That large clade, in turn, comprises two subclades, one containing represent-

atives of three tribes, *Aptosimeae*, *Leucophylleae* and *Myoporeae* in their current updated circumscriptions, and another grouping the remaining representatives of *Scrophulariaceae*.

The tribe *Aptosimeae*, as outlined in recent studies (Olmsted & Reeves 1995; Olmsted & al. 2001; Fischer 2004; Oxelman & al. 2005; Tank & al. 2006; Takhtajan 2009), is a group represented by three genera (*Anticharis* Endl., *Aptosimum* Burch. ex Benth., and *Peliostomum* E. Mey. ex Benth.) and c. 40 species geographically restricted to tropical and southern Africa and southern Asia from the Arabian Peninsula to Malesia. Two genera, *Ap-*

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tosimum with c. 20 species and *Peliostomum* with seven species, occur only in tropical and southern Africa, and representatives of *Anticharis* (with c. 14 species) also occur in southern Asia. South African taxa of *Aptosimeae* have a distinctive pollination syndrome. These species are reported to be pollinated by several taxa of a specialized group of pollen wasps (*Hymenoptera*, tribe *Masariini*), which are behaviourally distinct from all other aculeate wasps, approaching in that respect the true bees. Widespread association of *Aptosimeae* with these pollen wasps and floral specialization of species of wasp genera *Celonites* and *Masarina* have been established (Gess & Gess 2010). The tribe is also of special interest because one of its genera, *Anticharis*, contains the only known group of species of *Scrophulariaceae* having C₄ photosynthesis (Sage & al. 2011; Khosravesht & al. 2012), which occurs very rarely in *Lamiales* (also registered in *Blepharis* Juss. in the *Acanthaceae*).

The *Aptosimeae* clade is sister to the clade *Androya+Leucophylleae+Myoporeae* (Oxelman & al. 2005; Tank & al. 2006). In the working version of the system of *Lamiales* (Olmstead 2012), *Aptosimeae* also contain *Stemodiopsis* Engl. (perhaps erroneously). Oxelman & al. (2005) placed *Stemodiopsis* in *Linderniaceae*, “[a] novel strongly supported clade of taxa earlier assigned to *Scrophulariaceae*...” with “unclear relationships to the rest of *Lamiales*.” Later, Rahmzadeh & al. (2005) placed this genus in *Gratiolaceae* (tribe *Stemodieae*, validly published in Reveal 2012). In publications that followed (Tank & al. 2006; Schäferhoff & al. 2010) *Stemodiopsis* was placed in *Linderniaceae*. The newest molecular phylogenetic data by Fisher & al. (2013) confirmed the close relationships of *Stemodiopsis* with other genera of *Linderniaceae*.

The *Myoporeae* clade in a strict sense currently includes the genera *Eremophila* R. Br. (more than 200 species, Australia, mostly arid regions in the west), *Myoporum* Sol. ex G. Forst. (c. 30 species, mostly Australian, with some extending to southeastern Asia, islands of the Pacific and Indian oceans), *Bontia* L. (monotypic, the Caribbean area), *Pentacoelium* Zucc. (monotypic, from southern Japan through the Ryukyu Islands and Taiwan to southeastern mainland China), and three recently described genera endemic to a small southwestern region in Western Australia: *Diocirea* Chinnock (four species), and monotypic *Calamphoreus* Chinnock and *Glycosystis* Chinnock (Chinnock 2007; Olmstead 2012). In his detailed taxonomic revision of the group, Chinnock (2007) preferred to treat it at the family rank (as *Myoporaceae*) but admitted that combining *Myoporaceae* and *Leucophylleae* is a reasonable option and that the combined “Myoporaceous clade” is “...possibly best treated at subfamily level within the *Scrophulariaceae* s.str.” (Chinnock 2007: 84). Considering generic relationships, Chinnock (l.c.) indicated that “*Diocirea* and *Calamphoreus* can be considered derivatives of *Eremophila*” and assumed that *Glycosystis* is closely related to one of the endemic Australian sections of *Myoporum*.

Androya H. Perrier, the monotypic genus represented by *A. decaryi* H. Perrier, is sometimes placed in *Myoporeae* or treated as a genus unassigned to any tribe (see Olmstead 2012). In earlier versions of his system, Takhtajan (1987, 1997) placed *Androya* in *Buddlejaceae*. Takhtajan (2009) later moved it to *Myoporaceae*, together with *Bontia*, *Eremophila* (incl. *Pholidia* R. Br. and *Stenochilus* R. Br.) and *Myoporum*.

The *Leucophylleae* clade contains *Capraria* L. (four species), *Eremogeton* Standl. & L. O. Williams (monotypic) and *Leucophyllum* Bonpl. (c. 16 species, including *Faxonanthus* Greenm.; see Henrickson & Flyr 1985). Geographically this group is restricted to Central America and the Caribbean, southernmost regions of North America, and some parts of South America (see the map in Gándara & Sosa 2013). In all recent versions of Takhtajan’s system (Takhtajan 1987, 1997, 2009), *Leucophylleae* contained just two genera, *Eremogeton* and *Leucophyllum*, while *Capraria* was placed in the tribe *Gratiolaceae* of *Scrophulariaceae* s.l. Rahmzadeh & al. (2005) included *Capraria* in *Gratiolaceae* tribe *Gratiolaceae*, which contradicts the placement suggested by Oxelman & al. (2005) and Gándara & Sosa (2013). The generic status of *Faxonanthus* and the phylogenetic relationships of *Eremogeton* remained unresolved until recently, due to the lack of molecular data (Oxelman & al. 2005). Gándara & Sosa (2013) filled that gap and included in their molecular study representatives of all three genera, paying special attention to *Leucophyllum*. They demonstrated that this genus in its traditional circumscription is not monophyletic, since both *Capraria* and *Eremogeton* are rooted within it. The alternative taxonomic options they suggested are (1) to submerge all three entities in one genus or (2) to split *Leucophyllum* into three genera, preserving the generic rank for *Capraria* and *Eremogeton*.

Molecular studies (Oxelman & al. 2005; Tank & al. 2006; Gándara & Sosa 2013) convincingly revealed that the sister clades corresponding to re-circumscribed tribes *Leucophylleae* and *Myoporeae* are together, in turn, sister to *Androya*. Reveal (2012) united tribes *Aptosimeae*, *Leucophylleae* and *Myoporeae* in *Scrophulariaceae* subfam. *Myoporoideae*.

Published data on pollen morphology

Scarce information is available on pollen grains of representatives of *Aptosimeae*. As far as we know, only pollen grains of *Aptosimum depressum* Burch. were studied by Erdtman (1952) using light microscopy. The situation with data on pollen morphology of *Myoporeae* and *Leucophylleae* is better. In particular, Erdtman (1952, light microscopy only) studied pollen of *Bontia daphnoides* L., *Myoporum laetum* G. Forst., *M. sandwicense* A. Gray, *Pholidia adenotricha* F. Muell. ex Benth. (now *Eremophila adenotricha* (F. Muell. ex Benth.) F. Muell.), *P. alternifolia* (R. Br.) Wettst. (now *E. alternifolia* R. Br.) and *Capraria biflora* L. and mentioned some affinity of pollen

grains of *C. biflora* to those in some *Myoporaceae* (recognized then as a separate family). Punt & Leenhouts (1967; light microscopy only) studied pollen grains of the monotypic genus *Androya* restricted to Madagascar. Minkin & Eshbaugh (1989) provided information on pollen grains of *Leucophyllum texanum* Benth. (light and scanning electron microscopy). Niezgodna & Tomb (1975) studied pollen grains of 14 species of *Scrophulariaceae* (tribe *Leucophylleae* and *C. biflora*) and 14 species of *Myoporaceae* using light, scanning and transmission electron microscopy. The most extensive palynomorphological study of *Myoporeae* (Australian taxa) was recently carried out by Chinnock (2007), who studied pollen grains of 28 species, including four species of *Diocirea*, two species of the monotypic *Calamphoreus* and *Glycocystis*, 19 species of *Eremophila* and three species of *Myoporum*. Pollen grains of *Bontia daphnoides* and *Pentacoelium bontioides* Siebold & Zucc. were also studied for comparison.

