WILEY-BLACKWELL

Cladistics (2013) 1–31

Cladistics

10.1111/cla.12036

A phylogenetic analysis of morphological and molecular characters of Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution

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Accepted 22 April 2013

Abstract

The angiosperm family Boraginaceae includes ca. 1600 species distributed among ca. 110 genera. Some floral features are constant within the family, but many vegetative, floral, pollen, and nutlet traits vary. Utilizing 224 species of Boraginaceae and related taxa, five matrices were constructed with various combinations of morphological characters, three chloroplast DNA regions, and one nuclear ribosomal DNA region. Phylogenetic analyses were conducted for these matrices, and patterns of character evolution were examined. Boraginaceae is resolved as monophyletic, with *Wellstedia* as its sister. *Codon* is sister to Boraginaceae + *Wellstedia*. Although most of the investigated morphological characters have a low consistency index, particular character states are synapomorphies for large clades in each of the tribes of the family. In Boraginaceae, the breeding system heterostyly evolved at least 12 times, which is the largest number of origins resolved in any family; therefore Boraginaceae can serve as a model for the evolution and development of heterostyly. Nutlet ornamentation is most diverse in Cynoglosseae and Trichodesmeae, while pollen and floral features are most variable in Borageneae and Lithospermeae. Phylogenetic relationships and patterns of character evolution identified in the present study set the stage for future work creating an updated taxonomic system of Boraginaceae.

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Introduction

The angiosperm family Boraginaceae includes ca. 1600 species distributed among ca. 110 genera. The family is characterized by a scorpioid cymose inflorescence (Buys and Hilger, 2003), a gynobasic style, and a two-part ovary that breaks into four nutlets. This circumscription is equivalent to, and has in the past been referred to as, Boraginaceae s.s. or Boraginoideae (Small, 1913; Gottschling et al., 2001; Diane et al., 2002). Boraginaceae has also been circumscribed in a broader context, which has been referred to as Boraginaceae s.l. or Boraginales. This broader circumscription has included four taxa treated as either subfamilies (Boraginoideae, Cordioideae, Ehretioideae, and Heliotropioideae) or families that are characterized by a scorpioid cyme and two-parted gynoecium (style position and fruit type vary) (Lawrence, 1937; Cronquist, 1981; Al-Shehbaz, 1991; Takhtajan, 1997). In the present study, the former circumscription is treated as Boraginaceae, while the latter is treated as Boraginales, which currently includes: the four traditionally recognized families (Boraginaceae, Cordiaceae, Ehretiaceae, and Heliotropiaceae); Hydrophyllaceae, which has been recognized as closely related to the aforementioned four taxa (Cronquist, 1981; Gottschling et al., 2001; Soltis et al., 2011); and three small families (Codonaceae, Lennoaceae, and Wellstediaceae) (Gottschling et al., 2001; Weigend and Hilger, 2010) that have yet to be critically studied in a phylogenetic context. Of the eight families in Boraginales, Boraginaceae is the most speciose, and although the inflorescence type, gynoecium position, and fruit type

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are consistent within the family, other vegetative, floral, pollen, and nutlet traits vary. The objective of the present study is two-fold: (i) to utilize morphological characters and DNA sequence data to reconstruct phylogenetic relationships within Boraginaceae; and (ii) to investigate patterns of morphological character evolution in the family.

During the past 17 years, researchers have conducted several phylogenetic studies on Boraginaceae. Most have focused on relationships within a genus or among closely related genera (e.g. Böhle et al., 1996; Boyd, 2003; Långström and Oxelman, 2003; Hilger et al., 2004; Buys, 2006; Selvi et al., 2006; Cohen and Davis, 2009, 2012; Weigend et al., 2009; Khoshsokhan et al., 2010; Hasenstab-Lehman and Simpson, 2012; Trinh et al., 2012; Huang et al., in press), although some (Långström and Chase, 2002; Mansion et al., 2009; Weigend et al., 2010; Nazaire and Hufford, 2012) have addressed higher-level relationships. The lack of overlapping taxon samples across multiple studies has made it difficult to cobble together a phylogeny of Boraginaceae, and ca. 40% of the genera of the family have yet to be included in a phylogenetic analysis. Questions remain concerning the placement of the many small genera (< 5 species) in the family as well as the monophyly of large, geographically widespread genera (e.g. *Anchusa* L., *Cynoglossum* L., *Myosotis* L., and *Onosma* L.) and tribes. Moreover, Boraginaceae remains unplaced among the lamiids in the latest treatment of the Angiosperm Phylogeny Group (APG III, 2009).

In Boraginaceae, tribes frequently have been recognized based on a combination of style division, stigma number, position of nutlet attachment, and nutlet ornamentation (e.g. Al-Shehbaz, 1991). This has led to the acceptance of between four (Långström and Chase, 2002) and 13 tribes (Popov, 1953), depending on the author, and has resulted in increased taxonomic complexity within the family. Recent phylogenetic analyses (Långström and Chase, 2002; Mansion et al., 2009; Weigend et al., 2010; Nazaire and Hufford, 2012) have led to the identification of four to five tribes—Boragineae, Cynoglosseae, Echiochileae Lithospermeae, and Trichodesmeae—that are congruent with the traditional taxonomic system of Boraginaceae. Phylogenetic relationships among tribes are becoming better resolved and better supported, but relationships within each tribe remain largely unresolved (e.g. Hilger et al., 2004), although the phylogeny of one tribe, Lithospermeae, has begun to be elucidated (Böhle et al., 1996; Buys, 2006; Thomas et al., 2008; Cecchi and Selvi, 2009; Cohen and Davis, 2009, 2012; Ferrero et al., 2009; Weigend et al., 2009).

Phylogenetic analyses of genera of Boraginaceae suggest that morphological character evolution provides intriguing patterns (Långström and Oxelman, 2003; Buys, 2006; Ferrero et al., 2009; Cohen, 2011; Hasenstab-Lehman and Simpson, 2012; Huang et al., in press), but these patterns have yet to be explored throughout the entire family. This is unfortunate because Boraginaceae is well suited to serve as a model for the study of particular morphological features. For example, heterostyly, a complex and elegant breeding system that involves morphological and physiological components (Fig. 1g), is present in Boraginaceae in at least nine genera scattered among three tribes (Ganders, 1979; Naiki, 2012). Within these tribes, Thomas et al. (2008), Ferrero et al. (2009), Cohen (2010, 2011), and Hasenstab-Lehman and Simpson (2012) provide evidence for multiple origins of heterostyly, but patterns of this breeding system have yet to be studied critically throughout the family. Additionally, because Boraginaceae only produces one type of fruit—nutlets (Fig. 1a–c)—it is possible to focus investigations of fruit evolution on the modifications of one type of fruit rather than, as is the case in many taxa of comparable size (Clausing et al., 2000; Knapp, 2002), the origin of different types of fruit as well as modifications of each type of fruit. The present study provides a family-level phylogenetic investigation of Boraginaceae that includes both DNA sequence data and morphological characters, which allows for phylogenetic relationships to be elucidated and patterns of character evolution to be examined.

Materials and methods

Taxon sampling

The present study includes 224 species (Appendix 1). Two hundred and six species from across 80 genera belong to the ingroup. This sampling comprises ca. 70% of the genera of Boraginaceae, and represents both the morphological and geographic range of variation in the family. The outgroup comprises 18 species from related families of Boraginales and Lamiidae (Gottschling et al., 2001; Luebert and Wen, 2008; Mansion et al., 2009; Soltis et al., 2011), including Codonaceae, Cordiaceae, Ehretiaceae, Heliotropiaceae, Hydrophyllaceae, Vahliaceae, and Wellstediaceae. No members of Lennoaceae were included in the present study, but this family has been resolved as nested within, or sister to, Ehretiaceae (Gottschling et al., 2001; Hilger et al., 2005).

DNA sequence data

Sequence data from four DNA regions were included in the present study: two protein-encoding plastid DNA (cpDNA) regions (matK and ndhF), one cpDNA intergenic spacer (trnL–trnF), and the nuclear

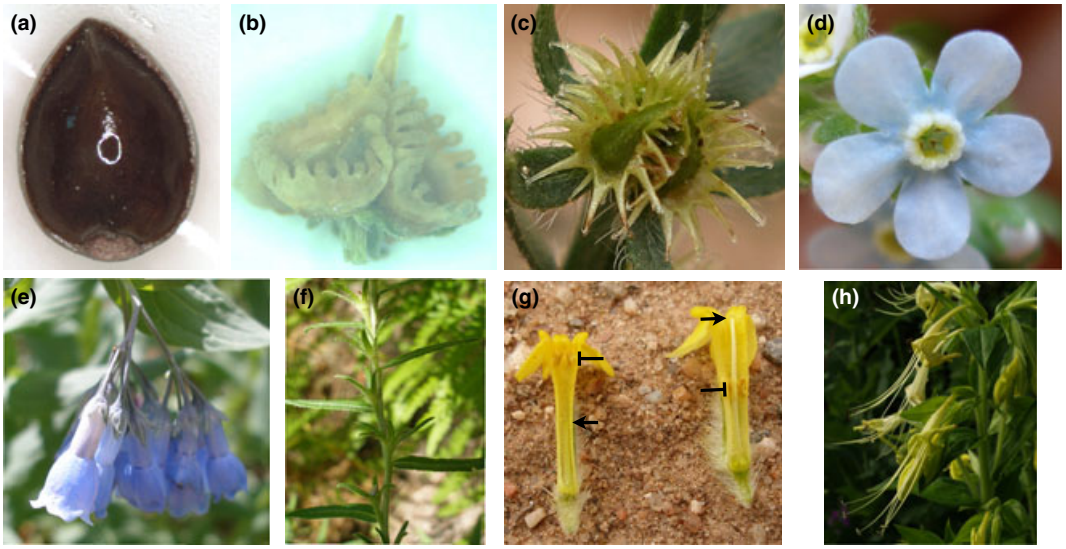

Fig. 1. Morphological features of species of Boraginaceae. (a) Smooth nutlet of *Myosotis* sp. (b) Nutlets with marginal wings, of *Omphalodes aliena*. (c) Nutlet with marginal glochids, of *Lappula redowskii*. (d) Actinomorphic corolla of *Hackelia micrantha*, note faucal appendages. (e) Ebracteate inflorescence of *Mertensia ciliata*. (f) Bracteate inflorescence of *Lithospermum multiflorum*. (g) Long-style (right) and short-style (left) morphs of heterostylous species of *Oreocarya flava*, arrows denote stigma position, blunt-ended arrows indicate anther position. (h) Zygomorphic corolla of *Lithospermum exsertum*.

ribosomal DNA (nrDNA) internal transcribed spacer (ITS). Specimens were collected from wild populations (voucher specimens deposited at BH or TAMIU herbarium) and leaves stored in silica gel, obtained from gardens (e.g. Cornell Plantations, Missouri Botanical Garden, and National Botanic Garden of Belgium) as leaf samples preserved in silica gel, or acquired as DNA isolations from the DNA bank of Royal Botanic Gardens, Kew, the South African National Biodiversity Institute (SANBI), or the DNA Bank Network (www.dnabank-network.org). Additional sequence data for multiple species were obtained from GenBank. Appendix 1 includes GenBank numbers for all species in the present study as well as voucher information for new sequences.

DNA extraction was performed with dried plant tissue using a modified CTAB extraction method (Doyle and Doyle, 1990) that included 2% PVP-40 in the CTAB extraction buffer. PCR amplifications of the DNA regions were conducted using either published primers or those designed by the author (Table 1). PCR mixtures, 25 μL in volume, consisted of 67 mM Tris–HCl with 2.1% DMSO and 0.01% TritonX per reaction or 1 × Ex Taq Buffer (Takara Bio Inc., Otsu, Japan), 2 mM $MgCl_2$, 0.2–0.25 mM dNTPs, 1 μM of primers, 0.125–1 μL Taq polymerase, and 0.1–2.5 μL DNA sample, depending on the DNA concentration. Amplifications were performed with an Eppendorf Mastercycler Pro, using the primers and annealing temperatures listed in Table 1. PCR products were separated on a 1–1.5% agarose gel and stained with ethidium bromide to determine if amplification had occurred. Prior to sequencing, some PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany).

Sequencing reactions were performed with BigDye 3.1 terminators [Applied Biosystems (ABI), Foster City, CA, USA] and locus-specific amplification primers. Sequencing products were precipitated using a modification of the ethanol/EDTA/sodium acetate method (ABI), and automated cycle sequencing was performed, with an ABI 3730 DNA Analyzer, by the Life Sciences Core Laboratory Center at Cornell University. Alternatively, sequencing reactions and subsequent steps were performed by the Life Science Core Laboratory Center at Cornell University, using Big Dye terminators and either an ABI 3700 or an ABI 3730. Sequence trace files were compiled, examined, and edited with *Codon*Code Aligner (*Codon*Code Corporation, Deadham, MA, USA). Sequences were deposited in GenBank (Appendix 1), and the matrix is available at Treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S14332).

