

A Broad Phylogenetic Analysis of Boraginaceae: Implications for the Relationships of *Mertensia*

Mare Nazaire^{1,3} and Larry Hufford^{1,2}

¹School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236, U. S. A

²hufford@mail.wsu.edu

³Author for correspondence (mnazaire@wsu.edu)

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Abstract—The phylogenetic relationships of *Mertensia* (Boraginaceae), which comprises approximately 45 species in both Asia and North America, have been uncertain, and taxonomists have placed the genus in various tribes of subfamily Boraginoideae, with the most recent placements in Trigonotideae and Cynoglosseae. Our study applies molecular phylogenetic methods to test the monophyly and relationships of *Mertensia*. We used DNA sequence data from the nuclear ribosomal nrITS region and four cpDNA regions (*matK*, *ndhF*, *rbcL*, *trnL-trnF*) to examine the placement of *Mertensia* among a sampling of accessions from approximately 70% of the genera of Boraginaceae s. l. Phylogeny reconstructions using maximum parsimony, maximum likelihood, and Bayesian inference were largely congruent with previous molecular phylogenetic analyses of Boraginaceae that had applied far fewer taxa. We recovered five deep clades that correspond to Boraginaceae subfamilies Boraginoideae, Cordioideae, Heliotropioideae, Hydrophylloideae, and Ehretioideae (including *Lennoa* and *Pholisma*). In subfamily Boraginoideae, we recovered clades that correspond to the tribes Echiochilieae, Lithospermeae, Cynoglosseae, and Boragineae, although several tribes previously circumscribed on the basis of morphological data were not recovered as monophyletic in our results. Based on the sister relationship between the genus *Codon* and subfamily Boraginoideae found in our phylogeny reconstructions, we propose **Codoneae** as a new tribe of Boraginoideae. We recovered strong support for the monophyly of *Mertensia* and the placement of the monotypic genus *Asperugo* as its sister. *Mertensia* and *Asperugo* were strongly supported as members of Cynoglosseae.

Keywords—Asteridae, Boraginaceae, Boraginoideae, maximum likelihood, *Mertensia*, molecular phylogenetics.

Mertensia Roth (Boraginaceae, subfamily Boraginoideae) comprises approximately 45 species distributed in the Northern Hemisphere of both North America and Asia (Williams 1937; Popov 1953a; Al-Shehbaz 1991). *Mertensia* shares many characteristics with other Boraginoideae, including hispid to strigose vestiture of stout, eglandular, unicellular trichomes, pentamerous sympetalous corolla bearing faucal appendages, and fruit a schizocarp of four one-seeded nutlets (Al-Shehbaz 1991). *Mertensia* is distinguished from other members of the Boraginoideae by undivided stigmas and a ventral and suprabasal (attached above the base) position of the oblique attachment scar of abscised nutlets (Johnston 1924a; Popov 1953b; Al-Shehbaz 1991). However, it is uncertain whether the stigma form or the nutlet attachment scar states are synapomorphies for the genus.

Since first described by Linnaeus (1753), *Mertensia* has been variously assigned to five different tribes within subfamily Boraginoideae: Symphyteae, Lithospermeae, Eritrichieae, Trigonotideae, and Cynoglosseae (Table 1). Its early placement in Symphyteae (Don 1838, p. 307) was based on the presence of a tubular corolla, included stamens, and “carpels or nuts fixed to the bottom of the calyx.” This latter observation that the nutlets are adnate to the calyx is morphologically unlikely, given that the flowers are hypogynous and have the gynoecium separated from the calyx by an encircling corolla. *Mertensia* was later placed in Lithospermeae (De Candolle 1846; Bentham 1876; Baillon 1890; Gürke 1897), but this was questioned by Johnston (1924a, 1924b), who placed it in Eritrichieae, based on nutlet attachment and simple stigmas. In contrast, Popov (1953b) placed *Mertensia* in the subtribe Trigonotidinae of Lithospermeae, stating that the subtribe was intermediate between Eritrichieae and typical Lithospermeae. Riedl (1967, 1968) disagreed with Popov’s placement of *Mertensia* in Lithospermeae and included both *Mertensia* and *Trigonotis* Stev. in subtribe Trigonotidinae of the newly recognized tribe Trigonotideae. (Both Popov and Riedl denoted the subtribe Trigonotidinae erroneously as “Trigonotideae.”

Although the Riedl and Popov subtribe name was adopted by subsequent workers, we follow the International Code of Botanical Nomenclature and use subtribe Trigonotidinae to denote this group in our study). Recent investigations of the phylogenetic relationships of Trigonotideae using molecular data suggest the tribe is polyphyletic and that *Mertensia* resides as a distinct lineage deeply nested in the tribe Cynoglosseae (Weigend et al. 2010).

Problems with the tribal placement of *Mertensia* are exacerbated by uncertainty about its closest relatives. Several authors have suggested relationships to other borage genera on the basis of morphological characters. For example, Johnston (1924b) suggested *Anoplocaryum* Ledeb., a genus of about five species in Asia (Nasir 2006), was closely allied to *Mertensia*, with the two genera differing only in corolla shape and nutlet attachment. Popov (1953b) suggested the monotypic genus *Brachybotrys* Maxim. ex Oliv. was most closely related to *Mertensia*. Al-Shehbaz (1991) asserted *Trigonotis* was closely related to *Mertensia*, from which it differed in flower size, corolla shape, and nutlet shape. Recent molecular studies based on chloroplast and nuclear data have inferred close relationships with other genera. For example, *Mertensia* was placed as sister to *Lappula* Moench and *Hackelia* Vasey ex Beal in Olmstead and Ferguson (2001; Olmstead, pers. comm.). In contrast, Mansion et al. (2009) inferred a sister relationship to *Omphalodes* Mill. More recently, Weigend et al. (2010) found *Mertensia* to be sister to a clade consisting of *Eritrichium* Schrad. ex Gaudin, *Hackelia*, and *Lappula*, but this relationship had limited support. These varying placements of *Mertensia* in molecular phylogenetic studies may reflect the different taxon sampling of each study as well as the relatively few borage genera sampled. For example, the three studies that have sampled the most borage genera (Långström and Chase 2002; Mansion et al. 2009; Weigend et al. 2010) included no more than 40 of the approximately 150 genera within Boraginaceae (sensu APG 2003, 2009). The limited sampling of putative close relatives

TABLE 1. Tribal treatment of Boraginoideae. Subtribes within tribes are indicated in bold. All taxa discussed or sampled by authors have been included. Tribes circumscribed by earlier authors (e.g., Borageae, Cordieae, Ehretieae, Heliotropieae; Don 1838; Bentham 1876) are now typically recognized as subfamilies (e.g., Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae). Bentham (1876) treated Boragineae (= Anchuseae), Cynoglosseae, Eritrichieae, and Lithospermeae as subtribes of Borageae. For consistency, we have included them here as tribes within Boraginoideae.

Author	Asperugeae	Boragineae (= Anchuseae)	Buglosseae	Cerintheae	Craniospermeae	Cynoglosseae	Echieae	Echiochileae
Don 1838		Borageae <i>Borago</i> <i>Trachystemon</i>	Anchuseae <i>Achusa</i> <i>Bothriospermum</i> <i>Exharrhena</i> <i>Myosotis</i>					
		Rindereae <i>Mattia</i> <i>Rindera</i> <i>Solenanthus</i> <i>Trichodesma</i>	Cynoglosseae <i>Asperugo</i> <i>Cynoglossum</i> <i>Echinosperrum</i> <i>Omphalodes</i> <i>Rochelia</i>					
DeCandolle 1846		<i>Anchusa</i> <i>Borago</i> <i>Caryolopha</i> <i>Lycopsis</i> <i>Moritzia</i> <i>Nonnea</i> <i>Psilostemon</i> <i>Stomotechium</i> <i>Symphytum</i>		<i>Cerinthe</i>		<i>Amsinckia</i> <i>Antiphytum</i> <i>Asperugo</i> <i>Caccinia</i> <i>Craniospermum</i> <i>Cynoglossum</i> <i>Diploma</i> <i>Echinosperrum</i> <i>Eritrichium</i> <i>Gruvelia</i> <i>Heterocaryum</i> <i>Krynitzia</i> <i>Mattia</i> <i>Omphalodes</i> <i>Pectocarya</i> <i>Plagiobothrys</i> <i>Rindera</i> <i>Solenanthus</i> <i>Suchtelenia</i> <i>Trichodesma</i> <i>Actinocarya</i> <i>Cynoglossum</i> <i>Harpagonella</i> <i>Heliocarya</i> <i>Lindelofia</i> <i>Myosotidium</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Pectocarya</i> <i>Rindera</i> <i>Solenanthus</i> <i>Suchtelenia</i> <i>Thyrocarpus</i> <i>Trichodesma</i>	<i>Lobostemon</i> <i>Echium</i> <i>Macrotomia</i>	
Bentham 1876		<i>Alkanna</i> <i>Anchusa</i> <i>Borago</i> <i>Lycopsis</i> <i>Nonnea</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Trachystemon</i> <i>Trigonocaryum</i>						
Baillon 1890		<i>Alkanna</i> <i>Anchusa</i> <i>Borago</i> <i>Lycopsis</i> <i>Oskampia</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Trachystemon</i> <i>Trigonocaryum</i>				<i>Caccinia</i> <i>Cynoglossum</i> <i>Heliocarya</i> <i>Kuschakewiczia</i> <i>Lindelofia</i> <i>Myosotidium</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Pectocarya</i> <i>Rindera</i> <i>Selkirkia</i> <i>Solenanthus</i> <i>Suchtelenia</i> <i>Thyrocarpus</i> <i>Trichodesma</i>	<i>Echiochilon</i> <i>Echium</i> <i>Zwackia</i>	

Eritrichieae	Heliotropieae	Heterocaryeae	Lithospermeae	Myosotideae	Rochelieae	Symphyteae	Trichodesmeae	Trigonotideae
	<i>Canala</i>		<i>Batschia</i>			<i>Cerithe</i>		
	<i>Coldenia</i>		<i>Craniospermum</i>			<i>Colsmannia</i>		
	<i>Euploca</i>		<i>Echiochilon</i>			<i>Mertensia</i>		
	<i>Halgania</i>		<i>Echium</i>			<i>Onosma</i>		
	<i>Heliotropium</i>		<i>Lithospermum</i>			<i>Onosmodium</i>		
	<i>Messerschmidia</i>		<i>Lobostemon</i>			<i>Pulmonaria</i>		
	<i>Piptoclaina</i>		<i>Lycopsis</i>			<i>Stomotechium</i>		
	<i>Preslaea</i>		<i>Macromeria</i>			<i>Symphytum</i>		
	<i>Tiaridium</i>		<i>Moltkia</i>					
	<i>Tournefortia</i>		<i>Nonea</i>					
			<i>Stribila</i>					
			<i>Alkanna</i>					
			<i>Arnebia</i>					
			<i>Bothriospermum</i>					
			<i>Colsmannia</i>					
			<i>Lithospermum</i>					
			<i>Macromeria</i>					
			<i>Maharanga</i>					
			<i>Meratia</i>					
			<i>Mertensia</i>					
			<i>Moltkia</i>					
			<i>Myosotis</i>					
			<i>Onosma</i>					
			<i>Onosmodium</i>					
			<i>Pentalophus</i>					
			<i>Pumonia</i>					
			<i>Stenosolenium</i>					
<i>Amsinckia</i>			<i>Ancistrocarya</i>					
<i>Asperugo</i>			<i>Antiphytum</i>					
<i>Bothriospermum</i>			<i>Arnebia</i>					
<i>Craniospermum</i>			<i>Cerithe</i>					
<i>Echidiocarya</i>			<i>Echium</i>					
<i>Echinospermum</i>			<i>Lithospermum</i>					
<i>Eritrichium</i>			<i>Lobostemon</i>					
<i>Gastrocotyle</i>			<i>Macromeria</i>					
<i>Microula</i>			<i>Macrotomia</i>					
<i>Rochelia</i>			<i>Megacaryon</i>					
			<i>Mertensia</i>					
			<i>Moltkia</i>					
			<i>Moritzia</i>					
			<i>Myosotis</i>					
			<i>Onosma</i>					
			<i>Onosmodium</i>					
			<i>Sericostoma</i>					
			<i>Trigonotis</i>					
			<i>Zwackhia</i>					
<i>Actinocarya</i>			<i>Ancistrocarya</i>					
<i>Allocarya</i>			<i>Antiphytum</i>					
<i>Amsinckia</i>			<i>Arnebia</i>					
<i>Asperugo</i>			<i>Brachybotrys</i>					
<i>Bothriospermum</i>			<i>Cerithe</i>					
<i>Craniospermum</i>			<i>Cystistemon</i>					
<i>Cryptantha</i>			<i>Lithospermum</i>					
<i>Eremocarya</i>			<i>Macromeria</i>					
<i>Eritrichium</i>			<i>Macrotomia</i>					
<i>Gastrocotyle</i>			<i>Mertensia</i>					
<i>Lappula</i>			<i>Moltkia</i>					
<i>Microula</i>			<i>Moritzia</i>					
<i>Oreocarya</i>			<i>Myosotis</i>					
<i>Piptocalyx</i>			<i>Onosma</i>					
<i>Plagiobothrys</i>			<i>Onosmodium</i>					
<i>Rochelia</i>			<i>Sericostoma</i>					

(Continued)

TABLE 1. Continued.

Author	Asperugeae	Boragineae (= Anchuseae)	Buglosseae	Cerinthae	Craniospermeae	Cynoglosseae	Echieae	Echiochileae
Baillon 1890								
Gürke 1897		<i>Alkanna</i> <i>Anchusa</i> <i>Borago</i> <i>Lycopsis</i> <i>Nonnea</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Trachystemon</i> <i>Trigonocaryum</i>				<i>Actinocarya</i> <i>Brachybotrys</i> <i>Caccinia</i> <i>Cynoglossum</i> <i>Heliocarya</i> <i>Kuschakewiczia</i> <i>Lindelofia</i> <i>Myosotidium</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Pectocarya</i> <i>Rindera</i> <i>Selkirkia</i> <i>Solenanthus</i> <i>Suchtelenia</i> <i>Thyrocarpus</i> <i>Trichodesma</i> <i>Tysonia</i>	<i>Echiochilon</i> <i>Echium</i> <i>Lobostemon</i> <i>Megacaryon</i> <i>Zwackhia</i>	
Johnston 1924b		<i>Anchusa</i> <i>Borago</i> <i>Brunnera</i> <i>Caryolopha</i> <i>Elizaldia</i> <i>Lithodora</i> <i>Lycopsis</i> <i>Nonea</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Trachystemon</i> <i>Trigonocaryum</i>				<i>Actinocarya</i> <i>Bothriospermum</i> <i>Caccinia</i> <i>Cynoglossum</i> <i>Lacaitaea</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Rindera</i> <i>Suchtelenia</i> <i>Thyrocarpus</i> <i>Trichodesma</i> <i>Tysonia</i>		
Popov 1953b	<i>Asperugo</i>	<i>Anchusa</i> <i>Borago</i> <i>Brunnera</i> <i>Gastrocotyle</i> <i>Lycopsis</i> <i>Nonea</i> <i>Phyllocara</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Trachystemon</i> <i>Trigonocaryum</i>		<i>Cerithe</i>	<i>Craniospermum</i>	<i>Bilegnum</i> <i>Cynoglossum</i> <i>Lindelofia</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Paracynoglossum</i> <i>Rindera</i> <i>Solenanthus</i> <i>Trachelanthus</i>	<i>Echium</i>	
Riedl 1967, 1968		<i>Anchusa</i> <i>Brunnera</i> <i>Gastrocotyle</i> <i>Nonnea</i> <i>Phyllocara</i> <i>Symphytum</i>				<i>Cynoglossum</i> <i>Lindelofia</i> <i>Mattiastrum</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Rindera</i> <i>Solenanthus</i> <i>Trachelanthus</i>		

