



Multiple origins for Hound's tongues (*Cynoglossum* L.) and Navel seeds (*Omphalodes* Mill.) – The phylogeny of the borage family (Boraginaceae s.str.)



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ABSTRACT

Recent studies all indicated that both the affinities and subdivision of Boraginaceae s.str. are unsatisfactorily resolved. Major open issues are the placement and affinities of Boraginaceae s.str. in Boraginales and the major clades of the family, with especially the large tribes Cynoglosseae and Eritrichieae repeatedly retrieved as non-monophyletic groups, and the doubtful monophyly of several larger genera, especially *Cynoglossum* and *Omphalodes*. The present study addresses and solves these questions using two plastid markers (*trnL-trnF*, *rps16*) on the basis of a sampling including 16 outgroup taxa and 172 ingroup species from 65 genera. The phylogeny shows high statistical support for most nodes on the backbone and on the individual clades. Boraginaceae s.str. are sister to African Wellstediaceae, Wellstediaceae–Boraginaceae s.str. is sister to African Codonaceae. Echiochileae are retrieved as sister to the remainder of Boraginaceae s.str., which, in turn, fall into two major clades, the Boragineae–Lithospermeae (in a well-supported sister relationship) and the Cynoglosseae s.l. (including Eritrichieae). Cynoglosseae s.l. is highly resolved, with *Trichodesmeae* (incl. *Microcaryum*, *Lasiocaryum*) as sister to the remainder of the group. Eritrichieae s.str. (*Eritrichium*, *Hackelia*, *Lappula*) are resolved on a poorly supported polytomy together with the *Omphalodes*-clade (incl. *Myosotidium*, *Cynoglossum* p.p.), and the *Mertensia*-clade (incl. *O. scorpioides*, *Asperugo*). The *Myosotideae* (*Myosotis*, *Trigonotis*, *Pseudomertensia*) are retrieved in a well-supported sister-relationship to the core-Cynoglosseae, the latter comprising all other genera sampled. *Cynoglossum* is retrieved as highly para- and polyphyletic, with a large range of generic segregates embedded in *Cynoglossum*, but other species of *Cynoglossum* are sister to *Microula* or to the American “Eritrichieae” (*Cryptantha* and allied genera). Representatives of the genus *Cynoglossum* in its current definition are segregated onto six independent lineages, members of *Omphalodes* onto three independent lineages. At least 11 of the genera here sampled are deeply nested in other genera. The data show that individual details of nutlet morphology (e.g., winged margins, glochidia) are highly homoplasious. Conversely, a complex of nutlet characters (e.g., characters of the gynobase and cicatrix together with nutlet orientation and sculpturing) tends to circumscribe natural units. Geographical distribution of major clades suggests that the family originated in Africa and western Asia and radiated to eastern Eurasia, with several independent dispersal events into Australia and the New World.

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1. Introduction

Boraginaceae s.str. are a subcosmopolitan plant family with a center of diversity in the northern temperate zone. Their gynoe-

cium morphology with a four-parted ovary and a gynobasic style is rare amongst angiosperms and is considered as the crucial diagnostic character in combination with alternate phyllotaxy, generally hispid indument and radially symmetrical corollas (Al-Shehbaz, 1991; Gleason and Cronquist, 1991; Gürke, 1893–1897; Popov, 1953). Inflorescence morphology is also quite characteristic, although not unique: Flowers are arranged in “boragoids”, i.e., dichasial partial inflorescences with monochasial, scorpioid branches (Buys and Hilger, 2003). Boraginaceae s.str. have been considered as a natural group for a very long time. They have been variously treated as an exclusive family (Boraginaceae s.str.)

Abbreviations: ML, maximum likelihood; BA, Bayesian analysis; BPPs, Bayesian Posterior Probabilities; MLBS, Maximum Likelihood Bootstrap Support.

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or – classically – as the typical subfamily (subfam. Boraginoideae, Gürke, 1893–1897) of a more widely defined family, which then included members lacking the typical ovary morphology and/or the typical inflorescence structure such as Cordiaceae, Heliotropiaceae and Hydrophyllaceae (APG, 2009). Delimitation of the group itself, irrespective of its taxonomic rank, has thus varied little in the past.

However, the subdivision of Boraginaceae s.str. (=Boraginaceae subfam. Boraginoideae) has been subject to highly divergent treatments and there is little agreement on the relationships within the family: Up to 13 tribes and eight subtribes have been recognized (Popov, 1953), including many monospecific genera and subtribes. Additional tribes were proposed subsequently, such as Trigonotideae (Riedl, 1967), Asperugeae Zakirov ex Ovczinnikova (Ovczinnikova, 2007), Echiochileae (Långström and Chase, 2002), Heterocaryeae Zakirov ex Ovczinnikova (Ovczinnikova, 2007), Trigonocaryeae Kerimov (Kerimov and Askerova, 2005). These and other infrafamilial classifications concentrated on removing individual “aberrant” genera into species-poor tribes or subtribes, but leaving the bulk of genera in the established tribes, leading to an equally complex and confusing infrafamilial classification. The definitions of these small (sub-)tribes are mostly based on evidently apomorphic characters such as twin-nutlets (Cerintheae Dumort.), zygomorphic flowers (Echieae Dumort.), reduction to one or two nutlets (Rochelieae DC.), or united sepals curving over the fruit (Harpagonelleae Baill.). The most recent listing of tribes was provided by Riedl (1997), recognizing the six tribes Boragineae, Cynoglosseae DC., Eritrichieae Benth. & Hook., Lithospermeae Dumort., Myosotideae Reichenb., and Trigonotideae Riedl. This subdivision is still generally accepted (Ariza-Espinar, 2006). Recent molecular studies (Långström and Chase, 2002; Långström and Oxelman, 2003; Weigend et al., 2010a), however, retrieve representatives of Eritrichieae, Myosotideae, and Trigonotideae as nested within Cynoglosseae s.l. The molecular data currently available indicate that the recognition of four major groups is justified: Boragineae, Echiochileae (Riedl) Långström & M.W. Chase, Lithospermeae and Cynoglosseae (including Eritrichieae). Trigonotideae were shown to represent a haphazard assemblage of unrelated genera in a recent study (Weigend et al., 2010a).

The relationships within three of these tribes are relatively well understood and have been studied with a combination of molecular and morphological characters: Lithospermeae (Böhle et al., 1996; Cecchi and Selvi, 2009; Seibert, 1978; Thomas et al., 2008; Weigend et al., 2009), Boragineae (Bigazzi et al., 1999; Guşuleac, 1923, 1928; Hilger et al., 2004; Selvi et al., 2006; Weigend et al., 2010a), and Echiochileae (Långström and Chase, 2002; Lönn, 1999). However, the largest and taxonomically and morphologically most complex group, Cynoglosseae s.l. (incl. Eritrichieae), is still very poorly understood. A recent attempt (Nazaire and Huford, 2012) at clarifying relationships fails to provide a resolved and supported backbone within Cynoglosseae and retrieves odd placements for several genera. Cynoglosseae s.l. comprise more than half of the species of the family, many of them in large and/or widespread and/or heterogeneous genera such as *Cryptantha* Lehm. ex G.Don (ca. 190 spp., Americas), *Cynoglossum* L. (ca. 100 spp., subcosmopolitan), *Eritrichium* Schrad. ex Gaudin (ca. 40 spp., Eurasia, North America), *Microula* (ca. 30 spp., E Asia), *Lappula* Moench (ca. 50–60 spp., Eurasia, Americas), *Hackelia* Opiz (ca. 45 spp., Eurasia, Americas), *Omphalodes* Mill. (20–30 spp., Eurasia, N America) and *Plagiobothrys* Fisch. & C.A. Mey. (ca. 70 spp., Americas, Australia, NE Asia). A publication providing insights on the relationships within *Cryptantha* and its immediate allies was published recently (Hasenstab-Lehman and Simpson, 2012), which shows the large genus *Cryptantha* to be paraphyletic and the genera *Amsinckia* and *Plagiobothrys* deeply nested in *Cryptantha*. However, the placement of this expanded *Cryptantha*-clade

(*Amsinckia*, *Cryptantha*, *Plagiobothrys*, *Pectocarya*) in Boraginaceae as a whole is still unresolved.

Numerous small, often monotypic genera have been described, such as *Amblynotus* I.M. Johnst., *Austrocynoglossum* Popov ex R.R.Mill., *Embadium* J.M.Black, *Gyrocarium* Valdés, *Metaeritrichium* W.T.Wang, *Mimophytum* Greenm., *Omphalolappula* Brand, *Sinojohnstonia* Huu, or *Tianschaniella* B.Fedtsch. These are generally poorly defined and have been segregated from larger genera without a critical study of the “parent taxa”.

Cynoglossum and its satellite genera are particularly problematic. *Cynoglossum*, in its current circumscription, is subcosmopolitan, with a clear center of diversity in the Mediterranean and western Asia, but with native species in the North America, western South America, East Africa and South Africa and Madagascar, the Himalayas, Japan and Australia. Additionally, numerous small, often monotypic genera have been segregated from *Cynoglossum* across the globe, such as African *Afrotysonia* Rauschert and *Cynoglossopsis* Brand, Australian *Austrocynoglossum* Popov ex R.R.Mill., Mexican *Oncaglossum* Sutorý and a whole range of Eurasian genera such as *Adelocaryum* Brand, *Ivanjohnstonia* Kazmi, *Lindelofia* Lehm., *Mattiastrum* (Boiss.) Brand, *Paracaryopsis* (Riedl) R.R.Mill., *Paracaryum* Boiss., *Paracynoglossum* Popov, *Pardoglossum* Barbier & Mathiez, *Rindera* Pall., *Solenanthus* Ledeb. and *Trachelanthus* Kunze. There have been several attempts at tidying up the group, by either the segregating and redefining individual genera (Mill and Miller, 1984; Mill, 2010; Riedl, 1971, 1981) or creating an infrageneric classification within *Cynoglossum*, without however touching the “classical” segregates such as *Lindelofia*, and *Paracaryum* (Riedl, 1962). Greuter and Burdet (in Greuter (1981)) reduced most of these segregate genera in a very widely defined genus *Cynoglossum*, without, however, contributing to the phylogenetic understanding of these morphologically divergent lineages.