Initial alignments were performed with MUSCLE (Edgar, 2004) as implemented by the European Bioinformatics Institute's MUSCLE server (www.ebi.ac.uk/Tools/msa/muscle) using the default settings. Subsequent adjustments were made in Bioedit ver. 7.0.5.3 (Hall, 1999) and Winclada ver. 1.7 (Nixon, 2002). Gaps were coded using simple indel coding (Simmons and Ochoterena, 2000). For the molecular matrix, 25% of the sequence data is missing. Because the resulting phylogenies are well supported and, in

Table 1
Primers used in the present study, for amplification (A) and sequencing (S), and annealing temperature for PCR reactions

Region and primer	Sequence	T_m (°C)	Amplification/sequencing
matK		48, 56–57	
2F	CAC TTG CTC AYG ATC ACG ATT*		A, S
390F	CGA TCT ATT CAT TCA ATA TTT C		A, S
590F	AAG ATG CCT CTT CTT TGC AT*		S
806R	TTG TGT TTC CGA GCC AAA GT*		S
881F	AAC CCT TCA ATG GTA CGG AGT C*		S
1069F	CCC TTC AAT GGT ACG GAG TC*		S
1107R	AGT TTT AGC ACA AGA AAG CGA AGT*		S
1389R	TTG TGT TTC CGA GCC AAA GT		S
1551R	TTT TCA TTG CAC ACG GCT TT*		A, S
1710R	GCT TGC ATT TTT CAT TGC ACA CG		A, S
ndhF		59	
1F	GTG GAT CAT ACC CTT GCT TCC*		A, S
37F	CTA TGT TAA TAG GAG TGG GGC TTC*		A, S
274R	ATT AAT ATT GAC ATA ATA GAA GTA AG		S
463R	GTC GTG CAA ACC AAA ATC CT*		S
536R	TCC CCT ACA CGA TTA GTT ACA A		S
803R	GAA AAA TTC CCG CCG CTA CCA TAG		S
803F	CTA TGG TAG CGG CGG GAA TTT TTC		S
934F	AAA GGG GCT TAG CTT ATT CCA C*		S
975F	TAT AAC CCA ATT GAG ACA TTG TGG*		S
1318F	GGA TTA ACY GCA TTT TAT ATG TTT CG		S
1318R	CGA AAC ATA TAA AAT GCR GTT AAT CC		S
1603F	CCT YAT GAA TCG GAC AAT ACT ATG C		S
1603R	GCA TAG TAT TGT CCG ATT CAT RAG G		S
2110R	CAT AAC CCC AAC GCT ATT TGT AAT*		A, S
trnL–trnF		59	
Tab C	CGA AAT CGG TAG ACG CTA CG		A, S
Tab D	GGG GAT AGA GGG ACT TGA AC		S
Tab E	GGT TCA AGT CCC TCT ATC CC		S
Tab F	ATT TGA ACT GGT GAC ACG AG		A, S
ITS		58	
ITS4	TCC TCC GCT TAT TGA TAT GC		A, S
ITS5	GGA AGT AAA AGT CGT AAC AAG G		A, S

Place of publication for previously designed primers indicated in text.
*Primer designed for present study.

general, relationships are congruent among various analyses, this amount of missing data did not appear to considerably affect relationships in the resulting phylogenies.

Morphological coding

The morphological matrix includes 27 characters (Table 2). Sixteen characters are binary, while the other 11 are multi-state. Morphological character data were gathered from observations of living plant material, herbarium specimens from BH, NY, TEX/LL, and US, and digital images of species. For species for which only a limited number of herbarium specimens were available, published descriptions were also consulted (Johnston, 1952, 1953a, b, 1954a, b; Popov, 1953; Riedl, 1967, 1997; Valentine and Chater, 1972; Sahay, 1979; Xi, 1984; Ahn and Lee, 1986; Díez et al., 1986; Al-Shehbaz, 1991; Díez and Valdés, 1991; Thulin and Johansson, 1994; Jian-Chang et al., 1995; Perveen et al., 1995; Zhu et al., 1995; Scheel et al., 1996; Retief and Van Wyk, 1997, 2002; Biggazi and Selvi, 1998, 2000; Lönn, 1999; Khatamsaz, 2001; Gagnidze et al., 2002; Boyd, 2003; Selvi and Bigazzi, 2003; Bigazzi et al., 2006; Aytas Akçin and Ulu, 2007; Maggi et al., 2008; Nikiforova, 2008; Thomas et al., 2008; Ferrero et al., 2009; Liu et al., 2010; Rabaey et al., 2010; Coutinho et al., 2012; Fokuda and Ikeda, 2012). When ample material was available, I observed at least 20 specimens for each species. If a species included multiple states for a character, the species was scored with all applicable states for that character. In a few cases, it was not possible to collect data (often related to pollen features or vestured pits) for a particular species. In this situation, if all the species in a genus had been observed to possess the same character state, scores for the specific character were based on data from congeneric species. However, if a genus is polymorphic for the character, then the character state was scored as missing. For

Table 2
Morphological characters, their states, additional information, and length and consistency index on trees from matrices that included morphological characters

Character	Character states	Comments	Length and consistency index (combined matrix/combined cpDNA matrix)
1 Naphthoquinones	(0) Present (1) Absent		16/14; 0.06/0.07
2 Vestured pits	(0) Present (1) Absent		2/2; 0.50/0.50
3 Leaf position	(0) Cauline (1) Cauline and basal (2) Cauline and pseudobasal	"A pseudobasal rosette is defined as a rosette that is sometimes present and may be ephemeral. This type of rosette includes leaves that, although they may have short internodes between them, are not necessarily from the base of the stem." (Cohen, 2011)	42–43/39; 0.04/0.05
4 Leaf venation	(0) Midvein (1) Midvein and secondary veins		31/26; 0.03/0.03
5 Cordate leaves	(0) Present (1) Absent		5/4; 0.20/0.25
6 Floral bracts	(0) Present (1) Absent (2) Only at the base		28/27; 0.07/0.07
7 Corolla shape	(0) Salverform (1) Campanulate—*Mertensia*-type (2) Funnelform (3) Rotate (4) Salverform-funnelform (5) Long-funnelform (6) Campanulate—*Cerinthe*-type (7) Urceolate (8) Campanulate—*Trichodesma*-type (9) Tubular	Three different types of campanulate corolla are identified in Boraginaceae, and each is associated with a genus characteristic of it.	52/49; 0.17/0.18
8 Corolla lobes	(0) Flared (ca. 90°) (1) Erect/ascending (< 45°) (2) Reflexed (ca. 180)		26/25–27; 0.07/0.07–0.08
9 Corolla symmetry	(0) Actinomorphic (1) Zygomorphic		7/7; 0.14/0.14
10 Corolla color	(0) Blue (1) Purple (2) Orange (3) Yellow (4) White (5) Red (6) Pink (7) Green	If a species is coded as polymorphic for this character, are two possible reasons: (i) the corolla includes multiple colors, or (ii) the species includes some individuals that develop corollas of one colour, but other individuals that produce corollas of another colour.	56–57/50–51; 0.08/0.09–0.10
11 Abaxial trichomes on corolla	(0) Present (1) Absent		17/15–16; 0.05/0.06
12 Adaxial trichomes on corolla	(0) Present (1) Absent		11/11; 0.09/0.09
13 Faucal appendages	(0) Present (1) Absent		17/14; 0.05/0.07
14 Glands inside corolla	(0) Present (1) Absent		31/25–26; 0.03/0.03–0.04
15 Type of herkogamy	(0) Approach herkogamy (1) Reverse herkogamy (2) Non-herkogamy (3) Reciprocal herkogamy	Multiple types of herkogamy are observed in some species.	45/42; 0.06/0.07
16 Anther position	(0) Inserted (1) Exserted		20–21/20; 0.04–0/05/0.05
17 Androecial apical projection	(0) Absent (1) Borago-type (2) *Myosotis*-type (3) *Onosma*-type (4) *Lobostemon*-type (5) *Trichodesma*-type	Multiple states of androecial apical projections are recognized because those in each genus differ from the others.	6/6; 0.83/0.83
18 Stigma position	(0) Inserted (1) Exserted		27–28/24–25; 0.03/0.04
19 Stigma location	(0) Terminal (1) Subterminal		11–13/8–9; 0.07–0.09/ 0.11–0.12
20 Conical stigmas	(0) Present (1) Absent	The presence of conical stigmas is restricted to species of Heliotropiaceae.	1–2/1; 1–0.50/1
21 Pollen shape	(0) Ovoid (1) Prolate with a constricted equator (Hourglass) (2) Cylindrical (3) Ellipsoid (4) Oblate-square (5) Triangular-prism (6) Spherical		31/30; 0.16/0.16
22 Pollen pore number	(0) 2—5 (1) 6—8 (2) 8—12		16/14; 0.12/0.14
23 Pollen pore position	(0) Equatorial (1) Sub-equatorial		9/9; 0.11/0.11
24 Heterocolpate pollen	(0) Present (1) Absent		3/2; 0.33/0.50
25 Fruit type	(0) Drupe (1) Capsule (2) Nutlet		4–5/4; 0.40–0.50/0.50
26 Nutlet surface ornamentation	(0) Tuberculate (1) Glochidiate (2) Marginal glochids (3) Rugose (4) Smooth (5) Marginal wings (6) Scaly	Nutlets of some species bear multiple types of surface ornamentation, such as marginal glochids and glochids.	49–51/46–47; 0.11–0.12/ 0.12–0.13
27 Nutlet attachment	(0) Basal (1) Not basal	Nutlet attachment that is not basal can vary from sub-medial to medial.	6/5; 0.16/0.20

Table 3
Type of data included, statistics, and phylogenetic characteristics of matrices used in present study

	Type of data				Matrix characteristics			Phylogeny characteristics		
Matrix	cpDNA	nrDNA ITS	Structural characters (gaps and inversions)	Morphology	Number of taxa	Number of characters	Number of informative characters	Number of MP trees	Length of MP trees	CI/RI
Combined	X	X	X	X	224	5924	1923	>1 000 000	8791	0.37/0.77
Molecular	X	X	X		224	5897	1896	>1 000 000	8199	0.39/0.78
Combined cpDNA	X		X	X	212	5414	1598	>1 000 000	5531	0.46/0.83
cpDNA	X		X		212	5387	1571	>1 000 000	4983	0.50/0.84
Morphology				X	224	27	27	>1 000 000	408	0.14/0.78

the morphological matrix, 10% of the cells are scored as missing.

Matrix construction and phylogenetic analysis

Five matrices were constructed (Table 3): (i) the combined matrix (morphological and all molecular data), (ii) the molecular matrix (only molecular data), (iii) the combined cpDNA matrix (morphological and cpDNA sequence data), (iv) the cpDNA matrix (only cpDNA sequence data), and (v) the morphological matrix (only morphological data). Taxon sampling differs among matrices because 13 species include only DNA sequence data for ITS as well as scores for morphological character data. Without the inclusion of ITS, it was not possible to resolve the phylogenetic placement of these 13 species. In order to reconstruct a resolved phylogeny, it was necessary to remove these species from the combined cpDNA and cpDNA matrices. Despite the decrease in taxon samples, the strict consensus trees of all the matrices, except that of the morphological matrix, are largely congruent. For cladistic analysis, all characters were treated as non-additive and weighted equally.

Maximum parsimony phylogenetic analyses were conducted with each of the five data matrices. The following search strategy was applied in all analyses: the data were analysed using TNT (Goloboff et al., 2008), with 1 000 000 trees held in memory, and five independent iterations of 1000 parsimony ratchet iterations (Nixon, 1999), with 20 trees held per iteration and a 10% probability of upweighting and a 10% probability of downweighting, followed by 1000 cycles of tree drifting; afterwards, 100 rounds of tree fusion and random sectorial searches were performed (Goloboff, 1999). This search strategy was repeated 100 times and then followed by TBR-max, swapping among all the most-parsimonious trees until completion.

Clade support for all matrices was measured with TNT (Goloboff et al., 2008). Ten thousand jackknife replicates (36% removal probability) (Farris et al., 1996) were performed. For each replicate, 10 TBR searches were conducted, with 10 trees held after each replicate, and a total of 99 999 trees held in memory for the duration of the entire jackknife resampling. Using TNT, SPR distance (Goloboff, 2007) between strict consensus trees was calculated with 100 replications. Consistency indices (CI) and retention indices (RI) were calculated after removal of parsimony-uninformative characters.

Patterns of character evolution were investigated using Fitch optimization (Fitch, 1971), as implemented in Winclada ver. 1.7 (Nixon, 2002). The number of gains and losses and CI were recorded for each character (Table 2).

Results

Sequence variation

Four DNA regions are included in the present analyses (Table 4). Together, the three cpDNA regions comprise a total of 5331 aligned nucleotides, 1571 of which are parsimony informative. This number includes 1515 informative nucleotides and 56 informative gaps and inversions (structural features) (Table 4). The matK gene provides the greatest number of informative nucleotide sites, 581; and of the cpDNA regions, it yields the largest percentage of informative nucleotide sites, 33.2%. However, the trnL–trnF spacer includes the greatest number of informative structural features, 26. The longest cpDNA region, ndhF, yields 40% more informative characters than trnL–trnF, but due to the large length of ndhF (ca. 50% longer than trnL–trnF), ndhF bears the smallest percentage of informative characters. The nrDNA ITS yields 566 aligned nucleotides, and 325 (57.4%) are parsimony informative. In total, the combined and molecular matrices include 5897 aligned nucleotides, with 1840 informative nucleotide sites and 56 informative structural features.

Table 4
Four DNA regions included in analyses

Region	Number of taxa	Aligned length (bp)	Informative nucleotide site characters	Informative nucleotide site characters (%)	Structural characters	Total number of informative characters	Total informative characters (%)
matK	160	1815	581	32	21	602	33.2
ndhF	154	2139	551	25.8	8	559	26.
trnL–trnF	181	1377	383	27.8	26	409	29.7
ITS	181	566	325	57.4	0	325	57.4
Mean	169	1474	460	31.2	14	474	32.1
Total	676	5897	1840	31.2	56	1896	32.1

Phylogenetic analyses

For each matrix, the number of most parsimonious (MP) trees, tree length, CI, and RI are presented in Table 3. The strict consensus trees from analyses of the combined and molecular matrices are provided in Figs 2 and 3, respectively, and those from analyses of the combined cpDNA, cpDNA, and morphological matrices are in Appendix 2. In general, analyses of the combined matrix resulted in the most resolution, and analyses of the morphological matrix resulted in the least resolution. The SPR distance between the strict consensus trees of the combined and molecular matrices is 13 moves, and between the consensus trees of the combined cpDNA and cpDNA matrices, the SPR distance is three moves.

The phylogeny is rooted with *Vahlia capensis* Thunb., and in all analyses members of Cordiaceae, Ehretiaceae, Heliotropiaceae, and Hydrophyllaceae form a well supported clade, with > 90% jackknife support (jk). *Wellstedia dinteri* Pilg. is resolved as sister to Boraginaceae (> 91% jk), and *Codon schenkii* Schinz. is sister to the clade composed of *Wellstedia* Balf.f. and Boraginaceae (> 88% jk).

In all analyses, Boraginaceae is resolved as monophyletic (> 99% jk). Three traditionally recognized tribes—Boragineae, Echiochileae, and Lithospermeae—are recovered as monophyletic, with Echiochileae sister to the rest of the family (> 92% in combined and molecular matrices, and > 71% in combined cpDNA and cpDNA matrices). The small tribe Trichodesmeae is not monophyletic because one of its members, *Suchtelenia* Kar. ex Meisn., is nested within Cynoglosseae; however, the remaining two genera of Trichodesmeae are sisters (> 91% jk). The clade that includes the other two members of Trichodesemeae may be nested within Cynoglosseae, but in most analyses a polytomy that includes Trichodesmeae, a clade comprising *Chionocharis hookeri* I. M. Johnst. and *Lasiocaryum munroi* I. M. Johnst., and at least one other clade of species of Cynoglosseae is resolved at the base of the clade composed of Cynoglosseae and Trichodesmeae. In analyses of the combined matrix, a clade of *Myosotidum* Hook. and *Omphalodes* Mill. is sister to all other species of Cynoglosseae, but in analyses of the combined cpDNA and cpDNA matrices, this clade is sister to *Asperugo* L. + *Mertensia* Roth. Analyses of the molecular matrix did not resolve the relationship between these two clades. In most analyses, the following genera in Cynoglosseae are recovered as non-monophyletic: *Cryptantha* Lehm. ex G.Don, *Cynoglossum*, *Lappula* Moench, *Myosotis*, *Paracaryum* Boiss., and *Trigonotis* Steven.