Eritrichieae	Heliotropieae	Heterocaryeae	Lithospermeae	Myosotideae	Rochelieae	Symphyteae	Trichodesmeae	Trigonotideae
<i>Sonnea</i>			<i>Thaumatocaryon</i>					
<i>Tretocarya</i>			<i>Trigonotis</i>					
<i>Allocarya</i>			<i>Ancistrocarya</i>					
<i>Amsinckia</i>			<i>Antiphytum</i>					
<i>Asperugo</i>			<i>Arnebia</i>					
<i>Bothriospermum</i>			<i>Cerinthe</i>					
<i>Craniospermum</i>			<i>Cystistemon</i>					
<i>Cryptantha</i>			<i>Lithospermum</i>					
<i>Eremocarya</i>			<i>Macromeria</i>					
<i>Eritrichium</i>			<i>Macrotomia</i>					
<i>Gastrocotyle</i>			<i>Mertensia</i>					
<i>Lappula</i>			<i>Moltkia</i>					
<i>Microula</i>			<i>Moritzia</i>					
<i>Oreocarya</i>			<i>Myosotis</i>					
<i>Piptocalyx</i>			<i>Onosma</i>					
<i>Plagiobothrys</i>			<i>Onosmodium</i>					
<i>Schistocaryum</i>			<i>Sericostoma</i>					
<i>Sonnea</i>			<i>Trigonotis</i>					
<i>Tretocarya</i>								
<i>Amblynotus</i>			<i>Aipyanthus</i>					
<i>Anoplocaryum</i>			<i>Alkana</i>					
<i>Asperugo</i>			<i>Ancistrocarya</i>					
<i>Brachybotrys</i>			<i>Arnebia</i>					
<i>Chionocharis</i>			<i>Cerinthe</i>					
<i>Craniospermum</i>			<i>Cystostemon</i>					
<i>Eritrichium</i>			<i>Echiochilon</i>					
<i>Hackelia</i>			<i>Echium</i>					
<i>Lappula</i>			<i>Lithospermum</i>					
<i>Megastoma</i>			<i>Macrotomia</i>					
<i>Mertensia</i>			<i>Maharanga</i>					
<i>Microcaryum</i>			<i>Megacaryon</i>					
<i>Microula</i>			<i>Moltkia</i>					
<i>Myosotidium</i>			<i>Onosma</i>					
<i>Myosotis</i>			<i>Sericostoma</i>					
<i>Oreogenia</i>			<i>Vaupelia</i>					
<i>Plagiobothrys</i>			<i>Zwackhia</i>					
<i>Rochelia</i>								
<i>Trigonotis</i>								
Cynoglossinae	<i>Heliotropium</i>	<i>Heterocaryum</i>	Eulithospermeae	<i>Myosotis</i>	<i>Rochelia</i>		<i>Caccinia</i>	
<i>Eritrichium</i>	<i>Tournefortia</i>		<i>Arnebia</i>				<i>Suchtelenia</i>	
<i>Hackelia</i>			<i>Lithospermum</i>				<i>Trichodesma</i>	
<i>Lappula</i>			<i>Macrotomia</i>					
<i>Lepechinella</i>			<i>Stenoselenium</i>					
<i>Stephanocaryum</i>								
<i>Tianschaniella</i>			Onosmeae					
			<i>Onosma</i>					
Lithosperminae			Alkanneae					
<i>Allocarya</i>			<i>Alkana</i>					
<i>Amblynotus</i>								
<i>Anoplocaryum</i>			Moltkieae					
			<i>Moltkia</i>					
			Bothriospermeae					
			<i>Bothriospermum</i>					
			Trigonotidinae					
			<i>Brachybotrys</i>					
			<i>Mertensia</i>					
			<i>Trigonotis</i>					
Echiochilinae			Moltkiinae	<i>Myosotis</i>			<i>Caccinia</i>	Antiphytinae
<i>Echiochilon</i>			<i>Moltkia</i>				<i>Heliocarya</i>	<i>Amblynotus</i>
							<i>Suchtelenia</i>	<i>Amphibologyne</i>
Pseudomertensiinae			Lithosperminae				<i>Trichodesma</i>	<i>Antiphytum</i>
<i>Pseudomertensia</i>			<i>Arnebia</i>					<i>Decalepidanthus</i>
			<i>Echioides</i>					<i>Sericostoma</i>
Eritrichiinae			<i>Lithospermum</i>					<i>Thaumatocaryon</i>
<i>Eritrichium</i>								
<i>Lappula</i>			Onosminae					Moltkiopsidinae
<i>Lepechinella</i>			<i>Cerinthe</i>					<i>Mairetis</i>
<i>Oreogenia</i>			<i>Chorianta</i>					<i>Moltkiopsis</i>

(Continued)

TABLE 1. Continued.

Author	Asperugeae	Boragineae (= Anchuseae)	Bugloseae	Ceritheae	Craniospermeae	Cynoglosseae	Echieae	Echiochileae
Riedl 1967, 1968								
Riedl 1997		<i>Anchusa</i> <i>Borago</i> <i>Elizalidia</i> <i>Gastrocotyle</i> <i>Nonea</i> <i>Pentaglottis</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Trachystemon</i>				<i>Actinocarya</i> <i>Antiotrema</i> <i>Cynoglossum</i> <i>Gyrocarpum</i> <i>Lindelofia</i> <i>Omphalodes</i> <i>Paracaryum</i> <i>Pardoglossum</i> <i>Pectocarya</i> <i>Rindera</i> <i>Solenanthus</i> <i>Thyrocarpus</i>		
Långström & Chase 2002		<i>Anchusa</i> <i>Borago</i> <i>Nonea</i> <i>Pentaglottis</i>				<i>Cryptantha</i> <i>Cynoglossum</i> <i>Lappula</i> <i>Lindelofia</i> <i>Myosotis</i> <i>Pardoglossum</i> <i>Trichodesma</i> <i>Trigonotis</i>		<i>Antiphytum</i> <i>Echiochilon</i> <i>Ogastemma</i> <i>Sericostoma</i>
Weigend et al. 2010		<i>Anchusa</i> <i>Borago</i> <i>Brunnera</i> <i>Cynoglottis</i> <i>Moritzia</i> <i>Pentaglottis</i> <i>Pulmonaria</i> <i>Symphytum</i> <i>Thaumatocaryon</i> <i>Trachystemon</i>				<i>Amsinckia</i> <i>Bothriospermum</i> <i>Caccinia</i> <i>Cryptantha</i> <i>Cynoglossum</i> <i>Eritrichium</i> <i>Hackelia</i> <i>Lappula</i> <i>Mertensia</i> <i>Myosotis</i> <i>Plagiobothrys</i> <i>Trichodesma</i> <i>Trigonotis</i>		<i>Echiochilon</i> <i>Ogastemma</i>

to *Mertensia* in phylogenetic studies to date (Olmstead and Ferguson 2001; Mansion et al. 2009; Weigend et al. 2010) makes further study essential to understand not only its evolutionary relationships but also its taxonomic placement.

The monophyly of *Mertensia* is uncertain. For example, Popov (1953a) called attention to six Himalayan species in *Mertensia* section *Oreocharis* that he considered to warrant possible generic segregation. Riedl (1967) later described *Pseudomertensia* Riedl to accommodate these species. No phylogenetic analyses, however, have tested whether this taxonomic change is consistent with evolutionary relationships. We question whether *Mertensia* is paraphyletic to *Pseudomertensia*.

To address the uncertainties and questions raised above, our primary objectives are to test the monophyly and phylogenetic relationships of *Mertensia*. Our approach is to reconstruct broadly the evolutionary relationships in Boraginaceae to infer major clades of subfamily Boraginoideae to address monophyly and relationships of *Mertensia* and provide insights on the tribal taxonomy of Boraginoideae. We apply DNA sequence data for approximately 70% of the genera

of Boraginaceae (sensu APG 2003, 2009) to provide the most comprehensive phylogeny reconstruction to date for the family.

MATERIALS AND METHODS

Taxon Sampling—To investigate the monophyly and closest relatives of *Mertensia*, we used PhyLoTA (Sanderson et al. 2008; <http://phylota.net/>) to assemble a phylogenetic dataset of DNA sequences available for Boraginaceae from GenBank. PhyLoTA returned a molecular data matrix consisting of 318 Boraginaceae accessions for 25 genes. We refined this initial matrix to (1) reduce the number of species within some monophyletic genera to fewer exemplars; and (2) maximize gene coverage and taxon sampling while limiting the amount of missing data. The refined matrix based on PhyLoTA had 171 accessions and markers from both nuclear and plastid DNA. The nuclear ribosomal 5.8S gene and internal transcribed spacers (= ITS region) and plastid *matK*, *ndhF*, *rbcl*, and *trnL-trnF* (including the *trnL* intron) were chosen for phylogeny reconstructions because they currently have the broadest and most complete sampling across Boraginaceae. Voucher specimens for sequences obtained from GenBank were not examined for verification of identification. In addition to the data assembled from GenBank, our matrix also consisted of data for 29 new accessions, representing eight species of

Eritrichieae	Heliotropieae	Heterocaryeae	Lithospermeae	Myosotideae	Rochelieae	Symphyteae	Trichodesmeae	Trigonotideae
<i>Tianschaniella</i>			<i>Onosma</i>					<i>Neatostema</i>
Heterocaryinae		Echiinae						Bothriosperminae
<i>Heterocaryum</i>			<i>Echium</i>					<i>Bothriospermum</i>
Rocheliinae			Alkanninae					Trigonotidinae
<i>Rochelia</i>			<i>Alkanna</i>					<i>Mertensia</i>
								<i>Trigonotis</i>
Asperuginae								
<i>Asperugo</i>								
<i>Amsinckia</i>			<i>Alkanna</i>	<i>Myosotis</i>			<i>Caccinia</i>	<i>Bothriospermum</i>
<i>Asperugo</i>			<i>Arnebia</i>				<i>Suchtelenia</i>	<i>Brachybotrys</i>
<i>Craniospermum</i>			<i>Buglossoides</i>				<i>Trichodesma</i>	<i>Mertensia</i>
<i>Cryptantha</i>			<i>Cerinthe</i>					<i>Moltkiopsis</i>
<i>Eritrichium</i>			<i>Cystostemon</i>					<i>Neatostema</i>
<i>Hackelia</i>			<i>Echiochilon</i>					<i>Ogastemma</i>
<i>Lappula</i>			<i>Echium</i>					<i>Omphalotrigonotis</i>
<i>Microula</i>			<i>Lasiarrhenum</i>					<i>Sericostoma</i>
<i>Myosotidium</i>			<i>Lithodora</i>					<i>Sinojohnstonia</i>
<i>Nesocaryum</i>			<i>Lithospermum</i>					<i>Trigonotis</i>
<i>Plagiobothrys</i>			<i>Lobostemon</i>					
<i>Rochelia</i>			<i>Macromeria</i>					
<i>Selkirkia</i>			<i>Maharanga</i>					
			<i>Moltkia</i>					
			<i>Onosma</i>					
			<i>Onosmodium</i>					
			<i>Buglossoides</i>					
			<i>Cerinthe</i>					
			<i>Cystostemon</i>					
			<i>Echium</i>					
			<i>Lithodora</i>					
			<i>Lithospermum</i>					
			<i>Lobostemon</i>					
			<i>Alkanna</i>					
			<i>Buglossoides</i>					
			<i>Cerinthe</i>					
			<i>Echium</i>					
			<i>Glandora</i>					
			<i>Lithospermum</i>					
			<i>Mairetis</i>					
			<i>Neatostema</i>					
			<i>Onosma</i>					
			<i>Podonosma</i>					

Mertensia and 21 species of *Amblynotus* I. M. Johnst., *Amsinckia* Lehm., *Asperugo* L., *Brachybotrys*, *Eritrichium*, *Hackelia*, *Lappula*, *Myosotis* L., *Omphalodes*, *Pseudomertensia*, and *Trigonotis*. Material of *Anoplocaryum* was not available, and, therefore, we are unable currently to address questions about relationships between it and *Mertensia*. Samples of new accessions included in this study were obtained through both field collections and herbaria (Appendix 1). Our final molecular data matrix consisted of 197 accessions representing 106 genera and 196 species of Boraginaceae (Appendix 1). Each of the six subfamilies of Boraginaceae (Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, Hydrophyloideae, and Lennooideae; sensu APG 2003, 2009) and the tribes of subfamily Boraginoideae were represented in the matrix.

Outgroups were chosen from among the Solanales, Lamiales, Gentianales, Garryales, and Vahliaceae based on inferred relationships of Boraginaceae in previous phylogenetic analyses of asterids (Savolainen et al. 2000; Bremer et al. 2002, 2004; Martínez-Millán 2010). Taxa selected include: *Solanum dulcamara* L. and *Nicotiana tabacum* L. from Solanales; *Acanthus ilicifolius* L., *Verbena rigida* Spreng., and *Lamium purpureum* L. from Lamiales; *Cinchona calisaya* Wedd., *Rhabdadenia biflora* (Jacq.) Müll. Arg., and *Apocynum cannabinum* L. from Gentianales; *Garrya elliptica* Douglas ex Lindl. and *Eucommia ulmoides* Oliv. from Garryales; and *Vahlia capensis* (L. f.) Thunb. from Vahliaceae (Appendix 1).

DNA Extraction, Amplification, and Sequencing—For new sequences generated for this study, total genomic DNA was isolated from approximately 10 mg silica-gel-dried or herbarium specimen leaf material using a

modified CTAB protocol (Doyle and Doyle 1987). We used an ammonium acetate precipitation step in the extraction procedure for cases in which secondary compounds appeared to affect negatively the quality of the DNA extraction (Cullings 1992).

The ITS region and three chloroplast markers (*matK*, *ndhF*, *trnL-trnF*) were amplified by PCR (primers used in this study are listed in Table 2). The PCR protocol for nuclear and plastid markers consisted of a 25- μ L sample containing 13.8 μ L sterile H₂O, 2.5 μ L 10 \times Thermopol reaction buffer with 20 mM Mg²⁺ (New England Biolabs, Ipswich, Massachusetts), 2.5 μ L 5/5 μ M primer, 2.5 μ L 3/5 μ M primer, 1.5 μ L 2.5mM dNTP, 0.2 μ L 5 U/ μ L *Taq* polymerase (New England Biolabs) and 2.0 μ L diluted DNA template of an unknown concentration. The PCR conditions in a Biometra® thermocycler (Whatman, Göttingen, Germany) for the ITS region included initial denaturation at 94°C for 2 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 sec, with a final extension at 72°C for 10 min. The PCR conditions for plastid markers *matK* and *ndhF* included initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 4 min, with a final extension at 72°C for 7 min. For plastid marker *trnL-trnF* and *trnL* intron, PCR conditions included initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 50.6°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 5 min. All PCR products were visualized by 1% agarose gel electrophoresis and purified using ExoSAP-IT (USB Corp., Cleveland, Ohio).

TABLE 2. Primers used for PCR and cycle sequencing of plastid and nuclear regions. References are given for previously published primer sequences; sequences for new primers developed for this study are noted below.

Region	Primers used	Reference/primer sequence (5'-3')
<i>matK</i>	matK710F	Plunkett et al. 1996
	matK1470R	Johnson and Soltis 1994
	matKmer1F	GAAAACKARYCTTCTTTTCAA
	matKmer1R	YTCMGTGGATTAAACCGTA
	matKmer2R	TGCACACAGCTTTCYCTATG
<i>ndhF</i>	ndhF1F	Olmstead and Sweere 1994
	ndhF972R	Olmstead and Sweere 1994
	ndhF803F	Olmstead and Sweere 1994
	ndhF972F	Olmstead and Sweere 1994
	ndhF2110R	Olmstead and Sweere 1994
<i>trnL-trnF</i>	trnLUAAR (TabC)	Taberlet et al. 1991
	trnFUAAAR (TabF)	Taberlet et al. 1991
ITS	N-nc18s10	Wen and Zimmer 1996
	C26A	Wen and Zimmer 1996

The 10- μ L cycle sequencing reactions contained between 3.33 and 6.33 μ L sterile H₂O, 0.67 μ L 5 μ M primer, 1.0 μ L 5 \times sequencing buffer (Applied Biosystems, Foster City, California), 1.0 μ L BigDye Terminator ver.3.1 (Applied Biosystems) and between 1.0 and 4.0 μ L cleaned PCR product. Cycle sequencing reactions included 25 cycles of 96°C for 10 sec, 50°C for 5 sec, with a final extension at 60°C for 4 min. Cycle sequence products were purified using 75% isopropanol precipitation, and DNA sequences were visualized on a 48-capillary 3730 DNA Analyzer (Applied Biosystems). Contigs were assembled and edited using Sequencher™ ver. 4.6 (Gene Codes Corp., Ann Arbor, Michigan).

Alignment and Phylogenetic Analysis—Initial sequence alignments for each gene region were prepared using MUSCLE ver. 3.8.31 (Edgar 2004) using the default settings. Subsequent alignments were manually adjusted using Se-Al ver. 2.0a11 (Rambaut 1996). For the ITS region, taxa were initially divided into groups based on sequence similarity, and separate alignments were made for each group. Subsequently, these groups were iteratively aligned with one another using the profile-to-profile alignment method implemented in MUSCLE ver. 3.8.31 (Edgar 2004). This approach of aligning sequences has been shown to be successful in other large-scale phylogenetic studies (e.g. Smith and Donoghue 2008; Tank and Donoghue 2010; Smith et al. 2011). For each molecular marker, regions that had ambiguous alignments were excluded from phylogenetic analyses. Concatenation of aligned gene regions and conversion of file types (e.g. FASTA to PHYLIP) were performed in Phyutility ver. 2.2 (Smith and Dunn 2008). All nuclear and plastid sequences generated in this study were deposited in GenBank (see Appendix 1) and alignments were deposited in TreeBASE (study number S11979). Due to the inclusion of partial sequences, there were 25.7% missing data in the *matK* dataset, 41.0% in the *ndhF* dataset, 13.8% in the *rbcl* dataset, 31.7% in the *trnL-trnF* dataset, 29.4% in the ITS dataset, 60.1% in the overall plastid dataset, and 60.6% in the overall combined dataset.

Although the inclusion of partial sequence data in large datasets may limit phylogenetic inference through uncertainty (e.g. Galtier and Daubin 2008), it is still possible to infer a large fraction of phylogenetic relationships among taxa (Sanderson et al. 2010).

