

Pollen morphology of the genus *Omphalodes* Mill. (Cynoglosseae, Boraginaceae)

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Abstract

To better understand the taxonomy and phylogeny of the genus *Omphalodes* (Boraginaceae), the pollen morphology of 23 species encompassing all major geographical and morphological groups was studied from acetolysed pollen grains using light and scanning electron microscopy. Three pollen types were distinguishable through apertural morphology and sculpture. A dichotomous key to the pollen types and pollen descriptions are provided. Despite being fairly homogenous, palynological data provided useful information to understand the relationships of *Omphalodes*, considering the uselessness of most floral and fruit characters, karyological homogeneity and striking disjunct geographical distribution. At the suprageneric level, pollen morphology supports the traditional tribal classification of *Omphalodes* within Cynoglosseae, in contrast to published molecular phylogenetic analyses. At the infrageneric level, pollen morphology agrees with phylogenetic analyses suggesting that *Omphalodes* could be a polyphyletic group. Palynological data would support the segregation of a monophyletic group comprising the Japanese species, corroborating recent phylogenetic results and define two different pollen types for the *Omphalodes* s. str. group. Biogeographical considerations are also discussed.

Keywords: *Acetolysis, dichotomous key, disjunct biogeographic pattern, ring-like aperture, phylogenetic relationships, pollen types, taxonomy*

Omphalodes (Boraginaceae, Boraginoideae) comprises 28 species of herbs from temperate habitats, including mostly narrow endemics but also some ornamental species like *O. verna*. The genus has a striking disjunct distribution in the Northern Hemisphere, occurring in three distant areas: Europe and adjacent areas of western Asia, northern Mexico and Texas, and Japan, being an unusual variant of the north temperate disjunct biogeographic pattern (*sensu* Raven, 1972). The genus shows great variation in habit (perennial, biennial and obligate annual species), ecology (ranging from coastal dunes to mountain summits or mesic habitats) and morphology (e.g. fruit characters). Still, some species seem to form natural subgroups as, for example the Japanese species or the annual species from south-western Europe. Several sections have been described for

the western Euro-Asiatic taxa (de Candolle, 1846; Brand, 1921; Popov, 1953), but their evolutionary significance has not been assessed. It has been suggested that the Japanese species should be considered a subgenus (Popov, 1953) and that *O. scorpioides* could even deserve an independent genus (Johnston, 1924), albeit no formal proposals have been made so far. Despite its biogeographic singularity and the evidence for a certain infrageneric structure (Popov, 1953), no attempts have been made to find morphological characters that could clarify the internal relationships of the genus.

The subfamily Boraginoideae has been divided into several tribes that vary from four to eight, depending on the author (de Candolle, 1846; Riedl, 1997). *Omphalodes* has been traditionally ascribed to the tribe Cynoglosseae (Brand, 1921), mainly

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because of the nutlet apical attachment to the gynobase (Johnston, 1924). Indeed, the internal systematics of the Boraginoideae, and in particular of the Cynoglosseae (Johnston, 1924; Riedl, 1997), heavily relies on fruit traits. However, this classification has been considered weak (Al-Shehbaz, 1991) mainly because fruits from this group are suggested to be under evolutionary selective pressures, thus, being labile characters (Johnston, 1924; Selvi et al., 2011) unsuitable for taxonomic purposes. Molecular analyses of Boraginaceae including all Boraginoideae tribes (Långström & Chase, 2002) and a representative of *Omphalodes* (the type species *O. verna*) have been performed to shed light on the phylogenetic relationships of the family. Interestingly, the phylogeny recovered merged the tribes Cynoglosseae, Eritrichieae, Myosotideae and Trigotideae and located *Omphalodes* outside of the Cynoglosseae *s. l.* clade at an unresolved position among all tribes of the subfamily Boraginoideae. Recent molecular phylogenetic analyses of *Omphalodes* species (Serrano et al., unpublished data) recovered five main clades: the American species, the annual European species, the western Eurasian perennial species, the Japanese species, and *O. scorpioides*. Two clades, the Japanese group and *O. scorpioides*, are separately nested among other genera of Cynoglosseae, therefore making the genus polyphyletic as currently circumscribed. However, morphological and karyological support for the inferred relationships is scarce. Floral characters are relatively homogeneous throughout the genus and fruit characters, albeit very variable, hardly show discontinuities that could be clearly correlated with the inferred phylogenetic groups. Karyological information is also relatively useless to clarify the internal evolutionary relationships of the genus, since the base number of the Boraginaceae ($x = 12$; Lewis, 1980) is much conserved within the Cynoglosseae (Luque & Valdés, 1986; Coppi et al., 2006; Selvi et al., 2011) and is present as $2n = 24$ in both western Eurasian (Grau, 1967) and Japanese (Kadota, 2009) species of *Omphalodes*. The exceptions are the European annual taxa, having a derived chromosome number $2n = 28$, (Grau, 1967; Valdés, 1987) and *O. caucasica* Brand with $2n = 22$ (Grau, 1967).

The Boraginaceae show great diversity in pollen ornamentation, size, shape and number of apertures and the family is considered one of the most eurypalynous among the angiosperms (Díez & Valdés, 1991). Several palynologists have demonstrated the importance of the palynological characters to understand the taxonomy, ecology and evolution of Boraginaceae (e.g. Díez et al., 1986; Ning et al., 1992; Scheel et al., 1996; Retief & Van Wyk, 1997; Liu et al., 2010). Pollen morphology has

been used as a valuable tool in delimiting genera within Cynoglosseae (Barbier & Mathez, 1973) and in suggesting several evolutionary hypotheses (Clarke et al., 1979; Bigazzi et al., 2006). A few authors published some significant studies of the pollen morphology of *Omphalodes* using light microscopy (LM) and, in some cases, scanning electron microscopy (SEM) (Barbier & Mathez, 1973; Díez, 1984; Ahn & Lee, 1986; Díez & Valdés, 1991). Still, these are partial studies including only a few species and, thus, pollen morphology of *Omphalodes* is insufficiently known.

The goal of this work was to investigate for the first time the variability of pollen morphology in *Omphalodes*, studying the majority of the species recognised and encompassing all major geographical and morphological groups, through light and scanning electron microscopy of acetolysed material. Since pollen is highly variable in Boraginaceae, it was hypothesised that the extremely disjunct distribution range of *Omphalodes* spp. suggests an ancient origin of the genus that may have allowed enough evolutionary time for accumulation of informative morphological changes. Palynological information was used to address two main questions in order to shed light to the complex internal systematics and the striking geographical pattern of *Omphalodes*. First, considering that molecular phylogenetic analyses question the position of *Omphalodes* within the Cynoglosseae (Långström & Chase, 2002), palynological data was studied to test whether pollen morphology supports the traditional tribal affiliation of the genus. Second, considering the homogeneity and continuum of variation of plant morphology, palynological data was explored to test if there is correlation between intra-generic variation in pollen morphology and the recent phylogenetic results that singularise, among others, the Japanese group.

Material and methods

Pollen samples were collected from a total of 34 herbarium specimens of the genus *Omphalodes* (see 'Specimens investigated') from the following institutions: COI, IEB, K, MA, P, SALAF, SANT, TEX, TI and TNS (abbreviations follow Holmgren et al., 1990). The samples included 26 *Omphalodes* taxa with representatives from all the geographic regions. All pollen samples were subjected to acetolysis according to the method proposed by Erdtman (1960). The terminology used for pollen descriptions follows Punt et al. (2007) and Hesse et al. (2009).