The clade of Cynoglosseae + Trichodesmeae is sister to one composed of Boragineae + Lithospermeae. This latter clade receives strong support (> 87% jk) in all analyses. In analyses of the combined matrix, a clade comprising species of *Elizaldia* Willk., *Melanortocarya* Selvi, Bigazzi, Hilger & Papini, *Nonea* Medik., *Paraskevia* W.Sauer & G.Sauer, and *Pulmonaria* L. is sister to the rest of Boragineae, but in analyses of the molecular, combined cpDNA, and cpDNA matrices, a clade composed of *Moritzia* DC. ex Meisn. and *Thaumatocaryon* Baill., two South American genera, is resolved as sister to the remainder of the tribe. In analyses of the combined matrix, this clade of South American species is nested within Boragineae and sister to a clade composed of *Anchusa*, *Gastrocotyle* Bunge, and five other genera. *Borago* L. is recovered as monophyletic (> 92% jk). *Pentaglottis* Tausch is resolved as sister to a clade composed of *Symphytum* L. and *Procopiania* Guşul. (> 78% jk). In analyses of the combined matrix, *Procopiania* is sister to *Symphytum*, while in others the former is nested among the latter. *Anchusa* is also resolved as non-monophyletic due to species of *Anchusella* Bigazzi, E.Nardi & Selvi, *Cynoglottis* (Guşul) Vural & Kit Tan, *Hormuzakia* Guşul., *Lycopsis* L., *Phyllocara* Guşul., and *Gastrocotyle* (except in analyses of the combined matrix) scattered among its members. The clade composed of all of these genera receives > 81% jk in analyses.

A clade comprising *Alkanna* Tausch and *Podonosma* Boiss. (> 99% jk) is sister to the rest of the Lithospermeae (> 91% jk). In most analyses, Lithospermeae is

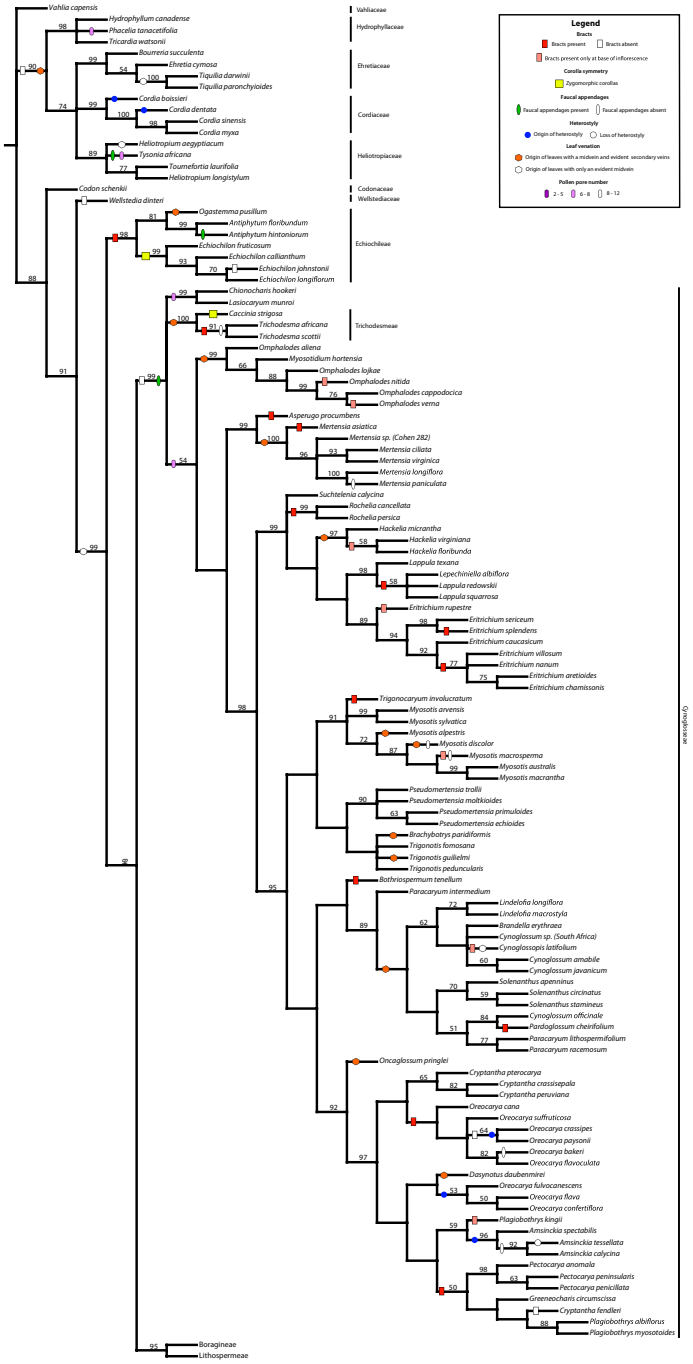

Fig. 2. Strict consensus of combined matrix (L = 8791, CI = 0.37, RI = 0.77) and phylogenetic distribution of characters. Numbers above branches are jackknife values > 50%. Bracts: red rectangles, bracts present; white rectangles, bracts absent; light red rectangles, bracts present only at base of inflorescence. Corolla symmetry: yellow square, zygomorphic corollas. Faucal appendages: green ellipse, faucal appendages present; white ellipse, faucal appendages absent. Heterostyly: blue circle, origin of heterostyly; white circle, loss of heterostyly. Leaf venation: orange hexagon, origin of leaves with midrib and evident secondary veins; white hexagon, origin of leaves with only evident midrib. Pollen pore number: purple rounded rectangle, two to five pores; light purple rounded rectangle, six to eight pores; white rounded rectangle, eight to 12 pores. Shapes with two colours indicate two possible ancestral states resolved.

well resolved; however, in analyses of the cpDNA matrix, the tribe has little resolution. A clade composed of *Halacsya* Dörfl., *Lithodora* Griseb., *Mairetis* I. M. Johnst., *Moltkiopsis* I. M. Johnst., *Neatostema* I. M. Johnst., and *Paramoltkia* Greuter is present in all analyses, but with varying degrees of support (49–99%

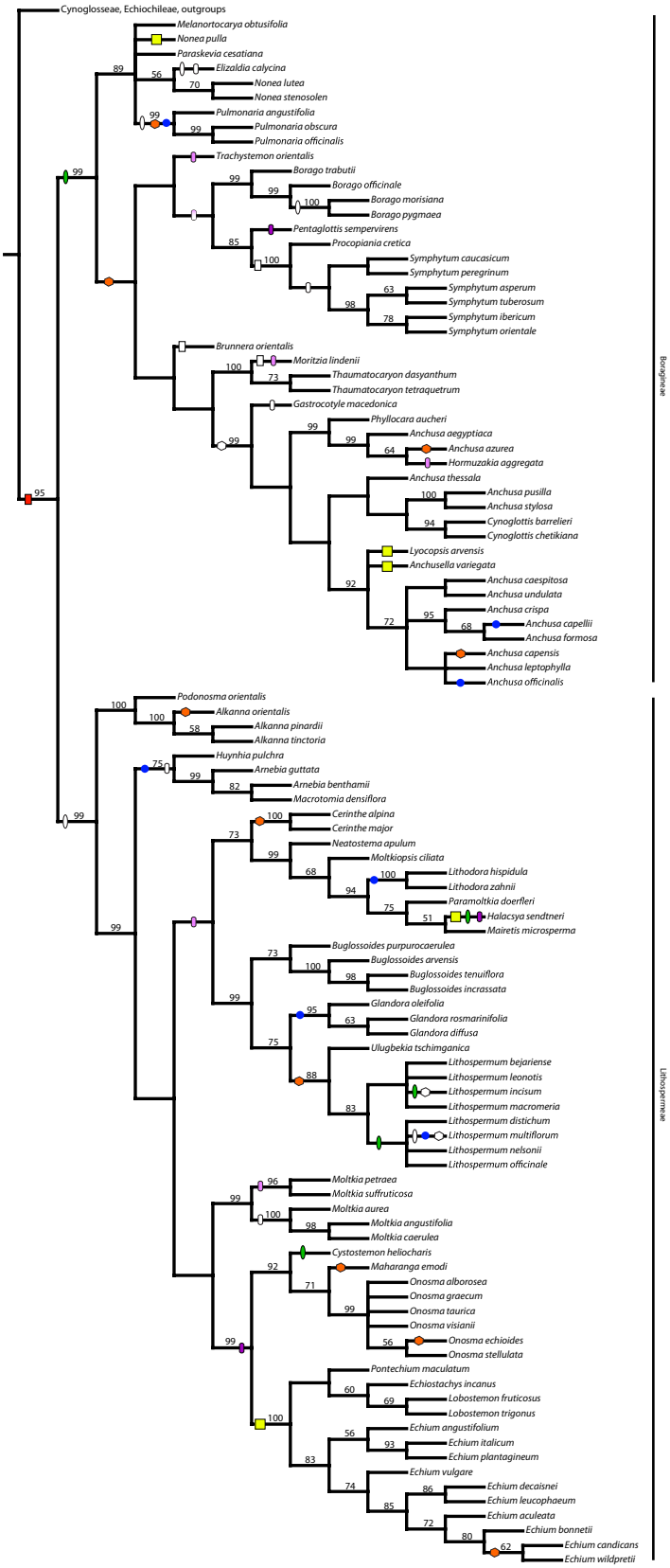

Fig. 2. (Continued).

Fig. 3. Strict consensus of molecular matrix (L = 8199, CI = 0.39, RI = 0.78). Numbers above branches are jackknife values > 50%.

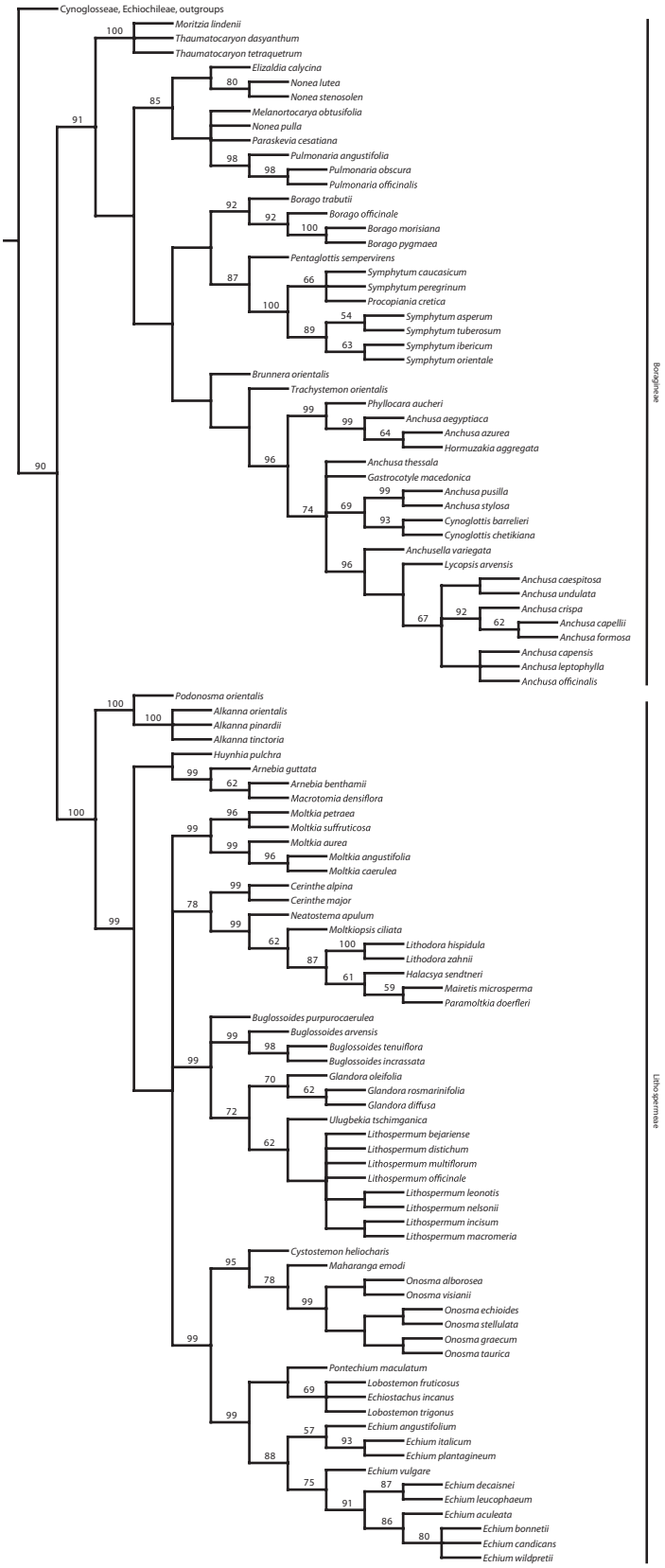

Fig. 3. (Continued).

jk). In analyses of the combined and molecular matrices, this clade is sister to one that includes two species of *Cerinthe* L. (73–78% jk). *Buglossoides* Moench, *Glandora* D.C. Thomas, Weigend & Hilger, *Lithospermum* L., and *Ulugbekia* Zakirov are resolved in a well supported clade (> 94% jk), and so are *Echiostachys* Levyns, *Echium* L., *Lobostemon* Lehm., and *Pontechium* U.-R. Böhle & Hilger (> 78% jk). In Lithospermeae, *Arnebia* Forssk. and *Onosma* are resolved as non-monophyletic in at least one analysis. *Arnebia* is non-monophyletic because *Macrotomia densiflora* (Ledeb.) J.F. Macbr. is nested among members of *Arnebia*. In analyses of the combined cpDNA matrix, *Maharanga* DC. is resolved among species of *Onosma*, but this relationship receives weak support (< 50% jk) and is only recovered in this particular analysis. In analyses of other matrices, *Maharanga* is sister to *Onosma*, and this relationship receives moderate support (71–78% jk).

