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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 *trn*L–*trn*F 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* Bentham (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 Palaearctic (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		
O. brassicifolia Sweet.[1,2]	CW and S Iberian Peninsula	Annual
O. cappadocica D.C. [1,2]	Caucasus	Perennial
O. caucasica Brand[1,2]	Caucasus	Perennial
O. commutata G.López[1,2]	S Iberian Peninsula	Annual
O. kusnetzovii Kolak[1,2]	Caucasus	Perennial
O. kuzinskyanae Wilk. [1,2]	CW Portugal	Annual
O. linifolia Moench [1,2]	Iberian Peninsula, W France to Crimea and W Caucasus	Annual
O. littoralis Lehm. [1,2]	NW Iberian Peninsula, SW France	Annual
O. lojkae Sommier & Levier[1,2]	Caucasus	Perennial
O. luciliae Boiss. [1,2]	Greece, N Iraq, W Iran	Perennial
O. nitida Hoffmanns. & Link[1,2]	NW Iberian Peninsula	Perennial
O. ripleyana P.H.Davis	Anatolia	Perennial
O. rupestris Rupr ex Boiss.	Caucasus	Perennial
O. runemarkii Strid & Kit Tan	Greece	Perennial
O. scorpioides Schrank. [1,2]	C and NE Europe	Biennal
O. verna Moench[1,2]	E Mediterranean and C Europe	Perennial
Japan		
O. akiensis Kadota[1]	Honshu, Hiroshima	Perennial
O. japonica Maxim.[1,2]	Honshu	Perennial
O. krameri Franch. & Sav.	Hokkaido, N to C. Honshu	Perennial
O. laevisperma Nakai	C Honshu	Perennial
O. prolifera Ohwi	C and W Honshu	Perennial
N. America		
O. aliena A.Gray ex Hemsl.[1,2]	S Texas and Mexico (Nuevo León and C Coahuila)	Annual
O. alienoides Nesom	S Texas and Mexico (Cohauila)	Annual/Perennial
O. australis Nesom	Mexico (Puebla)	Perennial
O. cardiophylla A.Gray ex Hems[1,2]	Mexico (Coahuila to Nuevo León and CW Tamaulipas)	Perennial
O. carranzae Nesom	Mexico (NW Cohauila, Sierra del Carmen)	Perennial
O. chiangii L.C.Higgins[2]	Mexico (CN Coahuila)	Perennial
O. erecta I.M. Johnston	Mexico (C Nuevo León to CW Tamaulipas)	Perennial
O. mexicana 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 *Myosotydium 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 *trn*L–*trn*F region (including the *trn*L intron and the *trn*L–*trn*F 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 (*trn*L–*trn*F) sequences taken from previous studies and downloaded from the Genbank database, plus 82 (ITS) and 68 (*trn*L–*trn*F) 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 Paleartic 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 *trn*L–*trn*F *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 Epgradient 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 *trn*L–*trn*F spacer are the same as those of the nuclear ones except for the annealing temperature (50 °C). The volume of genomic DNA was 1μl in both regions. Nested PCRs were needed to amplify old herbarium specimens. The amplifications of the *trn*L–*trn*F 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 *trn*L–*trn*F region: '*trn*L–*trn*F BOR F' (5' CCC GCA ATT AAT AAA AAT GGGC 3') and '*trn*L–*trn*F 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 *trn*L–*trn*F matrix comprised 180 accessions, representing 170 species and included 1218 characters (hereafter called "plastid matrix"). Two additional ITS and *trn*L–*trn*F 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 (*trn*L–*trn*F 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 $50x10^6$ 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 *trn*L–*trn*F majority-rule consensus trees obtained from the BI analyses were compared using 10^5 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 obtained 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

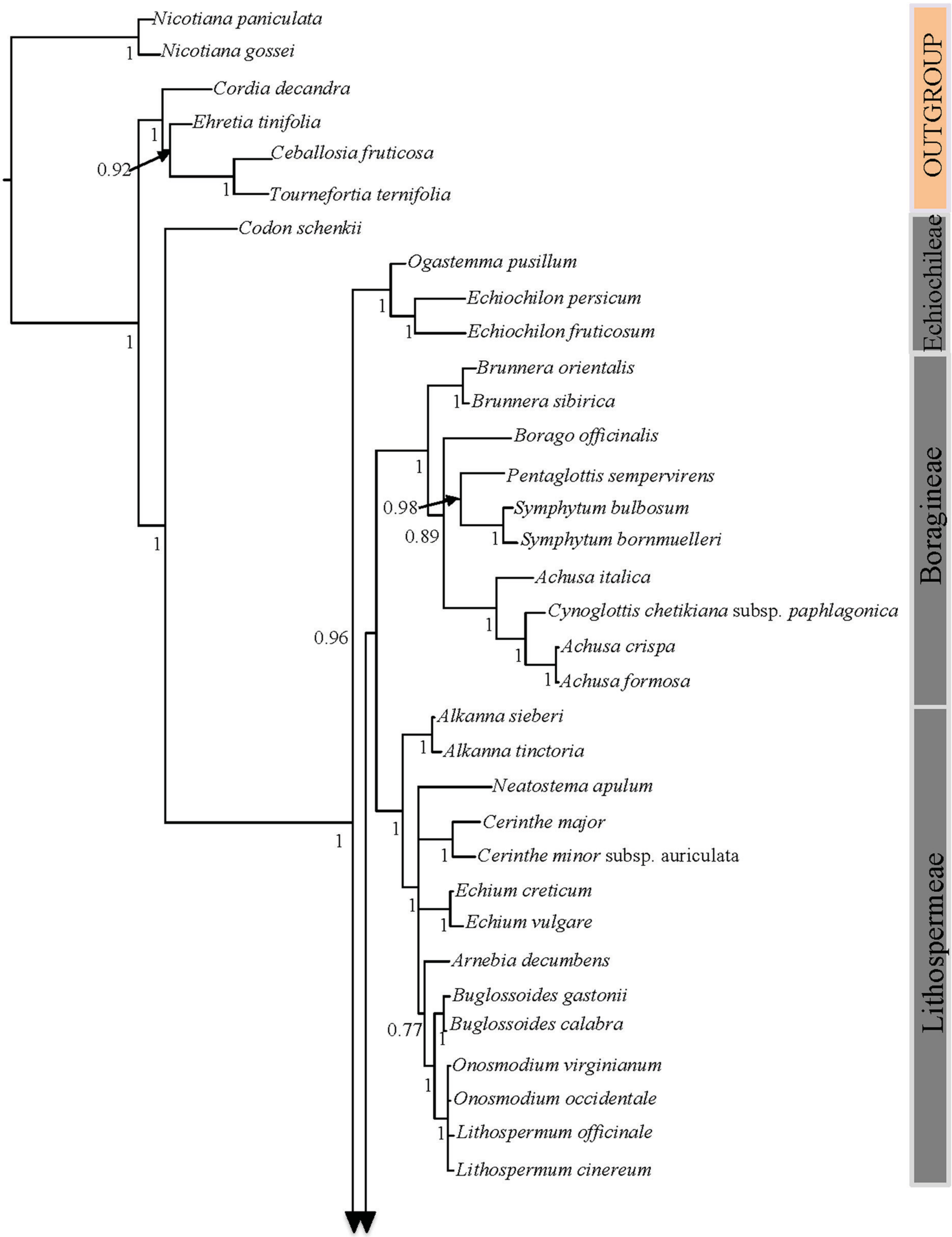

FIGURE 1 (continued)