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were performed for three datasets: (1) nuclear data; (2) concatenated plastid data (herein plastid dataset); and (3) combined nuclear and plastid data (herein combined dataset). The partition homogeneity test (Farris et al. 1995), implemented in PAUP* ver. 4.0b10 (Swofford 2002), was used to test for topological incongruence between the nuclear and plastid datasets. To reduce potential incongruence, non-overlapping taxa from the nuclear and plastid datasets were removed prior to the test. The analysis applied 100 test replicates, each with 100 random order entry heuristic searches and one tree saved per replicate.

The MP analyses were performed using PAUP* ver. 4.0b10 (Swofford 2002). All characters were weighted equally, and gaps were treated as missing data. Heuristic MP searches were performed using random taxon addition with branch swapping by tree bisection-reconnection (TBR), saving one tree per replicate. One thousand replicated searches were conducted to search for islands of equally most parsimonious trees. For analyses that did not run to completion, MaxTrees was set to 50,000. Bootstrap analysis (Felsenstein 1985) was conducted to examine support for clades. Bootstrap analysis used 1,000 random taxon addition replicates and TBR branch swapping. For bootstrap searches that did not run to completion, MaxTrees was set at 10,000; otherwise, MaxTrees automatically increased by 100 until the searches were completed.

For ML and BI analyses, models of molecular evolution that best fit the data were tested in jModeltest (Guindon and Gascuel 2003; Posada 2008), using hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC). For the nuclear and combined datasets, hLRT and AIC selected the GTR + I + G model. For the plastid dataset, TVM + I + G was selected by both hLRT and AIC. However, the TVM model cannot be implemented in RAxML (Stamatakis 2006; Stamatakis et al. 2008) or MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The GTR + I + G model, which is similar to TVM + I + G model, but specifies eight parameters rather than six (Posada and Crandall 2001), was implemented in ML and BI searches of the nuclear, plastid, and combined datasets, as well as in alternative phylogenetic hypothesis testing.

The ML analyses were performed using RAxML ver. 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) with each analysis consisting of 1,000 rapid bootstrap replicates. Each plastid region was treated as a separate partition. The *trnL-trnF* intergenic spacer and *trnL* intron in the plastid and combined datasets were treated as one partition. Every bootstrap tree generated by the rapid bootstrap analyses was used as a starting tree for all ML searches. Trees with the highest ML scores were selected. Bootstrap values were summarized with Phyutility (Smith and Dunn 2008).

The BI analyses were conducted using MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) treating each gene region as a separate partition and employing the substitution models as noted in Table 3. The plastid dataset was analyzed with separate models for each chloroplast partition. As in the ML analysis, the *trnL-trnF* intergenic spacer and *trnL* intron were treated as one partition. Analyses were initiated using random starting trees and two runs for 35,000,000 generations using four Markov Chains (three incrementally heated, one cold)

TABLE 3. Results of the Shimodaira-Hasegawa (SH) tests and Approximately Unbiased (AU) tests of alternate phylogenetic hypotheses. *p* values are based on analyses with distributions of candidate trees that included 10,000 trees sampled from the posterior distribution of BI searches using the combined molecular dataset. Asterisks next to *p* values indicate significant values (*p* = 0.05).

Tested Hypothesis	Rationale	-ln L score	SH test <i>p</i> value	AU test <i>p</i> value
Unconstrained full analysis		70,402.839865	NA	NA
Monophyly of <i>Mertensia</i> and <i>Pseudomertensia</i>	<i>Pseudomertensia</i> treated as part of <i>Mertensia</i> suggested by Bentham (1876), Clark (1883), and Popov (1953a)	70,465.978705	0.310	0.029
Monophyly of <i>Mertensia</i> and <i>Brachybotrys</i>	Close relationship suggested by Popov (1953b)	70,436.290989	0.733	0.206
Monophyly of <i>Mertensia</i> and <i>Trigonotis</i>	Close relationship suggested by Riedl (1967, 1968), and Al-Shehbaz (1991)	70,514.935104	0.019	< 0.001
Monophyly of <i>Mertensia</i> and <i>Omphalodes</i>	Close relationship suggested by Mansion et al. (2009)	70,556.733493	0.001	0.002
Monophyly of <i>Mertensia</i> and <i>Hackelia</i> + <i>Lappula</i>	Sister relationship inferred by Olmstead and Ferguson (2001)	70,646.163436	< 0.001	< 0.001
Monophyly of <i>Mertensia</i> and <i>Hackelia</i> + <i>Lappula</i> + <i>Eritrichium</i>	Sister relationship inferred by Weigend et al. (2010)	70,751.036242	< 0.001	< 0.001

that were sampled every 1,000 generations. The number of trees required to reach stationarity was determined using Tracer ver. 1.5.0 (Rambaut and Drummond 2009). The first 10,000,000 trees were discarded as burn-in and excluded from the analysis. The remaining trees were used to calculate a 50% majority rule consensus tree to represent posterior probabilities for each node. Bootstrap and posterior probability values are defined in the text as strong (> 90), moderate (60–90), and weak (< 60).

Alternative Phylogenetic Hypotheses—Alternative hypotheses for relationships of *Mertensia* were tested using the Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa 1999) and the approximately unbiased (AU; Shimodaira 2002) tests. The SH test is biased in terms of tree selection, but performs well in reducing the number of type-1 errors (Shimodaira 2002). In contrast, the AU test reduces the bias present in the SH test, but under certain circumstances, may not perform well in reducing the number of type-1 errors (Shimodaira 2002). For SH and AU tests, previous hypotheses of relationships were modeled using ML searches based on the combined dataset using topological constraints (Table 3). The topologies were constrained to force (1) the monophyly of *Mertensia* and *Pseudomertensia*, (2) the monophyly of *Mertensia* and *Brachybotrys*, (3) the monophyly of *Mertensia* and *Trigonotis*, (4) the monophyly of *Mertensia* and *Omphalodes*, (5) the monophyly of *Mertensia*, *Hackelia*, and *Lappula*, and (6) the monophyly of *Mertensia*, *Hackelia*, *Lappula*, and *Eritrichium*. Searches consisted of 250 bootstrap replicates with the GTR + G substitution model in RAxML ver. 7.04 (Stamatakis 2006; Stamatakis et al. 2008). A distribution of candidate trees was constructed by combining the ML tree, constrained tree, and 10,000 trees sampled from the posterior probability distribution generated from BI. Likelihood scores of the best ML tree and BI trees were compared to those of the constrained trees with the ML score of each constrained tree using the SH and AU tests with the resampling-estimated log-likelihood (RELL) optimization in CONSEL (Shimodaira and Hasegawa 2001). Alternative hypotheses were rejected if they were significantly worse than the unconstrained tree ($p < 0.05$).

Scanning Electron Microscopy—Representative specimens of *Mertensia maritima* (L.) Gray (voucher: *Kines s. n.* [WS]), *Pseudomertensia echioides* Riedl (voucher: *Stewart 26343* [UC]), and *Asperugo procumbens* L. (voucher: *Hafercamp 68* [WS]) were sampled for morphological study of outlet characteristics using scanning electron microscopy (SEM). Nutlets were obtained from herbarium specimens, mounted directly on metal stubs without prior treatment and sputter coated with gold. Specimens were examined and photographed using a Hitachi S-570 SEM.

RESULTS

Phylogenetic Data—The combined matrix consisted of 6,888 characters, including 598 nuclear and 6,290 plastid characters (Table 4). The plastid dataset provided far more parsimony-informative characters than the nuclear dataset (Table 4). Models of molecular evolution selected by AIC were applied to ML and BI analyses because these estimations of log likelihoods (-lnL) were better than or equal to those resulting from the hLRT criterion (Table 4).

Nuclear Phylogenetic Reconstructions—The ML analysis of the nuclear dataset resulted in a single most likely tree (-lnL score = 15,857.466586; Fig. 1; Table 4). The majority rule consensus tree (not shown) generated by BI analysis was similar to the ML topology, except that in the BI results some of the deeper nodes in subfamily Boraginoideae were not resolved. The MP analysis resulted in nine equally parsimonious trees of 3,243 steps (Table 4) and differed from the topologies generated in the ML and BI analyses in subfamilial relationships in Boraginaceae and in the placement of the tribe Cynoglosseae in subfamily Boraginoideae. Cynoglosseae were monophyletic and sister to Lithospermeae in topologies generated from the MP analysis; however, Cynoglosseae was paraphyletic to the remaining tribes within Boraginoideae in topologies from the ML and BI analyses, but with little support.

Plastid Phylogenetic Reconstructions—The ML analysis of the plastid dataset resulted in a single most likely tree (-lnL score = 52,633.597017; Fig. 2; Table 4). The MP analysis of the plastid dataset resulted in 82 most parsimonious trees of 7,836 steps (Table 4). Searches using the plastid dataset with MP, ML, and BI analyses recovered similar topologies, except that MP topologies differed in the placement of some subfamilies of Boraginaceae. The MP topologies recovered subfamily Hydrophyllloideae as monophyletic, and ML and BI analyses recovered Hydrophyllloideae as paraphyletic to Ehretioideae, Lennooideae, Cordioideae, and Heliotropioideae.

Combined Molecular Phylogenetic Reconstructions—Results from the partition homogeneity test showed that topologies from the nuclear and plastid datasets were significantly incongruent ($p = 0.01$). Incongruence between the nuclear and plastid dataset was largely centered in the placement of subfamilies Cordioideae, Ehretioideae, Heliotropioideae, Hydrophyllloideae, and Lennooideae, and in the placement of tribe Cynoglosseae in subfamily Boraginoideae; however, many of the relevant nodes in the nuclear dataset had limited support. The results obtained from analyses of the combined dataset provided greater topological resolution and more robust branch support than analyses of either the independent nuclear or plastid datasets.

The MP analysis of the combined dataset resulted in eight equally most parsimonious trees of 11,205 steps (Table 4; strict consensus tree in Fig. 3). ML analysis resulted in one most likely tree (-lnL score = 69,451.131176; Fig. 4; Table 4). Limited

TABLE 4. Summary of phylogenetic datasets, likelihood model parameters, and statistics from MP and ML searches. MP and ML analyses were not conducted for individual plastid markers (n/a).

	IIS	matK	ndhF	rbcL	trnL-trnF	plastid	combined
Number of accessions sampled (of 208)	180	115	114	73	154	183	208
Number of characters (after alignment)	598	1,825	2,096	1,405	964	6,290	6,888
Parsimony							
Variable characters	101	n/a	n/a	n/a	n/a	1,000	1,101
Parsimony-informative characters	332	n/a	n/a	n/a	n/a	1,940	2,272
Max tree setting	50,000	n/a	n/a	n/a	n/a	50,000	50,000
Number of MP trees	9	n/a	n/a	n/a	n/a	82	8
Length of MP tree	3,243	n/a	n/a	n/a	n/a	7,836	11,205
Likelihood							
Model of molecular evolution (hLRT criterion)	GTR + I + G	HKY	HKY	GTR + I + G	GTR + I + G	TVM + I + G	GTR + I + G
log likelihood score	18,409.0022	20,894.4551	20,138.8663	8,207.6253	13,016.1054	59,196.0078	76,048.5078
Model of molecular evolution (AIC criterion)	GTR + I + G	TVM + G	GTR + I + G	TVM + I + G	TVM + G	TVM + I + G	GTR + I + G
log likelihood score	16,099.6067	17,273.2317	16,325.4414	7,478.4029	10,162.259	118,410.0156	152,117.0156
Number of ML trees	1	n/a	n/a	n/a	n/a	1	1
Likelihood score (-lnL)	15,857.466586	n/a	n/a	n/a	n/a	52,633.597017	69,451.131176



FIG. 1. Best phylogenetic tree of Boraginaceae based on the maximum likelihood analysis of the nuclear dataset. Outgroup taxa and clades that correspond to subfamilial taxa are indicated. Numbers above branches indicate bootstrap proportions above 50%. A. Basal grade of Boraginaceae. B. Boraginoideae.

support was recovered for several of the deeper nodes in both the ML and MP topologies (Figs. 3, 4). The BI analyses converged on similar log likelihood values after 6,000,000 generations, and the resulting majority rule consensus topology (not shown) was congruent with the most likely topology generated from the ML analysis, but less resolved.

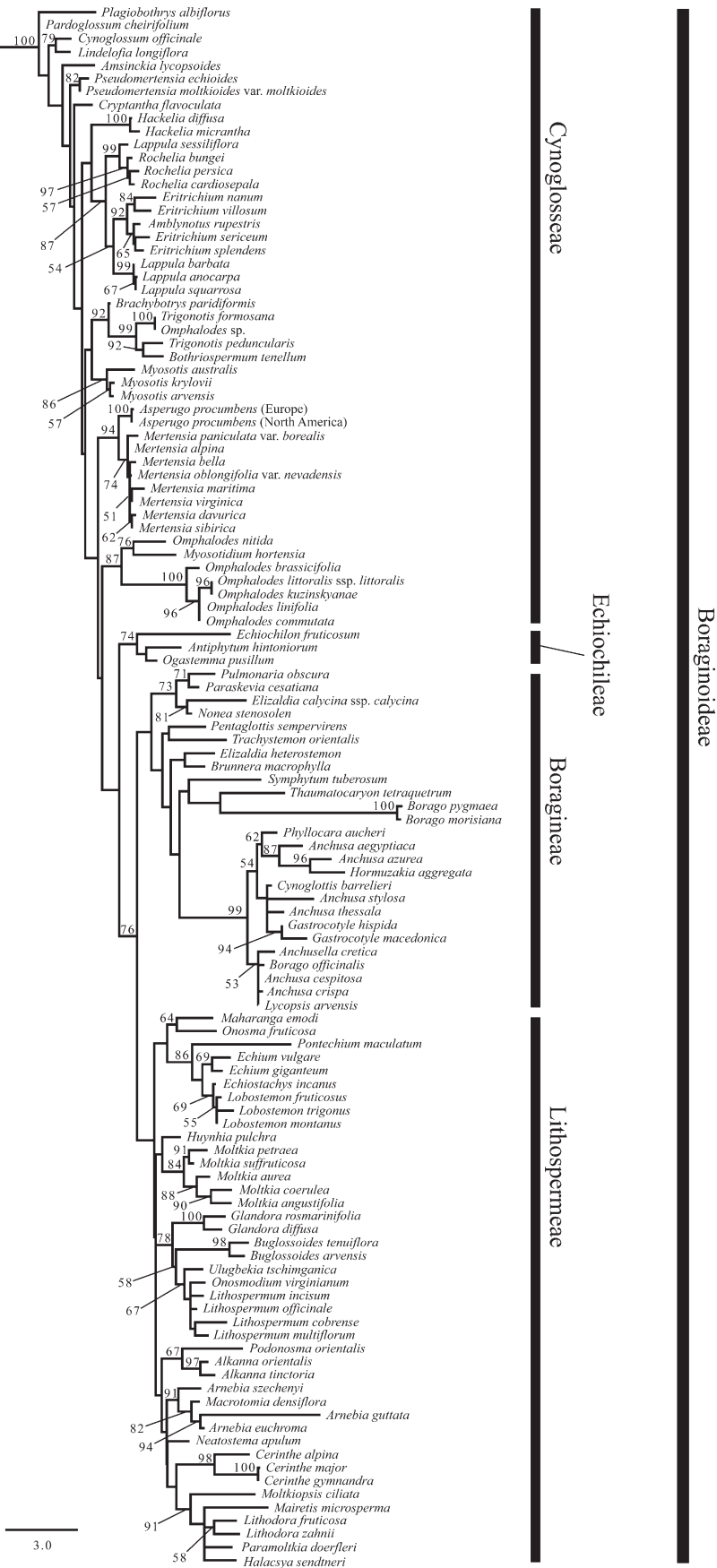
The MP, ML, and BI analyses of the combined dataset recovered several well-supported clades that were consistent across analyses (Figs. 3, 4). Each recovered a strongly supported, monophyletic Boraginaceae. The ML topology (Fig. 4) has in Boraginaceae five moderate to well supported clades that corresponded to five of the six subfamilies recognized in recent phylogenetic studies: Boraginoideae, Cordioideae, Heliotropioideae, Hydrophyllloideae, and Lennooideae. The sixth subfamily, Ehretioideae, was recovered as paraphyletic to subfamily Lennooideae in the ML and BI results (Fig. 4; in the MP results separate clades of Ehretioideae were part of a polytomy that also included other lineages, see Fig. 3). The paraphyly of Ehretioideae to Lennooideae was also recovered in analyses of the independent plastid dataset (Fig. 2). Notably, independent analyses of the nuclear dataset recovered Ehretioideae as monophyletic and placed Lennooideae as sister to subfamily Cordioideae (Fig. 1). In analyses of all three datasets, however, the placement of Lennooideae had

limited support (Figs. 1–4). In addition to the placement of Lennooideae, some MP topologies (not shown) differed from the ML and BI results in the placement of subfamily Cordioideae as sister to Heliotropioideae + Ehretioideae + Hydrophyllloideae. In both ML and BI topologies, Cordioideae was placed as sister to the Lennooideae + Ehretioideae clade (Fig. 4).