Traditionally, Eritrichieae were differentiated from Cynoglosseae based on the shape of the gynobase: taxa with a narrowly pyramidal to subulate gynobase and mostly small nutlets were placed in Eritrichieae, whereas taxa with broadly pyramidal gynobase and mostly larger nutlets were placed into Cynoglosseae (Brand, 1914, 1925; Gürke, 1893–1897). There are several genera with a more or less flat gynobase (as in Lithospermeae and Boragineae), but with nutlets more similar to taxa in Eritrichieae or Cynoglosseae (i.e., sharing dorsiventrally flattened or angular fruits, having a median nutlet attachment or glochidiate or pubescent fruit), such as *Asperugo*, *Myosotis* or *Mertensia*. These genera have been difficult to place in the established tribes and were therefore either variously placed into different tribes by different authors, or were removed into monotypic tribes or subtribes. Recent molecular data (Långström and Chase, 2002; Weigend et al., 2010a; Khoshokhan Mozaffar et al., 2013) then clearly retrieved them within a more broadly defined Cynoglosseae, mixed with some members of Eritrichieae, indicating the paraphyly of the two tribes as previously proposed. Recently, Ovczinnikova addressed the systematics of tribe Eritrichieae based on palynological and fruit characters in a series of eight papers, which are summarized in a new infratribal classification (Ovczinnikova, 2009), but this mainly led to the revival or description of additional subtribes (e.g., Eritrichieae subtr. Anoplocaryiinae Ovczinnikova, Eritrichieae subtr. Echinosperrinae Ovczinnikova, Asperugeae Zakirov ex Ovczinnikova, Lithospermeae subtr. Pseudomertensiinae Riedl), and did not resolve the problems of the delimitation of the tribe or its internal relationships. An addition, the circumscription and subdivision of Eritrichieae of Ovczinnikova (2009) was shown to be completely at odds with phylogeny at least for American taxa in a recent paper (Hasenstab-Lehman and Simpson, 2012) including *Cryptantha*, *Pectocarya*, *Plagiobothrys* and *Pectocarya*. *Pectocarya*, placed in Cynoglosseae by Ovczinnikova, 2009, is here retrieved as sister to a *Cryptantha*-clade (Eritrichieae subtribe *Cryptanthiinae*

in Ovczinnikova (2009)) which is paraphyletic to the genera *Plagiobothrys* (Eritrichieae subtribe Allocaryiinae in Ovczinnikova (2009)) and *Amsinckia* (Eritrichieae subtribe Amsinckiinae in Ovczinnikova (2009)). Some insights were recently provided on the affinities of *Omphalodes* to *Myosotidium* and *Mertensia* to *Asperugo*, however on an entirely unresolved backbone (Nazaire and Hufford, 2012).

Thus, all recent studies indicate that there is still a lot of confusion with regards to infrafamilial classification of Boraginaceae s.str. and that the various partial classifications published in the last decades have largely created unnatural units. In the present study we attempt to identify the major clades of Boraginaceae s.str. and their immediate sister groups, with a special emphasis on the largest and most poorly understood subgroups Cynoglosseae and Eritrichieae. We also aim at clarifying some questions with regards to genus delimitation in the problematical complexes of *Cynoglossum* s.l., *Omphalodes* s.l. and *Eritrichium* s.l., using two plastid DNA markers and a wide taxonomic and geographical sampling largely based on personal field collections over the last two decades,

2. Material and methods

2.1. Plant material and taxon sampling

Several dozen field trips and the cultivation of a wide range of taxa provided much of the plant material for the study here presented. Plant material from the field and cultivation was silica-gel dried. Additionally, much plant material was provided by colleagues (see Acknowledgments). Several large herbarium loans allowed us to further complement the sampling. Overall, our sampling includes 172 of ca. 1700 species, representing 64 of the ca. 85 genera as currently recognized. All plant material was checked for correct determination, primarily with the following floristic treatments: Argentina (Ariza-Espinar, 2006), Bhutan (Mill, 1999), China (Ge-Ling et al., 1995), East Africa (Verdcourt, 1991), Europe (Tutin et al., 1972), Iran (Riedl, 1967), Malesia (Riedl, 1997), Pakistan and Kashmir (Ali and Nasir, 1989; Kazmi, 1970, 1971; Stewart, 1972), Russia/SSSR (Popov, 1953), Somalia (Thulin and Warfa, 2006), Turkey (Davis, 1979), USA (Gleason and Cronquist, 1991; Hickman, 1993).

Starting from the sampling in Weigend et al. (2009) and Weigend et al. (2010a,b) sampling was expanded to include a wide range of representatives of the bulk of the genera of Boraginaceae s.str. The present study includes a total of 188 taxa, 16 outgroup taxa from Ehretiaceae, Hydrophyllaceae, Heliotropiaceae, Codonaceae and Wellstediaceae and Vahliaceae and 172 ingroup taxa. Most sequences for outgroup taxa were obtained from Hilger & Diane (2003), Luebert & Wen (2008), Luebert et al. (2011a,b), and Mansion et al. (2009). Larger genera were represented with a range of species wherever possible. Intercontinentally disjunct genera (*Cynoglossum*, *Hackelia*, *Plagiobothrys*, *Omphalodes*) were represented by species from all parts of their range wherever possible. A list of the plant material used in this study including voucher numbers and GenBank accession numbers is provided in the Appendix. All taxa are present with complete sequences.

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from samples of silica-gel-dried leaves or herbarium material with a modified CTAB method (Doyle and Doyle, 1987). The PCR amplifications were performed in a Trio-Thermoblock thermal cycler (Biometra, Göttingen, Germany) in a 25 µl volume containing 0.6 U of Taq Polymerase, 5.0 mM MgCl₂, 100 µM of each dNTP, 0.2 µM of each primer and about 50 ng of genomic DNA. Amplification primers and cycling conditions followed Moore

and Jansen (2006) for *rps16*. Primers 'c' and 'f' were used for the amplification of the *trnL-trnF* region (Taberlet et al., 1991) and the thermal cycling conditions were the same as that for *rps16*. PCR products were purified with the peqGold Cycle-Pure Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany) or the QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) following manufacturer's instructions. Cycle sequencing was performed with Big-Dye Terminator v3.1 (Applied Biosystems, Foster City, California, USA). The resulting sequences were assembled using Chromas Pro v.1.33 (Technelysium Pty Ltd., Tewantin, QLD, Australia).

2.3. Alignment and phylogenetic analyses

Automatic alignments were conducted using the software Mafft v.6.603 (Katoh et al., 2002) with default settings, followed by manual adjustments using Se-AL 2.0a11 (Rambaut, 1996). The combined data set contained 2520 aligned positions (*rps16*: 1208, *trnL-trnF*: 1312) and 1754 distinct alignment patterns (*rps16*: 840, *trnL-trnF*: 914). Phylogenetic analyses were carried out with maximum likelihood (ML, Felsenstein, 1981) and Bayesian (BA, Mau et al., 1999) methods. ML was run in RAxML 7.2.8 (Stamatakis et al., 2008); and BA was conducted using MrBayes v.3.1 (Ronquist and Huelsenbeck, 2003). The nucleotide substitution model that best fits each data partition (*rps16* and *trnL-trnF*) was determined with Modeltest v.3.7 (Posada and Crandall, 1998) prior to the analyses. Selected models were GTR + Γ for *rps16* and GTR + I + Γ for *trnL-trnF*. The ML analyses were performed under the selected substitution models, with partitions unlinked. Branch support was calculated with 1000 non-parametric bootstrap replicates using the same settings as described above. BA was carried out, under the selected models, with partitions unlinked, for 2×10^6 generations/run with a sampling frequency every 1000 generations in four independent runs. After inspection of convergence in Tracer v.1.5 (available at <http://tree.bio.ed.ac.uk/software/tracer/>, accessed 12 May 2011), the 1×10^6 first generations were discarded as burn-in. BA analyses were conducted at the High-Performance Computing of the Freie Universität Berlin. We used the genus *Vahlia* (Vahliaceae) to root the final trees, because this genus has been suggested to be sister to the Boraginales (Bremer et al., 2002; Moore and Jansen, 2006).

3. Results

3.1. Major clades – Fig. 1

Fig. 1 displays the major clades of Boraginaceae here retrieved. Boraginaceae s.str. is found on a well-supported clade (1 BPP, 100 MLBS) with the monogeneric African families Wellstediaceae (1 BPP, 100 MLBS) and Codonaceae (1 BPP, 99 MLBS) as consecutive sister taxa. This (Codonaceae (Wellstediaceae–Boraginaceae))–clade is retrieved as sister to the remainder of Boraginales, i.e., the families Heliotropiaceae, Hydrophyllaceae and Cordiaceae/Ehretiaceae, which in turn are retrieved as a monophylum.

Echiochileae, a mostly deserticolous group with a center of diversity in N Africa and W Asia, is retrieved as the first branch of Boraginaceae s.str. (1 BPP, 100 MLBS). The remainder of the families falls into two sister-clades, one comprising Lithospermeae and Boragineae (0.92 BPP, 62 MLBS), as well-supported sister groups (1 BPP, 89 MLBS), the other Cynoglosseae incl. Eritrichieae (1 BPP, 97 MBS), i.e., comprising all genera not subsumed under the other three tribes.

