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Molecular phylogenetics and morphology support two new genera (*Memoremea* and *Nihon*) of Boraginaceae s.s.

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Abstract

Omphalodes (Boraginaceae s.s., Cynoglosseae s.l.) comprises ca. 29 species of annual and perennial plants distributed in three main disjunct areas: Western Palearctic, Japan and SW of North America. This paper uses micromorphological and DNA data to re-assess the monophyly and taxonomy of the genus. Morphological characters of 15 species of *Omphalodes* and four closely-related genera were analysed using SEM. A total of 82 ITS and 68 *trnL-trnF* sequences were newly sequenced, including 14 species of *Omphalodes* and three genera of the tribe Cynoglosseae. Phylogenetic analyses of 57 genera (186 species) of Boraginaceae indicated that *Omphalodes* as currently circumscribed is formed by three independent lineages, which were supported by morphological characters of the fruit. As a result, and in the interest of a more natural classification, two new genera are described to accommodate *Omphalodes scorpioides* (*Memoremea*) from Europe and the Japanese species (*Nihon*). *Memoremea* is distinguished from all the other species previously included in *Omphalodes* by the apical attachment scar and the hollow nutlet margin. *Nihon* is easily discriminated by the abrupt change of margin ornamentation towards the nutlet aperture. We also provided a taxonomic treatment that proposes the lowest number of nomenclature changes, although six new combinations are required.

Key words: Carpology, DNA sequences, Scanning Electron Microscopy, Systematics

Introduction

Boraginaceae s.s. (=Boraginaceae subfam. Boraginoideae; Al-Shehbaz, 1991; Gürke, 1893) comprises 112 genera and about 1600 species (Stevens 2001 onwards) of herbaceous plants and shrubs. Between four and 13 tribes have been traditionally recognized within the Boraginaceae s.s. based on morphological characters (De Candolle 1846, Baillon 1890, Gürke 1893, Johnston 1924, Popov 1953, Al-Shehbaz 1991, Riedl 1997). Nutlet macromorphology has traditionally been used to divide Boraginaceae into either 13 (Popov 1953) or six (Riedl 1997) tribes. However, molecular phylogenies of the family are largely congruent with synthetic treatments that reduce tribal division into four tribes: Lithospermeae, Boragineae, Echiochileae and Cynoglosseae s.l. (Långström & Chase 2002, Långström & Oxelman 2003, Weigend *et al.* 2010, Nazaire & Hufford 2012, Weigend *et al.* 2013, Cohen 2014). The three first tribes are each supported by autoapomorphic carpological characters: Echiochileae is characterized by a basal or submedial attachment scar and a flat to pyramidal gynobase (Långström & Chase 2002); Lithospermeae mostly present ovoid, keeled, slightly compressed and strongly incurved nutlets with a broad basal attachment scar and a flat gynobase (Långström & Chase 2002, Weigend *et al.* 2010); and Boragineae is basally attached with planar gynobase and basal annulus surrounding the scar (Hilger *et al.* 2004). The fourth tribe (Cynoglosseae s.l.), recognized based on molecular phylogenetics, includes a set of morphologically heterogeneous subtribes, showing the widest variety of nutlet morphology and ornamentation, including deeply dentate margins, glochidia, papillae or even surface completely smooth, and gynophore configuration from nearly flat to pyramidal. Some genera also have a more or less thickened wing. The most recent phylogenetic reconstructions (Cohen 2014; Weigend *et al.* 2013) recovered six well-supported major groups within Cynoglosseae s.l.: Trichodesmeae, Eritrichieae, Myosotideae, *Omphalodes* s.s., *Mertensia* clade, and Cynoglosseae s.s. This latter comprises *Cynoglossum* Linnaeus (1753:134) and related genera (*e.g.* *Paracaryum* Boissier (1849: 128), *Rindera* Pallas (1771: 486), *Solenanthus* Ledebour (1829: 8) and *Trachelanthus* Kunze (1850: 665)), several East Asian genera (*e.g.* *Bothriospermum* Bunge (1831: 47), *Microula* Benthham (1876: 853)), and the taxa previously considered within the subtribe Cryptanthinae.

Omphalodes Miller (1754: 968) is a genus of the family Boraginaceae, known by the common English name of “navelwort” or “navel seeds” (Weigend *et al.* 2013), traditionally included in the tribe Cynoglosseae s.s. (De Candolle 1846, Gürke 1893, Johnston 1924, Riedl 1997, Valdés 2004; see revision in Nazaire & Hufford 2012). *Omphalodes* comprises ca. 29 herbaceous annual and perennial species (Table 1). They are distributed in three disjunct areas: the Western Palearctic (Popov 1953, Tutin *et al.* 1972, Davis 1978, Nasir 1989, Fernández & Talavera 2012), Japan (Ka 1965, Yamazaki 1993), and SW North America (Nesom 2013). The largest number of species is found in the Mediterranean Region (11 spp.). The fruit of *Omphalodes* is a tetranutlet, with shortly cylindrical or ovoid, glabrous or hairy nutlets showing a great variation in the epidermis ornamentation (Fernández & Talavera 2012). The abaxial surface of the fruit is flattish, limited by a conspicuous winged margin that is entire, lobulated or dentate and inwardly curved or erect. The insertion of the fruit is elliptic, flat, without an appendage and attached to a more or less conical receptacle on its upper half, leaving an ovate to deltoid scar. The embryo is usually erect or exceptionally curved as in *O. scorpioides* Schrank (1812: 222) (Popov 1953).

TABLE 1: List of the species of *Omphalodes* recognized in the most recent taxonomic treatments (Popov (1953), Nesom (2013), Ka (1965), Tutin *et al.* (1972), Yamazaki (1993), Strid and Tan (2005), Kadota (2009), Fernández and Talavera (2012) and Euro+Med (2006–present)). Superindices 1 and 2 indicate those species included in the DNA and SEM study respectively.

Species	Distribution	Annual/Perennial
Western Palearctic		
<i>O. brassicifolia</i> Sweet. ^{1,2}	CW and S Iberian Peninsula	Annual
<i>O. cappadocica</i> D.C. ^{1,2}	Caucasus	Perennial
<i>O. caucasica</i> Brand ^{1,2}	Caucasus	Perennial
<i>O. commutata</i> G.López ^{1,2}	S Iberian Peninsula	Annual
<i>O. kusnetzovii</i> Kolak ^{1,2}	Caucasus	Perennial
<i>O. kuzinskyanae</i> Wilk. ^{1,2}	CW Portugal	Annual
<i>O. linifolia</i> Moench ^{1,2}	Iberian Peninsula, W France to Crimea and W Caucasus	Annual
<i>O. littoralis</i> Lehm. ^{1,2}	NW Iberian Peninsula, SW France	Annual
<i>O. lojkae</i> Sommier & Levier ^{1,2}	Caucasus	Perennial
<i>O. luciliae</i> Boiss. ^{1,2}	Greece, N Iraq, W Iran	Perennial
<i>O. nitida</i> Hoffmanns. & Link ^{1,2}	NW Iberian Peninsula	Perennial
<i>O. riplejana</i> P.H.Davis	Anatolia	Perennial
<i>O. rupestris</i> Rupr ex Boiss.	Caucasus	Perennial
<i>O. runemarkii</i> Strid & Kit Tan	Greece	Perennial
<i>O. scorpioides</i> Schrank. ^{1,2}	C and NE Europe	Biennial
<i>O. verna</i> Moench ^{1,2}	E Mediterranean and C Europe	Perennial
Japan		
<i>O. akiensis</i> Kadota ¹	Honshu, Hiroshima	Perennial
<i>O. japonica</i> Maxim. ^{1,2}	Honshu	Perennial
<i>O. krameri</i> Franch. & Sav.	Hokkaido, N to C. Honshu	Perennial
<i>O. laevisperma</i> Nakai	C Honshu	Perennial
<i>O. prolifera</i> Ohwi	C and W Honshu	Perennial
N. America		
<i>O. aliena</i> A.Gray ex Hemsl. ^{1,2}	S Texas and Mexico (Nuevo León and C Coahuila)	Annual
<i>O. alienoides</i> Nesom	S Texas and Mexico (Cohauila)	Annual/Perennial
<i>O. australis</i> Nesom	Mexico (Puebla)	Perennial
<i>O. cardiophylla</i> A.Gray ex Hemsl. ²	Mexico (Coahuila to Nuevo León and CW Tamaulipas)	Perennial
<i>O. carranzae</i> Nesom	Mexico (NW Cohauila, Sierra del Carmen)	Perennial
<i>O. chiangii</i> L.C.Higgins ²	Mexico (CN Coahuila)	Perennial
<i>O. erecta</i> I.M. Johnston	Mexico (C Nuevo León to CW Tamaulipas)	Perennial
<i>O. mexicana</i> S.Watson	Mexico (C Nuevo León)	Perennial

Miller (1754) described *Omphalodes* following polynomial nomenclature. The first author using binomial nomenclature was Moench who described *O. linifolia* Moench (1794: 719) and *O. verna* Moench (1794: 420). *Omphalodes verna* was designated as the type species by Stafleu in Flora Neerlandica (van Ooststroom *et al.* 1961). De Candolle (1846) recognized four sections within the genus. Section *Eu-Omphalodes* De Candolle (1846:11) comprised the perennial European taxa *O. cappadocica* De Candolle (1846: 161), *O. luciliae* Boissier (1844: 41), *O. nitida* Hoffmannsegg & Link (1811: 194) and *O. verna*, and section *Maschalanthus* De Candolle (1846:11) included only *O. scorpioides*. With the exception of *O. scorpioides* which he retained in the monotypic section *Maschalantus*, Brand (1921) included taxa currently considered in other genera such as *Microula* and *Sinojohnstonia* Hu (1936: 201), together with all species of *Omphalodes* then known within section *Eu-Omphalodes*. The treatment of Popov (1953) proposed three sections based on habit and the shape and development of the nutlet gynophore. The section *Arctotertiariae* Popov (1953: 609) included perennials with small gynophores. This section was divided into two series: *Vernales* Popov (1953: 609) comprising Eurasian species from forest habitats (*O. verna*, *O. nitida*, *O. cappadocica*); and *Rupestres* Popov (1953: 613) comprising species from rocky subalpine habitats in Asia Minor and the Caucasus (*O. kusnetzovii* Kolakovsky (1948: 62), *O. lojkae* Sommier & Levier (1892: 157) and *O. rupestris* Ruprecht ex Boissier (1879: 267)). The second section *Pseudoparacaryum* Popov (1953: 616) included annual species with large pyramidal gynophores (represented in the former USSR only by *O. linifolia*). The third section was the monotypic *Maschalanthus* that included only the biennial *O. scorpioides*, distributed in Eastern Europe and possessing a small gynophore.

Recent phylogenetic studies of the Boraginaceae including nine species of *Omphalodes* (Weigend *et al.* 2013) have revealed its polyphyly, since *O. scorpioides* and the Japanese taxon *O. akiensis* Kadota (2009: 342) appear in independent lineages. The split of *Omphalodes* had already been noted based on morphology and palynology (Popov 1953, Pereira Coutinho *et al.* 2012). Additionally, the morphologically dissimilar *Myosotyidium hortensia* (Decaisne) Baillon (1890: 333), a subantarctic megaherb from Chatham Island, was also placed within *Omphalodes* s.s. in phylogenetic studies (Heenan *et al.* 2011). All these studies imply the need for further investigation of the systematics of Cynoglosseae. Furthermore, it is notable that to date, little effort has been made to look into morphological characters that support monophyletic groups and boundaries within this tribe.

In the present study we performed a phylogenetic analysis of Cynoglosseae s.l. to evaluate the polyphyly of *Omphalodes* and provided a review of the taxonomy of the genus. In order to accurately analyze key taxonomic characters of *Omphalodes*, such as nutlet structure and ornamentation, we performed a scanning electron microscopy (SEM) study with representative sampling of fruits of Cynoglosseae s.l. based on clades, number of species, distribution areas and nutlet diversity. The main objectives were to: (1) identify monophyletic groups of *Omphalodes* and relatives, (2) find key morphological characters supporting those groups, and (3) propose taxonomic rearrangements needed for a more natural classification of the species.

Materials and methods

Phylogenetic study

DNA and taxon sampling

Two DNA regions were selected for the phylogenetic study, the nuclear ITS region and the plastid *trnL-trnF* region (including the *trnL* intron and the *trnL-trnF* spacer). Selection of both regions was based on previous studies (Weigend *et al.* 2010, Hasenstab-Lehman & Simpson 2012, Nazaire & Hufford 2012, Mozaffar *et al.* 2013, Cohen 2014, Weigend *et al.* 2013). Forty genera (157 spp.) of the tribe Cynoglosseae s.l. were analysed (Appendix S1), which represents 71% of the total number of genera recognised. In total, we analyzed 67 (ITS) and 78 (*trnL-trnF*) sequences taken from previous studies and downloaded from the Genbank database, plus 82 (ITS) and 68 (*trnL-trnF*) samples sequenced specifically for this study (Appendix S1). As a result, taxon sampling was increased and 41 species and three genera that had not previously sequenced were included in our analysis (Appendix S1). Fourteen species of *Omphalodes* were sampled, which represents 50% of the total number of species recognised. Special effort was made to represent *Omphalodes* distributed in the western Palearctic where the main diversity centre occurs, resulting in the inclusion of 11 out of the 16 species in this region. Three more species of *Omphalodes* were analysed (one of eight from America; two of five from Japan). For outgroup samples, we included ITS sequences from 35 species of 23 genera and *trnL-trnF* sequences from 32 species of 22 genera representing the other Boraginaceae tribes and subfamilies. We also included three species of *Nicotiana* Linnaeus (1753: 180) (Solanaceae) from Genbank to root the tree, based on previous results (Nazaire & Hufford 2012) (Appendix S1).

Plant material for DNA extractions was obtained from herbarium specimens and field collections (Appendix S1).

All taxa were newly identified using the available taxonomic bibliography and verified with local floras: Europe (Tutin *et al.* 1972, Talavera *et al.* 2012); SW Asia (Riedl 1967, Davis 1978, Nasir 1989), former USSR (Popov 1953), China (Ge-ling *et al.* 1995), New Zealand (Allan 1961), Australia (Toelken 1986; Jeanes 1999), E Africa (Riedl & Edwards 2006; Thulin & Warfa 2006) and N America (Nesom 2013).

DNA extraction, amplification and sequencing

DNA was extracted from leaf tissue using Dneasy Plant Mini Kits (Qiagen, Valencia, California, USA) following the manufacturers protocol. PCR amplifications were performed in an Eppendorf Mastercycler Eppgradient S (Hamburg, Germany). PCR conditions for ITS consisted of initial denaturation at 95 °C for 5 mins followed by 35 cycles of 95 °C for one minute, 48 °C for one minute, 72 °C for one minute, and a final elongation stage of 72 °C for ten minutes. PCR conditions for the *trnL–trnF* spacer are the same as those of the nuclear ones except for the annealing temperature (50 °C). The volume of genomic DNA was 1 µl in both regions. Nested PCRs were needed to amplify old herbarium specimens. The amplifications of the *trnL–trnF* region were done with primers ‘c’ and ‘f’ (Taberlet *et al.* 1991). Two internal primers were designed using *Geneious* 5.4. (Drummond *et al.* 2011) for nested PCR of the *trnL–trnF* region: ‘*trnL–trnF* BOR F’ (5’ CCC GCA ATT AAT AAA AAT GGGC 3’) and ‘*trnL–trnF* BOR R’ (5’ ATA ATC AGG GGT CTA TGT 3’). The external primers ‘17SE’ and ‘26SE’ (Sun *et al.* 1994) were used for the amplification of the ITS region, with ‘ITS1’ and ‘ITS4’ (White *et al.* 1990) used for nested PCRs. PCR products were sequenced using the Macrogen Europe sequencing service (Amsterdam, The Netherlands).

Alignment and phylogenetic analyses

Two matrices were compiled. The ITS matrix included 186 accessions representing 163 spp and included 774 characters (hereafter called “nuclear matrix”). The *trnL–trnF* matrix comprised 180 accessions, representing 170 species and included 1218 characters (hereafter called “plastid matrix”). Two additional ITS and *trnL–trnF* reduced matrices were compiled including only the 146 samples for which sequence data was available for both regions.

Sequences were automatically aligned using Fast Fourier transform (MAFFT, Katoh *et al.* 2002) on the website platform EMBL-EBI (EMBL-EBI, 2013) and manually reviewed using *Geneious* 5.1.7. (Drummond *et al.* 2011).

Phylogenetic reconstructions were performed under Bayesian Inference (BI) using MrBayes v.3.2 (Ronquist & Huelsenbeck, 2003) in Bioportal (Kumar *et al.* 2009). The nucleotide substitution model that best fitted each region (*trnL–trnF* and ITS1, 5.8S and ITS2) was inferred using JModelTest v. 0.1.1. (Posada 2008) The substitution model selected using the Akaike information criterion with correction (AICc) was SYM+G for ITS1 and ITS2, and GTR+G for the 5.8S region. This latter model was also selected for the plastid matrix. Bayesian inference was run for 50x10⁶ generations, sampling every 1000 generations in four independent Markov chain Monte Carlo (MCMC). Four BI analyses were run, two with the complete nuclear and plastid matrices and two with the reduced ones.

The Approximate Unbiased test (AU; Shimodaira 2002) was used to explore discordance between the nuclear and plastid phylogenies and test for combinability. The AU test was performed in Treefinder (Jobb *et al.* 2004, Jobb 2007). The ITS and *trnL–trnF* majority-rule consensus trees obtained from the BI analyses were compared using 10⁵ replicates. Competing hypotheses were rejected at a significance level of 0.05.

Fruit morphology

A carpological study was carried out using Scanning Electron Microscopy (SEM) in order to evaluate morphological support for monophyletic groups in *Cynoglosseae* s.l. and *Omphalodes*. An exhaustive description of different fruit traits was performed for the 15 species of *Omphalodes* studied based on a total of 45 samples (Appendix S2). Although only one of the five Japanese *Omphalodes* was sampled, documented morphological variation for the species of *Omphalodes* in the archipelago is very limited (Ka 1965, Yamazaki 1993, Kadota 2009). Three mature nutlets were sampled per specimen, in order to obtain three views: abaxial and adaxial sides, and a cross section (to observe the inner side of the aperture). No prior treatment was done. The specimens were mounted directly onto metal stubs and metalized with gold-coating. Specimens were photographed with a Hitachi S3000N SEM. All photographs generated were revised and described focusing on six major characters: shape (mm), adaxial surface, scar, abaxial aperture (mm), margin and nutlet abaxial side epidermis (Table 2). In addition, representatives of the four genera recovered as the most closely related to *Omphalodes* s.s., *O. scorpioides* and the Japanese clade respectively (*Asperugo* Linnaeus (1753: 138), *Bothriospermum*, *Myosotidium*, *Thyrocarpus* Hance (1862: 225); see Results and Figs 1–2) were also characterized in order to find shared traits. Unfortunately, no sample of *Mertensia* Roth (1797: 34) could be included because we found little material in good condition. We used the exhaustive descriptions of the fruits of this genus given by Popov (1953), as well as SEM photographs from Nazaire & Hufford (2012) that contributed to nutlet descriptions.