Patterns of morphological character evolution

Patterns of morphological character evolution are quite variable among the 27 investigated characters. Most characters have a very small CI (< 0.15); however, seven characters—vestured pits, cordate leaves, androecial apical projection, conical stigma, heterocolpate pollen, fruit type, and nutlet attachment—have a CI > 0.20 (Table 2). Despite the small CI for the other 20 characters, most have states diagnostic of particular clades, even if the character exhibits much homoplasy across the family. For example, the CI of stigma position is 0.03–0.04, but stigmas exserted from the corolla tube are a synapomorphy for at least one large and one small clade in each of three tribes: Boragineae, Cynoglosseae, and Lithospermeae. Additionally, stigma insertion is a synapomorphy for Echiochileae, while stigma exsertion is a synapomorphy for Trichodesmeae. Evolutionary patterns for each character are presented in Appendix 3.

Discussion

Phylogenetic relationships

Outgroup relationships. The phylogenies reconstructed in the present study are largely congruent with each other as well as those from previous investigations (Gottschling et al., 2001; Luebert and Wen, 2008; Nazaire and Hufford, 2012). The trees are rooted with *Vahlia capensis* because previous analyses have resolved Vahliaceae as closely related or sister to Boraginales (Bremer et al., 2002; Luebert and Wen, 2008; Nazaire and Hufford, 2012). Among the outgroups, Hydrophyllaceae is sister to a clade composed of Cordiaceae, Ehretiaceae, and Heliotropiaceae, with different phylogenetic relationships resolved among the latter three families, depending on the inclusion or exclusion of *ITS* (Figs 2 and 3). The clade comprising Cordiaceae, Ehretiaceae, Heliotropiaceae, and Hydrophyllaceae is sister to one composed of Boraginaceae, *Codon* L., and *Wellstedia*.

Wellstedia is resolved as sister to Boraginaceae, and *Codon* is sister to the clade composed of *Wellstedia* and Boraginaceae. Some authors, such as Gürke (1897), Pilger (1912), and Takhtajan (1997), have recognized the affinity between *Wellstedia* and Boraginaceae, with the latter two authors placing the genus as a subfamily of Boraginaceae s.l., and *Codon* often has been placed as a member of Hydrophyllaceae (Cronquist, 1981; Takhtajan, 1997; Ferguson, 1998). Although *Wellstedia*, *Codon*, and Boraginaceae are resolved as a monophyletic group, it is not advisable to treat the three taxa as members of one family. Instead, the two genera should each be recognized as separate families, as previous authors have suggested (Novák, 1943; Merxmüller, 1960; Weigend and Hilger, 2010). Although Boraginaceae, *Codon*, and *Wellstedia* share some morphological features, such as alternate leaves, the flowers (four-merous in *Wellstedia* and 10- to 12-merous in *Codon*) and fruit (capsules in both genera) differ. The present study is the first to include *Wellstedia*, but arguments have been made that *Codon* be included in Boraginaceae (Nazaire and Hufford, 2012). These arguments have centred on the phylogenetic position of the taxon, not on the creation of a utilitarian manner in which to circumscribe Boraginaceae, a family currently diagnosed by multiple floral and fruit features. Including these two genera in Boraginaceae would necessitate expanding the circumscription of a recognizable family and, in doing so, make diagnostic characters for a broader Boraginaceae difficult to identify. Until phylogenetic results place these genera within Boraginaceae or a useful manner is identified in which to circumscribe a broader Boraginaceae, the author recommends that *Wellstedia* and *Codon* be members of Wellstediaceae and Codonaceae, respectively.

Ingroup relationships. In the present analyses, clades corresponding to five tribes are resolved. Of these, Echiochileae is sister to the rest of Boraginaceae, and two large clades each composed of two tribes—Cynoglosseae + Trichodesmeae and Boragineae + Lithospermeae—are also recovered. Two of these tribes are not monophyletic. Two genera of the small tribe Trichodesmeae, *Caccinia* Savi and *Trichodesma* R.Br., are sisters, but the third, *Suchtelenia*, is nested within Cynoglosseae, the tribe to which de Candolle (1846), Gürke (1897), Johnston (1924), and others assigned the genus. Therefore, in order to circumscribe

a monophyletic Cynoglosseae, future taxonomic systems should include *Suchtelenia* in Cynoglosseae. The acceptance of Trichodesmeae, even if composed of only *Caccinia* and *Trichodesma*, may make Cynoglosseae paraphyletic because the former is either resolved as sister to the latter or nested within it. The two tribes share some features, such as nutlets with non-basal attachment, but the pollen differs. The pollen of Trichodesmeae has two to five pores and is isocolpate, while that of Cynoglosseae has six to eight pores and is heterocolpate. The pollen of *Suchtelenia* is identical to that of Cynoglosseae, so it is unsurprising that the genus is resolved in the tribe. Because of the pollen differences and ambiguous phylogenetic position, Trichodesmeae should be retained, for the time being, as a distinct tribe (perhaps to be recognized as subtribe in future classifications).

In Cynoglosseae, the relationships resolved from analyses of the five different matrices are, in general, congruent. A clade composed of two species, *Chionocharis hookeri* and *Lasiocaryum munroi*, is resolved towards the base of the tribe. These two species have not been included in prior phylogenetic analyses and, unlike most members of the tribe, these two are restricted to higher elevations of the Himalayan Mountains and adjacent areas (Zhu et al., 1995). The flowers of *Chionocharis hookeri* and *Lasiocaryum munroi* resemble those of other members of Cynoglosseae, but their small habit, which may be specialized for alpine environments (Körner, 2003), differs from many other members of the tribe. This habit may provide information as to that of the ancestor of Cynoglosseae, or it may represent a derived characteristic of these montane species.

Myosotidium and *Omphalodes* are resolved in the same clade, which is characterized by cordate leaves and nutlets with marginal wings and/or marginal spines. This clade is of particular interest from a biogeographic perspective because species of *Omphalodes* are native to Eurasia and North America, while *Myosotidium* is restricted to the Chatham Islands located 800 km east of New Zealand. Although taxon sampling of these two genera is limited in the present study, analyses of the combined and molecular matrices resolve *Omphalodes aliena* A.Gray ex Hemsl., a New World member of the genus, sister to the other species in this clade. This reconstruction suggests that *Omphalodes* may have originated in the New World and subsequently colonized the Chatham Islands and Eurasia. If this is the case, this pattern would be opposite most others in Boraginaceae, in which members originate in the Old World and subsequently colonized the New World (Raven and Axelrod, 1974; Långström and Chase, 2002; Cohen and Davis, 2009, 2012). However, results of the combined cpDNA and cpDNA matrices resolve *Omphalodes lojkae* Sommier & Levier, a Eurasian species, as sister to the other members of the clade (Appendix 2). This reconstruction is congruent with a Eurasian origin followed by migration to the other regions. Regardless of the pattern of migration, *Myosotidium* appears to be nested among species of *Omphalodes*, resulting in the latter being paraphyletic. Additional taxon sampling of *Omphalodes* will help elucidate phylogenetic relationships in order to clarify biogeographic patterns and taxonomy in this clade.

Eritrichium Schrad. ex Gaudin, *Hackelia* Opiz, *Lappula*, *Lepechiniella* Popov, *Rochelia* Rchb., and *Suchtelenia* comprise a clade. Students of Boraginaceae have long recognized the close relationship among the first five genera (Johnston, 1923; Popov, 1953; Boivin, 1966; Gentry and Carr, 1976; Ovchinnikova, 2009; Khoshsokhan et al., 2010; Mozaffar et al., 2013; Huang et al., in press), which have often been included in Eritricheae. However, this clade is nested within Cynoglosseae. Consequently, the recognition of Eritricheae, while it is monophyletic, results in the non-monophyly of Cynoglosseae and therefore should not be accepted (perhaps to be recognized as subtribe in future classifications). Of the genera in this clade, *Lappula* is resolved as non-monophyletic because *Lepechiniella albiflora* Riedl is nested among species of the genus, a result also recovered by Mozaffar et al. (2013). These authors also resolved *Eritrichium* as non-monophyletic, but the present study, which includes greater taxon sampling of the genus, reconstructs *Eritrichium* as monophyletic. Previous researchers have proposed a close relationship between *Suchtelenia* and *Cynoglossum* (Valdés, 2004), but not between *Suchtelenia* and members of Eritricheae. However, the nutlets of the latter two taxa are similar. Indeed, some individuals of *Suchtelenia* develop nutlets with small marginal spines (Popov, 1953), and a similar type of nutlet is present in many species of Eritricheae.

In Cynoglosseae, a clade composed of *Brachybotrys* Maxim. ex Oliv., *Myosotis*, *Pseudomertensia* Riedl, *Trigonocaryum* Trautv., *Trigonotis*, and in some

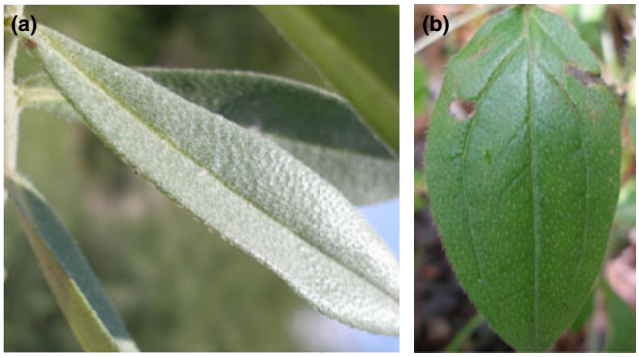

Fig. 4. Leaf venation in Boraginaceae. (a) Leaf with only evident midrib. (b) Leaf with evident midrib and secondary veins.

analyses *Bothriospermum* Bunge is characterized by smooth nutlets. With the exception of *Myosotis*, all species of this clade are endemic to Eurasia. The presented phylogenetic analyses suggest a Eurasian origin of *Myosotis*, with subsequent colonizations of the New World, Australia, and New Zealand. One member of this clade, *Trigonocaryum*, a monotypic genus restricted to the Caucasus (Gagnidze et al., 2002), is nested within *Myosotis* (Fig. 3). Although the two genera differ in chromosome number, their close relationship has previously been recognized due to the presence of similar nutlets (Popov, 1953; Gagnidze et al., 2002).

A large clade in Cynoglosseae includes the species-rich genus *Cryptantha* and its relatives, and this clade is the largest radiation of Boraginaceae in the New World. The present results are congruent with those of Hasenstab-Lehman and Simpson (2012), who report that *Cryptantha* is not monophyletic because species of multiple New World genera, including *Amsinckia* Lehm. and *Plagiobothrys* Fisch. & C.A. Mey., are nested among its species. Hasenstab-Lehman and Simpson (2012) resurrected the genus *Oreocarya* Greene, a genus with many heterostylous species, which was resolved as monophyletic in their study; however, the present analyses recover at least two separate clades that include species of the genus (Figs 2 and 3). These disparate results may be due to different taxon sampling of species of *Oreocarya*, as the overlap among included species is minimal between the two studies. It also is possible that *Oreocarya* is not monophyletic, and the genus may need to be divided into two genera.

Another explanation for the different phylogenetic results may be due to the inclusion, in the present analyses, of *Dasynotus* I. M. Johnst., a highly derived monotypic genus endemic to Idaho, USA. *Dasynotus* has a unique morphology, which includes large white salverform corollas with long horn-like faucal appendages and large nutlets covered with sparse trichomes, that is not present in other species of Boraginaceae. The phylogenetic position of *Dasynotus* varies depending on the matrix analysed, and this makes it difficult to identify closely related species and to infer the origin of the unusual morphology. One closely related species identified in analyses of the combined matrix, *Oreocarya fulvocanescens* (S.Watson) Greene, seems a likely candidate as it develops corollas that are white, > 1 cm in length, and bear faucal appendages (Fig. 2). However, given the ambiguous phylogenetic placement of *Dasynotus*, it seems that the best approach to clarify close relatives is via sampling additional taxa.

Oncaglossum pringlei (Greenm.) Sutorý, a species endemic to Mexico (Sutorý, 2010), is resolved as sister to the clade of *Cryptantha* and its relatives. *Oncaglossum pringlei* develops nutlets with glochids, which are not common among *Cryptantha* and its relatives. Selvi et al. (2011) suggest that nutlets with glochids, such as those present in *Oncaglossum* and *Cynoglossum*, may travel great distances because the glochids allow the nutlets to become tangled in the hair of migrating animals. This dispersal strategy may have helped the ancestral species of this clade colonize North America, with alternate dispersal strategies developing in most species of *Cryptantha* and its relatives.

Recently, Selvi et al. (2011) recognized that *Cynoglossum* is not monophyletic because *Pardoglossum* Barbier & Mathez and *Solenanthus* Ledeb. are nested among its species. The present analyses resolve similar relationships, and provide evidence that species from other genera, such as *Brandella* R.R.Mill, *Cynoglossopsis* Brand, *Lindelofia* Lehm., and *Paracaryum*, are also interdigitated among species of *Cynoglossum*. This clade, in which glochidiate nutlets are a synapomorphy, should be the subject of lower-level phylogenetic investigations in order to establish diagnosable, monophyletic genera.

Boragineae and Lithospermeae are resolved as sisters, and, with few exceptions, the species of these two tribes are characterized by the presence of floral bracts and nutlets with basal attachment. In analyses of the combined matrix, a clade composed of *Melanortocarya*, *Nonea*, and three other genera is resolved as sister to the remainder of Boragineae, but in analyses of the other three matrices, a clade of two South American genera, *Moritzia* and *Thaumatocaryon*, is reconstructed in this position. This latter placement is consistent with Weigend et al. (2010) and some analyses of Nazaire and Hufford (2012), studies that utilized only DNA sequence data. It seems well established that these South American genera are members of Boragineae, and their phylogenetic placement has implications for the time at which the tribe colonized the New World. If the clade that includes *Moritzia* and *Thaumatocaryon* is sister to the rest of the tribe, this provides evidence that members of Boragineae were present in the New World early in the diversification of the tribe, but this separate New World lineage did not radiate to the same extent as the Old World members. Alternatively, if the clade of South American species is resolved as sister to *Anchusa* and its relatives, then the colonization of the New World by species of Boragineae may not have occurred until later in the evolution of the tribe. The clade of *Moritzia* and *Thaumatocaryon* then would represent an offshoot of the tribe, not a separate New World lineage the same age as the clade of Old World species.