Subfamily Boraginoideae were recovered as monophyletic, with strong support for the four clades that corresponded to tribes recognized in recent molecular phylogenetic studies: Boragineae, Cynoglosseae, Echiochileae, and Lithospermeae (Fig. 4). Boragineae and Lithospermeae were sister clades, with Cynoglosseae as their closest relatives. Echiochileae were recovered as sister to the other three tribes. Relationships among some of the deepest clades within tribes Boragineae, Cynoglosseae, and Lithospermeae were poorly supported; however, several more terminal clades had strong support. The MP, ML, and BI analyses of the combined dataset recovered *Codon royenii* L. as sister to subfamily Boraginoideae, with strong support for this sister relationship (Fig. 4).

Analyses of the combined dataset recovered a strongly supported, monophyletic *Mertensia*, excluding *Pseudomertensia*, as a lineage of Cynoglosseae (Figs. 3, 4). *Mertensia* was strongly supported to be sister to the monotypic genus *Asperugo*

Figure 1A



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FIG. 1. (continued)

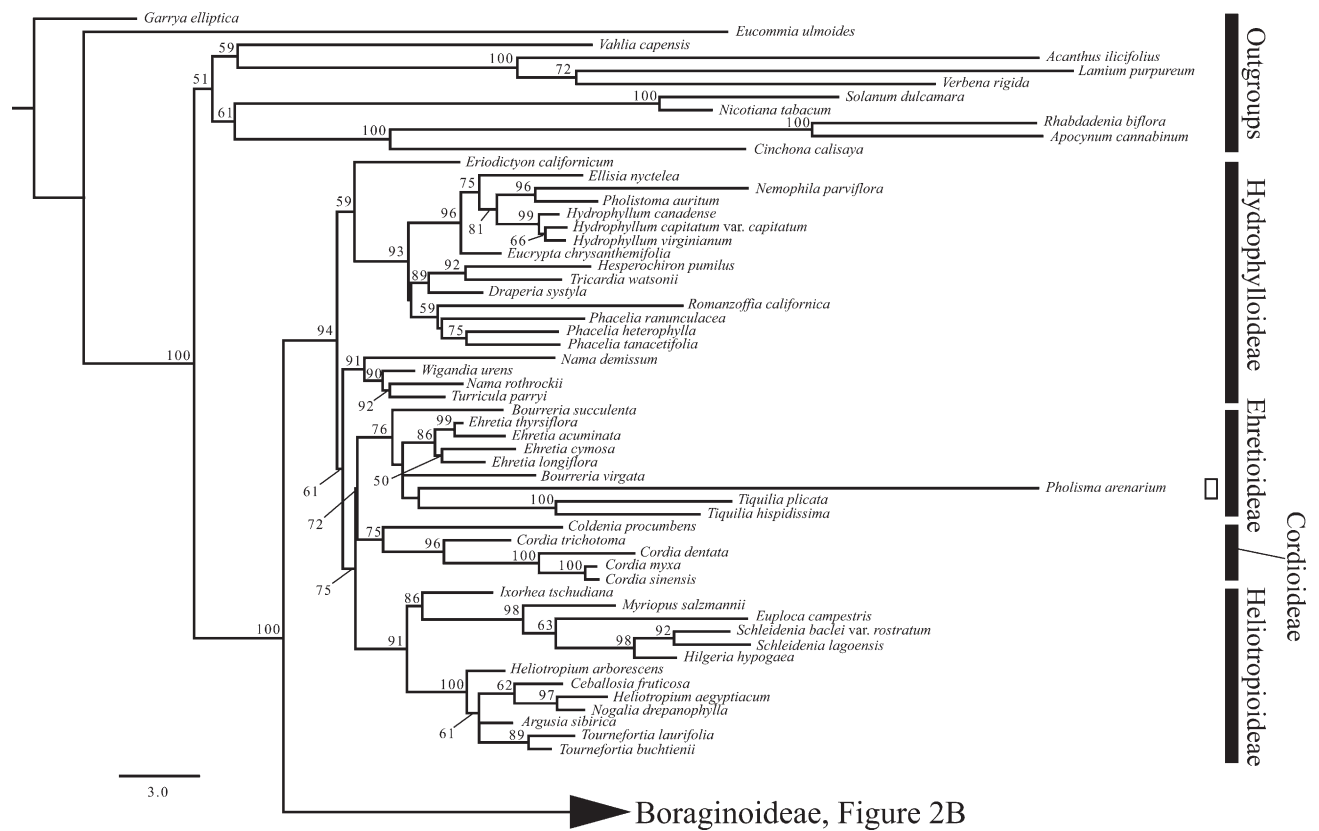


FIG. 2. Best phylogenetic tree of Boraginaceae based on the maximum likelihood analysis of the plastid dataset. Outgroup taxa and clades that correspond to subfamilial taxa are indicated. Open box denotes Lennooideae nested in Ehretioideae. Numbers above branches indicate bootstrap proportions above 50%. A. Basal grade of Boraginaceae. B. Boraginoideae.

(Figs. 3, 4). Phylogenetic analyses of both the independent nuclear and plastid datasets also recovered strong support for the sister relationship of *Mertensia* + *Asperugo* (Figs. 1, 2). *Pseudomertensia* was recovered in analyses of the combined and independent nuclear and plastid datasets as more closely related to *Myosotis* than to *Mertensia*, and there was strong support for this sister relationship (Figs. 1–4).

Alternative Phylogenetic Hypotheses—Previous hypotheses of relationships of *Mertensia* were modeled using topological constraints (Table 3). The six topologies constrained to model alternative hypotheses all had less optimal $-\ln L$ scores than the unconstrained tree (Table 3). Topologies that forced the monophyly of *Mertensia* and *Trigonotis*; *Mertensia* and *Omphalodes*; *Mertensia*, *Hackelia*, and *Lappula*; and *Mertensia*, *Hackelia*, *Lappula*, and *Eritrichium* were rejected by both the SH test and the AU test (Table 3). Both SH and AU tests failed to reject hypotheses of monophyly for *Mertensia* and *Brachybotrys*. The SH test did not reject constraint topologies forcing the monophyly of *Mertensia* and *Pseudomertensia*; however, this hypothesis of monophyly was rejected by the AU test (Table 3).

DISCUSSION

Monophyly of *Mertensia*—Results from our phylogenetic analyses of the nuclear, plastid, and combined molecular datasets recovered a strongly supported, monophyletic *Mertensia* (Figs. 1–4) that excludes sampled *Pseudomertensia*. Our phylogenetic sampling included two of the six species of *Pseudomertensia* that had been previously circumscribed

as part of *Mertensia* (Bentham 1876; Clark 1883; Popov 1953a). *Pseudomertensia echioides* and *P. moltkioides* (Royle ex Benth.) Kazmi var. *moltkioides* were recovered as monophyletic and more closely related to *Myosotis* than to *Mertensia* in our analyses. We tested the strength of our results by constraining *Mertensia* and *Pseudomertensia* to be monophyletic, and the best topology resulting under this constraint (tree not shown) was rejected by the AU test, but not by the SH test (Table 3). Conservatism of the SH test (Buckley 2002) and/or the amount of missing data in our datasets may account for the SH test result.

Relationships of *Pseudomertensia*—There has been disagreement among authors about the placement of *Pseudomertensia*. Initially, *Pseudomertensia* was treated as subgenus *Oreocharis* of *Lithospermum* L. (Decaisne 1844) and subsequently placed in *Eritrichium* section *Oreocharis* (DeCandolle 1846). Bentham (1876) and Clark (1883) suggested that the exerted styles of these Himalayan species more closely resembled *Mertensia* than *Lithospermum*, *Eritrichium*, or *Trigonotis* and placed them in *Mertensia*. Popov (1953a) called attention to differences between *Mertensia* and these Himalayan taxa but treated them as section *Oreocharis* in *Mertensia*. Riedl (1967) elevated the Himalayan taxa to *Pseudomertensia*, emphasizing that the two genera differed in characteristics of the gynobase and positioning of nutlets (and subsequent form of the attachment scar on the nutlet). Our observations indicate that *Mertensia* has a distinctly conical gynobase (Fig. 5A), but it is shorter and more pyramidal in *Pseudomertensia* (Fig. 5B). Nutlets of *Mertensia* have a minute, poorly defined, and raised attachment scar, located in a suprabasal position below a winged

Figure 2A

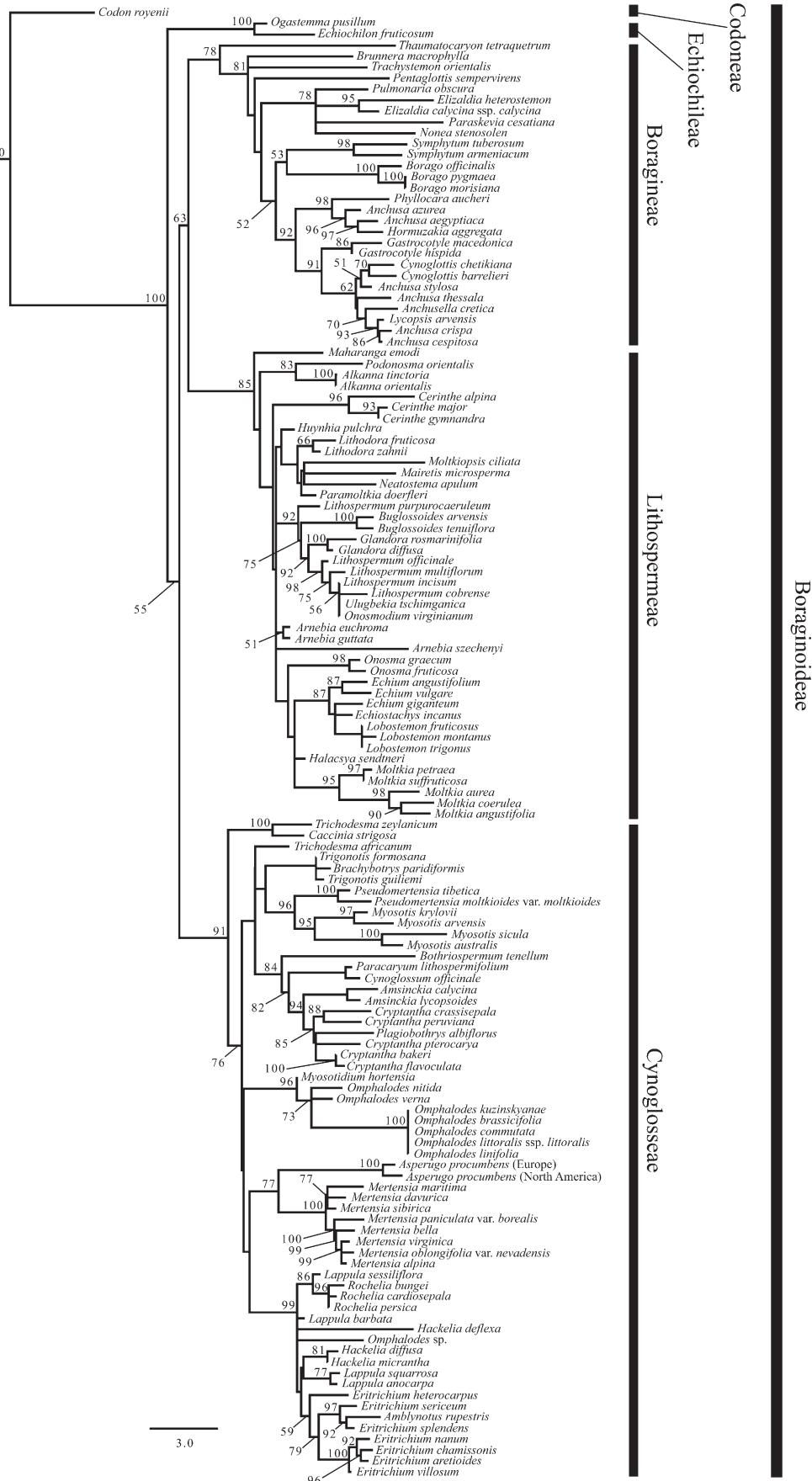


FIG. 2. (continued)

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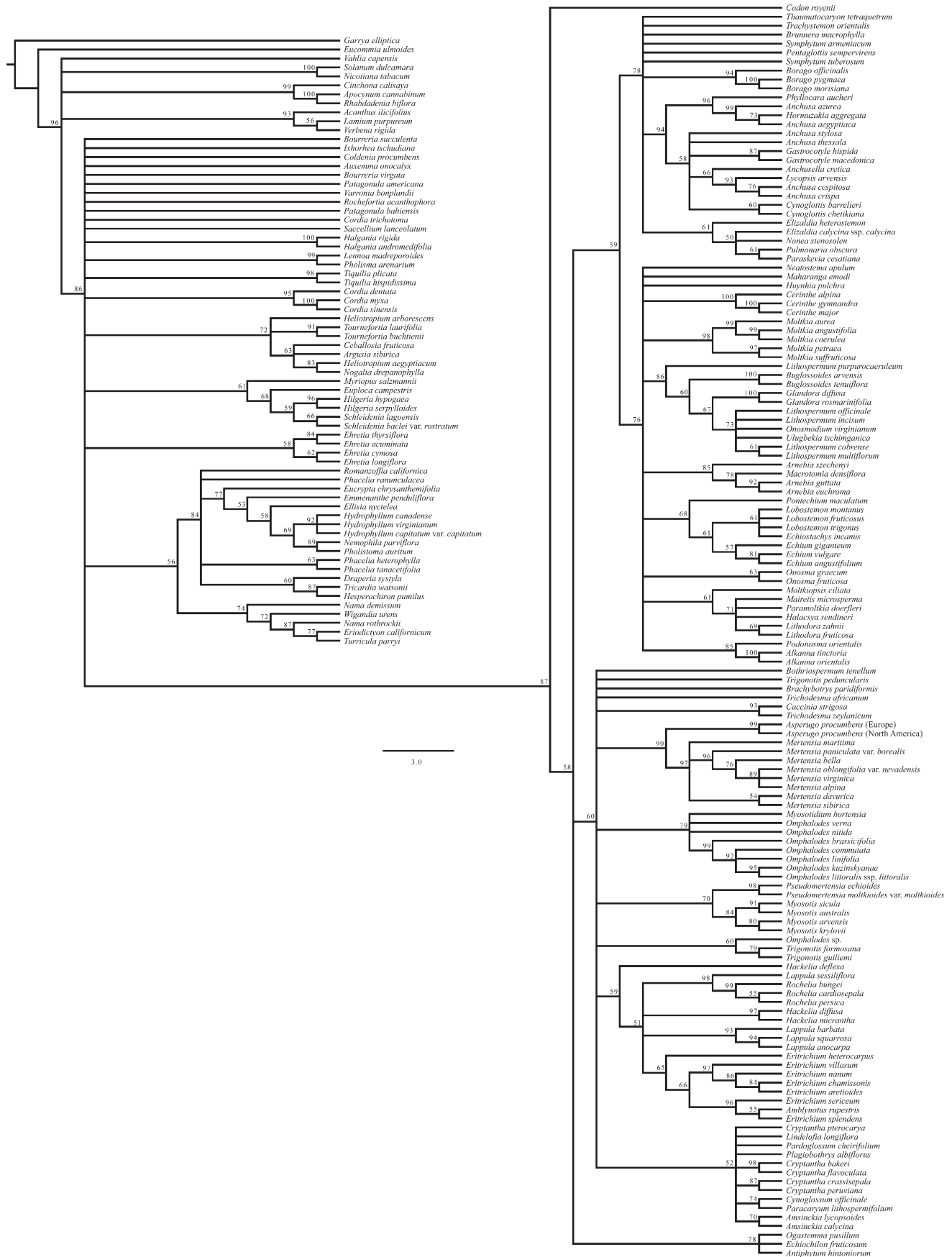


FIG. 3. Strict consensus tree of the equally most parsimonious trees from maximum parsimony analysis of the combined nuclear and plastid datasets for Boraginaceae. Numbers above branches indicate bootstrap proportions above 50%.