Cynoglosseae s.l. again fall into two clades, a large clade comprising the bulk of the taxa (1 BPP, 93 MLBS), and a smaller clade with mainly *Caccinia* and *Trichodesma* (Trichodesmeae: 0.95 BPP, 92 MLBS). The major clade is not completely resolved, but has four

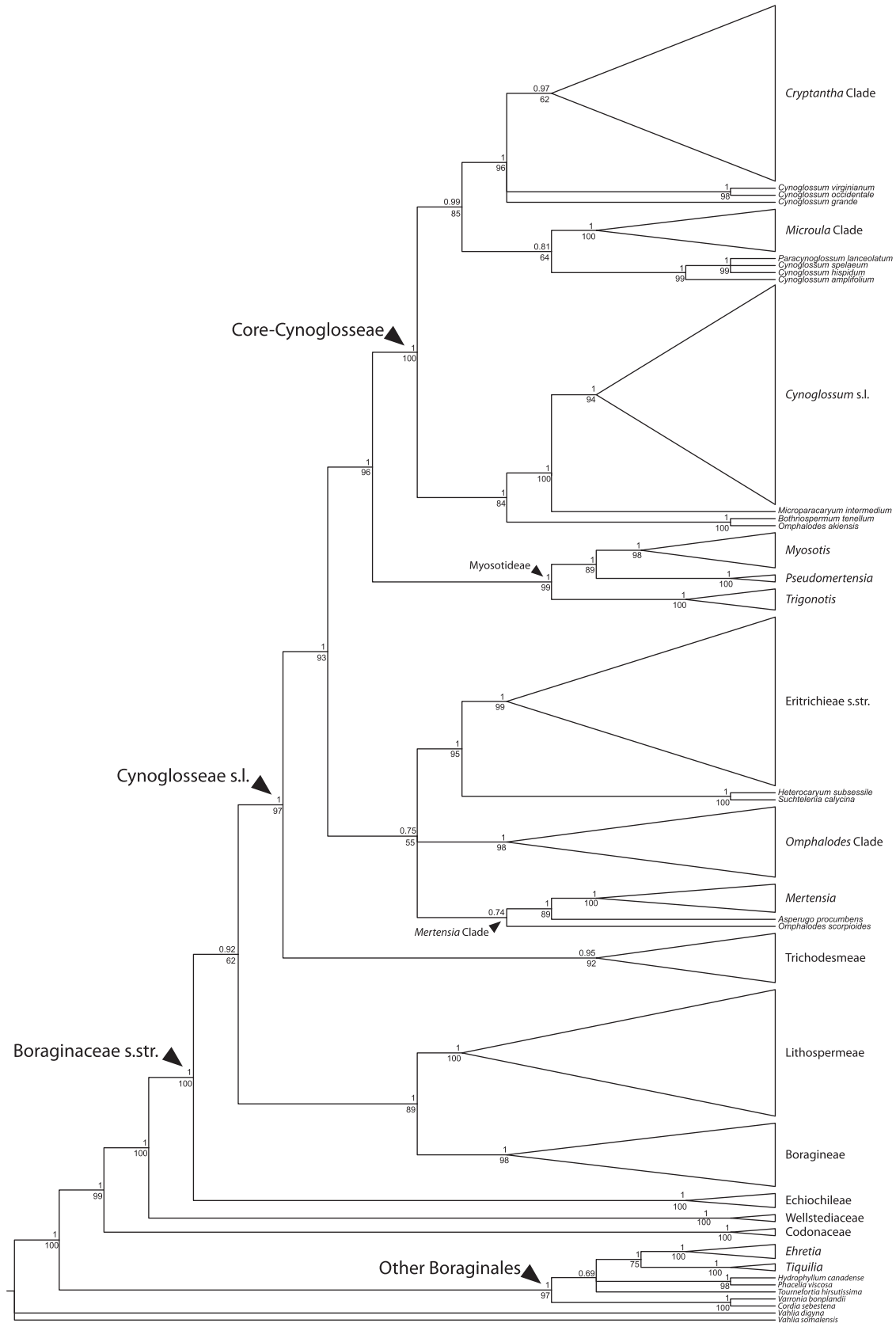


Fig. 1. Simplified phylogeny of Boraginaceae s.str. obtained from a Bayesian analysis of *rps16* and *trnL-trnF*. Major clades are named according to their largest genus or established names of tribes. Numbers above branches are Bayesian Posterior Probabilities and numbers below branches are Maximum Likelihood Bootstrap values.

well-supported subclades. Three of the clades are retrieved together with very low support (0.75 BPP, 55 MLBS): Named after their largest genera these are the *Mertensia*-clade, which has

Omphalodes scorpioides weakly supported (0.74 BPP) as sister to a well supported (1 BPP, 89 MLBS) clade comprising *Asperugo* and *Mertensia*; the *Omphalodes*-clade principally contains most species

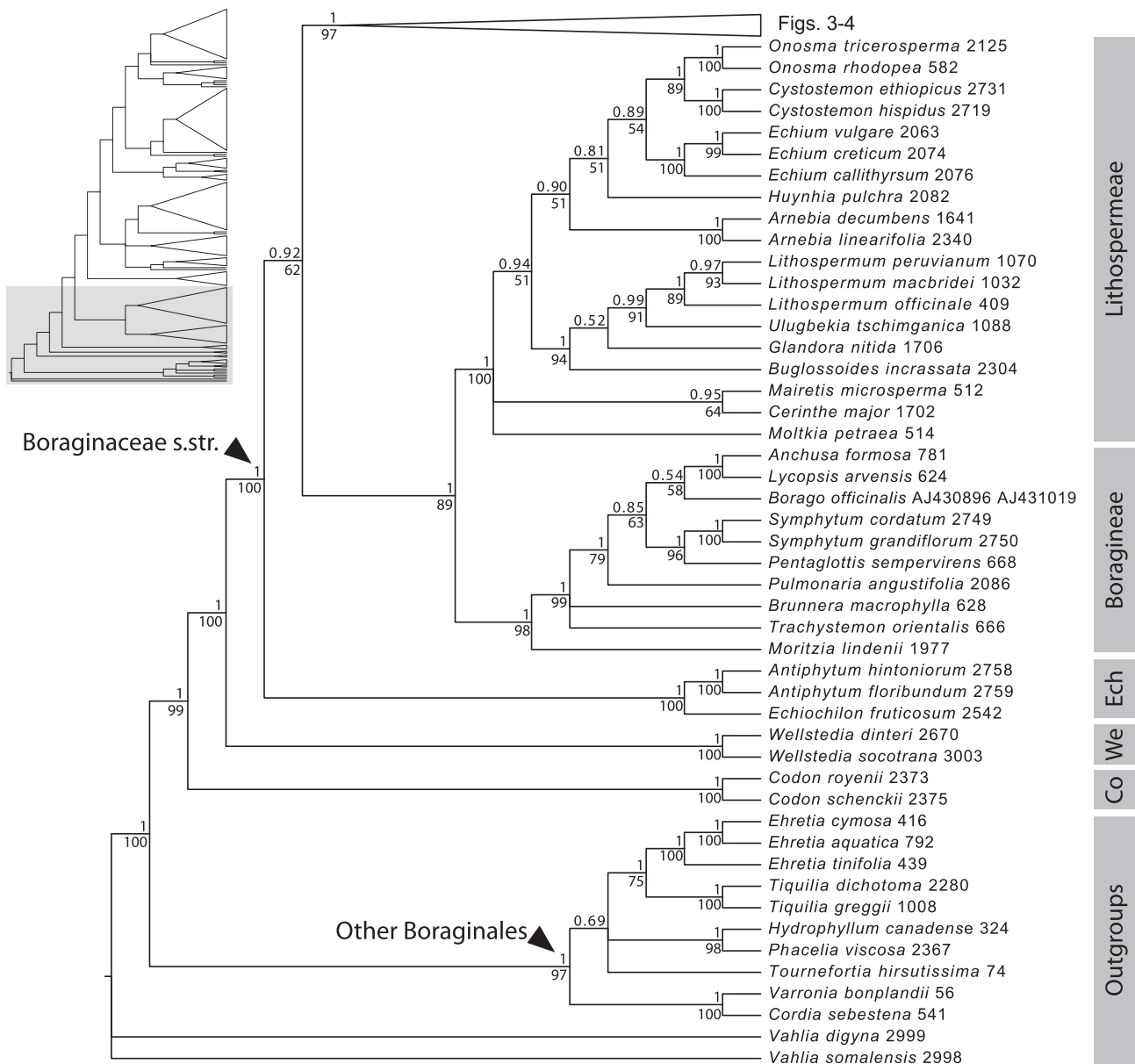


Fig. 2. Cladogram of Boraginaceae s.str. showing the other families of Boraginales and the basal grade of the family (Echiochileae, Boragineae, Lithospermeae), obtained from a Bayesian analysis of *rps16* and *trnL-trnF*. Top-left figure indicates the position of the displayed phylogeny in Fig. 1. Major clades are indicated (Co, Codonaceae; We, Wellstediaceae; Ech, Echiochileae). Numbers above branches are Bayesian Posterior Probabilities and numbers below branches are Maximum Likelihood Bootstrap values.

of *Omphalodes* sampled (1 BPP, 98 MLBS); the third major clade consists of Eritrichieae s.str. and in a well-supported sister relationship to *Heterocaryum* + *Suchtelenia* (1 BPP, 95 MLBS). The sister relationship between *Heterocaryum* and *Suchtelenia* (1 BPP, 100 MLBS) and the monophyly of Eritrichieae s.str. as here retrieved are strongly supported (1 BPP, 99 MLBS). The fourth, well-supported clade (1 BPP, 96 MLBS) comprises Myosotideae (*Myosotis* and allied genera, 1 BPP, 99 MLBS) as sister to a core-Cynoglosseae clade (*Cynoglossum* plus segregate genera plus American and East Asian Cynoglosseae and Eritrichieae, i.e., *Microula*, *Cryptantha* and allied genera) with high support (1 BPP, 100 MLBS).

In the Myosotideae *Myosotis* is recovered as a monophyletic group (1 BPP, 98 MLBS) and sister to *Pseudomertensia* (1 BPP, 98 MLBS), which are in turn sister to *Trigonotis* (1 BPP, 100 MLBS).

The *Cynoglossum* s.l. to *Omphalodes akiensis* clade (1 BPP, 84 MLBS) contains most species of the highly polyphyletic and paraphyletic genus *Cynoglossum* mixed with different species across its subclades. *Bothriospermum* plus *Omphalodes akiensis* are

recovered as sister (1 BPP, 84 MLBS) to a clade comprising *Microparacaryum* and the bulk Old World *Cynoglossum* and its segregate genera (1 BPP, 100 MLBS). These Old World *Cynoglossum* and its segregate genera form a well-supported clade (1 BPP, 94 MLBS), falling into two, moderately supported subclades.