Considering new data on phylogenetic relationships of early-branching (basal) lineages of *Scrophulariaceae* and the virtual absence of information on pollen grains of *Aptosimeae*, we decided to partially fill this gap. In order to obtain additional data for comparison, we also studied some species of *Myoporeae*. The main objective of the study was to analyse and compare data on pollen morphology of early-branching lineages of *Scrophulariaceae* with molecular phylogenetic evidence for revealing possible ways of morphological evolution and hypothetical ancestral character states of pollen in this family.

Material and methods

Pollen from six species belonging to all three genera of *Aptosimeae* was sampled in the herbarium of the Missouri Botanical Garden, St Louis, Missouri, U.S.A. (MO). Pollen from six species representing two genera (*Eremophila* and *Myoporum*) of *Myoporeae* was sampled in the herbarium of the M.G. Kholodny Institute of Botany, the National Academy of Sciences of Ukraine, Kyiv (Kiev), Ukraine (KW); herbarium codes according to Thiers (2015+). Investigated specimens are cited according to the label information.

Pollen morphology was studied using light microscopy (LM) and scanning electron microscopy (SEM). For light microscopy studies (LM, Biolar, $\times 700$), the pollen was acetolysed following Erdtman (1952). For size determinations, 20 measurements were taken along the polar and equatorial axes for each species.

For scanning electron microscopy (SEM, JSM-6060LA), pollen grains were treated with 96% ethanol, then the samples were sputter-coated with gold at the Center of Electron Microscopy of the M.G. Kholodny Institute of Botany (National Academy of Sciences of Ukraine, Kyiv). The micrographs were minimally edited with Adobe Photoshop 6.0 to enhance the images. Terminology used in descriptions of pollen grains mainly

follows the glossaries by Punt & al. (1994) and Tokarev (2002), with minor adjustments.

Results

General description of pollen grains

Aptosimeae

Pollen grains in monads, radially symmetrical, isopolar, 3-syncolporate, prolate, sometimes oblate-spheroidal; mainly medium-sized, occasionally in some taxa small: P[olar axis]=25.3–45.2 μm , E[quatorial diameter]=15.9–33.2 μm . Outline in polar view 3-lobate, in equatorial view elliptic, sometimes circular. Colpi 2.4–6.6 μm wide, fused on apocolpia, with distinct, strict and slightly thickened margins; colpus membrane smooth.

Endoapertures mainly distinct, rarely indistinct, lalongate or sometimes lolongate, elliptic or circular, 4–7.9 μm long, 2.7–9.3 μm wide, sometimes covered by margins of colpi.

Exine thin, 0.7–2 μm thick. Exine layers invisible, sometimes tectum two times thinner than lower layers. Exine sculpture striate, rarely with microperforations and small granules; apertures membrane psilate, granulate and granulate-verrucate.

Myoporeae

Pollen grains in monads, radially symmetrical, isopolar, 3-colpate-diorate, spheroidal, rarely oblate-spheroidal or prolate; mainly medium-sized, occasionally in some taxa small: P=17.3–35.9 μm , E=14.6–33.2 μm . Outline in polar view 3-lobate, in equatorial view elliptic or circular. Colpi 2.7–6.6 μm wide, with distinct \pm strict thickenings, or with slight to indistinct thickenings; margins uneven, slightly tapering to pointed or rounded ends; colpus membrane smooth.

Endoapertures distinct or indistinct, 4–13.3 μm long, 1.3–5.3 μm wide, lalongate, elliptic, margins uneven.

Exine 1.3–2.7 μm thick. Tectum two times thinner than infratectum. Columellae indistinct or distinct, short, arranged sparsely. Exine sculpture foveolate, microreticulate, rugulate-microperforate, rugulate, sometimes with small granules. Colpus membrane smooth, sometimes granulate.

Pollen types and subtypes

Pollen grains in the studied taxa can be subdivided into two basic types, based on their aperture types, with three and five subtypes, respectively. The subtypes are separated mainly according to the pollen size, exine sculpture, and details of the colpi and endoapertures.

Type I: 3-syncolporate.

This type includes all three genera of *Aptosimeae*.

Subtype Ia: P=25.3–33.2 μm , E=15.9–22.6 μm . Sculpture striate, rarely with microperforations. Colpi 2.7–4 μm wide. *Anticharis*.

1. Endoapertures distinct, elliptic, 4–6.6 μm long, 2.7–5.3 μm wide. Colpus membrane smooth. *Anticharis imbricata*.

2. Endoapertures indistinct, mainly circular, rarely elliptic, 4–6.6 μm long, 4–6.6 μm wide. Colpus membrane smooth and granulate. *Anticharis linearis*.

Subtype Ib: P=25.3–34.6 μm , E=18.6–23.9 μm . Sculpture striate, rarely with microperforations and small granule. Colpi 2.4–4 μm wide. *Peliostomum*.

1. Endoapertures distinct, circular or elliptic, 4–5.3 μm long, 2.7–6.6 μm wide, covered by margins of colpi. Colpus membrane smooth, rarely granulate- verrucate. *Peliostomum leucorrhizum*.

2. Endoapertures distinct, circular or elliptic, 4–6.6 μm long, 2.7–4 μm wide, covered by margins of colpi. Colpus membrane smooth, rarely granulate. *Peliostomum virgatum*.

Subtype Ic: P=34.6–45.2 μm , E=22.6–33.2 μm . Sculpture striate. *Aptosimum*.

1. Colpi 2.4–2.7 μm wide. Endoapertures distinct, lologate, elliptic, 5.3–7.9 μm long, 7.9–9.3 μm wide. Colpus membrane smooth or granulate-verrucate. *Aptosimum spinescens*.

2. Colpi 2.7–6.6 μm wide. Endoapertures distinct, circular, 6.6–7.9 μm long, 6.6–7.9 μm wide. Colpus membrane granulate and granulate-verrucate. *Aptosimum indivisum*.

Type II: 3-colpate-diorate.

This type includes all studied members of *Myoporeae*.

Subtype IIa: P=17.3–23.9 μm , E=18.6–23.9 μm . Sculpture foveolate. Colpi 2.7–5.3 μm wide, with pointed ends. Endoapertures indistinct, elliptic, 5.3–7.9 μm long, 1.3–2.4 μm wide. *Eremophila rotundifolia*, *E. sturtii*.

Subtype IIb: P=18.6–22.6 μm , E=18.6–21.3 μm . Sculpture microreticulate. Colpi 2.7–3.3 μm wide, with pointed ends. Endoapertures distinct or indistinct, elliptic, 4–6.6 μm long, 2.7–5.3 μm wide. *Myoporum oppositifolium*.

Subtype IIc: P=21.3–25.3 μm , E=22.6–23.9 μm . Sculpture microreticulate. Colpi 3.3–5.3 μm wide, with pointed ends. Endoapertures distinct, elliptic, 5.3–9.3 μm long, 2.7–3.3 μm wide. *Eremophila elderi*.