Additional observations were performed on 311 herbarium specimens at different states of ripening from eight

herbaria (A, B, M, MA, MBK, MSB, RSA, TEX) using a stereomicroscope in order to extend the SEM sampling. Key characters were evaluated based on taxonomic and phylogenetic results.

FIGURE 1

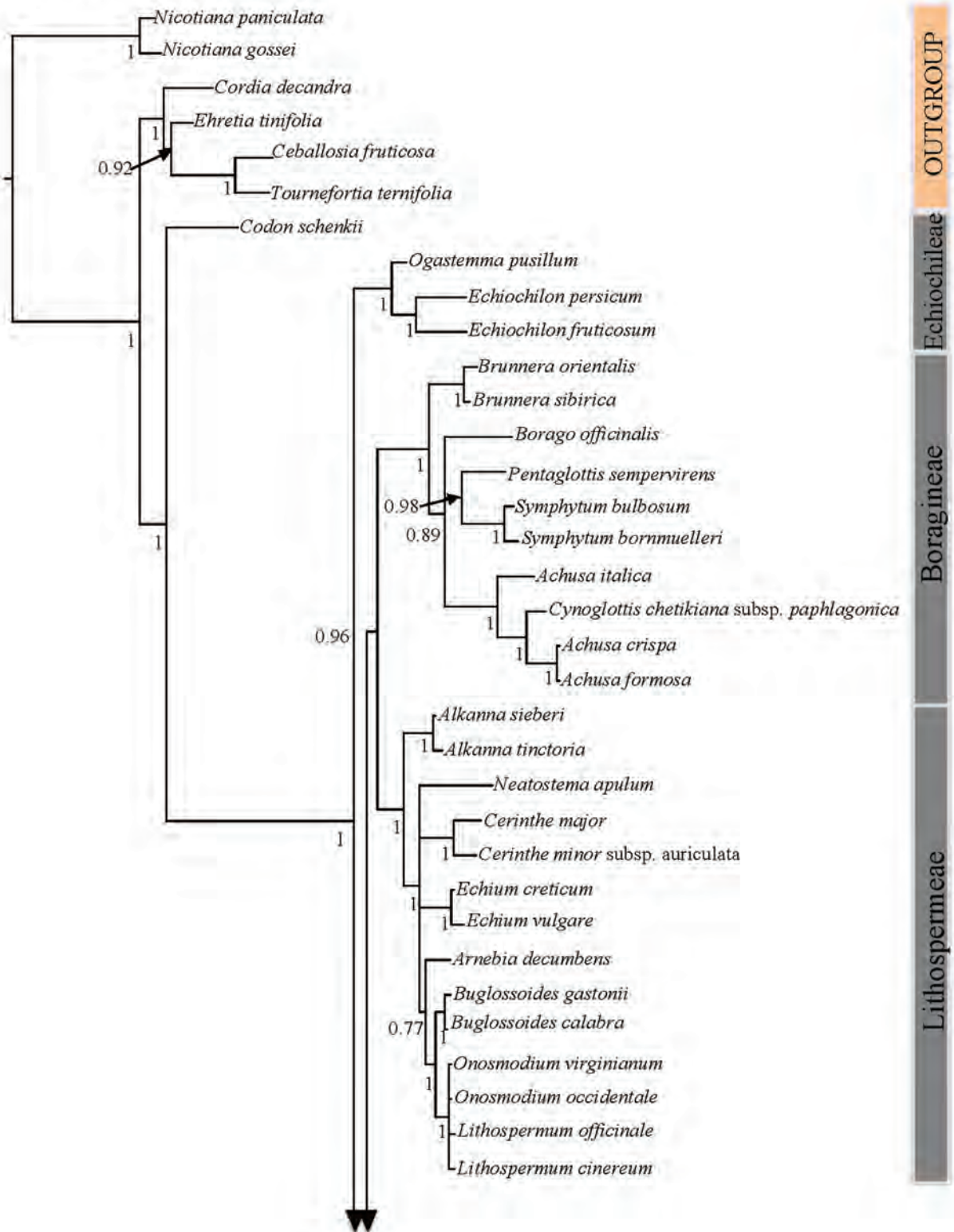


FIGURE 1 (continued)

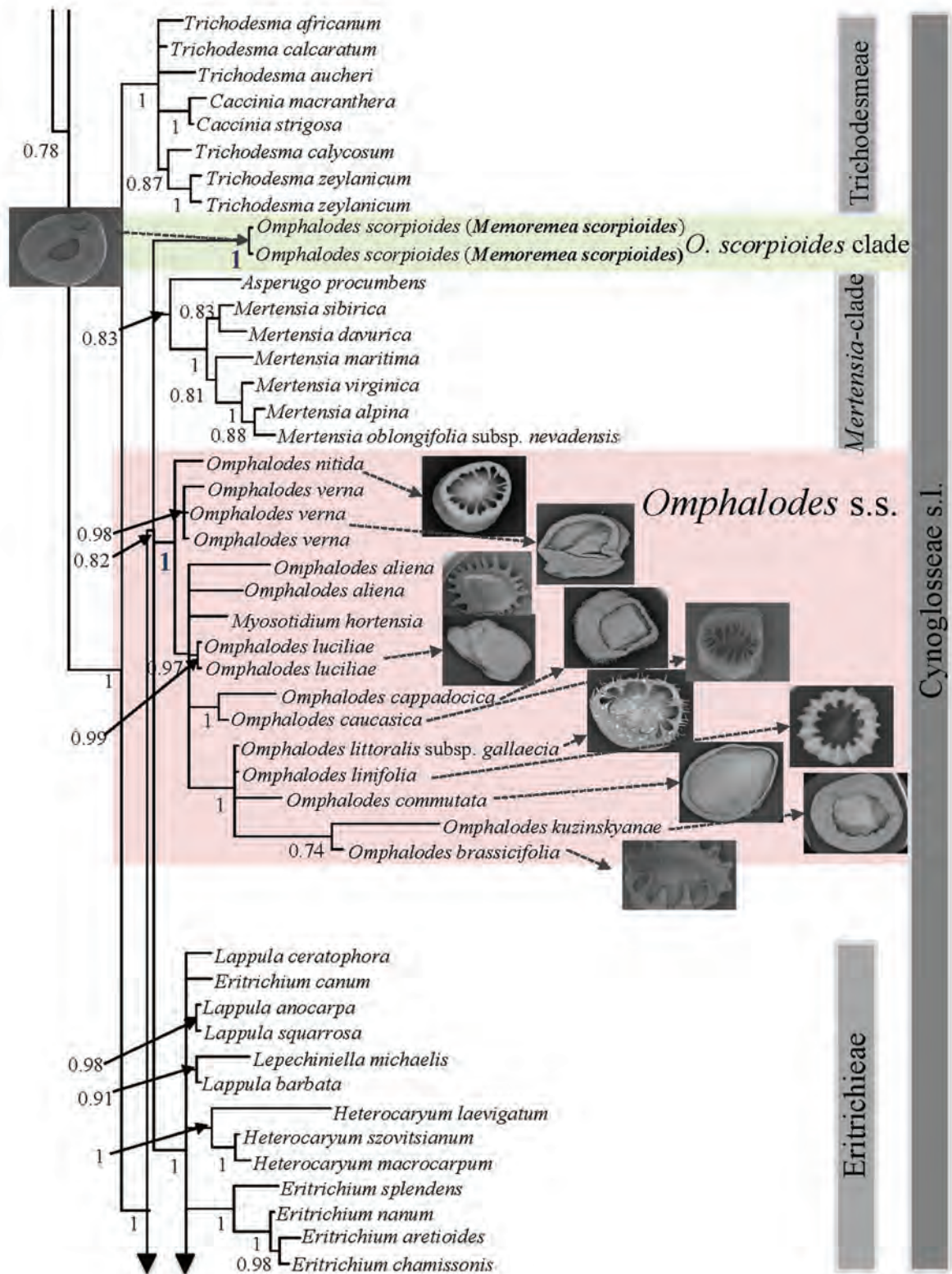


FIGURE 1 (continued)

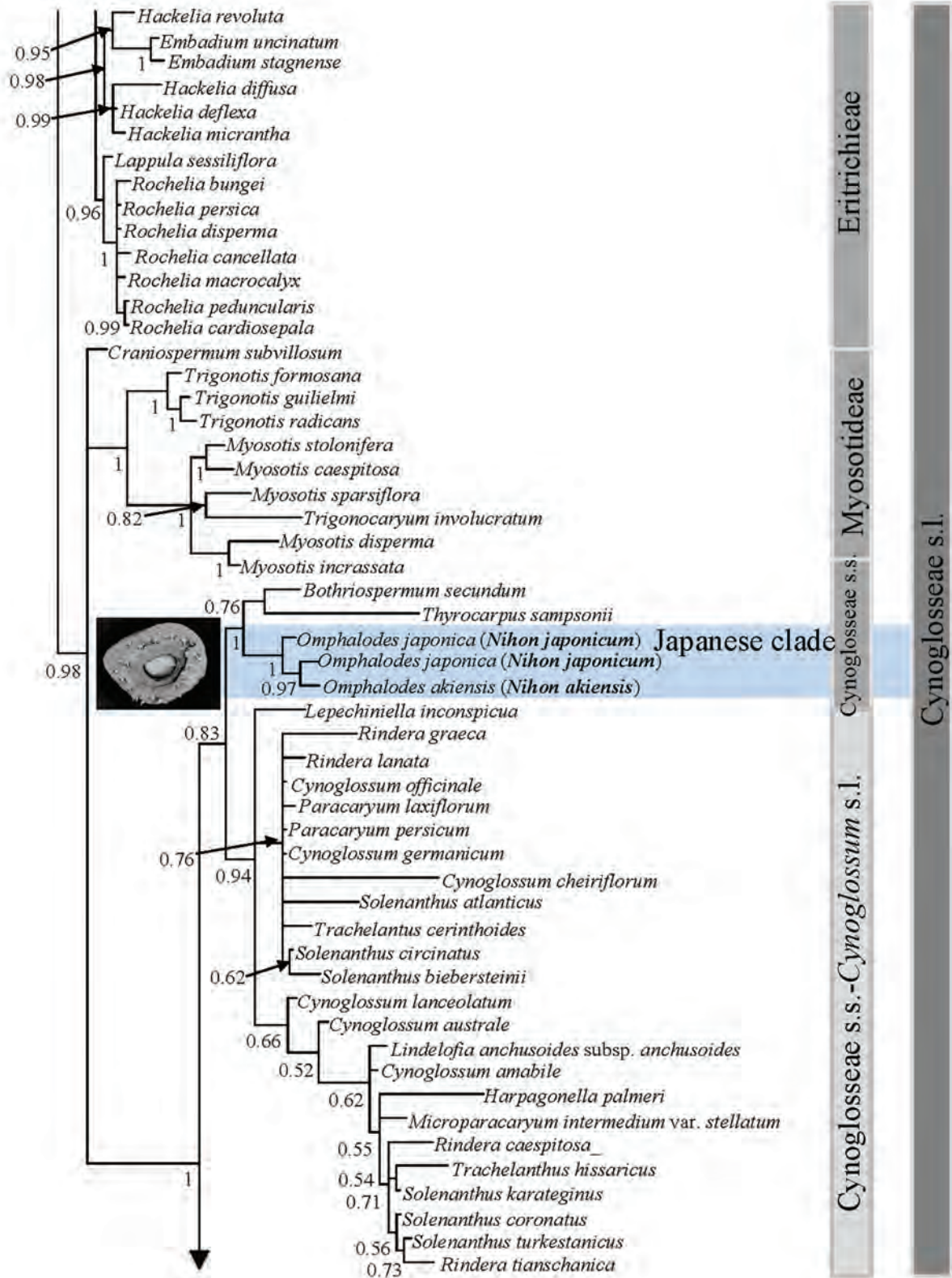


FIGURE 1 (continued)

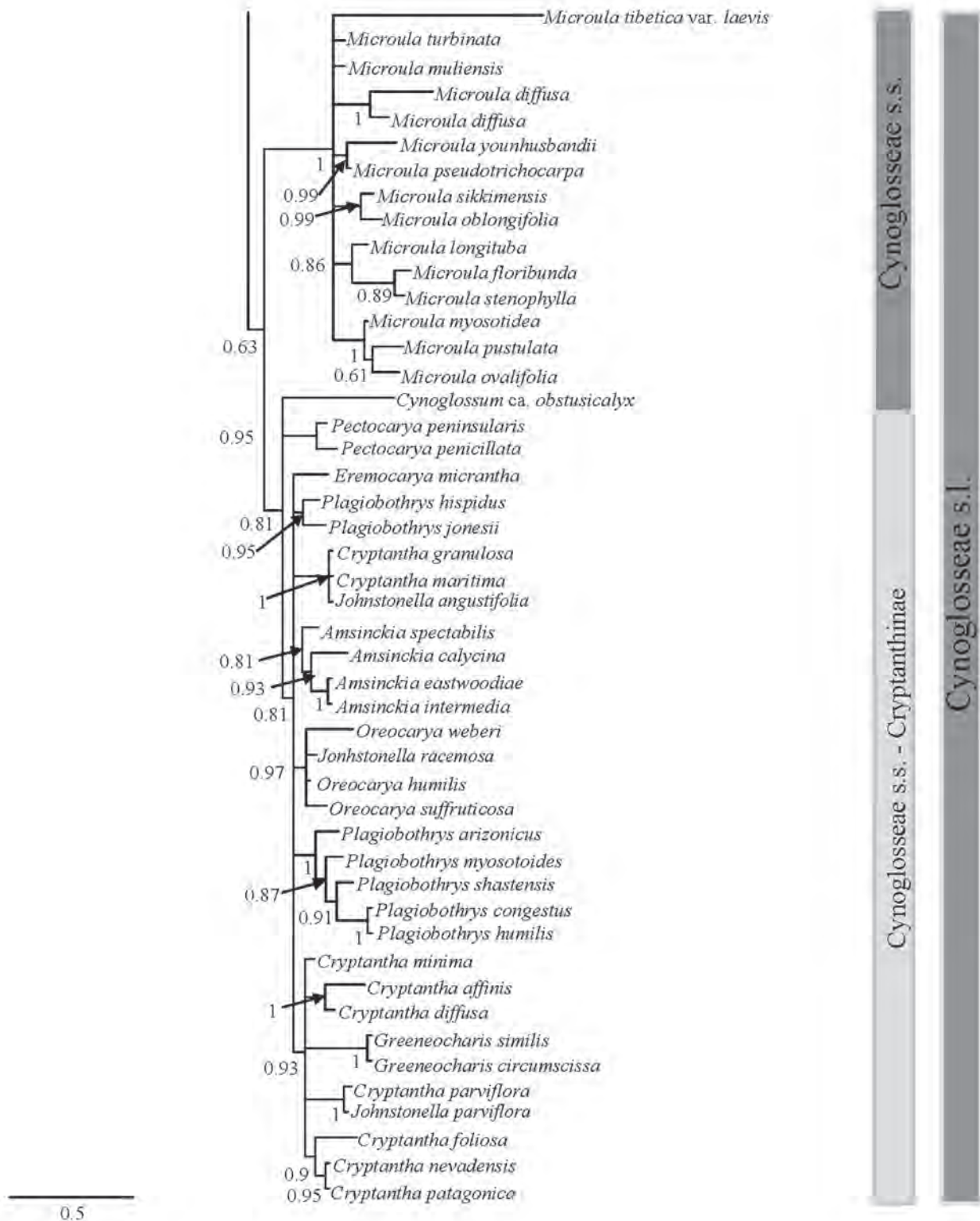


FIGURE 1. Bayesian majority rule consensus tree based on plastid (*trnL-trnF*) sequences. Numbers below nodes are Bayesian posterior probabilities, some indicated by solid arrows. Major clades are indicated. SEM photographs of species of *Omphalodes* are shown in their respective clades, some indicated by lined arrows. Scale bar represents the number of substitutions per site and is positioned at the end of the figure.

TABLE 2. Main carpological features of the different species of *Omphalodes* studied and the three sister taxa *Asperugo procumbens*, *Bothriospermum*, *Mertensia*, *Myosotidium* and *Thyrocarpus*. The measures provided correspond to the largest sides. Superindex 1 indicates the description of *Mertensia* is based on Popov (1953).