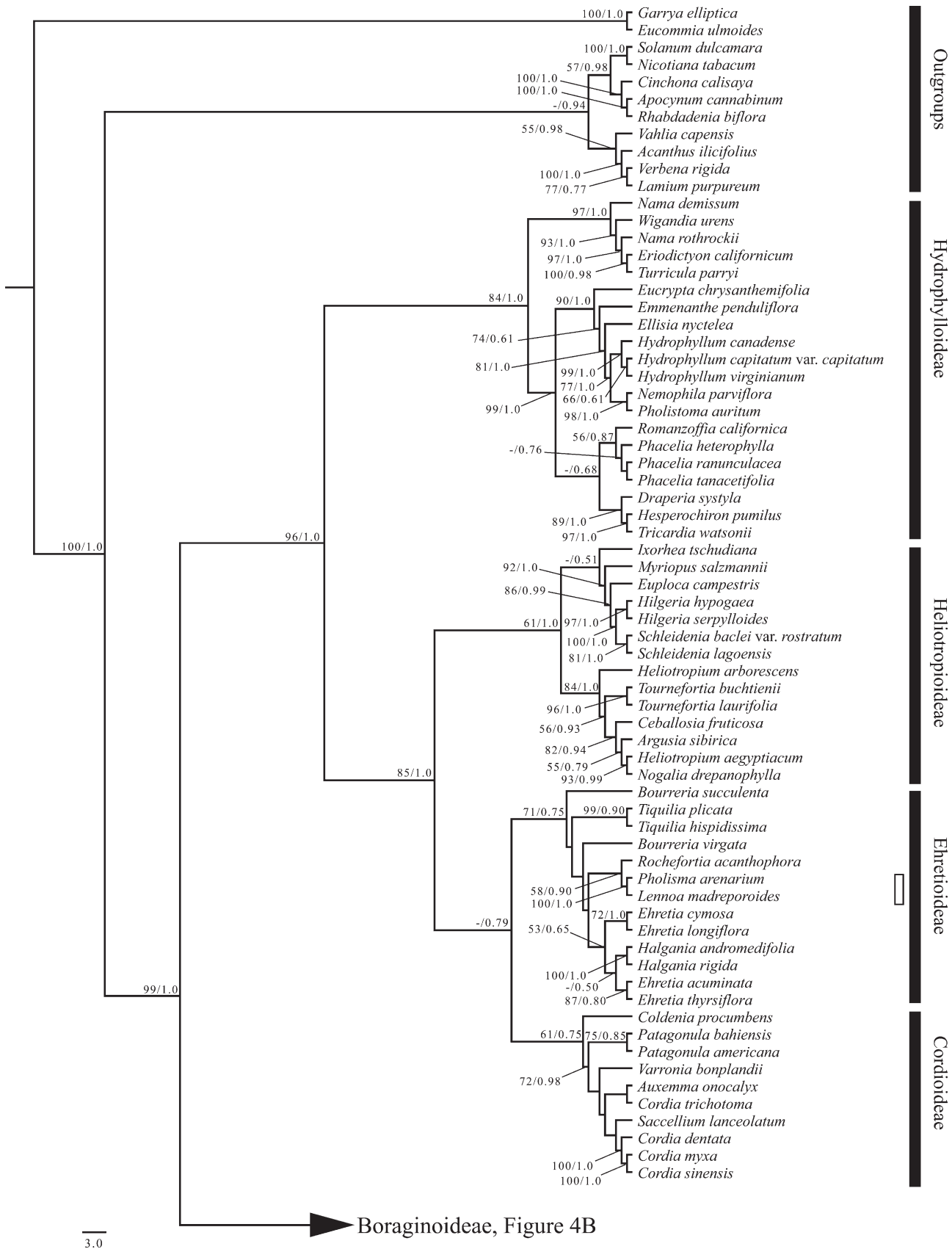


FIG. 4. Best phylogenetic tree of Boraginaceae based on the maximum likelihood analysis of the combined nuclear and plastid dataset. Numbers above branches indicate ML bootstrap proportions/BI posterior probabilities, respectively. Patterned boxes refer to tribal classifications of Johnston (= J; 1924b), Popov (= P; 1953b), and Riedl (= R; 1997). Solid lines identify subfamilial taxa and outgroups. Open box denotes Lennooideae nested in Ehretioideae. A. Basal grade of Boraginaceae. B. Boraginoideae tribes Codoneae, Echiochileae, Lithospermeae, and Boragineae. C. Boraginoideae tribe Cynoglosseae.

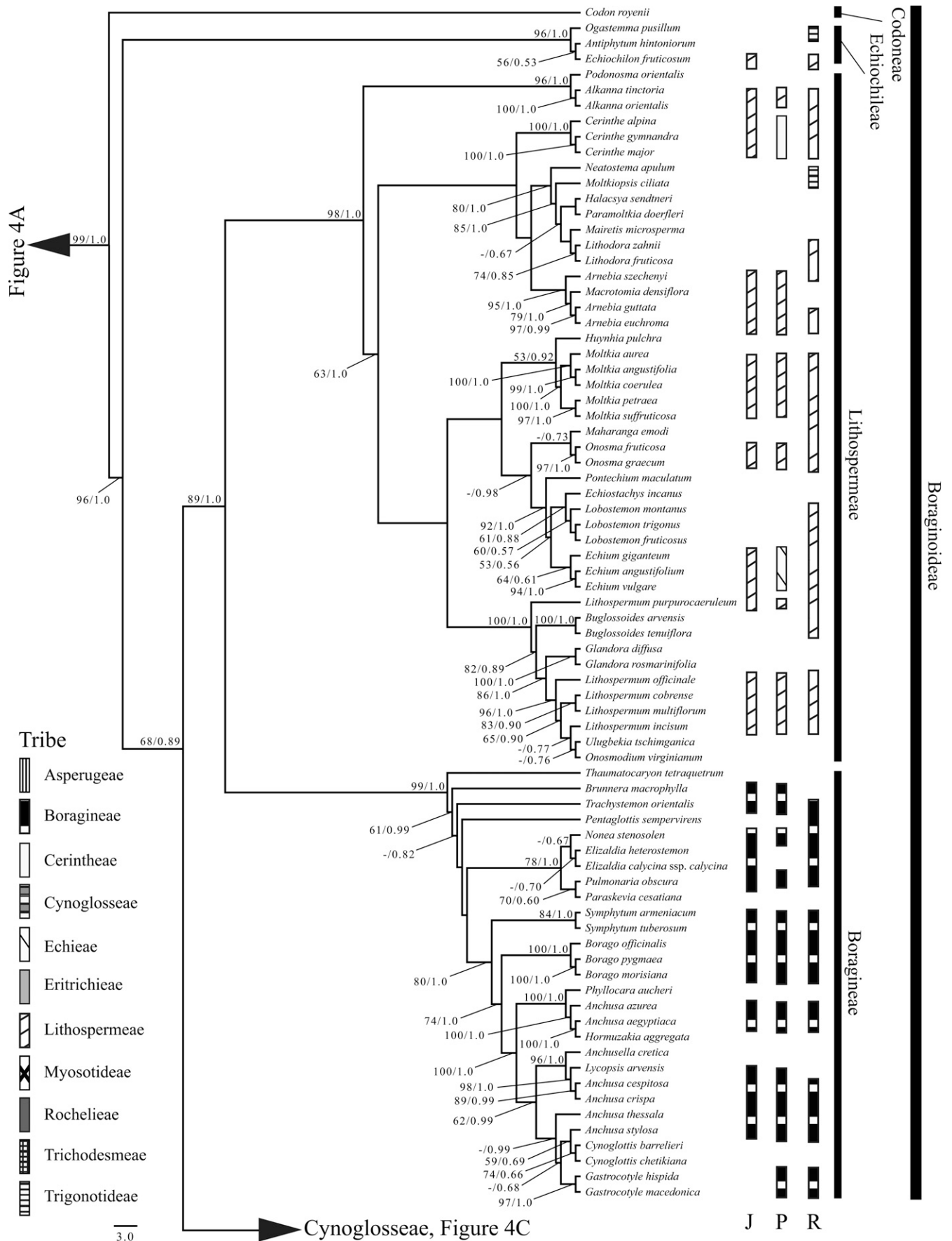


FIG. 4. (continued)

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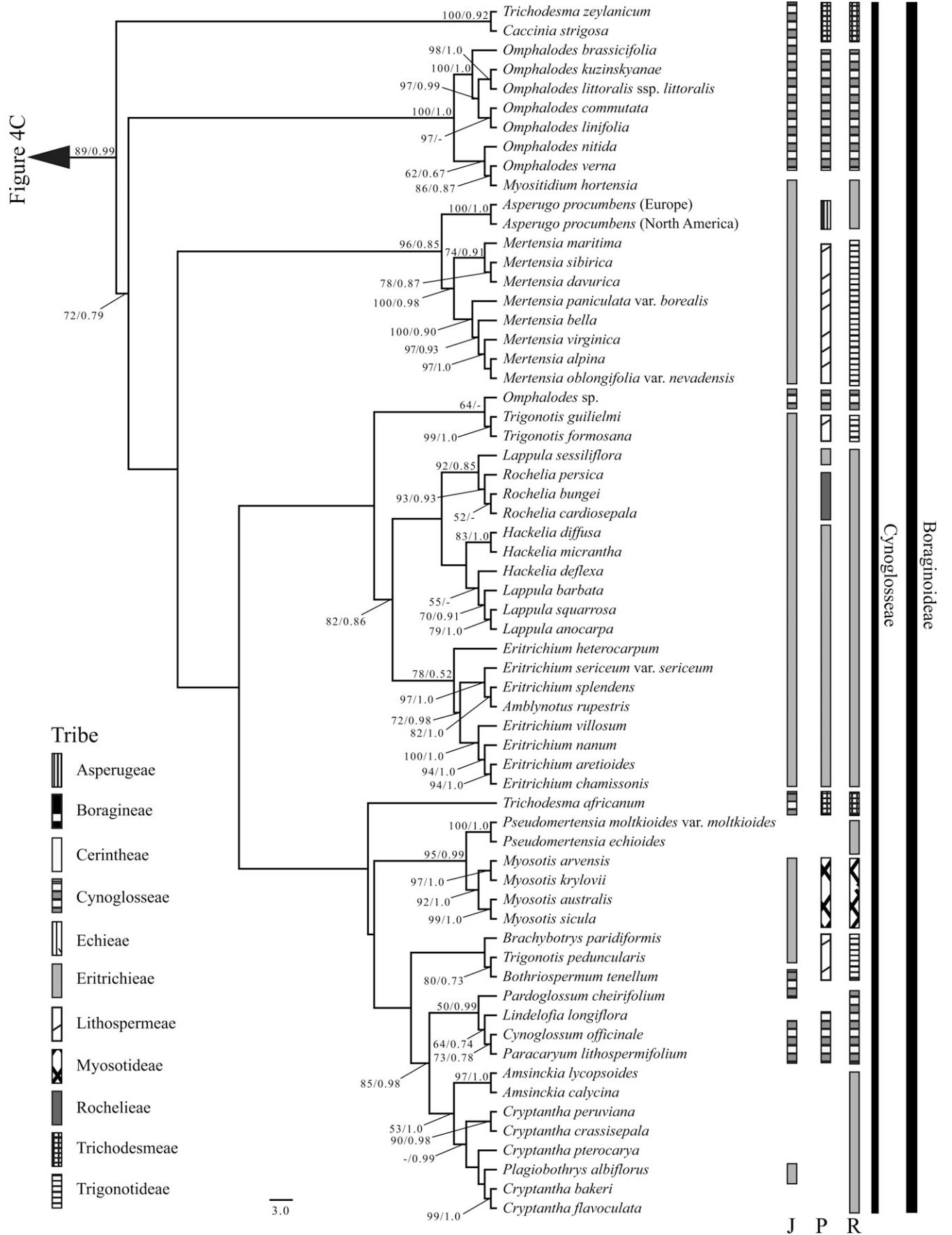


FIG. 4. (continued)

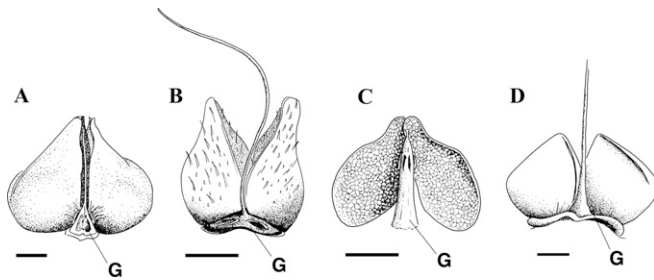


FIG. 5. Nutlets and attachment to gynobase (G) in *Mertensia*, *Pseudomertensia*, *Asperugo*, and *Brachybotrys*. For each taxon, two nutlets are shown attached to the gynobase and two nutlets have been removed. A. *Mertensia maritima* (Kines s. n. [WS]). B. *Pseudomertensia echioides* (Stewart 26343 [UC]). C. *Asperugo procumbens* (Cusick 3441 [WS]). D. *Brachybotrys paridiformis* (Lee & Lee 149 [US]). Scale bars: 1 mm. Drawn by M. Nazaire.

keel (Fig. 6A). The larger and more distinct attachment scar of nutlets of *Pseudomertensia* occupies nearly the entire basal surface (Fig. 6B). Riedl (1967) also suggested that growth habit, ecology, and geographic distribution differed greatly between *Mertensia* and *Pseudomertensia*. He noted that *Mertensia* is distinguished by its leafy stems, mesophytic habitat, and North American/Siberian distribution; conversely, *Pseudomertensia* is characterized by shoots with only basal leaf rosettes, alpine habitat, and restricted Himalayan distribution.

Although not previously hypothesized to be closely related, our phylogenetic results recovered a strongly supported sister relationship between *Pseudomertensia* and *Myosotis* (Figs. 2–4). Earlier phylogenetic studies of Boraginaceae, in which *Pseudomertensia* had not been sampled, recovered a sister relationship between *Myosotis* and *Trigonotis* (Långström and Chase 2002; Weigend et al. 2010) or found *Myosotis* to be paraphyletic to *Lappula* (Mansion et al. 2009). Both *Pseudomertensia* and *Myosotis* have nutlets characterized by a basally positioned attachment scar, although the two genera are distinguished by ventrally keeled nutlets in *Pseudomertensia* but unkeeled in *Myosotis* and a short pyramidal gynobase in *Pseudomertensia* vs. a flat gynobase in *Myosotis* (Johnston 1924b; Riedl 1967).

Closest Relatives of *Mertensia*—Our results point toward a new understanding of the relationships of *Mertensia* that contrast with those hypothesized prior to phylogenetic

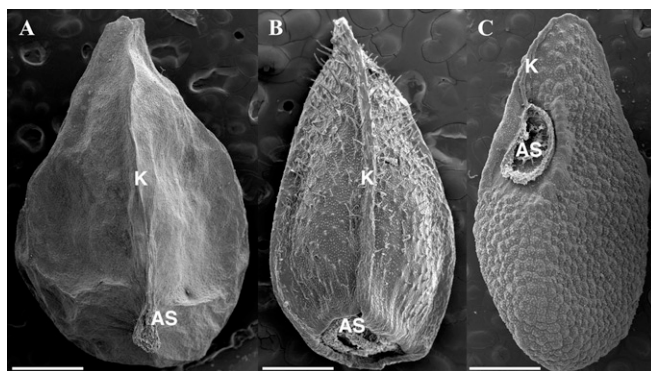


FIG. 6. Nutlet morphology of *Mertensia*, *Pseudomertensia*, and *Asperugo*. A. Ventral view of mature nutlet of *Mertensia maritima*, showing raised attachment scar (AS) in suprabasilar position below keel (K). B. Ventral view of mature nutlet of *Pseudomertensia echioides*, showing basal attachment scar (AS). C. Oblique ventral view of mature nutlet of *Asperugo procumbens*, showing circular attachment scar (AS) below keel (K). Scale bars: 0.5 mm.

studies. Subsequent to its initial placement in Symphyteae (Don 1838), *Mertensia* was treated by early workers as Lithospermeae (DeCandolle 1846; Bentham 1876; Baillon 1890; Gürke 1897) because it shared a suprabasilar nutlet attachment (Fig. 6A) with other members this tribe. Johnston (1924b) questioned this placement and suggested that *Mertensia* was closely related to members of Anchuseae or Eritrichieae. Johnston (1924b; p. 57) considered *Mertensia* among “the most primitive members” of Eritrichieae because of its oblique and suprabasilar position of the flat, undefined attachment scar (termed “areole” by Johnston 1924b; Popov 1953b; Riedl 1967) on the nutlet, the simple stigma, and its geographic distribution centered in western North America. Popov (1953b) emphasized that the large corolla in *Mertensia* closely resembled typical *Lithospermum* and placed it with *Brachybotrys* and *Trigonotis* in the newly recognized subtribe Trigonotidinae of Lithospermeae. Riedl (1967, 1968) disagreed with Popov’s placement and recognized both *Mertensia* and *Trigonotis* as members of subtribe Trigonotidinae of the new tribe Trigonotideae. He argued that of all the subtribes in Trigonotideae, Trigonotidinae were “the most highly specialized” (Riedl 1968; p. 319). Our molecular phylogenetic results are not consistent with the placement of *Mertensia* made by earlier workers; in contrast, our results recovered *Mertensia* as a lineage in the tribe Cynoglosseae (Figs. 1–4), which was also found recently in molecular phylogenetic studies by Weigend et al. (2010).

Our phylogenetic analyses recovered *Asperugo*, a monotypic genus native to Europe and introduced to North America, as sister to *Mertensia*, with strong support for this sister relationship (Figs. 1–4). The sister relationship of *Asperugo* and *Mertensia* is not easily inferred from morphology. Some authors had placed *Asperugo* in its own tribe (e.g. Asperugeae; Popov 1953b) or subtribe (e.g. Asperuginae; Riedl 1968) because they considered it to be morphologically distinct from other borages (Table 1). *Asperugo* is a weakly prostrate to climbing annual with retrorsely prickly-hispid stems and a plicate, prominently veined, irregularly toothed and lobed calyx that becomes strongly accrescent in fruit. In contrast, *Mertensia* is perennial, lacking retrorsely prickly-hispid stems, the calyx is only slightly to moderately accrescent in fruit, and shares none of the other calyx features of *Asperugo*. The nutlets in *Asperugo* are attached to an elongate and strongly compressed gynobase (Fig. 5C), while in *Mertensia* the gynobase is short and conical (Fig. 5A). In *Asperugo* the nutlets are strongly flattened, with an attachment scar that is circular and positioned above the middle of the nutlet (Fig. 6C); in *Mertensia*, the nutlets are tetrahedral, with a winged keel on the ventral side, and the attachment scar is flat, poorly defined, and located in a suprabasilar position (Fig. 6). In our analyses, we have not found any obvious morphological synapomorphies between *Mertensia* and *Asperugo*, but we note that the characters that distinguish *Asperugo* are somewhat unusual for borages, which may explain why earlier workers had understood neither its placement in Boraginoideae nor its relationships to other genera.

