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Morphology of pollen in Apiales (Asterids, Eudicots)

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Abstract

In this monograph, for the first time, the pollen morphology was analysed in the context of modern taxonomic treatment of the order and statistically evaluated in search of traits that could be utilised in further taxonomic and evolutionary studies. Our research included pollen sampled from 417 herbarium specimens representing 158 species belonging to 125 genera distributed among all major lineages of Apiales. The pollen was mechanically isolated, acetolysed, suspended in pure glycerine and mounted on paraffin-sealed slides for light microscopy investigation.

Although most of the analysed traits were highly homoplastic and showed significant overlap even between distantly related lineages, we were able to construct a taxonomic key based on characters that bear the strongest phylogenetic signal: P/E ratio, mesocolpium shape observed in polar view and ectocolpus length relative to polar diameter. All the investigated traits are easy to observe with light microscopy and defined by clear and well-documented typology.

Early diverging lineages of Apiales constitute a distinct group due to subprolate pollen grains (P/E ratio < 1.25). Among four subfamilies of Apiaceae, Mackinlayoideae can be easily identified based on a combination of traits shared with *Klotzschia* and *Platysace*—enigmatic umbllifers with highly uncertain phylogenetic position. Pollen of Azorelloideae is much more diverse but retains many plesiomorphic traits found in early-diverging Apioideae. In contrast, Saniculoideae and most representatives of Apioideae are characterised by evolutionary advanced morphology (perprolate pollen grains with relatively short ectocolpus and bone-shaped outline in colpus view). However, it remains unclear whether similarities between Saniculoideae and higher apioids are an example of convergent evolution or reflect common ancestry. Pollen of *Hermas* shows a unique combination of traits some of which are typical for Azorelloideae while others resemble Saniculoideae.

Keywords: Apiaceae, Araliaceae, Griseliniaceae, Myodocarpaceae, palynology, Pennantiaceae, phylogenetics, Pittosporaceae, systematics, Torricelliaceae

Introduction

The angiosperm order Apiales comprises about 6,000 species assigned to 522 genera (Bittrich & Kadereit 2018). According to the results of molecular phylogenetic studies, its early-diverging lineages include families Pennantiaceae, Torricelliaceae, Griseliniaceae and Pittosporaceae (Fig. 1; Calviño & Downie 2007, Nicolas & Plunkett 2009, Downie *et al.* 2010, Banasiak *et al.* 2013, Wen *et al.* 2020). These relatively small taxa comprising 11–14 genera in total constitute a basal grade with respect to three remaining families: Araliaceae, Myodocarpaceae and Apiaceae (=Umbelliferae) (Kårehed 2003, Chandler & Plunkett 2004, Nicolas & Plunkett 2009, 2014, Chase *et al.* 2016). The umbellifers with 466 genera and over 3,800 species leave behind the second-largest family, i.e., Araliaceae, comprising ca. 1900 species distributed among 40 genera (Plunkett *et al.* 2018).

Although molecular taxonomy has been in use for years, many clades of Apiales still miss well-defined synapomorphies. Studies focusing on life form, morphology and anatomy (Lowry *et al.* 2001, Plunkett *et al.* 2004, Liu *et al.* 2006, 2010, Magee *et al.* 2010) revealed that many traits are highly homoplastic and thus have little value in taxonomy. Palynology could potentially provide additional characters for such investigations. However, it has never been thoroughly revised in the phylogenetic context.

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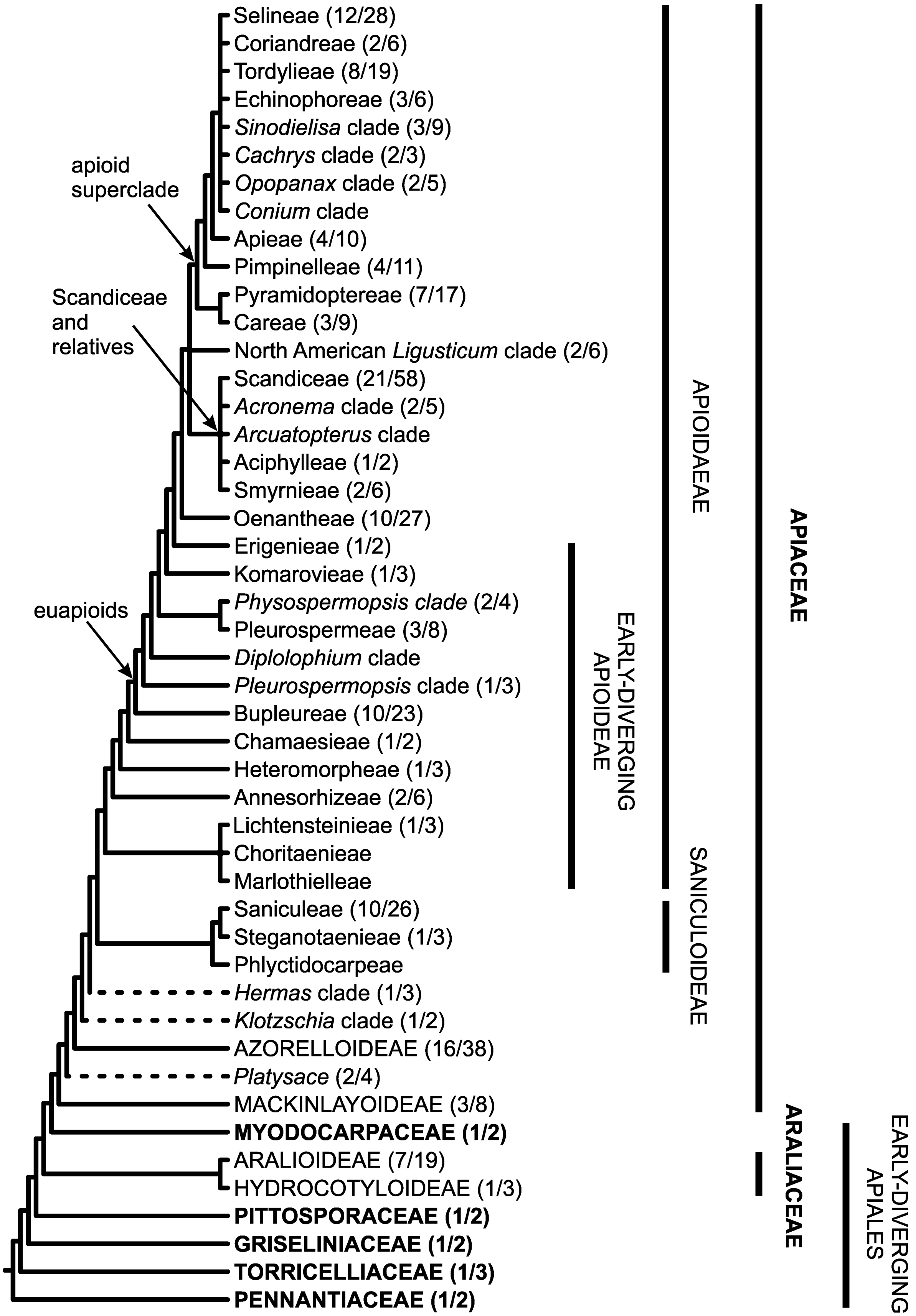

FIGURE 1. Phylogenetic supertree illustrating relationships between major clades of Apiales. Arrows indicate informal taxonomic units. Branches leading to clades with highly uncertain phylogenetic position are marked with dashed lines. The number of species/specimens sampled from the particular clade is given in brackets. For references, see the main text.

Before the rise of molecular phylogenetics, incorrect assumptions regarding the relationships of taxa within the order and purely descriptive character of conducted studies have often led to irrelevant conclusions regarding the taxonomic significance of pollen morphology. For instance, pollen of *Pennantia* J.R.Forst. & G.Forst. was described during the revision of family Icacinaceae to which the genus was erroneously assigned (Cranwell 1942, Dahl 1952) while most other representatives of early-diverging lineages (e.g., *Griselinia* G.Forst, *Torricellia* DC., *Aralidium* Miq.) were analysed as a part of broadly defined Cornaceae (Chao 1954, Tseng 1980, Wang & Chen 1990). Similar problem can be noted for Araliaceae subfamily Hydrocotyloideae, Myodocarpaceae and basal lineages of Apiaceae as symplesiomorphies shared by representatives of these groups did not allow for their clear delineation (Ting *et al.* 1964) before the rise of molecular systematics. Tseng provided a detailed description of pollen grains for numerous representatives of Araliaceae (Tseng 1971, 1973, 1974, Tseng & Shoup 1978, Tseng *et al.* 1983) and inferred the utility of palynological data for the taxonomy of the group but incorrect phylogenetic assumptions led him to consider *Klotzschia* Cham. (actually nested within Apiaceae) as a 'link' between Apiaceae and Araliaceae (Shoup & Tseng 1977). Cerceau-Larrival who throughout her lifetime described over 1,500 contemporary and fossil pollen types met the same obstacles considering their evolutionary relationships, which were highly speculative (Cerceau-Larrival 1962, 1967, 1971, Gruas-Cavagnetto & Cerceau-Larrival 1984). Over 100 of species belonging to Apiaceae and Araliaceae were described as a part of *Northwest European pollen flora* series (van Helvoort & Punt 1984, Punt 1984) but these studies were strongly biased geographically and in effect also taxonomically, as many groups of Apiales, particularly some early-branching lineages, do not occur in Europe.

Apart from inadequate sampling, large-scale comparative palynological analyses in the order Apiales are complicated due to incompatible typologies used by various authors over the years to describe pollen, what led to different diagnostic features proposed for artificial dichotomous keys. For instance Punt (1984) considered the external and internal outline of exine being the most important diagnostic character for Apiaceae and criticised Cerceau-Larrival (1962), who put at the first place in her key the proportion of polar and equatorial diameter of pollen grain (P/E ratio).

The aim of this study is to describe pollen morphology in Apiales in the context of modern taxonomy based on molecular phylogenetics facilitating further taxonomic and evolutionary studies. To achieve this goal, we provide: (1) unified well-documented typology applicable across whole order, (2) diagnoses of major taxonomic groups and (3) taxonomic key for pollen identification based on statistical analyses of categorical and continuous traits.

Materials & methods

Sampling strategy

Pollen was sampled from 417 herbarium specimens representing 158 species distributed among all major lineages of Apiales (Appendix S1). Whenever possible, in order to circumvent taxonomic confusion and to avoid examining closely related species (e.g., presumably morphologically redundant sister taxa), sampling included the species that had already been subject to molecular phylogenetic studies (Calviño & Downie 2007, Nicolas & Plunkett 2009, Downie *et al.* 2010, Banasiak *et al.* 2013). Most of the species (91%) were represented by two or more specimens preferably from different collections, minimising the risk of misidentification, and only 14 species were included in the study with one pollen sample each. All but three specimens with missing locality descriptions were georeferenced according to the protocol proposed by Wieczorek *et al.* (2011) and using an online calculator (Wieczorek & Wieczorek 2015). The sampled specimens represented the entire geographic distribution of Apiales (Fig. 2), and most of them were collected in Eurasia, which was recovered as the major diversification centre of its largest family, Apiaceae (Banasiak *et al.* 2013).

Pollen preparation and microscopic observations

The pollen was isolated from well-dried herbarium specimens by removing either whole parts of inflorescences or, rarely, individual flowers. Subsequently, the samples were shaken in the water, mechanically homogenised, and acetolysed according to the protocol proposed by Jones (2014). During acetolysis, a small amount of previously acetolysed *Lilium* L. pollen was added to each sample as the marker of successful pollen isolation. Finally, pollen was suspended in pure glycerine and mounted on paraffin-sealed slides for light microscopy investigation.

Depending on pollen size the observations were made under either 600× or 1000× magnification using a Nikon Eclipse E600 microscope equipped with Nomarski Inference Contrast (NIC). Digital photographs and measurements were taken using NIS-Elements imaging software ver. 4.0 (The NIS-Elements Development Team 2020). The photos were adjusted for even background brightness and white balance using ImageMagick ver. 6.8.9-9 (The ImageMagick Development Team 2019). The procedure ensures that differences in saturation between specimens are not artefacts due to changed lighting, but reflect the true properties of pollen grains.

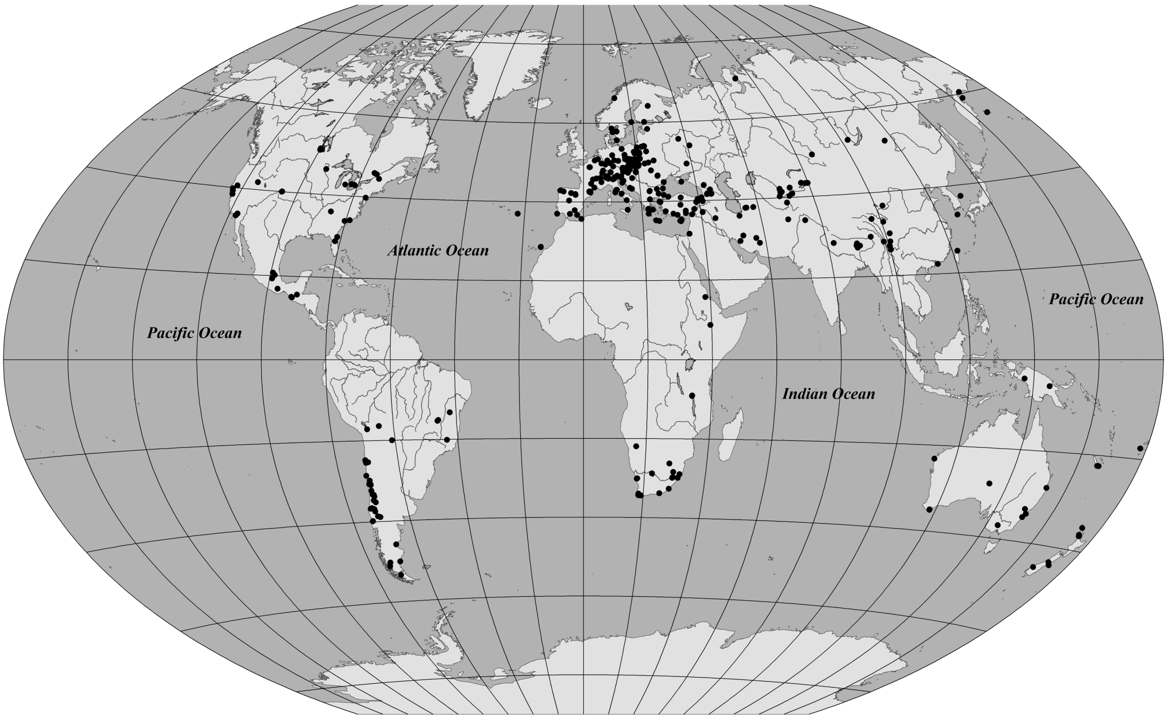

FIGURE 2. Distribution of sampled specimens on the world map drawn using Winkel triple projection with 20° graticule.

Typology for pollen morphology in Apiales

In order to analyse the pollen morphology and provide its accurate description, grains should be observed in three views because each of them gives information about different traits. In **side view** (Fig. 3A) pollen grain has its polar axis parallel to the surface of a microscope slide with colpus visible at one side of the pollen (colpus side) and mesocolpium at the other (mesocolpium side) both visible in optical cross-section. In this view, exine stratification can be analysed in detail. **Colpus view** (Fig. 3B) differs in that pollen grain is rotated 60° around its polar axis. This view allows focusing on colpus while the surface of two adjacent mesocolpia is visible at an angle and somewhat distorted. **Polar view** (Fig. 3C) shows pollen grain standing edgeways with its apocolpium or polar region facing the observer, i.e. its longitudinal axis is perpendicular to the surface of a microscope slide. Pollen grains with strongly elongated polar axis require to be braced on other grains or debris to observe them in this view.