Taxon	Shape (mm)	Adaxial surface	Scar	Abaxial aperture (mm)	Margin	Nutlet abaxial side epidermis
North American Taxa						
<i>O. aliena</i>	Ovoid, 2 × 2.5	Densely covered by short papillae and dense short papillose trichomes	Central, deltoid	2 × 1.7	Flat, wide; edge deeply dentate-lobate; both outer and inner sides with short rigid papillose trichomes, crowded in the tips of the lobes	Densely covered by short papillae and short sparse papillose trichomes, flattish
<i>O. cardiophylla</i>	Ovoid, 3.5 × 3	Densely covered by short papillae, and dense long smooth trichomes	Central, deltoid	3.5 × 3	Curved inward, wide; edge dentate-lobate; outer side densely covered by long smooth trichomes, inner side densely covered by short papillae	Very densely covered by short papillae and dense long smooth trichomes, with a scarcely prominent central rib
<i>O. chiangii</i>	Orbicular, 2.3 × 1.3	Densely covered by scale-like papillae	Central, deltoid	1.5 × 1.3	Strongly curved inward, wide, delimiting an air chamber, but not hollow; edge entire; outer side densely covered by scale-like papillae and long papillae at the top, inner side densely covered short papillae	Densely covered by short papillae, glabrous, flattish
Annual European Taxa						
<i>O. brassicifolia</i>	Ovoid, 3.5 × 3	Densely covered by short papillae, and dense long smooth trichomes	Central, deltoid	3.5 × 3	Curved inward, wide; edge dentate-lobate; outer side with dense long smooth trichomes, inner side densely covered by short papillae	Very densely covered by short papillae and dense long smooth trichomes, with a scarcely prominent central rib
<i>O. commutata</i>	Subdeltoid, 2.1 × 2.1	Densely covered by short papillae and dense long smooth trichomes	Central, deltoid	1.3 × 1.3	Curved inward, narrow; edge entire; outer side densely covered by short papillae and sparse long smooth trichomes, inner side glabrous and smooth	Densely covered by short papillae, glabrous, flattish

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TABLE 2. (Continued)

Taxon	Shape (mm)	Adaxial surface	Scar	Abaxial aperture (mm)	Margin	Nutlet abaxial side epidermis
<i>O. kuzinskyanae</i>	Orbicular, 4.9 × 4.6	Densely covered by short papillae and sparse hooked trichomes	Central, deltoid	3 × 2.4	Curved inward, wide; edge slightly undulated; outer side densely covered by short papillae and sparse hooked trichomes, inner side smooth and glabrous	Densely covered by short papillae and sparse trichomes at the center, glabrous and wrinkled towards the edges, flattish
<i>O. linifolia</i>	Orbicular, 2.78 × 2.68	Densely covered by short papillae and disperse smooth trichomes, with dense long smooth trichomes around the scar	Central, deltoid	1.58 × 1.56	Curved inward, wide, hollow in its upper edge and delimiting a small air chamber; edge dentate, with ribs that ends on each tooth; outer side densely covered by short papillae, and long papillae towards the tips of the teeth, inner side glabrous, somewhat rough at the innermost border	Densely covered by short papillae, glabrous, with a scarcely prominent central rib
<i>O. littoralis</i>	Ovoid to deltoid, 2.8 × 2.7	Reticulate, with sparse long flat trichomes	Central, deltoid	1.9 × 2.2	Curved inward, wide; edge dentate; outer side densely covered by short papillae and sparse hooked trichomes; inner side smooth and glabrous	Densely covered by short papillae towards the edges, sparse long hooked trichomes at the center, flattish
Perennial Western Palearctic Taxa						
<i>O. caucasica</i>	Orbicular, 2.1 × 2.1	Densely covered by short papillae, and sparse long papillose trichomes	Central, deltoid	1.9 × 1	Curved inward, wide; edge deeply dentate-lobate; outer side densely covered by long papillae and dense long trichomes crowded at the tips, inner side glabrous and smooth	Densely covered by short papillae, glabrous, flattish
<i>O. cappadocica</i>	Subdeltoid, 2.1 × 2.1	Densely covered by short papillae and dense long smooth trichomes	Central, deltoid	1.3 × 1.3	Curved inward, narrow; edge entire; outer side densely covered by short papillae and sparse long smooth trichomes, inner side glabrous and smooth	Densely covered by short papillae, glabrous, flattish

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TABLE 2. (Continued)

Taxon	Shape (mm)	Adaxial surface	Scar	Abaxial aperture (mm)	Margin	Nutlet abaxial side epidermis
<i>O. luciliae</i>	Ovoid, 2.9 × 1.6	Densely covered by short papillae and very sparse short papillose trichomes	Central, deltoid	2.7 × 1.5	Curved inward, narrow; edge entire; both outer and inner sides densely covered by short papillae	Densely covered by short papillae, glabrous, flattish
<i>O. nitida</i>	Ovoid, 2.8 × 2.3	Densely covered by short papillae and papillose spinulae that make transition to sparse papillose trichomes	Central, deltoid	2.7 × 1.9	Curved inward, wide; edge deeply dentate-lobate; outer side with sparse short papillose trichomes and spinules, crowded at the tips of the lobes, inner side glabrous and smooth	Densely covered by short papillae and sparse spinulae, glabrous, flattish.
<i>O. verna</i>	Orbicular, 2.1 × 1.7	Densely covered by short papillae and sparse long smooth trichomes	Central, deltoid	1.5 × 1.3	Curved inward, narrow; edge entire; outer side densely covered by short papillae and sparse long smooth trichomes, inner side reticulate and glabrous	Densely covered by short papillae, disperse long smooth trichomes, flattish
Japanese Taxon						
<i>O. japonica</i>	Orbicular, 2.4 × 2.1	Densely covered by short papillose trichomes	Apical, deltoid	0.7 × 0.6	Strongly curved inward, hollow and delimiting an air chamber in its entire width, very wide; edge entire; outer side densely covered by short papillose trichomes and disperse long spines in its external half, which is dark when ripe, that sharply changes towards the aperture to wrinkled and with sparsely covered by short papillae, whitish when ripe, inner side smooth and glabrous	Densely covered by short papillae, glabrous, flattish
Biennial European Taxon						
<i>O. scorpioides</i>	Orbicular, 2.8 × 2.5	Sparse short papillae and short, nearly smooth, trichomes	Apical, deltoid	2.5 × 1.5	Curved inward, wide, hollow and delimiting an air chamber in its entire width; edge entire; both outer and inner side with, sparse trichomes, rough in the innermost border	Sparsely covered by short papillae and long, nearly smooth, trichomes, flattish

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TABLE 2. (Continued)

Taxon	Shape (mm)	Adaxial surface	Scar	Abaxial aperture (mm)	Margin	Nutlet abaxial side epidermis
Other taxa associated						
<i>Asperugo</i>	3.5 × 2	Dense short papillae, compound papillae	Subapical, rounded	Absent	Flat narrow keel, densely covered by short papillae	Densely covered by short papillae, compound papillae
<i>Bothriospermum</i>	Ovoid to reniform, 2 × 1	Dense short and long papillae, compound papillae	Apical, linear	0.5 × <0.4	Curved inward, narrow, edge entire, both outer and inner side densely covered by short papillae. Presence of an inner free layer wrinkled with disperse short smooth trichomes	Densely covered by short papillae, glabrous, flattish
<i>Mertensia</i> ¹	Tetrahedral, 3 to 5 in length	Rugose or grumose, glabrous, keeled.	Basal, rounded	Absent	Rarely narrow wing with prickles	Rugose or grumose, glabrous
<i>Myosotidium</i>	Deltoid, 10 × 14	Slightly wrinkled	Central deltoid	9 × 10	Flat or curved outward, irregular in width; edge entire, irregular; both outer and inner sides smooth, slightly wrinkled, and glabrous	Slightly wrinkled, glabrous, flattish
<i>Thyrocarpus</i>	Ovoid to reniform, 2 × 1.4	Dense short and long papillae, compound papillae	Apical, linear	1.75 × 0.5	Curved inward, wide, edge dentate, both outer and inner side densely covered by short papillae, longer in tips. Presence of an innermost free layer wrinkled with sparse short smooth trichomes	Apparently missed

Results

Phylogenetic reconstructions

Our plastid (Fig. 1) and nuclear (Fig. 2) phylogenetic reconstructions are mainly in agreement with previous phylogenies of Boraginaceae (Långström & Chase 2002, Långström & Oxelman 2003, Weigend *et al.* 2010, Nazaire & Hufford 2012, Weigend *et al.* 2013, Cohen 2014). Results from the AU test reveal that the ITS topology is not rejected by the plastid dataset (difference in $-\text{LnL} = 32.171$, $p = 0.9$), whereas the plastid topology is rejected by the ITS dataset (difference in $-\text{LnL} = 748.790$, $p < 0.001$). Four topological incongruences were detected between the plastid and nuclear trees. One of these incongruences affects the internal resolution of our study group (*O. verna*, *O. nitida*, see below; Figs. 1–2). The remaining three incongruences will not be further discussed since they do not affect our study group (see *Symphytum* Linnaeus (1753: 136), *Borago* Linnaeus (1753: 136) and *Harpagonella* A. Gray (1876: 88) in Figs. 1–2). Because of these results, we did not perform a combined analysis. Accordingly, the phylogenetic results based on the nuclear and plastid datasets are shown independently.

The polyphyly of *Omphalodes* (*Omphalodes* s.l.) is supported by our phylogenetic analyses since three well-supported clades are consistently recovered in the nuclear and plastid trees (Figs. 1–2): (1) the *Omphalodes* s.s. clade, (2) *O. scorpioides*, and (3) the Japanese *Omphalodes* clade.

FIGURE 2

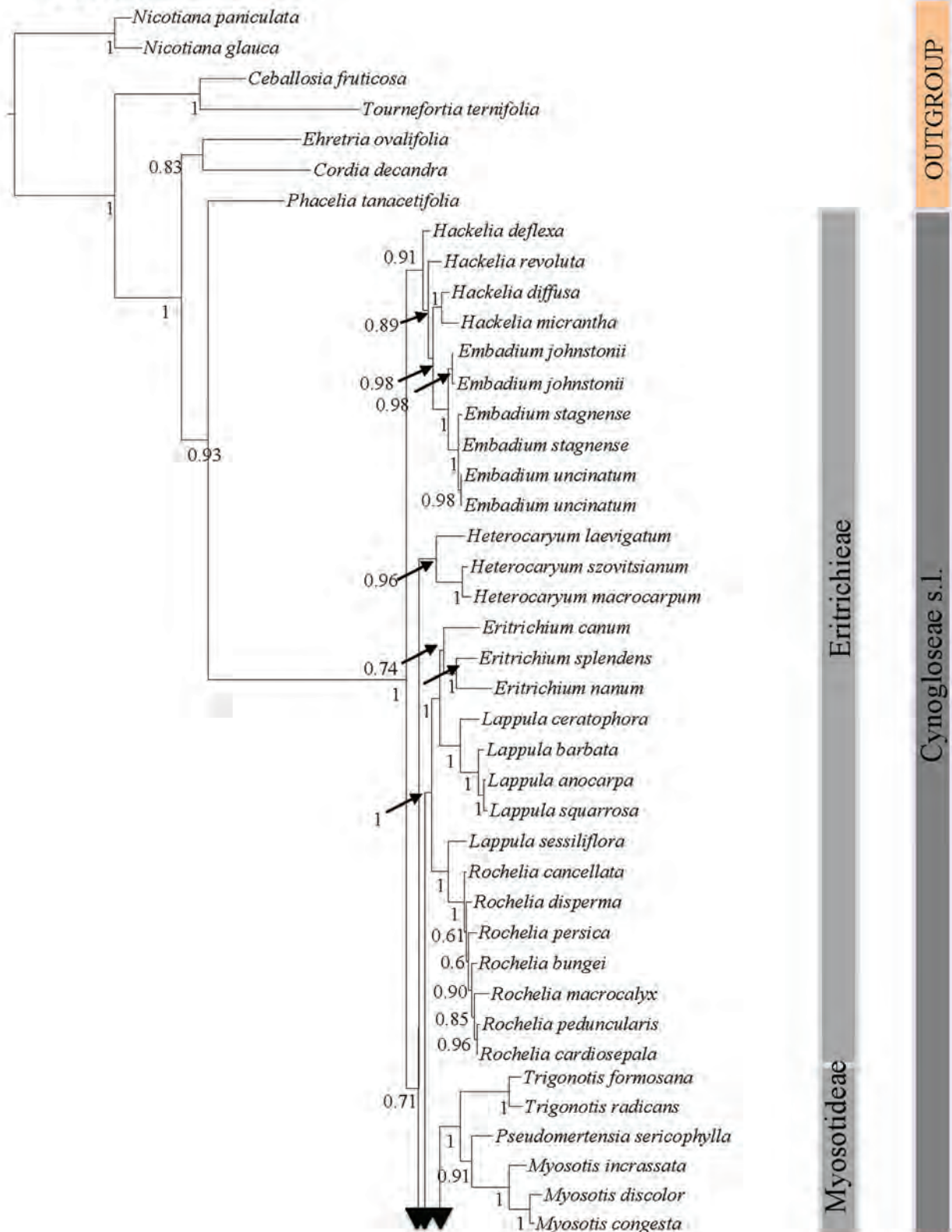


FIGURE 2 (continued)

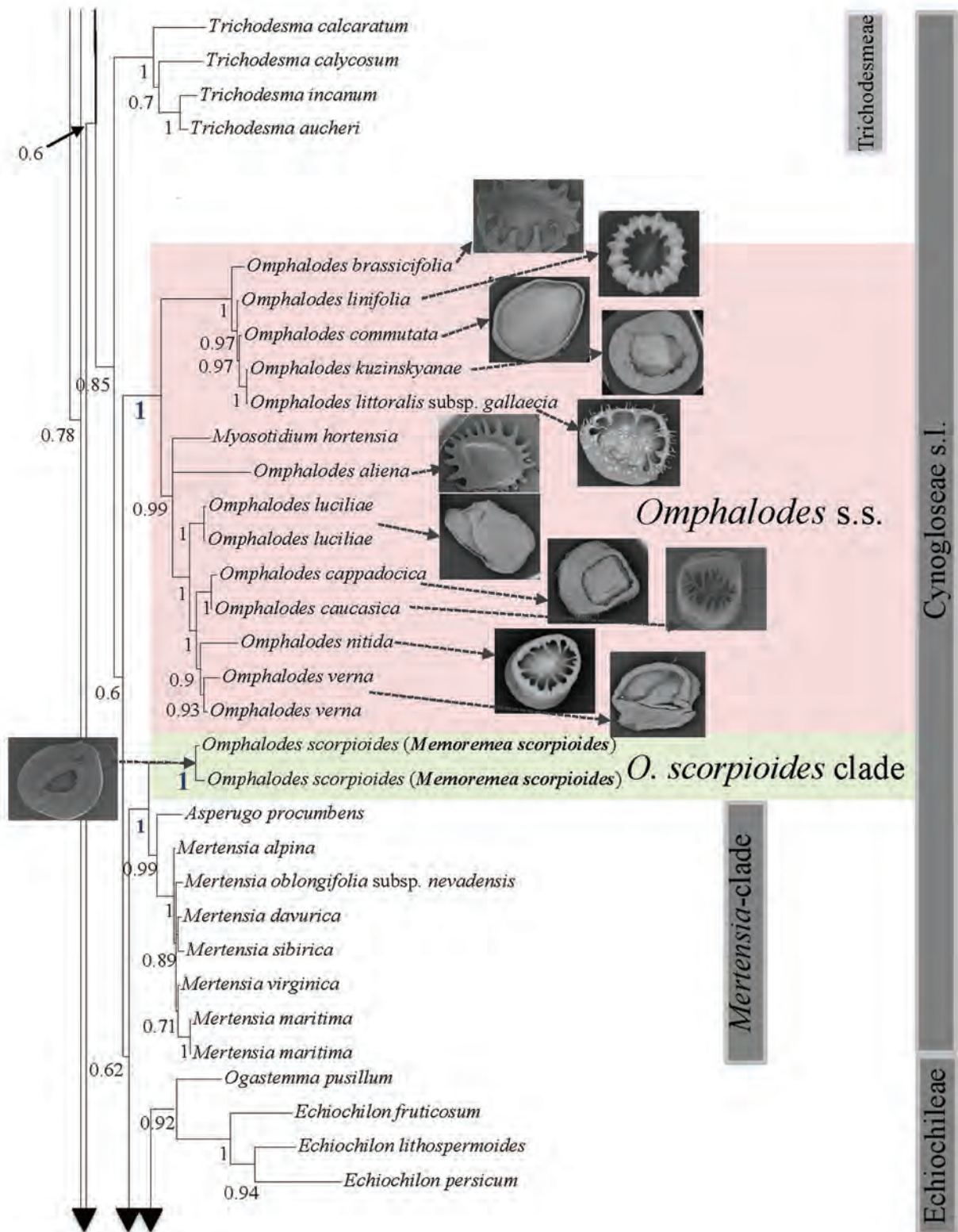


FIGURE 2 (continued)

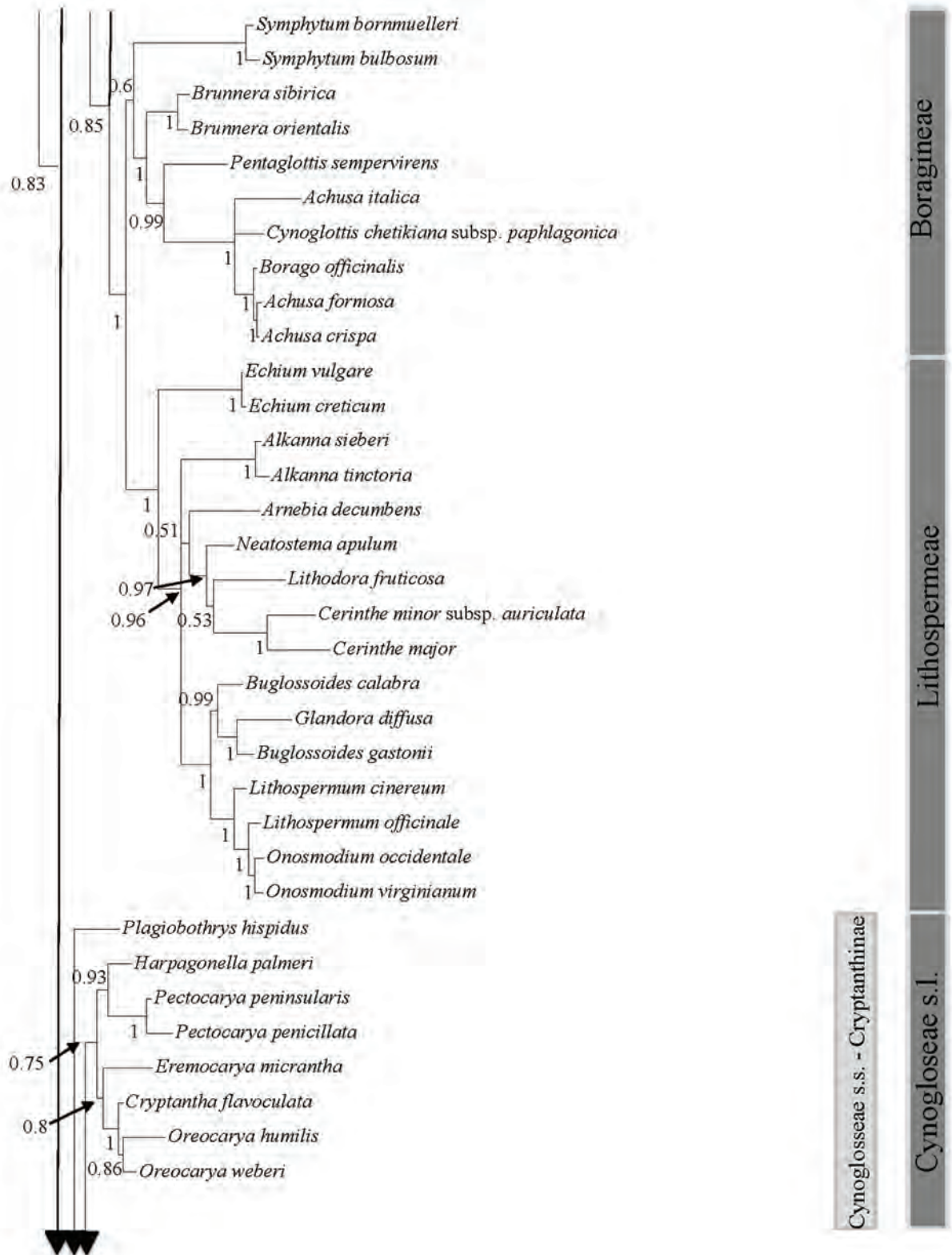


FIGURE 2 (continued)