Subtype IIId: P=27.9–35.9 μm , E=26.6–31.9 μm . Sculpture rugulate-microperforate. Colpi 4–5.3 μm wide, with pointed ends. Endoapertures distinct, elliptic, 7.9–10.6 μm long, 2–2.4 μm wide. *Eremophila glabra*.

Subtype IIe: P=27.9–34.6 μm , E=25.3–33.2 μm . Sculpture rugulate. Colpi 2.7–6.6 μm wide, with rounded ends. Endoapertures distinct or indistinct, elliptic, 4–13.3 μm long, 2–4 μm wide. *Eremophila debilis*.

Descriptions of pollen grains

Tribes, genera within tribes and species within genera are listed alphabetically. The summary of pollen meas-

urements for all studied taxa is provided in Table 1. Main pollen morphology characters are additionally summarized in Table 2.

Aptosimeae

Anticharis Endl.

Anticharis imbricata Schinz (Fig. 1A, G; 3A–D).

LM — Pollen grains 3-syncolporate, prolate, in polar view 3-lobed, in equatorial view elliptic. P=25.3–33.2 μm , E=15.9–22.6 μm . Colpi 2.7–4 μm wide, with distinct, strict margins, slightly thickened, fused on apocolpia; colpus membrane smooth, sometimes with granules over endoaperture. Endoapertures distinct, elliptic, 4–6.6 μm long, 2.7–5.3 μm wide. Exine 0.7–1.3 μm thick. Exine layers invisible. Exine sculpture invisible.

SEM — Sculpture striate, rarely with microperforations. Colpus membrane smooth, sometimes granular over endoaperture.

Specimen investigated — NAMIBIA: grid ref. 2114 AA, flats SW of Brandberg West Mine, sandy stony open flats with scattered *Welwitschia* plants, 14 May 1976, *Oliver & Müller 6666* (MO).

Anticharis linearis Hochst. ex Asch. (Fig. 1B, H; 3E–H).

LM — Pollen grains 3-syncolporate, prolate, in polar view 3-lobed, in equatorial view elliptic. P=26.6–30.6 μm , E=19.9–22.6 μm . Colpi 2.7–4 μm wide, with distinct, strict margins, slightly thickened, fused on apocolpia; colpus membrane smooth, sometimes with granules over endoaperture. Endoapertures indistinct, mainly circular, rarely elliptic, 4–6.6 μm long, 4–6.6 μm wide. Exine 0.7–1.3 μm thick. Exine layers invisible. Exine sculpture invisible.

SEM — Sculpture striate, rarely with microperforations. Colpus membrane smooth and granulate, sometimes granular over endoaperture.

Specimen investigated — NAMIBIA: grid ref. 2618 AA, low bush plains c. 45 km N of Keetmanshoop, 13 Mar 1988, *P. Goldblatt & J. Manning 8748* (MO).

Aptosimum Burch. ex Benth.

Aptosimum indivisum Burch. ex Benth. (Fig. 1C, I; 3I–L).

LM — Pollen grains 3-syncolporate, prolate, in polar view 3-lobed, in equatorial view elliptic. P=37.2–45.2 μm , E=27.9–31.9 μm . Colpi 2.7–6.6 μm wide, with distinct, strict margins, slightly thickened, fused on apocolpia; colpus membrane smooth, sometimes with granules over endoaperture. Endoapertures distinct, circular, 6.6–7.9 μm long, 6.6–7.9 μm wide. Exine 1.1–2 μm thick. Exine layers invisible. Exine sculpture indistinct.

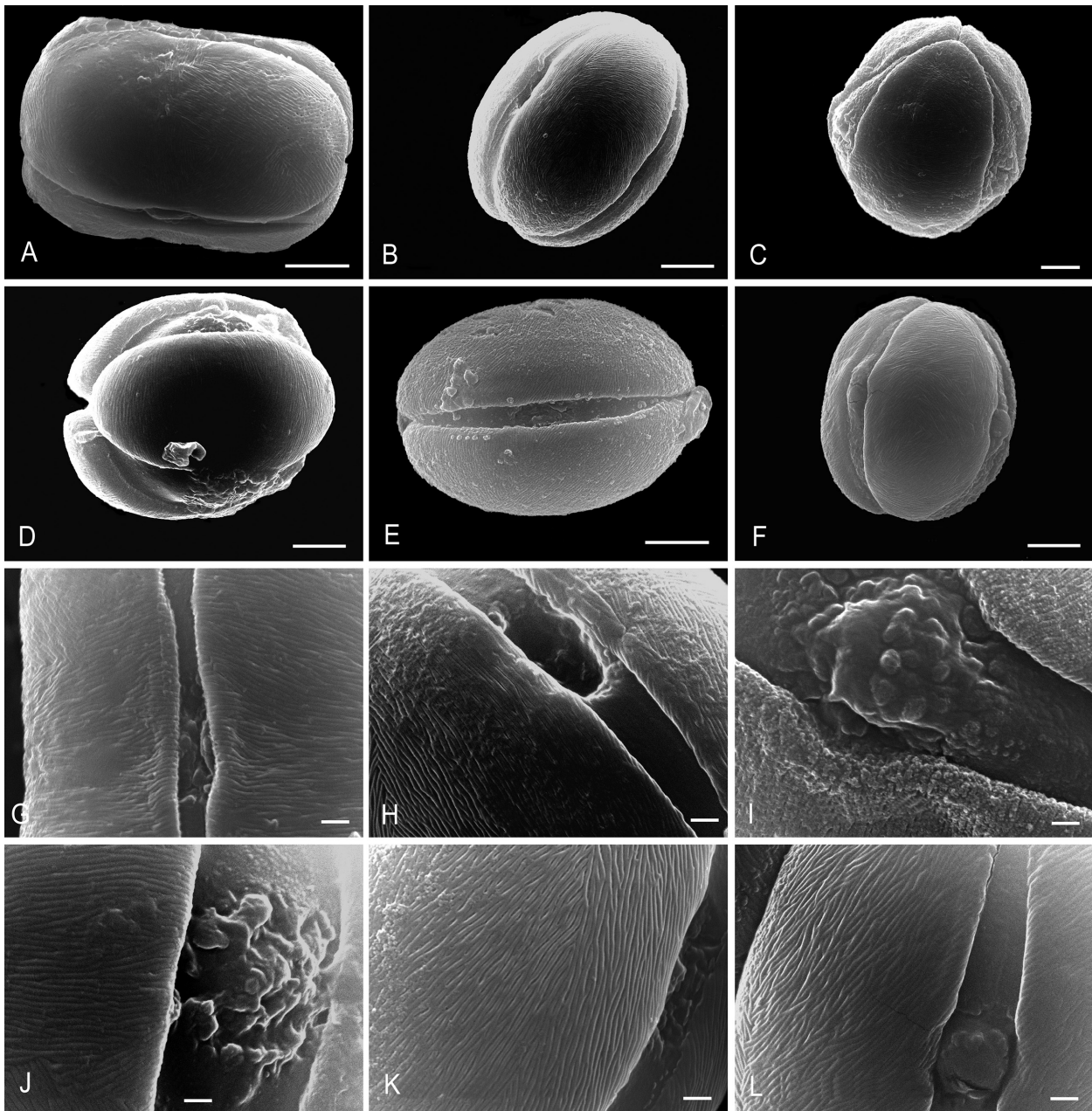


Fig. 1. Pollen grains of *Aptosimeae* (SEM) – A, G: *Anticharis imbricata*; B, H: *A. linearis*; C, I: *Aptosimum indivisum*; D, J: *A. spinescens*; E, K: *Peliostomum leucorrhizum*; F, L: *P. virgatum*; A–B, D–F: equatorial view; C: equatorial and polar view; exine sculpture: G, J, L: striate; H, K: striate with microperforations; I: granulate and granulate-verrucate aperture membranes. – Scale bars: A–F = 5 μ m; G–L = 1 μ m.