The other large clade of core-Cynoglosseae is highly heterogeneous including a range of genera from both Eritrichieae and Cynoglosseae as traditionally defined, but well-supported (0.99 BPP, 85 MLBS) and shows a sister relationship between a highly supported American clade (American *Cynoglossum*, *Cryptantha* and allied genera, 1 BPP, 96 MLBS) and a weakly supported Asian-African clade (mainly *Microula*, 0.81 BPP, 64 MLBS). The American clade retrieves the North American species of *Cynoglossum* (*C. virginianum*, *C. occidentale*, *C. grande*) in an unresolved position with *Cryptantha* and allied genera. The Asian-African clade retrieves several species of *Paracynoglossum* and *Cynoglossum* with high branch support (1 BPP, 99 MLBS) as sister to Central Asian *Microula* and allied genera (1 BPP, 100 MLBS).

3.2. Echiochileae–Boraginaceae–Lithospermeae – Fig. 2

Fig. 2 shows the details of the tribes Echiochileae, Boraginaceae, and Lithospermeae. Echiochileae are represented by a total of three species of the larger genera *Echiochilon* and *Antiphytum*, which are retrieved together (1 BPP, 100 MLBS). Boraginaceae are here retrieved with South American *Moritzia* as sister to the rest of the tribe (1 BPP, 99 MLBS), followed by *Brunnera* and *Trachystemon* in an unresolved position relative to a well-supported clade comprising the genera *Pulmonaria*, *Pentaglottis*, *Symphytum*, *Borago*,

Lycopsis and *Anchusa* (1 BPP, 79 MLBS). Lithospermeae are retrieved with *Moltkia*, *Cerinth* and *Mairetis* largely unresolved, but with *Mairetis* sister to *Cerinth* (0.95 BPP, 64 MLBS). Core-Lithospermeae then show a sister relationship between a *Buglossoides*–*Lithospermum*-clade (1 BPP, 94 MLBS) and a larger, moderately supported clade with *Arnebia* in a basal position (0.94 BPP, 51 MLBS). In this *Arnebia*-clade, *Arnebia* (= *Huynhia*) *pulchra* is retrieved separate from the two other species of *Arnebia* sampled. *Onosma* and *Cystostemon* are retrieved as sister taxa (1 BPP, 89 MLBS) and are in turn sister to *Echium* (0.89 BPP, 54 MLBS).

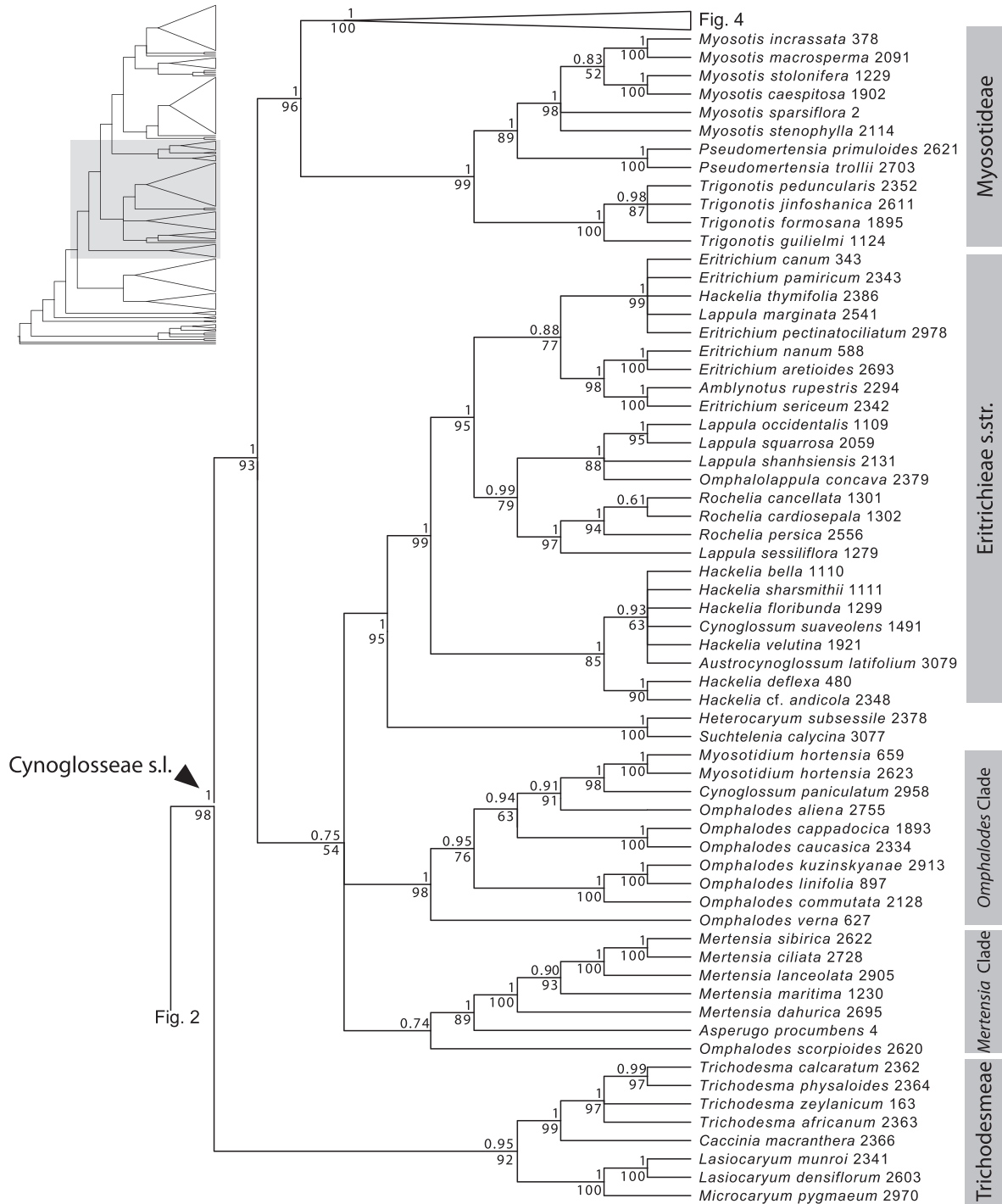


Fig. 3. Cladogram of Boraginaceae s.str. showing the basal grade of “Cynoglosseae/Eritrichieae” – Trichodesmeae, Mertensia, Omphalodes Clade, Eritrichieae s.str. and Myosotideae, obtained from a Bayesian analysis of *rps16* and *trnL-trnF*. Top-left figure indicates the position of the displayed phylogeny in Fig. 1. Major clades are indicated. Numbers above branches are Bayesian Posterior Probabilities and numbers below branches are Maximum Likelihood Bootstrap values.

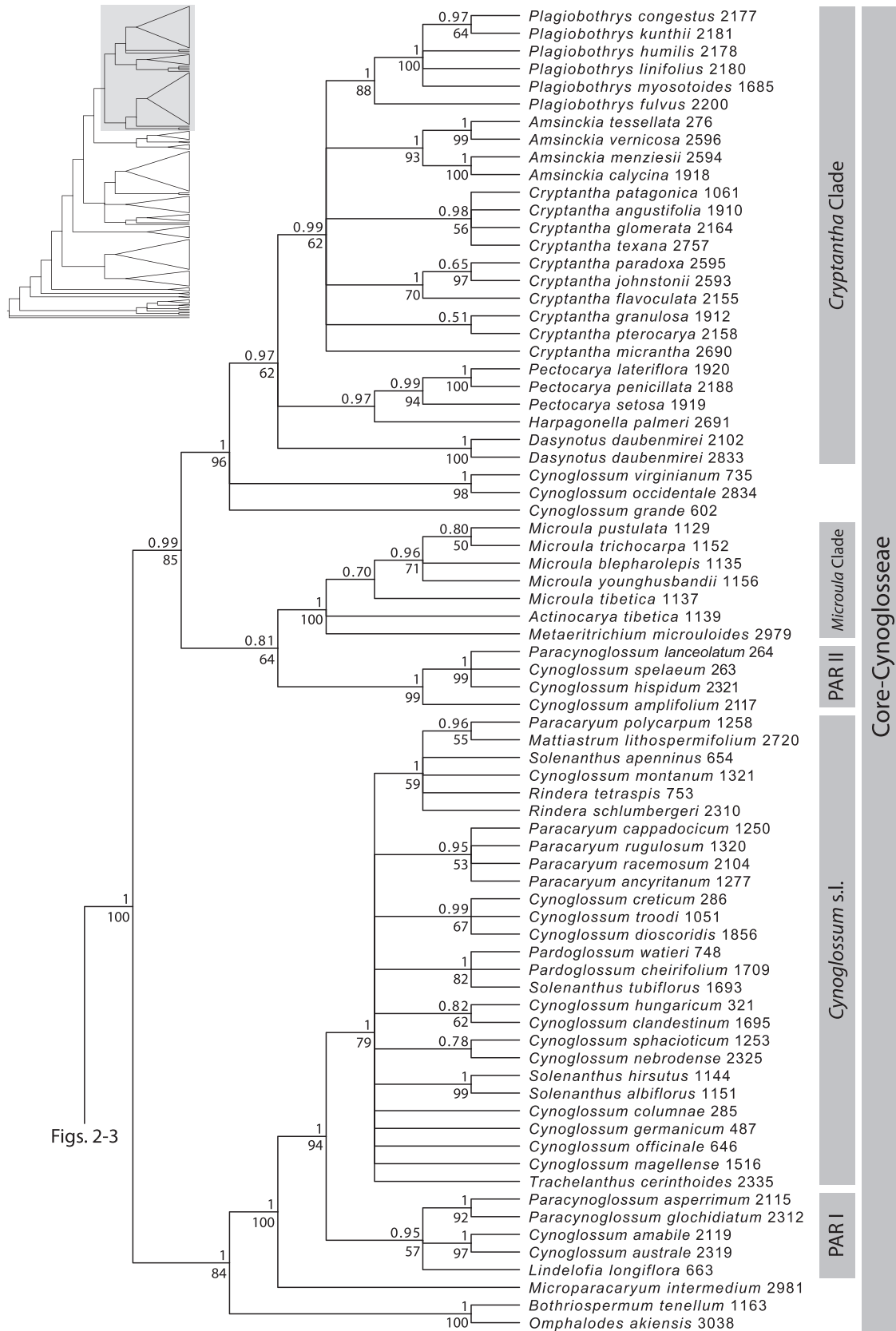


Fig. 4. Cladogram of Boraginaceae s.str. showing the core-Cynoglosseae, obtained from a Bayesian analysis of *rps16* and *trnL-trnF*. Top-left figure indicates the position of the displayed phylogeny in Fig. 1. Major clades are indicated (Par I and Par II: *Paracynoglossum* p.p.). Numbers above branches are Bayesian Posterior Probabilities and numbers below branches are Maximum Likelihood Bootstrap values.