General pollen morphology in Apiales—background and terminology

Pollen class:—According to the typology provided by Erdtman & Straka (1961) and later by Punt *et al.* (2007) pollen grains of Apiales can be classified as zonocolporate, based on a presence of compound apertures situated only at the equator (along polar axis). The only exception from that pattern is probably the genus *Torricellia* (Torricelliaceae), which lacks endoaperture and thus is zonocolpate (Chao 1954). Pollen grains with these types of morphology bear three apparent regions—aperture (apertural area), mesocolpium and apocolpium.

Polar/Equatorial (P/E) ratio:—P/E ratio varies from spheroidal, prolate spheroidal to prolate and perprolate with relatively shorter polar axis being common in non-umbelliferous Apiales. Perprolate pollen is found exclusively in the family Apiaceae.

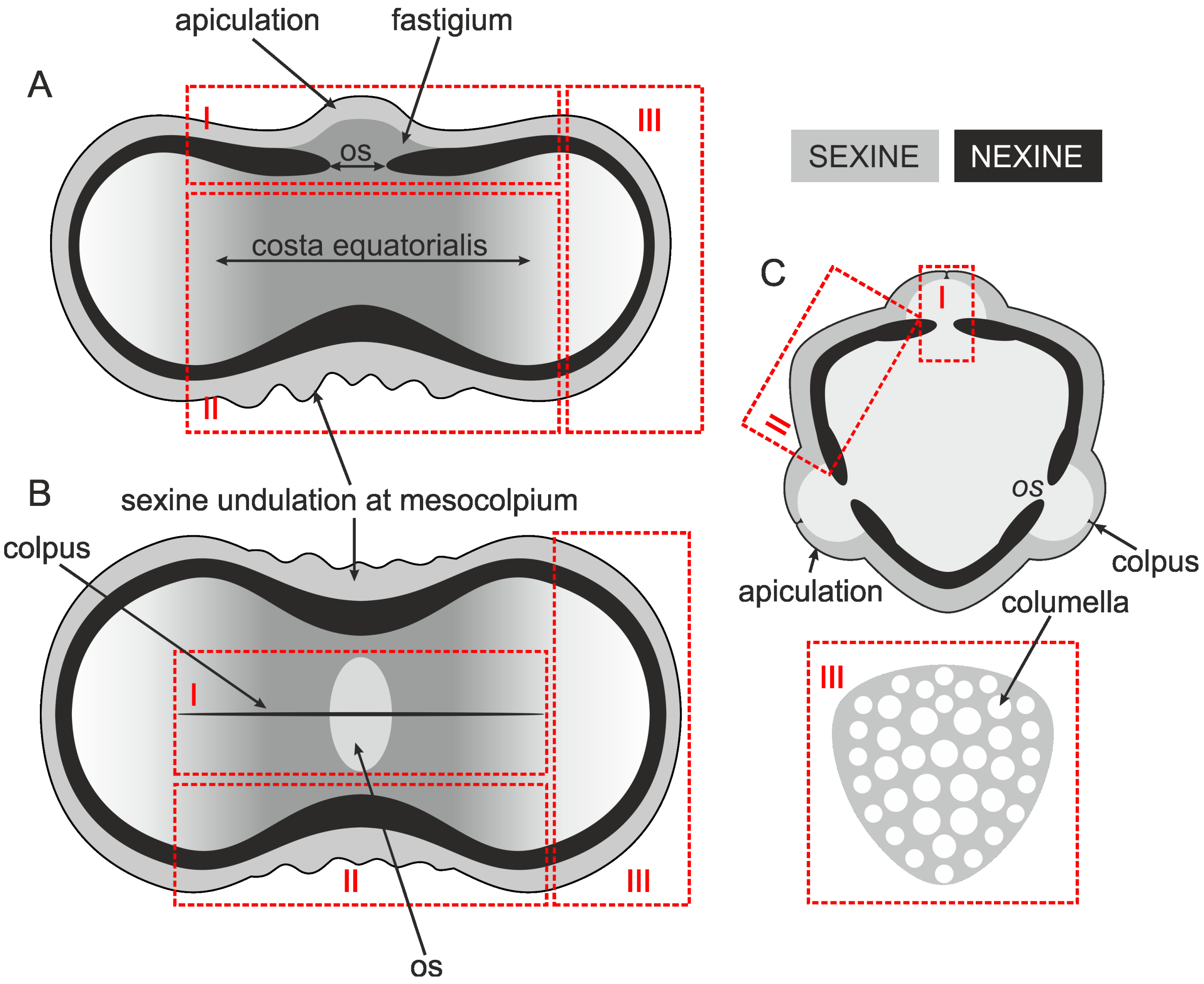

FIGURE 3. Schematic general pollen morphology in Apiales showed in the side (A), colpus (B) and polar view (C). Red dashed rectangles highlight apertural area (I), mesocolpium (II) and apocolpium (III) seen at different angles or in different focal planes.

Apertural area:—Compound aperture is always composed of colpus (ectoaperture, located in sexine) and os (endoaperture, located in nexine). Ectoaperture may be relatively broad or slit-like; in the latter may sometimes dilate or become discontinuous at the crossing with endoaperture (Cerceau-Larrival 1962, Erdtman 1963). Shape and size of endoaperture show high intraspecific variation. However, it always runs at the equator perpendicularly to ectoaperture, forming transverse furrow (Cerceau-Larrival 1962, Ting *et al.* 1964). Pollen in Apiales usually has three apertures while aberrant grains departing from this rule appear very rarely within samples. Structurally dicolporate pollen has been found only in two genera from Apiaceae-Apioideae—namely *Echinophora* L. (Cerceau-Larrival 1971) and *Tordylium* Tourn. ex L. *sensu lato* (Al-Eisawi & Jury 1988), whereas some representatives of Apiaceae–Azorelloideae (e.g., *Bowlesia* Ruiz & Pav.) are known to produce tetra- or pentacolporate grains (Ting *et al.* 1964).

Pollen wall stratification:—In non-umbelliferous Apiales nexine is usually only slightly thinner than sexine, but in Apiaceae, the difference is more pronounced. In this family, sexine tends to grow thicker towards poles creating a characteristic ‘bone-shaped’ outline of the entire grain. Two layers of sporoderm usually follow each other’s outline but sometimes may delaminate in the apertural area creating fastigium. The appearance of this structure may result from apiculation of sexine but does not necessarily have to.

Costae endocolpi are usually present in various developmental stages. Some are less pronounced or restricted only to surroundings of endoaperture, while others may even form prominent, broad costa equatorialis running all around the equator. The latter scenario seems to be specific for the evolutionary advanced representatives of family Apiaceae (Iversen & Troels Smith 1950, Punt 1984).

Ornamentation:—Grains are usually tectate with collumellar infratectum but semitectate (Tseng & Shoup 1978), partially (Tseng 1974) or completely (Tseng & Shoup 1978, Henwood 1991) intectate ones are relatively common in the family Araliaceae. Columellae (if present) are usually simple, occasionally digitate as in *Heracleum* L. (Punt 1984) or *Turgenia* Hoffm. (Cerceau-Larrival 1962). The ornamentation in the early-diverging lineages of Apiales is usually striate (Tseng 1980, Williams & Adam 1999), while in Araliaceae and Myodocarpaceae the reticulate type (sometimes with irregular gemmate pattern) is the most common one. In Apiaceae, one may find pertectate, perforate, rugulate and striate patterns.

Qualitative traits scored in this study

Each of the following traits was scored for a given species based on examination of all available specimens in order to accommodate for variation between specimens or even between pollen grains within a single specimen. Trait descriptors (C1–C17) correspond to the preferred order of microscopic observations and refer to the columns in the data matrix (Appendix S2).

C1–C4 Exine outline (Fig. 4A):—The term was first used by Cerceau-Larrival (1962) and later adapted by Punt (1984). Exine outline can be concave (0), straight (1) or convex (2) at both colpus and mesocolpium side. As layers of sporoderm tend to follow each other, the outer and inner outline at respective side usually does the same, but it is not a strict rule. If apiculation of the exine above endoaperture is present, then it should not be taken into account when describing exine outline at colpus side but considered a separate trait. Exine outline is to some extent variable at the species level.

C5 Exine apiculation (Fig. 4B):—Apiculation occurs when the exine bulges at the crossing of ecto- and endoaperture. This trait is independent of the exine outline at colpus side. Apiculation can be absent (0), affecting sexine only (1) or concerning both sexine and nexine (2).

C6 Fastigium (Fig. 4C):—The term describes a space created by delamination of sexine and nexine in the apertural area. It usually arises as a result of pronounced apiculation of sexine or combination of strongly concave inner contour and convex outer contour at colpus side of the grain. Minor delaminations found in some grains within a sample are considered absent (0), and the term applies only for clearly visible structures (1).

C7 Sexine undulation at mesocolpium side (Fig. 4D):—Sexine undulation describes a structure of tectum or sexine 2 as visible in optical cross-section at mesocolpium side. Depending on the degree of undulation, it is smooth (0), crenated (1) or undulated (2). In Araliaceae, one can distinguish the fourth type—budding (3), which corresponds to the gemmate ornamentation of the exine.

C8 Polar caps (Fig. 4E):—Polar caps are synonymous with apocolpium observed in the side view. They can be either obtuse (0) or subacute (1) when unequal expansion of columellae creates a sharpened tip at the peak of apocolpium.

C9 General outline (Fig. 4F):—General outline can be described by looking at the outer contour of the grain in the colpus view. Three shapes are distinguished in this way—rhombic (0), elliptic (1) and bone-shaped (2).

C14 Mesocolpium shape (Fig. 4G):—In the polar view, mesocolpium is visible as an area between adjacent apertures. By observing the external contour of the exine, one can classify its shape as straight (0), rounded (1), distinctly rounded (2) or sharply-bent (3).

C15 Number of apertures:—Pollen grains of Apiales are almost exclusively tricolp(or)ate (0), but exceptions from that rule occur sporadically including both reduced (1) and increased number of apertures (2).

C16 Ectocolpus infolding (Fig. 4H):—Species with absent (0) or weakly pronounced infolding (1) are found predominantly in early-diverging families of Apiales and Apiaceae subfamily Azorelloideae. Most representatives of Apiales have their apertures sunken inside the sporoderm groove, and hence can be described as having strongly pronounced ectocolpus infolding (2).

C17 Columellae at poles (Fig. 4I):—Columellae at poles can be visible as 'dots' covering the area of apocolpium. They may be either minute (0), fusing (1), separate (2) or forming muri (3). In order to correctly identify them, the LO-analysis and/or use of DIH microscope is advised.

C10 Ectocolpus above the equator (Fig. 5A):—At the side where ectoaperture crosses with endoaperture, the former can remain continuous (0), dilated (1) or discontinuous (2) creating demicolpi.

C11 Margins of endoaperture (Fig. 5B):—Margins of endoaperture are its contours perpendicular to ectoaperture, which can be concave (0), straight (1) or convex (2).

C12 Ends of endoaperture (Fig. 5C):—Contours of the endoaperture running parallel to ectoaperture are called ends of endoaperture. They can be diffuse (0), acute (1), rounded (2) or truncate (3).

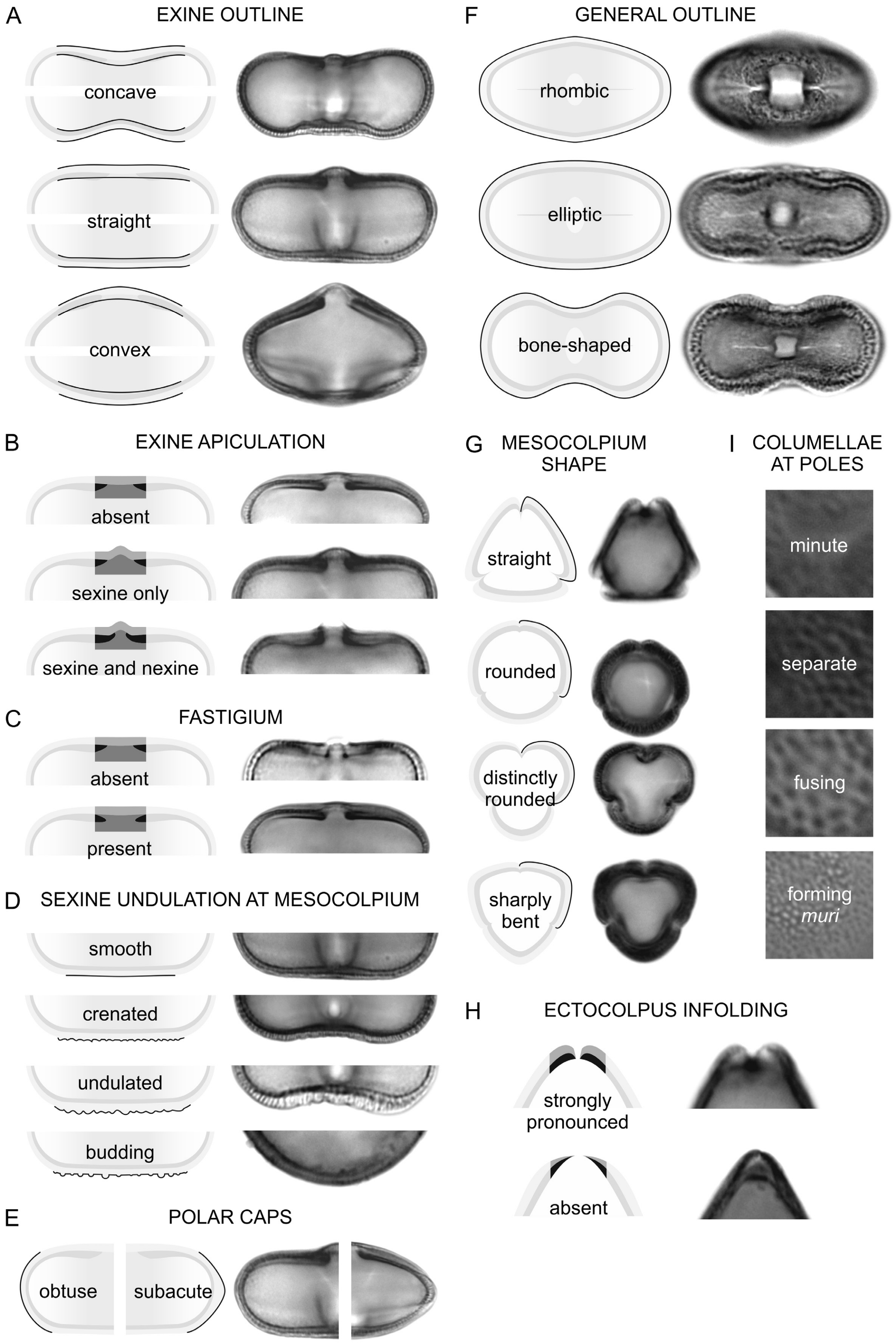

FIGURE 4. Qualitative traits related to sporoderm outline and ornamentation observed in the side (A–E), colpus (F), and polar view (G–I). Thickened lines or intensified colouring indicate the location of each trait on a pollen grain.

C13 Horned ends of endoaperture (Fig. 5D):—Horns are H-shaped, distinct apical extensions of endoaperture ends running towards the poles of a pollen grain or sometimes even arching towards its centre. They can be either absent (0) or present (1).