For LM, pollen grains were pre-treated with *t*-butanol, mounted in silicone oil (Andersen, 1960) and observed using a Motic BA 310 light

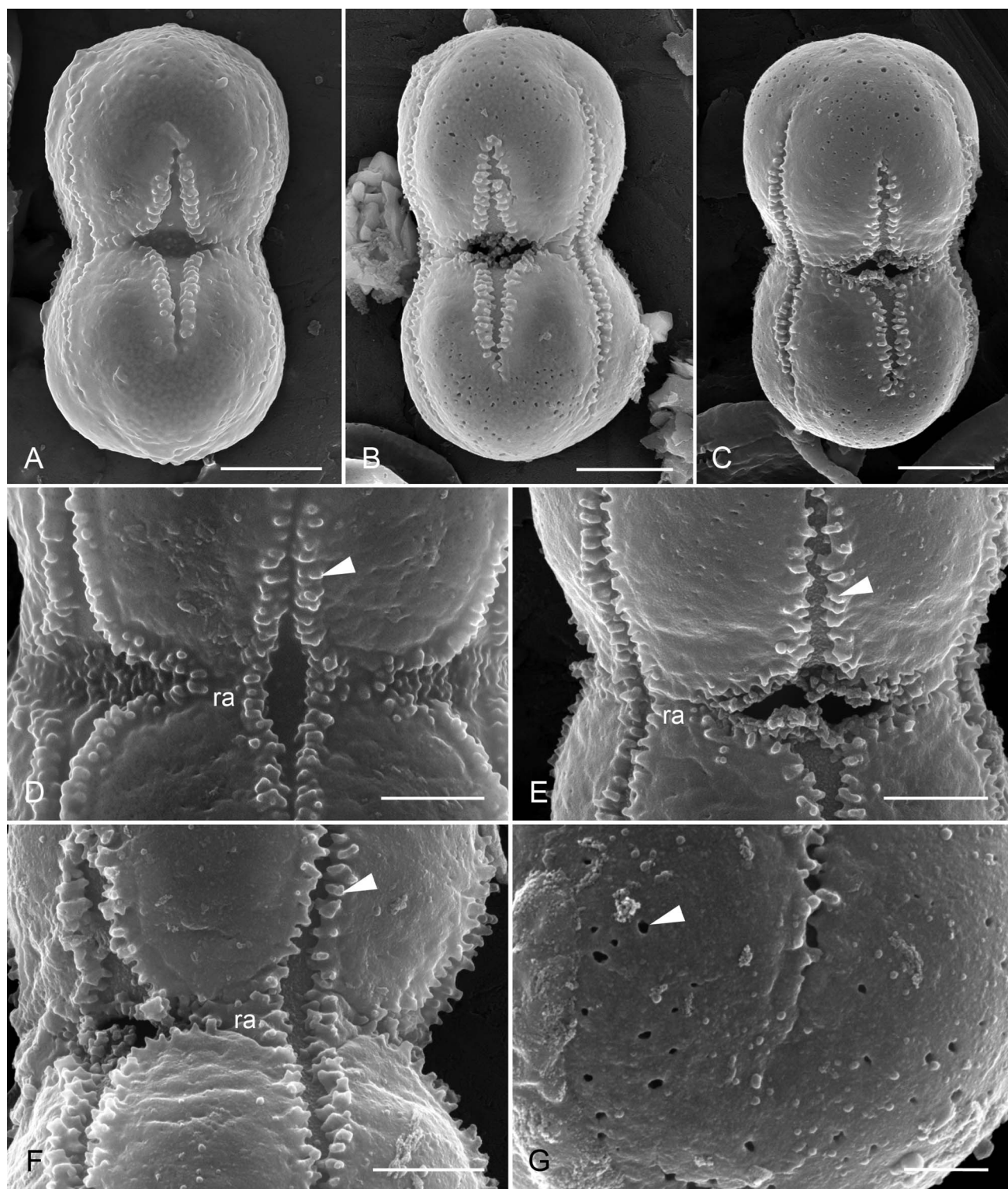


Figure 1. SEM micrographs of pollen grains of *Omphalodes*. **A–G.** *Omphalodes japonica* pollen type: **A.** *Omphalodes akiensis*, equatorial view; **B.** *Omphalodes japonica*, equatorial view; **C.** *Omphalodes krameri*, equatorial view; **D.** *Omphalodes akiensis*, detail of apertural system and granula in the margins of colporus and pseudocolpus (arrow); **E.** *Omphalodes krameri*, detail of apertural system and granula in the margins of colporus and pseudocolpus (arrow); **F, G.** *Omphalodes japonica*, detail of apertural system and granula in the margins of colporus and pseudocolpus (**F**, arrow) and detail of the exine showing the perforations (**G**, arrow). ra – ring-like aperture. Scale bars – 2 μm (A–C); 1 μm (D–F); 500 nm (G).

microscope with an oil immersion objective lens (magnification = 1000 $\times$). The exine thickness was measured in 15 pollen grains from each taxon using a micrometer.

For SEM, the acetolysed pollen grains were air dried (Ahn & Lee, 1986), mounted on aluminium stubs and coated with a 30 nm layer of gold/palladium for eight minutes at high vacuum in a sputtering chamber (Jeol JFC-1100 Ion Sputter). Pollen grains were then observed with a Zeiss FESEM ULTRA plus scanning electron microscope (operating at 15 kV) and micrographs of at least 15 pollen grains were taken using ImageTool (v.3.0 for Windows, University of Texas Health Science Centre, San Antonio, TX, USA). Due to the small pollen size, micrographs were then used for morphometric analysis. The following characters were measured in 15 pollen grains from each taxon: polar axis (*P*), equatorial axis (*E*), maximum width of the grain, pseudocolpi length, colpi ectoaperture length and endoaperture width. The *P:E* ratio was then calculated. The general pollen morphology and particularly the type of sculpture of the margins of the pseudocolpi and colpi and the presence/absence of a ring-like aperture were studied also from the SEM micrographs. The ring-like aperture is an outer circumferential aperture at the equator of the pollen grain (following Hesse et al., 2009).

Descriptive statistics (mean and standard deviation of the mean) of the quantitative variables were calculated for each species (subspecies were not considered due to the low variability observed within species). Multivariate analyses were performed to investigate the structural organisation of the species studied based on all the quantitative and qualitative palynological characters combined. Principal component analysis (PCA) was performed using the specimen mean values for all the measurements, except exine thickness.

Palynological description of pollen types were made based on the quantitative and qualitative morphologic results.