3.3. Trichodesmeae–Eritrichieae s.str.–Mertensia–Omphalodes–Myosotideae – Fig. 3

Fig. 3 shows the details of the clades of Trichodesmeae and the Eritrichieae s.l., comprising genera such as *Mertensia*, *Lappula*, *Eritrichium*, *Hackelia*, *Omphalodes* and *Myosotis*.

Trichodesma and *Caccinia* are retrieved in a well-supported sister relationship (1 BPP, 99 MLBS) and are in turn sister (0.95 BPP, 92 MLBS) to the genera *Microcaryum* and *Lasiocaryum* (1 BPP, 100 MLBS).

Asian, American, and European species of *Mertensia* are retrieved as monophyletic (1 BPP, 100 MLBS) and sister to Eurasian *Asperugo procumbens* (1 BPP, 89 MLBS).

Most species of *Omphalodes* are retrieved on the *Omphalodes*-clade, but the genus as such is both paraphyletic and polyphyletic: Western Eurasian *O. scorpioides* is retrieved, albeit with low support (0.74 BPP), as sister to the *Asperugo–Mertensia*-clade, Japanese *O. akiensis* is retrieved with *Bothriospermum* in core-Cynoglosseae (Fig. 4). The western Eurasian and American species of *Omphalodes* are retrieved on one clade, but the western Eurasian taxa appear to represent the basal grade, with American *O. aliena* resolved as sister to Chatham Island endemic *Myositidium* and Chilean *Cynoglossum paniculatum*.

Heterocaryum and *Suchtelenia*, two enigmatic central Asian genera, are suggested to be sister to each other (1 BPP, 100 MLBS) and form the sister clade to Eritrichieae s.str., centered around the genera *Eritrichium*, *Hackelia* and *Lappula*. Eritrichieae s.str. in turn fall into two clades, one of them (1 BPP, 85 MLBS) dominated by *Hackelia*, but also including the Australian taxa *Cynoglossum suaveolens* and *Austrocynoglossum*. This *Hackelia*-clade is sister to an *Eritrichium–Lappula*-clade (1 BPP, 95 MLBS), which in turn falls into one subclade dominated by *Lappula* (with monophyletic *Rochelia* and Australian *Omphalolappula* nested in *Lappula*) and one dominated by *Eritrichium* (with *Amblynotus rupestris* and *Hackelia thymifolia* nested in *Eritrichium*).

Subcosmopolitan *Myosotis* is clearly recovered as sister to central Asian *Pseudomertensia* (1 BPP, 89 MLBS) and these two in turn are sister to eastern Asian *Trigonotis* (1 BPP, 99 MLBS).

3.4. Core-Cynoglosseae – Fig. 4

Fig. 4 shows the detailed relationships within core-Cynoglosseae in a narrower sense, including American “Eritrichieae” (i.e., *Cryptantha* and its allies). The core-Cynoglosseae fall into two large sister groups, one dominated by Eurasian taxa, mainly western Eurasian *Cynoglossum/Paracynoglossum* and segregate genera, the

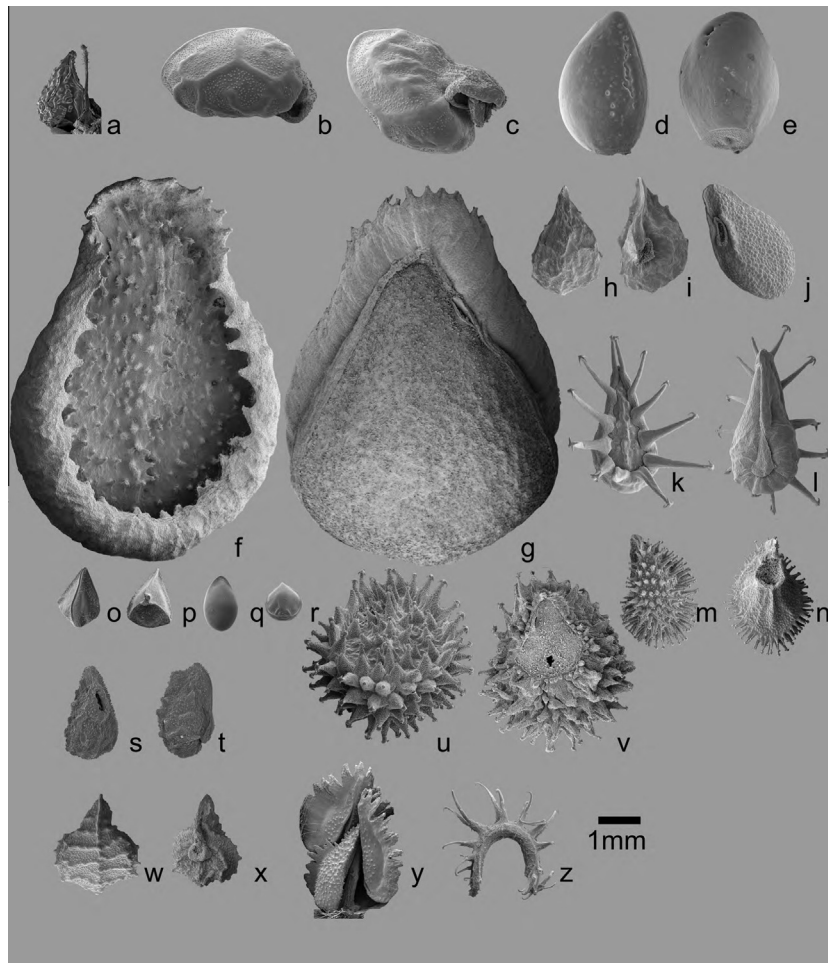


Fig. 5. Nutlets the major clades of Boraginaceae s.str. (SEM-pictures), magnification identical. Echiochileae: (a) *Antiphytum floribundum* (Lott et al. 5574, HUH); Boragineae: (b, c) *Pentagottis sempervirens* (Weigend 9065, BSB); Lithospermeae: (d, e) *Lithospermum erythrorhizon* (Weigend 8127, BSB); Trichodesmeae: (f, g) *Trichodesma boissieri* (Weigend 9197, BSB); *Mertensia*-clade: (h, i) *Mertensia sibirica* (Weigend 9069, BSB), (j) *Asperugo procumbens* (Weigend 7018, BSB); Eritrichieae: (k, l) *Lappula patula* (Dürbye 1680, B), (m, n) *Austrocynoglossum latifolium* (Weigend 9441, BSB); Myosotideae: (o, p) *Trigonotis tibetica* (Boufford et al. 34270, HUH); (q, r) *Myosotis arvensis* (Weigend 9179, BSB); *Microoula*-clade: (s, t) *Microoula ovalifolia* (Boufford et al. 39420, HUH); *Cynoglossum* s.l.: (u, v) *Cynoglossum amabile* (Weigend 9115, BSB); *Cryptantha* clade: (w, x) *Plagiobothrys nothofulvus* (Weigend 9178, BSB), (y) *Cryptantha pterocarya* (Weigend 9128, BSB), (z) *Pectocarya recurvata* (Weigend 9055, BSB).

other comprising two larger subclades, one dominated by Asian *Cynoglossum*/*Paracynoglossum* together with *Microula* (and its segregates), the other comprising the bulk of American Cynoglosseae (i.e., *Cynoglossum* and *Pectocarya*) and *Eritrichieae* (*Plagiobothrys*, *Amsinckia*, *Cryptantha*).

Cynoglossum s.l.: The type species (*C. officinale* L.) and the “typical” western Eurasian species of *Cynoglossum* s.l. are all retrieved on one, well-supported clade (1 BPP, 94 MLBS), which includes the majority of generic segregates that have been proposed, such as *Paracaryum*, *Solenanthus*, *Mattiastrum*, *Rindera*, *Pardoglossum*, *Solenanthus*, *Trachelanthus* and *Lindelofia*. These genera are deeply nested and mixed with each other and species of *Cynoglossum* on both subclades. The only clear resolution found is between an eastern Asian *Paracynoglossum*-I-clade (PAR I) including central Asian *Lindelofia* and Asian/Australian *Cynoglossum* (0.95 BPP, 57 MLBS) and a western Eurasian–Mediterranean *Cynoglossum*-clade including all western Eurasian and Mediterranean segregate genera (1 BPP, 79 MLBS). These two clades are sister to (western Eurasian) *Microparacaryum* (1 BPP, 100 MLBS) and these together to eastern Asian *Bothriospermum* and *Omphalodes akiensis* (1 BPP, 84 MLBS).

The second large clade of Cynoglosseae has two subclades, both of which have sister subclades representing species of *Cynoglossum*: The *Microula*-clade is sister to (0.81 BPP, 64 MLBS) a clade with African species of *Cynoglossum* and *Paracynoglossum* (PAR II – 1 BPP, 100 MLBS). Eastern Asian *Microula* itself is retrieved together with eastern Asian *Metaeritrichium* and *Actinocarya* (1 BPP, 100 MLBS), but without internal resolution.

Cryptantha is retrieved together with the American genera *Dasynotus*, *Harpagonella*, *Pectocarya*, *Plagiobothrys* as monophyletic group on a polytomy with all three North American species of *Cynoglossum* sampled. The *Cryptantha*-clade then again has a polytomy with three subclade: (a) Idaho-endemic *Dasynotus daubermirei*, (b) *Pectocarya* and *Harpagonella* (0.97 BPP), and (c) all species of *Cryptantha*, *Amsinckia* and *Plagiobothrys* sampled (0.99 BPP, 62 MLBS). In this latter *Cryptantha*-subclade the genera *Amsinckia* and *Plagiobothrys* (incl. *Allocarya*) are retrieved as monophyletic (1 BPP, 93 MLBS respectively 1 BPP, 88 MLBS), but *Cryptantha* is unresolved and retrieved in 4 separate clades.

