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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*, *rps*16) 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 Borageineae–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.

1. Introduction

Boraginaceae s.str. are a subcosmopolitan plant family with a center of diversity in the northern temperate zone. Their gynoecium 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 Hufford, 2012) at clarifying relationships fails to provide a resolved and supported backbone within Cynoglosseae and retrieves odd placements for several genera.space 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 s 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, *Gyrocaryum* 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 & Mathez, *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; Khoshsokhan 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. Echinosperminae 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