Results

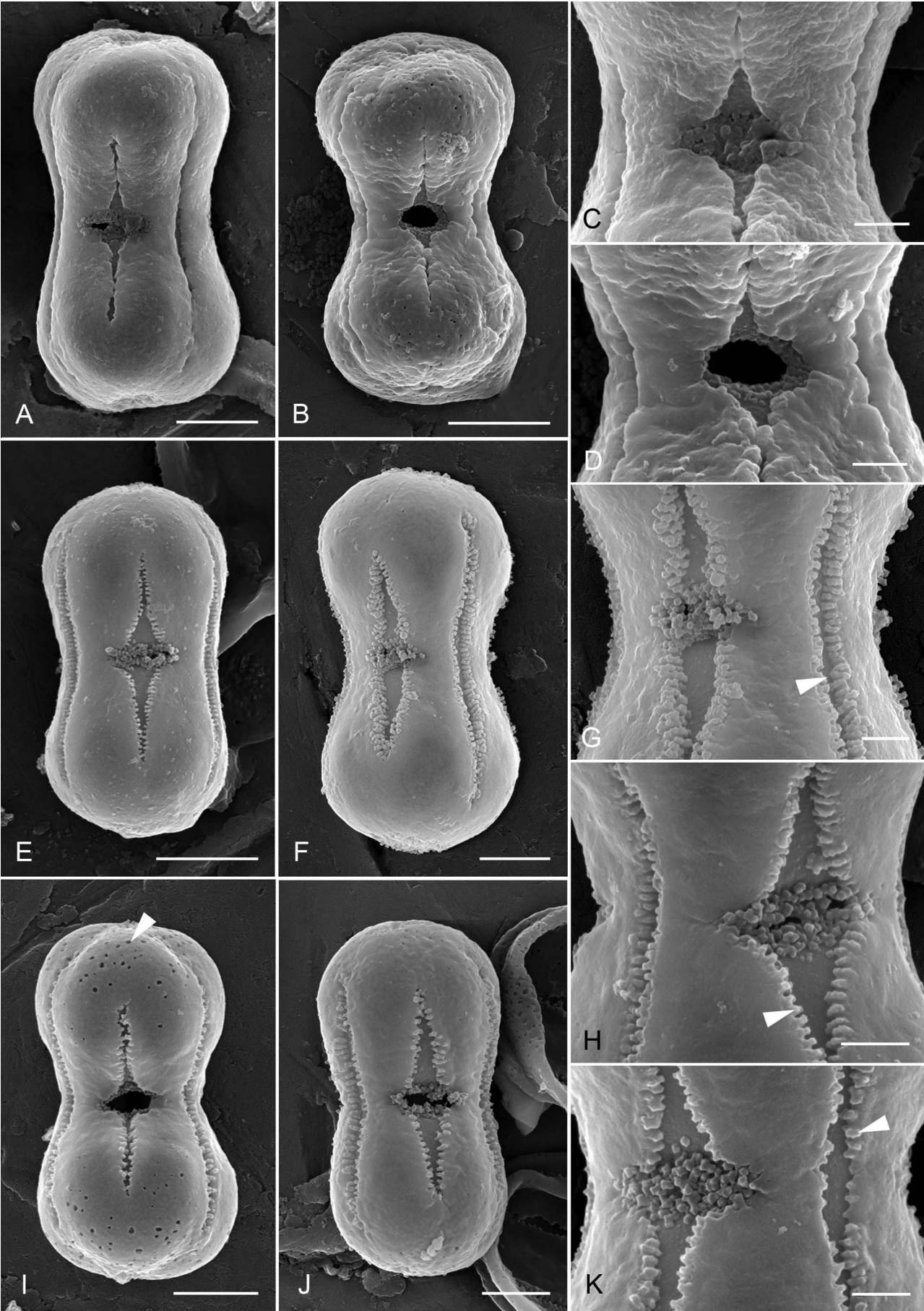
The morphology of the pollen belonging to 23 species of *Omphalodes* (covering 82% of the genus)

was studied using LM and SEM (Figures 1, 2; Table I). This study constitutes the first survey of the pollen morphology for 19 of the species studied.

Overall, pollen morphological features, including polarity, symmetry, shape, size and exine thickness of the species of *Omphalodes* studied were revealed to be fairly constant (Figures 1, 2). The most important characters for pollen type delimitation were qualitative: the sculpture of the margins of the colpi and pseudocolpi and the presence/absence of a ring-like aperture. Despite the continuous gradient of transition observed among the species for all the quantitative characters (Table I), it was possible to recognise three pollen types based on the presence/absence of a ring-like aperture and on the presence/absence of conspicuous granula in the apertural margins: *O. verna* pollen type, *O. japonica* pollen type and *O. chiangii* pollen type (see descriptions later). Interestingly, the abundance of these sculpture elements seems to be positively correlated with annual habits and negatively with perennial American species, and the presence of a ring-like aperture is restricted to the Japanese perennial species (Figures 1, 2; Table I).

The first three axes accounted for 86.1% of the total variation (55.1, 17.2 and 13.8% of variation explained by the first, second and third components, respectively) as obtained via PCA of the specimens studied (Figure 3). The first component had a high negative loading for all the palynological characters except *P:E* ratio, while the second component had negative loadings for all the characters except *E*, pseudocolpi length and the presence/absence of granula in the ectoaperture margins, for which positive loadings were obtained (Figure 3). The multivariate analysis enabled us to separate the three pollen types across the two first components (Figure 3). Clearly, a group including the Japanese species (*Omphalodes japonica* pollen type) separates from the remaining species in both axes due to their greater pollen size (reflected in the characters measured) and qualitative characters (presence of a ring-like aperture and granula in the apertural margins; Figure 3). The remaining two groups (*O. verna* and *O. chiangii* pollen types) show a continuum, but can be separated from each other across the

Figure 2. SEM micrographs of pollen grains of *Omphalodes*. **A–D.** *Omphalodes chiangii* pollen type: **A.** *Omphalodes cardiophylla*, equatorial view; **B.** *Omphalodes chiangii*, equatorial view; **C.** *Omphalodes cardiophylla*, detail of apertural system and margins of colpi and pseudocolpus without granula; **D.** *Omphalodes chiangii*, detail of apertural system and margins of colpi and pseudocolpus without granula. **E–K.** *Omphalodes verna* pollen type: **E.** *Omphalodes linifolia*, equatorial view; **F, G.** *Omphalodes littoralis*, equatorial view (**F**) and detail of apertural system and granula in the margins of colpi and pseudocolpus (**G**, arrow); **H.** *Omphalodes commutata*, detail of apertural system and granula in the margins of colpi and pseudocolpus (arrow); **I.** *Omphalodes luciliae*, equatorial view with visible perforations (arrow); **J.** *Omphalodes verna*, equatorial view; **K.** *Omphalodes nitida*, detail of apertural system and granula in the margins of colpi and pseudocolpus (arrow). Scale bars – 1.5 μ m (A, B, E, F, I, J); 500 nm (C, D, G, H, K).



second component (Figure 3). These observations corroborate the pollen types defined later.

General palynological description of *Omphalodes*

Pollen grains are radially symmetrical, isopolar, rectangular-elliptic and more or less constricted at the equator (dumbbell-shaped), more or less rounded at the poles, sub-circular to sub-hexagonal in polar view; prolate to perprolate; $P:E = 1.3-2.8$ (2.3 ± 0.4), $P = 6.4-10.9$ (8.6 ± 0.9) μm , $E = 2.2-6.2$ (3.8 ± 0.2) μm , maximum width of the grains $= 2.2-7.5$ (4.8 ± 0.6) μm . Apertural system is 6-zono-heterocolpate: three pseudocolpi $3.6-9.0$ (6.3 ± 0.9) μm long, three colpi elongated-rhomboidal, operculate with ectoaperture $2.7-6.6$ (4.5 ± 0.8) μm long and endoaperture lalongate, $0.9-3.8$ (1.7 ± 0.5) μm wide; margins of the pseudocolpi and of the colpi without granula or granulate; colpal and pseudocolpal membranes psilate; ring-like aperture absent or present; opercula granulate. Exine is $0.5-1.0$ μm thick, costae slightly developed. Sculpture finely granulate, perforate.