SEM — Sculpture striate. Colpus membrane granulate and granulate-verrucate, sometimes granular over endoaperture.

Specimen investigated — SOUTH AFRICA: NORTHERN CAPE: grid ref. 3119 (Calvinia) BD, along rail tracks near Downes Siding, 31°29'03"S, 19°57'04"E, 3606 ft, 12 Sep 2004, Peter Goldblatt & L. J. Porter 12415 (MO).

Aptosimum spinescens (Thunb.) Weber (Fig. 1D, J; 3M–P).

LM — Pollen grains 3-syncolporate, prolate, rarely oblate-spheroidal, in polar view 3-lobed, in equatorial view elliptic, sometimes circular. P=34.6–39.9 μ m, E=22.6–33.2 μ m. Colpi 2.4–2.7 μ m wide, with distinct, strict margins, slightly thickened, fused on apocolpia; colpus membrane smooth, sometimes with granules over endoaperture. Endoapertures distinct, elliptic, 5.3–7.9 μ m long, 7.9–9.3 μ m wide. Exine 1.1–1.3 μ m thick. Exine layers invisible. Exine sculpture indistinct.

SEM — Sculpture striate. Colpus membrane smooth or granulate-verrucate, sometimes granular over endoaperture.

Specimen investigated — NAMIBIA: grid ref. 2416 DB, Maltehöhe district, N of Maltehöhe, about 45 km on road to Walvis Bay, 24°40'495"S, 16°49'513"E, 1341 m, dry grassland, sandy loam soil, dryland (veld), level flat slope, 11 Mar 1995, *P. M. Burgoyne 3472* (MO).

Peliostomum E. Mey. ex Benth.

Peliostomum leucorrhizum E. Mey. ex Benth. (Fig. 1E, K; 3Q–T).

LM — Pollen grains 3-syncolporate, prolate, in polar view 3-lobed, in equatorial view elliptic. P=25.3–31.9 µm, E=18.6–22.6 µm. Colpi 2.7–4 µm wide, with distinct, strict margins, slightly thickened, fused on apocolpia; colpus membrane smooth, with granules over endoaperture. Endoapertures distinct, circular or elliptic, sometimes lolongate, 4–5.3 µm long, 2.7–6.6 µm wide, covered by margins of colpi. Exine 1.1–1.3 µm thick. Exine layers usually invisible; sometimes visible, with tectum two times thinner than lower layers. Exine sculpture invisible.

SEM — Sculpture striate, rarely with microperforations and small granules. Colpus membrane smooth, rarely granulate-verrucate.

Specimen investigated — SOUTH AFRICA: NORTHERN CAPE PROVINCE: 31 km N of Britstown (on N 13), karroid veld and sandy lax shrub steppe, 29 Sep 1974, *B. Nordenstam & J. Lundgren 2106* (MO).

Peliostomum virgatum E. Mey. ex Benth. (Fig. 1F, L; 3U–X).

LM — Pollen grains 3-syncolporate, prolate, in polar view 3-lobed, in equatorial view elliptic. P=27.9–34.6 µm, E=19.9–23.9 µm. Colpi 2.4–4 µm wide, with distinct, strict margins, slightly thickening, fused on apocolpia; colpus membrane smooth, with granules over endoaperture. Endoapertures distinct, circular or elliptic, 4–6.6 µm long, 2.7–4 µm wide, covered by margins of colpi. Exine 0.7–1.3 µm thick. Exine layers usually invisible; sometimes visible, with tectum two times thinner than lower layers. Exine sculpture invisible.

SEM — Sculpture striate, rarely with microperforations and small granules. Colpus membrane smooth, rarely granulate.

Specimen investigated — SOUTH AFRICA: NORTHERN CAPE: grid ref. 2917 DC, Namaqua National Park, Keerom, Keurbos homestead, 29°59'39"S, 17°39'38.5"E, 464 m, level succulent karroo, well-drained sand soil, granite, full sun, 12 Aug 2009, *Bester S. P. 9438* (MO), det. J. Ready, Sep 2009.

Myoporeae

Eremophila R. Br.

Eremophila debilis (Andrews) Chinnock (= *Myoporum debile* (Andrews) R. Br.) (Fig. 2E, K; 4Q–T).

LM — Pollen grains 3-colpate-diorate, prolate, oblate-spheroidal, rarely spheroidal, in polar view 3-lobed, in equatorial view elliptic or circular. P=27.9–34.6 µm, E=25.3–33.2 µm. Colpi 2.7–6.6 µm wide, with distinct, strict and thickened, or indistinct margins, slightly tapering to rounded ends; aperture membranes smooth. Endoapertures distinct or indistinct, elliptic, 4–13.3 µm long, 2–4 µm wide. Exine 2–2.7 µm thick. Tectum two times thinner than infratectum. Columellae distinct, short, sparsely arranged. Exine sculpture distinct, microreticulate.

SEM — Sculpture rugulate, sometimes with small granules. Colpus membrane smooth, sometimes also with small granules.

Specimen investigated — AUSTRALIA: QUEENSLAND: Darling Downs district, Yelarbon, around cemetery, c. 1 km from P.O., 28°34'S, 150°45'E, 240 m, topography flat, brown loamy soil, damaged *Eucalyptus* woodland, 10 Oct 1983, *E. M. Canning 5820 & B. Rimes* (KW).

Eremophila elderi F. Muell. (Fig. 2A, G; 4A–D).

LM — Pollen grains 3-colpate-diorate, spheroidal, rarely oblate-spheroidal and prolate, in polar view 3-lobed, in equatorial view circular. P=21.3–25.3 µm, E=22.6–23.9 µm. Colpi 3.3–5.3 µm wide, with distinct margins, slightly tapering to pointed ends; aperture membranes smooth. Endoapertures distinct, elliptic, 5.3–9.3 µm long, 2.7–3.3 µm wide. Exine 2–2.4 µm thick. Tectum two times thinner than infratectum. Columellae distinct or indistinct, short, arranged sparsely. Exine sculpture distinct, reticulate.

SEM — Sculpture microreticulate, rarely with small granules near colpi. Lumina of reticulum small, rounded, elongated or rounded-angular. Colpus membrane smooth, sometimes with small granules.

Specimen investigated — AUSTRALIA: SOUTH AUSTRALIA: NW region, N-facing slopes of N-most extremity of groups of prominent inselbergs between Deering Hills and Mann Ranges, c. 18 km NE of Mt Cooperinna, 26°15'00"S, 130°05'30"E, common between rocks on hillside, 8 Nov 1978, *N. N. Donner 6619* (KW).

Eremophila glabra (R. Br.) Ostenf. (Fig. 2B, H; 4E–H).

LM — Pollen grains 3-colpate-diorate, prolate, rarely spheroidal and oblate-spheroidal, in polar view 3-lobed, in equatorial view elliptic, rarely circular. P=27.9–35.9 µm, E=26.6–31.9 µm. Colpi 4–5.3 µm wide, with distinct, ± strict, thickened margins, slightly tapering to pointed ends; aperture membranes smooth. Endoapertures distinct, elliptic, with uneven margins, 7.9–10.6 µm long, 2–2.4 µm wide. Exine 2.4–2.7 µm thick. Tectum two times thinner than infratectum. Columellae mainly indistinct or distinct, short, sparsely arranged. Exine sculpture distinct, microreticulate.