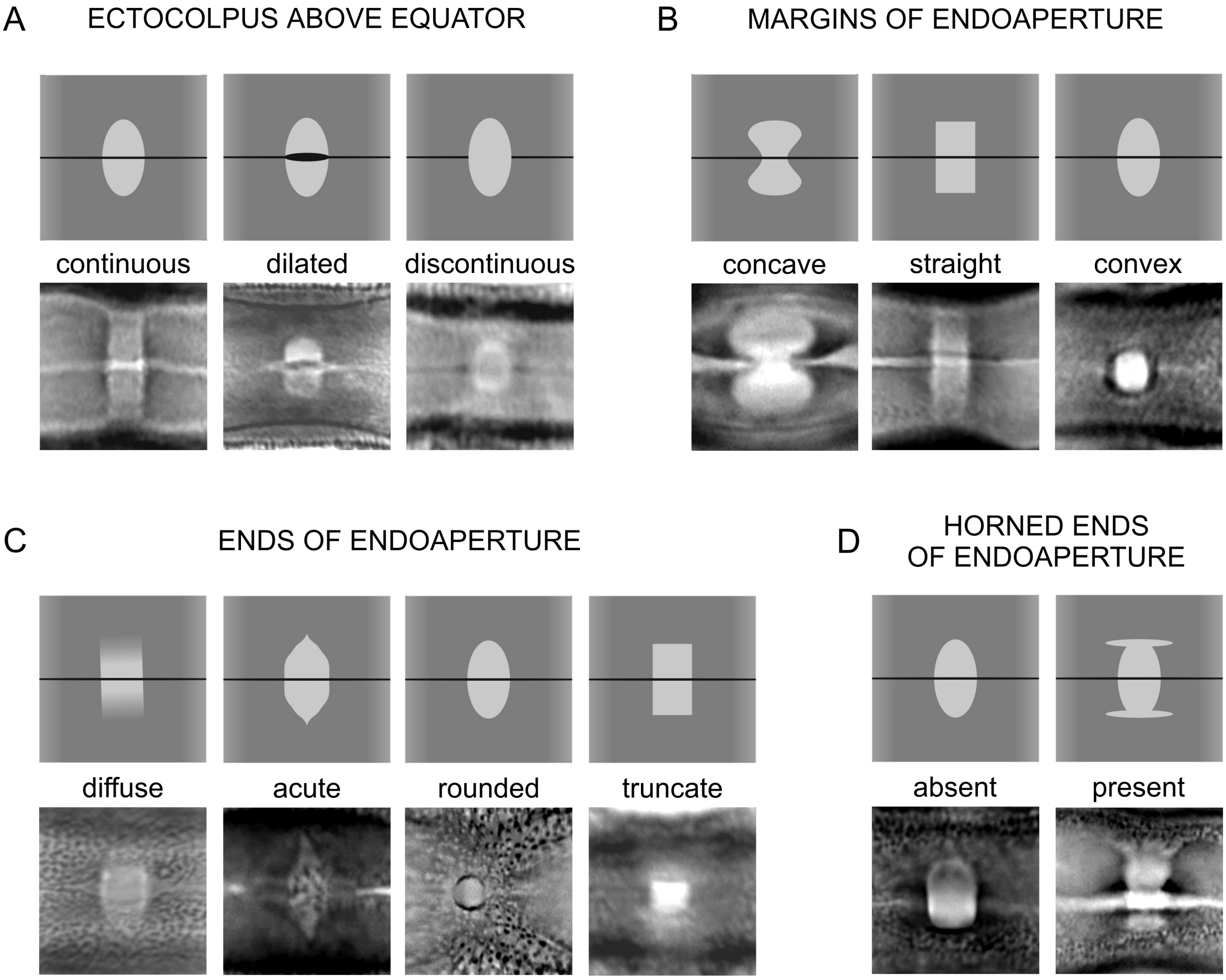

FIGURE 5. Qualitative traits related to the morphology of endoaperture.

Quantitative traits measured in this study

Ten representative pollen grains were selected for each specimen: five in side and colpus view each. Then measurements of each of the following traits were taken and averaged before subsequent analyses.

Polar and equatorial lengths (P & E):—Polar axis of the grain is understood as a line joining peaks of opposed apocolpia. Each of three equatorial axes runs through the centre of endoaperture and is perpendicular to the polar axis. Measurements of equatorial length include apiculation of exine above endoaperture (Fig. 6A).

Sexine and nexine thickness at poles and equator (SP, NP, SE, NE):—Sexine and nexine thickness is measured in the side view, separately at the peak of apocolpium (pole) and the median part of mesocolpium (equator), i.e. at its intersection with the equatorial axis (Fig. 6A).

Costae width (COSW):—The thickness of costae is established by measuring the width of nexine at its widest part in the apertural area in the side view (Fig. 6A).

Costae length (COSL):—Costae are visible in colpus view as the darker area of sporoderm around the equator, which colour fades away towards poles. Sometimes costae are thinning gradually and there is no distinct boundary between costae and the rest of nexine and thus measurements are to some extent arbitrary (Fig. 6B).

Ectoaperture length (ECT):—Ectoaperture is visible in colpus view as a bright line of variable width running parallel to the polar axis. Ectoaperture is usually very narrow (slit-like) but in some species can be widened above endoaperture or at the whole length (Fig. 6B). When the aperture is very long, i.e. extending from one pole to the other, and exine outline at the equator is distinctly convex (Fig. 6A) the measurement considerably underestimates the actual length of endoaperture as one actually measures the projection of curve onto a plane.

Endoaperture length and width (ENDL & ENDW):—Length of endoaperture is measured along its axis perpendicular to the ectoaperture while its width is measured along the ectoaperture (Fig. 6B).

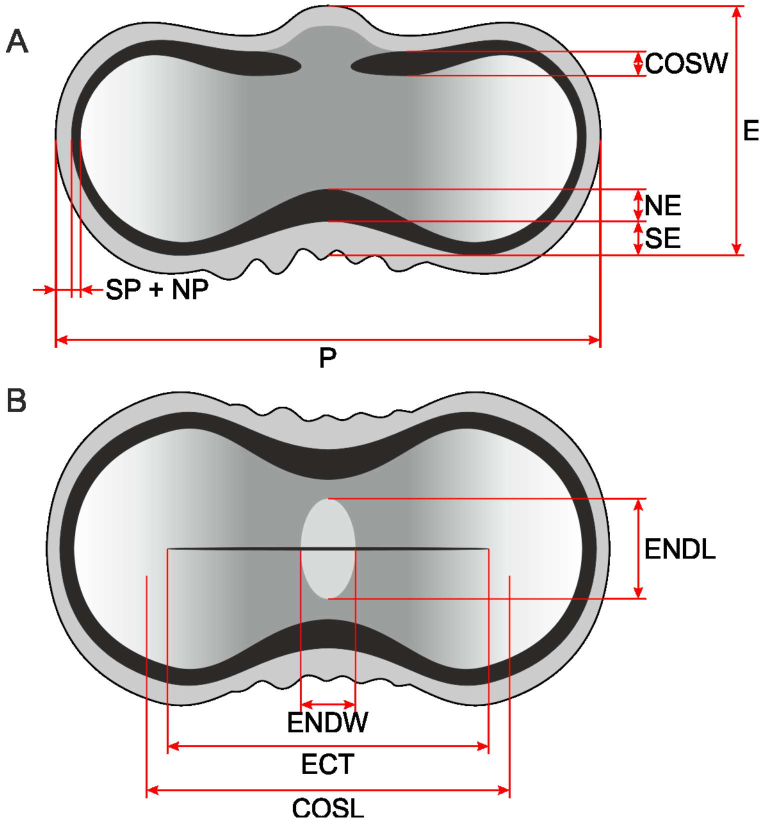

FIGURE 6. Quantitative traits measured in the side (A), and colpus view (B). P: polar length, E: equatorial length, SP: sexine thickness at poles, NP: nexine thickness at poles, SE: sexine thickness at the equator, NE: nexine thickness at the equator, COSW: costae width, COSL: costae length, ECT: ectoaperture length, ENDL: endoaperture length, ENDW: endoaperture width.

Statistical analyses

As the measurements of quantitative traits tend to be correlated with the size of a pollen grain, we decided to use a set of standardised variables instead of raw measurements in all the analyses (Table 1). The utilised ratios reflect the shape better than raw measurements and are usually much less correlated with each other, i.e. they can be considered approximately independent traits. For each new variable and taxonomic group, the following parameters were calculated: empirical range and median, kernel density estimate of its distribution, and nonparametric tolerance interval covering at least 80% of the future observations with 90% confidence (Young & Mathew 2014). Degree of correlation between variables was assessed employing Principal Component Analysis (PCA) based on the correlation matrix.

The dichotomous key for pollen identification was constructed by fitting a classification (regression) tree to a dataset consisting of merged standardised quantitative traits and qualitative traits transformed into binary dummy variables (Breiman *et al.* 1984).

All the above calculations and visualisations were performed using R environment for statistical computing (R Core Team 2016) with packages ‘tolerance’ ver. 1.3.0 (Young 2010), ‘tree’ ver. 1.0-40 (Ripley 2019), and ‘ggplot2’ ver. 3.2.1 (Wickham 2016).

TABLE 1. The list of standardised quantitative traits used in the analyses with their morphological interpretation. See Fig. 6 for the explanation of raw measurements.

1. P: polar length (pollen size).
2. P/E: the ratio of polar length to equatorial length (pollen grain elongation: general shape).
3. WP/P: the ratio of wall (exine) thickness at poles to polar length (relative exine thickness at poles).
4. WE/P: the ratio of wall (exine) thickness at the equator to polar length (relative exine thickness at the equator).
5. NP/WP: the ratio of nexine thickness at poles to wall (exine) thickness at poles (nexine contribution to the cell wall at poles).
6. NE/WE: the ratio of nexine thickness at the equator to wall (exine) thickness at the equator (nexine contribution to the cell wall at the equator).
7. COSW/NP: the ratio of costae width to nexine thickness at poles (relative costae thickness).
8. ECT/P: the ratio of ectocolpus length to polar length (relative ectoaperture length).
9. COSL/P: the tratio of costae length to polar length (relative costae length).
10. ENDW/P: the ratio of endoaperture width to polar length (relative endoaperture size).
11. ENDL/ENDW: the ratio of endoaperture length to its width (endoaperture elongation: general shape).

Results

Characteristics of datasets

Qualitative traits were evaluated for all 158 species, but with two exceptions (Appendix S2). First, as *Torricellia tiliifolia* DC. is the only species in this study with a simple aperture instead of compound one, we could not determine C11–C13 traits related to the endoaperture. Secondly, *Azorella andina* (Phil.) Drude has exceptionally protruding sexine above endoaperture and, due to very distorted picture, we were not able to determine whether the species has horned aperture (trait C13).

Quantitative traits were measured for 410 out of 417 specimens as seven were redundant, i.e. there were more than three specimens for a given species (Appendix S3). Again, in the case of *Torricellia tiliifolia* we could not measure traits referring to endoaperture (ENDL, ENDW) and costae colpi (COSL, COSW), which are missing in this species. Moreover, exceptionally large pollen of *Turgenia latifolia* L. was excluded from the analyses as an outlier.

With the exception of well-isolated phylogenetic lineages represented by the genera *Klotzchia* Cham., *Platysace* Bunge and *Hermas* L., the other measured specimens were aggregated before statistical analyses into eight mono- or paraphyletic groups encompassing (3–)10–28(–50) species (Table 2).

All raw quantitative traits were positively correlated to a varying degree with the first principal component interpreted as pollen size (Fig. 7A). Especially costae width and length (COSW & COSL) could be easily predicted based on polar length (P). From the other hand, exine thickness at the equator (SE & NE) were almost independent of pollen size. After standardisation of quantitative traits, the variance explained by the first two principal components was reduced from 63% to 46% and the loadings spread at different angles indicating a substantial decrease in correlation among traits (Fig. 7B). However, some standardised traits (e.g., ENDW/P & ENDL/ENDW) were still highly positively or negatively correlated with each other and thus redundant for the construction of the dichotomous key.

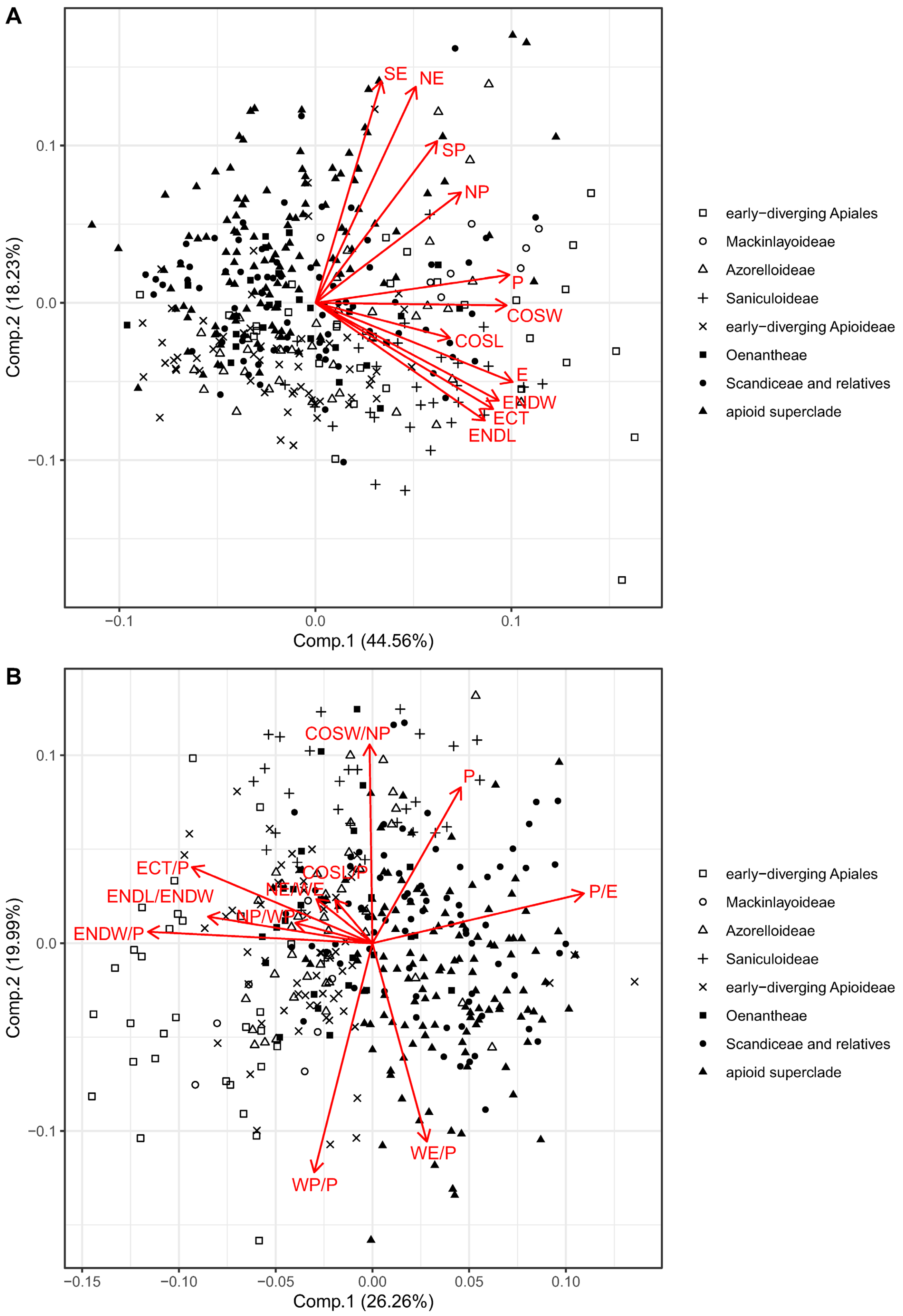

FIGURE 7. Principal component analysis of raw measurements (A) and standardised quantitative traits (B) with loadings visualised as red arrows. The numbers along axes indicate the percentage of variance explained by each principal component.