Dichotomous key to pollen types

- 1 Margins of the pseudocolpi and colpi conspicuously granulate 2
 Margins of the pseudocolpi and colpi not granulate *Omphalodes chiangii* pollen type
- 2 Ring-like aperture present
 Omphalodes japonica pollen type
- Ring-like aperture absent
 Omphalodes verna pollen type

Pollen type descriptions

Omphalodes japonica pollen type. — Species included: *Omphalodes akiensis*, *O. japonica*, *O. krameri* and *O. laevisperma*. Pollen grains radially symmetrical; isopolar; rectangular-elliptic and more or less constricted at the equator, more or less rounded at the poles; sub-circular to sub-hexagonal in polar view; prolate to perprolate, $P:E = 1.8-2.5$ (2.2 ± 0.3), $P = 8.1-10.7$ (9.7 ± 0.5) μm , $E = 3.2-5.6$ (4.4 ± 0.6) μm , maximum width of the grains $= 4.4-6.0$ (5.4 ± 0.4) μm . Apertural system 6-zono-heterocolpate: three pseudocolpi $4.7-8.5$ (7.0 ± 0.8) μm long, three colpi elongated-rhomboidal with the ectoaperture $2.7-6.5$ (4.9 ± 0.9) μm long and the endoaperture lalongate, $0.9-3.3$ (2.2 ± 0.7) μm wide; margins of the pseudocolpi and colpi conspicuously granulate; colpal and pseudocolpal membranes psilate; ring-like aperture present. Exine $0.5-1.0$ μm thick, costae slightly

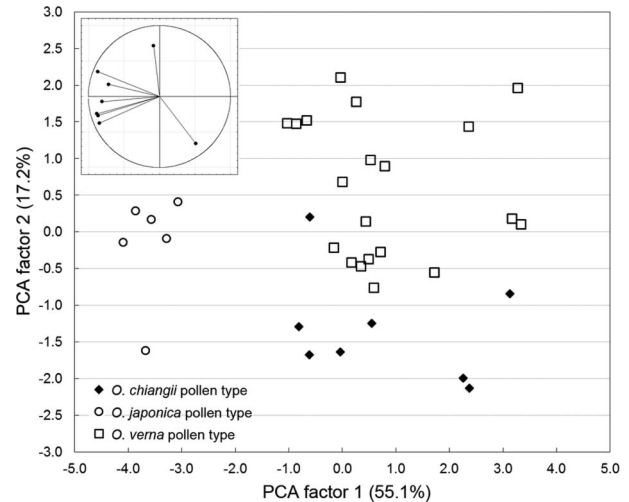


Figure 3. Principal component analysis (PCA) performed with the palynological characters and the specimens of *Omphalodes*. Specimens are represented in the first and second PCA components and were marked according to the pollen type: *Omphalodes chiangii* pollen type (◆), *O. japonica* pollen type (○) and *O. verna* pollen type (□). Percentage of variance explained by the component factors is also provided in parenthesis in each axis. Inset gives the projection of the variables on the same factor axes with the following variables in a clockwise direction: $P:E$, colporus; P , maximum width of the grain, ring-like aperture, pseudocolpus; E and margin ornamentation.

developed. Sculpture finely granulate, imperforate (Figure 1).

Omphalodes chiangii pollen type. — Species included: *Omphalodes acuminata*, *O. cardiophylla*, *O. chiangii*, *O. erecta*, *O. lojkae* and *O. mexicana*. Pollen grains radially symmetrical; isopolar; rectangular-elliptic and more or less constricted at the equator, more or less rounded at the poles; sub-circular to sub-hexagonal in polar view; prolate to perprolate, $P:E = 31.2-3.8$ (2.4 ± 0.4), $P = 7.0-10.7$ (8.5 ± 0.7) μm , $E = 2.3-6.2$ (3.7 ± 0.8) μm , maximum width of the grains $= 3.7-6.3$ (4.9 ± 0.6) μm . Apertural system 6-zono-heterocolpate: three pseudocolpi $4.6-8.4$ (6.4 ± 0.8) μm long, three colpi elongated-rhomboidal, operculate, with the ectoaperture $2.7-6.1$ (4.3 ± 0.7) μm long and the endoaperture lalongate, $1.1-2.7$ (1.6 ± 0.3) μm wide; margins of the pseudocolpi and colpi not granulate; colpal and pseudocolpal membranes psilate; ring-like aperture absent; opercula granulate. Exine $0.5-1.0$ μm thick, costae slightly developed. Sculpture finely granulate, perforate (Figure 2A–D).

Omphalodes verna pollen type. — Species included: *Omphalodes aliena*, *O. commutata*, *O. kuzinskyanae*, *O. linifolia*, *O. littoralis*, *O. luciliae*, *O. brassicifolia*, *O. nitida*, *O. richardsonii*, *O. ripleiana*, *O. rupestris*,

Table I. Summary of the quantitative and qualitative pollen morphological characters for the *Omphalodes* species examined.