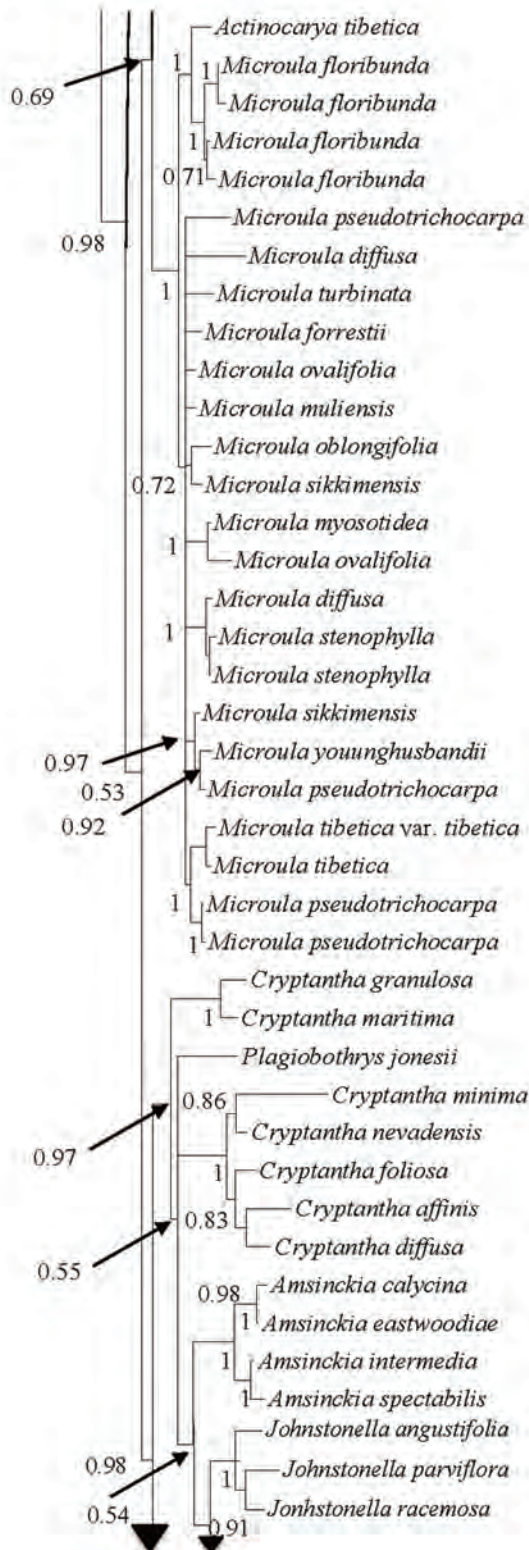


FIGURE 2 (continued)

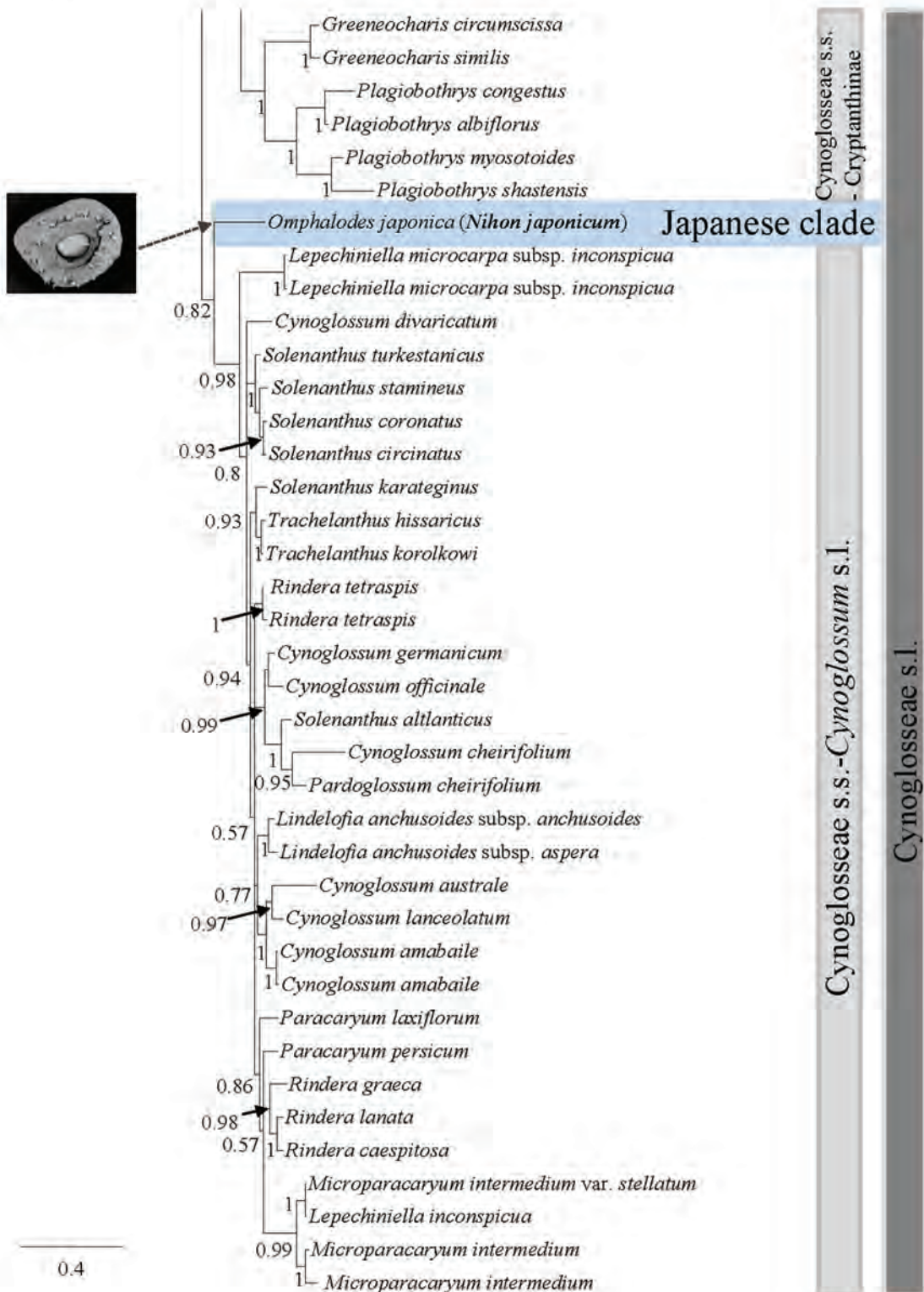


FIGURE 2. Bayesian majority rule consensus tree based on nuclear (ITS) sequences. Numbers below nodes are Bayesian posterior probabilities, some indicated by solid arrows. Major clades are indicated. SEM photographs of species of *Omphalodes* are shown in their respective clades, some indicated by lined arrows. Scale bar represents the number of substitutions per site and is positioned at the end of the figure.

The plastid reconstruction reveals that *Omphalodes* accessions are placed in three independent well-supported clades (1 BPP each, Fig. 1). In particular, the Japanese clade is placed within Cynoglosseae s.s. and the remaining two clades (*Omphalodes* s.s. and *O. scorpioides*) appear in a basal polytomy of Cynoglosseae s.l. together with the Eritrichieae clade and the *Mertensia* clade (*Mertensia* and *Asperugo*). The *Omphalodes* s.s. clade includes all eleven of the Mediterranean and western Asia annual and perennial species studied plus *Myosotidium* (1 BPP, Fig. 1). Within this clade, the Mediterranean perennial species *O. verna* and *O. nitida* form a monophyletic group in a basal polytomy, together a subclade including the remaining species of *Omphalodes* s.s. It contains the five annual species from the Western Mediterranean clustering in a well-supported group (1 BPP, Fig. 1), while the Mediterranean and West Asian perennial species, *O. aliena* A.Gray ex Hemsley (1882: 377) and *Myosotidium* are unresolved. The second main clade of *Omphalodes* s.l. includes the two samples of the biennial species *O. scorpioides* (1 BPP, Fig. 1). Finally, the two Japanese species of *Omphalodes* form a clade (1 BPP, Fig. 1), and they group together with two Asian species (*Thyrocarpus sampsonii* Hance (1862: 225) and *Bothriospermum secundum* Maximowicz (1859: 202); 1 BPP, Fig. 1).

The nuclear tree also shows three independent clades congruent with those of the plastid phylogeny (Fig. 2): the *O. scorpioides* clade is sister to the *Mertensia* clade (1 BPP, Fig. 2), *Omphalodes* s.s. is shown to be an independent clade and the Japanese clade is related to nine genera of Cynoglosseae s.s. (Fig. 2). In contrast to the plastid topology, *Omphalodes* s.s. has a Mediterranean annual subclade (1 BPP, Fig. 2) sister to a subclade of the remaining species of *Omphalodes* s.s. (Mediterranean perennials, America, western Asia) plus *Myosotidium hortensia* (New Zealand) (0.99 BPP, Fig. 2).

Fruit morphology

Descriptions of nutlets of the 15 species of *Omphalodes* sampled, as well as those of the five closest relative genera are shown based on the three *Omphalodes* s.l. clades obtained in the phylogenetic analyses (see above).

Nutlets in Boraginaceae s.s. shows two markedly differentiated adaxial and abaxial surfaces. The nutlet is inserted into the gynobase producing an attachment scar. Its shape and position along the length of the adaxial face, remains constant among most the species of the genus except for the Japanese *Omphalodes* and *O. scorpioides* (see below). The abaxial side of the fruits of all *Omphalodes* displays a discontinuity of the exocarp resulting in a rounded aperture of varying sizes and a more or less curved aperture margin (Fig. 3A–AW). However, the North American *O. aliena* and *O. cardiophylla* A. Gray ex Hemsley (1882: 377), and Eurasian *O. brassicifolia* Sweet (1826: 293) and *O. luciliae* Boissier (1844: 41) lack such a discontinuity and thus the mesocarp is completely covered by the exocarp. This carpological characteristic was used to define and group species of *Omphalodes*. In the present study, we refer to the base of abaxial aperture as abaxial surface. All the structures mentioned can appear heterogeneously ornamented with different types and densities of trichomes, papillae or spines. In addition to traits already reported in previous studies, for the first time, an air chamber has been observed within the margin of the nutlets in four species of *Omphalodes* (*O. linifolia*, *O. chiangii* Higgins (1976: 412), *O. scorpioides* and *O. japonica* Maximowicz (1872: 452)). *Omphalodes linifolia* forms a small chamber (Fig. 3U) by the ripping of the mesocarp from the exocarp at the top of the margin. In *Omphalodes scorpioides* and *Omphalodes japonica* the air chamber completely occupies the margin, which resembles a floater (Fig. 3AV, 3AS). In contrast, in *Omphalodes chiangii* the margin is actually solid, but it is strongly incurved delimiting the air chamber (Fig. 3H). The air chamber is delimited by a mesocarpic ‘wrapping tissue’-like surface, except in *O. chiangii* that seems to be entirely exocarpic. The significant SEM traits commented above, were also observed on the herbarium specimens studied under the stereomicroscope, with special attention paid to three additional species from Japan. The characteristics of the three clades of *Omphalodes* s.l. are discussed below. A summary table with all the six traits described from the 15 species is provided (Table 2).

Omphalodes s.s. clade

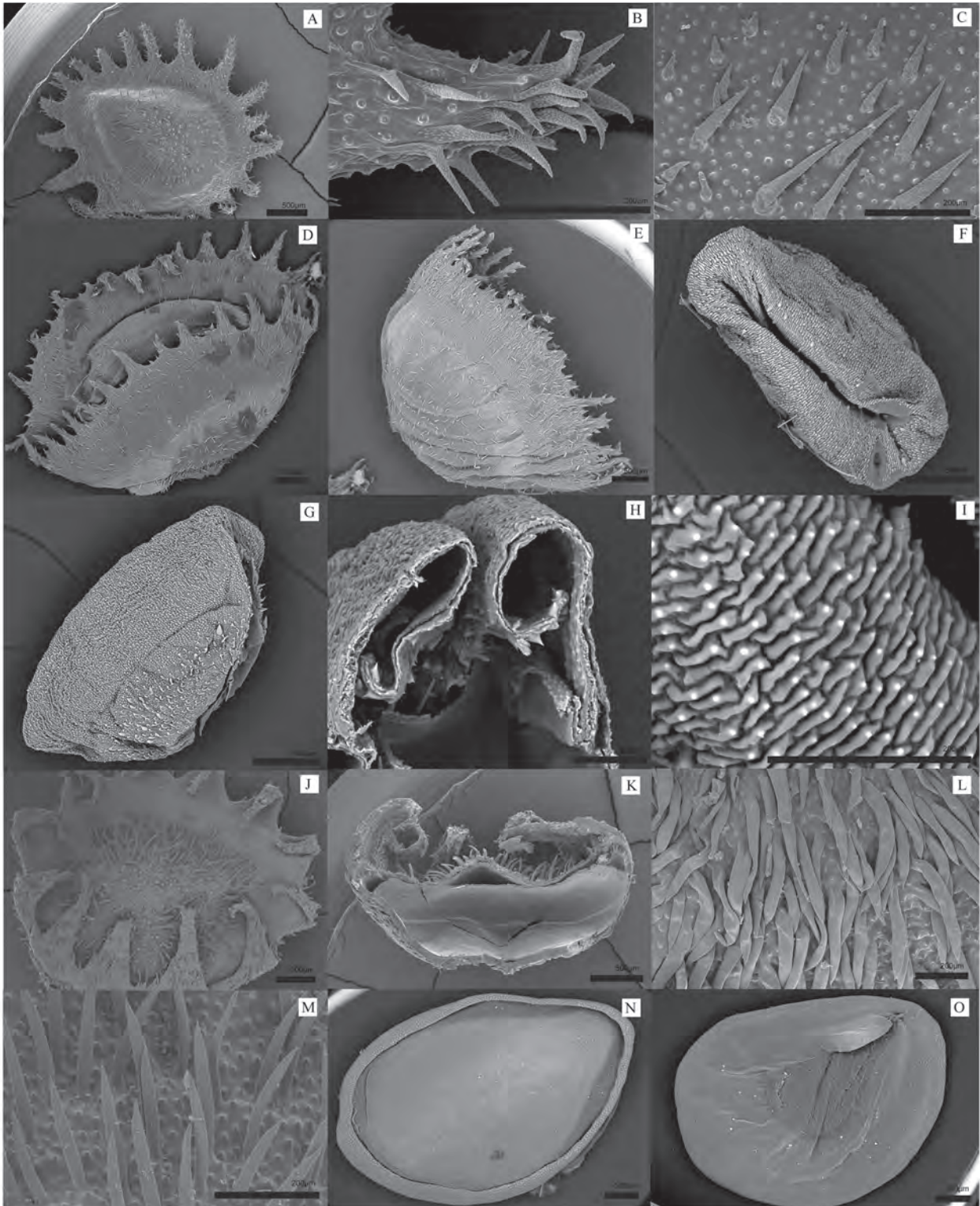
The nutlets of the *Omphalodes* s.s. clade are suborbicular (or ovoid-subdeltoid), 2–3 (4) × 1–3 (4) mm (Fig. 3A–3AW). Annual European taxa have bigger nutlets (c. 3 mm in diameter) than those of European perennials (2 mm) (Fig. 3J–X, 3Y–AP). The nutlets of North American species are ovoid to orbicular, 2 (3) × 1–3 mm. The New Zealand genus (*Myosotidium hortensia*) embedded in this clade is morphologically larger in all its parts, including bigger deltoid nutlets (10 × 14 mm). The adaxial nutlet surface of taxa in the *Omphalodes* s.s. clade is densely to sparsely covered by papillae (e.g. Fig. 3L, 3T, 3AJ), and rigid (Fig. 3Z) to hooked (Fig. 3W–X) trichomes. The papillae are conspicuous and range from long towards the edges to short in the central parts of the nutlet (Fig. 3AJ). Trichomes can be papillose or more or less smooth (Fig. 3L, 3AF, 3AL). The endemic North American *Omphalodes chiangii*, unfortunately not sequenced for this study, displays papillae ridges densely covering both adaxial and abaxial sides (Fig. 3I). The nutlets of *M. hortensia* have smooth to slightly wrinkled surface either in the adaxial or abaxial sides as in its margin (Fig. 3BB–BC).

The scar is subdeltoid and it is placed in the centre of the adaxial side (Fig. 3O, 3R, 3T, 3W, 3Z, 3AJ). *Myosotidium hortensia* has the broadest deltoid scar covering nearly all of the adaxial side.

The abaxial aperture is 1–3 × 1–2 (3) mm in the species ascribed to *Omphalodes*, whereas the largest aperture is found in *Myosotidium* (*M. hortensia*, 9 × 10 mm). Major differences between species are found on the edge, curvature and width of the margin, including ornamentation of the epidermis. The edge of the margin varies from entire (Fig. 3F, 3N, 3Y, 3AG, 3AN) to dentate or lobate (Fig. 3A, 3D, 3J, 3S, 3V, 3AD, 3AI). The margin varies from narrow and slightly curved, barely covering the aperture borders (Fig. 3N, 3Y, 3AG, 3AN) to wider than the nutlet body (Fig. 3U), hardly curved and covering nearly one-third of the aperture (Fig. 3Q). The margin can be incurved and covering the aperture (Fig. 3H, 3J, 3AI) to nearly flat and exposing entirely the abaxial surface as in *Omphalodes aliena* (Fig. 3A). All *Omphalodes* taxa have a solid margin except *O. linifolia*, in which the upper part of the margin displays a small air chamber (Fig. 3U). The epidermis on the external surface is usually sparsely to densely covered by papillae and/or straight (Fig. 3AC) to hooked (Fig. 3V) trichomes. These trichomes vary from smooth (Fig. 3AE) to papillose. Sometimes dentate margins become crowded of trichomes (Fig. 3B) towards the edge. The internal surface of the margin is usually glabrous and smooth, especially in those species that have their margins curved inwards (Fig. 3U, 3AB, 3AK, 3AP). The ornamentation of the margin surfaces in the North American studied taxa and the Palearctic *O. brassicifolia* and *O. luciliae* is similar in both internal and external surface (Fig. 3A, 3D, 3H, 3J, 3K). The margin of *M. hortensia* is irregular in width, and is either flat or curved outwards.