4. Discussion

4.1. Placement of Boraginaceae in Boraginales

The phylogenetic data here presented for the first time provide an unambiguous position of Boraginaceae relative to their closest relatives in Boraginales. Boraginaceae are sister to African Wellstediaceae and Boraginaceae–Wellstediaceae are in turn sister to SW African Codonaceae. A sister-relationship of *Codon* to Boraginaceae s.str. was first indicated by Ferguson (1999), and is here confirmed based on a much broader sampling. With regard to the evolution of fruit characters, this clearly indicates that a reduction of seed number (from multi-seeded to generally four-seeded fruits) and transformation of fruit morphology (from dehiscent to indehiscent) took place at least twice independently in Boraginales, at least once in the Hydrophyllaceae–Heliotropiaceae–Cordiaceae-clade and once in the Codonaceae–Wellstediaceae–Boraginaceae-clade. In the latter clade there is a gradual transformation of fruit morphology and seed number: *Codon* has an entire, superior ovary with an apical style developing into conventional apically dehiscent capsules and ca. 60–70 seeds/fruit (own data), *Wellstedia* has bilaterally compressed, more or less bilocular and deeply two-lobed fruits, with the style inserting in a cleft between the two halves, and with to 2–4 seeds/fruit (Thulin and Johansson, 1996). In both these families the seeds are the dispersal units and the fruit (pericarp) remains on the plant. Boraginaceae have fruits strictly

subdivided into four mericarpids, with a gynobasic style inserted between them. The mature fruit usually falls into four mericarpids, which are dispersed separately. Only the central part of the gynoeceum, the so-called gynobase, remains on the plant.

The floral morphology of the three families also shows clear differences, with polymeric *Codon* (in calyx, corolla and androecium) different from Boraginaceae and Wellstediaceae, but also aberrant for Boraginales as a whole. The faucal and basal scales present in the corollas of typical Boraginaceae are absent (*Codon*) or very doubtfully developed as a basal corolla modification in Wellstediaceae. Faucal and basal scales are also very weakly developed in the tribe Echiochileae, where they are present only as ciliate or papillate faucal appendages or indistinct invaginations in the corolla throat. Boraginaceae themselves fall into the well-established clades Echiochileae, Lithospermeae–Boragineae and Cynoglosseae (including all the small tribes described). This result is congruent with what was found in previous molecular studies (Långström and Oxelman, 2003; Weigend et al., 2010a). The main differences are the larger extent of sampling and the much higher degree of supported resolution in Cynoglosseae s.l. (i.e., incl. Eritrichieae s.str., Myosotideae, etc.) reached in the present study.

4.2. Major clades of Boraginaceae s.str.

The present data confirms four major clades in the family: Echiochileae, Boragineae, Lithospermeae, and Cynoglosseae s.l. (incl. Eritrichieae). The delimitation of the former three groups is in line with recent molecular studies (Bigazzi et al., 1999; Cecchi and Selvi, 2009; Hilger et al., 2004; Khoshokhan Mozaffar et al., 2013; Långström and Chase, 2002; Lönn, 1999; Selvi et al., 2006; Thomas et al., 2008; Weigend et al., 2009, 2010a).

4.2.1. Echiochileae, Boragineae and Lithospermeae

The present data suggest a sister position of Echiochileae, which agrees with their aberrant fruit (Fig. 5a) and pollen morphology (Långström and Chase, 2002). The second larger clade consists of the pair Boragineae and Lithospermeae, with a well-supported sister relationship between these two tribes. This agrees well with morphology: the two groups have much similarity in fruit and flower morphology and are differentiated basically by opposite derivations of the fruit: Both have more or less ovoidal, often acuminate nutlets which are broadly sessile on a flat gynobase (Fig. 5b–e). Nutlets are often more or less distinctly keeled ventrally and/or dorsally. However, the pericarp in Lithospermeae is calcium-mineralized, and typically smooth and perforate, rarely verrucose (Fig. 5d, Hilger et al., 1993; Seibert, 1978). Conversely, the nutlets of Boragineae have a non-mineralized pericarp with a papillose surface, but usually possess a well-developed, plug-shaped elaiosome at base (Fig. 5c), which is absent in Lithospermeae (Guşuleac, 1928; Hilger et al., 2004; Johnston, 1924b; Riedl, 1963). The Cynoglosseae/Eritrichieae are here sampled broadly and concentrating on the problematic taxa for the first time, and the pattern emerging is far more detailed and far better supported than in previous studies. It clearly confirms the polyphyly of the two tribes. Furthermore, virtually all additional tribes and subtribes proposed are deeply nested in the Cynoglosseae s.l. (e.g., Trigonotideae, Myosotideae, Asperugeae, Anoplocaryinae, Pseudomertensiinae). Unlike in Nazaire and Hufford (2012) relationships between the clades are largely resolved and the major genera are grossly re-arranged, with Eritrichieae s.str. (essentially *Eritrichium*, *Lappula*, *Hackelia*) deeply nested in Cynoglosseae and sister to Heterocaryae including *Suchtelenia* (previously placed in Trichodesmeae). Eritrichieae in a wider sense are not retrieved at all, and its constituent genera are scattered throughout Cynoglosseae: The transformation from a broadly pyramidal to a narrowly subulate gynobase and a reduction of nutlet size has apparently taken

place numerous times independently. All recent attempts of reclassifying Cynoglosseae and Eritrichieae (Mill, 2010; Mill and Miller, 1984; Ovczinnikova, 2009; Riedl, 1962, 1971, 1981) failed to identify and name natural units.

4.2.2. *Trichodesmeae*

An expanded *Trichodesmeae* is retrieved as sister to the main clade of Cynoglosseae s.l., and *Trichodesma* and *Caccinia* resulted as sister groups. In contrast to Nazaire and Hufford (2012) *Trichodesma* is retrieved as clearly monophyletic. The close affinity between these two genera and their distinctness from *Cynoglossum* and its immediate allies are here confirmed, but the inclusion of *Suchtelenia* in *Trichodesmeae* (Al-Shehbaz, 1991; Riedl, 1997) or Cynoglosseae (Brand, 1921) is clearly refuted. *Trichodesma* and *Caccinia* have large, mostly flat, winged and glochidiate nutlets (Fig. 5f), which are dorsally very similar to those of *Cynoglossum* and some satellite genera, but differ in the very large cicatrix, i.e., are attached to the gynobase virtually with their entire adaxial surface (Fig. 5g), whereas there is a much narrower attachment region in *Cynoglossum* (Fig. 5v). *Trichodesma* and *Caccinia* are large and robust herbs or shrubs, with *Trichodesma scottii* from Socotra the only true tree in the family. It is therefore very surprising that they are retrieved as sister to the diminutive, mostly annual genera *Lasiocaryum* and *Microcaryum*, with minute, turbinate, often pubescent nutlets. The latter two genera were placed in Eritrichieae by Brand (1931), and due to their nutlet morphology, have been considered as part of their most typical subgroup until recently (Eritrichieae subtr. Eritrichiinae, Ovczinnikova, 2009). *Microcaryum* is the smallest species of Boraginaceae, barely reaching 3–5 cm in overall height. This first clade of Cynoglosseae s.l. thus already comprises the two extremes in life history and fruit morphology found in the tribe overall and comprises representatives previously assigned to the two major tribes Eritrichieae and Cynoglosseae. The morphological coherence of the two genus pairs *Lasiocaryum*/*Microcaryum* and *Caccinia*/*Trichodesma* is only borne out by their broad attachment to the gynobase, which is not found in any of the groups they have been traditionally associated with.

4.2.3. “Eritrichieae” (*Mertensia* clade + *Omphalodes* clade + *Eritricheae* s.str.)

There is only moderate support for the monophyly of a group of – individually well-supported – subclades which include Eritrichieae s.str. (*Eritrichium*, *Lappula*, *Hackelia* and satellite genera) as sister to Heterocarpaceae including *Suchtelenia*. This Eritrichieae-clade is retrieved on a polytomy with clades including the *Omphalodes*-clade (formerly Cynoglosseae) and the *Mertensia* clade (traditionally placed in Lithospermeae or Trigonotideae). While relationships between the clades are not fully resolved, all three clades show some surprising placements:

Mertensia clade: The members of *Mertensia* are retrieved as a monophylum, but surprisingly sister to *Asperugo* (traditionally placed in the monotypical tribe Asperugeae). This relationship was also shown by Nazaire and Hufford (2012). The climbing annual *Asperugo*, with its folded calyx and highly asymmetrical nutlets (Fig. 5j) shows no obvious morphological similarity to *Mertensia*, but nor does it show any clear affinity to other members of Boraginaceae, which would contradict its molecular placement. Closer examination shows that *Asperugo* and *Mertensia* share an oblique cicatrix and adaxial nutlet side (Fig. 5h–j), which is not elsewhere found in Boraginaceae, underscoring their otherwise surprising close relationship.

Omphalodes clade *Omphalodes* is characterized by nutlets with mostly incurved, membranaceous wings on a broadly pyramidal gynobase, with a few taxa showing reduced and/or coriaceous marginal wings (Nesom, 1988). Nutlets are always strongly dorsiventrally compressed and have a very large attachment region to

the broadly pyramidal gynobase. However, “typical” western Eurasian taxa (including the type species *O. verna*) are paraphyletic to the American representative *O. aliena*, and also to South American *Cynoglossum paniculatum* (with dorsally convex, densely glochidiate nutlets) and Chatham Island endemic *Myosotidium* (with broad, coriaceous winged nutlets). Nazaire and Hufford (2012) showed that *Myosotidium* is nested in *Omphalodes*, but they failed to include western hemisphere taxa of this clade in their study. *Omphalodes* is thus paraphyletic and includes *Myosotidium* and at least one species of *Cynoglossum*. At least *Myosotidium* agrees well with the remainder of *Omphalodes* based on its broadly sessile, coriaceous and dorsiventrally flattened nutlets. At the same time, two species of *Omphalodes* are retrieved outside the *Omphalodes*-subclade: *O. scorioides*, with superficially typical *Omphalodes*-fruit, but a much smaller, subcircular cicatrix, is retrieved in the *Mertensia*-clade, which otherwise lacks species with winged fruits. *Omphalodes scorioides* has been considered as isolated in *Omphalodes* for a long time, but no alternative placement have been suggested (Brand, 1921). The orientation of the nutlet and size and location of the areola are, however, well in line with a placement near *Mertensia* and *Asperugo*. Japanese *O. akiensis* appears to be completely unrelated to the remainder of *Omphalodes*. It belongs to the core-Cynoglosseae and is most closely allied to morphologically aberrant *Bothriospermum*. This confirms the findings of Popov (1953) and Coutinho et al. (2012) on closely allied *O. japonica* as not similar to the remainder of *Omphalodes*.