The largest genus in Boragineae, *Anchusa*, is resolved as non-monophyletic because *Anchusella*, *Cynoglottis*, *Hormuzakia*, *Lycopsis*, *Phyllocara*, and possibly *Gastrocotyle* are nested among its species, results similar to those of Hilger et al. (2004) and Mansion et al. (2009). As with *Cynoglossum*, *Anchusa*

and its relatives should be investigated as part of a lower-level phylogenetic study to reconstruct stable relationships and identify the best manner in which to circumscribe genera.

Of all the tribes in Boraginaceae, Lithospermeae has received the most attention recently (Böhle et al., 1996; Hilger and Böhle, 2000; Buys, 2006; Thomas et al., 2008; Cecchi and Selvi, 2009; Cohen and Davis, 2009, 2012; Ferrero et al., 2009; Selvi et al., 2009; Weigend et al., 2009; Cecchi et al., 2011; Cohen, 2011). The phylogenies presented provide additional resolution to clarify phylogenetic relationships for groups that have not been the subject of prior studies, such as *Arnebia* and *Onosma*. *Arnebia* is not monophyletic because *Macrotomia* is nested among its species, and *Huynhia*, a monotypic genus originally placed in *Arnebia* (Johnston, 1952), is resolved as sister to *Arnebia* + *Macrotomia*. Pollen with 8–12 subequatorial pores is a synapomorphy of the clade composed of these three genera, and this is the only clade in the tribe that bears this combination of pollen characteristics. *Cystostemon* Balf.f., *Maharanga*, and *Onosma* are members of a clade, and the close relationship among these genera was hypothesized by Johnston (1954a). The species of these three genera have very similar morphologies, with differences observed primarily in corolla shape. In analyses of the combined cpDNA matrix, *Maharanga* is nested within *Onosma*, but this relationship receives weak support. Cecchi et al. (2011) resolve a similar relationship, with *Maharanga* sister to an early diverging clade of *Onosma*, and this relationship is well supported in their analyses. Together, *Cystostemon* and *Maharanga* include 10 species, and greater taxon sampling in future studies will help resolve whether the genera are nested within *Onosma* or represent separate lineages with distinct corolla shapes. The clade composed of *Cystostemon*, *Maharanga*, and *Onosma* is sister to one that includes *Echiostachys*, *Echium*, *Lobostemon*, and *Pontechium*. Pollen with three pores is a synapomorphy for the clade that includes all seven genera, while zygomorphic funnelform corollas are a synapomorphy for the clade composed of the latter four genera.

Vegetative characters

Four vegetative characters were investigated in the present study, and two—vestured pits and pattern of leaf venation—provide noteworthy evolutionary patterns. In Boraginales, vestured pits are present in four families: Boraginaceae, Cordiaceae, Ehretiaceae, and Heliotropiaceae (Rabaey et al., 2010). In Boraginaceae, vestured pits originated at least twice, once in *Antiphytum* DC. ex Meisn. and once in Lithospermeae. Jansen et al. (2003, 2009) suggest that vestured pits may reduce embolism, particularly in alpine and arid regions. Species of *Antiphytum* and Lithospermeae inhabit these types of area, but they do not appear to do so at a greater frequency than species of the family that do not develop vestured pits (Zhu et al., 1995; Cohen, in review). Therefore, until additional studies shed light on the functional ecology of vestured pits in Boraginaceae, the character remains just a useful diagnostic feature.

Most species of Boraginaceae develop leaves with only an evident midrib (Fig. 4a), which is the ancestral condition for the family. In Boraginaceae, the pattern of leaf venation appears evolutionarily labile, with multiple origins of species that bear leaves with evident secondary venation (Fig. 4b), and this character state is a synapomorphy for numerous clades, particularly in Cynoglosseae (Fig. 2, hexagons). In this tribe, this type of leaf venation characterizes four clades—*Myosotidium* + *Omphalodes*, *Mertensia*, *Hackelia*, and *Cynoglossum* and related genera—as well as six species in other clades. The evolutionary pattern of leaf venation in Cynoglosseae is similar to that in vanilloid orchids. In this group, Cameron and Dickison (1998) were able to use leaf architecture to differentiate among genera. In Cynoglosseae, evident secondary leaf venation can help distinguish the species of *Hackelia* included in the present study from those of closely related genera, all of which only develop leaves with an evident midrib.

Leaves with more and larger veins can have several advantages, such as greater mechanical support, hydraulic conductance, and vascular redundancy (Roth-Nebelsick et al., 2001; Sack et al., 2008; McKown et al., 2010). Species of Boraginaceae that bear leaves with more veins tend to have larger leaves and habits, which may require greater biomechanical support and hydraulic conductance. The third advantage, vascular redundancy, may provide another explanation for the success of some geographically widespread groups of moderate size, such as *Hackelia*, *Omphalodes*, and *Symphytum*, that bear leaves with evident secondary veins. Leaves with more secondary veins have greater vascular redundancy, and this helps the plant tolerate more mechanical damage than leaves with only an evident midrib (Sack et al., 2008). However, some of the most speciose and cosmopolitan genera in the family, such as *Cryptantha* and *Onosma*, produce leaves with only an evident midrib. Many of the plants in these two genera are small in stature, develop abundant relatively narrow leaves, and have a thick indument, which could protect the plant from mechanical damage or herbivory (Chamberlain, 1979; Kelley and Wilken, 1993; Agrawal et al., 2009). Consequently, some species of Boraginaceae may have evolved a redundancy system of secondary venation to protect against damage, while others may have developed a dense indument for the same purpose, a feature

also observed in species of *Asclepias* L. (Agrawal et al., 2009).

Floral characters

Inflorescence bracts. With only three exceptions, all species of Boragineae, Echiochileae, and Lithospermeae develop bracteate inflorescences (Figs 1f and 2, rectangles). In contrast, most species of Cynoglosseae do not (Fig. 1e) and, unlike most tribes of Boraginaceae, the development of bracts appears quite evolutionarily labile in this tribe. Cynoglosseae is also the only tribe in which species develop bracts only at the base of the inflorescence. This state may seem intermediate between bracteate and ebracteate inflorescences, but in Boraginaceae it is resolved as a stable condition, not a transition between the two extremes (Fig. 2).

The function of the inflorescence bracts does not appear to relate directly to pollination biology or fruit dispersal, as is the case in other groups of plants, such as *Bougainvillea* Comm. ex Juss. or *Atriplex* L. (Mandák and Pyšek, 2001). Unlike these other groups, the bracts in species of Boraginaceae are green and resemble leaves. Therefore it seems likely that these bracts serve the same function as leaves and are advantageous to plants that bear them because the bracts will provide increased photosynthetic products to flowers and fruit (Hori and Tsuge, 1993; Zhao and Oosterhuis, 1999).

Corollas. Corolla shape is quite variable in Boragineae and Lithospermeae. In these two tribes, nine of the 10 identified corolla shapes are present, with three of them restricted to these two tribes. In contrast, seven corolla shapes are found in Cynoglosseae and Trichodesmeae. In Boragineae and Lithospermeae, corolla shape is more evolutionarily labile than in Cynoglosseae and Trichodesmeae, but most large clades in the former two tribes are characterized by a particular corolla shape. For example, in Boragineae the clade that includes *Brunnera* Steven and *Anchusa* is characterized by salverform corollas, which are a synapomorphy for the clade.

Although corolla shape is variable throughout the family, this is not the case for other corolla features, such as corolla symmetry (Figs 1h and 2, squares). Ninety per cent of the species of Boraginaceae included in the present analysis develop actinomorphic corollas, with zygomorphic corollas originating at least six times among the other 10%. This type of corolla symmetry often evolved in only one or two species, and no reversals to actinomorphic corollas are resolved (but see Buys, 2006). The repeated, yet uncommon, origin of zygomorphic corollas in Boraginaceae suggests that few species have developed the specialized pollination syndromes often associated with bilateral symmetry (Neal et al., 1998). However, one exception—the clade that includes *Echium* and *Lobostemon*—is notable. Species of this clade have diversified throughout the Canary Islands (*Echium*) and South Africa (*Lobostemon*), with many species possessing specialized pollination syndromes (e.g. Olesen, 1988; Van Wyk et al., 1997). Given the association between zygomorphic corollas and distinct pollinators, this corolla symmetry may have played a role for the species of this clade in their diversification and success in new habitats.

Faucal appendages. Faucal appendages (also referred to as "fornices") are thickenings or inward evaginations of the corolla that develop at the intersection of the base of the corolla lobes and the apex of the corolla tube (Cohen, 2011). These appendages have evolved multiple times in Boraginaceae, and most species of Boragineae and Cynoglosseae produce flowers with faucal appendages (Fig. 2, ellipses). These appendages are ancestral to each of these tribes, but the optimization is ambiguous as to whether or not the appendages of the two tribes are homologous. Other origins of faucal appendages also are resolved, including at least three in Lithospermeae and one in Echiochileae. Despite the numerous gains of faucal appendages, at least eight losses are resolved, with each occurring in only one species or in small clades.

On one hand, the prevalence of faucal appendages in Boraginaceae and the limited number of losses suggest that these appendages may provide an advantage for the plant, but on the other hand, most species of the large tribe Lithospermeae do not bear flowers with faucal appendages. Additionally, in this latter tribe most of the origins of faucal appendages are in single species, not speciose clades. Although most species of Lithospermeae lack faucal appendages, these appendages may be advantageous. This appears to be due to the various manners in which faucal appendages can be modified to better attract pollinators, such as bearing glands, having a different colour from the rest of the corolla, or developing specialized epidermal cells (Kelley and Wilken, 1993; Cohen, 2011), as well as the ability of faucal appendages to constrict the apex of the corolla tube, thus influencing the orientation of the pollinator.

The lack of faucal appendages in Lithospermeae may be explained by the diversity of corolla shapes in the tribe. Faucal appendages often are associated with particular corolla shapes, such as salverform, but not with others, including urceolate and funnelform (Cohen, 2011). These latter shapes are common in Lithospermeae, but not in other tribes. Consequently,

species of Lithospermeae may have evolved one manner to attract pollinators—specialized corolla shapes—while members of other tribes have developed faucal appendages for this function.

Heterostyly. In the present study, eight to 10 origins of the breeding system heterostyly are resolved, two to three in Cynoglosseae, two to three in Boragineae, and four in Lithospermeae (Fig. 2, circles), and, within *Lithospermum*, Cohen (2011) resolved at least four additional origins of heterostyly. Therefore the breeding system originated at least 12 times within Boraginaceae. The present study resolves only one loss of heterostyly, in *Amsinckia*, but additional losses have previously been reported in the same genus (Schoen et al., 1997; Li and Johnston, 2010). To date, representatives from all genera of Boraginaceae with heterostylous species have been included in phylogenetic analyses (Schoen et al., 1997; Thomas et al., 2008; Ferrero et al., 2009; Cohen, 2011; Hasenstab-Lehman and Simpson, 2012), but this is not the case for all heterostylous species of the family. As more heterostylous species, particularly those of *Cryptantha* and its relatives, are included in phylogenetic analyses, the number of origins will likely increase.

In Boragineae and Cynoglosseae, non-herkogamy (lack of spatial separation between anthers and stigmas) is common, and heterostylous species are resolved to have originated from a non-herkogamous ancestor. In Lithospermeae, approach herkogamy (stigmas positioned above the anthers) is common, and approach herkogamous species are ancestral to three origins of heterostyly (the fourth origin is ambiguous). The different types of herkogamy exhibited by the ancestral species suggest two distinct manners in which heterostyly may have arisen. In Boragineae and Cynoglosseae, the non-herkogamous ancestors are congruent with the evolution of heterostyly proposed by Charlesworth and Charlesworth (1979), in which a non-herkogamous ancestral population develops self- and intramorph-incompatibility prior to reciprocal herkogamy. In contrast, the approach herkogamous ancestors resolved in Lithospermeae are congruent with scenarios proposed by Anderson (1973) and Lloyd and Webb (1992a). These authors hypothesized that in an ancestral population of approach herkogamous species, reciprocal herkogamy evolved before self- and intramorph-incompatibility. Consequently, the manner in which heterostyly developed in Lithospermeae appears to differ from that in Boragineae and Cyngolosseae. In addition to the phylogenetic data, distinct floral developmental patterns and self- and intramorph-incompatibilities have been reported in each of the three tribes with heterostylous species (Ganders, 1979; Philipp and Schou, 1981; Schou and Philipp, 1983; Casper, 1985; Li and Johnston, 2010; Cohen et al., 2012). The present study is the first to report distinct manners (i.e. scenario of Charlesworth and Charlesworth, 1979 vs. scenarios of Anderson, 1973 and Lloyd and Webb, 1992a) in which heterostyly may have arisen in different clades of the same family.

Twenty years ago, Lloyd and Webb (1992b) stated that Boraginaceae is one of "the most obvious candidates for divergent routes to heterostyly." However, these authors may not have hypothesized that the 12–14 origins of heterostyly resolved in Boraginaceae would be the greatest number of origins presently identified in any family. Despite this large number, multiple origins of the breeding system are not uncommon. Heterostyly evolved independently two to 10 times in Linaceae (McDill et al., 2009), five times in Lythraceae (Morris, 2007), four times in Pontederiaceae (Kohn et al., 1996), and two to three times in Rubiaceae (Ferrero et al., 2012). Current research suggests that within angiosperm families, multiple origins of heterostyly are more common than is a single origin followed by multiple losses, a pattern resolved only in Menyanthaceae (Tippery and Les, 2008, 2011). Due to the large number of different origins of heterostyly, Boraginaceae can serve as a model for the investigation of the various manners in which the heterostylous breeding system can arise.

Pollen characters

In Boraginaceae, pollen varies in size, shape, pore number, pore position, and other features. Seven different pollen shapes are present in Boraginaceae. Ellipsoid pollen is resolved as ancestral for the family as well as for each tribe; however, this shape is not present in Echiochileae, the tribe sister to the rest of the family. Species from this early diverging tribe develop pollen shapes, such as oblate-square and triangular-prism, uncommon in the rest of the family. Of the five tribes, pollen shape is most diverse in Lithospermeae, and pollen shapes are diagnostic and synapomorphic for large clades in this tribe. For example, ovoid pollen is a synapomorphy for two clades: *Podonosma* + *Alkanna* and the clade that includes *Echiostachys*, *Echium*, *Lobostemon*, and *Pontechium*. Additionally, although heterostylous species are present in three tribes of Boraginaceae, Lithospermeae is the only one that includes heterostylous species that bear pollen dimorphic in both size and shape (Johnston, 1952), and this type of pollen originated at least twice within the tribe.