TABLE 2. The number of measured species and specimens aggregated by major taxonomic groups, i.e. excluding *Hermas*, *Klotzschia* and *Platysace*.

Taxon	**Species**	**Specimens**
Early-diverging Apiales	12	30
Mackinlayoideae	3	8
Azorelloideae	16	38
Saniculoideae	11	29
Early-diverging Apioideae	23	57
Oenantheae	10	27
Scandiceae and relatives	28	77
Apioid superclade	50	123
Total	**153**	**389**

Diagnoses of taxonomic groups

With some notable exceptions, identification of Apiales pollen to family or lower taxonomic level requires a detailed examination of pollen grain in all three positions, i.e., in side, colpus and polar view. Such identification can be challenging if an environmental sample contains little pollen. Depending on species, pollen grains tend to lay either in side or colpus view making observations in other views difficult or even impossible. Especially perprolate pollen grains, i.e. having an elongated polar axis, rarely can be observed in polar view. Thus, it is recommended to add some other relatively large, easily discernible and perceptible spores or pollen to the sample, e.g., *Lycopodium* L. or *Lilium,* during acetolysis. Apart from being the marker of successful pollen isolation, they provide support for smaller pollen grains, increasing the number of sporomorphs observed in polar view. Pollen of *Lilium* is large enough to make extra room between a microscope slide and a cover slip, and the additional space can be used to rotate pollen grains by tapping with a preparation needle. We cannot overstress how important it is to analyse multiple pollen grains obtained from at least two different populations whenever there is such possibility, as especially quantitative traits tend to vary slightly between individual plants.

Early diverging Apiales (non-Apiaceae; Plate 1**)**:—Early-diverging lineages of Apiales have subprolate pollen grains (P/E < 1.25) and this trait alone is usually enough to distinguish them from Apiaceae. However, *Pennantia*, *Griselinia* (Plate 1A&C), and some representatives of Araliaceae constitute notable exceptions, in which the P/E ratio can even reach value of 1.4. To differentiate them from early-diverging Apioideae (protoapioids and basal euapioids), one must take into account the presence of reticulate ornamentation (absent in Apiaceae) and shape of mesocolpium in polar view, which is always straight in early-diverging Apioideae but rounded in Griseliniaceae and Pennatiaceae.

Mackinlayoideae (Plate 2A–C):—Identification of this subfamily is rather straightforward based on the combination of four traits: prolate pollen grains (P/E ratio 1.25–1.7), distinctly rounded or sharply-bent mesocolpium in polar view coupled with an elliptic (not rhombic!) general outline in colpus view and separate columellae at poles. Polar caps are always subacute, but this trait is common also in Azorelloideae and Saniculoideae. Pollen of Mackinlayoideae reassembles *Platysace* and *Klotzschia* (Plate 2D–F), which have uncertain phylogenetic position.

Platysace (Plate 2D&E):—Pollen of *Platysace* is strongly reminiscent of Mackinlayoideae having distinctly rounded mesocolpium, elliptic general outline and separate columellae at poles. However, *Platysace* has a variable number of apertures in analysed pollen grains (tetracolpate ones are relatively frequent in samples of *Platysace*) and/or short costae (< 0.2 polar length).

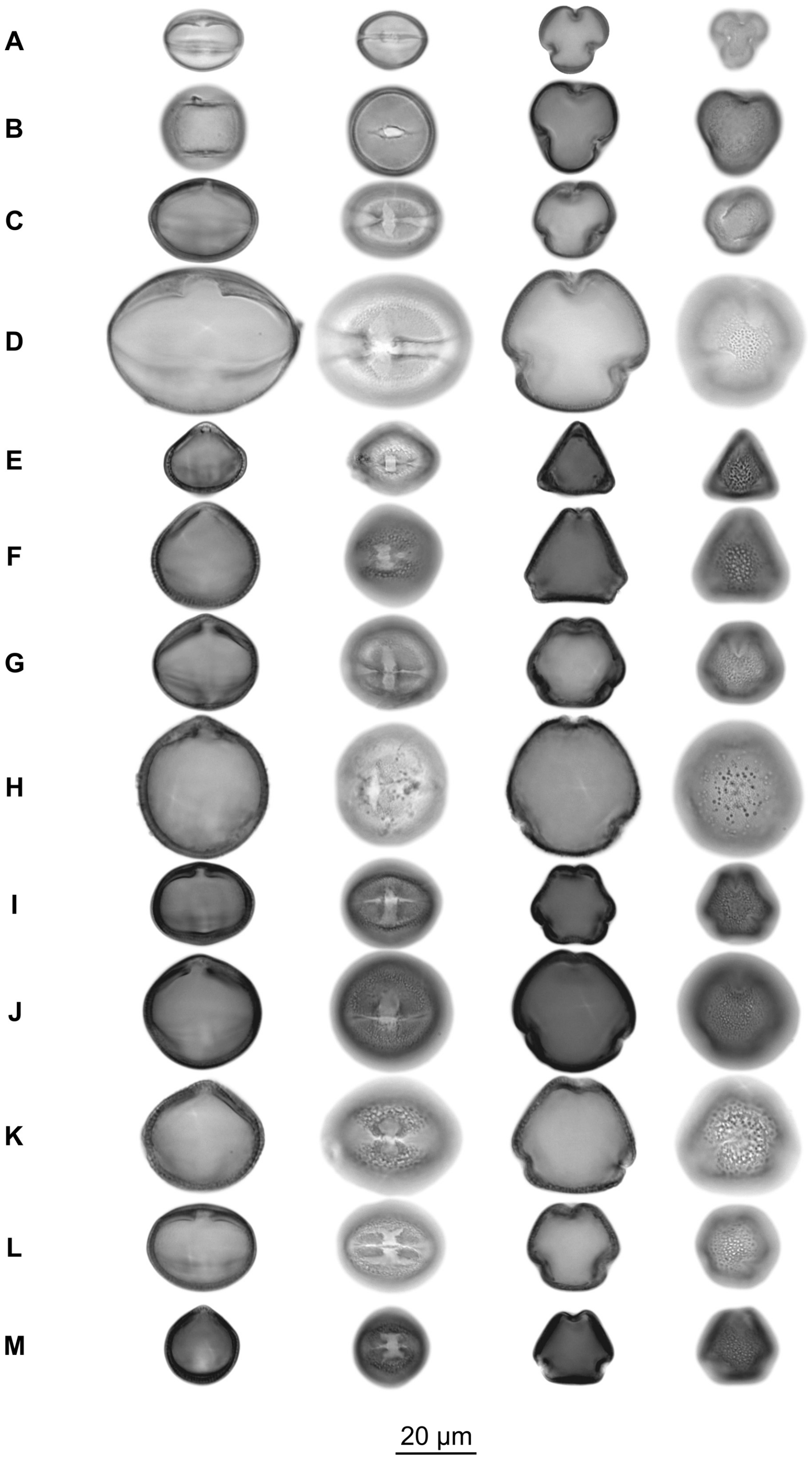

PLATE 1. Pollen grains of early-diverging Apiales. A: ***Pennantia corymbosa*** J.R.Forst. & G.Forst., B: ***Torricellia tiliifolia*** DC., C: ***Griselinia lucida*** (J.R.Forst. & G.Forst.) G.Forst., D: ***Hymenosporum flavum*** (Hook.) F.Muell., E: ***Hydrocotyle vulgaris*** L., F: ***Aralia spinosa*** L., G: ***Dendropanax arboreus*** (L.) Decne. & Planch., H: ***Fatsia japonica*** (Thunb.) Decne. & Planch., I: ***Harmsiopanax ingens*** Philipson, J: ***Panax quinquefolius*** L., K: ***Pseudopanax crassifolius*** (Sol. ex A.Cunn.) K.Koch, L: ***Schefflera heptaphylla*** (L.) Frodin, M: ***Myodocarpus fraxinifolius*** Brongn. & Gris.

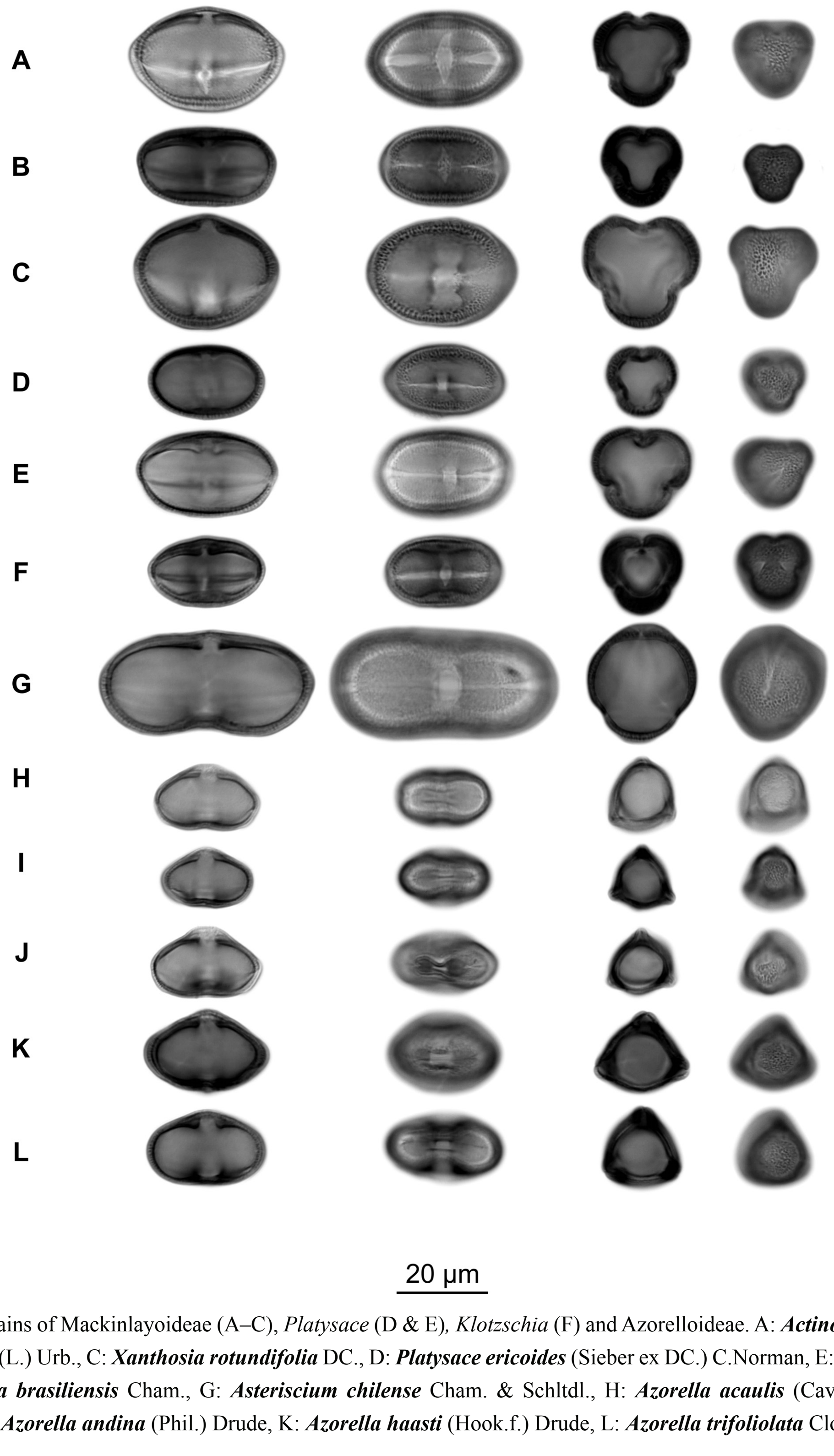

PLATE 2. Pollen grains of Mackinlayoideae (A–C), *Platysace* (D & E), *Klotzschia* (F) and Azorelloideae. A: ***Actinotus helianthi*** Labill., B: ***Centella asiatica*** (L.) Urb., C: ***Xanthosia rotundifolia*** DC., D: ***Platysace ericoides*** (Sieber ex DC.) C.Norman, E: ***Platysace lanceolata*** Druce, F: ***Klotzschia brasiliensis*** Cham., G: ***Asteriscium chilense*** Cham. & Schltdl., H: ***Azorella acaulis*** (Cav.) Drude, I: ***Azorella ameghinoi*** Speg., J: ***Azorella andina*** (Phil.) Drude, K: ***Azorella haasti*** (Hook.f.) Drude, L: ***Azorella trifoliolata*** Clos.

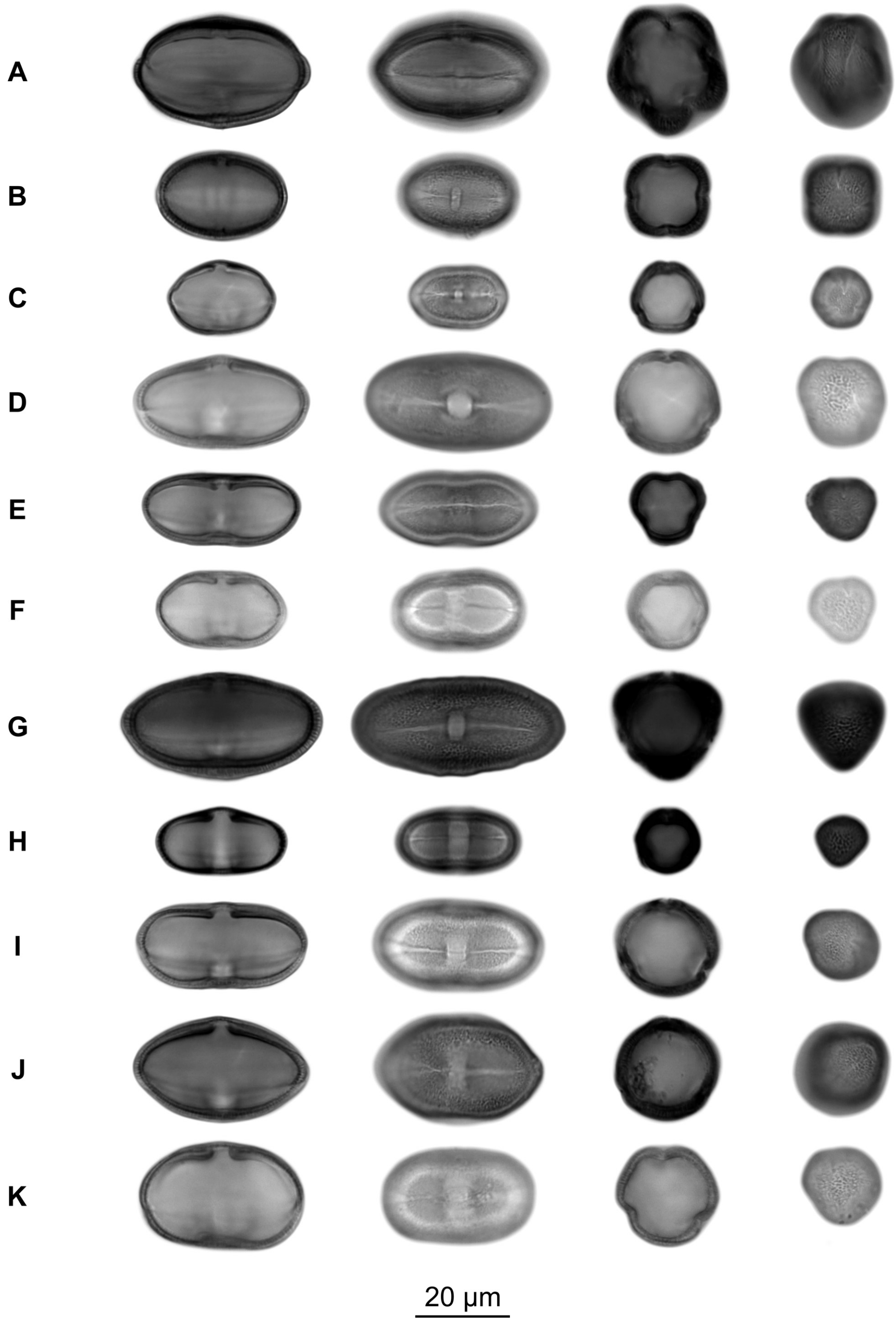

PLATE 3. Pollen grains of Azorelloideae (A–J) and *Hermas* (K). A: ***Bowlesia lobata*** Ruiz & Pav., B: ***Bowlesia tropaeolifolia*** Gillet & Hook., C: ***Domeykoa oppositifolia*** Phil., D: ***Eremocharis fruticosa*** Phil., E: ***Gymnophyton flexuosum*** Clos, F: ***Gymnophyton isatidicarpum*** (DC.) Mathias & Constance, G: ***Homalocarpus dichotomus*** (Poepp. ex DC.) Mathias & Constance, H: ***Mulinum spinosum*** (Cav.) Pers., I: ***Pozoa coriacea*** Lag., J: ***Spananthe paniculata*** Jacq., K: ***Hermas villosa*** (L.) Thunb.