The abaxial surface is generally flattish, except for *Omphalodes linifolia* and *Omphalodes brassicifolia* that display a scarcely prominent central rib (Fig. 3K, 3U). The ornamentation of the epidermis at the base of the aperture on the abaxial side usually consists of short papillae, sometimes with sparse trichomes (Fig. 3C, 3AA, 3AM, 3AO).

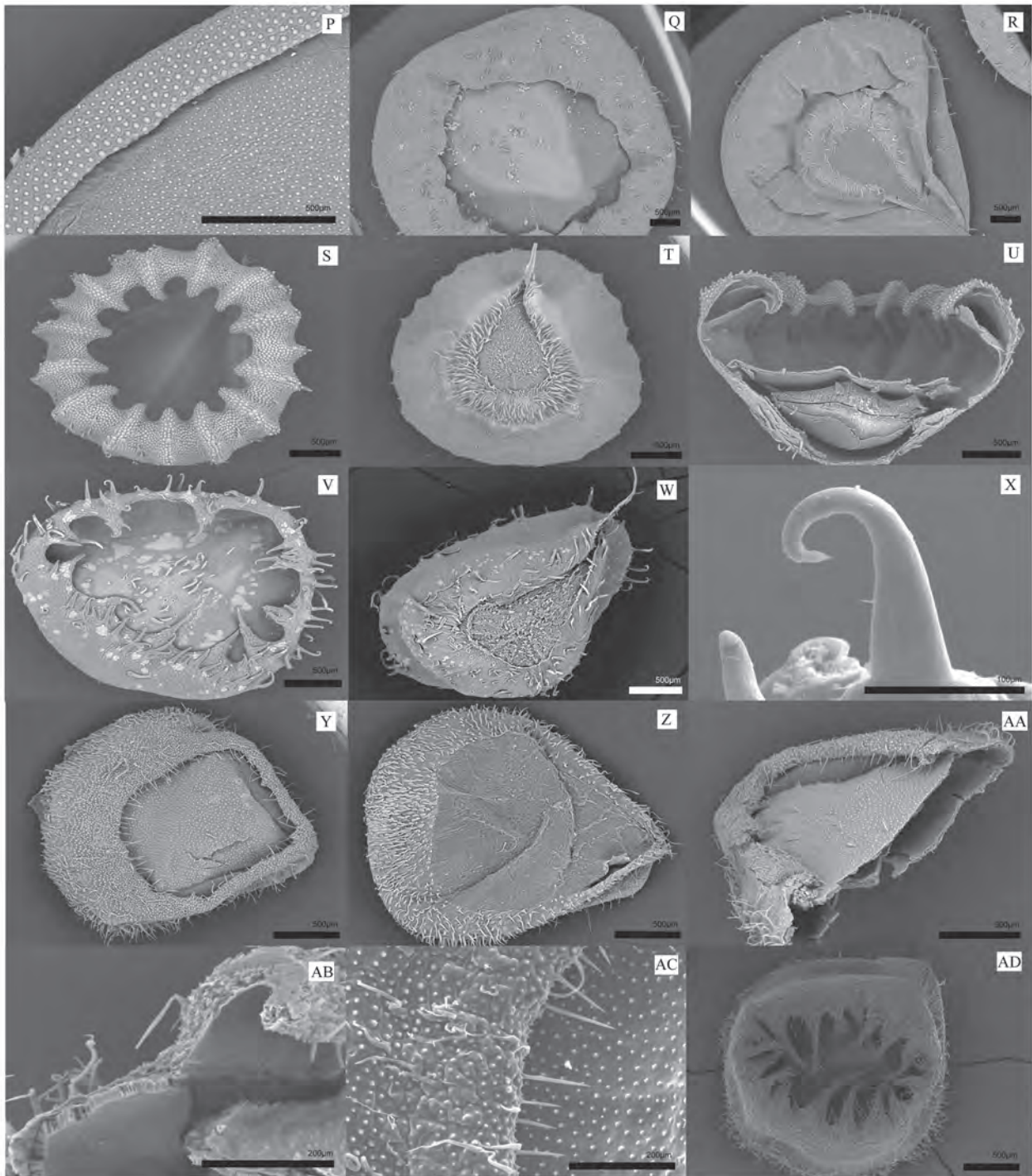
FIGURE 3. SEM photographs of the nutlets of the different species of *Omphalodes* and allied genera. **A–C.** *Omphalodes aliena*. **A.** Abaxial view. **B.** Detailed view of a tooth of the margin. **C.** Detailed view of the base of the abaxial aperture. **D–E.** *O. cardiophylla*. **D.** Abaxial view. **E.** Lateral view. **F–I.** *O. chiangii*. **F.** Abaxial view. **G.** Lateral view. **H.** Detailed view of the margin in cross-section. **I.** Detailed view of the ornamentation of the epidermis margin. **J–M.** *O. brassicifolia*. **J.** Abaxial view. **K.** Cross-section view. **L.** Detailed view of adaxial epidermis ornamentation. **M.** Detailed view of the ornamentation of the epidermis from the base of the abaxial aperture. **N–P.** *O. commutata*. **N.** Abaxial view. **O.** Adaxial view. **P.** Detailed view of the ornamentation of the epidermis at the margin of the abaxial aperture. **Q–R.** *O. kuzinskyanae*. **Q.** Abaxial view. **R.** Adaxial view. **S–U.** *O. linifolia*. **S.** Abaxial view. **T.** Adaxial view. **U.** Cross-section view. **V–X.** *O. littoralis* subsp. *gallaecia*. **V.** Abaxial view. **W.** Adaxial view. **X.** Detailed view of a hooked trichome. **Y–AC.** *O. cappadocica*. **Y.** Abaxial view. **Z.** Adaxial view. **AA.** Cross-section view. **AB.** Detailed cross-section view of the margin. **AC.** Detailed view of the margin edge and the base of the abaxial aperture. **AD–AF.** *O. caucasica*. **AD.** Abaxial view. **AE.** Margin in cross-section. **AF.** Detailed view of the trichomes and papillae from the adaxial epidermis. **AG–AH.** *O. luciliae*. **AG.** Abaxial view. **AH.** Adaxial view. **AI–AM.** *O. nitida*. **AI.** Abaxial view. **AJ.** Adaxial view. **AK.** Detailed cross-section view. **AL.** Detailed view of the transition between the nutlet body and the margin. **AM.** Detailed view of the ornamentation of the epidermis from the base of the abaxial aperture. **AN–AP.** *O. verna*. **AN.** Abaxial view. **AO.** Detailed view of the base of the abaxial aperture, the margin, and the nutlet body. **AP.** Detailed view of the inner side of the margin. **AQ–AT.** *O. japonica*. **AQ.** Abaxial view. **AR.** Adaxial view. **AS.** Cross-section view. **AT.** Detailed view of the ornamentation of the epidermis from the base of the abaxial aperture. **AU–AW.** *O. scorpioides*. **AU.** Abaxial view. **AV.** Detailed cross-section view. **AW.** Detailed view of the trichomes from the adaxial side. **AX–BA.** *Bothriospermum secundum*. **AX.** Abaxial view. **AY.** Cross-section view. **AZ.** Detailed view of the abaxial aperture and inner half of the margin. **BA.** Detailed view of the outer half of the margin and nutlet body. **BB–BC.** *Myosotidium hortensia*. **BB.** Detailed view of the epidermis of the adaxial side of the nutlet. **BC.** Detailed view of the epidermis of the inner side of the margin. **BD–BF.** *Thyrocarpus sampsonii*. **BD.** Abaxial view. **BE.** Adaxial view. **BF.** Cross-section view. **BG–BH.** *Asperugo procumbens*. **BG.** Adaxial view. **BH.** Detailed view of the epidermis.



Omphalodes scorpioides clade and sister group

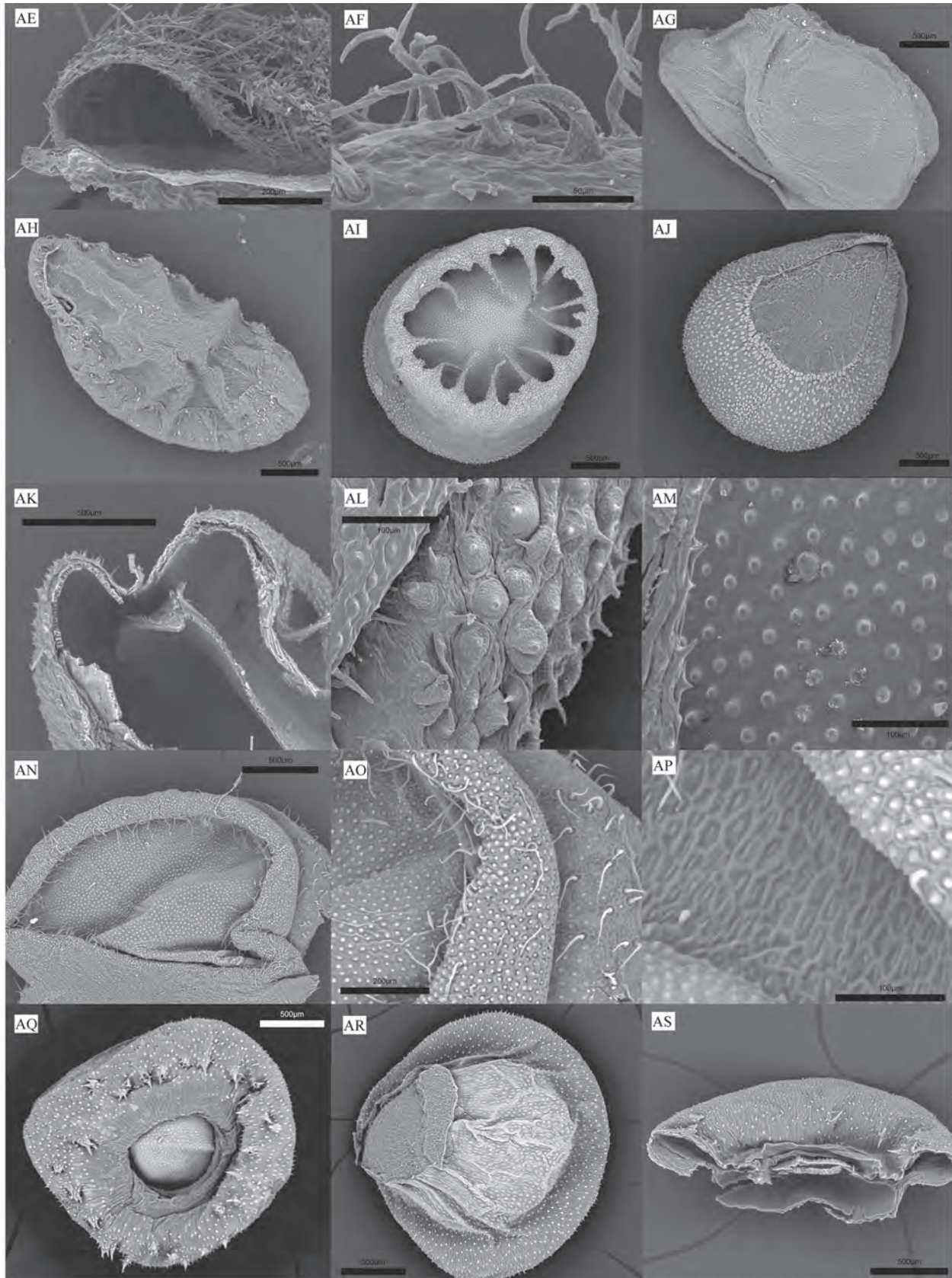
The nutlets of *Omphalodes scorpioides* (Fig. 3AU–AW) are orbicular and 2.8×2.5 mm. The adaxial side has sparse short papillae and short smooth trichomes (Fig. 3AW). The scar is deltoid in shape and it is in an apical position, which is distinct from the remaining European taxa of the *Omphalodes* s.s. clade. The margin of *O. scorpioides* nutlets is deeply inwardly curved, partially covering the abaxial aperture (Fig. 3AU, 3AV). Smooth trichomes occur sparsely on both the outer and inner sides of the margin (Fig. 3AV). Ornamentation of the abaxial aperture displays short papillae and smooth trichomes (Fig. 3AV). This species is also characterized by an air chamber that fills the entire margin (Figs. 3AU–AV).

Mertensia and *Asperugo* constitute the sister group of *O. scorpioides*. The nutlets of these taxa are laterally compressed and thus the adaxial and abaxial sides are substituted by ventral and dorsal sides respectively. The studied sample *Asperugo procumbens* Linnaeus (1753: 138) displays an ovoid, 3.5 × 2 mm nutlet. Both lateral sides are densely covered by fine papillae grouped together and forming prominences and wrinkles (Fig. 3BH). The rounded scar is subapical and ventrally positioned. The flat narrow margin is densely covered by short papillae on the dorsal side (Fig. 3BG). The nutlets of *Mertensia* are tetrahedral, 3–5 mm in length (Popov 1953, Nazaire & Hufford 2012). As indicated by these authors, both lateral sides are rugose or grumose. The rounded scar is basal and ventrally positioned. Rarely, a narrow wing with prickles is observed.



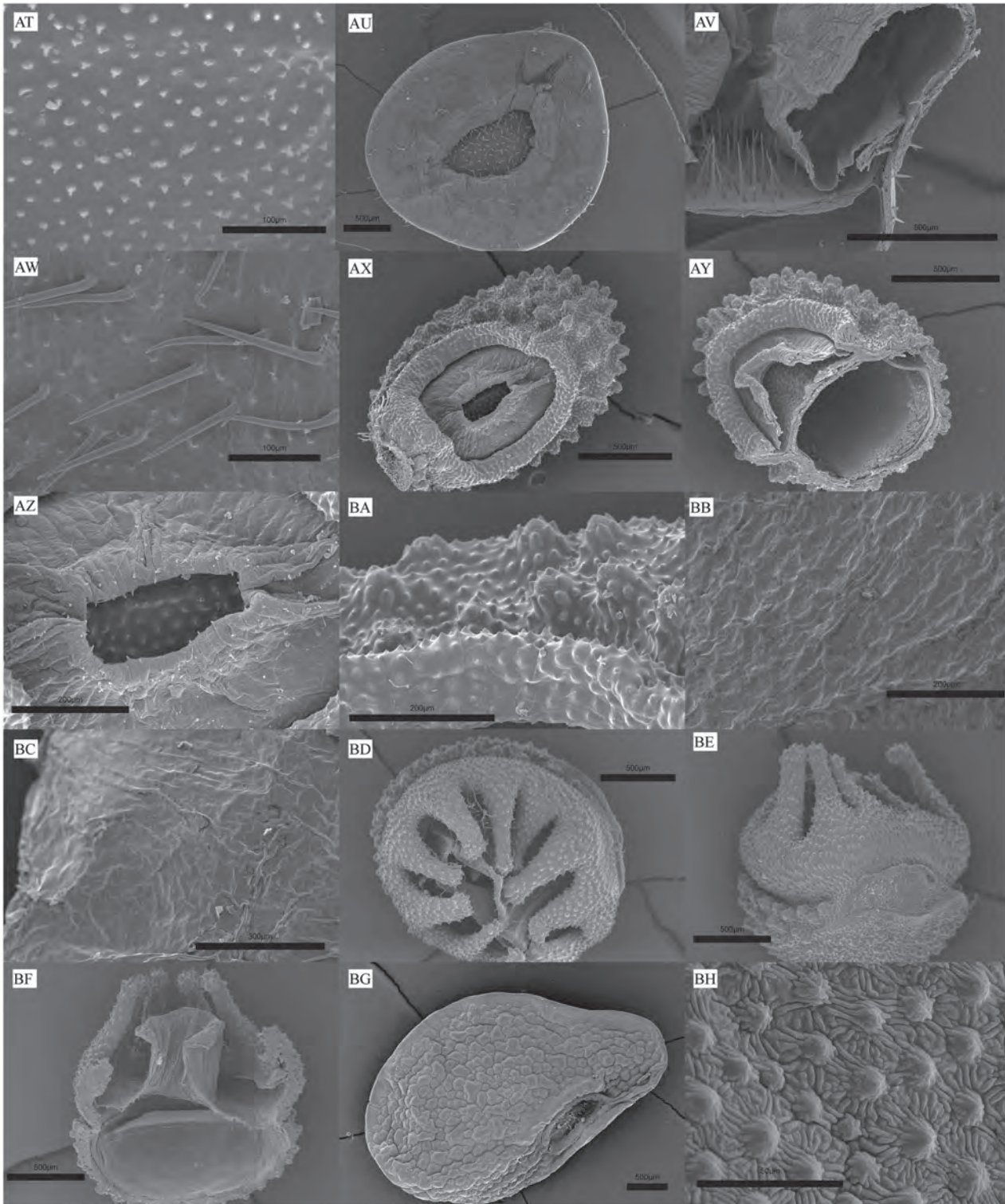
Japanese clade and its closest relatives

Significant morphological differences have been observed between the species of the Japanese clade and the species of the other two clades of *Omphalodes*. Despite the single specimen of this clade included in the SEM study, the diagnostic traits detected were confirmed in all the other Japanese samples in the stereomicroscope study.



The nutlets of *O. japonica* (Fig. 3AQ–AT) are orbicular and 2.4×2.1 mm. The adaxial side is homogeneously and densely covered by short papillose trichomes (Fig. 3AR). The scar is deltoid and located in an apical position (Fig. 3AR). The margin is strongly inwardly curved and covers most of the abaxial aperture (0.7×0.6 mm) (Fig. 3AQ, 3AS). A unique trait is the differentiation of two distinct parts of the margin: (1) the part that is closer to the body of the nutlet is characterized by a dark stony surface with dense short papillose trichomes and dispersed long spines on its external half, and (2) the part of the margin more distant from the body of the nutlet is whitish, papiraceous wrinkled

with sparse short papillae towards the aperture (Fig. 3AQ). The margin is hollow and filled by an air chamber in its entire width (Fig 3AS). The inner side of the margin is smooth and glabrous (Fig. 3AS). Ornamentation of the abaxial side is densely covered by short papillae (Fig. 3AT).