The advantage of pollen shape diversity within Boraginaceae could relate to a type of lock-and-key pollination (Ghorbel and Nabli, 1998; Biggazi and Selvi, 2000; Cohen, 2010). Biggazi and Selvi (2000) provide evidence that pollen of a particular shape can be

captured and retained between stigmatic papillae of a complementary shape. This lock-and-key pollination orients pollen of the correct shape while restricting access and retention of foreign pollen to the stigmatic surface. The extent of the interconnection between pollen and stigma papillae shape has been explored primarily in Boragineae (Ghorbel and Nabli, 1998; Biggazi and Selvi, 2000), but this type of pollination appears to be more widespread in the family, with Cohen (2010) providing evidence of this relationship in Lithospermeae. By placing in a phylogenetic context the shapes of both pollen and stigmatic papillae, it would be possible to identify the number of origins of this lock-and-key pollination and to test if shifts in the shape of one are associated with changes in the other.

As with pollen shape, pollen pore number is variable in Boraginaceae (Fig. 2, rounded rectangles), and this character is most evolutionarily labile within Boragineae and Lithospermeae. Within these tribes, seven and five transitions, respectively, are resolved for pollen pore number (Fig. 2). In Boragineae, most of these transitions are in single species, but in Lithospermeae, shifts in pollen pore number tend to characterize larger clades. In general, pollen pore number in Boraginaceae has increased from three pores to six or greater, a trend observed in other groups as well, such as *Cuscuta* L. (Welsh et al., 2010), *Dioscorea* L. (Schols et al., 2005), and Sanguisorbeae (Chung et al., 2010). Dajoz et al. (1991) and Furness and Rudall (2004) suggest that an increase in pollen pore number may be advantageous because a greater number of pores results in a greater number of germination sites, and therefore a greater probability that at least one of these sites will be in an area favourable for germination. This advantage provides an explanation for the trend of increasing pollen pore number in Boraginaceae. Despite this putative advantage, most species of the family, and many of the more speciose and geographically widespread genera such as *Anchusa* and *Onosma*, bear pollen with three to five functional pores. Dajoz et al. (1991) provide evidence that, although pollen with fewer pores may not germinate as quickly as pollen with more pores, pollen with fewer pores is longer lived and tends to produce pollen tubes with an increased growth rate. This helps explain the small number of pores in most species of the family as well as the presence of heterocolpate pollen in Cynoglosseae. Given that pollen with more pores and with fewer pores both have advantages, variation in pollen pore number may be expected, even if a consistent unidirectional trend is also identified.

Fruit characters

All species of Boraginaceae develop nutlets, as do some members of Ehretiaceae and Heliotropiaceae. In each of these three families, nutlets originated independently. Although the type of fruit in Boraginaceae is constant, variation occurs in nutlet ornamentation (Fig. 1a–c). In most of the tribes of Boraginaceae, this variation is limited, with species bearing nutlets that are rugose, tuberculate, or smooth. In contrast, in Cynoglosseae and Trichodesmeae nutlet ornamentation is diverse. Species develop nutlets that range from rugose, tuberculate, or smooth to glochidiate, with marginal wings, and/or with marginal glochids (Fig. 1b,c). These latter three types of nutlet are exclusive to these two tribes, and specific types of nutlet ornamentation characterize particular clades. For example, nutlets with marginal glochids or wings (Fig. 1b) characterize the clade that includes *Omphalodes* and *Myosotidium*, and glochidiate nutlets are a synapomorphy for the clade that includes *Paracaryum* and *Cynoglossum*. Unlike the nutlets of most species of Boraginaceae, which appear to be barochorious (Mora-Vicente et al., 2009), those with glochids or wings have adaptations for additional types of dispersal, such as epizoochory (Ma et al., 2010; Gómez-González et al., 2011; Selvi et al., 2011) or anemochory (Thorsen et al., 2009). The greater dispersal ability of the nutlets of species of Cynoglosseae may help explain why, compared with other tribes of the family, this tribe has the most widespread geographic distribution as well as the greatest number of independent colonization events of the New World, South Africa, Australia, and New Zealand.

Despite the lack of glochids and wings present on the nutlets of most species in the family, other adaptations for nutlet dispersal are evident. In *Myosotis* (Thorsen et al., 2009), *Mortizia* (Melcher et al., 2000), *Arnebia*, and other genera, the calyx, which produces stiff trichomes, envelops the nutlets. This leads to all four nutlets being dispersed together as a unit inside the calyx. This method of dispersal is similar to that present in many species of Cynoglosseae, but it involves modification of different plant organs—the calyx rather than the ovary. Given the close spatial proximity of the sepals to the gynoecium, the similarities in fruit dispersal could be the result of transference of function (Baum and Donoghue, 2002). Rather than produce glochids on the surface of the nutlets, the development of these structures (i.e. stiff trichomes) may have shifted to the calyx. This hypothesis can be tested by investigating whether the same or different genes are involved in the development of each type of dispersal unit.

Conclusion

In Boraginaceae, Cynoglosseae has the greatest diversity of nutlet ornamentation, while floral and

pollen features are most diverse in Boragineae and Lithospermeae. Given these differences among tribes, it appears that specific features of the plant have been under fewer evolutionary and/or developmental constraints in particular clades: fruit in Cynoglosseae, and flowers and pollen in Boragineae and Lithospermeae. Consequently, species of Cynoglosseae have developed nutlets with diverse surface ornamentations, which appear to have provided members of the tribe with increased opportunities to colonize more areas more often. In contrast, Boragineae and Lithospermeae have developed greater variation in floral morphology and breeding systems, such as heterostyly (Schoen et al., 1997; Thomas et al., 2008; Ferrero et al., 2009; Cohen, 2011; Hasenstab-Lehman and Simpson, 2012), lock-and-key pollination (Biggazi and Selvi, 1998), and zygomorphy, allowing these two tribes to exploit more diverse pollination syndromes compared to members of Cynoglosseae.

The present study is the first to investigate phylogenetic relationships of the entire Boraginaceae using both molecular and morphological data. From these phylogenies, it is evident that additional species-level phylogenetic studies should be undertaken on specific clades in which large, widespread genera, such *Myosotis*, *Cynoglossum*, *Eritrichium*, and *Anchusa*, are resolved as non-monophyletic. Further analyses of these genera and their relatives will help to determine the most appropriate manners in which to circumscribe genera. In future family-level studies of Boraginaceae, it will be important to include more East Asian representatives of the family. Many genera, particularly small genera, are endemic to this region (Zhu et al., 1995), but to date, East Asian members have been poorly sampled in evolutionary studies of Boraginaceae. Including species from this region will provide critical data on phylogenetic relationships and character evolution, and will allow for a comprehensive reevalution of the taxonomy of the family, which is overdue.

Given the morphological diversity, as well as the patterns of evolution of vegetative, floral, pollen, and fruit features, Boraginaceae can serve as a model for the investigation of various morphological features, including heterostyly, corolla shape and symmetry, inflorescence bracts, leaf venation, pollen shape and pore number, and fruit ornamentation (Figs 1, 2 and 4). Future studies can focus further on the examination of the morphology, anatomy, development, genetics, and evolution of these and other variable characters within this diverse family.

Acknowledgements

The author would like to thank Caroline D. Kellogg, Janelle M. Burke, and two anonymous reviewers for helpful comments on the manuscript. The USDA, Denver Botanical Garden, National Botanic Garden of Belgium, Cornell Plantations, Brooklyn Botanical Garden, Oxford Botanical Garden, University of British Columbia Botanical Garden, Missouri Botanical Garden, and Royal Botanic Garden Edinburgh sent plant material that was used in this project. The Royal Botanic Garden, Kew, South African National Biodiversity Institute, the Missouri Botanical Garden DNA bank, and the DNA bank network provided DNA isolations. *Codon*Code Corporation granted a free version of *Codon*Code Aligner, which was very helpful for sequence viewing and assembly. Funding for this project was provided by start-up funds from Texas A&M International University.

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Appendix 1

Species	Collection	Location	ITS	matK	ndhF	trnL-trnF
Alkanna orientalis	56245-3	DNA bank network		KF287775	KF287859	KF288025
Alkanna pinardii		Genbank	EU919576	EU919612		
Alkanna tinctoria	B 10 0326075	DNA bank network	KF287943	KF287776	KF287860	FJ763304
Amsinckia calycina						GQ285246
Amsinckia spectabilis			JQ513393			JQ582295
Amsinckia tessellata	W6 27115	USDA	KF287944	KF287777	KF287861	KF288027
Anchusa aegyptiaca		Genbank	AY383294	EU599709	EU599797	EU599973
Anchusa azurea		Genbank	AY383293	EU599711	EU599799	
Anchusa capellii		Genbank	AY383297	EU599732	EU599819	AY383257
Anchusa capensis	022032	Denver Bot. Gard	KF287945	KF287778	KF287862	KF288028
Anchusa cespitosa		Genbank	AY383310	EU599721	EU599809	
Anchusa crispa		Genbank	AY071853	EU599733	EU599813	GQ285252
Anchusa formosa		Genbank	GQ285226	EU599724	EU599812	GQ285251
Anchusa leptophylla	2002 1090-96	Natl. Bot. Gard. Belgium	KF287946	KF287779	KF287863	KF288029
Anchusa officinalis	Cohen 174	Cornell Plantations	KF287947	KF287780	KF287864	KF288030
Anchusa pusilla		Genbank	AY045713	EU599716	EU599804	EU600068
Anchusa stylosa		Genbank	AY383308	EU599715	EU599803	EU600067
Anchusa thessala		Genbank	AF531084	EU599717	EU599805	AF530599
Anchusa undulata		Genbank	AY383300	EU599722	EU599810	
Anchusella variegata		Genbank	AY383306			AY383265
Antiphytum floribundum	Cohen 227	Michoacan, Mexico	KF287948			KF288031
Antiphytum hintoniorum		Genbank	AJ555899			
Arnebia benthamii	Chase 34887	RBG Kew DNA bank	KF287949	KF287781	KF287865	KF288032
Arnebia guttata		Genbank	EF199862			
Asperugo procumbens	B 10 0341981	DNA bank network	KF287950		KF287866	KF288033
Borago morisiana		Genbank	DQ657838	EU599705	EU599793	EU600057
Borago officinalis	Cohen 172	Cornell Plantations	KF287951	KF287782	KF287867	KF288034
Borago pygmaea		Genbank	DQ657844	EU599707	EU599795	GQ285274
Borago trabutii		Genbank	DQ657848	EU599703	EU599791	EU600055
Bothriospermum tenellum		Genbank	DQ320741			GQ285272
Bourreria succulenta		Genbank	DQ197285	DQ197229	DQ197257	
Brachybotrys paridiformis		Genbank	JQ388498	JQ388524	JQ388552	
Brandella erythraea	Collenette 9204	RBG Kew DNA bank	KF287952	KF287783		KF288035
Brunnera orientalis	Cohen 169	Cultivated in New York	KF287953	KF287784	KF287868	KF288036
Buglossoides arvense	19792083	Natl. Bot. Gard. Belgium	KF287954	KF287785	KF287869	KF288037
Buglossoides incrassata		Genbank	FJ763191		EU599765	FJ763255
Buglossoides purpurocaerulea	19792084, Chase 6055	Nati. Bot. Gard. Belgium and RBG Kew or Genbank	KF287955	EU599678	EU599766	FJ763308
Buglossoides tenuiflora		Genbank		EU599675	EU599763	EU600027
Caccinia strigosa	Chase 38129	RBG Kew DNA bank	KF287956	KF287786	KF287870	KF288039
Cerinthe alpina		Genbank	FJ541017	EU919615		
Cerinthe major	Cohen 91	Cultivated in New York	KF287957	KF287787	KF287871	KF288040
Chionocharis hookeri	Crawford et al. 571	RBG Kew DNA bank	KF287958		KF287872	KF288041
Codon schenkii		Genbank			AF047776	
Cordia boissieri	Cohen 417	TX, USA	KF287959	KF287788		
Cordia dentata		Genbank	EU862051	EU599654	EU599742	EU600006
Cordia myxa		Genbank	AF402578	EU599652	EU599740	EU600004

Appendix 1 (*Continued*)