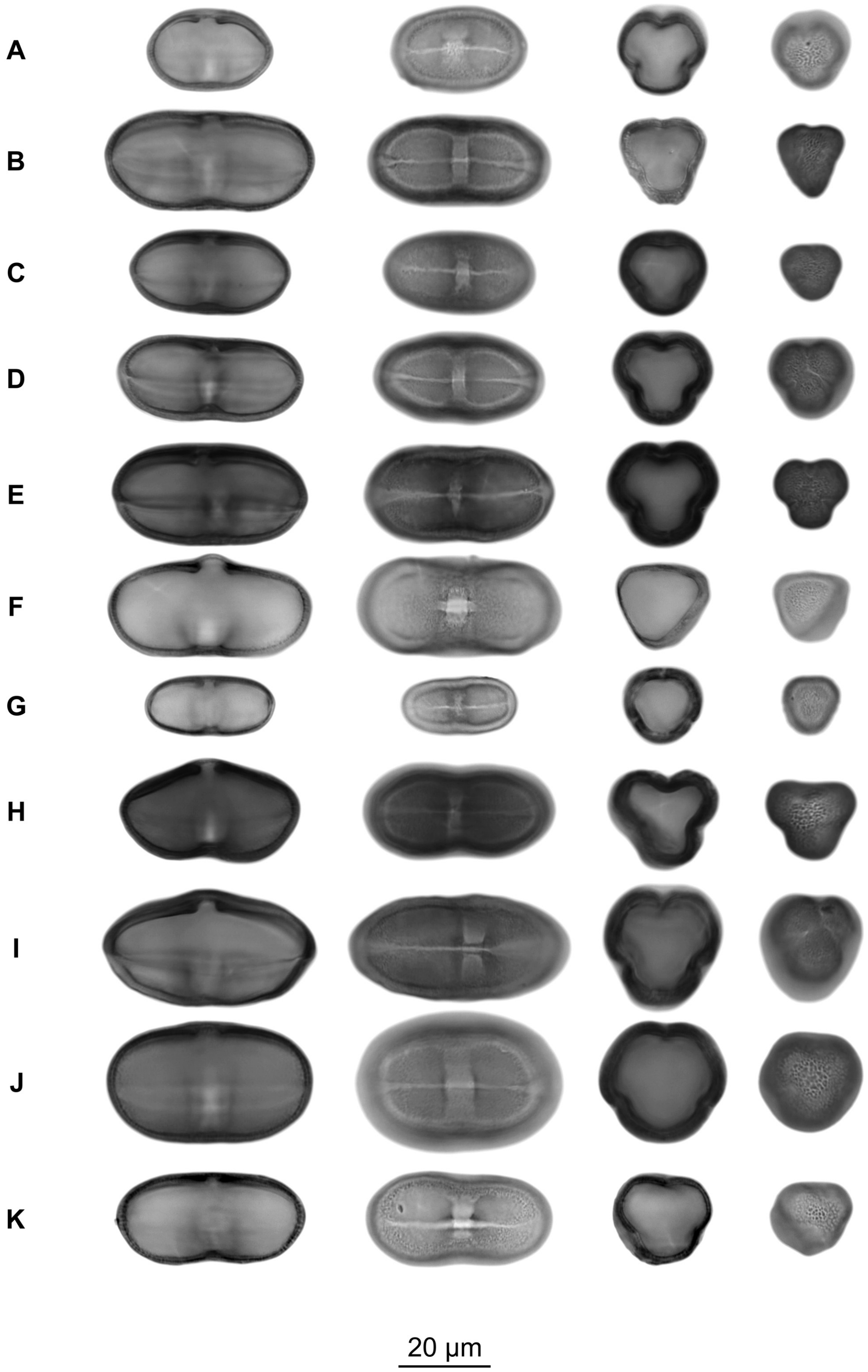

PLATE 4. Pollen grains of Saniculoideae. ***Steganotaenia araliacea*** Hochst., B: ***Actinolema eryngioides*** (Fenzl) Koso-Pol., C: ***Alepidea amatymbica*** Eckl. & Zeyh., D: ***Alepidea peduncularis*** Steud. ex A.Rich., E: ***Arctopus echinatus*** L., F: ***Eryngium campestre*** L., G: ***Eryngium ebracteatum*** Lam., H: ***Sanicula canadensis* var. *marilandica*** (L.) Hitchc., I: ***Sanicula crassicaulis*** Poepp. ex DC., J: ***Sanicula epipactis*** (Scop.) E.H.L.Krause, K: ***Sanicula europaea*** L.

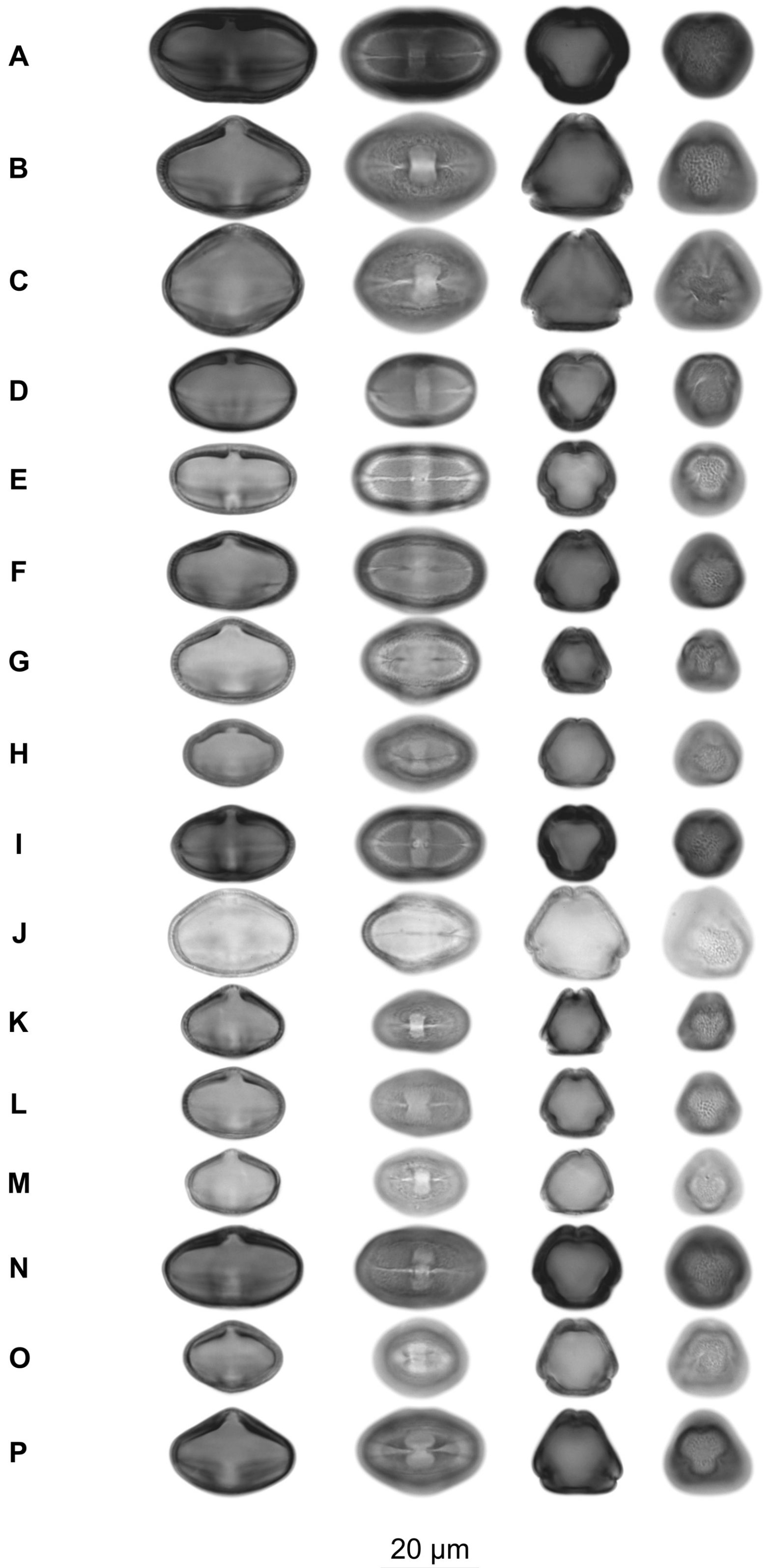

PLATE 5. Pollen grains of early-diverging Apioideae. A: ***Lichtensteinia trifida*** Cham. & Schltdl., B: ***Annesorhiza altiscapa*** Schltr. ex H.Wolff, C: ***Molopospermum peloponnesiacum*** (L.) W.D.J.Koch, D: ***Heteromorpha arborescens*** (Spreng.) Cham. & Schltdl., E: ***Chamaesium paradoxum*** H.Wolff, F: ***Bupleurum aureum*** Fisch. ex Hoffm., G: ***Bupleurum balansae*** Boiss. & Reut., H: ***Bupleurum falcatum*** L., H: ***Bupleurum falcatum* subsp. *cernuum*** (Ten.) Arcang., I: ***Bupleurum fruticosum*** L., J: ***Bupleurum longifolium*** L., K: ***Bupleurum praealtum*** L., L: ***Bupleurum ranunculoides*** L., M: ***Bupleurum rotundifolium*** L., N: ***Bupleurum stellatum*** L., O: ***Bupleurum tenuissimum*** L., P: ***Pleurospermopsis sikkimensis*** (C.B.Clarke) C.Norman.

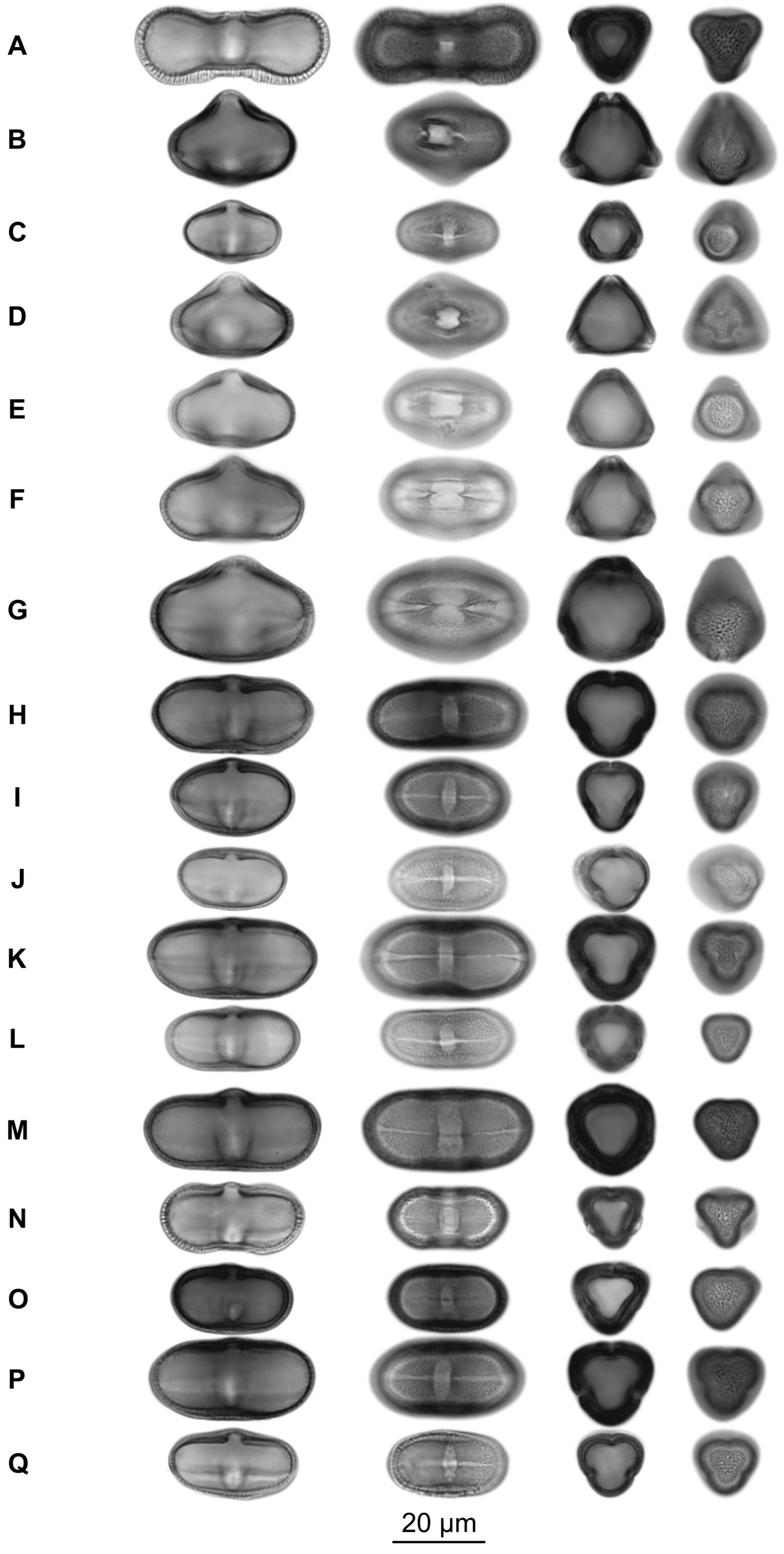

PLATE 6. Pollen grains of early-diverging Apioideae (A–G) and Oenantheae (H–Q). A: ***Eremodaucus lehmannii*** Bunge, B: ***Physospermum cornubiense*** (L.) DC., C: ***Pleurospermum uralense*** Hoffm., D: ***Cyclorhiza peucedanifolia*** (Franch.) Constance, E: ***Haplosphaera phaea*** Hand.-Mazz., F: ***Sinolimprichtia alpina*** H.Wolff, G: ***Erigenia bulbosa*** (Michx.) Nutt., H: ***Cicuta virosa*** L., I: ***Cryptotaenia canadensis*** (L.) DC, J: ***Helosciadium nodiflorum*** (L.) W.D.J.Koch, K: ***Neogoezia gracilipes*** (Hemsl.) Hemsl., L: ***Oenanthe aquatica*** (L.) Poir., M: ***Oxypolis fendleri*** (A.Gray) A.Heller, N: ***Perideridia kelloggii*** (A.Gray) Mathias, O: ***Ptilimnium capillaceum*** (Michx.) Raf., P: ***Sium latifolium*** L., Q: ***Trocdaris verticillatum*** (L.) Raf.