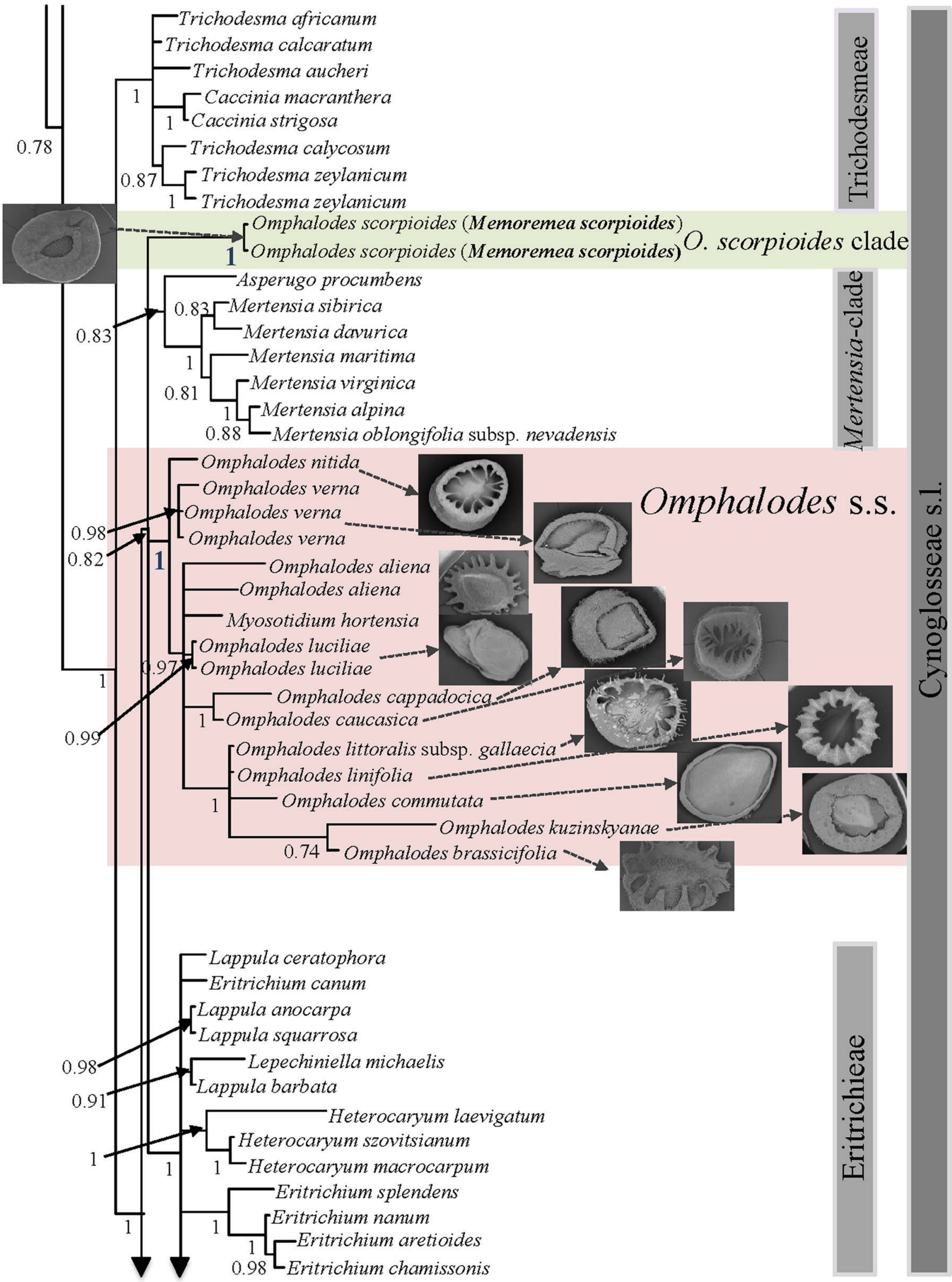

FIGURE 1 (continued)

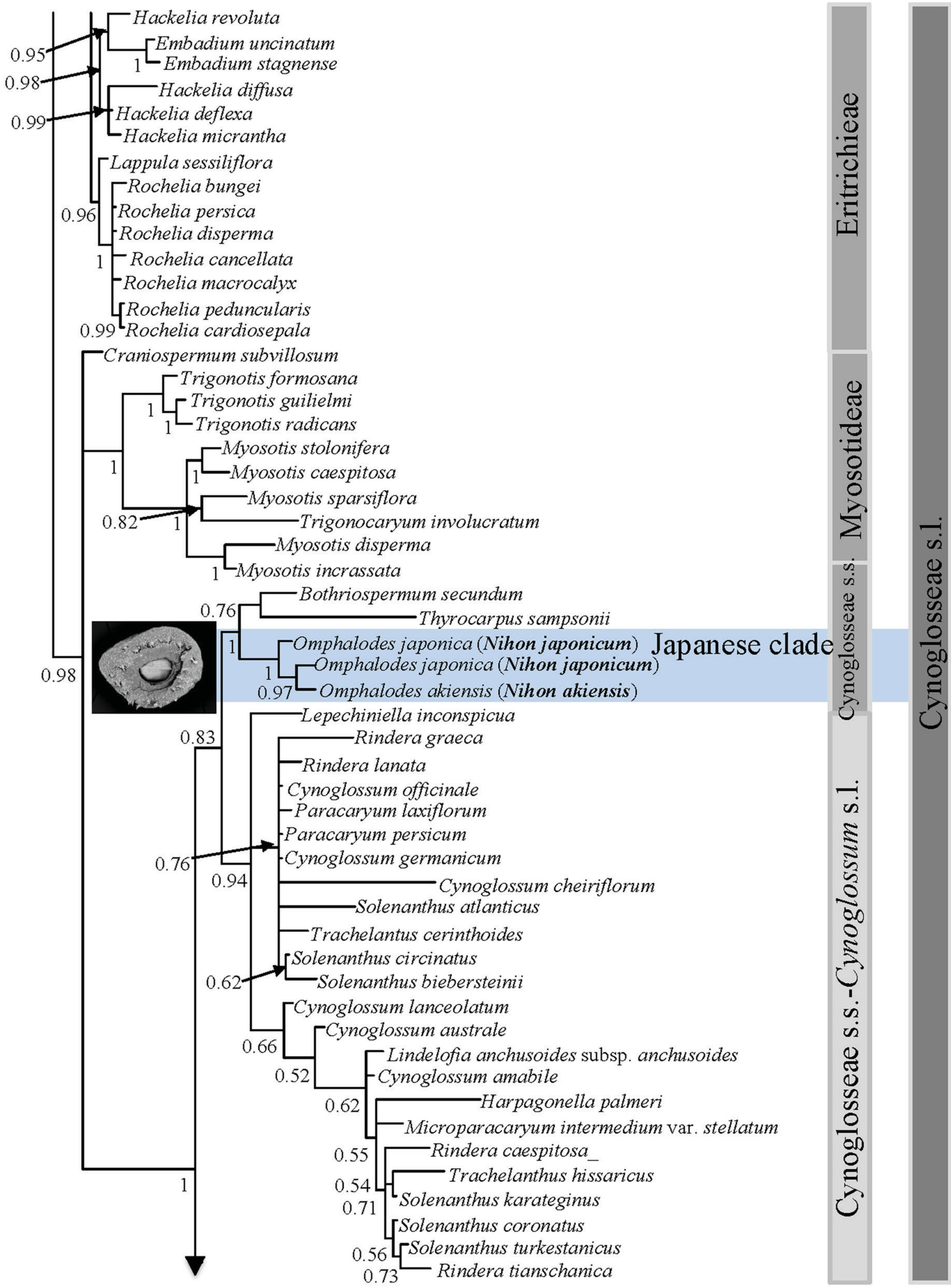

FIGURE 1 (continued)

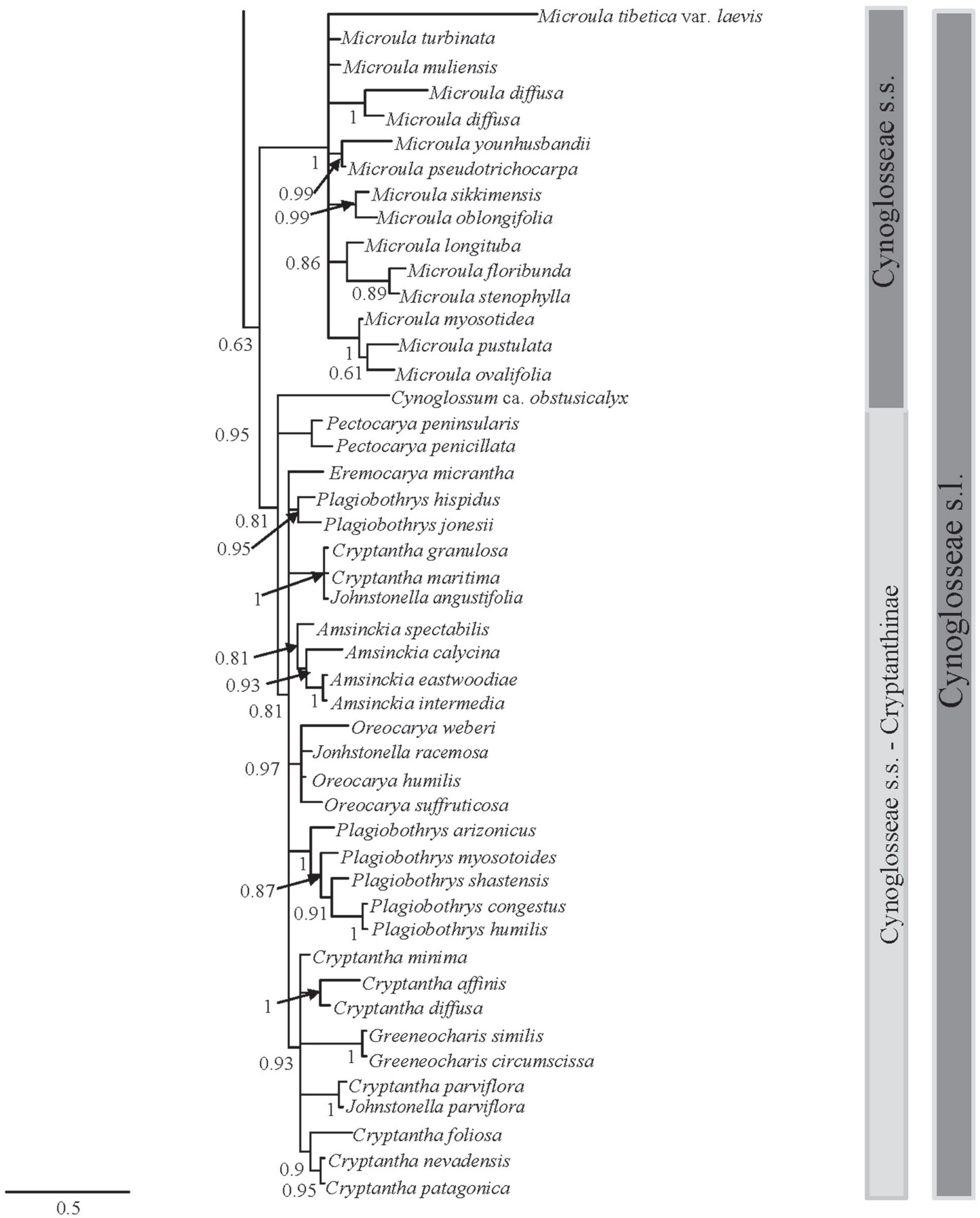

FIGURE 1. Bayesian majority rule consensus tree based on plastid (*trn*L–*trn*F) sequences. Numbers below nodes are Bayesian posterior probabilities, some indicated by solid arrows. Major clades are indicated. SEM photographs of species of *Omphalodes* are shown in their respectives clades, some indicated 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						
O. aliena	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
O. cardiophylla	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
O. chiangii	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						
O. brassicifolia	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
O. commutata	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
O. kuzinskyanae	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
O. linifolia	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
O. littoralis	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						
O. caucasica	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
O. cappadocica	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
O. luciliae	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
O. nitida	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.
O. verna	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						
O. japonica	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
Biennal European Taxon						
O. scorpioides	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						
Asperugo	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
Bothriospermum	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
Mertensia[1]	Tetrahedral, 3 to 5 in length	Rugose or grumose, glabrous, keeled.	Basal, rounded	Absent	Rarely narrow wing with prickles	Rugose or grumose, glabrous
Myosotidium	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
Thyrocarpus	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