Taxa	P (μm)	E (μm)	P:E	Maximum grain width (μm)	Pseudocolpus (μm)	Colporus (μm)	Endo-aperture (μm)	Margins ornamentation	Ring-like aperture	Habit and geographical distribution
<i>Omphalodes japonica</i> pollen type										
<i>O. aikiensis</i>	9.2–9.6 (9.4±0.2)	3.9–4.8 (4.4±0.4)	1.9–2.4 (2.2±0.3)	4.9–5.2 (5.0±0.1)	7.05–7.06 (7.1±0.0)	5.8–6.1 (6.0±0.1)	2.9–3.1 (3.0±0.1)	Yes	Yes	Perennial, Japan
<i>O. japonica</i>	9.1–10.7 (9.8±0.4)	4.1–5.6 (4.7±0.5)	1.8–2.5 (2.1±0.2)	4.8–6.0 (5.4±0.3)	6.1–7.8 (7.1±0.6)	4.7–5.9 (5.4±0.5)	1.5–3.3 (2.3±0.5)	Yes	Yes	Perennial, Japan
<i>O. krameri</i>	9.1–10.6 (9.9±0.6)	4.2–4.9 (4.6±0.2)	2.0–2.4 (2.1±0.2)	5.2–6.0 (5.6±0.3)	6.9–8.5 (7.6±0.5)	3.6–6.5 (4.7±0.9)	1.1–1.3 (1.2±0.1)	Yes	Yes	Perennial, Japan
<i>O. laevisperma</i>	8.7–10.9 (9.8±0.4)	3.6–5.8 (4.6±0.6)	1.7–2.6 (2.2±0.2)	4.8–5.9 (5.4±0.4)	6.8–9.0 (7.6±0.5)	4.4–7.1 (5.4±0.7)	0.7–2.1 (1.5±0.4)	Yes	Yes	Perennial, Japan
<i>Omphalodes chiangii</i> pollen type										
<i>O. acuminata</i>	7.0–8.7 (7.8±0.4)	2.2–3.5 (3.0±0.3)	2.2–3.7 (2.7±0.4)	3.7–4.9 (4.4±0.3)	5.2–7.1 (6.0±0.6)	2.9–5.0 (3.9±0.6)	1.1–2.3 (1.4±0.3)	No	No	Perennial, Mexico
<i>O. cardiophylla</i>	7.6–8.9 (8.1±0.4)	2.3–3.6 (3.0±0.4)	2.1–3.8 (2.8±0.4)	3.9–5.2 (4.4±0.4)	5.1–7.8 (6.2±0.5)	3.6–6.1 (4.4±0.6)	1.1–1.6 (1.3±0.2)	No	No	Perennial, Mexico
<i>O. chiangii</i>	7.3–9.5 (8.6±0.7)	4.9–6.2 (4.7±0.8)	3.6–6.2 (4.7±0.8)	1.3–2.4 (1.9±0.3)	4.1–6.3 (5.2±0.5)	4.7–7.5 (6.0±0.9)	1.4–2.1 (1.8±0.3)	No	No	Perennial, America
<i>O. erecta</i>	8.4–9.9 (9.0±0.4)	3.0–4.4 (3.9±0.4)	2.1–2.9 (2.4±0.2)	4.1–5.4 (4.9±0.4)	5.2–7.8 (6.6±0.7)	4.3–5.6 (4.7±0.4)	1.1–2.1 (1.8±0.5)	No	No	Perennial, Mexico
<i>O. lojkae</i>	8.1–9.8 (8.9±0.5)	3.3–4.4 (3.9±0.3)	2.0–2.7 (2.3±0.2)	4.5–6.1 (5.5±0.4)	5.8–8.0 (6.7±0.6)	4.0–5.1 (4.3±0.4)	1.1–2.1 (1.6±0.4)	No	No	Perennial, Caucasus
<i>O. mexicana</i>	7.9–10.7 (8.5±0.8)	2.8–4.2 (3.6±0.4)	1.9–3.0 (2.4±0.3)	4.9–5.6 (5.1±0.2)	5.7–8.4 (6.9±0.7)	3.8–5.5 (4.6±0.5)	1.3–1.7 (1.5±0.1)	No	No	Perennial, Mexico
<i>Omphalodes verna</i> pollen type										
<i>O. alvena</i>	7.8–10.8 (8.8±0.6)	2.9–4.6 (3.4±0.4)	2.2–3.0 (2.6±0.2)	4.2–6.3 (4.8±0.4)	5.2–7.8 (6.2±0.6)	3.7–5.5 (4.4±0.59)	0.9–2.0 (1.4±0.3)	Yes	No	Annual/perennial, Mexico, Texas
<i>O. comutata</i>	8.0–9.7 (8.1±0.4)	3.1–4.5 (3.7±0.4)	2.0–2.6 (2.4±0.2)	4.1–5.9 (4.6±0.5)	5.9–7.2 (6.5±0.4)	3.6–5.5 (4.6±0.5)	1.4–2.1 (1.7±0.2)	Yes	No	Annual, Iberian Peninsula
<i>O. kuzinskyanae</i>	7.4–9.1 (8.4±0.5)	3.0–4.6 (4.0±0.5)	1.8–2.4 (2.1±0.3)	4.0–5.2 (4.6±0.4)	5.5–7.8 (6.2±0.7)	3.1–5.9 (5.0±0.7)	1.1–1.7 (1.4±0.2)	Yes	No	Annual, Iberian Peninsula

(Continued)

Table I. (Continued).

Taxa	<i>P</i> (μm)	<i>E</i> (μm)	<i>P:E</i>	Maximum grain width (μm)	Pseudocolpus (μm)	Colporus (μm)	Endo-aperture (μm)	Margins ornamentation	Ring-like aperture	Habit and geographical distribution
<i>O. linifolia</i>	8.1–10.7 (9.1 ± 0.7)	2.7–4.8 (3.5 ± 0.5)	1.9–3.5 (2.6 ± 0.3)	2.2–5.0 (4.6 ± 0.6)	5.4–8.1 (6.6 ± 0.6)	2.9–6.0 (4.6 ± 1.0)	1.1–2.3 (1.6 ± 0.3)	Yes	No	Annual, Iberian Peninsula
<i>O. litoralis</i>	7.8–10.0 (8.9 ± 0.7)	3.4–4.5 (3.8 ± 0.3)	2.1–2.6 (2.3 ± 0.1)	4.1–5.0 (4.5 ± 0.3)	5.1–7.4 (6.1 ± 0.6)	4.0–6.6 (5.4 ± 0.8)	1.5–1.9 (1.6 ± 0.2)	Yes	No	Annual, Iberian Peninsula
<i>O. litoralis</i>	8.1–10.2 (9.4 ± 0.5)	3.2–4.3 (3.8 ± 0.3)	2.2–3.0 (2.5 ± 0.2)	4.4–5.4 (5.0 ± 0.3)	4.7–7.4 (6.2 ± 0.7)	2.7–5.1 (3.9 ± 0.7)	0.9–1.6 (1.3 ± 0.2)	Yes	No	Annual, West France
<i>O. litoralis</i>	6.4–7.7 (7.1 ± 0.4)	2.8–4.0 (3.5 ± 0.4)	1.8–2.8 (2.1 ± 0.3)	3.4–4.9 (4.0 ± 0.3)	3.6–6.2 (5.0 ± 0.7)	2.9–4.1 (3.4 ± 0.4)	1.3–2.2 (1.6 ± 0.2)	Yes	No	Perennial, Greece, West Asia
<i>O. brassicifolia</i>	7.4–9.8 (8.3 ± 0.6)	3.3–4.3 (4.0 ± 0.3)	1.7–2.5 (2.1 ± 0.2)	4.3–5.3 (4.7 ± 0.4)	4.8–8.0 (6.2 ± 0.8)	2.8–5.2 (4.2 ± 0.7)	1.1–1.7 (1.4 ± 0.2)	Yes	No	Annual, Iberian Peninsula
<i>O. nitida</i>	7.4–9.3 (8.4 ± 0.5)	3.0–4.2 (3.5 ± 0.3)	1.9–2.9 (2.4 ± 0.2)	4.3–5.7 (4.7 ± 0.4)	6.5–8.5 (7.0 ± 0.7)	3.2–4.7 (4.1 ± 0.4)	2.4–3.8 (2.9 ± 0.5)	Yes	No	Perennial, Iberian Peninsula
<i>O. richardsonii</i>	7.3–8.7 (7.7 ± 0.4)	2.6–3.2 (2.9 ± 0.2)	2.4–3.1 (2.7 ± 0.2)	3.8–4.4 (4.1 ± 0.2)	4.8–6.3 (5.3 ± 0.5)	3.3–5.4 (4.0 ± 0.6)	1.0–1.7 (1.3 ± 0.2)	Yes	No	Perennial, Mexico
<i>O. riplejana</i>	6.4–8.6 (6.8 ± 0.7)	2.7–4.0 (3.4 ± 0.4)	2.1–2.5 (2.3 ± 0.1)	3.4–4.5 (3.9 ± 0.3)	4.2–5.8 (5.2 ± 0.4)	3.8–6.1 (4.8 ± 0.6)	1.1–1.7 (1.4 ± 0.2)	Yes	No	Perennial, Anatolia
<i>O. rupestris</i>	6.5–8.9 (7.6 ± 0.8)	3.7–4.8 (4.7 ± 0.3)	1.5–2.1 (1.8 ± 0.2)	4.2–5.2 (4.6 ± 0.4)	5.5–7.8 (6.4 ± 0.7)	4.1–6.5 (5.0 ± 0.6)	1.5–2.5 (1.9 ± 0.3)	Yes	No	Perennial, Caucasus
<i>O. scorpioides</i>	7.4–10.5 (8.4 ± 0.7)	3.7–4.9 (4.4 ± 0.3)	1.7–2.4 (1.9 ± 0.2)	4.4–5.7 (4.8 ± 0.4)	5.4–7.3 (6.2 ± 0.6)	4.0–4.9 (4.4 ± 0.3)	1.1–2.4 (2.6 ± 0.5)	Yes	No	Biennial, Europe
<i>O. verna</i>	8.4–9.3 (8.9 ± 0.3)	4.2–4.9 (4.5 ± 0.3)	1.8–2.2 (2.0 ± 0.2)	4.5–7.5 (5.3 ± 0.8)	4.7–7.3 (5.8 ± 0.9)	4.1–6.5 (5.2 ± 0.7)	2.1–3.1 (2.7 ± 0.4)	Yes	No	Perennial, Europe