The sister group of the Japanese clade (*Bothriospermum* and *Thyrocarpus*), displays some similar traits. The fruits of *Bothriospermum zeylanicum* Druce (1917: 610) are reniform and 1 × 1 mm (Fig. 3AX). The nutlet of this species is adaxially orientated contrary to the rest of the species. The scar is apical but completely displaced to the top of the nutlet, which together with the turning of the nutlet has been erroneously interpreted as a basal position (Ge-ling *et al.* 1995). The abaxial side is densely covered by short and long grouped papillae (Fig. 3BA). The margin is narrow, curved inwards with the entire edge densely covered by short papillae (Fig. 3AY, 3BA). As in *O. japonica*, the nutlet has a wrinkled layer that surrounds the aperture, with some short, sparse and smooth trichomes (Fig. 3AZ). This structure

of *Bothriospermum* is similar to the inner side of the margin observed in *O. japonica* (see above), but in this genus the layer is free. The ornamentation of the adaxial side is glabrous, flattish and also densely covered by short papillae. The fruit of *Thyrocarpus glochidiatus* is from ovoid to reniform and 2×1.4 mm (Fig. 3BD). The ornamentation of the adaxial side is similar to that of *B. zeylanicum* (Fig. 3BE). The margin is wide, curved inwards, with a deeply dentate edge (Fig. 3BD) and densely covered by short papillae that are longer at tips (Fig. 3BD). As in *Bothriospermum*, there is an inner free layer of wrinkled tissue, with sparsely short, smooth trichomes (Fig. 3BF). Interestingly, the abaxial side of the pericarp seems to be lost or extremely reduced, unlike all other samples of the studied genera (Fig. 3BF).

Discussion

Our phylogenetic results agree with most recent molecular phylogenies of Boraginaceae s.s. (Långström & Chase 2002, Långström & Oxelman 2003, Weigend *et al.* 2010, Nazaire & Hufford 2012, Weigend *et al.* 2013, Cohen 2014). The tribe Cynoglosseae s.l., as recently conceived (Långström & Chase 2002, Långström & Oxelman 2003, Weigend *et al.* 2010, Nazaire & Hufford 2012, Weigend *et al.* 2013, Cohen 2014), is formed by morphologically heterogeneous groups that were consistently recovered in the plastid (Fig 1), but not in the nuclear trees (Fig 2). The accessions of *Omphalodes* included in our analyses are placed in Cynoglosseae s.l. in the plastid tree, but not all of them are resolved in this group in the nuclear tree.

Three independent lineages of Omphalodes

Omphalodes is split into three independent lineages in agreement with Weigend *et al.* (2013). Previous studies already noted the heterogeneity of the genus. Indeed, De Candolle (1846) and Brand (1921) already segregated *O. scorpioides* from the remaining European taxa at sectional level. Later, Popov (1953) indicated that the Japanese taxa were morphologically distinct to the remaining species of the genus, and *O. scorpioides* was dissimilar to the remaining European *Omphalodes* in morphological and embryological features (see below). Pereira Coutinho *et al.* (2012) also noted the distinctiveness of the pollen of the Japanese species which have a ring-like equatorial aperture, absent in the other taxa. They also found differences in the pollen morphology between the Old World species of *Omphalodes* s.s. (margins granulate) and the New World taxa (margins smooth). However, they did not observe significant differences between the pollen of *O. scorpioides* and that of the other European species except that the pollen of *O. scorpioides* is more compact and globose.

A minor incongruence between plastid and ITS reconstructions was detected within *Omphalodes* s.s. Concerning the different placement of two European perennial species (*O. verna* and *O. nitida*). Only the clade containing the annual taxa of *Omphalodes* is recovered in both phylogenetic reconstructions (Figs. 1–2). However, no unique characters can be readily found to define any internal grouping within the *Omphalodes* s.s. clade (see Fig. 3A–AP). In contrast, the central deltoid attachment scar and the solid margin are found in all the species (Table 2). The low number of American species herein included (one (molecular) and three (carpological) species studied out of the total six American species)—prevented us from proposing a more solid phylogenetic hypothesis. Pereira Coutinho *et al.* (2012) reported common traits in the pollen for all the American species. In contrast, more variability was found in the nutlets of American species, in which *O. chiangii* has the most dissimilar ornamentation while *O. cardiophylla* and *O. aliena* are very similar (Fig 3A–E). The unique carpological traits displayed by *O. chiangii* (Fig. 3F–I), and the distinctive morphology of *O. erecta* from northeastern Mexico (erect habit, greater nutlet size (8 mm in width), three of four nutlets aborted and a distinct slightly dentate spreading wing; Johnston 1935), indicate that further taxonomic studies of North American species are necessary as previously suggested by Nesom (2013). The phylogenetic placement of the Oceanic megaherb *Myosotidium hortensia* within the *Omphalodes* s.s. clade is in agreement with previous molecular results (Heenan *et al.* 2011, Nazaire & Hufford 2012, Cohen 2014, Mozaffar *et al.* 2013, Weigend *et al.* 2013). There is a single trait shared between *Myosotidium* and the *Omphalodes* s.s. clade, namely the marginal wing of the nutlet. The findings of Weigend *et al.* (2013) and Nesom (2013), in which South American members of *Cynoglossum* (with no marginal wing) are nested within the *Omphalodes* s.s. clade, prevented us from proposing the transference of *Myosotidium* to *Omphalodes* s.s. One more argument against proposing a new nomenclatural combination is the poor molecular sample of North American species (one of eight). This is an important issue since a great morphological heterogeneity has been found within North American species (Nesom 2013). An extended sample in terms of species and DNA regions will help elucidate the systematics of this group.

The biennial European *O. scorpioides* is distantly related to *Omphalodes* s.s. (Fig. 2) because it forms an independent

lineage sister to the *Mertensia* clade in the ITS tree (Fig. 2). Dense low papillae on the nutlet epidermis is the single character shared by *O. scorpioides* and the other two genera of its sister group (*Asperugo* and *Mertensia*). Indeed, the nutlets of *O. scorpioides* are fairly distinct from those of *Asperugo* and *Mertensia* (Fig. 3AU, 3BG; Nazaire & Hufford 2012). In addition, a set of fruit characters such as the apical attachment scar, the hollow margin forming an air chamber that fills its entire width (Fig. 3AV) and the nearly smooth trichomes (Fig. 3AU-AW) readily distinguish this taxon from the species of the *Omphalodes* s.s. clade. Some morphological characters, such as the flower disposition along the stems in contrast to terminal inflorescences, and the curved rather than erect embryo (Popov 1953), are additional characters that support the independence of *O. scorpioides* from *Omphalodes* s.s. The distinction of *O. scorpioides* from the remaining taxa of the genus was already indicated by previous authors on the basis of morphological (De Candolle 1846, Brand 1921, Popov 1953) and molecular studies (Cohen 2014; Weigend *et al.* 2013). Our results help to resolve the sister group and morphological support for the lineage *Asperugo-Mertensia-O. scorpioides*. *O. scorpioides* is readily distinguished from *Asperugo* and *Mertensia* by characters such as nutlet shape, ornamentation and presence of aperture.

The third clade comprises the Japanese species of *Omphalodes*. The sample of the plastid phylogeny, which is more complete, reveals its monophyly (Fig. 1). The plastid tree reveals a sister-group relationship of the Japanese *Omphalodes* with a lineage of two Asian genera (*Bothriospermum secundum* and *Thyrocarpus sampsonii*) (Fig. 1), all three of which are placed within Cynoglosseae s.s., in agreement with Weigend *et al.* (2013). In addition, they displayed a unique character within *Omphalodes* s.l.: the two distinct parts of the nutlet margin (see above, Fig. 3AQ). This character was already emphasized in the taxonomic treatment for the Flora of Japan (Yamakazi 1993). Such smooth prolongation seems to be analogous to that found in *B. secundum* and *T. sampsonii* as a free inner layer. Other traits that differentiate the fruit of the Japanese *Omphalodes* from the taxa included in *Omphalodes* s.s. are the hollow margin completely filled by an air chamber, and the upper position of the attachment scar. Similarly, *Thyrocarpus* and *Bothriospermum* have an apical scar. Remarkably, the attachment scar of *Bothriospermum* has been interpreted to be in a basal position (Ge-ling *et al.* 1995). However, a close observation shows that the nutlet is completely turned inward, leaving the aperture in an adaxial position, and thus apparently attached by its base.

Taxonomic proposal splitting Omphalodes s.l.

This study is based on a representative sample of *Omphalodes*. Three independent lineages consistently found in the nuclear and plastid phylogenetic reconstructions (Figs. 1–2) are supported by morphological characters, which should be taxonomically acknowledged. The fruit study provided a detailed source of information that support the partition of *Omphalodes*, as character states are shared by taxa within each of the three lineages. As a result, a reorganization of *Omphalodes* s.l. is partly feasible at least for *O. scorpioides* and the Japanese taxa.

Other names used at the generic level to refer to *Omphalodes* taxa are *Picotia* Roemer & Schultes (1819: 10) and *Omphalium* (Wallr.) Roth (1827: 590). *Picotia* is an illegitimate name (*nomen superfluum*) since it explicitly cited *Omphalodes* as synonym and included *Omphalodes verna*, which is the type species of the genus *Omphalodes* (van Ooststroom *et al.* 1961). *Omphalium* has *Cynoglossum* sect. *Omphalium* Wallr. as basionym, which is a legitimate name. However, this name is also taxonomically superfluous at the genus level because the protologue of its basionym also cited *Omphalodes* as a synonym and included *O. verna*. *Omphalodes scorpioides* has been included in the monotypic section *Maschalanthus* (De Candolle 1846, Brand 1921, Popov 1953); however at the genus level *Maschalanthus* would be illegitimate, as it would be a later homonym of the moss genus *Maschalanthus* Sprengel ex Schultz (1806: 356). As far as we know, the Japanese species do not have any previous taxon recognition at the genus level, as most of the species are of recent description.

As a result, we propose the creation of two new genera to accommodate *O. scorpioides* from Europe (*Memoremea*) and the Japanese species of *Omphalodes* (*Nihon*).

Nomenclature

Omphalodes Miller (1794: 968). Lectotype (designated by Stafleu (van Ooststroom *et al.* 1961)):*—Omphalodes verna* Moench (1794: 420).

≡ *Picotia* Roemer & Schultes, (1819: 10), nom. superfl. Lectotype here designated:*—Picotia verna* (Moench) Roemer & Schultes (≡ *Omphalodes verna* Moench)

≡ *Cynoglossum* sect. *Omphalium* Wallroth (1822: 77). Lectotype here designated:*—Cynoglossum omphalodes* L. (≡ *Omphalodes verna* Moench)

≡ *Omphalium* (Wallr.) Roth (1827: 590)

TABLE 3. Key characters defining the three independent lineages of *Omphalodes* s.l.

Clade	Habit	Inflorescences	Pollen	Nutlet scar position	Nutlet margin	Embryo position
<i>Omphalodes</i> s.s.	Annual/ Perennial	Terminal	Oblong, no ring-like aperture	Central	Solid, differently ornamented	Erect
<i>Omphalodes scorpioides</i> (<i>Memoremea scorpioides</i>)	Biennial	Axilar	Globose, no ring-like aperture	Apical	Hollow, homogeneously ornamented (smooth trichomes)	Curved
Japanese species (<i>Nihon</i>)	Perennial	Terminal	Oblong, ring-like aperture	Apical	Hollow, abrupt ornamentation change towards the edge (hairy-spiny to wrinkled and nearly smooth)	Unknown

Memoremea Otero, Jim.-Mejías, Valcárcel & P. Vargas *gen. nov.* Type:—*Memoremea scorpioides* (Haenke) Otero, Jim.-Mejías, Valcárcel & P. Vargas \equiv *Omphalodes* sect. *Maschalanthus* De Candolle (1846:161)

Description. This new genus resembles *Omphalodes* in nutlet shape and size, from which it can be distinguished by the apical attachment scar, the strongly incurved entire, wide and hollow margin of the nutlet with an air chamber, and the presence of smooth trichomes on both adaxial and abaxial sides of the nutlet. In addition, the more compact and globose pollen grains, and axillary inflorescence distinguish *Memoremea* from *Omphalodes* s.s.

Etymology. The Latin phrase “*Memore me*” which means “Remember me”, which would be complementary to “Forget-me-not”, the common name that refers to some species of the tribe Cynoglosseae in many languages, especially species of the genus *Myosotis*.

Memoremea scorpioides (Haenke) Otero, Jim.-Mejías, Valcárcel & P. Vargas, *comb. nov.*

\equiv *Cynoglossum scorpioides* Haenke (1788: 3) (basionym)

\equiv *Omphalodes scorpioides* (Haenke) Schrank (1812: 222)

Nihon Otero, Jim.-Mejías, Valcárcel & P. Vargas *gen. nov.* Type:—*Nihon japonicum* (Maxim.) Otero, Jim.-Mejías, Valcárcel & P. Vargas

Description. This new genus resembles *Omphalodes* in nutlet shape and size, from which it can be distinguished by the ornamentation of the margin of the nutlet, which abruptly changes towards the aperture from hairy-spiny to wrinkled and nearly smooth resulting in two distinctive parts. In addition, the nutlet scar is found apically and the margin is completely hollow by an extensive air chamber. An additional key character to distinguish this new genus is the presence of a ring-like equatorial aperture on the pollen grains, that is absent in the rest of species of *Omphalodes*.

Etymology. *Nihon* is the name of Japan in Japanese as written in Latin alphabet.

Nihon japonicum (Thunb.) Otero, Jim.-Mejías, Valcárcel & P. Vargas *comb. nov.*

\equiv *Cynoglossum japonicum* Thunberg (1784: 187) (basionym)

\equiv *Omphalodes japonica* (Thunb.) Maximowicz (1872: 452)

Nihon akiensis (Kadota) Otero, Jim.-Mejías, Valcárcel & P. Vargas *comb. nov.*

\equiv *Omphalodes akiensis* Kadota (2009: 342) (basionym).

Nihon krameri (Franch. & Sav.) Otero, Jim.-Mejías, Valcárcel & P. Vargas *comb. nov.*

\equiv *Omphalodes krameri* Franchet & Savatier (1879: 452) (basionym).

Nihon laevispermum (Nakai) Otero, Jim.-Mejías, Valcárcel & P. Vargas *comb. nov.*

\equiv *Omphalodes laevisperma* Nakai (1949: 17) (basionym).

Nihon proliferum (Ohwi) Otero, Jim.-Mejías, Valcárcel & P. Vargas *comb. nov.*

\equiv *Omphalodes prolifera* Ohwi (1956: 98) (basionym).

Conclusions

The phylogenetic reconstructions of our study, coupled with morphological characters of the nutlet, help us to propose a more natural classification of *Omphalodes* species. The inclusion of 14 of the 29 recognized species of *Omphalodes* in our phylogenetic study, clearly supports the polyphyly of the genus. Indeed, we found three independent lineages, which were consistent with results from recent publications. The morphological nutlet differences herein found provided further support for the three lineages of *Omphalodes*. In addition, vegetative and reproductive (inflorescence, pollen ornamentation) characters used in previous taxonomic treatments give solid grounds to recognise three genera, two of them newly proposed: *Memoremea* and *Nihon*. Despite the considerable sampling effort made for this study, additional investigations are needed to infer phylogenetic relationships of all the species of *Omphalodes* from North America and within *Nihon*. Our study has also provided an extended phylogenetic reconstruction of Boraginaceae s.s., especially tribe Cynoglosseae s.l., which also needs further sample of species and DNA sequencing regions.

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Appendix S1

List of material used for the molecular study. Asterisks indicate new Genbank accession numbers (this study).

Species	Country (Locality)	Voucher	ITS	<i>trnL-trnF</i>
<i>Actinocarya tibetica</i> Benth.	China (Xizang)	G. & S. Miehe 03-030-08 (MSB)	*KF849172	
<i>Alkanna sieberi</i> A.DC.	Greece (Crete)	A. Kagiampaki 2000/10 (BSB)	FJ763199	FJ763263
<i>Alkanna tinctoria</i> (L.) DC.	Spain	H.H.Hilger s.n. 05.06.2006 (BSB)	FJ763250	FJ763304
<i>Amsinckia calycina</i> (Moris) Cagter	Spain (Zamora)/Perú	Bariego, PB-1516 (MA)/ M. Weigend & Ch. Schwarzer 8031 (BSB)	*KF849140	GQ285246
<i>Amsinckia eastwoodiae</i> J. F. Macbride	UNKNOWN	Helmkamp 6530 (SD)	JQ513391	JQ582293
<i>Amsinckia intermedia</i> Fisch. & C. A. Mey.	UNKNOWN	Simpson 2791 (SDSU 17575)	JQ513392	JQ582294
<i>Amsinckia spectabilis</i> Fisch. & C. A. Mey. var. <i>microcarpa</i> (Greene) Jeps. & Hoover	UNKNOWN	Helmkamp 8336 (SD)	JQ513393	JQ582295
<i>Anchusa crispa</i> Viv.	France (Corse)	F. Selvi & M. Bigazzi 99.005 (FI)	GQ285227	GQ285252
<i>Anchusa italica</i> Retz.	Spain	H.H. Hilger s.n. (BSB)	GQ285233	GQ285268
<i>Anchusa formosa</i> Selvi, Bigazzi & Bacchetta	Italy	M. Bigazzi & F. Selvi 97.006 (FI)	GQ285226	GQ285251
<i>Arnebia decumbens</i> (Vent.) Cosson & Kral.	Tunesia	D. Podlech 32857 (M)	FJ763239	FJ763294
<i>Asperugo procumbens</i> L.	Sweden (Uppsala)/ China (Xinjiang)	Alm 1283 (WS)/ J. F. Huang 20090190 (XJBI)	JQ388497	JX976911
<i>Borago officinalis</i> L.	Germany	O. Mohr 600 (BSB)	FJ763248	FJ763302
<i>Bothriospermum secundum</i> Maxim.	China (Shandong, Lianchnghzhen)	Goucheng-yo 20063-603-8 (MA)		*KF849184
<i>Brunnera orientalis</i> (Schenk) I.M. Johnst.	Turkey (Gümüshane)	Bigazzi & Selvi 00.28 (BSB, FI)	AF531087	GQ285253
<i>Brunnera sibirica</i> Steven	Cultivated	M. Weigend 9066 (BSB)	GQ285234	GQ285273
<i>Buglossoides calabra</i> (Ten.) I.M. Johnst.	Italy (Calabria)	A. Coppi & L. Cecchi 07/56 (FI, BSB)	FJ763251	FJ763305
<i>Buglossoides gastonii</i> (Benth.) I.M. Johnst.	Spain (Pyrenees)	BG München-Nymphenburg G/1125 (M)	FJ788930	FJ788929
<i>Caccinia macranthera</i> Brand	Armenia (Kotayk)	Fayvush <i>et al.</i> 05-0683790641 (MA)		*KF849215
<i>Caccinia strigosa</i> Boiss.	Germany (Berlin Cult. Syst. Bot.)	O. Mohr M592 (BSB)		GQ285241
<i>Ceballosia fruticosa</i> (L.f.) Kunkel ex Förther	UNKNOWN	Weigend & Weigend 8703 (B)	HQ286133	HQ286165
<i>Cerintho minor</i> L. subsp. <i>auriculata</i> (Ten.) Domac	Italy	M. Bigazzi & F. Selvi 03.06 (BSB)	FJ763223	FJ763281
<i>Cerintho major</i> L.	Spain	M. Bigazzi & F. Selvi 04.22 (BSB)	FJ763244	FJ763298
<i>Codon schenckii</i> Schinz	Namibia	H. & E. Walter 118 (B)		GQ285270
<i>Cordia decandra</i> Hook. & Arn.	UNKNOWN	Luebert & Kritznern 1873 (SGO EIF)	EF688903	EF688851
<i>Craniospermum subvillosum</i> Lehm. (Lac. Baikal)	UNKNOWN	M. Popov 3848 (MO)		*KF849214
<i>Cryptantha affinis</i> (A. Gray) Greene	UNKNOWN	Reiser s. n. (SDSU)	JQ513395	JQ582297
<i>Cryptantha diffusa</i> (Phil.) I. M. Johnst.)	UNKNOWN	Munoz 2745 (MO)	JQ513408	JQ582310
<i>Cryptantha flavoculata</i> Payson	UNKNOWN	UNKNOWN	AF091154	
<i>Cryptantha foliosa</i> (Greene) Greene	UNKNOWN	Rebman 6803 (SD)	JQ513413	JQ582315
<i>Cryptantha granulosa</i> (Ruiz & Pav.) I. M. Johnst.	UNKNOWN	Weigend 2000/642 (MO)	JQ513416	JQ582318
<i>Cryptantha maritima</i> Greene	UNKNOWN	Simpson 3043 (SDSU)	JQ513425	JQ582327
<i>Cryptantha minima</i> Rydberg	UNKNOWN	Freeman 14292 (COLO)	JQ513429	JQ582331
<i>Cryptantha nevadensis</i> A. Nels. & P. B. Kennedy var. <i>nevadensis</i>	UNKNOWN	Gregory 1305 (SD)	JQ513432	JQ582334
<i>Cryptantha patagonica</i> (Speg.) I. M. Johnst.	Argentina	M. Weigend <i>et al.</i> 5957 (BSB)		GQ285256
<i>Cryptantha racemosa</i> (A. Gray) Greene	UNKNOWN	Rebman 6305 (SDSU)	JQ513436	JQ582338