SEM — Sculpture rugulate-microperforate, rare-

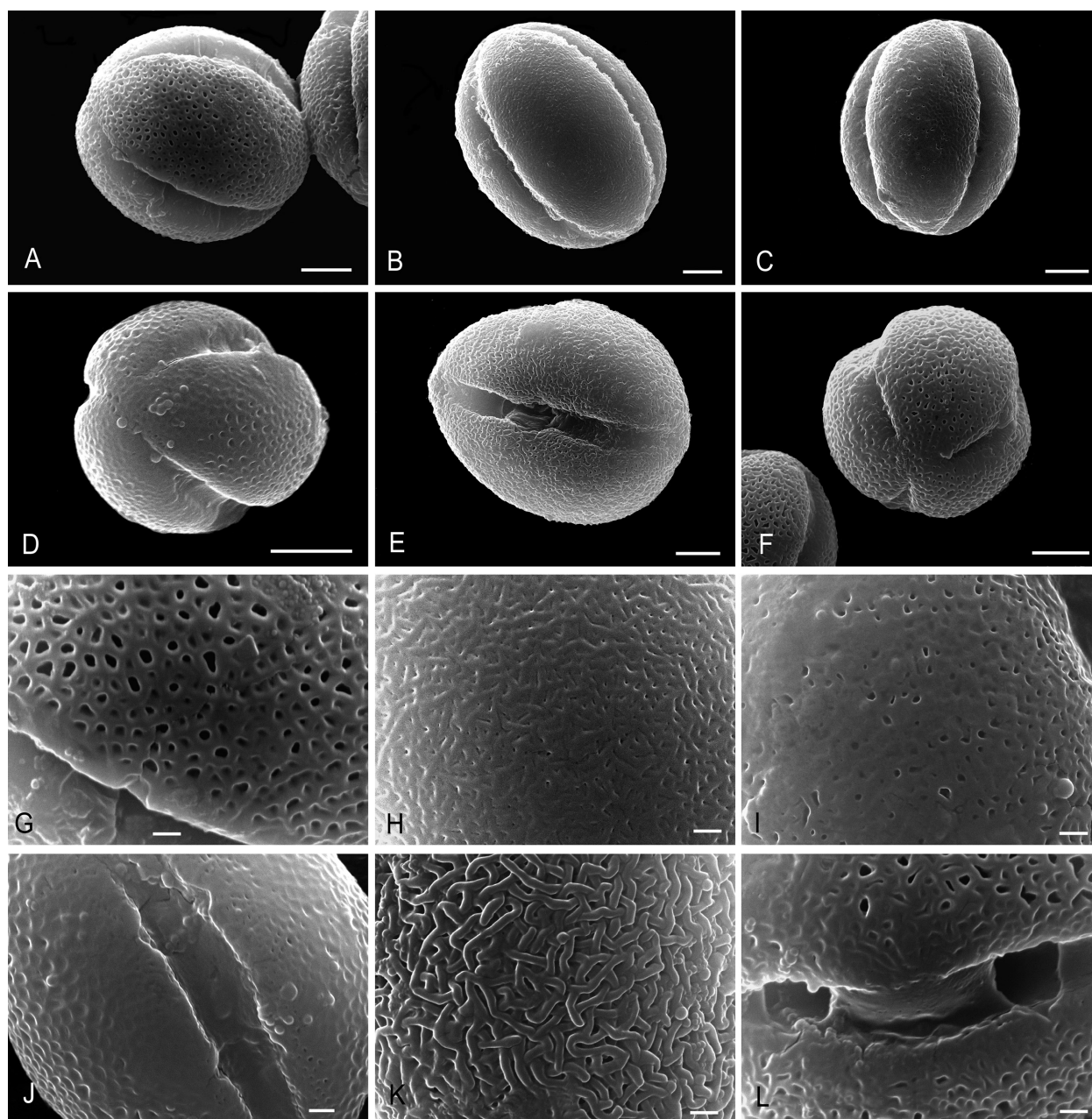


Fig. 2. Pollen grains of *Eremophila* and *Myoporum* (SEM) – A, G: *E. elderi*; B, H: *E. glabra*; C, I: *E. rotundifolia*; D, J: *E. sturtii*; E, K: *E. debilis*; F, L: *M. oppositifolium*; A–C, E: equatorial view; D, F: polar view; exine sculpture: G, L: microreticulate; H: rugulate-microperforate; I–J: foveolate; K: rugulate. – Scale bars: A–F = 5 μm ; G–L = 1 μm .

ly with small granules near colpi. Colpus membrane smooth, sometimes with small granules.

Specimen investigated — AUSTRALIA: SOUTH AUSTRALIA: N Eyre Peninsula, foot of Iron Duke, at S end of Middleback Range, c. 50 km SW of Whyalla, growing in deep red sand, 29 Dec 1970, A. *E. Orchard* 2942 (KW).

Eremophila rotundifolia F. Muell. (Fig. 2C, I; Fig. 4 I–L).

LM — Pollen grains 3-colpate-diorate, prolate and oblate-spheroidal, rarely spheroidal, in polar view 3-lobed, in equatorial view elliptic or circular. P=18.6–23.9 μm , E=18.6–23.9 μm . Colpi 4–5.3 μm wide, with indistinct,

\pm strict margins, slightly tapering to pointed ends; aperture membranes smooth. Endoapertures indistinct, elliptic, with uneven margins, 5.3–6.6 μm long, 1.3–2.4 μm wide. Exine 1.1–1.6 μm thick. Tectum two times thinner than infratectum. Columellae distinct, short, sparsely arranged. Exine sculpture distinct, microreticulate.

SEM — Sculpture foveolate. Colpus membrane smooth.

Specimen investigated — AUSTRALIA: SOUTH AUSTRALIA: Lake Eyre Basin, Beresford Hill, 29°16'S, 136°40'E, 80–100 m, extinct spring mound, limestone formation, 4 Oct 1978, K. *Chorney* 1020 (KW).

Eremophila sturtii R. Br. (Fig. 2D, J; 4M–P).

LM — Pollen grains 3-colpate-diorate, prolate, rarely spheroidal, in polar view 3-lobed, in equatorial view elliptic, rarely circular. $P=17.3\text{--}21.3\ \mu\text{m}$, $E=14.6\text{--}18.6\ \mu\text{m}$. Colpi $2.7\text{--}5.3\ \mu\text{m}$ wide, with indistinct, uneven margins, slightly tapering to pointed ends; aperture membranes smooth. Endoapertures indistinct, elliptic, with uneven margins, $5.3\text{--}7.9\ \mu\text{m}$ long, $1.3\text{--}2.4\ \mu\text{m}$ wide. Exine $1.1\text{--}1.6\ \mu\text{m}$ thick. Tectum two times thinner than infratectum. Columellae indistinct. Exine sculpture mainly indistinct, microreticulate.

SEM — Sculpture foveolate, rarely with granules near colpi. Colpus membrane smooth, sometimes with small granules.

Specimen investigated — AUSTRALIA: QUEENSLAND: Warrego district, c. 25 km SW of Eulo on Hungerford road, flood plain of Paroo River, $28^{\circ}20'S$, $144^{\circ}54'E$, grey sandy clay soil, *Eucalyptus largiflorens* open woodland with well-developed shrubby layer of *Eremophila mitchellii* and *E. sturtii*, 13 Sep 1973, R. J. Henderson H2048 & D. E. Boyland (KW).