Notes: *P*, polar axis; *E*, equatorial axis; *P:E*, ratio between polar and equatorial axes; margins ornamentation refers to the presence/absence of conspicuous granula in pseudocolpi and colpi margins. For each taxon and character the following information is provided: minimum and maximum, followed by mean ± standard deviation of the mean in parenthesis. Quantitative variables are provided in micrometres, except *P:E*. Dichotomous key for the pollen types is provided in the 'Results' section.

O. scorpioides and *O. verna*. Pollen grains radially symmetrical; isopolar; rectangular-elliptic and more or less constricted at the equator, more or less rounded at the poles; sub-circular to sub-hexagonal in polar view; prolate to perprolate, $P:E = 1.5-3.5$ (2.3 ± 0.4), $P = 6.4-10.9$ (8.5 ± 0.9) μm , $E = 2.6-5.8$ (3.8 ± 0.6) μm , maximum width of the grains $= 2.2-7.5$ (4.6 ± 0.6) μm . Apertural system 6-zono-heterocolpate: three pseudocolpi $3.6-9.0$ (6.2 ± 0.9) μm long, three colpi elongated-rhomboidal, operculate, with ectoaperture $2.8-6.6$ (4.6 ± 0.8) μm long and endoaperture alongate, $0.9-3.8$ (1.7 ± 0.5) μm wide; margins of the pseudocolpi and colpi conspicuously granulate; colp and pseudocolpal membranes psilate; ring-like aperture absent; opercula granulate. Exine $0.5-1.0$ μm thick, costae slightly developed. Sculpture finely granulate, perforate (Figure 2E-K).

Discussion

The results of this study revealed that most of the pollen morphological features (including polarity, symmetry, shape, size and exine thickness) of *Omphalodes* species are quite constant, though the sculpture of the aperture margins and presence/absence of a ring-like aperture varies among the taxa. Overall, the results obtained agreed with previous descriptions (Liu et al., 2001; Hargrove & Simpson, 2003; Bigazzi et al., 2006), despite some varied values, most probably due to different methodologies (e.g. Barbier & Mathez, 1973; Díez & Valdés, 1991; Buchner & Halbritter, 2011). Regardless of the fairly continuous gradient observed across the species studied for all the quantitative characters, it was possible to recognise three pollen types (each one including several species) that differed mostly in qualitative characters related with the presence/absence of a ring-like aperture and of conspicuous granula in the apertural margins. The need to include several con-generic species in one pollen type has been already described for the Boraginaceae (e.g. Barbier & Mathez, 1973; Clarke, 1977; Díez, 1984; Díez & Valdés, 1991; Scheel et al., 1996) and some palynologists have even considered the genus, more than the species, as the basic palynosystematic unit (e.g. Pons, 1970). This view is valid especially when studying taxonomic groups with low sculptural variability such as Cynoglosseae. In addition to the three pollen types described, two main patterns arose from the palynological data obtained in this study. First, the Japanese taxa form a group strongly characterised by its greater size, presence of a ring-like aperture and presence of granula in the apertural margins, supporting recent phylogenetic

analyses. Second, though the perennial species from both the Old and the New World could belong either to the *O. chiangii* or *O. verna* pollen types, the pollen of all annual species belongs exclusively to the *O. verna* pollen type. It is also interesting to note that within the *O. verna* pollen type, the pollen grains of *O. scorpioides* are more compact and globose than the pollen grains of the other species.

Among the pollen characters studied, the occurrence of a ring-like aperture was of major importance in the discrimination of pollen types, namely the Japanese taxa. The existence of a ring-like aperture has already been described for some genera of Cynoglosseae (e.g. *Cryptantha*, *Paracaryum*, *Rindera* and *Solenanthus*; Hargrove & Simpson, 2003; Bigazzi et al., 2006), but was never reported for *Omphalodes* up to now (e.g. Barbier & Mathez, 1973). The heterocolpate condition was constant in all the species of *Omphalodes* studied, as well as in other taxa of Cynoglosseae (Anh & Lee, 1986; Valdés, 1987; Díez & Valdés, 1991; Hargrove & Simpson, 2003; Coutinho et al., unpublished results) being, most probably, an apomorphic condition for *Omphalodes* and Cynoglosseae (Hargrove & Simpson, 2003). Three functions have been proposed for the heterocolpate condition: Points for pollen tube growth, points for the exchange of physiologically active substances and harmomegathy (Muller, 1979), the latter being solely associated with pseudocolpi (Hargrove & Simpson, 2003). In *Omphalodes*, harmomegathic reactions are indeed facilitated by the presence of pseudocolpi that enable the accommodation of volume changes after (de)hydration, and by a thin exine that increases the flexibility of the pollen grain (Bolick, 1981; Payne, 1981). The perforations and opercula observed in *Omphalodes* pollen grains may also constitute important regulatory structures for the exchange of physiologically active substances (Bolick, 1981; Payne, 1981).

Phylogenetic analysis based on the *atpB* plastid marker places *Omphalodes* in an isolated position within Boraginoideae and suggests no relation with the Cynoglosseae (Långström & Chase, 2002). However, the pollen morphological data collected in this study does not support this molecular inference; instead, palynological data corroborates the classical inclusion of the genus within Cynoglosseae. In fact, Cynoglosseae s. l. is the only tribe of the subfamily Boraginoideae that possess heterocolpate pollen, with colpate pollen being restricted to Boragineae and Lithospermeae. Still, independent evolution of heterocolpate pollen in *Omphalodes* and Cynoglosseae should be tested in a phylogenetic context using more molecular markers. It is also interesting to observe that the *O. japonica* pollen type is similar to the *Cynoglossum officinale* L. pollen type

(Clarke, 1977) and the *C. creticum* Mill. pollen type (Díez, 1984), while the *O. verna* pollen type resembles the *Myosotis arvensis* (L.) Hill and *M. stolonifera* J. Gay pollen types (Clarke, 1977; Díez, 1984).