Species	Collection	Location	ITS	matK	ndhF	trnL-trnF
Cordia sinensis		Genbank	AY321613	EU599653	EU599741	EU600005
Cryptantha crassisepala		Genbank		EU599666	EU599754	EU600018
Cryptantha fendleri		Genbank	JQ513411			JQ582313
Cryptantha peruviana		Genbank		EU599667	EU599755	EU600019
Cryptantha pterocarya		Genbank		EU599665	EU599753	EU600017
Cynoglossopsis latifolia	Edwards 3748	RBG Kew DNA bank	KF287965			
Cynoglossum amabile	Cohen 89	Cultivated in New York	KF287966	KF287796	KF287880	KF288048
Cynoglossum javanicum	Chase 38132	RBG Kew DNA bank	KF287967	KF287797	KF287881	KF288049
Cynoglossum sp.	DGA Styles 2280	SANBI		KF287795	KF287879	
Cynoglossum officinale		Genbank	AF402582	EU599664	EU599752	GQ285248
Cynoglottis barrelieri		Genbank	AF531081	EU599713	EU599801	EU600065
Cynoglottis chetikiana		Genbank	GQ285228	EU599714	EU599802	EU600066
Cystostemon heliocharis	Chase 6545	RBG Kew	KF287968	KF287799	KF287883	KF288051
Dasynotus daubenmirei	Cohen 403	ID, USA	KF287969	KF287800	KF287884	KF288052
Echiochilon callianthum	Thulin et al. 8272	RBG Kew	KF287970			KF288053
Echiochilon fruticosum		Genbank	AJ555908			EU044881
Echiochilon johnstonii	Thulin and Dahir 6717	RBG Kew	KF287971			KF288054
Echiochilon longiflorum	Chase 6168	Genbank	AJ555913			
Echiostachys incanus		Genbank	AF284112			AF284110
Echium aculeatum		Genbank	EU048849	EU599692	EU599780	L43166
Echium angustifolium		Genbank		EU599695	EU599783	EU600047
Echium bonnetii		Genbank	L43184	EU599688	EU599776	L43182
Echium candicans		Genbank	EU048856	AF543610		
Echium decaisnei		Genbank	EU048852	EU599691		L43210
Echium italicum		Genbank	L43236	EU599699	EU599787	EU600051
Echium leucophaeum		Genbank	L43240	EU599689	EU599777	L43238
Echium plantagineum		Genbank	L43272	EU599697	EU599784	L43270
Echium vulgare	Cohen 212	Cornell Plantations	KF287972			KF288056
Echium wildpretii	Cohen 255	Brooklyn Bot. Gard.	KF287973	KF287802	KF287886	
Ehretia cymosa		Genbank	AF385790	EU599660	EU599748	EU600012
Elizaldia calycina		Genbank	AY383305			DQ269662
Eritrichium aretioides		Genbank			JQ388553	JQ388579
Eritrichium caucasicum	20091732	RBG Edinburgh	KF287974	KF287803	KF287887	KF288057
Eritrichium chamissonis		Genbank		JQ388526	JQ388554	JQ388580
Eritrichium nanum		Genbank	AY092901	AY092894	JQ388556	JQ388581
Eritrichium rupestre	B 10 0209718	DNA bank network		KF287804	KF287888	
Eritrichium sericeum		Genbank	JQ388500	JQ388529	JQ388557	GQ244954
Eritrichium splendens		Genbank	JQ388501	JQ388530	JQ388558	JQ388582
Eritrichium villosum		Genbank		JQ388531	JQ388559	GQ244957
Gastrocotyle macedonica		Genbank	AY045715			AY045706
Glandora diffusa	Chase 6063	RBG Kew DNA bank				FJ763300
Glandora oleifolia	Chase 34889, 34890	RBG Kew DNA bank	FJ789869	JF488878		JF489064
Glandora rosmarinifolia		Genbank	FJ789872	EU599682	EU599771	FJ763291
Greeneocharis circumscissa		Genbank	JQ513403			
Hackelia floribunda	Cohen 256	UT, USA				KF288058
Hackelia micrantha	Cohen 262	UT, USA	KF287975	KF287805	KF287889	KF288059
Hackelia virginiana	Townsmith and Guest 253	MO Bot. Gard. DNA Bank	KF287976	KF287806	KF287890	KF288060
Halacsya sendtneri		Genbank	EU919588	EU919618		EU044885
Heliotropium aegyptiacum		Genbank	AF396918	EU599646	EU599734	EU599998

Appendix 1 (*Continued*)

Species	Collection	Location	ITS	matK	ndhF	trnL-trnF
Heliotropium longistylum		Genbank	EF688901		EF688936	
Hormuzakia aggregata	Plume 71	Italy	KF287977	KF287807	KF287891	KF288061
Huynhia pulchra	Cohen 260	Cultivated in New York	KF287978	KF287808	KF287892	KF288062
Hydrophyllum canadense		Genbank		EU599649	EU599737	EU600001
Lappula redowskii	Cohen 161	WY, USA	KF287979		KF287893	KF288063
Lappula squarrosa	10000906	Natl. Bot. Gard. Belgium			KF287894	
Lappula texana	Cohen 368	TX USA	KF287980		KF287895	KF288064
Lasiocaryum munroi	Cuttin and Vernay 81	RBG Kew DNA bank	KF287981			
Lepechiniella albiflora	Rechinger 31424	RBG Kew DNA bank	KF287982	KF287809		KF288065
Lindelofia longiflora	19981513-95	Natl. Bot. Gard. Belgium	KF287983	KF287810	KF287896	KF288066
Lindelofia macrostyla	Chase 38131	RBG Kew DNA bank	KF287984	KF287811	KF287897	KF288067
Lithodora hispidula	Chase 34888	RBG Kew DNA bank	KF287985	KF287812	KF287898	KF288068
Lithodora zahnii	Chase 34891	RBG Kew	KF287986	KF287813		KF288069
Lithospermum bejariense	Cohne 375	TX, USA	KF287987	KF287814	KF287899	KF288070
Lithospermum distichum	Cohen 192, 202	Nuevo León and D. F., Mexico	KF287988	KF287815	KF287900	KF288071
Lithospermum incisum	Cohen 371	TX, USA	KF287989	KF287816	KF287901	KF288072
Lithospermum leonotis	Cohen 195	Nuevo León, Mexico		KF287817		
Lithospermum macromeria	Cohen 141, 151	Arizona, USA			KF287902	KF288073
Lithospermum multiflorum	Cohen 81	TX USA	KF287990	KF287818	KF287903	KF288074
Lithospermum nelsoniii	Cohen 184	Nuevo León, Mexico		KF287819		KF288075
Lithospermum officinale	Cohen 171	Cornell Plantations	KF287991	KF287820	KF287904	KF288076
Lobostemon fruticosus	Cohen 23748, 6090	RBG Kew	KF287992	KF287821	KF287905	KF288077
Lobostemon trigonus		Genbank	FJ789876			FJ789858
Lycopsis arvensis		Genbank	AY045711	EU599718	EU599806	EU600070
Macrotomia densiflora		Genbank	EU919591			
Maharanga emodi		Genbank	FJ763207			FJ763269
Mairetis microsperma		Genbank	FJ763193	EU919620		FJ763257
Melanortocarya obtusifolia		Genbank	DQ269681			AY627874
Mertensia sp.	Cohen 282	UT, USA	KF287993	KF287822	KF287906	KF288078
Mertensia asiatica	Cohen 258	Cultivated in New York		KF287823	KF287907	KF288079
Mertensia ciliata	Cohen 261	UT, USA		KF287824	KF287908	KF288080
Mertensia longiflora	Cohen 407	ID, USA	KF287994	KF287825	KF287909	KF288081
Mertensia paniculata	Cohen 405	ID, USA	KF287995		KF287910	KF288082
Mertensia virginica		NY, USA	KF287996	KF287826	KF287911	KF288083
Moltkia angustifolia		Genbank	FJ763252	EU919621		
Moltkia aurea		Genbank	EU919594	EU919622		
Moltkia caerulea		Genbank	EU919595	EUE919623		
Moltkia petraea	2000 1260-54	Natl. Bot. Gard. Belgium	KF287997	KF287827	KF287912	FJ763258
Moltkia suffruticosa		Genbank	EU919597	EU919625		EU044893
Moltkiopsis ciliata		Genbank	EU919598	EU919626		
Moritzia lindenii		Genbank	GQ285231			GQ285255
Myosotidium hortensia	20020498	Oxford Bot. Gard.	AY092902	KF287828	KF287913	KF288085
Myosotis alpestris		Genbank	AY092907		AY092854	
Myosotis arvensis	B 10 0340905	DNA bank network			KF287914	KF288086
Myosotis australis		Genbank	AY092911		AY092844	
Myosotis discolor		Genbank	AY092919		AY092852	
Myosotis macrantha		Genbank	AY092924		AY092853	
Myosotis macrosperma		Genbank	AY092925		AY092855	
Myosotis sylvatica	B 10 0340390	DNA bank network		KF287829	KF287915	

Appendix 1 (*Continued*)

Species	Collection	Location	ITS	matK	ndhF	trnL-trnF
Neatostema apulum		Genbank	EU919599	EU599686	EU599774	FJ763262
Nonea lutea	Cohen 257	Cultivated in New York	KF287999	KF287830	KF287916	KF288087
Nonea pulla		Genbank				AY383275
Nonea stenosolen		Genbank		EU599701	EU599789	EU600053
Ogastemma pusillum	Chase 6546	RBG Kew DNA bank and Genbank	KF288000	KF287831	KF287917	KF288088
Omphalodes aliena	Cohen 367	TX, USA	KF288001	KF287832	KF287918	KF288089
Omphalodes cappadocica	1995-3595	Missouri Bot. Gard.		KF287833	KF287919	KF288090
Omphalodes lojkae	071569	Denver Bot. Gard	KF288002	KF287834	KF287920	KF288091
Omphalodes nitida	1996001.1	Oxford Bot. Gard.	KF288003	KF287835	KF287921	KF288092
Omphalodes verna	19830183	Natl. Bot. Gard. Belgium	KF288004	KF287836	KF287922	KF288093
Oncaglossum pringlei	Cohen 219	Michoacan, Mexico		KF287798	KF287882	KF288050
Onosma alborosea	010628-6156-1974	UBC Bot. Gard.	KF288005	KF287837		
Onosma echioides		Genbank	EU919601	EU919628		
Onosma graecum		Genbank		EU599684	EU599772	EU600036
Onosma stellulata	1992 1317-39	Natl. Bot. Gard. Belgium	KF288006		KF287923	KF288094
Onosma taurica		Genbank	GU827151	EU599685	EU5997773	EU600037
Onosma visianii		Genbank	EU919603			
Oreocarya bakeri		Genbank		EU599668	EU599756	EU600020
Oreocarya cana	King and Graves 12436	MO Bot. Gard. DNA Bank	KF288007	KF287838	KF287924	KF288095
Oreocarya confertiflora	Cohen 401	NV, USA	KF287961	KF287790	KF287874	KF288043
Oreocarya crassipes		TX, USA	KF287962	KF287791	KF287875	KF288044
Oreocarya flava	Cohen 389	NM, USA	KF287963	KF287792	KF287876	KF288045
Oreocarya flavoculata		Genbank	AF091154	EU599669	EU599757	EU600021
Oreocarya fulvocanescens	Cohne 391	NM, USA	KF287964	KF287793	KF287877	KF288046
Oreocarya paysonii	Cohen 386	NM, USA		KF287794	KF287878	KF288047
Oreocarya suffruticosa	Cohen 308	AZ, USA	KF287960	KF287789	KF287873	KF288042
Paracaryum intermedium	Collenette 858	RBG Kew DNA bank	KF288008	KF287839		KF288096
Paracaryum lithospermifolium		Genbank		EU599663	EU599751	EU600015
Paracaryum recemosum	Cohen 259	Cultivated in New York	KF288009	KF287840	KF287925	KF288097
Paramoltkia doerfleri		Genbank	KF288010	KF287841		KF288098
Paraskevia cesatiana		Genbank	AY383318			AY383276
Pardoglossum cheirifolium	Chase 6065	RBG Kew DNA bank	KF288011	KF287842	KF287926	KF288099
Pectocarya anomala		Genbank	JQ513449			JQ582348
Pectocarya penicillata		Genbank	JQ513450			JQ582349
Pectocarya peninsularis		Genbank	JQ513451			JQ582350
Pentaglottis sempervirens	Chase 6058	RBG Kew DNA bank	KF288012	KF287843	KF287927	KF288100
Phacelia tanacetifolia		Genbank		EU599650	EU599738	EU600002
Phyllocara aucheri		Genbank	AY383290	EU599710	EU599798	EU600062
Plagiobothrys albiflorus		Genbank	AY092899		AY092891	
Plagiobothrys kingii		Genbank	JQ513457			JQ582354
Plagiobothrys myosotoides		Genbank	JQ513459			JQ582356
Podonosma orientalis	B 10 0326644	DNA bank network and Genkbank	FJ763253	EU599674	KF287928	FJ763307
Pontechium maculatum	990415	Denver Bot. Gard. and Genbank	EU919608	KF287801	KF287885	KF288055
Procopiania cretica		Genbank	AY383284			AY383246
Pseudomertensia echioides		Genbank	JQ388517	JQ388546		
Pseudomertensia moltkioides		Genbank	JQ388518	JQ388547	JQ388573	
Pseudomertensia primuloides	19751894	RBG Edinburgh	KF288014	KF287844	KF287929	KF288102
Pseudomertensia trollii	19391024	RBG Edinburgh		KF287845	KF287930	KF288103
Pulmonaria angustifolia	981057	Denver Bot. Gard	KF288015	KF287846	KF287931	KF288104

Appendix 1 (*Continued*)

Species	Collection	Location	ITS	matK	ndhF	trnL-trnF
Pulmonaria obscura		Genbank	FJ763200	EU599700	EU599788	FJ763264
Pulmonaria officinalis	B 10 0209607	DNA bank network		KF287847	KF287932	KF288105
Rochelia cancellata		Genbank	AB564702			AB564712
Rochelia persica		Genbank	AB564697			AB564707
Solenanthus apenninus		Genbank	FR715322			
Solenanthus circinatus		Genbank	FR715324			
Solenanthus stamineus		Genbank	FR715325			
Suchtelenia calycina	Russian collector s.n.	RBG Kew DNA bank	KF288016	KF287848		KF288106
Symphytum asperum	Cohen 221A	Chicago Bot. Gard.	KF288017	KF287849	KF287933	KF288107
Symphytum caucasicum	032862-0647-1996	UBC Bot. Gard.		KF287850	KF287934	KF288108
Symphytum ibericum	026940-0304-1988	UBC Bot. Gard.	KF288018	KF287851	KF287935	KF288109
Symphytum orientale	0001865	Oxford Bot. Gard.		KF287852	KF287936	KF288110
Symphytum peregrinum	233329	USDA		KF287853	KF287937	KF288111
Symphytum tuberosum	032863-0647-1996	UBC Bot. Gard.	KF288019	KF287854	KF287938	KF288112
Thaumatocaryon dasyanthum		Genbank	GQ285230			GQ285271
Thaumatocaryon tetraquetrum		Genbank				GQ285260
Tiquilia darwinii		Genbank	DQ197542	DQ197248	DQ197276	
Tiquilia paronychioides		Genbank	EF688860	DQ197249	EF688912	
Tournefortia laurifolia		Genbank		EU599648	EU599736	EU600000
Trachystemon orientalis	1978-1795	Missouri Bot. Gard.	KF288020	KF287855	KF287939	KF288113
Tricardia watsonii		Genbank	AF091209		AF047775	
Trichodesma africana	LHMS 1606	SANBI	KF288021	KF287856	KF287940	
Trichodesma scottii	Chase 2912/Cohen 418	RBG Kew DNA bank and cultivated in California	KF288022	KF287857	KF287941	KF288114
Trigonocaryum involucratum	Merello, Schmidt, and Shetekauri 2173	MO Bot. Gard. DNA Bank	KF288023	KF287858	KF287942	KF288115
Trigonotis formosana		Genbank	JQ388519		JQ388574	GQ285261
Trigonotis guiliielmi		Genbank				GQ285257
Trigonotis peduncularis		Genbank	DQ320750			
Tysonia africana	Hilliard and Burtt 25595	SANBI	KF288024			
Ulugbekia tschimganica		Genbank	FJ763220			FJ763279
Vahlia capensis		Genbank		AJ429316	AJ236273	AJ430904
Wellstedia dinteri		Genbank		HQ384575	HQ384862	HQ412983

Appendix 2

Strict consensus trees of the (A) combined cpDNA (L = 5531, CI/RI = 0.46/0.83), (B) cpDNA (L = 4983, CI/RI = 0.50/0.84), and (C) morph matrices (L = 408, CI/RI = 0.14/0.78). Numbers above branches are jackknife values

Fig. A1.