Myoporum Sol. ex G. Forst.

Myoporum oppositifolium R. Br. (Fig. 2F, L; 4U–X).

LM — Pollen grains 3-colpate-diorate, spheroidal, rarely oblate-spheroidal and prolate, in polar view 3-lobed, in equatorial view circular. $P=18.6\text{--}22.6\ \mu\text{m}$, $E=18.6\text{--}21.3\ \mu\text{m}$. Colpi $2.7\text{--}3.3\ \mu\text{m}$ wide, with distinct, slightly thickening or indistinct margins, slightly tapering to \pm pointed ends; aperture membranes smooth. Endoapertures distinct or indistinct, elliptic, $4\text{--}6.6\ \mu\text{m}$ long, $2.7\text{--}5.3\ \mu\text{m}$ wide. Exine $2\text{--}2.7\ \mu\text{m}$ thick. Tectum two times thinner than infratectum. Columellae indistinct, short, sparsely arranged. Exine sculpture distinct, reticulate.

SEM — Sculpture microreticulate, sometimes with small granules. Lumina of reticulum small, rounded, elongated or rounded-angular. Colpus membrane smooth, sometimes with small granules.

Specimen investigated — AUSTRALIA: WESTERN AUSTRALIA: SW division, Augusta c. 115 km SSW of Bunbury, near golf links, 12 Oct 1967, A. M. Ashby 2377 (KW).

Discussion

Comparative pollen morphology of *Aptosimeae*

The palynomorphological data showed that pollen grains of the studied taxa of *Aptosimeae* have similar aperture types and sculpture; however, they differ in their size and some features of apertures (Table 1, 2). Thus, species of *Aptosimum* have the largest pollen grains and endoapertures as compared to two other genera, *Anticharis* and

Peliostomum (Table 1). Pollen grains of the two studied species of *Peliostomum* have distinctive features in the structure of their apertures: endoaperture edges are covered with edges of colpi, a feature not observed in *Anticharis* and *Aptosimum*.

The two studied species of *Aptosimum* differ in their pollen size and structure of apertures. Pollen grains of *A. indivisum* are larger, with wider colpi and circular endoapertures, while pollen grains of *A. spinescens* are smaller, with narrower colpi and elliptic endoapertures.

The studied species of *Anticharis* differ in the shape of endoapertures (Table 2).

Species of *Peliostomum* have pollen grains similar in size, colpi structure and exine sculpture. In both *P. leucorrhizum* and *P. virgatum* we observed both circular and elliptic endoapertures; however, in addition to that, longitudinal endoapertures also sometimes occur in *P. leucorrhizum*.

Probably the specialized pollination by pollen wasps (Gess & Gess 2010) mentioned above is partly responsible for at least some peculiar pollen morphology features of *Aptosimeae* revealed in the present study.

Comparative pollen morphology of *Myoporeae*

Our data showed that pollen grains in the studied species of tribe *Myoporeae* are similar by their 3-colpate-diorate type of apertures but differ in their size, exine sculpture and structural features of apertures (Table 1, 2).

The studied species of *Eremophila* differ in their pollen size, exine sculpture and structural features of apertures. The smallest pollen grains are characteristic of *Eremophila elderi*, *E. rotundifolia* and *E. sturtii*. Pollen grains of *E. rotundifolia* and *E. sturtii* are similar in having narrow endoapertures and foveolate sculpture; in contrast, pollen grains of *E. elderi* have wider endoapertures and microreticulate exine sculpture. Pollen grains of *E. debilis* and *E. glabra* are larger than those in the other three species. These two species differ by their colpi structure: colpi in *E. debilis* have rounded ends, whereas in *E. glabra* the ends are pointed. Pollen grains of these two taxa also differ from those in all other species by their exine sculpture (Table 2).

Pollen of *Myoporum oppositifolium* is similar to pollen of *Eremophila rotundifolia* and *E. sturtii* in size and colpi structure; however, it differs by exine sculpture and endoaperture structure (Table 1, 2).

In general, our data are comparable to data provided by Niezgoda & Tomb (1975), with some additional features revealed. In particular, we found in members of *Eremophila* some exine sculpture types that were not reported earlier: foveolate, rugulate-microperforate and rugulate. Niezgoda & Tomb (1975) mentioned that rugulate exine sculpture occurs only in two studied species, *Capraria biflora* and *Leucophyllum pruinosum* I. M. Johnst. Our studies demonstrated that this sculpture pattern also occurs in some representatives of *Eremophila*.



Fig. 3. Pollen grains of Aptosimeae (LM) – A–D: *Anticharis imbricata*; E–H: *A. linearis*; I–L: *Aptosimum indivisum*; M–P: *A. spinescens*; Q–T: *Peliostomum leucorrhizum*; U–X: *P. virgatum*; A, B, E, F, I, J, M, N, Q, R, U, V: equatorial view; C, D, G, H, K, L, O, P, S, T, W, X: polar view. – Scale bars: A–X = 10 μ m.