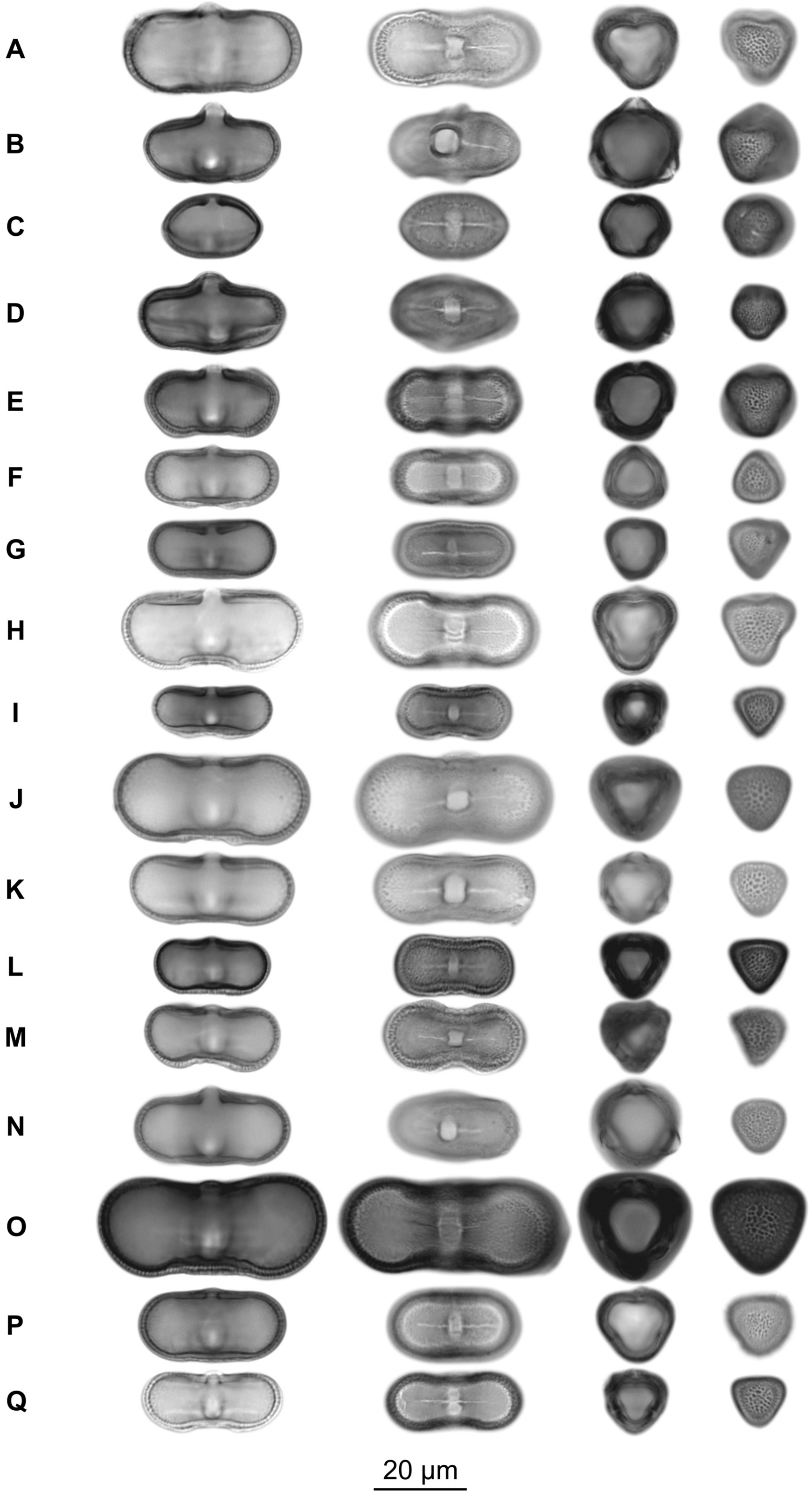

PLATE 7. Pollen grains of Scandiceae and relatives. A: ***Lecokia cretica*** (Lam.) DC., B: ***Smyrnium olusatrum*** L., C: ***Aciphylla aurea*** W.R.B.Oliv., D: ***Ligusticum scoticum*** L., E: ***Pleurospermum hookeri*** C.B.Clarke, F: ***Anthriscus cerefolium*** (L.) Hoffm., G: ***Anthriscus sylvestris* subsp. *sylvestris*** (L.) Hoffm., H: ***Athamanta cretensis*** L., I: ***Chaerophyllum aromaticum*** L., J: ***Chaerophyllum aureum*** L., K: ***Chaerophyllum hirsutum*** L., L: ***Chaerophyllum temulum*** L., M: ***Conopodium majus*** (Gouan) Loret, N: ***Osmorhiza aristata*** (Thunb.) Makino & Jabe, O: ***Scandix pecten-veneris*** L., P: ***Scandix stellata*** Banks & Sol., Q: ***Sphallerocarpus gracilis*** (Besser ex Trevir.) Koso-Pol.

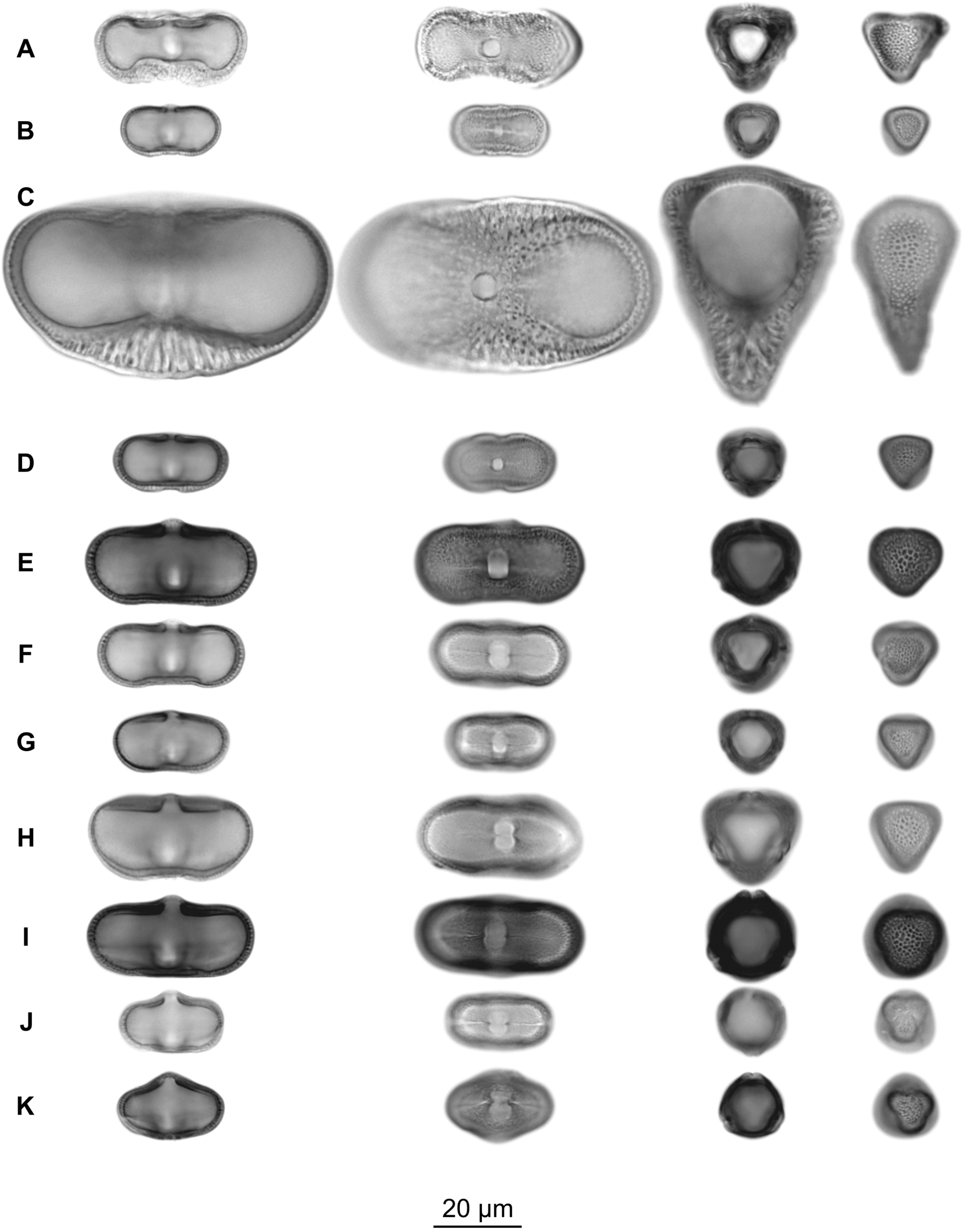

PLATE 8. Pollen grains of Scandiceae (A–I) and representatives of North American *Licusticum* clade (J & K). A: ***Astrodaucus orientalis*** (L.) Drude, B: ***Torilis japonica*** (Houtt.) DC., C: ***Turgenia latifolia*** (L.) Hoffm., D: ***Daucus carota*** L., E: ***Laser archangelica*** (Wulfen) Spalik & Wojew., F: ***Laserpitium latifolium*** L., G: ***Silphiodaucus prutenicus*** (L.) Spalik, Wojew., Banasiak, Piwczyński & Reduron, H: ***Thapsia villosa*** L., I: ***Ferula glauca*** L., J: ***Meum athamanticum*** Jacq., K: ***Mutellina purpurea*** (Poir.) Reduron, Charpin & Pimenov.

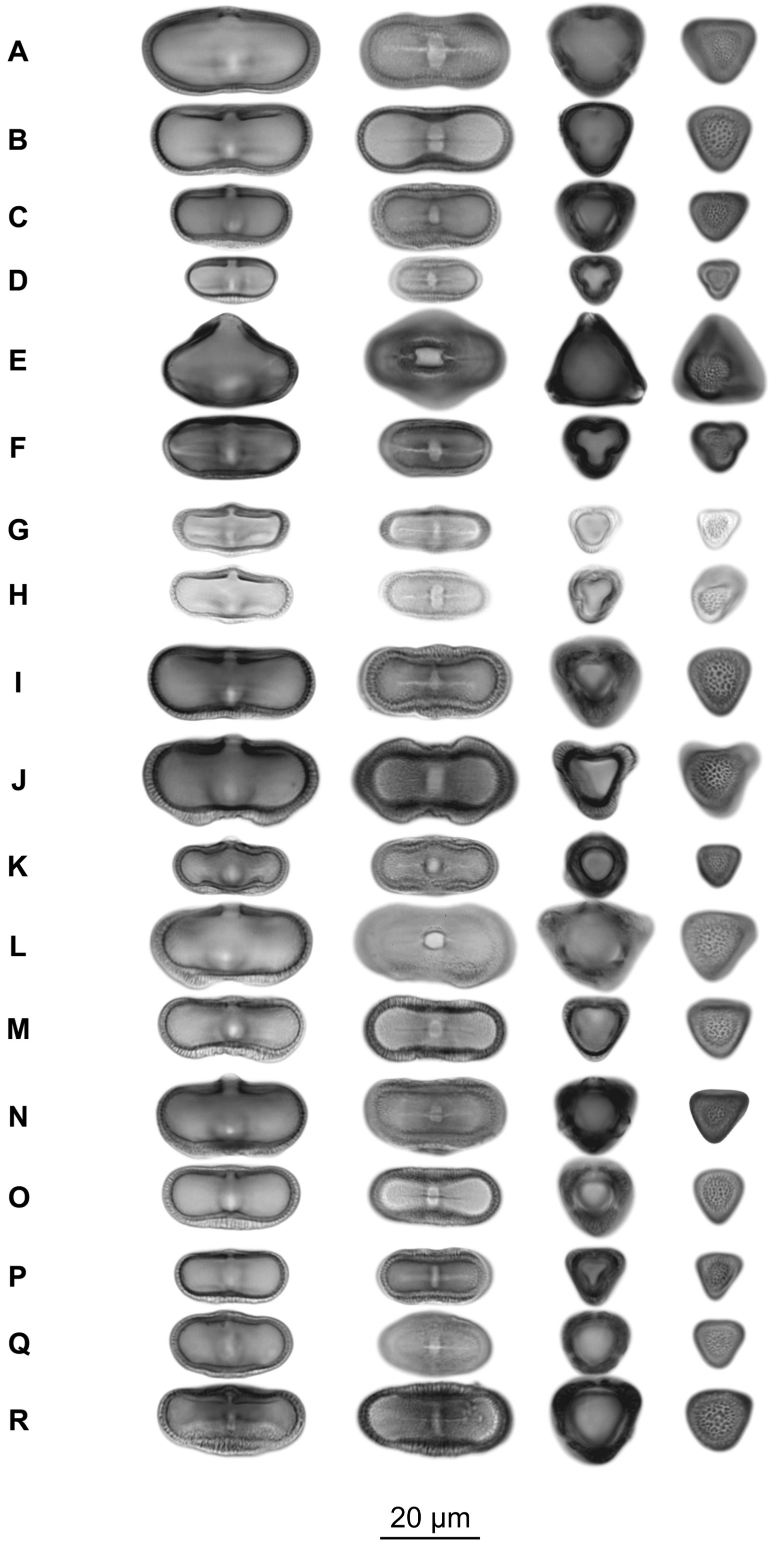

PLATE 9. Pollen grains of apioid superclade. A: ***Aegopodium podagraria*** L., B: ***Carum carvi*** L., C: ***Falcaria vulgaris*** Bernh., D: ***Ammoides pusilla*** (Brot.) Breistr., E: ***Carum buriaticum*** Turcz., F: ***Crithmum maritimum*** L., G: ***Elaeosticta allioides*** (Regel & Schmalh.) Kljuykov, Pimenov & V.N.Tikhom., H: ***Elwendia persica*** (Boiss.) Pimenov & Kljuykov, I: ***Scaligeria napiformis*** (Spreng.) Grande, J: ***Schrenkia vaginata*** (Ledeb.) Fisch. & C.A.Mey., K: ***Aphanopleura capillifolia*** (Regel & Schmalh.) Lipsky, L: ***Pimpinella anisum*** L., M: ***Pimpinella peregrina*** L., N: ***Pimpinella saxifraga*** L., O: ***Ammi majus*** L., P: ***Anethum graveolens*** L., Q: ***Petroselinum crispum*** (Mill.) Fuss, R: ***Rutheopsis herbanica*** (Bolle) A.Hansen & G.Kunke.