Fig. A1. (Continued).

Fig. A1. (Continued).

Appendix 3

Patterns of evolution for each of the 27 morphological characters investigated in the present study

Patterns of morphological character evolution

Morphological characters – vegetative

Naphthoquinones. Most species of Boraginaceae do not produce naphthoquinones, which result in a red or purple color in the roots, and the lack of these secondary compounds is ancestral for the family. At least 11 independent origins of naphthoquinones are resolved, along with no unambiguous losses. All tribes, except Trichodesmeae, include species that synthesize naphthoquinones, and Lithospermeae has the greatest number of species that produce these secondary compounds. Naphthoquinones are a synapomorphy for many species pairs as well as the clade that includes *Arnebia*, *Huynia*, and *Macrotomia.*

Vestured pits. Vestured pits are not present in most species of Boraginaceae, but the presence of vestured pits characterizes species of *Antiphytum* and Lithospermeae. This feature is a synapomorphy for the tribe.

Position of leaves. The development of only cauline leaves is the ancestral condition for the family and for Echiochileae, and most species of Lithospermeae develop only cauline leaves. Most species of Cynoglosseae and Boragineae are characterized by both basal and cauline leaves, and this is the ancestral condition for the latter. Although most species of a tribe develop a particular leaf position, the alternate type of leaf position originated multiple times. With the exception of the clade that includes *Gastrocotyle* and *Anchusa*, most of these origins in Boragineae and Cynoglosseae occur in individual species.

Pattern of leaf venation. Most species of the outgroup are characterized by leaves with an evident midvein and secondary veins, but this is not the ancestral condition for Boraginaceae. Most species of Boraginaceae develop leaves with only an evident midvein, although multiple origins of leaves with both a midvein and secondary veins occur in the family. In *Cynoglosseae*, this type of leaf venation is a synapomorphy for four medium to large clades: *Myosotidium* + *Omphalodes*, *Mertensia*, *Hackelia*, and the one that includes *Cynoglossum*, *Lindelofia*, and four other genera. In Boragineae, leaves with both a midvein and secondary veins is a synapomorphy for the clade that includes *Trachystemon* and *Moritzia*. As with the position of leaves, a reversal is resolved in the tribe, with leaves that only include a midvein being a synapomorphy for the clade that includes *Gastrocotyle* and *Anchusa.*

Cordate leaves. A cordate leaf shape is uncommon in Boraginaceae. Most origins of this type of leaf are in single species, but cordate leaves are a synapomorphy for the clade that includes *Myosotidium* and *Omphalodes.*

Morphological characters – floral

Floral bracts. Although most species of Echiochileae develop floral bracts, which is a synapomorphy for the tribe, the ancestral

condition for this character is ambiguous. Most members of Cynoglosseae lack floral bracts, although floral bracts originated at least 14 times within the tribe. Additionally, Cynoglosseae is the only tribe that includes species with floral bracts present only at the base of the inflorescence. All species of Lithospermeae develop floral bracts, and this is also the case for almost all species of Boragineae. In this tribe, three losses of floral bracts are resolved: in *Moritzia*, in *Brunnera*, and in the clade that includes *Procopiania* and *Symphytum*.

Corolla shape. I identified ten different corolla shapes. The salverform shape is most common, but funnelform corollas are resolved as ancestral for the family. This corolla shape is common in Echiochileae, but not in other tribes, although this shape is a synapomorphy for the clade that includes *Echiostachys*, *Echium*, *Lobostemon*, and *Pontechium*. Salverform corollas are ancestral in all other tribes. The funnelform-salverform shape is a synapomorphy for the clade comprising species of *Buglossoides* and *Ulugbekia* as well as for other species pairs. Most other corolla shapes tend to originate in individual species. Most species of Cynoglosseae develop salverform corollas, but this is not the case in Boragineae and Lithospermeae. In these latter two tribes, corolla shape is quite diverse with multiple origins of different shapes of corollas as well as more diversity in corolla shape than is observed in Cynoglosseae.

Corolla lobes. Most species of Boraginaceae bear corollas with flared lobes, and this is the ancestral condition for the family. Multiple independent origins of erect corolla lobes are resolved in the present phylogenies. Erect corolla lobes are a synapomorphy for small clades consisting of one or two genera, such as *Asperugo* + *Mertensia*, as well as the large clade composed of *Moltkia*, *Echium*, and six other genera. Many species are polymorphic for both erect and flared corolla lobes. Only a small number of species bear corollas with reflexed corolla lobes, with this condition most common in Lithospermeae. In most cases, reflexed corolla lobes originated independently in single species.

Corolla symmetry. Most species of Boraginaceae bear actinomorphic corollas, but zygomorphic corollas originated at least six times. These origins occurred in all tribes except Cynoglosseae. In general, zygomorphic corollas originate within a single species or genus, but this type of corolla symmetry is a synapomorphy for *Echiochilon* as well as the clade composed of *Echiostachys*, *Echium*, *Lobostemon*, and *Pontechium*.

Corolla color. Eight different corolla colors were identified. White is reconstructed as the ancestral corolla color for Boraginaceae. Although this color is common in Echiochileae, it is not as common among species of the other tribes. Corolla color is quite variable throughout Boraginaceae, and many species are scored as polymorphic. Despite this variability, particular corolla colors are synapomorphies for large clades, such as blue for Cynoglosseae, yellow for Lithospermeae, and white for *Cryptantha* and relatives.

Abaxial trichomes on the corolla. The absence of abaxial trichomes on the corolla is the ancestral condition for the family, but the majority of species of Echiochileae included in the present study bear corollas with abaxial trichomes. Within Boraginaceae, at least 10 independent origins of the presence of abaxial trichomes on corollas are reconstructed, with most occurring in Boragineae and Lithospermeae. In Boragineae, this state tends to originate in single species, but in Lithospermeae, this state is a synapomorphy, in analyses of the combined matrix, for the clade that includes all of the tribe except *Alkanna* + *Podonosma*. In analyses of the cpDNA combined matrix, the presence of abaxial trichomes on the corolla are is a synapomorphy for two large clades: *Arnebia*, *Lithospermum*, and three other genera as well as *Echium* and *Lobostemon*. In this tribe, at least two reversals are resolved to corollas that are glabrous abaxially.

Adaxial trichomes on the corolla. Most species of Boraginaceae do not bear corollas with adaxial trichomes, and this is the ancestral condition for the family. The presence of corollas with adaxial trichomes originated at least 10 times. In all but one instance – *Echiochilon* – this state arose in isolated species.

Faucal appendages. Although in Boraginaceae the absence of faucal appendages is the ancestral condition, faucal appendages are present in most of the sampled species of the family. All tribes of Boraginaceae include species that bear flowers with faucal appendages, and this is the case for most species of Boragineae and Cynoglosseae. However, it is ambiguous as to whether or not the faucal appendages in these two tribes are ancestral. In contrast to Boragineae and Cynoglosseae, most species of Lithospermeae develop flowers without faucal appendages. Faucal appendages have been lost at last seven times in Boraginaceae.

Glands inside corolla. Although most species of Boraginaceae bear corollas with glands, the absence of glands is resolved as the ancestral condition for the family, and this is the condition for all but one species of Echiochileae, *Antiphytum hintoniorum* L.C. Higgins & B.L. Turner. Most species of Cynoglosseae and Boragineae bear corollas with glands, but the opposite condition is most common in Lithospermeae, although the presence of corolla glands is a synapomorphy, in Lithospermeae, for the large clade that includes *Buglossoides* and *Lithospermum*. In Boraginaceae, at least 14 reversals to corollas without glands are resolved, with most of these reversals occurring in individual species or small clades.

Type of herkogamy. Non-herkogamy is ancestral for the family as well as for all tribes, except Trichodesmeae in which approach herkogamy is the ancestral state. Most species of Boragineae and Cynoglosseae are non-herkogamous, although approach herkogamy originated multiple times in each of these tribes. In Boragineae, approach herkogamy is a synapomorphy for the clade that includes *Trachystemon* and *Symphytum*. Approach herkogamy is most common in Lithospermeae, and it is a synapomorphy for the large clade composed of *Cerinthe* and *Echium*. Reverse herkgaomy as a fixed state is present only among isolated species in Boraginaceae. Reciprocal herkogamy originated at least eight times among species of Boraginaceae.

Anther position. Although most outgroup species develop flowers with anthers exserted from the corolla, most species of Boraginaceae do not, and this is the ancestral condition for the family as well as all tribes, except Trichodesmeae in which exserted anthers are a synapomorphy. Most instances of anther exsertion occur in isolated species or in species pairs, but this state is a synapomorphy for a large clade in Lithospermeae that includes *Moltkia* and *Echium* in analyses of the combined matrix and *Echium* and *Pontechium* in analyses of the combined cpDNA matrix.

Androecial apical projection. Five different types of androecial apical projections were identified in the present study. Each of these is characteristic of a particular genus or group of genera, and all but one state, *Myosotis*-type, is a synapomorphy for a clade. For example, different types of apical projections are synapomorphies for *Borago* and for *Trichodesma*.

Stigma position. Most species of Boraginaceae bear stigmas included in the corolla, and this is the ancestral condition for the family. Each tribe, except Echiochileae, includes one large clade characterized by exserted stigmas. For example, in Lithospermeae exserted stigmas are a synapomorphy for the clade that includes *Moltkia*, *Echium*, and six other genera, and in Cynoglosseae, this condition is a synapomorphy for the clade composed of *Paracaryum* and *Lindelofia*.

Stigma location. Terminal stigmas is both the ancestral and most common condition in Boraginaceae. Subterminal stigmas originated at least 11 times in the family. Most of these origins occur among individual species; however, two genera, *Buglossoides* and *Echiochilon*, are characterized by subterminal stigmas, and this state originated independently in each.

Conical stigmas. Stigmas with a conical shape only are present in species of *Heliotropium*. This type of stigma is resolved to have originated one to two times among members of the genus.

Pollen shape. Seven different pollen shapes have been identified in Boraginaceae. The ancestral condition for the family is ellipsoid, and this also is the most common shape in Boraginaceae, especially in Borageineae and Lithospermeae. Although ellipsoid pollen is resolved in Cynoglosseae as ancestral, many species in this tribe bear pollen that is prolate with a constricted equator (hourglass). However, a reversal to ellipsoid pollen is identified, and this type of pollen is a synapomorphy for the large clade that includes *Cynoglossum* and *Cryptantha*. Ovoid pollen is restricted to Lithospermeae, and it is a synapomorphy *Alkanna* + *Podonosma* as well as the clade that includes *Echium*, *Echiostachys*, *Lobostemon*, and *Pontechium*. Other pollen shapes characterize only a few species, and in most cases, each shape is a synapomorphy for a small group of species.

Pollen pore number. Pollen with two to five pores is the ancestral condition for Boraginaceae. Pollen with a more pores has originated multiple times. These origins are most common in Borageineae and Lithospermeae. In Borageineae, *Symphytum* is characterized by pollen with eight to twelve pores, and pollen with six to eight pores originated independently in *Trachystemon*, *Moritzia*, and *Hormuzakia*. In Lithospermeae, pollen with six to eight pores is a synapomorphy for a large clade that includes species of *Lithospermum* and *Neatostema*. Pollen with eight to twelve pores is a synapomorphy for two small clades in Lithospermeae: *Huynhia* + *Macrotomia* as well as three species of *Moltkia*. In Boraginaceae, only two instances are identified in which pollen pore number decreased, and both of these – *Halacsya* and, in analyses of the combined matrix, the clade that includes *Onosma* and *Echium* – occur in Lithospermeae

Pollen pore position. Pollen with equatorial pollen pores is the ancestral condition for the family, and most species develop pollen with equatorial pores. According to the present phylogenies, at least six independent origins of pollen with subequatorial pores are resolved throughout the family. All of these origins occur in Lithospermeae, except one in *Moritzia*.

Heterocolopate pollen. Heterocolpate pollen is a synapomorphy for Cynoglosseae, and species of Cynoglosseae are the only species in Boraginaceae that bear this type of pollen. Two outgroup species, *Phacelia tanacetifolia* Benth. and *Tysonia africana* Bolus, are the only other species included the present study that develop heterocolpate pollen, and this type of pollen originated independently in each.

Organismal characters – fruit

Fruit type. All species of Boraginaceae bear nutlets, and for the family, this fruit type is a synapomorphy. Nutlets are also present in Heliotropiaceae and Ehretiaceae, and phylogenetic results suggest that nutlets originated independently in each of these two families. Capsules are a present in Hydrophyllaceae, *Codon*, and *Wellstedia*, and drupes are a synapomorphy for the clade that includes Cordiaceae, Ehretiaceae, and Heliotropiaceae.

Nutlet surface ornamentation. I identified eight different types of nutlet surface ornamentation. The ancestral type is ambiguous for the family. In analyses of the combined matrix, rugose and tuberculate nutlets are ancestral for Borageineae and Lithospermeae, respectively, while in analyses of the combined cpDNA matrix, smooth nutlets are resolved as ancestral for the clade that includes both of these tribes. Nutlets with tuberculate or rugose ornamentation arose independently in Borageineae, Cynoglosseae, and Lithospermeae. Nutlets with glochids, marginal glochids, or marginal wings are restricted to Cynoglosseae and Trichodesmeae. Each of these nutlet types is a synapomorphy for a medium to large clade. For example, the presence of marginal glochids is a synapomorphy for the clade consisting of *Eritrichium*, *Hackelia*, *Lappula*, and *Lepechiniella*. Additionally, in Cynoglosseae smooth nutlets are a synapomorphy for the clade that includes *Bothriospermum*, *Brachybotrys*, *Myosotis*, *Pseudomertensia*, *Trigonocaryum*, and *Trigonotis*.

Nutlet attachment. All species of Borageineae and Lithospermeae included in the present analyses develop nutlets with basal attachment, and this condition is a synapomorphy for the clade composed of these two tribes. In contrast, all species of Cynoglosseae and Trichodesmeae, with the exception of species of *Brachybotrys* and *Pseudomertensia*, bear nutlets with non-basal attachment. In Echiochileae, nutlet attachment is more variable. Although most members of the tribe develop nutlets with basal attachment, two independent origins of non-basal attachment are resolved.