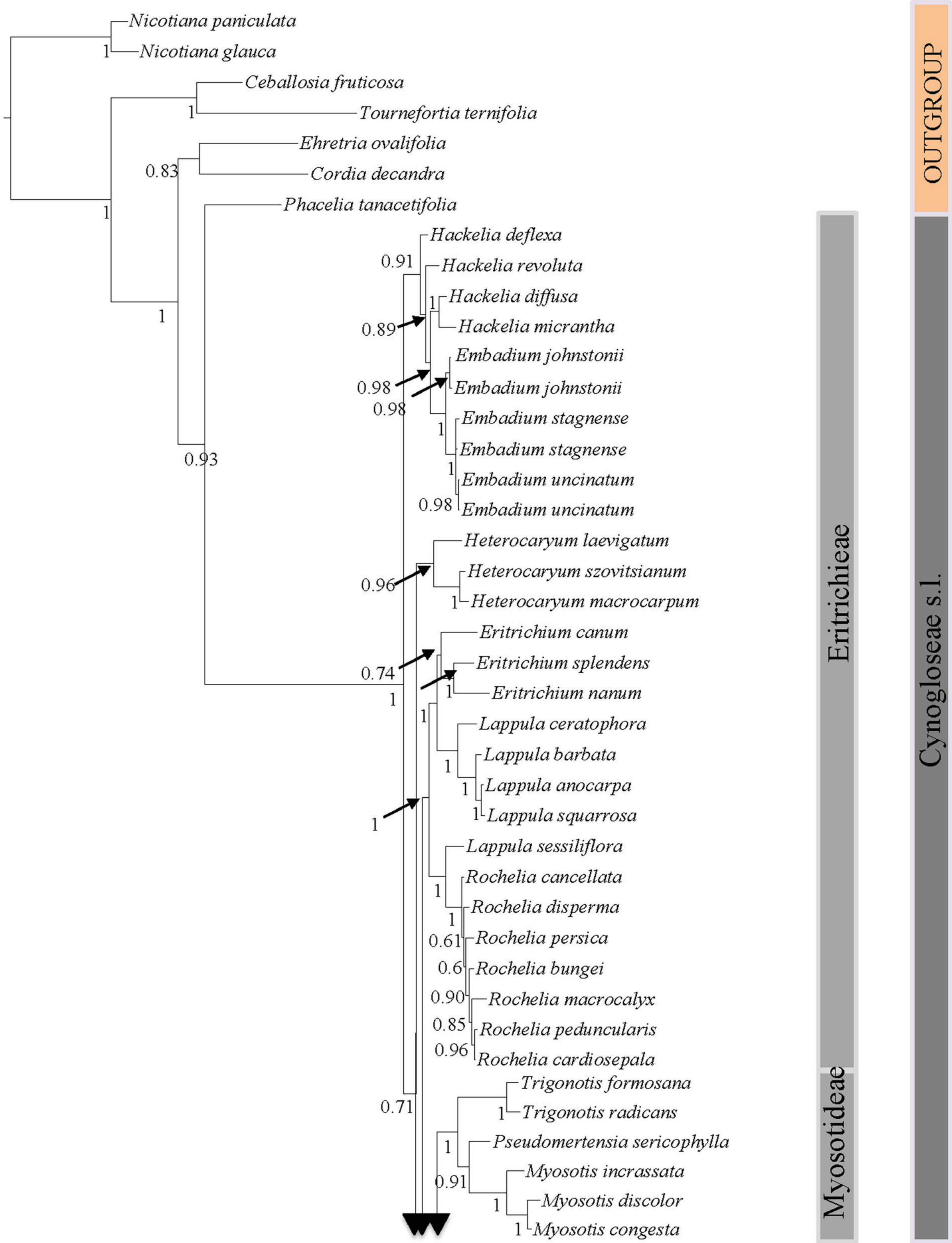
Results

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 – LnL = 32.171, p = 0.9), whereas the plastid topology is rejected by the ITS dataset (difference in – 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



Nicotiana paniculata
Nicotiana glauca
Ceballosia fruticosa
Tournefortia ternifolia
Ehretria ovalifolia
Cordia decandra
Phacelia tanacetifolia
Hackelia deflexa
Hackelia revoluta
Hackelia diffusa
Hackelia micrantha
Embadium johnstonii
Embadium johnstonii
Embadium stagnense
Embadium stagnense
Embadium uncinatum
Embadium uncinatum
Heterocaryum laevigatum
Heterocaryum szovitsianum
Heterocaryum macrocarpum
Eritrichium canum
Eritrichium splendens
Eritrichium nanum
Lappula ceratophora
Lappula barbata
Lappula anocarpa
Lappula squarrosa
Lappula sessiliflora
Rochelia cancellata
Rochelia disperma
Rochelia persica
Rochelia bungei
Rochelia macrocalyx
Rochelia peduncularis
Rochelia cardiosepala
Trigonotis formosana
Trigonotis radicans
Pseudomertensia sericophylla
Myosotis incrassata
Myosotis discolor
Myosotis congesta
0.83
0.93
0.91
0.89
0.98
0.98
0.98
0.96
0.74
0.61
0.6
0.90
0.85
0.96
0.71
0.91
OUTGROUP
Eritrichieae
Myosotideae
Cynogloseae s.l.

FIGURE 2 (continued)