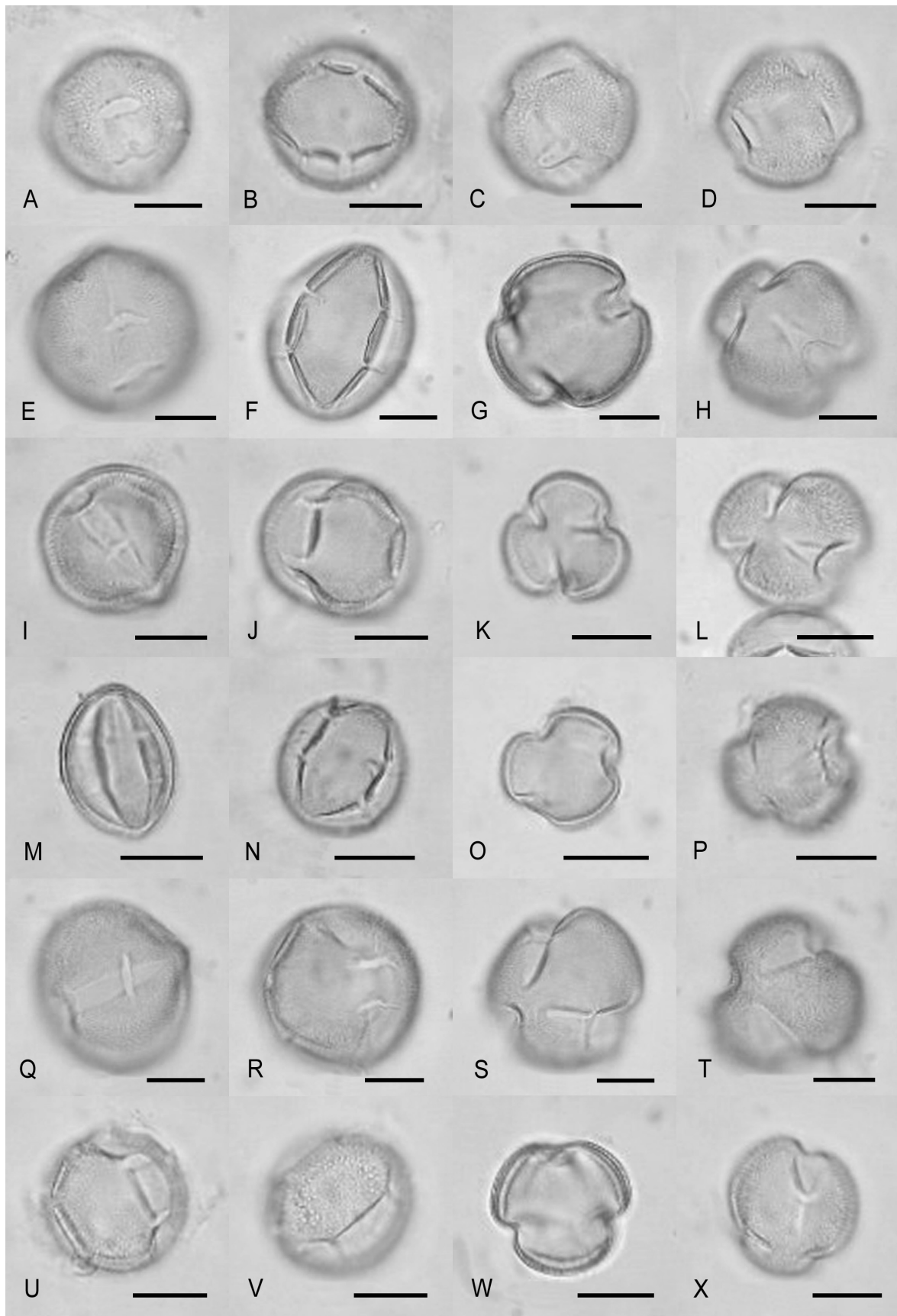


Fig. 4. Pollen grains of *Eremophila* and *Myoporum* (LM) – A–D: *E. elderi*; E–H: *E. glabra*; I–L: *E. rotundifolia*; M–P: *E. sturtii*; Q–T: *E. debilis*; U–X: *M. oppositifolium*; A, B, E, F, I, J, M, N, Q, R, U, V: equatorial view; C, D, G, H, K, L, O, P, S, T, W, X: polar view. – Scale bars: A–X = 10 μ m.

Table 1. Summary of pollen measurements. All dimensions are in μm .

Taxon	Polar axis	Equatorial axis	Colpi width	Endoaperture length	Endoaperture width	Exine thickness
<i>Anticharis imbricata</i>	25.3–33.2	15.9–22.6	2.7–4.0	4.0–6.6	2.7–5.3	0.7–1.3
<i>Anticharis linearis</i>	26.6–30.6	19.9–22.6	2.7–4.0	4.0–6.6	4.0–6.6	0.7–1.3
<i>Aptosimum indivisum</i>	37.2–45.2	27.9–31.9	2.7–6.6	6.6–7.9	6.6–7.9	1.1–2.0
<i>Aptosimum spinescens</i>	34.6–39.9	22.6–33.2	2.4–2.7	5.3–7.9	7.9–9.3	1.1–1.3
<i>Peliostomum leucorrhizum</i>	25.3–31.9	18.6–22.6	2.7–4.0	4.0–5.3	2.7–6.6	1.1–1.3
<i>Peliostomum virgatum</i>	27.9–34.6	19.9–23.9	2.4–4.0	4.0–6.6	2.7–4.0	0.7–1.3
<i>Eremophila debilis</i>	27.9–34.6	25.3–33.2	2.7–6.6	4.0–13.3	2.0–4.0	2.0–2.7
<i>Eremophila elderi</i>	21.3–25.3	22.6–23.9	3.3–5.3	5.3–9.3	2.7–3.3	2.0–2.4
<i>Eremophila glabra</i>	27.9–35.9	26.6–31.9	4.0–5.3	7.9–10.6	2.0–2.4	2.4–2.7
<i>Eremophila rotundifolia</i>	18.6–23.9	18.6–23.9	4.0–5.3	5.3–6.6	1.3–2.4	1.1–1.6
<i>Eremophila sturtii</i>	17.3–21.3	14.6–18.6	2.7–5.3	5.3–7.9	1.3–2.4	1.1–1.6
<i>Myoporum oppositifolium</i>	18.6–22.6	18.6–21.3	2.7–3.3	4.0–6.6	2.7–5.3	2.0–2.7

Chinnock (2007) reported that pollen grains of almost all genera (selected representatives of *Calamphoreus*, *Eremophila*, *Glycocyttis* and *Myoporum*) have reticulate exine (coarsely and finely reticulate), and only all four species of *Diocirea* have punctitegillate (perforate) exine sculpture. However, our new data indicated that an exine sculpture pattern very similar to punctitegillate (perforate) also occurs in some species of *Eremophila*, in particular, *E. rotundifolia* and *E. sturtii*. Because of that, we believe that this peculiar exine pattern cannot be regarded as a reliable character distinguishing *Diocirea* and *Eremophila*.

Species of *Eremophila* studied by us belong to different sections (following the system proposed by Chinnock 2007) and differ by their pollen morphology. However, at present we have insufficient palynological data to analyse whether these characters support the sections, and pollen studies of additional taxa of that genus are needed.

Comparison of palynomorphological and molecular phylogenetic evidence

According to molecular phylogenetic data (Oxelman & al. 2005; Khosravesh & al. 2012), *Anticharis* and *Peliostomum* are sister groups within *Aptosimeae*. Palynomorphological data confirm the close relationship between these two genera, as shown above. It has been also shown that within *Anticharis* three clades can be outlined reflecting the gradual transition to C_4 photosynthesis (Khosravesh & al. 2012). *Aptosimum* is sister to the *Anticharis*+*Peliostomum* clade, and pollen morphology data also support that pattern, since the morphological differences between pollen grains of *Anticharis* and *Peliostomum* are exceeded by the differences between those two genera and *Aptosimum*.

According to molecular phylogenetic data (Oxelman & al. 2005), the genera *Bontia*, *Eremophila* and *Myopo-*

rum (*Myoporeae* in the strict sense) form a clade sister to the *Capraria*+*Leucophyllum* clade (*Leucophylleae*). Our palynomorphological data, as well as data of other authors (Niezgoda & Tomb 1975), are compatible with the molecular phylogenetic data. Pollen grains of all studied representatives of these genera are 3-colpate-diorate, with foveolate, microreticulate, reticulate, rugulate-perforate and/or rugulate exine sculpture.

The monotypic Madagascan genus *Androya* has been revealed by molecular phylogenetic analysis (Oxelman & al. 2005) as the most early-branching clade, which is sister to the clade containing all other genera of *Myoporeae* and *Leucophylleae*. According to Punt & Leenhouts (1967), pollen grains of *Androya* are 3-colporate, with smooth exine sculpture, thus differing considerably from pollen grains of other taxa of *Myoporeae* and *Leucophylleae*.

Oxelman & al. (2005) indicated that members of *Leucophylleae* (*Capraria* and *Leucophyllum*) are closely related to *Myoporeae* according to anatomical and palynomorphological evidence. Considering possible relationships of *Aptosimeae*, they mentioned that “[m]onophyly of *Aptosimeae* is strongly supported by our chloroplast DNA sequences, and a sister group relation with *Androya*, *Leucophylleae*, and *Myoporeae* is moderately supported. All have 3-colpate, diporate [sic!] pollen (Erdtman 1952; Niezgoda & Tomb 1975; Punt 1980)” (Oxelman & al. 2005: 419). The word “diporate” in their text is most probably an error for “diorate”.

In fact, our data indicate that pollen grains in the clades *Aptosimeae*, *Leucophylleae*+*Myoporeae* and *Androya* evidently differ by their aperture types: 3-syncolporate, 3-colpate-diorate, and 3-colporate, respectively. There are also some differences in exine sculpture patterns: striate in *Aptosimeae*; rather diverse (but not striate nor smooth) in *Leucophylleae*+*Myoporeae*, a more diverse and species-rich group; and smooth in *Androya* (Table 2).

Table 2. Summary of pollen morphological characters: original data compared with literature data. “—” = no data reported.