Eritrichieae s.str.: The third major clade consists of (*Heterocaryum* + *Suchtelenia*) and Eritrichieae in the narrowest sense in a well-supported sister relationship. Khoshokhan Mozaffar et al. (2013) have very recently shown that *Rochelia* and *Lepechiniella* are deeply nested in *Lappula* and that *Heterocaryum* is sister to a clade of *Eritrichium* and *Lappula*. We can further show a sister relationship between *Heterocaryum* and *Suchtelenia* (the latter usually placed in *Trichodesmeae*) as first suggested by Popov (1953). Popov (1953) recognized that both genera share the presence of two different pairs of nutlets in each fruit with the ventral side of the nutlet deeply immersed into the gynobase and only two of the nutlets becoming detached, whereas the other two are dispersed with the gynobase. This unique derived condition thus underscores their exclusive relationship. Eritrichieae essentially include the three major genera *Eritrichium*, *Lappula* (triangular nutlets with elongated cicatrix, Fig. 5k and l), and *Hackelia*. All segregate genera in this complex are clearly nested in one of these major genera, *Amblynotus* (in *Eritrichium*), *Omphalolappula* and *Rochelia* (in *Lappula*) and *Austrocynoglossum* (*Cynoglossum*-like nutlet with rounded cicatrix, Fig. 5n, in *Hackelia*). *Cynoglossum suaveolens* is also retrieved in *Hackelia* and can be transferred to that genus. This reduces the number of genera recognized from seven to three, greatly simplifying genus delimitation.

4.2.4. *Myosotideae*

Myosotideae are not retrieved as monophyletic by Nazaire and Hufford (2012), who also retrieve the genus *Trigonotis* on two widely distant, albeit unsupported clades. Our data clearly contradict this finding, retrieving *Myosotideae* including the genera *Trigonotis* and *Pseudomertensia* (formerly placed in Lithospermeae) as well supported clade and sister to Core-Cynoglosseae. All three genera have small, non-glochidiate, lentil-shaped or tetrahedral nutlets on an essentially flat gynobase and are thus clearly allied (Fig. 5o–r). With their relatively small, softly pubescent leaves, small, mostly hypocrateriform corollas it is often difficult to even distinguish the genera at first sight, and *Trigonotis* and *Myosotis* can only be keyed out by differences in petal aestivation, so that the monophyly of this group of genera does not come as a surprise and it is rather surprising that the close coherence of these three genera was not recognized previously.

4.2.5. Core-Cynoglosseae

The core-Cynoglosseae represent a mixture of elements formerly placed in Eritrichieae (e.g., *Microula*, *Bothriospermum*, *Cryptantha*, *Plagiobothrys*), the bulk of the species of *Cynoglossum* and its satellite genera, and some previously unplaced taxa such as *Dasynotus* or *Omphalodes akiensis*, totalling 74 of the 172 accessions investigated. Core-Cynoglosseae consists of several, well-resolved subclades which are characterized by the predominance of, or at least presence of several species of *Cynoglossum*. The genus *Cynoglossum* as currently defined is highly poly- and paraphyletic, with some species retrieved already in *Hackelia* (*C. suaveolens* from Australia) and *Omphalodes* (*C. paniculatum* from Chile). In core-Cynoglosseae, species of *Cynoglossum* are scattered over a total of four different subclades:

- (a) several Asian and one Australian species of *Paracynoglossum* and *Cynoglossum* together with Himalayan *Lindelofia* (PAR I) as sister to
- (b) all western Eurasian *Cynoglossum* mixed with representatives of a total of five segregate genera (*Paracaryum*, *Pardoglossum*, *Solenanthus*, *Mattiastrum*, *Trachelanthus*), which have been segregated based on details of nutlet morphology (*Cynoglossum* s.l.).
- (c) several Asian and African species together with *Paracynoglossum* (PAR II) as sister to the central Asian *Microula*-subclade, and
- (d) the three native North American species sampled on a clade together with monotypic North American *Dasynotus* and the three predominantly North American genera *Plagiobothrys*, *Pectocarya*, *Amsinckia* and *Cryptantha*.

Overall, the species currently placed in *Cynoglossum* thus belong to a total of six, more or less independent lineages and represent a grade, not a clade. The genus circumscription and subdivision of Riedl (1962) created entirely unnatural units and the taxa included in *Cynoglossum* by Greuter and Burdet (in Greuter (1981)) do not belong to a monophyletic group. At the same time, *Cynoglossum* even in its narrowest circumscription (*Cynoglossum* s.l. in Fig. 4) is paraphyletic to *Mattiastrum*, *Paracaryum*, *Pardoglossum*, *Rindera*, *Solenanthus*, *Trachelanthus*. The results of Selvi et al. (2011) are here underscored, who found that even some of the small, mainly Mediterranean segregate genera, such as *Solenanthus*, *Paracynoglossum* and *Paracaryum*, are polyphyletic and the narrow morphological definitions based on details of the fruit or flower morphology are evidently incorrect. The species separated as *Paracynoglossum* fall into two different clades (PAR I and PARII). The clade including *P. glochidiatum* (= *P. denticulatum*) as type species of *Paracynoglossum* (Ge-Ling et al., 1995; Popov, 1953; Riedl, 1963) could be taxonomically redefined as *Paracynoglossum*, but at present no morphological data have been brought forward with which to define this clade.

The Asian genus *Microula* and closely allied *Metaeritrichium* and *Actinocarya*, the latter two recently synonymised to *Actinocarya* (Han et al., 2012) with mostly small, herbaceous species and small, often eglochidiate fruits on a more or less flat gynobase (Fig. 5s and t) are retrieved as sister to larger species of *Cynoglossum*/*Paracynoglossum* with conventional *Cynoglossum* fruit (dorsally convex, densely glochidiate, with more or less distinct rim, on pyramidal gynobase, Fig. 5u and v). The same phenomenon is again encountered on the *Cryptantha*-clade, where the perennial species of North American *Cynoglossum*, with large, dorsally convex fruits on a pyramidal gynobase, represent the basal grade, and the smaller, often annual and ephemeral taxa of *Cryptantha*, *Amsinckia* and *Plagiobothrys* with their narrow, usually eglochidate nutlets on a subulate gynobase (Fig. 5w–y) represent the derived condition. *Dasynotus*, an enigmatic

monotypic genus from Idaho, is here retrieved on the crown-clade with *Cryptantha* and its allied genera. With its perennial habit, large flowers and fruits (similar to North American *Cynoglossum*), but nutlets with a dorsal keel (similar to *Cryptantha* and allied genera) it represents the perfect connecting link between these two, superficially disparate groups.

An isolated position of North American *Cynoglossum* within the genus has never been suggested. Riedl (1963) even placed it into the typical section of *Cynoglossum* as putatively closely allied to Central European species. However, closer examination shows that the North American species differ in their much shorter, broadly triangular areola from the other subgroups in the genus. *Pectocarya*, which has always been placed in Cynoglosseae because of its pyramidal gynobase and recurved, glochidiate nutlets (Fig. 5z), is here shown to be closely allied to the florally and vegetatively similar taxa of *Cryptantha* and *Plagiobothrys*, indicating that fruit morphology is here not a good indicator of relationship. Hasenstab-Lehman and Simpson (2012) retrieved *Pectocarya* in an unresolved position relative to *Cryptantha*/*Amsinckia*/*Plagiobothrys*. Our data clearly show that the latter three genera represent a monophyletic group (as would be expected from morphology), but that *Pectocarya* represents an independent albeit closely allied lineage and is sister to monotypic *Harpagonella*, at least with the species here included.

4.3. Phylogeography

The consecutive sister taxa of Boraginaceae, Codonaceae and Wellstediaceae, are both restricted to Africa, and the sister clade of Boraginaceae, Echiochileae, is largely restricted to northern Africa and western/central Asia (only *Antiphytum* in the Americas). Both Lithospermeae and Boragineae are predominantly western Eurasian, in the case of Boragineae only two small genera are found in South America (Weigend et al., 2010a), in *Lithospermeae* numerous species of *Lithospermum* are found in the Americas, but these are nested in the Eurasian species and none of the other genera of the tribe is present in the New World (Weigend et al., 2009, 2010b). The same overall pattern repeats itself virtually across all clades and subclades: Trichodesmeae are predominantly western and central Asian, with a few species of *Trichodesma* in Africa and Australia. On the *Mertensia*-clade, the two first-branching taxa (*Omphalodes scorpioides* and *Asperugo*) are western Eurasian, the same is true for the *Omphalodes*-clade. The sister-clade to the Eritrichieae s.str. (with the bulk of the species in Asia and only a few species in Australia, Europe and Africa) are the Heterocaryeae, an exclusively central Asian group. In Myosotideae, only representatives of the most derived genus, *Myosotis*, are found outside Asia. Core-Cynoglosseae have their center of diversity in western Eurasia, with a few taxa of *Cynoglossum* in Africa and Asia, and some clades predominantly eastern Asian. Only a single lineage of core-Cynoglosseae appears to have colonized the New World, the *Cryptantha*-clade, which is in turn sister to an Asian clade with *Cynoglossum* and *Microula*. The primary diversification of Boraginaceae thus appears to have taken place in Africa and western Eurasia, with an early and massive diversification of several lineages in eastern Asia. Dispersal to Australia appears to have occurred repeatedly, with *Cynoglossum suaveolens* and *Austrocynoglossum* arising from within *Hackelia*, *Cynoglossum australe* from SE Asian *Cynoglossum* s.l. and *Omphalolappula* arising from *Lappula*. Dispersal from America seems to have occurred in *Plagiobothrys*. However, there was no radiation of Boraginaceae in Australia and New Zealand, with the only exception of *Myosotis* (Myosotideae) in New Zealand (Winkworth et al., 2002). Conversely, there have been several dispersal events to the Americas as already documented in the literature: in the Boragineae (*Thaumatocaryum* and *Moritzia*, Weigend et al., 2010a), *Lithospermum* (Weigend et al., 2009), *Myosotis*

Table 1

The tribal (or informal) placement of the genera of Cynoglosseae/Eritrichieae as here included compared to most recent placements in the literature (Tribe abbreviations: Cynoglosseae = CYN, Eritrichieae = ERI, Harpagonelleae = HAR, Heterocaryeae = HET, Lithospermeae = LIT, Myosotideae = MYO, Rochelieae = ROC, Trichodesmeae = TRI, Trigonotideae = TRG, subtribe abbreviations: Allocaryinae = ALLI, Amsinckinae = AMSI, Asperuginae = ASPI, Cryptanthinae = CRYI, Echinosperrinae = ECSI, Eritrichiinae = ERII, Harpagonellinae = HARI, Heterocaryinae = HETI, Rochelliinae = ROCI, Pseudomertensiinae = PSEI).