Based on palynological data, it was possible to clearly discriminate the Japanese species from the remaining species of *Omphalodes*. This corroborates the segregation of the monophyletic group of Japanese species from *Omphalodes* proposed by recent molecular phylogenetic analyses (Serrano et al., unpublished data). Our results revealed that *Omphalodes* is a quite stenopalynous taxon (except for the aperture margin sculpture and presence of a ring-like aperture), in contrast to other genera of the Cynoglosseae, in which the high variability of pollen morphology significantly assisted taxonomic delimitations within the tribe (Barbier & Mathez, 1973). The observed low variability within *Omphalodes* does not support the hypothesis of a relatively ancient diversification of the genus, as suggested by the disjunct distribution. Alternatively, a possible trend to palynological stasis in the genus needs to be tested in a dated phylogenetic framework.

Finally, the results obtained have also significant biogeographic implications. Pollen morphology gives support to a reduced concept of *Omphalodes* that would include only species with *O. chiangii* or *O. verna* pollen types. These taxa display a disjunct distribution range between West Eurasia and North America that fits in the well-known Madrean-Tethyan Flora pattern (Raven & Axelrod, 1974; Wen & Ickert-Bond, 2009). However, whether the *Omphalodes* disjunction originated in the first half of the Tertiary, as proposed by the Madrean-Tethyan hypothesis (Axelrod, 1975), or resulted from more recent long distance dispersal events still remains an open question.

Conclusion

This study investigated for the first time the variability of pollen morphology in *Omphalodes*, encompassing 23 species (82% of the recognised species) and all major geographical and morphological groups. Palynological data provided useful information to assist the complex systematics of the genus *Omphalodes* considering the uselessness of most floral and fruit characters, karyological homogeneity and striking geographical distribution. At the suprageneric level, pollen morphology supports the traditional tribal classification of the genus, in contrast to previously published molecular phylogenetic analyses. At the infrageneric level, pollen morphology corroborates the recent phylogenetic results for *Omphalodes* and clearly separates, among others, the Japanese taxa in a distinct group.

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Specimens investigated

- Omphalodes acuminata* B. L. Rob. Mexico: Nuevo León, Monterrey. Ford M186 (TEX 218154); Mexico: Nuevo León, Villa Santiago. Johnson & Barkley 1609M (TEX 120476).
- Omphalodes akiensis* Kadota. Japan: Hiroshima pref., Hiramidani gorge. Kubota s.n. (TNS 737082).
- Omphalodes aliena* A. Gray ex Hemsl. USA: Texas, Presidio. Correll & Rollins 23704. (TEX 35421); Mexico: Nuevo León, Minas Manto Blanco. Johnston, Wendt & Chiang 10248F (TEX); Mexico: Nuevo León, Rayones. Hinton 20173 (TEX 223071).
- Omphalodes cardiophylla* A. Gray ex Hemsl. México: Coahuila, General Cepeda. Encina 228 & Závala. (TEX 223076).
- Omphalodes chiangii* Higgins. Mexico: Sierra del Carmen. Carranza s. n. (IEB 15903).
- Omphalodes commutata* G. López. Spain: Málaga, Serranía de Ronda. Fuertes, Ladero, G. López & Navarro s.n. (SALAF 12451).
- Omphalodes erecta* I. M. Johnst. Mexico: Galeana. Hinton s.n. (IEB 22275); Mexico: Aramberri. Hinton s.n. (IEB 23145).
- Omphalodes japonica* (Thunb.) Maxim. Japan: Kyoto, Yamashiro. Togashi s.n. (TI); Japan: Cultivated in Karuizawa Botanical Garden. Arai s.n. (SANT 59953).
- Omphalodes krameri* Franch et Sav. Japan: Nagano Pref., Kitasakugun. Yahara et al. s.n. (TI).
- Omphalodes kuzinskyanae* Willk. Portugal: Cascais, Quinta do Espírito Santo. Torre s.n. COI; Portugal: Cascais, near Guincho. Carballal & Serrano s.n. (SANT 50016).
- Omphalodes laevisperma* Nakai. Japan: Murakamishi. Togashi s.n. (TI).
- Omphalodes linifolia* (L.) Moench. Spain: Málaga, El Burgo. Perez & Molero s.n. (SANT 12850); Portugal: Serra da Arrábida. Carballal & Serrano s.n. (SANT 50020).
- Omphalodes littoralis* Lehm. subsp. *gallaecica* M. Lánz. Spain: Ferrol, Covas. Carballal & Serrano s.n. (SANT 51847).
- Omphalodes littoralis* Lehm. subsp. *littoralis*. France: Charente-Inf., La Rochelle. Giraudias s.n. (P 271712).
- Omphalodes lojkae* Sommier et Levier. Georgia: Svanethi. Schethekauzi s.n. (MA 575919).
- Omphalodes luciliae* Boiss. Greece: Parnaso massif, Gorge Gouna. Maire & Petitmengin s.n. (MA 94701).
- Omphalodes brassicifolia* Sweet. Spain: Cáceres, Arroyomolinos de la Vera. Ladero & Amor s.n. (SALAF 23592).
- Omphalodes mexicana* S. Watson. Mexico: Nuevo León, Galeana, Cerro el Gallo. Hinton 21036 (IEB 137190).