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Appendix S1. (Continued)

Species	Country (Locality)	Voucher	ITS	trnL-trnF
<i>Cynoglossum amabile</i> Stapf. & Drum.	Bolivia (La Paz)	Beck 8670 (M)	*KF849142	*KF849232
<i>Cynoglossum amabile</i> Stapf. & Drum.	Germany (Culltiv Munich)/	H. Förther s.n (MSB)	*KF849141	
<i>Cynoglossum australe</i> R. Br.	Australia (Tasmania, Lookout)	M. Visoiu 102 (MA)	*KF849126	*KF849187
<i>Cynoglossum cheiriflorum</i> L.	Spain (Granada)	C. Aedo, 9749 (MA)	*KF849129	*KF849203
<i>Cynoglossum divaricatum</i> Steph. ex Lehm.	Russia (Burytia)	I. Chan, E. Balde s.n. (NSK)	*KF849131	
<i>Cynoglossum germanicum</i> Jacq.	Germany (Harz, Sachsen-Anhalt)/ Cult. Botanischer Garten Berlin-Dahlem	Hilger 1999 (BSB, FI)/H. H. Hilger s. n. (BSB)	FR715306	GQ285245
<i>Cynoglossum lanceolatum</i> Forssk.	Pakistan (Karakorum)/ South africa republic (Kwazulu-Natal)	B. Dickoré 12183 (MSB)/C. Aedo <i>et al.</i> 15048 (MA)	*KF849143	*KF849202
<i>Cynoglossum ca. obtusicalix</i> Retief & A.E.van Wyk.	South africa republic (Eastern Cape, Rhodes)	Aedo <i>et al.</i> 15140 (MS)		*KF849196
<i>Cynoglossum officinale</i> L.	Iran/ Cult. Botanical Garden Berlin-Dahlem	Assadi s.n. (TARI)/H. H. Hilger s. n. (BSB)	AB758292	GQ285248
<i>Cynoglottis chetikiana</i> Vural & Kit Tan subsp. <i>paphlagonica</i> (Hauskn. ex Bornm.) Vural & Kit Tan	Turkey	F. Selvi & M. Bigazzi (BSB, FI)	GQ285228	GQ285254
<i>Echium vulgare</i> L.	Germany	O. Mohr No. 597 (BSB)	FJ763247	FJ763301
<i>Echium creticum</i> L.	(cult.) BG München;	H. Foerther s.n. (BSB)	FJ763249	FJ763303
<i>Echiochilon fruticosum</i> Desf.	Egypt/Libya	Lundquist 5655 (UPS)/Kagiampaki s.n. (BSB)	AJ555908	FJ763310
<i>Echiochilon lithospermoides</i> (S. Moore) I.M. Johnst.	Ethiopia	Gilbert & Sebsebe 8700 (UPS)	AJ555912	
<i>Echiochilon persicum</i> (Burm.f.) Johnst.	Iran	Mozaffarian 49917 (TARI)	AB758293	AB758322
<i>Ehretia tinifolia</i> L.	UNKNOWN	Gottschling CUB52 (BSB)		HQ286270
<i>Ehretia ovalifolia</i> Hassk.	UNKNOWN	UNKNOWN	AF091156	
<i>Embadium stagnense</i> J.M. Black	Australia (Gairdner-Torrens, Kokatha)	F.J. Badman 8530 (AD)	*KF849113	
<i>Embadium stagnense</i> J.M. Black	Australia (Gairdner-Torrens, Mt Wallaby)	R.J. Bater 57455B (AD)	*KF849114	*KF849241
<i>Embadium johnstonii</i> Ising	Australia (Lake Eyre)	R.J.-P. Davies 694 (AD)	*KF849117	
<i>Embadium johnstonii</i> Ising.	Australia (Lake Eyre)	R.J.-P. Davies 695 (AD)	*KF849118	
<i>Embadium uncinatum</i> Ising	Australia (Eyre peninsula, Scrubby Peak)	M.J. Thorpe 48 <i>et al.</i> (AD)	*KF849115	*KF849222
<i>Embadium uncinatum</i> Ising	Australia (Gawler ranges)	M.J. Thorpe 50 <i>et al.</i> (AD)	*KF849116	
<i>Eremocarya micrantha</i> Greene	UNKNOWN	Rebman 11358 (SD)	JQ513427	JQ582329
<i>Eritrichium aretioides</i> (Cham.) DC.	USA (Alaska Noatak Quad)	Parker, Elven & Solstad 14806 (O)		JQ388579
<i>Eritrichium canum</i> (Benth.) Kitam.	Germany (Cult. in Munich Botanical Garden)/Germany (Cult. Botanischer Garten Muenchen-Nymphenburg)	-/H. Foerther s.n. (M)	AB758294	GQ285242
<i>Eritrichium chamissonis</i> DC.	Canada (Yukon Territory)	Solstad & Elven 03/0601A (O)		JQ388580
<i>Eritrichium nanum</i> (L.) Schrader ex Gaudin	Switzerland/ USA (Colorado, El Paso Co.)	Hertel 25764 (MSB)/ Nazaire 1809 (WS)	AY092901	JQ388581
<i>Eritrichium splendens</i> Kearney ex W. Wight	Alaska (Noatak Quad)	Solstad & Elven 03/1216 (O)	JQ388501	JQ388582
<i>Greeneocharis circumscissa</i> (Hook. & Arn.) Rydb.	UNKNOWN	Simpson 3108 (SDSU)	JQ513403	JQ582305
<i>Greeneocharis similis</i> (K.Mathew & P.H.Raven) Hasenstab & M.G.Simpson	UNKNOWN	Henrickson 17339 (RSA)	JQ513439	JQ582341

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Appendix S1. (Continued)

Species	Country (Locality)	Voucher	ITS	trnL–trnF
<i>Hackelia deflexa</i> (Wahlenb.) Opiz	China (Xinjiang)/Italy	J. F. Huang 20090109 (XJBI)/W. Frey s. n. (BSB)	JX976808	GQ285244
<i>Hackelia diffusa</i> (Lehm.) I. M. Johnst.	USA (Washington, Kittitas Co.)	Lopushinsky 07-6 (WS)	JQ388503	JQ388583
<i>Hackelia micrantha</i> (Eastw.) J. L. Gentry,	USA (Oregon, Grant Co.),	Hinchliff 869 (WS)	JQ388504	JQ388584
<i>Hackelia revoluta</i> I.M. Johnst.	Argentina (La Rioja)	C. Aedo 15407 (MA)	*KF849119	*KF849224
<i>Harpagonella palmeri</i> A. Gray	USA (California)	S. Boyd 3045 (MSB)	*KF849150	*KF849234
<i>Heterocaryum laevigatum</i> (Kar. et Kir.) DC.	Iran	Faghihna & Zangooei 25349 (FUMH)	AB758296	AB758325
<i>Heterocaryum macrocarpum</i> Zak.	Iran	Joharchi & Zangooei, 19639 (TMUH)	AB758300	AB758329
<i>Heterocaryum szoviisianum</i> (Fisch. & Mey.) DC.	Iran	Kazempour Osaloo, 2007-5 (TMUH)	AB758298	AB758327
<i>Johnstonella angustifolia</i> (Torr.) Hasenstab & M.G.Simpson	UNKNOWN	Simpson 8III98A (SDSU)	JQ513397	JQ582299
<i>Johnstonella parviflora</i> (Phil.) Hasenstab & M.G.Simpson	UNKNOWN	Van der Werff 20532 (MO)	JQ513433	JQ582335
<i>Lappula anocarpa</i> C. J. Wang	China (Xinjiang)/China(Xinjiang)	J. F. Huang & B. C. Han 201008016(XJBI)/Juan Qiu 08-0007 (XJA)	JX976775	JQ388585
<i>Lappula barbata</i> (M. B.) Gürke	Iran	Kazempour Osaloo 2008-1(TMUH)	AB564703	AB564713
<i>Lappula ceratophora</i> (M. Pop.) M. Pop.	Iran	Mozaffarian 58407(TARI)	AB758301	AB758330
<i>Lappula sessiliflora</i> (Boiss.) Gürke	Iran	Kazempour Osaloo 2007-3 (TMUH)	AB564704	AB564714
<i>Lappula squarrosa</i> (Retz.) Dumort.	USA (Utah, First Water Canyon)/Cult. Syst. Bot. Berlin	S. L. Welsh & E. Ne 21211 (BRY)/O. Mohr 591 (BSB)	JX976797	GQ285265
<i>Lepechiniella michaelis</i> Golosk.	Kazakhstan (Aalatau Dshungaricus)	V. Goloskokov s.n. (MO)		*KF849223
<i>Lepechiniella inconspicua</i> (Brand) Riedl	Iran	Joharchi & Ayatollahi (FUMH)	AB758311	AB758338
<i>Lepechiniella microcarpa</i> (Boiss.) Riedl subsp. <i>inconspicua</i> (Brand) F.Sadat	Afghanistan (Futur-Tal)	Roemer 110 (M)	*KF849127	
<i>Lepechiniella microcarpa</i> (Boiss.) Riedl subsp. <i>inconspicua</i> (Brand) F.Sadat	Afghanistan. (Kapisa)	A. Dieterle 52 (M)	*KF849128	
<i>Lindelofia anchusoides</i> subsp. <i>anchusoides</i> (Lindl.) Lehmann	Afghanistan (Badakhshan)	C.Schloeder & M.Jacobs 1478 (MSB)	*KF849145	*KF849230
<i>Lindelofia anchusoides</i> (Lindl.) Lehm. subsp. <i>aspera</i> (Rech.f.) F.Sadat	Afghanistan (Ghazni)	D.Podlech 31875 (MSB)	*KF849146	
<i>Lithodora diffusa</i> (Lag.) I.M.Johnst.	Spain (Pl. de Toro)	SANT58512	FJ789863	
<i>Lithodora fruticosa</i> Griseb.	Spain (Montefrío)	SANT58519	FJ789864	
<i>Lithospermum cinereum</i> DC.	Republic of South Africa	O.A. Leistner 2109 (M)	FJ763240	FJ763295
<i>Lithospermum officinale</i> L.	Germany	A. Werres & M. Ristow s.n. (BSB)	FJ763189	FJ763254
<i>Mertensia alpina</i> (Torr.) G.	USA (DonColorado, El Paso Co.)	Nazaire 1810 (WS)	JQ388507	JQ388587
<i>Mertensia davorica</i> (Sims) G. Don.	China (Hebei)	Nazaire 1889 (WS)	JQ388509	JQ388589
<i>Mertensia maritima</i> (L.) Gray	Canada (Nunavut)/UK (Shetlands)	Kines s.n. (WS)/H.H. Hilger s.n.(BSB)	JQ388510	GQ285259
<i>Mertensia maritima</i> (L.) Gray	Canada (Quebec)	J.A. Churchill 7572013 (RSA)	*KF849122	
<i>Mertensia oblongifolia</i> (Nutt.) G. Don var. <i>nevadensis</i> (A. Nelson) L. O. Williams	USA (Oregon, Harney Co.)	Nazaire & Bunch 1748 (WS)	JQ388511	JQ388591
<i>Mertensia sibirica</i> (L.) G. Don	China (Shanxi)	Nazaire 1892 (WS)	JQ388513	JQ388593
<i>Mertensia virginica</i> (L.) Pers. ex Link.	USA (Kentucky, Jefferson Co.)/cult. Institut für Biologie – Systematische Botanik und Pflanzengeographie, FU Berlin	Collins ch3 (WS)/M. Weigend 8134 (BSB)	JQ388514	GQ285267

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Appendix S1. (Continued)

Species	Country (Locality)	Voucher	ITS	<i>trnL-trnF</i>
<i>Microparacaryum intermedium</i> (Fresen.) Hilger & Podlech	Egypt (Sinai)	D.Podlech 49702 (MSB)	*KF849124	*KF849240
<i>Microparacaryum intermedium</i> (Fres.) Hilger & Podlech <i>var. stellatum</i> (H.Riedl) f. <i>paracaryoides</i> Hilger & Podlech	Afghanistan (Kandahar)	Freitag & Breckle 4629 (MSB)	*KF849125	
<i>Microula diffusa</i> W.T. Wang	China (Gande Xian)	T.N. Ho <i>et al.</i> 937 (A)		*KF849183
<i>Microula diffusa</i> W.T. Wang	China (Gonde Xian)	T.N. Ho <i>et al.</i> 937 (MO)		*KF849180
<i>Microula diffusa</i> W.T. Wang	China (Xizang, S Tibet)	B. Dickoré 9895 (MSB)	*KF849160	
<i>Microula diffusa</i> W.T. Wang	China (Xizang, E Tibet)	B. Dickoré 9150 (MSB)	*KF849153	
<i>Microula floribunda</i> W.T. Wang	China (Xizang, E Tibet)	B. Dickoré 9149 (MSB)	*KF849157	
<i>Microula floribunda</i> W.T. Wang	China (Qinghai, Xindu Xian)	D.E. Boufford <i>et al.</i> 26990 (A)	*KF849155	
<i>Microula floribunda</i> W.T. Wang	China (Qinghai, Yushu Xian)	D.E. Boufford <i>et al.</i> 26746 (A)	*KF849156	
<i>Microula floribunda</i> W.T. Wang	China (Qinghai, Nangqen Xian)	T.N. Ho <i>et al.</i> 2715 (MO)	*KF849158	*KF849242
<i>Microula forrestii</i> I.M. Johnst.	China (Xizang, SE Tibet)	B. Dickoré 10759 (MSB)	*KF849159	
<i>Microula longituba</i> W.T. Wang	China (Qinghai, Chindy Xian)	T.N. Ho <i>et al.</i> 1811 (MO)		*KF849181
<i>Microula muliensis</i> W.T. Wang	China (Sichuan Sêrtar Xian)	D.E. Boufford <i>et al.</i> 27850 (A)	*KF849170	*KF849201
<i>Microula myosotidea</i> I.M. Johnst. (with <i>Microula ovalifolia</i>)	China (Qinghai, Nangqên Xian)	T.N. Ho <i>et al.</i> 2926 (A)	*KF849164	*KF849199
<i>Microula oblongifolia</i> Hand.-Mazz.	China (Qinghai, Jiuzhi Xian)	D.E. Boufford <i>et al.</i> 39457 (MBK)	*KF849163	*KF849190
<i>Microula ovalifolia</i> I.M. Johnst. (with <i>Microula myosotidea</i>)	China (Qinghai, Nangqên Xian)	T.N. Ho <i>et al.</i> 2926 (A)	*KF849165	*KF849200
<i>Microula ovalifolia</i> I.M. Johnst.	China (Yunnan, Zhongdian)	D.Podlech 54539 (MSB)	*KF849169	
<i>Microula pseudotricocarpa</i> W.T. Wang	China (Sichuan, Ruoergai Xian)	D.E. Boufford <i>et al.</i> 40204 (A)	*KF849152	
<i>Microula pseudotricocarpa</i> W.T. Wang	China (Sichuan, Xiaojin Xian)	D.E. Boufford <i>et al.</i> 38545 (MBK)	*KF849173	
<i>Microula pseudotricocarpa</i> W.T. Wang	China (Sichuan, Serxu Xian)	D.E. Boufford <i>et al.</i> 33630 (MBK)	*KF849177	*KF849197
<i>Microula pseudotrichocarpa</i> W.T. Wang	China (Qinghai, Gande Xian)	T.N. Ho <i>et al.</i> 939 (MO)	*KF849174	
<i>Microula pustulosa</i> W.T. Wang	China (Qinghai, Nangqên Xian)	T.N. Ho <i>et al.</i> 2926 (MO)		*KF849182
<i>Microula sikkimensis</i> (C.B. Clarke) Hemsley	China (Sichuan, Aba Xian)	D.E. Boufford <i>et al.</i> 39444 (MBK)	*KF849166	*KF849189
<i>Microula sikkimensis</i> Hemsley	China (Qinghai, Yushu Xian)	T.N. Ho <i>et al.</i> 2326 (MO)	*KF849176	
<i>Microula stenophylla</i> W.T. Wang	China (Qinghai, Yushu Xian)	D.E. Boufford <i>et al.</i> 26810 (A)	*KF849161	
<i>Microula stenophylla</i> W.T. Wang	China (Qinghai, Dari Xian)	T.N. Ho <i>et al.</i> 1138 (MO)	*KF849162	*KF849243
<i>Microula tibetica</i> Benth.	China (Xizang, Tibet)	D.E. Boufford <i>et al.</i> 32026 (MSB)	*KF849168	
<i>Microula tibetica var. tibetica</i> Maxim.	China (Qinghai, C Tibet)	B. Dickoré 4394 (MSB)	*KF849167	
<i>Microula tibetica var. laevis</i> W.T. Wang	China (Xizang)	G. & S. Miede 03-052-16 (MSB)		*KF849193
<i>Microula turbinata</i> W.T. Wang	China (Sichuan, Honguan Xian)	D.E. Boufford 40079 (MBK)	*KF849154	*KF849198
<i>Microula younghusbandii</i> Duthie	China (Qinghai, Chindu Xian)	T.N. Ho <i>et al.</i> 1724 (MO)	*KF849175	*KF849185
<i>Myosotidium hortensia</i> (Decne.) Baill.	New Zealand (Chatham Islands)	PB Heenan s.n. (CHR)	*KF849096	*KF849208
<i>Myosotis caespitosa</i> DC.	Finland	H. H. Hilger 1575 (BSB)		GQ285262
<i>Myosotis congesta</i> Shuttlew. ex Alb. & Reynier	Greece	Phitos M-33 (MSB)	AY092916	
<i>Myosotis discolor</i> Pers.	New Zealand (Kahuterawa Valley, North Island) (naturalized introduction)/-	MPN 11910/Ahart 9593 (JEPS)	AY092919	JQ582358
<i>Myosotis incrassata</i> Guss.	Greece/Greece (Crete)	Merxtnüller & Wiedmann 20130 (MSB)/H. H. Hilger Kreta 1998/5 (BSB)	AY092922	GQ285243
<i>Myosotis sparsiflora</i> Mikan	Cult. Syst. Bot. Berlin	M. Weigend 8138 (BSB)		GQ285239
<i>Myosotis stolonifera</i> J. Gray	UK (Shetlands)	Hilger s. n. (BSB)		GQ285258
<i>Neatostema apulum</i> (L.) I.M. Johnst.	USA (Hawaii, Maui)	AMWF7	GQ478099	
<i>Nicotiana glauca</i> Graham	Hawaii, Maui, Kahului airport	AMWF7	GQ478099	