In Popov’s (1953b) treatment of Boraginaceae, he placed *Brachybotrys*, *Mertensia*, and *Trigonotis* in subtribe Trigonotidinae of tribe Lithospermeae and suggested that *Brachybotrys*, a monotypic genus of Asia, was unquestionably closely related to *Mertensia*. Popov emphasized that both genera shared similarities in stamen position and length (not extending beyond the apex of the corolla) and in the attachment scar on the nutlet. Al-Shehbaz (1991) hypothesized that *Trigonotis* was

closely related to *Mertensia*, and, following the treatments of Popov (1953b) and Riedl (1967, 1968), included *Mertensia* and *Trigonotis* in Trigonotideae. Results from our phylogenetic analyses of the nuclear, plastid, and combined molecular datasets recovered *Brachybotrys* and species of a paraphyletic *Trigonotis* as more closely related to each other than to *Mertensia* (Figs. 1–4). In analyses of the combined dataset, *Brachybotrys* was recovered as sister to a clade consisting of *T. peduncularis* (Trevis.) Benth. ex Baker & S. Moore and *Bothriospermum* Bunge but with little support for this relationship (Fig. 3). In independent analyses of the plastid dataset, *Brachybotrys* was moderately supported as nested between *T. formosana* Hayata and *T. guilielmi* (A. Gray) Guerke (Fig. 3). Independent analyses of the nuclear dataset indicated *Brachybotrys* was sister to a clade consisting of *Bothriospermum*, one accession of an *Omphalodes* species (collector unknown 090 [XJA]), *T. peduncularis*, and *T. formosana*, with strong support for this relationship (Fig. 1). Our observations of morphological characters, also noted by Johnston (1924b), indicate that *Brachybotrys* (Fig. 5D) and *Trigonotis* share a flat to depressed gynobase, tetrahedral-shaped nutlets, and a basal positioning of the attachment scar on the nutlet. Constraint topologies that forced the monophyly of *Mertensia* and *Trigonotis* were rejected by the SH test and the AU test. However, both SH and AU tests were unable to reject the monophyly of *Mertensia* and *Brachybotrys*. This result is not surprising, given the limited support for nearly all nodes between the placements of *Mertensia* and *Brachybotrys* in our results (Fig. 4C) and our incomplete nuclear and plastid sequences for *Brachybotrys*.

Mansion et al. (2009) inferred a sister relationship between *Omphalodes* and *Mertensia* but had limited taxon sampling in their study of Boraginoideae. Långström and Chase (2002) recovered *Omphalodes* as sister to the tribes Echiochileae, Boragineae, Lithospermeae, and Cynoglosseae in their phylogeny reconstruction based on plastid *atpB* sequences, although they emphasized that the position of *Omphalodes* was unresolved within Boraginoideae. All phylogeny reconstructions in our study recovered *Omphalodes* as polyphyletic. One accession of *Omphalodes* (collector unknown 090 [XJA]; specimen not examined by us) formed a clade with *Brachybotrys*, *Bothriospermum*, and *Trigonotis*; whereas the remaining sampled species of *Omphalodes* were paraphyletic to *Myosotidium* Hook. (Figs. 1–4). None of our results supported a sister relationship for *Mertensia* and *Omphalodes*, and both SH and AU tests rejected the topology constraining *Mertensia* and *Omphalodes* to be monophyletic.

In molecular phylogenetic studies based on plastid *trnL-trnF* and nuclear ITS sequences, Weigend et al. (2010) recovered *Mertensia* as sister to a clade consisting of *Eritrichium*, *Hackelia*, and *Lappula*; however, there was limited support for this relationship. With the exception of *Eritrichium*, similar relationships for *Mertensia* were also obtained in molecular phylogenetic studies of Olmstead and Ferguson (2001; Olmstead pers. comm.). Our study recovered a paraphyletic *Eritrichium* that formed a clade with *Amblynotus* (Figs. 3, 4C). Modest support was found for the sister relationship of *Eritrichium* + *Amblynotus* to *Hackelia* + *Lappula* + *Rochelia* Rchb. (Fig. 4C). Neither *Hackelia* nor *Lappula* were monophyletic in our analyses (Fig. 4C). Constraining the monophyly of *Mertensia*, *Hackelia*, and *Lappula* resulted in a best topology in which all three genera were placed in a clade with *Amblynotus*, *Eritrichium*, and *Rochelia* (tree not shown). A

similar result was also obtained when the monophyly of *Mertensia*, *Hackelia*, *Lappula*, and *Eritrichium* were constrained (tree not shown). The SH and AU tests rejected the best topologies under both of these constraints. In contrast to earlier molecular phylogenetic studies that had more limited taxon sampling (Olmstead and Ferguson 2001; Weigend et al. 2010), all phylogenies generated in our study found moderate to strong support for an *Amblynotus*, *Eritrichium*, *Hackelia*, *Lappula*, and *Rochelia* clade (Figs. 1–4). Our results are largely consistent with the treatment of Johnston (1924b), who emphasized similarities in nutlet characters as the basis for close relationships between *Hackelia* and *Eritrichium* and between *Lappula* and *Rochelia*. In contrast, our results are not consistent with Johnston's (1924b) assessment of *Amblynotus* as more closely related to *Cryptantha* than to *Eritrichium*.

Tribes of Subfamily Boraginoideae—Our phylogenetic analyses of the combined dataset recovered strong support for the monophyly of subfamily Boraginoideae and four constituent subclades that corresponded to tribes recognized in recent molecular phylogenetic studies (Långström and Chase 2002; Weigend et al. 2010; Figs. 2, 4). Our results for tribal relationships are largely consistent with those from previous phylogenetic studies that found Boragineae and Lithospermeae as sister clades, Cynoglosseae as their closest relative, and Echiochileae as sister to the Boragineae + Lithospermeae + Cynoglosseae clade (Mansion et al. 2009; Weigend et al. 2010). Although earlier phylogenetic studies using only plastid *atpB* sequence data found Echiochileae to be sister to Lithospermeae, Cynoglosseae as their closest relative, and Boragineae as sister to the Cynoglosseae + Echiochileae + Lithospermeae clade (Långström and Chase 2002), these relationships had poor support.

Tribal classification in Boraginoideae has been widely divergent among authors, ranging from four to 13 tribes (Table 1). Some tribes circumscribed by earlier authors (e.g. Borageae, Cordieae, Ehretieae, Heliotropieae; Don 1838; Bentham 1876) are now typically recognized as subfamilies (e.g. Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae; Al-Shehbaz 1991; Långström and Chase 2002; APG 2003, 2009). Prior to molecular studies, tribal classification primarily emphasized characteristics of the gynobase, nutlet attachment, and position of the attachment scar on the nutlet; however, this emphasis has led to considerable confusion and disagreement about tribal and generic circumscriptions. Our phylogenetic results demonstrate that several tribes previously circumscribed on the basis of these characters are not monophyletic. For example, Trigonotideae, first recognized by Riedl (1967, 1968) and maintained by some authors (Al-Shehbaz 1991; Takhtajan 1997), were delimited based on a flat gynobase with a slightly concave to slightly convex center and smooth or tuberculate nutlets that bear a sharp ventral ridge or keel above the suprabaasal and raised (described as "stipitate" by Riedl 1968, 1997) attachment scar. Riedl also referred to pollen characters (e.g. hetercolpate vs. colporate pollen) as important in delimiting members of Trigonotideae, in which most genera had hetercolpate pollen. He admitted, however, that some genera with colporate pollen (e.g. *Neatostema* I. M. Johnst., *Sericostoma* Stocks ex Wight) would be better placed in Lithospermeae. Recent molecular phylogenetic studies of Långström and Chase (2002) and Weigend et al. (2010) have shown that the genera circumscribed as Trigonotideae (sensu Riedl 1967, 1968, 1997) are distributed among all four tribes of Boraginoideae. Our

phylogenetic analysis, which included eight of the 12 genera treated as Trigonotideae by Riedl (Fig. 4; Table 1; 1967, 1968, 1997), corroborates the findings of Långström and Chase (2002) and Weigend et al. (2010).

Our results demonstrate that several genera previously circumscribed as the tribe Eritrichieae (sensu Bentham 1876; Baillon 1890; Gürke 1897; Johnston 1924b; Popov 1953b; Riedl 1967, 1968, 1997; Table 1) are nested within Cynoglosseae (Fig. 4). First described by Bentham (1876), Eritrichieae have often been considered closely related to Cynoglosseae (e.g. Hilger 1985), and authors have differed on the placement of some taxa in either Eritrichieae or Cynoglosseae (Bentham 1876; Baillon 1890; Gürke 1897; Johnston 1924b). Johnston (1924b), who refined tribal classification in Boraginoideae, considered Eritrichieae to be intermediate between Cynoglosseae and Lithospermeae, and regarded some genera, including *Mertensia*, as the “most primitive” Eritrichieae. Taxa such as *Eritrichium*, *Lappula*, and *Trigonotis* were regarded by Johnston as the most specialized Eritrichieae and closely approximating Cynoglosseae. Characters used to distinguish Eritrichieae from Cynoglosseae included erect to suberect nutlets with a basal to suprabaasal attachment scar in Eritrichieae vs. ascending or divergent nutlets, with an apical to subapical positioning of the attachment scar in Cynoglosseae (Johnston 1924b; Al-Shehbaz 1991). Our results recover an eritrichioid s. s. clade that is nested in Cynoglosseae, which is consistent with evidence from Långström and Chase (2002) and Weigend et al. (2010), and we do not find *Mertensia* placed in a manner consistent with Johnston’s (1924b) idea that it is the “most primitive” Eritrichieae.

Prior to the establishment of the monogeneric tribe Myosotideae (Popov 1953b), *Myosotis* had been treated as Lithospermeae (DeCandolle 1846; Bentham 1876; Baillon 1890; Gürke 1897) because it shared basally attached nutlets with that tribe. Johnston (1924b) argued for Eritrichieae rather than Lithospermeae as the placement for *Myosotis* based on its simple stigma, somewhat ridged nutlets, and habit. Popov (1953b) and subsequent workers (Riedl 1967, 1968; Al-Shehbaz 1991) contended that *Myosotis* should be placed within its own tribe, Myosotideae, on the basis of its contorted corolla lobes in aestivation, a unique character for borages. In contrast with these placements, our results show *Myosotis* is nested in Cynoglosseae, a result that is consistent with the molecular phylogenetic studies of Långström and Chase (2002) and Weigend et al. (2010).

Trichodesma R. Br. and *Caccinia* Savi have served as the core of the tribe Trichodesmeae (Popov 1953b; Riedl 1967, 1968) based on shared patelliform (patella-shaped) calyx, a pyramidal gynobase with longitudinal protuberances along the ribs, and dorsoventrally flattened nutlets. However, Popov (1953b) noted that the gynobase and nutlet attachment in both *Trichodesma* and *Caccinia* closely resembled that of *Cynoglossum* L. Phylogenies in Weigend et al. (2010) showed that *T. zeylanicum* (Burm. f.) R. Br. and *C. strigosa* Boiss. were sister to the remaining genera of Cynoglosseae, and they treated *Trichodesma* and *Caccinia* as Cynoglosseae, emphasizing that more work was needed to better understand the phylogenetic position of these taxa. Our results also placed *T. zeylanicum* and *C. strigosa* as sister to the rest of Cynoglosseae (Fig. 4). In contrast to other studies, however, we also sampled *T. africanum* R. Br. and found *Trichodesma* to be polyphyletic. We advocate sampling additional genera previously treated as Trichodesmeae to pro-

vide more evidence for their association with *Trichodesma* and *Caccinia* or other Cynoglosseae.

Taxonomy of Boraginaceae—Different views have long persisted regarding the subfamilial classification of Boraginaceae. While most authors have broadly treated Boraginaceae as consisting of four to five subfamilies, including Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, and Wellstedioideae (Cronquist 1981; Al-Shehbaz 1991; Långström and Chase 2002), others have regarded Boraginaceae in a strict sense and recognized Cordiaceae, Ehretiaceae, Heliotropiaceae, and Wellstediaceae as independent families (Novák 1943; Hutchinson 1969; Heywood 1993; Gottschling et al. 2001; Diane et al. 2002; Hilger and Diane 2003; Gottschling et al. 2004; Luebert and Wen 2008). Phylogenetic approaches led to an even broader treatment of Boraginaceae when it was recognized that Hydrophyllaceae were paraphyletic to Boraginaceae (Olmstead et al. 1992, 1993a; Chase et al. 1993; Ferguson 1999; Gottschling et al. 2001). Although Baillon (1890) was the first to include Hydrophyllaceae (as Hydrophyllae) in Boraginaceae, his treatment of Hydrophyllaceae was not adopted by subsequent workers. Each of these family level approaches for Boraginaceae could be seen as consistent with our phylogenetic results, although we apply here the treatment of APG (2003, 2009). In Boraginaceae sensu APG, we recovered five moderate to well-supported clades that correspond to five of the six subfamilies recognized in recent phylogenetic studies (Ferguson 1999; Gottschling et al. 2001): Boraginoideae, Cordioideae, Heliotropioideae, Hydrophyllaceae, and Lennooideae. The sixth subfamily, Ehretioideae, was recovered as paraphyletic to Lennooideae. With the exception of the placement of Lennooideae, relationships among the subfamilies of Boraginaceae in our results are consistent with those of Gottschling et al. (2001).

Confusion has surrounded the classification and relationships of the new world parasitic genera *Lennoa* Lex. and *Pholisma* Nutt. ex Hook. Since the first comprehensive study of these taxa by Solms-Laubach (1870), many workers have regarded the two genera as the family Lennoaceae (Hutchinson 1969; Cronquist 1981; Yatskievych and Mason 1986) and some have suggested close relationships with Orobanchaceae (Hooker 1844), Monotropaceae (Lindley 1853; Hutchinson 1969), and Ericaceae (Solms-Laubach 1870) among others. More recently, *Lennoa* and *Pholisma* have been treated as a subfamily within Boraginaceae s. l. (Lennooideae; Craven 2005; APG 2009). Earlier phylogenetic studies using only ITS1 differed in the placement of Lennooideae relative to Ehretioideae, finding strong support for a sister relationship between the two subfamilies (Gottschling et al. 2001); however, we recovered Lennooideae as nested in Ehretioideae (Fig. 4), and we provisionally recommend that the former be submerged in the latter.

The southern African genus *Codon* L., traditionally assigned to Hydrophyllaceae (Hydrophyllae; Baillon 1890) and recently treated as a monogeneric subfamily within Boraginaceae s. l. (Codonoideae; Retief and Van Wyk 2005) or as its own family (Codonaceae; Weigend and Hilger 2010), was recovered in our results as sister to Boraginoideae (Figs. 2–4). *Codon* is unique in Boraginaceae in that it has a 10- or 12-lobed corolla and bicarpellate gynoecium. Although Retief and Van Wyk’s (2005) assertion that *Codon* is closely related to Ehretioideae and Wellstedioideae based on shared similarities in pollen, inflorescence architecture, and trichome morphology, all of our phylogeny reconstructions show strong support for a

sister relationship between *Codon* and Boraginoideae, a result that is consistent with the molecular phylogenetic studies of Ferguson (1999) and Luebert and Wen (2008). Based on these findings, we advocate for the recognition of *Codon* as a new tribe of Boraginoideae, Codoneae.

In conclusion, our study provides the most comprehensive phylogeny reconstruction to date for Boraginaceae. Our results are consistent with a treatment of Boraginaceae that consists of the five subfamilies Boraginoideae, Cordioideae, Heliotropioideae, Hydrophyloideae, and Ehretioideae (including *Lennea* and *Pholisma*) that correspond to the deepest major lineages in our trees. We also recovered four clades in subfamily Boraginoideae that corresponded to the tribes Echiochilieae, Lithospermeae, Cynoglosseae, and Boragineae as recognized in recent phylogenetic studies that had applied far fewer taxa. Based on the sister relationship between the genus *Codon* and subfamily Boraginoideae, we advocate for the recognition of *Codon* as a new tribe of Boraginoideae, Codoneae. We found strong support for the monophyly of *Mertensia* and the placement of the monotypic genus *Asperugo* as its sister. Hypothesis testing has allowed us to reject some of the previous hypotheses of relationships with *Mertensia*. The *Mertensia* + *Asperugo* clade is well supported as part of Cynoglosseae. Further, our molecular phylogenetic approach to reconstruct the broad evolutionary relationships in Boraginaceae has demonstrated that several tribes in Boraginoideae that were previously circumscribed on the basis of morphological characters are not monophyletic.