Tribe (synonyms)	Placement as retrieved in present study	Al-Shehbaz (1991)	Riedl (1967, 1997)	Ovczinnikova (2007/2009)	Popov (1953)	Subtribes here included
<i>Trichodesmeae</i> Zakirov ex Riedl. (1967)	<i>Caccinia</i> Savi	TRI	TRI	–	TRI	
	<i>Heliocarya</i> Bunge	–	TRI	–	(TRI)	
	<i>Trichodesma</i> R. Br.	TRI	TRI	–	TRI	
	<i>Lasiocaryum</i> I.M. Johnst.	–	(ERI–ERII)	–	–	
<i>Omphalodes</i> -clade	<i>Microcaryum</i> I.M. Johnst.	–	–	ERI–ERII	–	
	<i>Omphalodes</i> Mill.	CYN	CYN	–	CYN	
	<i>Cynoglossum</i> L. p.p. – South American species	–	–	–	–	
Myosotideae Rchb.f. (1858)	<i>Myosotidium</i> Hook	ERI	ERI	–	–	
	<i>Pseudomertensia</i> Riedl	ERI	ERI–PSEI	–	–	Pseudomertensiinae H. Riedl. (1967)
Eritrichieae Gürke (1893). syn.	<i>Myosotis</i> L.	MYO	MYO	–	MYO	
	<i>Trigonotis</i> Steven	TRG	TRG	–	LIT–TRGI	
Eritrichieae Gürke (1893). syn.	<i>Lappula</i> Moench	ERI	ERI–ERII	ERI–ECSI	ERI–CYN	Echinosperrinae Ovczinnikova (2005)
	<i>Omphalolappula</i> Brand	ERI	–	–	–	
	<i>Sclerocaryopsis</i> Brand	–	–	–	(ERI–CYN)	
Rochelieae A.DC. (1846)	<i>Rochelia</i> Rchb.	ERI	ERI–ROCI	–	ROC	
	<i>Eritrichium</i> Schrad. ex Gaudin	ERI	ERI–ERII	ERI–ERII	ERI–CYN	
	<i>Amblynotus</i> I.M. Johnst.	–	–	ERI–ERII	ERI–LITN	
	<i>Hackelia</i> Opiz	ERI	ERI	ERI–ERII	ERI–CYN	
	<i>Austrocynoglossum</i> Popov ex R.R. Mill	ERI	–	–	–	
Heterocaryeae Zakirov ex Ovczinnikova (2007)	<i>Heterocaryum</i> A.DC.	–	ERI–HETI	HET	HET	Heterocaryinae Riedl (1967)
<i>Mertensia</i> -clade	<i>Suchtelenia</i> Karel. ex Meisn.	TRI	TRI	TRI	TRC	
	<i>Asperugo</i> L.	ERI	ERI–ASPI	ASP	ASP	Asperugeae Zakirov ex Ovczinnikova (2007)
<i>Microula</i> -clade	<i>Mertensia</i> Roth	TRG	TRG	–	LIT–TRGI	
	<i>Omphalodes</i> Moench p.p. (<i>O. scorpioides</i>)	–	–	–	–	
	<i>Cynoglossum</i> L. p.p.	–	–	–	–	
	<i>Microula</i> Benth.	ERI	ERI	ERI–ERII	–	
	<i>Actinocarya</i> Benth.	CYN	CYN	–	–	
Cryptanthaeae Brand (1925) nom. illeg. syn. Harpagonelleae Baill. (1890)	<i>Metaeritrichium</i> W.T. Wang	–	–	ERI–ERII	–	
	<i>Paracynoglossum</i> Popov p.p.	–	–	–	CYN	
	<i>Amsinckia</i> Lehm.	ERI	ERI	ERI–AMSI	–	Allocaryinae Grig. ex Ovczinnikova (2007)
	<i>Cryptantha</i> Lehm. ex G.Don	ERI	ERI	ERI–CRYI	–	
	<i>Cynoglossum</i> L. p.p. (North American)	–	–	–	–	
Cynoglosseae W.D.J. Koch (1837)	<i>Dasynotus</i> I.M. Johnst.	–	–	–	–	
	<i>Plagiobothrys</i> Fisch. & C.A. Mey.	ERI	ERI	ERI–ALLI	–	
	<i>Allocarya</i> Greene	–	–	ERI–ALLI	ERI–LITN	
	<i>Pectocarya</i> DC. ex Meisn.	CYN	CYN	CYN	–	
	<i>Harpagonella</i> A. Gray	–	–	–	–	
	<i>Lepechinella</i> Popov	ERI	(ERI–ERII)	ERI–ECSI	ERI–CYN	Cynoglossinae Dumort. (1827)
<i>Microparacaryum</i> (Popov ex Riedl) Hilger & Podlech	<i>Microparacaryum</i> (Popov ex Riedl)	–	(CYN)	–	(CYN)	
	<i>Cynoglossum</i> L. s.l.	CYN	CYN	CYN	CYN	
	<i>Lindelofia</i> Lehm.	CYN	CYN	–	CYN	
	<i>Mattiastrum</i> (Boiss.) Brand	CYN	CYN	CYN	CYN	
	<i>Paracaryum</i> Boiss.	–	–	–	CYN	
	<i>Paracynoglossum</i> Popov p.p.	CYN	CYN	CYN	–	

(continued on next page)

Table 1 (continued)

Tribe (synonyms)	Placement as retrieved in present study	Al-Shehbaz (1991)	Riedl (1967, 1997)	Ovczinnikova (2007/2009)	Popov (1953)	Subtribes here included
	<i>Pardoglossum</i> Barbier & Mathez	CYN	CYN	CYN	CYN	
	<i>Rindera</i> Pall.	CYN	CYN	CYN	CYN	
	<i>Solenanthus</i> Ledeb.	CYN	CYN	–	CYN	
	<i>Trachelanthus</i> Kunze	TRG	TRG– BOTI	TRG	LIT–BOTI	
	<i>Bothriospermum</i> Bunge					

(Winkworth et al., 2002), Eritrichieae s.str. (in *Hackelia*, *Eritrichium* and *Lappula* – Johnston, 1924a,b). The data here presented further indicate that western Eurasian *Omphalodes* gave rise to American *Omphalodes* and some “*Cynoglossum*”, Asian *Mertensia* reached North America and diversified there and the large *Cryptantha* clade originated in situ from ancestors in “*Cynoglossum*”, which are in turn sister to an Asian-African *Microula*/*Paracynoglossum* clade. The *Cryptantha* clade is by far the most speciose North American group of Boraginaceae, with several hundred species. The overall picture emerging from present-day distribution patterns would be that the primary divergence events in the Boraginaceae-lineage occurred in Africa/western Eurasia with subsequent major diversifications across temperate Asia and several late dispersal events to Australia and the New World.

5. Conclusions

Here, for the first time, detailed and well-supported relationships of Boraginaceae s.str. are presented, both with regards to their closest relatives and with regards to the major lineages in the order. The consecutive sister-relationships Codonaceae–Wells-ediaceae–Boraginaceae s.str. show a clear trend towards ovule reduction, segregation and finally the development of a gynobasic style and four separate nutlets, they also trace the gradual transformation of the corolla, with increasing elaboration of the faulcal and basal scales. Within Boraginaceae, the overall transformations of the fruit and gynobase morphology are very well reflected in the phylogenetic data and retrieve well-supported and morphologically plausible clades. The elevated gynobase with ventral (versus basal) attachment of the nutlet appears to be the key-synapomorphy characterizing the large Cynoglosseae/Eritrichieae clade (Cynoglosseae s.l.). Details of gynobase and nutlet shape, nutlet orientation and sculpturing are, conversely, highly homoplasious and have only limited value for the definition of groups. Small-fruited lineages with subulate gynobases (“Eritrichieae”) thus have arisen repeatedly independently. The data here presented permit the publication of a revised infra-familial classification of the family in the near future. Large-scale re-arrangements of generic limits will be required, concerning mainly the genus *Cynoglossum* s.l. (with several species transferred to other genera and numerous genera synonymised with *Cynoglossum* s.str.), but also genera such as *Hackelia*, *Omphalodes*, *Lappula*, and *Eritrichium*. Table 1 summarizes the most recent placement for the genera included in this study and the placement suggested in the study here presented.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.04.009>.

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Glossary

Areola: the scar on the gynobase, where the nutlet was attached during its development

Cicatrix: the scar on the nutlet, where it was attached to the gynobase during development, with/foraminated/pierced by the funicular canal

Gynobasic: due to the early subdivision of the ovary the style inserts not on the apex of the ovary (as is usually the case), but apparently directly on the receptacle between the four nutlets. This is termed gynobasic

Gynobase: the part of the ovary, the style and the receptacle which remains in the flower after dispersal of the nutlets. Depending on the orientation and attach-

ment of the nutlet (see areola, cicatrix) and the degree of development of style and receptacle the gynobase can be virtually flat, with four depressed scars, or variously pyramidal or subulate, then often with elongate scars

Nutlet: during ontogeny the basically bi-carpellate ovary of Boraginaceae subdivides and bulges out into four one-seeded mericarpids, which are usually dispersed separately, these are called nutlets