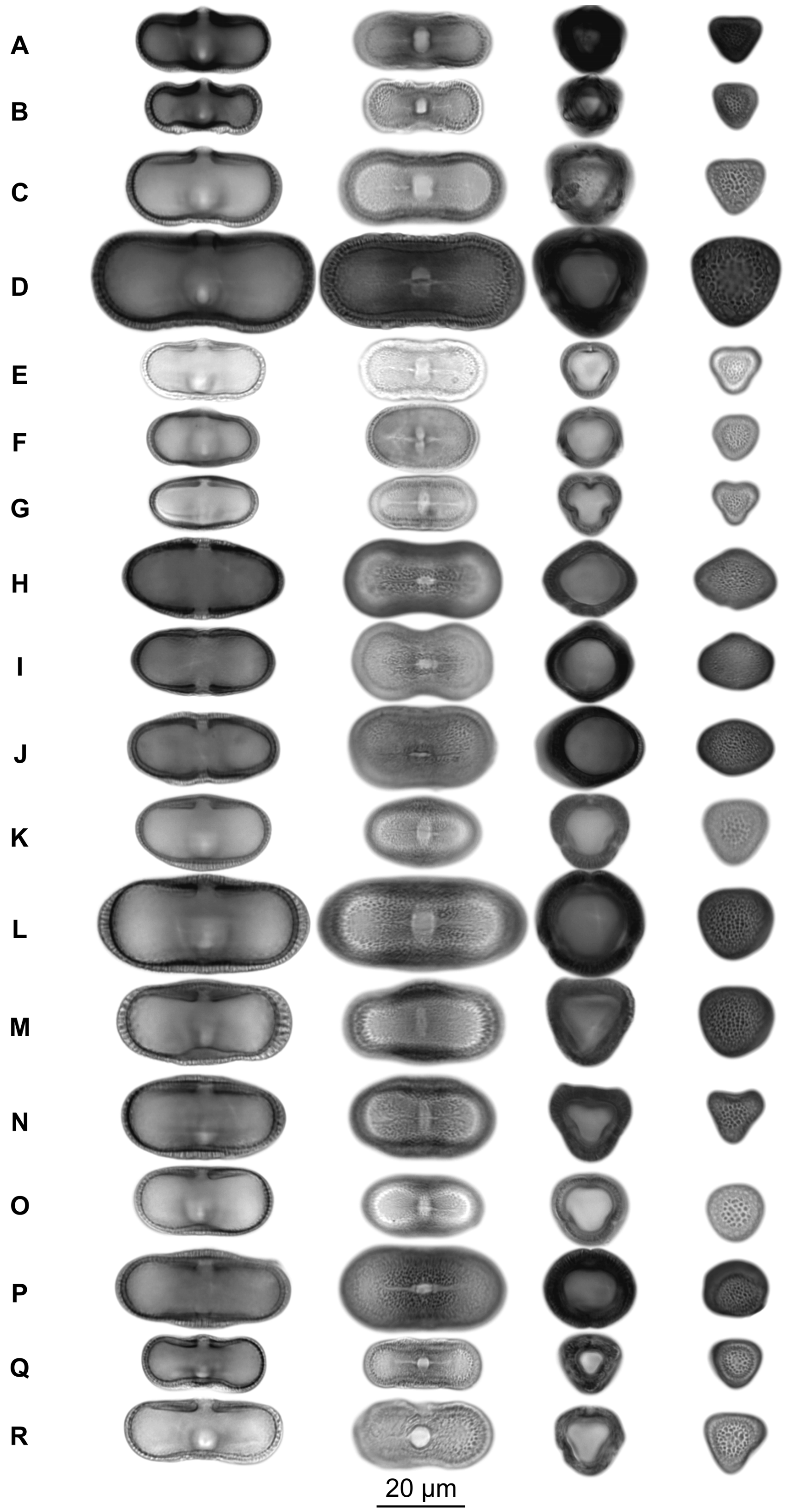

PLATE 10. Pollen grains of apioid superclade. A: ***Opopanax hispidus*** (Friv.) Griseb., B: ***Stefanoffia daucoides*** (Boiss.) H.Wolff, C: ***Ferulago macrosciadea*** Boiss. & Balansa, D: ***Prangos ferulacea*** (L.) Lindl., E: ***Conioselinum tataricum*** Hoffm., F: ***Levisticum officinale*** W.D.J.Koch, G: ***Silaum silaus*** (L.) Schinz & Thell., H: ***Echinophora chrysantha*** Freyn & Sint., I: ***Echinophora tenuifolia*** L., J: ***Pycnocycla spinosa*** Decne., K: ***Cymbocarpum erythraeum*** (DC.) Boiss., L: ***Heracleum pastinacifolium* subsp. *incanum*** (Boiss. & A. Huet) P.H.Davis, M: ***Heracleum sphondylium* subsp. *flavescens*** (Willd.) Soó, M: ***Heracleum sphondylium* subsp. *spondylium*** L., N: ***Pastinaca armena*** Fisch. & C.A.Mey., O: ***Pastinaca sativa*** L., P: ***Tordylium maximum*** L., Q: ***Tordylium trachycarpum*** (Boiss.) Al-Eisawi, R: ***Zosima orientalis*** Hoffm.

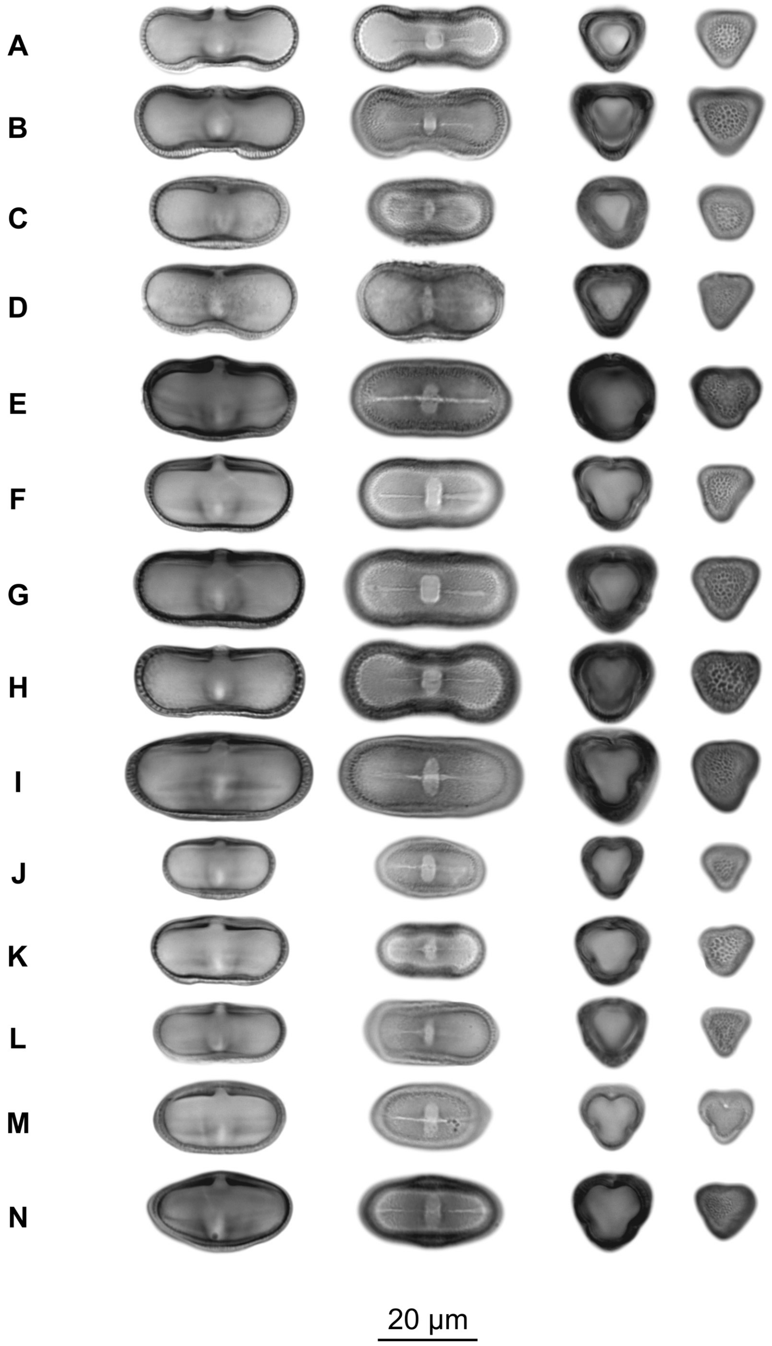

PLATE 11. Pollen grains of apioid superclade. A: ***Bifora radians*** M.Bieb, B: ***Coriandrum sativum*** L., C: ***Aethusa cynapium*** L., C: ***Aethusa cynapium* subsp. *segetalis*** (Boenn.) Schübl. & G.Martens, D: ***Angelica archangelica*** L., E: ***Arracacia aegopodioides*** (Kunth) J.M.Coult. & Rose, F: ***Arracacia longipedunculata*** J.M.Coult. & Rose, G: ***Arracacia tolucensis* var. *multifida*** (S.Watson) Mathias & Constance, H: ***Ligusticum mutellinoides*** (Crantz) Vill., I: ***Lomatium bicolor* var. *leptocarpum*** (Nutt. ex Torrey & A.Gray) Schlessman, J: ***Seseli annuum*** L., K: ***Seseli hippomarathrum*** Jacq., L: ***Thysselinum palustre*** (L.) Hoffm., M: ***Trinia glauca*** (L.) Dumort., N: ***Zizia aurea*** (L.) W.D.J.Koch.

Klotzschia (Plate 2F):—Although consistently proven to be a separate phylogenetic lineage, *Klotzschia* is yet another taxon of uncertain affinity (apart from *Platysace*) which pollen shares many apomorphies with Mackinlayoideae. Its grains are prolate, elliptic in general outline, with acute ends of aperture (similarly to *Actinotus* Labillardière and *Centella* L.) and distinctly rounded mesocolpium. Nevertheless, these two taxa can be differentiated by investigating columellae at poles which are fused in *Klotzschia* and separate in Mackinlayoideae.

Azorelloideae (Plates 2G–L & 3A–J):—Pollen of this subfamily is morphologically diverse, and the only trait shared among all representatives of the group is rounded mesocolpium in polar view. However, this trait is sometimes present in other groups, especially in early-diverging Apiales. Among others, broadly-defined genus *Azorella* Lam. including *Huanca* Cav. is exceptionally idiosyncratic and easy to identify (small, rhombic pollen, usually with concave mesocolpium side and apparent 'keeled' apiculation of exine above endoaperture; Plate 2H–L). Genus *Bowlesia* Ruiz & Pavón can be identified based on more than three apertures easy to count in polar view (Plate 3A&B). Other Azorelloideae (Plate 3C–J), due to relatively frequent presence of straight or concave mesocolpium contour, must be differentiated with higher apioids (particularly Scandiceae and relatives) based on the presence of continuous, slit-like colpus with horned ends of endoaperture.

Hermas (Plate 3K):—Pollen of *Hermas*, whereas generally plesiomorphic in appearance, shows a mixture of advanced traits normally found in other groups of Apiaceae. Potential synapomorphies shared with Azorelloideae may indicate its affinity to this group, most notably the rounded mesocolpium in polar view and horned endoaperture. These traits coupled by obtuse polar caps clearly distinguish the genus from representatives of Mackinlayoideae and Apioideae. On the other hand, thin pollen wall and relatively large size accounts on its resemblance to Saniculoideae.

Saniculoideae (Plate 4):—Similarly to higher apioids and some Azorelloideae, pollen of Saniculoideae is strongly prolate (lower tolerance limit for the P/E ratio: 1.6). Contrarily to the advanced apioids, ectocolpus is rarely shorter than 70% of polar length, and pollen grains are generally bigger with polar axis almost always longer than 30 μm. Even so, pollen grains in other groups can be as long as 40 μm making differentiation of Saniculoideae rather complicated. Horned ends of endoaperture are very frequent but less distinct than in Azorelloideae and the trait that easily separates two groups is mesocolpium shape in polar view—rounded in Azorelloideae and distinctly rounded in Saniculoideae.

Early diverging Apioideae (protoapioids and basal euapioids; Plates 5 & 6A–G):—Similarly to basalmost Apiaceae (Mackinlayoideae and Azorelloideae) and early-diverging Apiales, protoapioids and euapioids have prolate pollen grains (tolerance limit for P/E ratio: 1.3–1.8). However, they can usually be easily delimited by straight mesocolpium in polar view coupled with rhombic general outline in colpus view. *Eremodaucus lehmannii* Bunge (Pleurospermeae; Plate 6A) constitutes a notable exception. Its pollen is strongly convergent with advanced Apioideae (perprolate grains with bone-shaped outline and reduced relative length of ectocolpus), and it is impossible to distinguish the species from higher apioids solely based on pollen morphology.

Oenantheae (Plate 6H–Q):—Although the tribe has in general many distinct morphological features like glabrous stems and adventitious roots (being an adaptation to helophytic or amphiphytic habit), its identification based on palynological traits is challenging. Oenantheae pollen seems to be a mixture of plesiomorphic (relatively small P/E ratio, long ectocolpus and acute ends of endoaperture) and apomorphic traits (sharply-bent mesocolpium in polar view and bone-shaped outline in colpus view). Assignment of pollen to this group is done by means of exclusion rather than based on characteristic, self-sustaining traits, and such identification needs caution.

Scandiceae and relatives (tribes Smyrnieae, Aciphylleae, *Acronema* clade, North American *Ligusticum* clade), and apioid superclade (Plates 7–11):—Although members of these two groups are treated separately in our key, they share distinct, apomorphic morphology. Most of their representatives have perprolate pollen grains with relatively short ectocolpus (usually not exceeding 65% of polar length), bone-shaped outline in colpus view and crenate or undulate tectum at mesocolpium. Less elongated pollen or longer ectocolpus found in some species calls for differentiation with other groups, especially with Azorelloideae and Oenantheae. The former very rarely have dilated or discontinuous ectocolpus above the equator (endoaperture), but the latter cannot be adequately delineated (as already mentioned in the description of Oenantheae). Differentiation between Scandiceae and relatives and apioid superclade requires a detailed examination of exine stratification at mesocolpium. Representatives of apioid superclade tend to have more pronounced columellae increasing total wall thickness and sexine to nexine ratio. However, these traits have considerable overlap among the groups, and fully confident identification is possible only if the total wall thickness is lower than 5% of polar length (Scandiceae and relatives) or if it exceeds 10% (apioid superclade).

Identification key to pollen grains of Apiales

PCA results showed that quantitative traits analysed solely are not enough for delineation of taxonomic groups (Fig. 7) and qualitative traits must be employed as well. A single trait with the highest discriminative power is the P/E ratio and thus appears at the very beginning of the identification key. This trait alone is enough to delineate early-diverging Apiales and quite well separates Mackinlayoideae and early-diverging Apioideae from the remaining groups except for less elongated Azorelloideae (Fig. 8A). However, these three groups characterised by prolate pollen grains, can be differentiated by mesocolpium shape in polar view (Fig. 8B). From the other hand, for more elongated grains, ectocolpus length relative to polar length stands in the first place separating Saniculoideae and Oenantheae from higher euapioids. (Fig. 8C). General outline seen in colpus view (Fig. 8D) helps for delineation of early-diverging Apioideae, which are almost always rhombic. Although this feature rarely occurs also in early-diverging Apiales and Azorelloideae as well. Raw polar length, i.e. not standardised by equatorial length, is useful only for identification of the largest representatives of Saniculoideae (Fig. 8E). Among traits of endoaperture, presence of horns is of the highest discriminative power, facilitating delineation of Saniculoideae and Azorelloideae and excluding higher euapioids (Fig. 8F). Wall stratification, especially columellae height at mesocolpium, is useful only for differentiation between Scandiceae and relatives and some apioid superclade members (Fig. 9A&B). The remaining traits summarised in Appendix S4 are either redundant, i.e. highly correlated with those already used for the construction of the key, or characterised by high variation within the groups or simply invariant between the groups, and thus are of little importance in identifying pollen of Apiales.