TAXONOMIC TREATMENT

Codoneae (Retief & A. E. van Wyk) Nazaire & L. Hufford, tribe nov.

Basionym: Boraginaceae subfam. Codonoideae Retief & A. E. van Wyk (2005: 79).—TYPE: *Codon* L., generic type species: *Codon royerii* (Linnaeus 1767: 292).

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- APPENDIX 1. Accessions sampled for ingroup and outgroup phylogenetic data. For data obtained from GenBank, accession numbers and literature references for vouchers are provided. For each taxon, we provide information in the following order: taxon, literature reference, *matK*, *ndhF*, *rbcL*, *trnL-trnF*, *trnL* intron, ITS. Missing sequence data are indicated by —.
- Ingroup**—*Alkanna orientalis* (L.) Boiss., Mansion et al. (2009), EU599674, EU599762, EU599850, EU599938, EU600026, —. *Alkanna orientalis*, Cecchi and Selvi (2009), —, —, —, —, —, EU919575. *Alkanna tinctoria* Tausch, Mansion et al. (2009), EU599673, EU599761, EU599849, EU599937, EU600025, —. *Alkanna tinctoria*, Cecchi and Selvi (2009), —, —, —, —, —, EU919577. *Amsinckia calycinia* (Moris) Chater, Weigend et al. (2010), —, —, —, —, —, GQ285246, GQ285246, —. *Anchusa aegyptiaca* (L.) DC., Mansion et al. (2009), EU599709, EU599797, EU599885, EU599973, EU600061, —. *Anchusa aegyptiaca*, Hilger et al. (2004), —, —, —, —, —, AY383294. *Anchusa azurea* Mill., Mansion et al. (2009), EU599711, EU599799, EU599887, EU599975, EU600063, —. *Anchusa azurea*, Hilger et al. (2004), —, —, —, —, —, AY383293. *Anchusa caespitosa* Lam., Mansion et al. (2009), EU599721, EU599809, EU599897, EU599985, EU600073, —. *Anchusa caespitosa*, Hilger et al. (2004), —, —, —, —, —, AY383310. *Anchusa crispa* Viv., Mansion et al. (2009), EU599727, EU599815, EU599903, EU599991, EU600079, —. *Anchusa crispa*, Weigend et al. (2010), —, —, —, —, —, GQ285227. *Anchusa stylosa* M. Bieb., Mansion et al. (2009), EU599715, EU599803, EU599891, EU599979, EU600067, —. *Anchusa stylosa*, Hilger et al. (2004), —, —, —, —, —, AY383308. *Anchusa thessala* Boiss. & Sprun., Mansion et al. (2009), EU599717, EU599805, EU599893, EU599981, EU600069, —. *Anchusa thessala*, Selvi et al. (2004), —, —, —, —, —, AF531084. *Anchusella cretica* (Mill.) Bigazzi et al. (2002), —, —, —, —, —, AY045709, —. *Anchusella cretica*, Selvi et al. (2004), —, —, —, —, —, AF531085. *Antiphytum hintoniolum* L. C. Higgins & B. L. Turner, Långström and Oxelman (2003), —, —, —, —, —, AJ555899. *Argusia sibirica* L. Dandy, Hilger and Diane (2003), —, —, —, —, —, AY376169, AY377789. *Arnebia euchroma* (Royle) I. M. Johnston, Hu and Zhao (unpublished), —, —, —, —, —, EF199852, EF199874, EF199848. *Arnebia guttata* Bunge, Hu and Zhao (unpublished), —, —, —, —, —, EF199854, EF199872, EF199862. *Arnebia szechenyi* Kanitz, Hu and Zhao (unpublished), —, —, —, —, —, EF199855, EF199871, EF199863. *Auxemma onocalyx* (Allemão) Bail. Gottschling et al. (2005), —, —, —, —, —, AY321592. *Borago morisiana* Bigazzi & Ricceri, Mansion et al. (2009), EU599705, EU599793, EU599881, EU599969, EU600057, —. *Borago morisiana*, Selvi et al. (2006b), —, —, —, —, —, DQ657837. *Borago officinalis* L., Mansion et al. (2009), EU599704, EU599792, EU599880, EU599968, EU600056, —. *Borago officinalis*, Ferguson (unpublished), —, —, —, —, —, AF091151. *Borago pygmaea* Chater & Greuter, Mansion et al. (2009), EU599707, EU599795, EU599883, EU599971, EU600059, —. *Borago pygmaea*, Hilger et al. (2004), —, —, —, —, —, AY383282. *Bothriospermum tenellum* (Hornem.) Fisch. & C. A. Mey., Weigend et al. (2010), —, —, —, —, —, GQ285272, —. *Bothriospermum tenellum*, Serrano et al. (unpublished), —, —, —, —, —, DQ320741. *Borreria succulenta* Jacq., Moore and Jansen (2006), DQ197229, DQ197257, —, —, —, DQ197285. *Borreria virgata* (Sw.) G. Don, Albach et al. (2001), —, —, —, —, —, AF258345, —, —, —. *Brunnera macrophylla* I. M. Johnston, Böhle et al. (1994), —, —, —, —, —, L33351, L33350, —. *Brunnera macrophylla*, Weigend et al. (2010), —, —, —, —, —, GQ285223. *Buglossoides arvensis* (L.) I. M. Johnston, Mansion et al. (2009), EU599676, EU599764, EU599852, EU599940, EU600028, —. *Buglossoides arvensis*, Weigend et al. (2009), —, —, —, —, —, FJ763192. *Buglossoides tenuiflora* (L. f.) I. M. Johnston, Mansion et al. (2009), EU599675, EU599763, EU599851, EU599939, EU600027, —. *Buglossoides tenuiflora*, Thomas et al. (2007), —, —, —, —, —, EU044867. *Caccinia strigosa* Boiss., Weigend et al. (2010), —, —, —, —, —, GQ285241, GQ285241, —. *Ceballosia fruticosa* (L. f.) Kunkel ex Förther, Mansion et al. (2009), EU599647, EU599735, EU599823, EU599911, EU599999, —. *Ceballosia fruticosa*, Hilger and Diane (2003), —, —, —, —, —, AY377791. *Cerinthe alpina* Kit., Cecchi and Selvi (2009), EU919615, —, —, —, —, —, —. *Cerinthe alpina*, Selvi et al. (2009), —, —, —, —, —, FJ541016. *Cerinthe gymnandra* Gasp., Ferrero et al. (2009), —, —, —, —, —, FJ789878, FJ789842, FJ789860. *Cerinthe major* L., Cecchi and Selvi (2009), EU919616, —, —, —, —, —. *Cerinthe major*, Böhle et al. (1996), —, —, —, —, —, L43199, —. *Cerinthe major*, Weigend et al. (2009), —, —, —, —, —, FJ763244. *Codon royenii* L., Forest et al. (2007), —, —, —, —, —, AM234925, —, —. *Coldenia procumbens* L., Moore and Jansen (2006), DQ197227, —, —, —, —, —, DQ197284. *Cordia dentata* Poir., Mansion et al. (2009), EU599654, EU599742, EU599830, EU599918, EU600006, —. *Cordia dentata*, Gammon and Kesseli (unpublished), —, —, —, —, —, EU862051. *Cordia myxa* Endl., Mansion et al. (2009), EU599652, EU599740, EU599828, EU599916, EU600004, —. *Cordia myxa*, Gottschling et al. (2001), —, —, —, —, —, AF402578. *Cordia sinensis* Lam., Mansion et al. (2009), EU599653, EU599741, EU599829, EU599917, EU600005, —. *Cordia sinensis*, Gottschling et al. (2005), —, —, —, —, —, —, AY321613. *Cordia trichotoma* (Vell.) Arráb. ex Steud., Mansion et al. (2009), EU599651, EU599739, EU599827, EU599915, EU600003, —. *Cordia trichotoma*, Tillberg et al. (unpublished), —, —, —, —, —, —, AY701596. *Cryptantha bakeri* (Greene) Payson, Mansion et al. (2009), EU599668, EU599756, EU599844, EU599932, EU600020, —. *Cryptantha crassispala* (Torr. & A. Gray) Greene, Mansion et al. (2009), EU599666, EU599754, EU599842, EU599930, EU600018, —. *Cryptantha flavoculata* (A. Nelson) Payson, Mansion et al. (2009), EU599669, EU599757, EU599845, EU599933, EU600021, —. *Cryptantha flavoculata*, Ferguson (unpublished), —, —, —, —, —, AF091154. *Cryptantha peruviana* I. M. Johnston, Mansion et al. (2009), EU599667, EU599755, EU599843, EU599931, EU600019, —. *Cryptantha pterocarya* (Torr.) Greene, Mansion et al. (2009), EU599665, EU599753, EU599841, EU599929, EU600017, —. *Cynoglossum officinale* L., Mansion et al. (2009), EU599664, EU599752, EU599840, EU599928, EU600016, —. *Cynoglossum officinale*, Gottschling et al. (2001), —, —, —, —, —, AF402582. *Cynoglossum barrelieri* Vural & Kit Tan, Mansion et al. (2009), EU599713, EU599801, EU599889, EU599977, EU600065, —. *Cynoglossum barrelieri*, Bigazzi et al. (2002), —, —, —, —, —, —, AY045716. *Cynoglossum chetikiana* Vural & Kit Tan, Mansion et al. (2009), EU599714, EU599880, EU599978, EU600066, —. *Draperia systyla* (A. Gray) Torr., Ferguson (1999), —, —, —, —, —, —, AF047770, —, —, —. *Draperia systyla*, Hansen et al. (2009), —, —, —, —, —, —, FJ814620. *Echiochilon fruticosum* Desf., Weigend et al. (2009), —, —, —, —, —, —, FJ763310, FJ763310, —. *Echiochilon fruticosum*, Långström and Oxelman (2003), —, —, —, —, —, —, AJ555908. *Echiostachys incanus* Levyns, Forest et al. (2007), —, —, —, —, —, —, AM234927, —, —, —. *Echiostachys incanus*, Hilger and Böhle (2000), —, —, —, —, —, —, AF284111, —, —, —. *Echium angustifolium* Lam., Mansion et al. (2009), EU599695, EU599783, EU599871, EU599959, EU600047, —. *Echium giganteum* L. f., Mansion et al. (2009), EU599694, EU599782, EU599870, EU599958, EU600046, —. *Echium giganteum*, Böhle et al. (1996), —, —, —, —, —, —, L43224. *Echium vulgare* L., Winkworth et al. (2002), AY092893, AY092890, —, —, —, —. *Echium vulgare*, Weigend et al. (2009), —, —, —, —, —, —, FJ763301, FJ763301, FJ763247. *Ehretia acuminata* R. Br., Mansion et al. (2009), EU599656, EU599744, EU599832, EU599920, EU600008, —. *Ehretia acuminata*, Gottschling and Hilger (2001), —, —, —, —, —, —, AF385799. *Ehretia cymosa* Thonn., Mansion et al. (2009), EU599660, EU599748, EU599836, EU599924, EU600012, —. *Ehretia cymosa*, Gottschling and Hilger (2001), —, —, —, —, —, —, AF385790. *Ehretia longiflora* Champ. ex Benth., Mansion et al. (2009), EU599658, EU599746, EU599834, EU599922, EU600010, —. *Ehretia longiflora*, Gottschling and Hilger (2004), —, —, —, —, —, —, AY331400. *Ehretia thyrsiflora* Nakai, Mansion et al. (2009), EU599655, EU599743, EU599831, EU599919, EU600007, —. *Elizaldia calycina* Maire ssp. *calycina*, Selvi et al. (2006a), —, —, —, —, —, —, DQ269662, DQ269669. *Elizaldia heterostemon* I. M. Johnston, Selvi et al. (2006a), —, —, —, —, —, —, DQ269663, DQ269671. *Ellisia nyctelea* L., Ferguson (1999), —, —, —, —, —, —, AF047796, —, —, —, —, —, AF091157. *Emmenanthe penduliflora* Benth., Ferguson (unpublished), —, —, —, —, —, —, AF091158. *Eriodictyon californicum* (Hook. & Arn.) Torr., Olmstead et al. (1992), —, —, —, —, —, —, L01916, —, —, —. *Eriodictyon californicum*, Ferguson (unpublished), —, —, —, —, —, —, AF091159. *Eritrichium sericeum* (Benth.) A. DC. ssp. *sericeum*, Soininen et al. (2009), —, —, —, —, —, —, GQ244953, —. *Eritrichium villosulum* (Ledeb.) Bunge, Soininen et al. (2009), —, —, —, —, —, —, —, GQ244957, —. *Eucryphia chrysanthemifolia* (Benth.) Greene, Ferguson (1999), —, —, —, —, —, —, —, —, —, —, —, AF047814, —, —, —, —. *Eucryphia chrysanthemifolia*, Ferguson (unpublished), —, —, —, —, —, —, —, —, —, —, —, AF091165. *Euploca campestris* (Griseb.) Diane & Hilger,