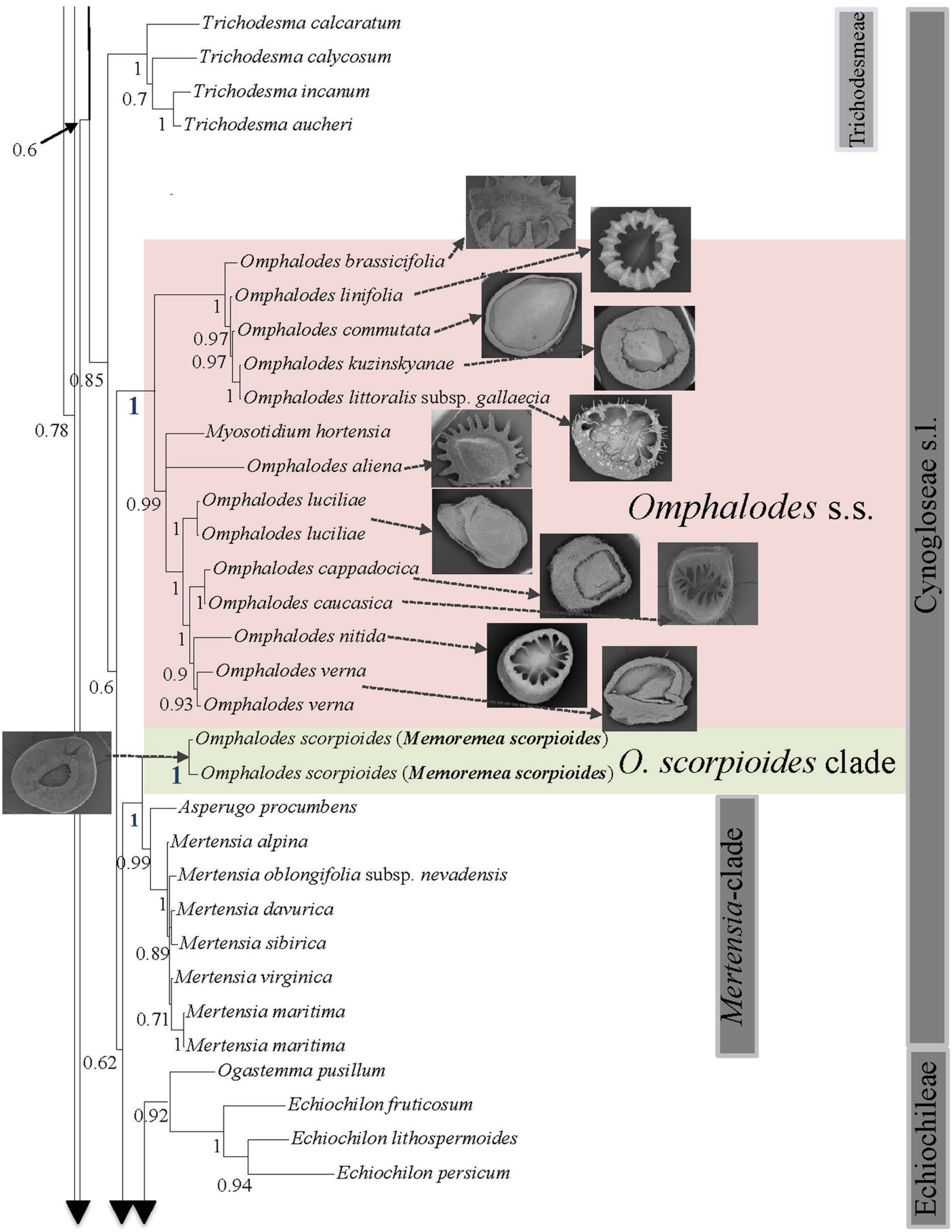
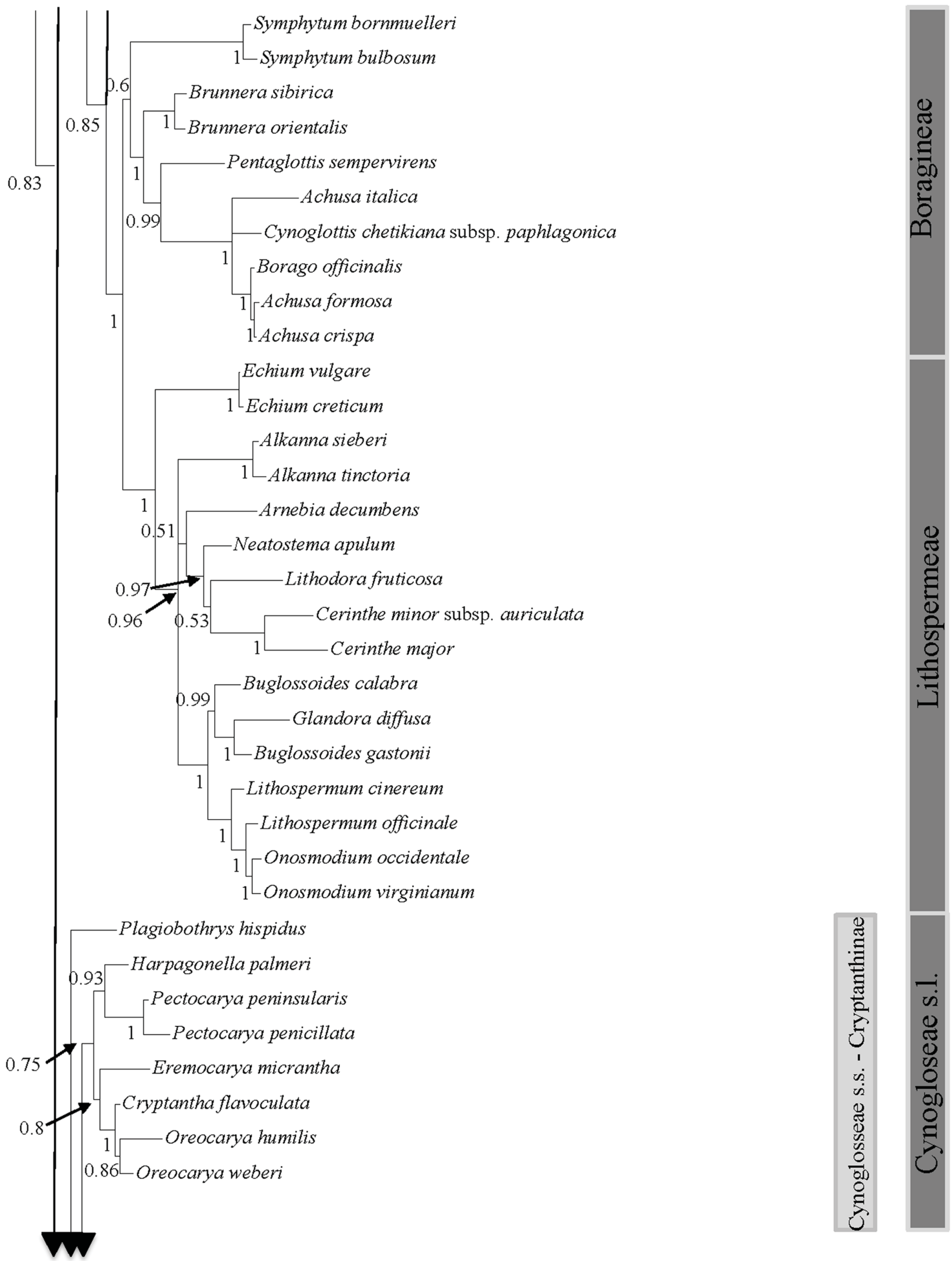


FIGURE 2 (continued)



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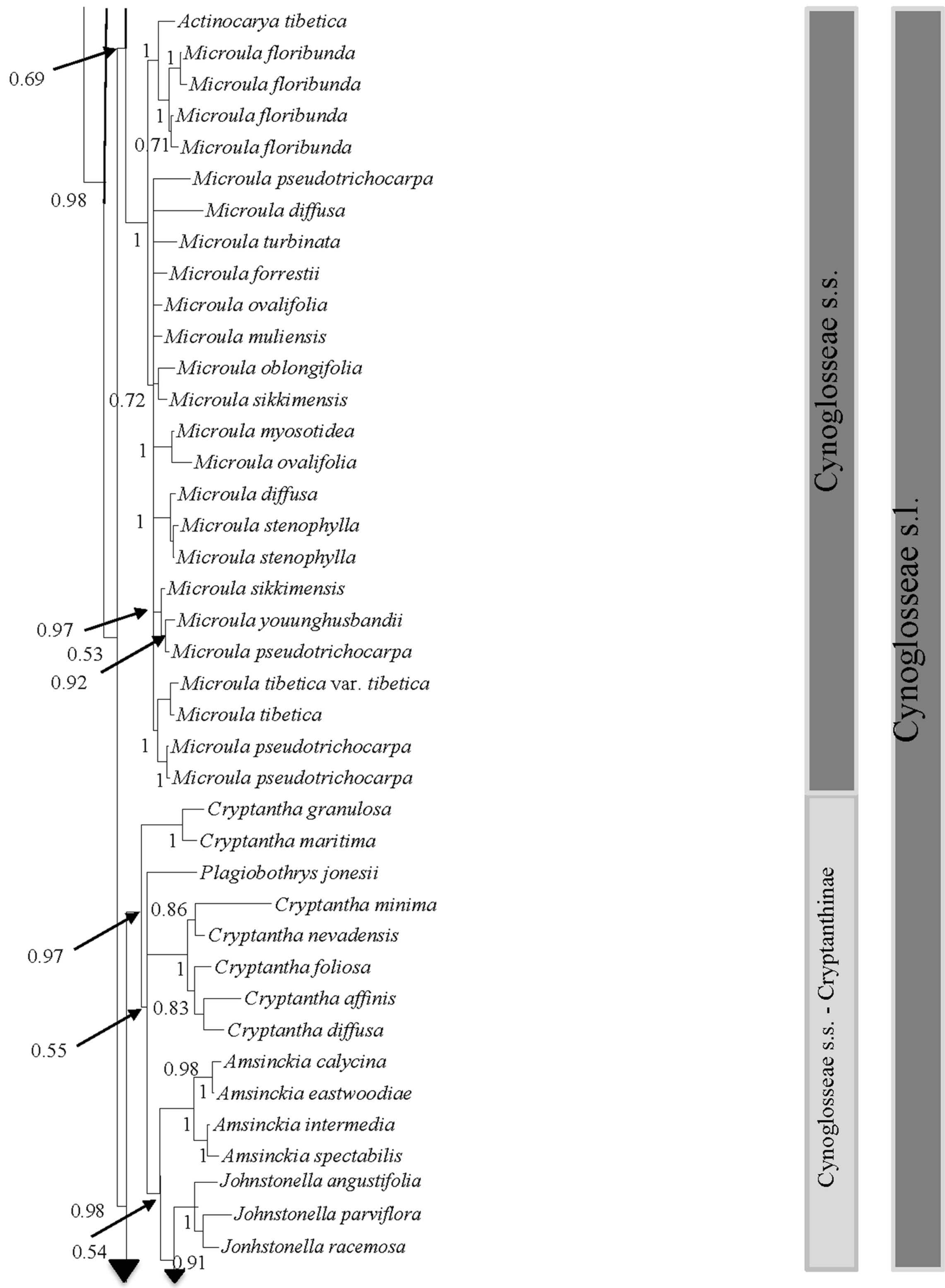


FIGURE 2 (continued)