1.	P/E ratio < 1.7	2
-	P/E ratio > 1.7	8
2.	P/E ratio < 1.3	*early-diverging Apiales*
-	P/E ratio > 1.3	3
3.	Mesocolpium rounded in polar view	4
-	Mesocolpium in polar view straight, distinctly or sharply-bent	5
4.	Ectocolpus discontinuous above equator	*Scandiceae and relatives*
-	Ectocolpus above equator slit-like or dilated	*Azorelloideae*
5.	General outline rhombic in colpus view	*early-diverging Apioideae*
-	General outline in colpus view elliptic or bone shaped	6
6.	Columellae separate in polar view	*Mackinlayoideae*
-	Columellae in polar view minute, fusing or forming muri	7
7.	Ends of endoaperture horned	*early-diverging Apioideae*
-	Ends of endoaperture without horns	*Oenantheae*
8.	Ectocolpus/P ratio < 0.65	9
-	Ectocolpus/P ratio > 0.65	13
9.	Wall thickness at equator/P < 0.05	*Scandiceae and relatives*
-	Wall thickness at equator/P > 0.05	10
10.	Inner outline at the colpus side concave	11
-	Inner outline at the colpus side straight or convex	12
11.	Endoaperture width/P ratio < 0.1	*apioid superclade*
-	Endoaperture width/P ratio > 0.1	*Scandiceae and relatives*
12.	Relative nexine thickness at equator < 0.5	*apioid superclade*
-	Relative nexine thickness at equator > 0.5	*Scandiceae and relatives*
13.	Ends of endoaperture horned and the outer outline at the colpus side convex	*Azorelloideae*
-	Ends of the endoaperture without horns or outer outline at the colpus side concave or straight	14
14.	Ends of the endoaperture horned	*Saniculoideae*
-	Ends of the endoaperture without horns	15
15.	Outer outline at the colpus side convex	16
-	Outer outline at the colpus side concave or straight	17
16.	Ectocolpus discontinuous above equator	*Scandiceae and relatives*
-	Ectocolpus above equator slit-like or dilated	*Azorelloideae*
17.	Polar length > 37 μm	*Saniculoideae*
-	Polar length < 37 μm	18
18.	Endoaperture ends acute	*Oenantheae*
-	Endoaperture ends diffused, rounded or truncate	*apioid superclade*

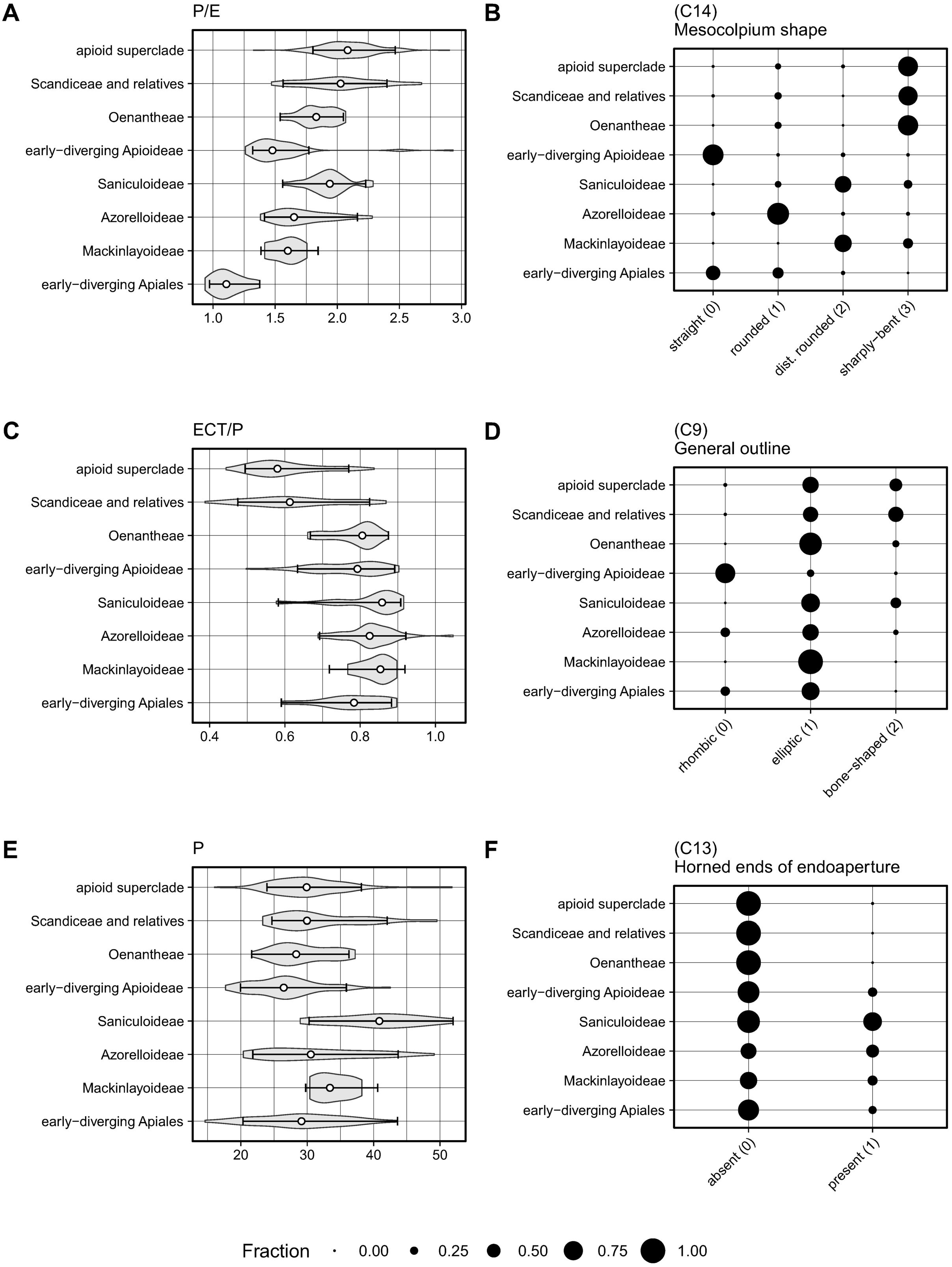

FIGURE 8. Traits with the highest importance for pollen identification. A, C, E: Empirical distributions, median and tolerance intervals for quantitative standardised traits. Grey violin plots represent truncated kernel density estimates, i.e., the lower and upper boundaries of a violin plot are empirical min. and max., respectively. White circles stand for sample median. Black bars represent nonparametric tolerance intervals covering at least 80% of the population with 90% confidence. B, D, F: Empirical distributions of qualitative traits. For a given taxon and trait, the radius of each circle is proportional to the fraction of species having a given state of the trait. A sum of the fractions larger than unity indicate the presence of polymorphic species within a given taxon. Trait symbols are the same as in Fig. 6 and in the text.

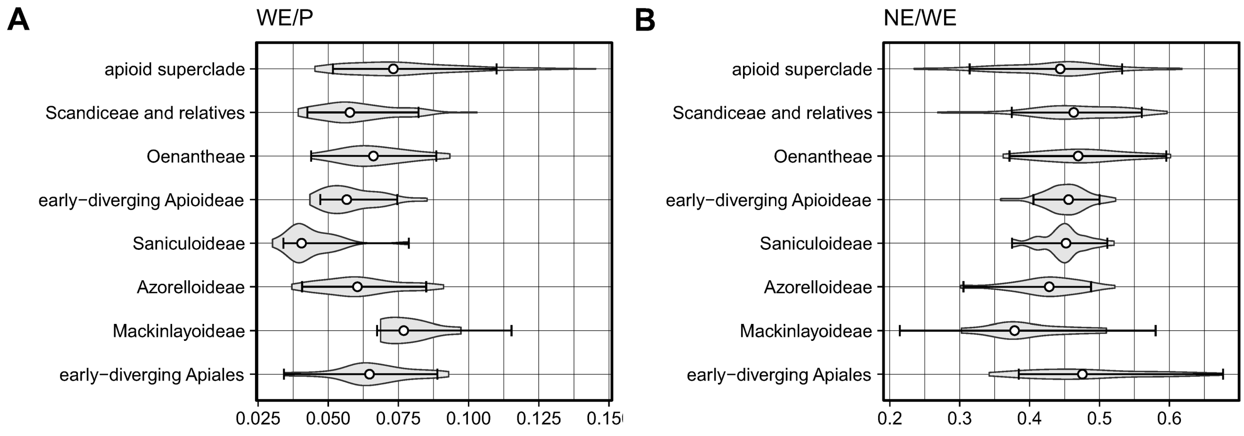

FIGURE 9. Traits with the highest importance for pollen identification, continued. Empirical distributions, median and tolerance intervals for quantitative standardised traits. For details, see caption to Fig. 8.

Discussion

Importance of particular traits for pollen identification

For many years, various authors were focusing on different sets of characters useful for the identification of pollen belonging to distinct groups of Apiales. In consequence, newly obtained morphological information was not related to comparative analyses of the pollen morphology, mostly due to the lack of a unified approach for the entire order. However, some essential traits were identified quite early. For instance, the P/E ratio introduced by Cerceau-Larrival (1962) for differentiation of Apiaceae was further successfully utilised in other systematic keys (Ting 1961, Cerceau-Larrival 1981, Punt 1984). Its utility was confirmed also in our study, i.e. for delineation of early-diverging Apiales and basal Apiaceae. The shape of endoaperture (Ting *et al.* 1964) and the presence of horns (sometimes referred to as ‘endonexinous breaks’ or ‘H-shaped os’) are remarkable for the systematic study of former Hydrocotyloideae (Moar 1960, Ting *et al.* 1964, Cerceau-Larrival 1981) and Araliaceae (Tseng 1971, 1973, Tseng & Shoup 1978). According to the results of our study, this trait alone, despite its variable presence in different lineages of Apiales, can be used to differentiate Azorelloideae from Saniculoideae, as well as advanced Apiaceae (Oenantheae, Scandiceae and relatives, and apioid superclade) from the rest of the order. Relative ectoaperture length has already been successfully utilised by Punt (1984), and this study confirms its utility for delineation of higher apioids, i.e. Scandiceae and relatives, and apioid superclade. Although in the previous study, this continuous trait was split into several discrete categories losing some important information.

Furthermore, many other traits proposed turned out to be less valuable. For example, the outline of exine seen in the side view, despite being used in both systematic and phylogenetic studies (Cerceau-Larrival 1967, Punt 1984), is highly prone to convergence. In consequence, the general shape of pollen can be similar even in distantly related groups of Apiaceae (e.g., Apioideae and Azorelloideae), while being almost uniform across the basal families of the order. More often than not, various authors paid much less attention to the traits seen only in polar view compared to those visible in either side or colpus view. This does seem to depend on the natural tendency of elongated pollen grains to stay parallel to a microscope slide. However, when comparing our results to those of older studies, it must be pointed out that mesocolpium shape seen in the polar view is of utmost importance for delineation of the subfamilies of Apiaceae.

Congruence of palynological data with modern taxonomy of Apiales

The results of this study show that palynological data constitute a valuable source of information for infrafamilial taxonomy of Apiales. Our data ties well with previous studies on the polyphyly of the former Hydrocotyloideae (Nicolas & Plunkett 2009) and inclusion of some of its members in Araliaceae. The results from this study go beyond previous reports, showing traits for delineation of recently described umbelliferous subfamilies—Mackinlayoideae and Azorelloideae. Differences between Apioideae and Saniculoideae are much more elusive and we were unable to find simple synapomorphies defining these two clades. Nevertheless, a combination of traits can be used to distinguish between these two groups. The basal African lineages of Apioideae (tribes Lichtensteinieae, Annesorhizeae and Heteromorpheae) are different from both saniculoids and more advanced apioids. They strongly resemble other taxa described under 'early-diverging Apioideae' (relatively small, prolate pollen grains with a rhombic outline and straight mesocolpium). In contrary to the taxonomic treatment of Saniculoideae as a part of Apioideae (Magee *et al.* 2010) our study of pollen morphology does not support this theory. Tribe Oenantheae shows a peculiar mixture of plesiomorphic (well-developed compound aperture) and apomorphic (elongation, bone-shaped outline and sharply-bent mesocolpium) traits that further support its delineation based on other data (Hardway *et al.* 2004). Two largest groups of subfamily Apioideae—apioid superclade and 'Scandiceae and relatives' show advanced pollen morphology (perprolate grains with a usually bone-shaped outline and reduced compound aperture) which is usually described as 'typical' of Apiaceae. Unfortunately, we could not identify clear single-trait synapomorphies for tribes/clades described within those two groups. We also were unable to conclude whether shared advanced pollen morphology of apioid superclade and 'Scandiceae and relatives' was acquired due to common ancestry or two times independently. The reason for that is the uncertain phylogenetic position of North American *Ligusticum* clade, which representatives show traits one would expect in early-diverging lineages of Apioideae (long ectocolpus, sometimes coupled with rhombic outline and straight mesocolpium in polar view). Based on that alone, we could assume that this small tribe constitutes a sister group to the large group uniting Scandiceae with its relatives and apioid superclade. However, relationships between these three groups have never been resolved based on molecular data and needs further examination. Our results neither support nor reject the recently postulated hypothesis on inclusion of *Acronema* clade in tribe Scandiceae (Wen *et al.* 2020), as pollen morphology does not give enough information to distinguish between these two groups. We are also aware that although this unexpected topology received high support as measured by both bootstrap support and posterior probability, it may be an artifact of sampling, as only these two clades from 'Scandiceae and relatives' were included in the transcriptome analyses.

Among *incertae sedis* genera analysed in this study, both *Platysace* and *Klotzschia* show many similarities with Mackinlayoideae. Pollen morphology cannot solely resolve their exact phylogenetic position, but it strongly contradicts some findings that they may be sister to Apioideae (Henwood & Hart 2001, Andersson *et al.* 2006, Nicolas & Plunkett 2014). Our data are more informative in the case of *Hermas*. The most recent analyses reconstruct this genus as a sister group to the clade Apioideae + Saniculoideae (Nicolas & Plunkett 2014, Calviño *et al.* 2016). Its phylogenetic position was predominantly supported based on of biogeography, as both Saniculoideae and *Hermas* are of African origin. Interestingly, palynological data also give credibility to this topology, as the pollen of *Hermas* shows a combination of traits otherwise typical for either Azorelloideae or Saniculoideae.

Limitations of this study in phylogeny dating

Fossil pollen of Apiales has been used as a source of calibration points for molecular dating (Banasiak *et al.* 2013, Nicolas & Plunkett 2014, Calviño *et al.* 2016). Pollen was crucial especially for Apiaceae, which representatives are hardly linked to any reasonably old macrofossils. Unfortunately, our results show that it is very hard or even impossible to unequivocally assign fossil pollen to extant taxa of Apiaceae. The most significant limitation is that pollen grains compressed during fossilisation can be seen only in the side or, rarely, colpus view. It is particularly true for Azorelloideae, Mackinlayoideae, and early-diverging lineages of Apioideae, which pollen grains lack evolutionary advanced morphological traits and their identification needs investigation of mesocolpium shape seen in the polar view. Because of that, the precise classification of upper Eocene palynomorphs as representatives of *Bupleurum* or *Pleurospermum* Hoffm. by Gruas-Cavagnetto & Cerceau-Larrival (1984) is rather doubtful. As these particular fossils were repetitively used to put constraints on the stem age for respective clades, the results of most calibrations up to date may be inadequate for Apiaceae. According to our current knowledge, the oldest fossilised pollen grains that can be admittedly linked to an extant group of umbellifers (higher apioids) come from the upper Oligocene sediments of Kars-Erzurum-Mus basin, Turkey (Sancay *et al.* 2006).

Summary

The unified typology for pollen of Apiales together with representative sampling based on recent advancement in the field of molecular taxonomy revealed some morphological patterns congruent with the modern classification of the group. However, for many taxa, we are still missing well-defined synapomorphies while some other groups seem to be apparently convergent. Further studies employing the comparative phylogenetic method are necessary to elucidate more detailed patterns of pollen evolution.

We were able to describe, validate and improve qualitative traits accommodating variation between specimens or even between pollen grains within a single specimen. Moreover, we determined the most important traits for pollen identification within Apiales. Those were: P/E ratio, mesocolpium shape observed in polar view and ectocolpus length relative to polar diameter. Our study proved that with the use of unified typology and the provided key, it is possible to successfully identify pollen of major taxa of Apiales.

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Supplementary material

Appendix S1:—List of herbarium specimens used in this study.
Appendix S2:—Scores of qualitative traits for each species.
Appendix S3:—Raw measurements of quantitative traits for each specimen.
Appendix S4:—Empirical distributions of quantitative and qualitative traits.

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