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Appendix S1. (Continued)

Species	Country (Locality)	Voucher	ITS	<i>trnL-trnF</i>
<i>Nicotiana gossei</i> Domin	UNKNOWN	R. G. Olmstead S-48 (WTU)		AY098700
<i>Nicotiana paniculata</i> L.	UNKNOWN	T. Helgason & A. Monro 502 (BM)/R. G. Olmstead S53 (WTU)	AJ492413	AY098701
<i>Ogastemma pusillum</i> (Coss. & Durieu ex Bonnet & Barratte) Brummitt	Saudi Arabia	Lady Rosemary Fitzgerald 73c (READ)	FJ763201	FJ763265
<i>Omphalodes akiensis</i> Kadota	Japan	Suga s.n. (B)		KC542616
<i>Omphalodes aliena</i> A.Gray ex Hemsl.	USA (Texas)	T.R. Van Devender <i>et al.</i> 85-86 (MO)	*KF849098	*KF849205
<i>Omphalodes aliena</i> A.Gray ex Hemsl.	Mexico	Hinton 28565 (TEX)		KC542600
<i>Omphalodes brassicifolia</i> Sweet	Spain (Extremadura, Cáceres)	M. Ladero & A. Amor 15416 (MA)	*KF849105	*KF849244
<i>Omphalodes cappadocica</i> DC	Georgia (Adjara)	A. Gröger <i>et al.</i> 1518 (M)	*KF849100	*KF849206
<i>Omphalodes caucasica</i> Brand	Russia (Abchasia)	A.K. Skvortsov s.n. (M)	*KF849101	*KF849207
<i>Omphalodes commutata</i> G. López	Spain (Cádiz, Grazalema)	A. Aparicio, García & Silvestre s.n. (MA)	*KF849108	*KF849225
<i>Omphalodes japonica</i> (Thunb.) Maxim.	Japan (Kochi, Nisimine)	K. Kamimure <i>et al.</i> FOK-065685 (MBK)		*KF849191
<i>Omphalodes japonica</i> (Thunb.) Maxim.	Japan (Kochi, Mt. Kojio-yama)	Kuroiwa <i>et al.</i> FOK-076224 (MBK)	*KF849151	*KF849192
<i>Omphalodes kuzinskyanae</i> Willk.	Portugal (Estremadura, Cascais)	D. Dracep 498936 (MA)	*KF849106	*KF849212
<i>Omphalodes linifolia</i> Moench	Spain (Jaén)/cultivated in Munich Botanical Garden	T. Carrera s.n. (MA)/-	*KF849109	AB758344
<i>Omphalodes littoralis</i> Lehm <i>subsp. gallaecia</i> Lainz	Spain (Galicia, Coruña)	R. Carbajal & M. Serrano s.n. (MA)	*KF849107	*KF849213
<i>Omphalodes luciliae</i> Boiss.	Greece (Makedonia)	M. Erben sn. (M)	*KF849097	*KF849204
<i>Omphalodes luciliae</i> Boiss.	Turkey (Antalya)	P.H. Davis 15609 (M)	*KF849099	*KF849211
<i>Omphalodes nitida</i> (Hoffmanns. & Link ex Willd.) Hoffmanns & Link	Spain (Galicia, Orense)	B. Casaseca 263 (MA)	*KF849171	*KF849237
<i>Omphalodes scorpioides</i> Schrank	Austria (Niederösterreich)	H. Merxmüller & O. Angerer 33286 (M)	*KF849120	*KF849221
<i>Omphalodes scorpioides</i> Schrank	Germany (Bayern, chwandorf)	M. & K. Weigend 7116 (M)	*KF849121	*KF849220
<i>Omphalodes verna</i> Moench	Italy (Liguria)	Podlech 1963 (M)		*KF849238
<i>Omphalodes verna</i> Moench	Germany (Bayern, berbayern)	F. Schuhwerk 06/265 <i>et al.</i> (M)	*KF849103	*KF849210
<i>Omphalodes verna</i> Moench	Slovenia (Primorsko)	E. Hörandl & F. Hadacek 5832 (MA)	*KF849102	*KF849209
<i>Onosmodium occidentale</i> Mack.	USA (Wyoming)	K.H. Dueholm 7141 (NY)	FJ763202	FJ763266
<i>Onosmodium virginianum</i> A.DC	USA (South Carolina)	J. Nelson 21082 (USCH)	FJ763197	FJ763261
<i>Oreocarya humilis</i> Greene	UNKNOWN	Honer 1089 (RSA)	JQ513418	JQ582320
<i>Oreocarya suffruticosa</i> (Torr.) Greene	US (Arizona)	Lambinon 03/US/315 (MA)		*KF849195
<i>Oreocarya weberi</i> (I.M.Johnst.) W.A.Weber	UNKNOWN	Rondeau s. n. (COLO)	JQ513444	JQ582346
<i>Paracaryum laxiflorum</i> Trautv.	Turkey (Zonguldak)	C. Aedo <i>et al.</i> 6272 (MA)	*KF849135	*KF849188
<i>Paracaryum persicum</i> (Boiss.) Boiss.	Iran	Kazempour Osaloo 2007-8 (TMUH)	AB758317	AB758345
<i>Pardoglossum cheirifolium</i> (L.) Barbier & Mathez	Tunisia (Kasserine)	Bigazzi & Selvi 04.25 (FI)	FR715320	
<i>Phacelia tanacetifolia</i> Benth.	USA (California, Inyo Co.)	Gilbert 108 (SFSU)	AY630332	
<i>Pectocarya penicillata</i> (Hook. & Arn.) A. DC.	UNKNOWN	Lauri 189 (SDSU 16855)	JQ513450	JQ582349
<i>Pectocarya peninsularis</i> I. M.Johnst.	UNKNOWN	Barth 135 (SD)	JQ513451	JQ582350
<i>Pentaglottis sempervirens</i> (L.) Tausch	Cult. Syst. Bot. Berlin	M. Weigend 9065 (BSB)	GQ285225	GQ285250
<i>Plagiobothrys albiflorus</i> (Griseb.) R.L. Pérez-Mor.	Argentina (Paso Cardenal Samore)	MPN 24689	AY092899	
<i>Plagiobothrys congestus</i> (Wedd.) I.M. Johnston	UNKNOWN	Beck s. n. (MO)	JQ513454	JQ582351
<i>Plagiobothrys hispidus</i> A. Gray	UNKNOWN	Taylor 16824 (UC)	JQ513455	JQ582352
<i>Plagiobothrys jonesii</i> A. Gray	UNKNOWN	Sanders 27585 (RSA)	JQ513456	JQ582353
<i>Plagiobothrys myosotoides</i> (Lehm.) Brand	UNKNOWN	Van der Werff 20645 (MO)	JQ513459	JQ582356
<i>Plagiobothrys shastensis</i> A. Gray	UNKNOWN	Ahart 11672 (JEPS)	JQ513460	JQ582357

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Appendix S1. (Continued)

Species	Country (Locality)	Voucher	ITS	<i>trnL-trnF</i>
<i>Pseudomertensia sericophylla</i> (Riedl) Y.J.Nasir	W Himalaya	U. Schickhoff 106A (MSB)	*KF849123	
<i>Rindera caespitosa</i> Bunge	Turkey (Maras)	Ekici 1616 (MA)	*KF849134	*KF849245
<i>Rindera graeca</i> Boiss. & Heldr.	Grecia (Korinthia)	Herrero <i>et al.</i> , AH3460 (MA)	*KF849132	*KF849178
<i>Rindera lanata</i> Bunge	Turkey (Gümüshane)	Sara Nisa <i>et al.</i> (MA)	*KF849133	*KF849179
<i>Rindera tetraspis</i> Pall.	Russia (Yergeni)	V. Sagalev & I. Rusanovih s.n. (MA)	*KF849148	
<i>Rindera tetraspis</i> Pall	Kazakhstan (Alma-Ata Region, Enbekshikazaksky)	A.Yu. Korolyuk, I.A. Shrustaleva s.n. (NSK)	*KF849149	
<i>Rindera tianschanica</i> M. Pop.	Kazakhstan (Tulkubas district, Sirdarinsky Alatau)	A.Yu. Korolyuk s.n. (NSK)		*KF849231
<i>Rochelia bungei</i> Trautv.	Iran	Assadi & Massoumi 55785 (TARI)	AB564695	AB564705
<i>Rochelia cancellata</i> Boiss. & Bal.	Turkey	Bani 4971 (TMUH)	AB564702	AB564712
<i>Rochelia cardiosepala</i> Bunge	Iran	Kazempour Osaloo 2006-1 (TMUH)	AB564701	AB564711
<i>Rochelia disperma</i> (L.F.) Koch.	Iran	Kazempour Osaloo 2007-2 (TMUH)	AB564698	AB564708
<i>Rochelia macrocalyx</i> Bunge	Iran	Freitag & Jadidi 29088 (TARI)	AB564700	AB564710
<i>Rochelia peduncularis</i> Boiss.	Iran	Abdolzadeh 20447 (FUMH)	AB564699	AB564709
<i>Rochelia persica</i> Bunge ex Boiss.	Iran	Kazempour Osaloo 2007-1	AB564697	AB564707
<i>Solenanthus atlanticus</i> Pit.	Morocco (El Hojib)	Lewalle, 12648 (MA)	*KF849130	*KF849226
<i>Solenanthus biebersteinii</i> DC.	Armenia (Syunik)	Quintanar <i>et al.</i> , 1604 (MA)		*KF849186
<i>Solenanthus circinatus</i> Ledeb	Kazakhstan (Tulkubas, Talassky)/Iran	A.Yu. Korolyuk s.n. (NSK)/ Khoshokhan s.n. (TMUH)	*KF849138	AB758346
<i>Solenanthus coronatus</i> Regel	Tadjikistan (Duschanbe)	S.V. Ovchinnikova 157 (NSK)	*KF849137	*KF849236
<i>Solenanthus karategimus</i> Lipsky	Kazakhstan (Tulkubast, Talassky)	A.Yu. Korolyuk s.n. (NSK)	*KF849139	*KF849233
<i>Solenanthus stamineus</i> (Desf.) Wettst.	Turkey (Erzurum)	Bigazzi & Selvi 02.72 (FI)	FR715325	
<i>Solenanthus turkestanicus</i> (Regel et Smim.) Kusn. (= <i>Kuschakewiczia</i> <i>turkestanica</i> Regel et Smim. Meling E.V.)	Tadjikistan (Chatlon)	S.V. Ovchinnikova 147 (NSK)	*KF849136	*KF849229
<i>Symphytum bornmuelleri</i> Bucknall.	Turkey	B. Tarikahya 2495 (B)	GQ285237	GQ285276
<i>Symphytum bulbosum</i> Schimp.	Turkey	B. Tarikahya 2369 (B)	GQ285235	GQ285275
<i>Thyrocarpus sampsonii</i> Hance	China (Gizhou, Yahne)	X.Jian-Xi 5573 (MO)		*KF849194
<i>Tournefortia ternifolia</i> Kunth.	UNKNOWN	Weigend <i>et al.</i> 5675 (BSB)	HQ286131	HQ286159
<i>Trachelanthus cerinthoides</i> Kunze	Iran (Isfahan)	Parishani s.n. (MA)		*KF849227
<i>Trachelanthus hissaricus</i> Lipsky	Tadjikistan (Duschanbe)	S.V. Ovchinnikova 157 (NSK)	*KF849144	*KF849228
<i>Trachelanthus korolkovii</i> Lipsky	Kazakhstan (Tulkubas, Talassky)	A.Yu. Korolyuk s.n. (NSK)	*KF849147	
<i>Trichodesma africanum</i> (L.) Lehm.	Chad (Bol)	J. Léonard 4493 (MA)		*KF849216
<i>Trichodesma aucheri</i> DC.	Iran	Mozaffarian, 57195 (TARI)	AB758319	AB758347
<i>Trichodesma calcaratum</i> Coss. & Batt.	Morocco (Tisnassemine)	T. Buira & J. Calvo JC 0447 (MA)	*KF849104	*KF849219
<i>Trichodesma calycosum</i> Collett & Hemsl.	Taiwan (Pingtung Hsien) / Taiwan (Kaohsiung Hsien)	C.H. Chen 06239 MO /Y.-Y. Huang 234 MO	*KF849111	*KF849218
<i>Trichodesma incanum</i> (Bunge) A.DC.	Afghanistan (Bamiyan)	C.Schloeder & M.Jacobs 1809 (MSB)	*KF849112	
<i>Trichodesma zeylanicum</i> (Burm. f.) R.Br.	Australia (Gairdner-Torrens)	F.J. Badman 2084 (MA)		*KF849217
<i>Trichodesma zeylanicum</i> (Burm. f.) R.Br.	Kenya	W. Schultka 12 (BSB)	-	GQ285240
<i>Trigonocaryum involucreatum</i> (Steven) Medw.	Kaukasus	M. Senser s.n. (M)		*KF849235
<i>Trigonotis formosana</i> Hayata	-/Taiwan	Bartholomew & Bouffourd 6160 (US)/ M. Weigend 8128 (BSB)	JQ388519	GQ285261
<i>Trigonotis guilielmi</i> A. Gray ex Gürke	Japan	T. Azuma 2001 (BSB)		GQ285257
<i>Trigonotis radicans</i> (Turcz.) Steven	China (Jilin)	H. Hertel 22497 (M)	*KF849110	*KF849239

Appendix S2

List of voucher specimens included in the fruit study.

<i>Asperugo procumbens</i> L.	Spain (Valencia, Piña de Esgueva)	J.A.Lázaro Bello s.n. (MA)
<i>Bothriospermum zeylanicum</i> Druce.	(label in Chinese)	Feb.2000 (4690883MO)
<i>Myosotidium hortensia</i> (Decne.) Baill.	New Zealand (Chatham Islands)	PB Heenan s.n. (CHR)
<i>Omphalodes aliena</i> A.Gray ex Hemsl.	USA (Texas)	T.R. Van Devender <i>et al.</i> 85-86 (MO)
<i>Omphalodes brassicifolia</i> Sweet	Spain (Salamanca, Aldeararcepreste)	J. Fdez Diez 58 (MA)
<i>Omphalodes cappadocica</i> DC.	Georgia (Adjara)	A. Gröger <i>et al.</i> 1518. (M)
<i>Omphalodes cardiophylla</i> Gray ex Hemsl.	Mexico (Ciudad Victoria, Tamaulipas)	Clausen & Edwards. 7376 (A)
<i>Omphalodes caucasica</i> Brand	Caucasus (Khosta Natural Reserve) 192902.	V.Vasak & A. Vzda sn.(M)
<i>Omphalodes chiangii</i> L.C.Higgins	Mexico (Galeana, Cerro El Gallo)	Hinton <i>et al.</i> 21036 (A)
<i>Omphalodes commutata</i> G. López	Spain (Cádiz, Grazalema)	A. Aparicio, García & Silvestre s.n. (MA)
<i>Omphalodes japonica</i> (Thunb.) Maxim.	Japan (Kochi, Mt. Kojio-yama)	Kuroiwa <i>et al.</i> FOK-076224 (MBK)
<i>Omphalodes kuzinskyanae</i> Willk.	Portugal (Estremadura Cascais)	E. Valdés Bermejo (MA)
<i>Omphalodes linifolia</i> Moench	Spain (Madrid, San Martín de la Vega)	J.C. Zamora s.n.
<i>Omphalodes littoralis</i> Lehm <i>subsp. gallaecia</i> Lainz	Spain (Galicia, Coruña)	R. Carbajal & M. Serrano s.n. (MA)
<i>Omphalodes luciliae</i> Boiss.	Turkey (Nigde, Ulukisla)	P.H. Davis 16537 (M)
<i>Omphalodes nitida</i> (Hoffmanns. & Link ex Willd.) Hoffmanns & Link	Spain (Galicia, Lugo)	C. García-Echave s.n.
<i>Omphalodes scorpioides</i> Schrank	Austria (Niederösterreich)	H. Merxmüller & O. Angerer 33286 (M)
<i>Omphalodes verna</i> Moench	Slovenia (Primorsko)	E. Hörandl & F. Hadacek 5832 (W)
<i>Thyrocarpus glochidiatus</i> Maxim.	(label in Chinese)	4.Apr.1999 (4707205MO)