- Omphalodes nitida* Hoffmanns et Link. Spain: Corunha, Ames. Fagúndez s.n. (SANT 53295).
- Omphalodes richardsonii* G. L. Nesom. Mexico: Tamaulipas, Gómez Farias. Richardson 1114 (TEX); Mexico: Tamaulipas, Gómez Farias. Woodruff 160 (TEX).
- Omphalodes ripleiana* P. H. Davis. Cultivated in Edinburgh. Davis s.n. (K).
- Omphalodes rupestris* Boiss. Caucasus region. Desoulavy s.n. (B 10 0293510).
- Omphalodes scorpioides* (Haenke) Schrank. Slovakia: Slovensky Kras. Zertova s.n. (MA 219178); Austria: Niederösterreich, Fuss des Rosalingebirges. Barta s.n. (MA 732115).
- Omphalodes verna* Moench. Italy: Udine, Valli del Natisone-Valle dell'Erbezzo. Feoli & Feoli s.n. (MA 350089).
- ## References
- Ahn, Y. M. & Lee, S. (1986). A palynotaxonomic study of the Korean Boraginaceae. *Korean Journal of Plant Taxonomy*, 16, 199–215.
- Al-Shehbaz, I. (1991). The genera of Boraginaceae in the southeastern United States. *Journal of the Arnold Arboretum, Supplement 1*, 1–169.
- Andersen, S. (1960). Silicon oil as a mounting medium for pollen grains. *Danmarks Geologiske Undersøgelse*, 4, 116–140.
- Axelrod, D. I. (1975). Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. *Annals of the Missouri Botanical Garden*, 62, 280–334.
- Barbier, E. & Mathez, J. (1973). Contribution à l'étude des Cynoglossées (Boraginacées): *Pardoglossum*, genre nouveau du Bassin Méditerranéen occidental. *Candollea*, 28, 281–323.
- Bigazzi, M., Nardi, E. & Selvi, F. (2006). Palynological contribution to the systematics of *Rindera* and the allied genera *Paracaryum* and *Solenanthus* (Boraginaceae-Cynoglosseae). *Willdenowia*, 36, 37–46.
- Bolick, M. R. (1981). Mechanics as an aid to interpreting pollen structure and function. *Review of Palaeobotany and Palynology*, 35, 61–69.
- Brand, A. (1921). Boraginaceae-Boraginoideae-Cynoglosseae. In A. Engler (Ed.), *Das Pflanzenreich Volume 78*, pp. 1–183. Leipzig: Engelmann.
- Buchner, R. & Halbritter, H. (2011). *Omphalodes verna*. In R. Buchner & M. Weber (Eds), *PalDat – a palynological database: Descriptions, illustrations, identification, and information retrieval*. <http://www.paldat.org/index.php?module=search&nav=sd&ID=103904&system=1> (accessed 1 November 2011).
- Clarke, G. C. S. (1977). The Northwest European pollen flora, 10. Boraginaceae. *Review of Palaeobotany and Palynology*, 24, 59–101.
- Clarke, G. C. S., Chanda, S. & Sahay, S. (1979). Pollen morphology in the genus *Pardoglossum* (Boraginaceae) with some observations on heterocolpate pollen. *Review of Palaeobotany and Palynology*, 28, 301–309.
- Coppi, A., Selvi, F. & Bigazzi, M. (2006). Chromosome studies in Mediterranean species of Boraginaceae. *Flora Mediterranea*, 16, 253–274.
- de Candolle, A. (1846). Borage. In A. de Candolle (Ed.), *Prodromus systematis naturalis regni vegetabilis, Pars decima*, pp. 1–178. Paris: Sumptibus Victoris Masson.
- Diez, M. J. (1984). Contribución al Atlas Palinológico de Andalucía Occidental, I. Boraginaceae. *Lagascalia*, 13, 147–171.
- Diez, M. J. & Valdés, B. (1991). Pollen morphology of the tribes Eritrichieae and Cynoglosseae (Boraginaceae) in the Iberian Peninsula and its taxonomic significance. *Botanical Journal of the Linnean Society*, 107, 49–66.
- Diez, M. J., Valdés, B. & Fernández, I. (1986). Pollen morphology of Spanish *Lithospermum* s. l. (Boraginaceae) and its taxonomic significance. *Grana*, 25, 171–176.
- Erdtman, G. (1960). The acetolysis method. *Svensk Botanisk Tidskrift*, 54, 561–564.
- Grau, J. (1967). Primäre und sekundäre Chromosomenbasis zahlen bei *Omphalodes*. *Österreichische Botanische Zeitschrift*, 114, 66–72.
- Hargrove, L. & Simpson, M. G. (2003). Ultrastructure of heterocolpate pollen in *Cryptantha* (Boraginaceae). *International Journal of Plant Sciences*, 164, 137–151.
- Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosh-Radivo, A. & Ulrich, S. (2009). *Pollen terminology – an illustrated handbook*. Wien: Springer.
- Holmgren, P. K., Holmgren, N. H. & Barnett, L. C. (1990). *Index herbariorum, Part I. The herbaria of the World*. New York: New York Botanical Garden.
- Johnston, I. M. (1924). Studies in the Boraginaceae, III. 1, The New World genera of the Boraginoideae. *Contributions from the Gray Herbarium of Harvard University*, 73, 42–78.
- Kadota, Y. (2009). *Omphalodes akiensis* (Boraginaceae), a new species from Hiroshima prefecture, western Japan. *Journal of Japanese Botany*, 84, 342–349.
- Lewis, W. H. (1980). Polyploidy in angiosperms: Dicotyledons. In W. H. Lewis (Ed.), *Polyploidy: Biological relevance*, pp. 241–268. New York: Plenum Press.
- Liu, J.-X., Li, J.-Y., Zhang, Y.-L. & Ning, J.-C. (2010). Pollen morphology of the tribe Lithospermeae of Boraginoideae in China and its taxonomic significance. *Plant Systematics and Evolution*, 290, 75–83.
- Liu, J.-X., Zhang, Y.-L., Ning, J.-C., Zhao, Y.-Y., Li, Y.-X., Zhang, J.-M. & Sun, X.-H. (2001). Pollen morphology of the tribe Cynoglosseae of Boraginoideae (Boraginaceae) in China. *Acta Phytotaxonomica Sinica*, 39, 515–522.
- Luque, T. & Valdés, B. (1986). Caryological study of Spanish Boraginaceae III. *Cynoglossum* L. s. str. *Willdenowia*, 15, 485–496.
- Långström, E. & Chase, M. W. (2002). Tribes of Boraginoideae (Boraginaceae) and placement of *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*: A phylogenetic analysis based on *atpB* plastid DNA sequence data. *Plant Systematics and Evolution*, 234, 137–153.
- Muller, J. (1979). Form and function in angiosperm pollen. *Annals of the Missouri Botanical Garden*, 66, 593–632.
- Ning, J.-C., Xi, Y.-Z. & Zhang, Y.-L. (1992). Pollen morphology of the tribe Anchuseae DC. (Boraginaceae) and its taxonomic significance. *Cathaya*, 4, 79–90.
- Payne, W. W. (1981). Structure and function in angiosperm pollen wall evolution. *Review of Palaeobotany and Palynology*, 35, 39–59.
- Pons, A. (1970). *Le pollen*. Paris: Presses Universitaires de France.
- Popov, M. G. (1953). Boraginaceae. In B. K. Shishkin (Ed.), *Flora of the USSR*, 19 pp. 97–691. Moscow: Izdatel'stvo Akademii Nauk SSR.
- Punt, W., Hoen, P. P., Blackmore, S., Nilsson, S. & Le Thomas, A. (2007). Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology*, 143, 1–81.
- Raven, P. H. (1972). Plant species disjunctions: A summary. *Annals of the Missouri Botanical Garden*, 59, 234–246.
- Raven, P. H. & Axelrod, D. I. (1974). Biogeography and post continental movements. *Annals of the Missouri Botanical Garden*, 61, 539–673.
- Retief, E. & Van Wyk, A. E. (1997). Palynology of southern Africa Boraginaceae: The genera *Lobostemon*, *Echiostachys* and *Echium*. *Grana*, 36, 271–278.

- Riedl, H. (1997). Boraginaceae. In C. Kalkman, H. P. Noteboom, W. J. de Wilde, D. W. Kirkup & P. F. Stevens (Eds), *Flora Malesiana, series 1, Spermatophyta*, pp. 43–144. Leiden: Rijksherbarium Publications Department.
- Scheel, R., Ybert, J.-P. & Barth, O. M. (1996). Pollen morphology of the Boraginaceae from Santa Catarina State (southern Brazil), with comments on the taxonomy of the family. *Grana*, 35, 138–153.
- Selvi, F., Coppi, A. & Cecchi, L. (2011). High epizoochorous specialization and low DNA sequence divergence in Mediterranean *Cynoglossum* (Boraginaceae): Evidence from fruit traits and ITS region. *Taxon*, 60, 969–985.
- Valdés, B. (1987). Boraginaceae. In B. Valdés, S. Talavera & E. Fernández-Galiano (Eds), *Flora vascular de Andalucía Occidental* 2, pp. 373–404. Barcelona: Ketres Editora.
- Wen, J. & Ickert-Bond, S. (2009). Evolution of the Madrean-Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution*, 47, 331–348.

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