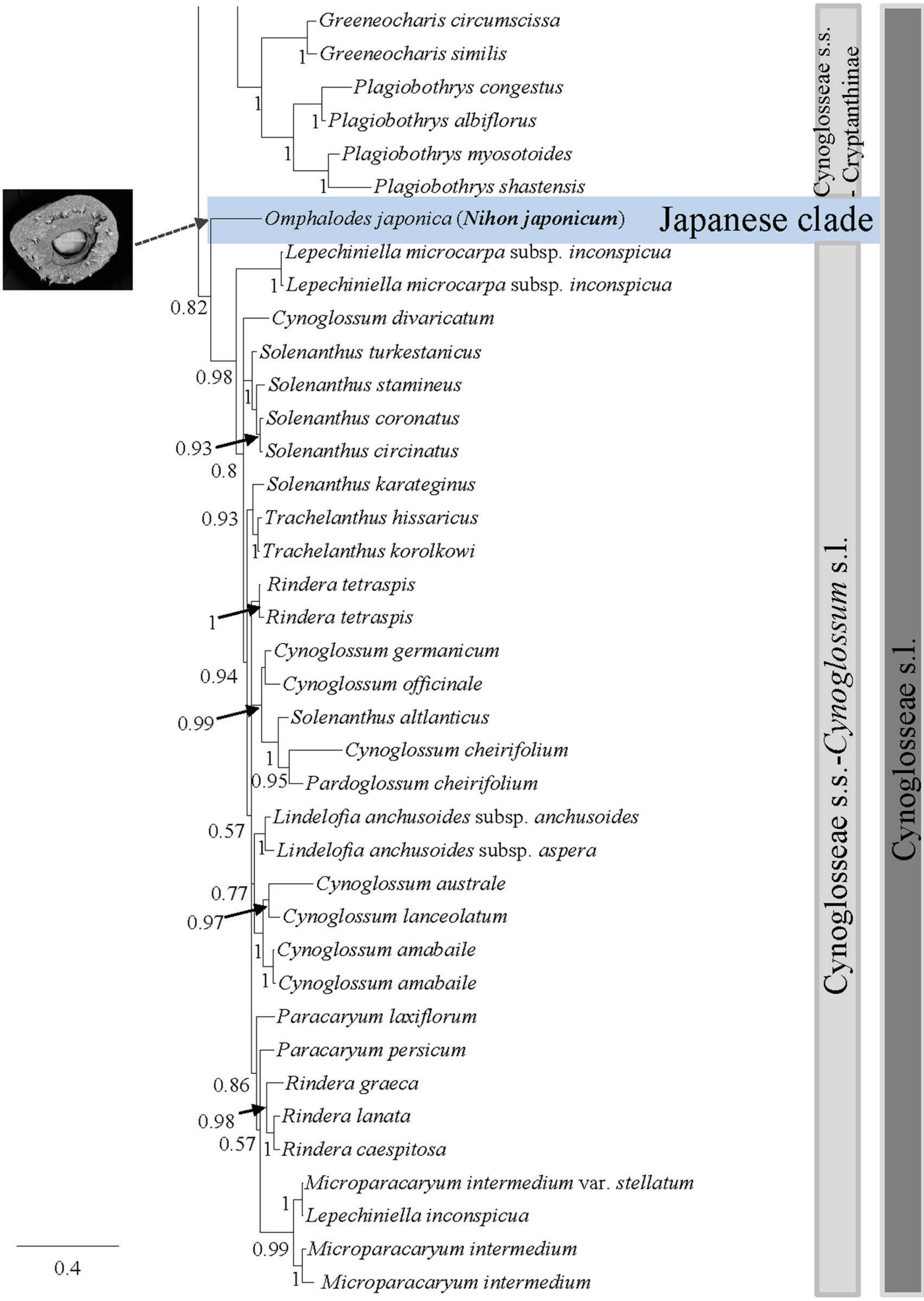


FIGURE 2. Bayesian majority rule consensus tree based on nuclear (ITS) sequences. Numbers below nodes are Bayesian posterior probabilities, some indicated by solid arrows. Major clades are indicated. SEM photographs of species of *Omphalodes* are shown in their respectives clades, some indicated by lined 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 biennal 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 nutles 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

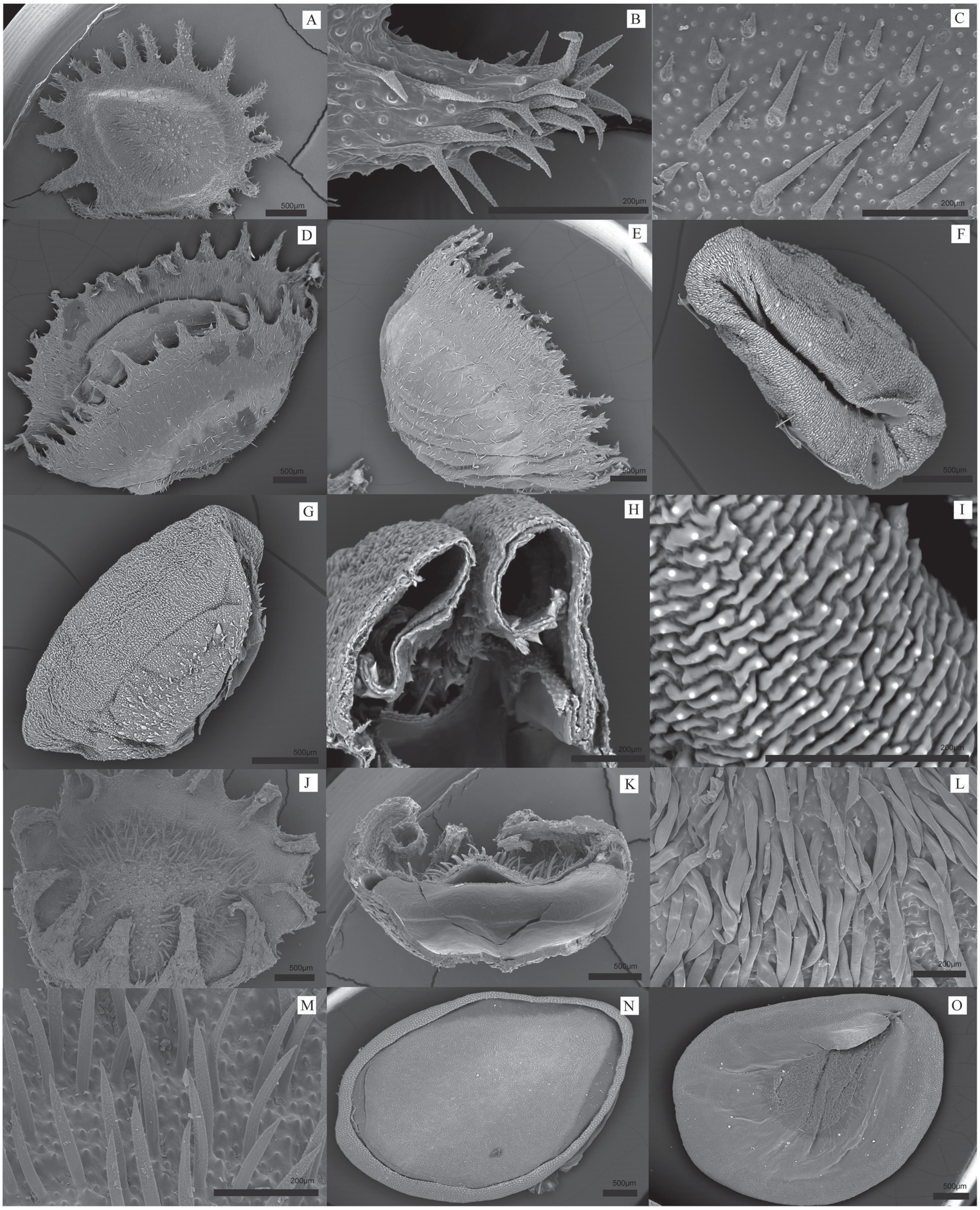
The nutlets of the *Omphalodes* s.s clade are suborbicular (or ovoid-subdeltoid), 2–3 (4) × 1–3 (4) mm (Fig. 3A–3AW). Annual European taxa have bigger nutlets (c. 3 mm in diameter) than those of European perennials (2 mm) (Fig. 3J–X, 3Y–AP). The nutlets of North American species are ovoid to orbicular, 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).