Taxon	Apertures	Colpus membrane	Endoaperture	Exine sculpture	Source of data
<i>Aptosimeae</i>					
<i>Anticharis imbricata</i>	3-syncolporate	smooth	elliptic	striate, rarely with microperforations	original data
<i>Anticharis linearis</i>	3-syncolporate	smooth and granulate	mainly circular, rarely elliptic	striate, rarely with microperforations	original data
<i>Aptosimum indivisum</i>	3-syncolporate	granulate and granulate-verrucate	circular	striate	original data
<i>Aptosimum spinescens</i>	3-syncolporate	smooth or granulate-verrucate	elliptic	striate	original data
<i>Peliostomum leucorrhizum</i>	3-syncolporate	smooth, rarely granulate-verrucate	circular or elliptic	striate, rarely with microperforations and small granules	original data
<i>Peliostomum virgatum</i>	3-syncolporate	smooth, rarely granulate	circular or elliptic	striate, rarely with microperforations and small granules	original data
<i>Myoporeae</i>					
<i>Eremophila debilis</i>	3-colpate-diorate	smooth, sometimes also with small granules	elliptic	rugulate, sometimes with small granules	original data
<i>Eremophila elderi</i>	3-colpate-diorate	smooth, sometimes with small granules	elliptic	microreticulate, rarely with small granules near colpi (small lumina)	original data
<i>Eremophila glabra</i>	3-colpate-diorate	smooth, sometimes also with small granules	elliptic	rugulate-microperforate, rarely with small granules near colpi	original data
<i>Eremophila rotundifolia</i>	3-colpate-diorate	smooth	elliptic	foveolate	original data
<i>Eremophila sturtii</i>	3-colpate-diorate	smooth, sometimes with small granules	elliptic	foveolate, rarely with granules near colpi	original data
<i>Eremophila</i> (all studied spp.)	3-colpate-diorate	—	elliptic	finely reticulate (lumina of irregular size)	Niezgoda & Tomb (1975)
<i>Eremophila</i> (all studied spp.)	3-colpate-diorate	—	—	finely reticulate	Chinnock (2007)
<i>Myoporum oppositifolium</i>	3-colpate-diorate	smooth, sometimes with small granules	elliptic	microreticulate, sometimes with small granules (small lumina)	original data
<i>Myoporum acuminatum</i> R. Br.	3-colpate-diorate	—	elliptic	finely reticulate (very large lumina)	Niezgoda & Tomb (1975)
<i>Myoporum</i> (all studied spp. except <i>M. acuminatum</i>)	3-colpate-diorate	—	elliptic	finely reticulate	Niezgoda & Tomb (1975)
<i>Myoporum</i> (all studied spp.)	3-colpate-diorate	—	—	finely or coarsely reticulate	Chinnock (2007)
<i>Pentacoelium</i>	3-colpate-diorate	—	—	finely reticulate	Chinnock (2007)
<i>Diocirea</i> (all studied spp.)	3-colpate-diorate	—	—	punctitegillate	Chinnock (2007)
<i>Calamphoreus</i>	3-colpate-diorate	—	—	finely reticulate	Chinnock (2007)
<i>Glycocystis</i>	3-colpate-diorate	—	—	finely reticulate	Chinnock (2007)
<i>Bontia daphnoides</i>	3-colpate-diorate	—	—	finely reticulate	Chinnock (2007)
<i>Bontia daphnoides</i>	3-colpate-diorate	—	elliptic	finely reticulate (lumina of irregular size)	Niezgoda & Tomb (1975)
<i>Leucophylleae</i>					
<i>Capraria biflora</i>	3-colpate-diorate	—	elliptic	rugulate	Niezgoda & Tomb (1975)
<i>Leucophyllum pruinosum</i>	3-colpate-diorate	—	elliptic	rugulate	Niezgoda & Tomb (1975)
<i>Leucophyllum</i> (all studied spp. except <i>L. pruinosum</i>)	3-colpate-diorate	—	elliptic	finely reticulate (lumina smaller near colpi)	Niezgoda & Tomb (1975)

Taxon	Apertures	Colpus membrane	Endoaperture	Exine sculpture	Source of data
<i>Leucophylleae</i> (continued)					
<i>Leucophyllum texanum</i>	3-colpate-diorate	psilate	—	reticulate	Minkin & Eshbaugh (1989)
<i>Faxonanthus pringlei</i>	3-colpate-diorate	—	elliptic	finely reticulate (small lumina)	Niezgoda & Tomb (1975)
<i>Eremogeton grandiflorus</i>	3-colpate-diorate	—	elliptic	finely reticulate (large irregular lumina)	Niezgoda & Tomb (1975)
<i>Androya</i> clade					
<i>Androya decaryi</i>	3-colporate	smooth	indistinct	smooth	Punt & Leenhouts (1967)

Our palynomorphological data, as well as other palynomorphological (Niezgoda & Tomb 1975) and molecular (Oxelman & al. 2005) evidence, indicate that the *Androya* clade probably merits recognition as a separate tribe, if *Leucophylleae* and *Myoporeae* are also recognized as separate tribes. An alternative logical solution would be an extended *Myoporeae*, re-circumscribed to include *Leucophylleae* and *Androya*.

Possible ancestral pollen character states in *Scrophulariaceae*

Pollen grains of representatives of all these tribes are characterized by the complex colporate type (combining ectoapertures and endoapertures). It may be assumed that this type is ancestral in the family *Scrophulariaceae*; however, the ancestral status of the colpate type cannot be excluded as well. If we postulate the colporate type as being ancestral in the family, then the 3-colporate type with smooth exine sculpture is probably the most ancient pattern, being also typical for many representatives of *Buddlejeae* and *Teedieae* (Tsymbalyuk & Mosyakin 2013; Mosyakin & Tsymbalyuk 2015), as well as for *Androya*. Further evolutionary changes of this type could have led to the formation of more advanced 3-colpate-diorate pollen with rugulate and reticulate exine sculpture (peculiar to *Leucophylleae+Myoporeae*) and 3-syncolporate pollen with mainly striate sculpture (typical for *Aptosimeae*, as shown in this article), either in parallel or consecutively.

Our original data on pollen morphology of *Hemimerideae*, most probably the basalmost clade of *Scrophulariaceae*, will be published later. However, it is already evident from our preliminary data that two subclades of that tribe have colpate and colporate pollen, respectively, which is consistent with our hypothesis on pollen evolution patterns in early-branching *Scrophulariaceae*.

Conclusions

Our analysis of available palynomorphological data (both published earlier and newly reported here) clearly outlines the same main clades as those revealed by molecu-

lar phylogenetic studies (Oxelman & al. 2005; Gándara & Sosa 2013): *Aptosimeae* (3-syncolporate pollen grains, striate exine sculpture); *Leucophylleae+Myoporeae* (3-colpate-diorate pollen grains, foveolate, microreticulate, rugulate-microperforate, rugulate and/or reticulate exine sculpture); and *Androya* (3-colporate pollen grains, smooth exine sculpture). The 3-colporate pollen type with smooth exine sculpture, typical for *Androya* and occurring also in many representatives of *Buddlejeae* and *Teedieae* (Tsymbalyuk & Mosyakin 2013; Mosyakin & Tsymbalyuk 2015), is hypothesized to be ancestral in *Scrophulariaceae*. The clades *Leucophylleae+Myoporeae* and *Aptosimeae* have more advanced pollen types: 3-colpate-diorate with mainly rugulate and reticulate exine in the former, and 3-syncolporate with mainly striate sculpture in the latter.

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