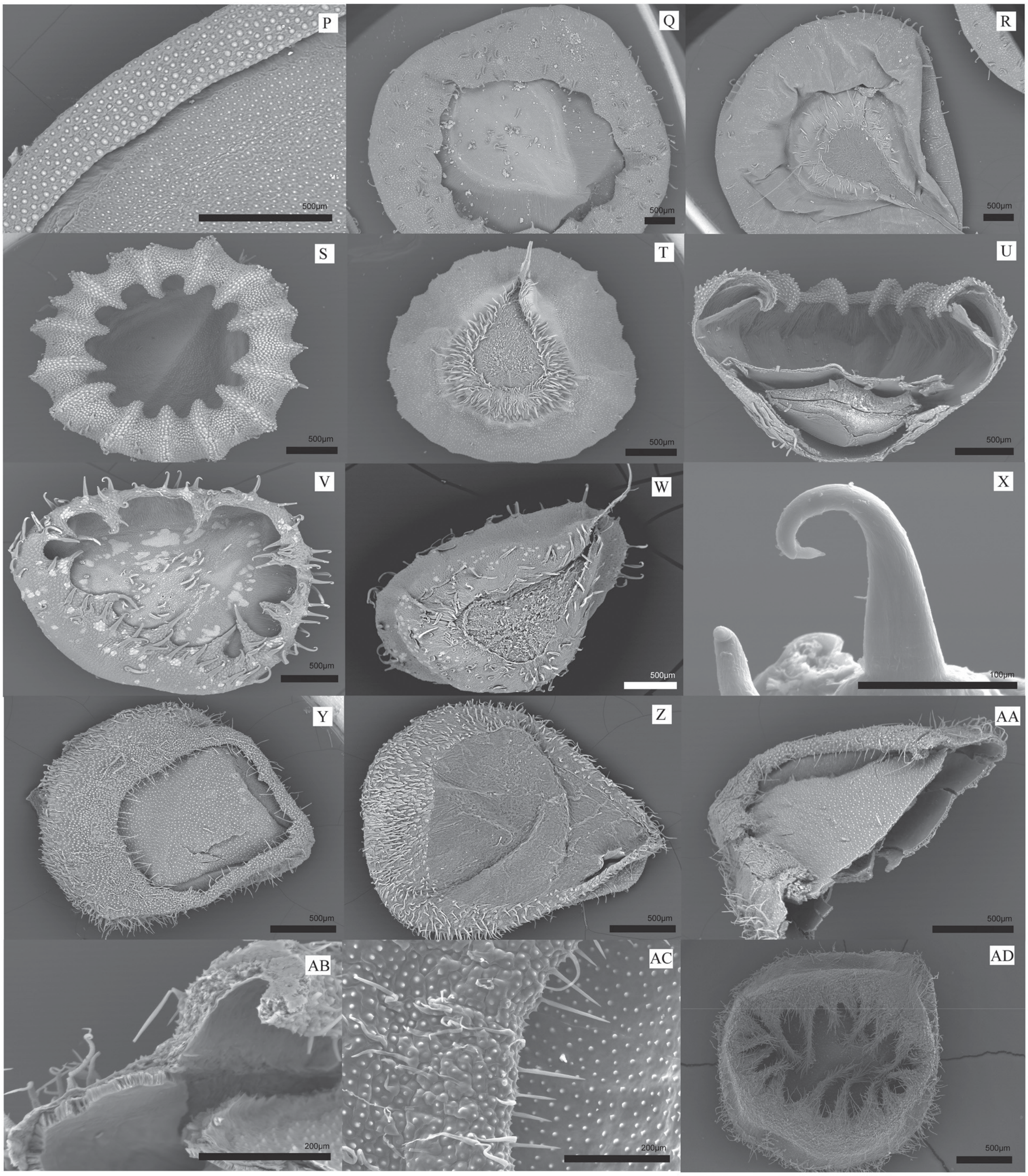
FIGURE 3. SEM photographs of the nutlets of the different species of *Omphalodes* and allied genera. **A–**C. *Omphalodes aliena*. **A**. Abaxial view. **B**. Detailed view of a tooth of the margin. **C** detailed view of the base of the abaxial aperture. **D–E**. *O. cardiophylla*. **D**. Abaxial view. **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.** *Botrhiospermum 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 nutletl body. **BB–BC.** *Myosotidium hortensia*. **BB**. Detailed view of the epidermis of the adaxial side of the nutlet. **BC**. Detaield 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

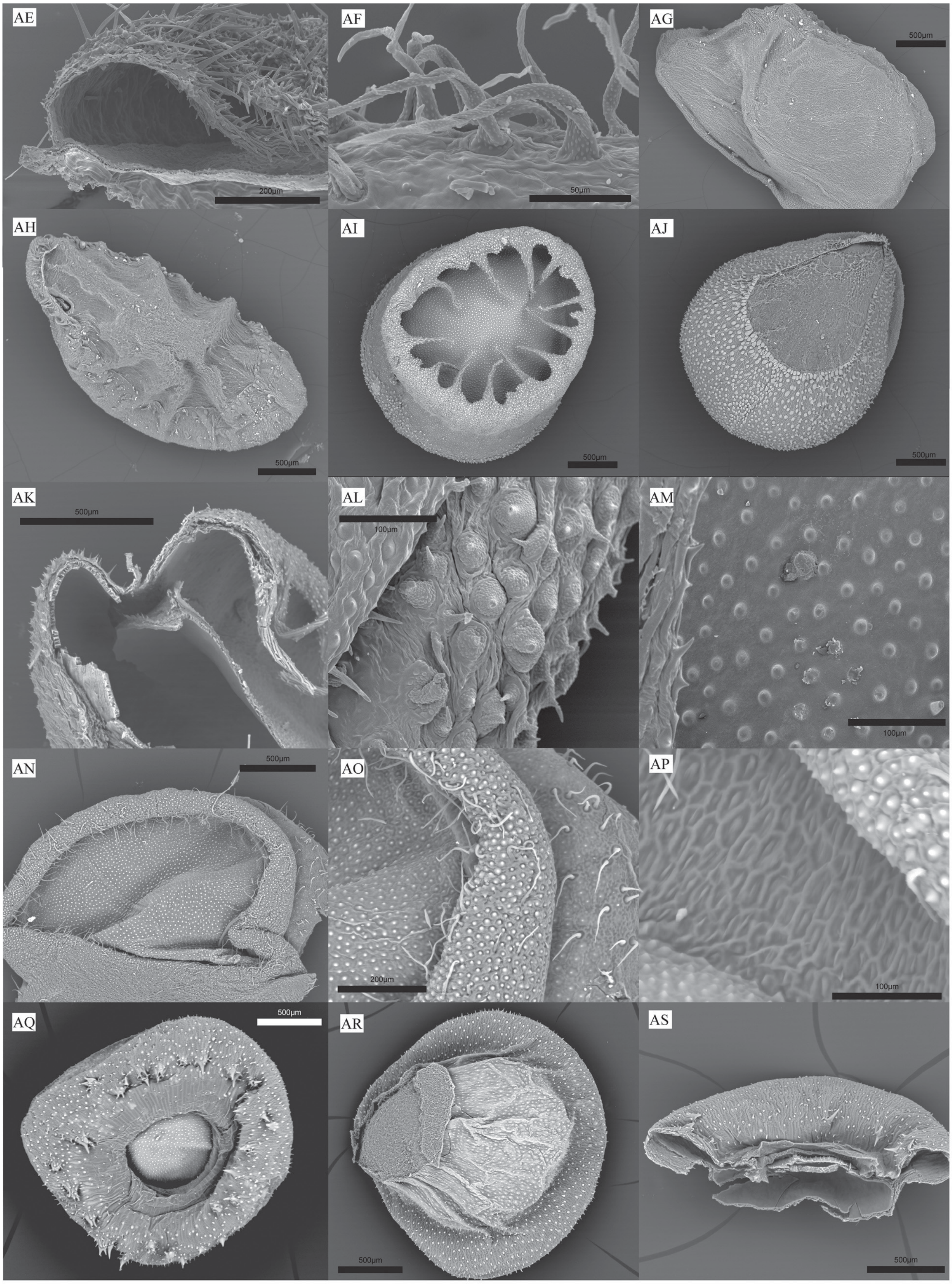
The nutlets of *Omphalodes scorpioides* (Fig. 3AU–AW) are orbicular and 2.8 × 2.5 mm. The adaxial side has sparse short papillae and short smooth trichomes (Fig. 3AW). The scar is deltoid in shape and it is in an apical position, which is distinct from the remaining European taxa of the *Omphalodes* 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 grummose. The rounded scar is basal and ventrally positioned. Rarely, a narrow wing with prickles is observed.

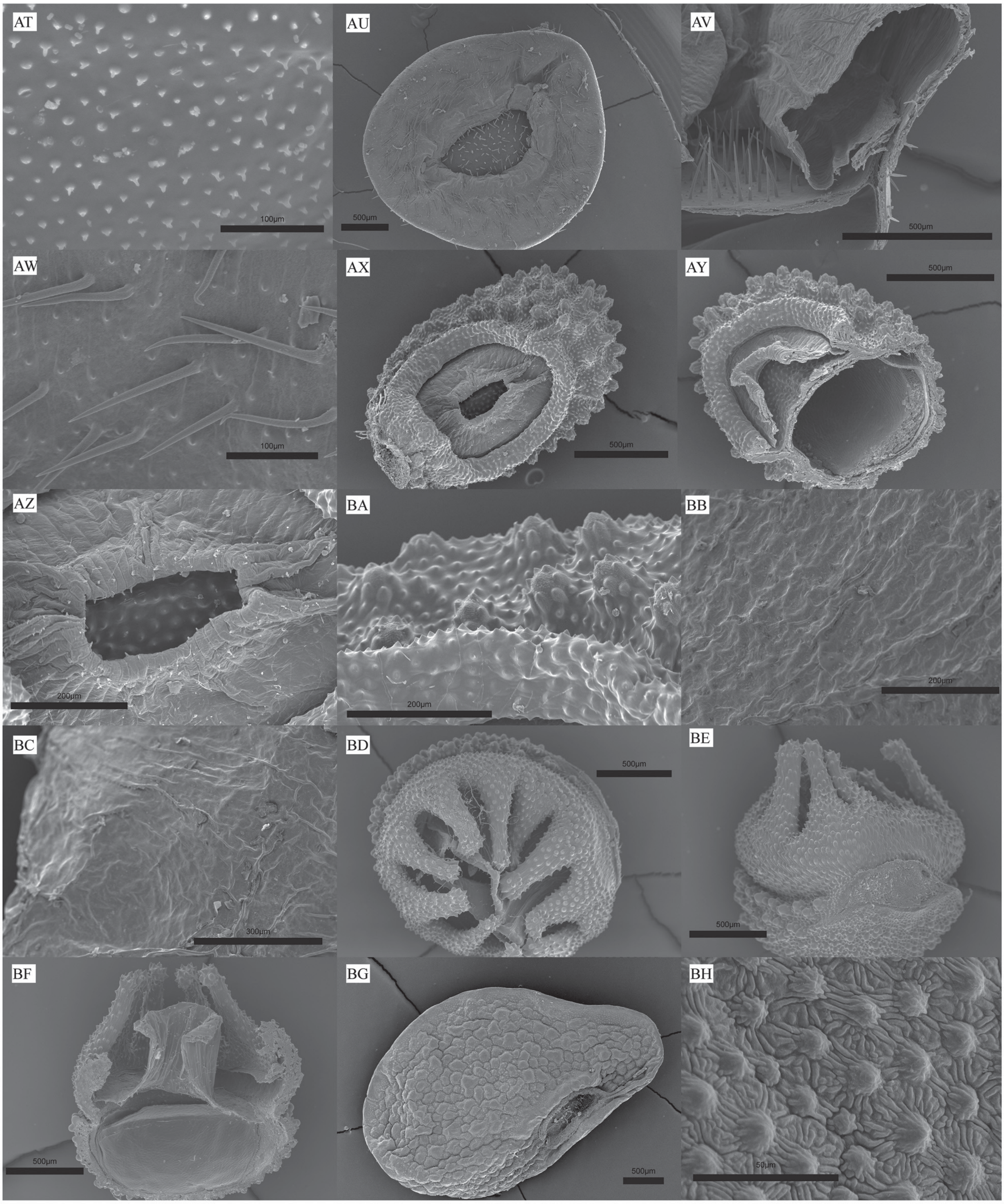


Japanese clade and its closest relatives

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 *Bothriospemum*, 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 biennal 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 explicitally 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 basiosym 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. superf. 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
Omphalodes s.s.	Annual/ Perennial	Terminal	Oblong, no ring-like aperture	Central	Solid, differently ornamented	Erect
Omphalodes scorpioides (*Memoremea scorpioides*)	Biennal	Axilar	Globose, no ring-like aperture	Apical	Hollow, homogeneously ornamented (smooth trichomes)	Curved
Japanese species (*Nihon*)	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 ≡ *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.*

≡ *Cynoglossum scorpioides* Haenke (1788: 3) (basionym)

≡ *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.*

≡ *Cynoglossum japonicum* Thunberg (1784: 187) (basionym)

≡ *Omphalodes japonica* (Thunb.) Maximowicz (1872: 452)

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

≡ *Omphalodes akiensis* Kadota (2009: 342) (basionym).

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

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

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

≡ *Omphalodes laevisperma* Nakai (1949: 17) (basionym).

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

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

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Appendix S1. (Continued)

Species	Country (Locality)	Voucher	ITS	*trnL–trnF*
Cynoglossum amabile Stapf. & Drum.	Bolivia (La Paz)	Beck 8670 (M)	*KF849142	*KF849232
Cynoglossum amabile Stapf. & Drum.	Germany (Culltiv Munich)/	H. Förther s.n (MSB)	*KF849141	
Cynoglossum australe R. Br.	Australia (Tasmania, Lookout)	M. Visoiu 102 (MA)	*KF849126	*KF849187
Cynoglossum cheiriflorum L.	Spain (Granada)	C. Aedo, 9749 (MA)	*KF849129	*KF849203
Cynoglossum divaricatum Steph. ex Lehm.	Russia (Burytia)	I. Chan, E. Balde s.n. (NSK)	*KF849131	
Cynoglossum germanicum Jacq.	Germany (Harz, Sachsen-Anhalt)/ Cult. Botanischer Garten Berlin-Dahlem	Hilger 1999 (BSB, FI)/H. H. Hilger s. n. (BSB)	FR715306	GQ285245
Cynoglossum lanceolatum Forssk.	Pakistan (Karakorum)/ South africa republic (Kwazulu-Natal)	B. Dickoré 12183 (MSB)/C. Aedo *et al*. 15048 (MA)	*KF849143	*KF849202
Cynoglossum ca. obtusicalix Retief & A.E.van Wyk.	South africa republic (Eastern Cape, Rhodes)	Aedo *et al.* 15140 (MS)		*KF849196
Cynoglossum officinale L.	Iran/ Cult. Botanical Garden Berlin-Dahlem	Assadi s.n. (TARI)/H. H. Hilger s. n. (BSB)	AB758292	GQ285248
Cynoglottis chetikiana Vural & Kit Tan subsp. *paphlagonica* (Hausskn. ex Bornm.) Vural & Kit Tan	Turkey	F. Selvi & M. Bigazzi (BSB, FI)	GQ285228	GQ285254
Echium vulgare L.	Germany	O. Mohr No. 597 (BSB)	FJ763247	FJ763301
Echium creticum L.	(cult.) BG München;	H. Foerther s.n. (BSB)	FJ763249	FJ763303
Echiochilon fruticosum Desf.	Egypt/Libya	Lundquist 5655 (UPS)/Kagiampaki s.n. (BSB)	AJ555908	FJ763310
Echiochilon lithospermoides (S. Moore) I.M. Johnst.	Ethiopia	Gilbert & Sebsebe 8700 (UPS)	AJ555912	
Echiochilon persicum (Burm.f.) Johnst.	Iran	Mozaffarian 49917 (TARI)	AB758293	AB758322
Ehretia tinifolia L.	UNKNOWN	Gottschling CUB52 (BSB)		HQ286270
Ehretia ovalifolia Hassk.	UNKNOWN	UNKNOWN	AF091156	
Embadium stagnense J.M. Black	Australia (Gairdner-Torrens, Kokatha)	F.J. Badman 8530 (AD)	*KF849113	
Embadium stagnense J.M. Black	Australia (Gairdner-Torrens, Mt Wallaby)	R.J. Bater 57455B (AD)	*KF849114	*KF849241
Embadium johnstonii Ising	Australia (Lake Eyre)	R.J.-P. Davies 694 (AD)	*KF849117	
Embadium johnstonii Ising.	Australia (Lake Eyre)	R.J.-P. Davies 695 (AD)	*KF849118	
Embadium uncinatum Ising	Australia (Eyre peninsula, Scrubby Peak)	M.J. Thorpe 48 *et al.* (AD)	*KF849115	*KF849222
Embadium uncinatum Ising	Australia (Gawler ranges)	M.J. Thorpe 50 *et al.* (AD)	*KF849116	
Eremocarya micrantha Greene	UNKNOWN	Rebman 11358 (SD)	JQ513427	JQ582329
Eritrichium aretioides (Cham.) DC.	USA (Alaska Noatak Quad)	Parker, Elven & Solstad 14806 (O)		JQ388579
Eritrichium canum (Benth.) Kitam.	Germany (Cult. in Munich Botanical Garden)/Germany (Cult. Botanischer Garten Muenchen-Nymphenburg)	-/H. Foerther s.n. (M)	AB758294	GQ285242
Eritrichium chamissonis DC.	Canada (Yukon Territory)	Solstad & Elven 03/0601A (O)		JQ388580
Eritrichium nanum (L.) Schrader ex Gaudin	Switzerland/ USA (Colorado, El Paso Co.)	Hertel 25764 (MSB)/ Nazaire 1809 (WS)	AY092901	JQ388581
Eritrichium splendens Kearney ex W. Wight	Alaska (Noatak Quad)	Solstad & Elven 03/1216 (O)	JQ388501	JQ388582
Greeneocharis circumscissa (Hook. & Arn.) Rydb.	UNKNOWN	Simpson 3108 (SDSU)	JQ513403	JQ582305
Greeneocharis similis (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*
Hackelia deflexa (Wahlenb.) Opiz	China (Xinjiang)/Italy	J. F. Huang 20090109 (XJBI)/W. Frey s. n. (BSB)	JX976808	GQ285244
Hackelia diffusa (Lehm.) I. M. Johnst.	USA (Washington, Kittitas Co.)	Lopushinsky 07-6 (WS)	JQ388503	JQ388583
Hackelia micrantha (Eastw.) J. L. Gentry,	USA (Oregon, Grant Co.),	Hinchliff 869 (WS)	JQ388504	JQ388584
Hackelia revoluta I.M. Johnst.	Argentina (La Rioja)	C. Aedo 15407 (MA)	*KF849119	*KF849224
Harpagonella palmeri A. Gray	USA (California)	S. Boyd 3045 (MSB)	*KF849150	*KF849234
Heterocaryum laevigatum (Kar. et Kir.) DC.	Iran	Faghihna & Zangooei 25349 (FUMH)	AB758296	AB758325
Heterocaryum macrocarpum Zak.	Iran	Joharchi & Zangooei, 19639 (TMUH)	AB758300	AB758329
Heterocaryum szovitsianum (Fisch. & Mey.) DC.	Iran	Kazempour Osaloo, 2007-5 (TMUH)	AB758298	AB758327
Johnstonella angustifolia (Torr.) Hasenstab & M.G.Simpson	UNKNOWN	Simpson 8III98A (SDSU)	JQ513397	JQ582299
Johnstonella parviflora (Phil.) Hasenstab & M.G.Simpson	UNKNOWN	Van der Werff 20532 (MO)	JQ513433	JQ582335
Lappula anocarpa C. J. Wang	China (Xinjiang)/China(Xinjiang)	J. F Huang & B. C. Han 201008016(XJBI)/ Juan Qiu 08-0007 (XJA)	JX976775	JQ388585
Lappula barbata (M. B.) Gürke	Iran	Kazempour Osaloo 2008-1(TMUH)	AB564703	AB564713
Lappula ceratophora (M. Pop.) M. Pop.	Iran	Mozaffarian 58407(TARI)	AB758301	AB758330
Lappula sessiliflora (Boiss.) Gürke	Iran	Kazempour Osaloo 2007-3 (TMUH)	AB564704	AB564714
Lappula squarrosa (Retz.) Dumort.	USA (Utah, First Water Canyon)/Cult. Syst. Bot. Berlin	S. L. Welsh & E. Ne 21211 (BRY)/O. Mohr 591 (BSB)	JX976797	GQ285265
Lepechiniella michaelis Golosk.	Kazakhstan (Aalatau Dshungaricus)	V. Goloskokov s.n. (MO)		*KF849223
Lepechiniella inconspicua (Brand) Riedl	Iran	Joharchi & Ayatollahi (FUMH)	AB758311	AB758338
Lepechiniella microcarpa (Boiss.) Riedl subsp. *inconspicua* (Brand) F.Sadat	Afghanistan (Futur-Tal)	Roemer 110 (M)	*KF849127	
Lepechiniella microcarpa (Boiss.) Riedl subsp. *inconspicua* (Brand) F.Sadat	Afghanistan. (Kapisa)	A. Dieterle 52 (M)	*KF849128	
Lindelofia anchusoides subsp. *anchusoides* (Lindl.) Lehmann	Afghanistan (Badakhshan)	C.Schloeder & M.Jacobs 1478 (MSB)	*KF849145	*KF849230
Lindelofia anchusoides (Lindl.) Lehm. subsp. *aspera* (Rech.f.) F.Sadat	Afghanistan (Ghazni)	D.Podlech 31875 (MSB)	*KF849146	
Lithodora diffusa (Lag.) I.M.Johnst.	Spain (Pl. de Toro)	SANT58512	FJ789863	
Lithodora fruticosa Griseb.	Spain (Montefrío)	SANT58519	FJ789864	
Lithospermum cinereum DC.	Republic of South Africa	O.A. Leistner 2109 (M)	FJ763240	FJ763295
Lithospermum officinale L.	Germany	A. Werres & M. Ristow s.n. (BSB)	FJ763189	FJ763254
Mertensia alpina (Torr.) G.	USA (DonColorado, El Paso Co.)	Nazaire 1810 (WS)	JQ388507	JQ388587
Mertensia davurica (Sims) G. Don.	China (Hebei)	Nazaire 1889 (WS)	JQ388509	JQ388589
Mertensia maritima (L.) Gray	Canada (Nunavut)/UK (Shetlands)	Kines s.n. (WS)/H.H. Hilger s.n.(BSB)	JQ388510	GQ285259
Mertensia maritima (L.) Gray	Canada (Quebec)	J.A. Churchill 7572013 (RSA)	*KF849122	
Mertensia oblongifolia (Nutt.) G. Don var. *nevadensis* (A. Nelson) L. O. Williams	USA (Oregon, Harney Co.)	Nazaire & Bunch 1748 (WS)	JQ388511	JQ388591
Mertensia sibirica (L.) G. Don	China (Shanxi)	Nazaire 1892 (WS)	JQ388513	JQ388593
Mertensia virginica (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	*trnL–trnF*
Microparacaryum intermedium (Fresen.) Hilger & Podlech	Egypt (Sinai)	D.Podlech 49702 (MSB)	*KF849124	*KF849240
Microparacaryum intermedium (Fres.) Hilger & Podlech *var. stellatum* (H.Riedl) *f. paracaryoides*Hilger & Podlech	Afghanistan (Kandahar)	Freitag & Breckle 4629 (MSB)	*KF849125	
Microula diffusa W.T. Wang	China (Gande Xian)	T.N. Ho *et al*. 937 (A)		*KF849183
Microula diffusa W.T. Wang	China (Gonde Xian)	T.N. Ho *et al*. 937 (MO)		*KF849180
Microula diffusa W.T. Wang	China(Xizang, S Tibet)	B. Dickoré 9895 (MSB)	*KF849160	
Microula diffusa W.T. Wang	China (Xizang, E Tibet)	B. Dickoré 9150 (MSB)	*KF849153	
Microula floribunda W.T.Wang.	China (Xizang, E Tibet)	B. Dickoré 9149 (MSB)	*KF849157	
Microula floribunda W.T.Wang	China (Qinghai, Xindu Xian)	D.E. Boufford *et al*. 26990 (A)	*KF849155	
Microula floribunda W.T.Wang	China (Qinghai, Yushu Xian)	D.E. Boufford *et al*. 26746 (A)	*KF849156	
Microula floribunda W.T.Wang	China (Qinghai, Nangqen Xian)	T.N. Ho *et al.* 2715 (MO)	*KF849158	*KF849242
Microula forrestii I.M. Johnst.	China (Xizang, SE Tibet)	B. Dickoré 10759 (MSB)	*KF849159	
Microula longituba W.T. Wang	China (Qinghai, Chindy Xian)	T.N. Ho *et al*. 1811 (MO)		*KF849181
Microula muliensis W.T. Wang	China (Sichuan Sêrtar Xian)	D.E. Boufford *et al*. 27850 (A)	*KF849170	*KF849201
Microula myosotidea I.M. Johnst. (with *Microula ovalifolia*)	China (Quinghai, Nangqên Xian)	T.N. Ho *et al.* 2926 (A)	*KF849164	*KF849199
Microula oblongifolia Hand.-Mazz.	China (Qinghai, Jiuzhi Xian)	D.E. Boufford *et al*. 39457 (MBK)	*KF849163	*KF849190
Microula ovalifolia I.M. Johnst. (with *Microula myosotidea*)	China (Qinghai, Nangqên Xian)	T.N. Ho *et al.* 2926 (A)	*KF849165	*KF849200
Microula ovalifolia I.M. Johnst.	China (Yunnan, Zhongdian)	D.Podlech 54539 (MSB)	*KF849169	
Microula pseudotricocarpa W.T.Wang	China (Sichuan, Ruoergai Xian)	D.E. Boufford *et al*. 40204 (A)	*KF849152	
Microula pseudotricocarpa W.T.Wang	China (Sichuan, Xiaojin Xian)	D.E. Boufford *et al*. 38545 (MBK)	*KF849173	
Microula pseudotricocarpa W.T.Wang	China (Sichuan, Serxu Xian)	D.E. Boufford *et al*. 33630 (MBK)	*KF849177	*KF849197
Microula pseudotrichocarpa W.T. Wang	China (Quinghai, Gande Xian)	T.N. Ho et al. 939 (MO)	*KF849174	
Microula pustulosa W.T.Wang	China (Quinghai, Nangqên Xian)	T.N. Ho *et al*. 2926 (MO)		*KF849182
Microula sikkimensis (C.B. Clarke) Hemsley	China (Sichuan, Aba Xian)	D.E. Boufford *et al*. 39444 (MBK)	*KF849166	*KF849189
Microula sikkimensis Hemsl.	China (Quinghai, Yushu Xian)	T.N. Ho *et al*. 2326 (MO)	*KF849176	
Microula stenophylla W.T.Wang	China (Qinghai, Yushu Xian)	D.E. Boufford *et al*. 26810 (A)	*KF849161	
Microula stenophylla W.T. Wang	China (Qinghai, Dari Xian)	T.N. Ho *et al.* 1138 (MO)	*KF849162	*KF849243
Microula tibetica Benth.	China (Xizang, Tibet)	D.E. Boufford *et al*. 32026 (MSB)	*KF849168	
Microula tibetica var. tibetica Maxim.	China Qinghai, C Tibet	B. Dickoré 4394 (MSB)	*KF849167	
Microula tibetica var. laevis W.T. Wang	China (Xizang)	G. & S. Miehe 03-052-16 (MSB)		*KF849193
Microula turbinata W.T.Wang	China (Sichuan, Honguan Xian)	D.E. Boufford 40079 (MBK)	*KF849154	*KF849198
Microula younghusbandii Duthie	China (Qinghai, Chindu Xian)	T.N. Ho *et al*. 1724 (MO)	*KF849175	*KF849185
Myosotidium hortensia (Decne.) Baill.	New Zealand (Chatham Islands)	PB Heenan s.n. (CHR)	*KF849096	*KF849208
Myosotis caespitosa DC.	Finland	H. H. Hilger 1575 (BSB)		GQ285262
Myosotis congesta Shuttlew. ex Alb. & Reynier	Greece	Phitos M-33 (MSB)	AY092916	
Myosotis discolor Pers.	New Zealand (Kahuterawa Valley, North Island) (naturalized introduction)/-	MPN 11910/Ahart 9593 (JEPS)	AY092919	JQ582358
Myosotis incrassata Guss.	Greece/Greece (Crete)	Merxtmüller & Wiedmann 20130 (MSB)/H. H. Hilger Kreta 1998/5 (BSB)	AY092922	GQ285243
Myosotis sparsiflora Mikan	Cult. Syst. Bot. Berlin	M. Weigend 8138 (BSB)		GQ285239
Myosotis stolonifera J. Gray	UK (Shetlands)	Hilger s. n. (BSB)		GQ285258
Neatostema apulum (L.) I.M.Johnst.	USA (Hawaii, Maui)	AMWF7	GQ478099	
Nicotiana glauca Graham	Hawaii, Maui, Kahului airport	AMWF7	GQ478099	

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Appendix S1. (Continued)

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

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Appendix S1. (Continued)

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

Appendix S2

List of voucher specimens included in the fruit study.

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