- Luebert and Wen (2008), —, EF688908, —, EF688803, EF688803, EF688856. *Gastrocotyle hispida* (Forssk.) Bunge, Bigazzi et al. (2002), —, —, —, AY045705, AY045714. *Gastrocotyle macedonica* (Degen & Dörfel) Bigazzi, Hilger & Selvi, Bigazzi et al. (2002), —, —, —, —, AY045706, —. *Gastrocotyle macedonica*, Selvi et al. (2004), —, —, —, —, AF531086. *Glandora diffusa* (Lag.) D. C. Thomas, Ferrero et al. (2009), FJ789899, —, —, FJ789881, —. *Glandora diffusa*, Weigend et al. (2009), —, —, —, —, —, FJ763246. *Glandora rosmarinifolia* (Ten.) D. C. Thomas, Mansion et al. (2009), EU599682, EU599770, EU599859, EU599946, EU600034, —. *Glandora rosmarinifolia*, Cecchi and Selvi (2009), —, —, —, —, —, EU919585. *Hackelia deflexa* Opiz, Weigend et al. (2010), —, —, —, —, GQ285244, —, —. *Halacsya sendtneri* Dörfel, Cecchi and Selvi (2009), EU919618, —, —, —, —, EU919586. *Halgania andromedifolia* Behr. and F. Muell. ex F. Muell., Gottschling et al. (2001), —, —, —, —, —, AF402584. *Halgania rigida* S. Moore, Gottschling et al. (2001), —, —, —, —, —, AF402585. *Heliotropium aegyptiacum* Lehm., Mansion et al. (2009), EU599646, EU599734, EU599822, EU599910, EU599998, —. *Heliotropium aegyptiacum*, Diane et al. (2002), —, —, —, —, —, AF396918. *Heliotropium arborescens* L., Olmstead et al. (1993a), —, —, —, —, —, L14399, —, —. *Heliotropium arborescens*, Hilger and Diane (2003), —, —, —, —, —, AY376177, —. *Heliotropium arborescens*, Luebert and Wen (2008), —, EF688911, —, —, —, EF688859. *Hesperochiron pumilus* (Griseb.) Porter, Ferguson (1999), —, AF047783, —, —, —, —, *Hesperochiron pumilus*, Ferguson (unpublished), —, —, —, —, —, AF091167. *Hilgeria hypogaea* (Urb. & Ekman) Förther, Hilger and Diane (2003), —, —, —, —, —, AY376217, AY377820. *Hilgeria serpyllodes* (Griseb.) Förther, Hilger and Diane (2003), —, —, —, —, —, AY377821. *Hormuzakia aggregata* Gusul., Mansion et al. (2009), EU599708, EU599796, EU599884, EU599972, EU600060, —. *Hormuzakia aggregata*, Hilger et al. (2004), —, —, —, —, —, AY383291. *Huynhia pulchra* (Willd. ex Roemer & Schultes) Grueter & Burdet, Weigend et al. (2009), —, —, —, —, —, FJ763278, —, FJ763219. *Hydrophyllum canadense* L., Mansion et al. (2009), EU599649, EU599737, EU599825, EU599913, EU600001, —. *Hydrophyllum capitatum* Douglas ex Benth. var. *capitatum*, Ferguson (1999), —, AF047785, —, —, —, —. *Hydrophyllum capitatum* var. *capitatum*, Ferguson (unpublished), —, —, —, —, —, AF091169. *Hydrophyllum virginianum* L., Ferguson (1999), —, AF019646, —, —, —, —. *Hydrophyllum virginianum*, Olmstead et al. (1992), —, —, —, —, —, L01927, —, —. *Ixorhea tschudiana* Fenzl, Hilger and Diane (2003), —, —, —, —, —, AY376218, —. *Ixorhea tschudiana*, Diane et al. (2002), —, —, —, —, —, AF396880. *Lappula barbata* (M. Bieb.) Gürke, Khoshokhan et al. (2010), —, —, —, —, —, AB564713, AB564713, AB564703. *Lappula sessiliflora* Gürke, Khoshokhan et al. (2010), —, —, —, —, —, AB564714, AB564714, AB564704. *Lennea madreporoides* Lex., Ferguson (unpublished), —, —, —, —, —, AF091171. *Lindelofia longiflora* (Benth.) Baill., Långström and Oxelman (2003), —, —, —, —, —, AJ555895. *Lithodora fruticosa* (L.) Griseb., Ferrero et al. (2009), —, —, —, —, —, FJ789882, FJ789884, FJ789864. *Lithodora zahnii* (Heldr. ex Halácsy) I. M. Johnst., Ferrero et al. (2009), —, —, —, —, —, FJ789891, FJ789855, FJ789873. *Lithospermum cobrense* Greene, Cohen and Davis (2009), FJ827262, —, —, —, —, —. *Lithospermum cobrense*, Weigend et al. (2009), —, —, —, —, —, FJ763224. *Lithospermum incisum* Lehm., Mansion et al. (2009), EU599681, EU599769, EU599857, FJ763283, FJ763283, —. *Lithospermum incisum*, Weigend et al. (2009), —, —, —, —, —, FJ763225. *Lithospermum multiflorum* Torr. ex A. Gray, Mansion et al. (2009), EU599680, EU599768, EU599856, EU599944, EU600032, —. *Lithospermum multiflorum*, Ferrero et al. (2009), —, —, —, —, —, FJ789874. *Lithospermum officinale* L., Mansion et al. (2009), EU599679, EU599767, EU599855, EU599943, EU600031, —. *Lithospermum officinale*, Weigend et al. (2009), —, —, —, —, —, FJ763189. *Lithospermum purpurocaeruleum* L., Mansion et al. (2009), EU599678, EU599766, EU599854, EU599942, EU600030, —. *Lobostemon fruticosus* (L.) H. Buek, Forest et al. (2007), —, —, —, —, —, AM234929, —, —. *Lobostemon fruticosus*, Böhle et al. (1996), —, —, —, —, —, L43243, —, —. *Lobostemon montanus* H. Buek, Hilger and Böhle (2000), —, —, —, —, —, AF284115, —, AF284113. *Lobostemon trigonus* H. Buek, Ferrero et al. (2009), FJ789912, —, —, —, —, —, FJ789894, FJ789858, FJ789876. *Lycopsis arvensis* L., Mansion et al. (2009), EU599718, EU599806, EU599894, EU599982, EU600070, —. *Lycopsis arvensis*, Bigazzi et al. (2002), —, —, —, —, —, AY045711. *Macrotomia densiflora* J. F. Macbr., Cecchi and Selvi (2009), —, —, —, —, —, EU919591. *Maharanga emodi* (Wall.) A. DC., Weigend et al. (2009), —, —, —, —, —, FJ763269, —, —. *Mairetis microsperma* (Boiss.) I. M. Johnst., Cecchi and Selvi (2009), EU919620, —, —, —, —, —. *Mairetis microsperma*, Weigend et al. (2009), —, —, —, —, —, FJ763257, —, FJ763193. *Moltkia angustifolia* DC., Cecchi and Selvi (2009), EU919621, —, —, —, —, EU919593. *Moltkia angustifolia*, Weigend et al. (2009), —, —, —, —, —, FJ763306, —, —. *Moltkia aurea* Boiss., Cecchi and Selvi (2009), EU919622, —, —, —, —, —, EU919594. *Moltkia coerulea* Lehm., Cecchi and Selvi (2009), EU919623, —, —, —, —, —, EU919595. *Moltkia petraea* Griseb., Cecchi and Selvi (2009), EU919624, —, —, —, —, —. *Moltkia petraea*, Weigend et al. (2009), —, —, —, —, —, FJ763258, —, FJ763194. *Moltkia suffruticosa* (L.) Brand, Cecchi and Selvi (2009), EU919625, —, —, —, —, —, EU919597. *Moltkia suffruticosa*, Thomas et al. (2007), —, —, —, —, —, EU044893, —. *Moltkiopsis ciliata*, I. M. Johnst., Cecchi and Selvi (2009), EU919626, —, —, —, —, —, EU919598. *Myosotidium hortensia* Baill., Winkworth et al. (2002), AY092895, —, —, —, —, —, AY092902. *Myosotis arvensis* (L.) Hill, Mansion et al. (2009), EU599671, EU599759, EU599847, EU599935, EU600023, —. *Myosotis arvensis*, Winkworth et al. (2002), —, —, —, —, —, AY092908. *Myosotis australis* R. Br., Winkworth et al. (2002), AY092884, AY092844, —, —, —, —, AY092933. *Myosotis sicula* Guss., Mansion et al. (2009), EU599672, EU599760, EU599848, EU599936, EU600024, —. *Myriopus salzmännii* (DC.) Diane & Hilger, Luebert and Wen (2008), —, EF688905, —, EF688800, —, EF688853. *Nama demissum* A. Gray, Ferguson (1999), —, AF047767, —, —, —, —. *Nama demissum*, Hilger and Diane (2003), —, —, —, —, —, AY376168, —. *Nama demissum*, Ferguson (unpublished), —, —, —, —, —, AF091174. *Nama rothrockii* A. Gray, Ferguson (1999), —, AF047765, —, —, —, —. *Nama rothrockii*, Ferguson (unpublished), —, —, —, —, —, AF091179. *Neatostema apulum* (L.) I. M. Johnst., Mansion et al. (2009), EU599686, EU599774, EU599862, EU599950, EU600038, —. *Neatostema apulum*, Weigend et al. (2009), —, —, —, —, —, FJ763198. *Nemophila parviflora* Douglas ex Benth., Ferguson (1999), —, AF047782, —, —, —, —. *Nemophila parviflora*, Ferguson (unpublished), —, —, —, —, —, AF091184. *Nogalia drepanophylla* (Baker) Verdc., —, —, —, —, —, AY376219, AY377822. *Nonea stenosen* Boiss. & Bal, Mansion et al. (2009), EU599701, EU599789, EU599877, EU599965, EU600053, —. *Nonea stenosen*, Selvi et al. (2006a), —, —, —, —, —, DQ269686. *Ogastemma pusillum* (Coss. & Durieu ex Bonnet & Barratte) Brummitt, Weigend et al. (2009), —, —, —, —, —, FJ763201. *Omphalodes brassicifolia* Sweet, Serrano et al. (unpublished), —, —, —, —, —, AY837607, —, AY837606. *Omphalodes commutata* G. López, Serrano et al. (unpublished), —, —, —, —, —, AY837610, —, AY837604. *Omphalodes kuzinskyanae* Willk., Serrano et al. (unpublished), —, —, —, —, —, AY837609, —, AY837601. *Omphalodes linifolia* (L.) Moench, Serrano et al. (unpublished), —, —, —, —, —, AY837612, —, AY837605. *Omphalodes littoralis* Lehm. ssp. *littoralis*, Serrano et al. (unpublished), —, —, —, —, —, AY837611, —, AY837603. *Omphalodes nitida* Hoffmanns. & Link, Serrano et al. (unpublished), —, —, —, —, —, AY837613, —, AY837615. *Omphalodes verna* Moench, Mansion et al. (2009), EU599662, EU599750, EU599838, EU599926, EU600014, —. *Onosma fruticosa* Sibth., Weigend et al. (2009), —, —, —, —, —, FJ763260, —, FJ763196. *Onosma graecum* Boiss, Mansion et al. (2009), EU599684, EU599772, EU599860, EU599948, EU600036, —. *Onosmodium virginianum* (L.) A. DC., Weigend et al. (2009), —, —, —, —, —, FJ763261, —, FJ763197. *Paracaryum lithospermifolium* Grande, Mansion et al. (2009), EU599663, EU599751, EU599839, EU599927, EU600015, —. *Paramoltkia doerfleri* (Wettst.) Greuter & Burdet, Cecchi and Selvi (2009), EU919630, —, —, —, —, —, EU919604. *Paramoltkia doerfleri*, Thomas et al. (2007), —, —, —, —, —, EU044886, —. *Paraskevia cesatiana* Sauer & Sauer, Hilger et al. (2004), —, —, —, —, —, AY383276, AY383318. *Pardoglossum cheirifolium* (L.) Barbier & Mathez, Serrano et al. (unpublished), —, —, —, —, —, DQ320748. *Patagonula americana* L., Gottschling et al. (2004), —, —, —, —, —, AY176080. *Patagonula bahiensis* Moric., Gottschling et al. (2005), —, —, —, —, —, AY321618. *Pentaglottis sempervirens* Tausch, Weigend et al. (2010), —, —, —, —, —, GQ285250, —, GQ285225. *Phacelia heterophylla* Pursh, Ferguson (1999), —, AF047805, —, —, —, —. *Phacelia heterophylla*, Albach et al. (2001), —, —, —, —, —, AF258344, —, —. *Phacelia heterophylla*, Ferguson (unpublished), —, —, —, —, —, AF091192. *Phacelia ranunculacea* (Nutt.) Constance, Kress et al. (2005), —, —, —, —, —, DQ006073, —, —, DQ005986. *Phacelia tanacetifolia* Benth., Mansion et al. (2009), EU599650, EU599738, EU599826, EU599914, EU600002, —. *Phacelia tanacetifolia*, Hansen et al. (2009), —, —, —, —, —, FJ814654. *Pholisma arenarium* Nutt. ex Hook., Bremer et al. (2002), AJ429309, —, AJ428894, —, AJ430897, —. *Pholisma arenarium*, Ferguson (unpublished), —, —, —, —, —, AF091203. *Pholistoma auritum* (Lindl.) Lilja ex Lindbl., Ferguson (1999), —, AF047799, —, —, —, —. *Pholistoma auritum*, Ferguson (unpublished), —, —, —, —, —, AF091204. *Phyllocara aucheri* Gusul., Mansion et al. (2009), EU599710, EU599798, EU599886, EU599974, EU600062, —. *Phyllocara aucheri*, Hilger et al. (2004), —, —, —, —, —, AY383290. *Plagiobothrys albiflorus* R. L. Pérez-Mor., Winkworth et al. (2002), AY092896, AY092891, —, —, —, —, —, AY092899. *Podonosma orientalis* (L.) Feinbrun, Weigend et al. (2009), —, —, —, —, —, FJ763307, —, FJ763253. *Pontechium maculatum* (L.) Böhle & Hilger, Cecchi and Selvi (2009), —, —, —, —, —, EU919608. *Pulmonaria obscura* Dumort., Mansion et al. (2009), EU599700, EU599788, EU599876,

EU599964, EU600052, —. *Pulmonaria obscura*, Weigend et al. (2009), —, —, —, FJ763200. *Rochefortia acanthophora* (DC.) Griseb., Gottschling et al. (2005), —, —, —, AY176081. *Rochelia bungei* Trautv., Khoshokhan et al. (2010), —, —, —, AB564705, AB564705, AB564695. *Rochelia cardiosepala* Bunge, Khoshokhan et al. (2010), —, —, —, AB564711, AB564711, AB564701. *Rochelia persica* Bunge, Khoshokhan et al. (2010), —, —, —, AB564707, AB564707, AB564697. *Romanzoffia californica* Greene, Ferguson (1999), —, AF047804, —, —, —, —. *Romanzoffia californica*, Hansen et al. (2009), —, —, —, —, FJ814619. *Saccellium lanceolatum* Bonpl., Gottschling et al. (2001), —, —, —, —, AF402592. *Schleidenia baclei* DC. var. *rostratum* I. M. Johnst., Hilger and Diane (2003), —, —, —, —, AY376220, DQ197268, —, —, —, —, AY376221, —. *Schleidenia lagoensis*, Diane et al. (2002), —, —, —, —, AF396892. *Symphytum armeniacum* Bucknall, Mansion et al. (2009), EU599790, EU599966, EU599978, EU600054, —. *Symphytum tuberosum* L., Hilger et al. (2004), —, —, —, —, AY383247, AY383285. *Thaumatocaryon tetraquetrum* I. M. Johnst., Weigend et al. (2010), —, —, —, GQ285260, —, GQ285229. *Tiquilia hispida* (Torr.) A. T. Richardson, Moore and Jansen (2006), DQ197240, DQ197268, —, —, —, —, DQ197246, DQ197274, —, —, —, —, DQ197570. *Tournefortia buchtienii* Killip, Luebert and Wen (2008), —, EF688910, —, EF688805, —, EF688858. *Tournefortia laurifolia* Vent., Mansion et al. (2009), EU599648, EU599736, EU599824, EU599912, EU600000, —. *Trachystemon orientalis* (L.) G. Don, Selvi et al. (2006b), DQ657836, —, —, —, —. *Trachystemon orientalis*, Weigend et al. (2010), —, —, —, GQ285249, —, GQ285224. *Tricardia watsonii* Torr. ex S. Watson, Ferguson (1999), —, AF047775, —, —, —, —, *Tricardia watsonii*, Ferguson (unpublished), —, —, —, —, —, AF091209. *Trichodesma africanum* (L.) Lehm., Forest et al. (2007), —, —, —, —, AM234930, —, —, —. *Trichodesma zeylanicum* (Burm. f.) R. Br., Weigend et al. (2010), —, —, —, GQ285240, GQ285240, —. *Trigonotis formosana* Hayata, Weigend et al. (2010), —, —, —, GQ285261, GQ285261, —. *Trigonotis guilielmi* (A. Gray) Gürke, Weigend et al. (2010), —, —, —, GQ285257, GQ285257, —. *Trigonotis peduncularis* (Trevis.) Benth. ex Baker & S. Moore, Serrano et al. (unpublished), —, —, —, —, —, DQ320740. *Turricula parryi* (A. Gray) J. F. Macbr., Ferguson (1999), —, AF047816, —, —, —, —, *Turricula parryi*, Ferguson (unpublished), —, —, —, —, —, AF091210. *Ulugbekia tschinganica* (B. Fedtsch.) Zakirov, Weigend et al. (2009), —, —, —, FJ763279, —, FJ763220. *Varronia bonplandii* Desv., —, —, —, —, —, AY321620. *Wigandia urens* (Ruiz & Pav.) Kunth, Ferguson (1999), —, AF047763, —, —, —. *Wigandia urens*, Luebert and Wen (2008), —, —, —, EF688846, —, —. *Wigandia urens*, Ferguson (unpublished), —, —, —, —, —, AF091212.

Outgroup—GARRYALES: *Eucommia ulmoides* Oliv., Chen et al. (2010), GQ434085, —, —, —, —. *Eucommia ulmoides*, Bremer et al. (2002), —, AJ429113, —, AJ430905, AJ430905, —. *Eucommia ulmoides*, Albert et al. (1992), —, —, L01917, —, —, —. *Eucommia ulmoides*, Ma et al. (unpublished), —, —, —, —, —, AY649995. *Garrya elliptica* Douglas ex Lindl., Bremer et al. (2002), AJ429319, —, —, —, —. *Garrya elliptica*, Olmstead et al. (2000), —, AF147714, —, —, —, —. *Garrya elliptica*, Soltis et al. (1990), —, —, L01919, —, —. *Garrya elliptica*, Setoguchi et al. (unpublished), —, —, —, —, —, AY061996. GENTIANALES: *Apocynum cannabinum* L., Simões et al. (2007), DQ660500, —, —, —, —. *Apocynum cannabinum*, Sennblad and Bremer (2002), —, AJ420113, —, —, —. *Apocynum cannabinum*, Sennblad and Bremer (1996), —, —, —, X91761, —, —, —. *Apocynum cannabinum*, Livshultz et al. (2007), —, —, —, EF456131, EF456131, —. *Apocynum cannabinum*, Kress et al. (2005), —, —, —, —, DQ005966. *Cinchona calisaya* Wedd., Andersson and Antonelli (2005), AY538379, —, AY538478, AY538447, AY538447, AY538352. *Cinchona calisaya*, Manns and Bremer (2010), —, GQ852293, —, —, —, —. *Rhabdadenia biflora* (Jacq.) Müll. Arg., Livshultz et al. (2007), EF456277, —, —, EF456150, —. *Rhabdadenia biflora*, Sennblad and Bremer (2002), —, AJ420123, AJ419759, —, —, —. *Rhabdadenia biflora*, Ionta and Judd (2007), —, —, —, —, DQ916832. LAMIALES: *Acanthus ilicifolius* L., McDade et al. (2005), —, —, —, DQ054852, DQ054852, DQ028412. *Lamium purpureum* L., Li et al. (2008), AM503816, —, —, —, —. *Lamium purpureum*, Wagstaff et al. (1998), —, —, U78694, —, —, —. *Lamium purpureum*, Wagstaff and Olmstead (1997), —, —, —, —, U75702, —, —. *Lamium purpureum*, Ferri et al. (2009), —, —, —, FJ490793, —, —. *Lamium purpureum*, Sudarmo (2008), —, —, —, —, —, AB266244. *Verbena rigida* Spreng., Müller et al. (2004), AF531820, —, —, —, —. *Verbena rigida*, Bremer et al. 2002, —, —,

—, AJ430940, AJ430940, —. *Verbena rigida*, O'Leary et al. 2009, —, —, —, —, —, FJ867403. SOLANALES: *Nicotiana tabacum* L., Kunnimalaiyaan and Nielsen (1997), NC_001879, —, —, —, —. *Nicotiana tabacum*, Olmstead et al. (1993b), —, —, —, —, —. *Nicotiana tabacum*, Shinozaki and Sugiura (1982), —, —, —, —, —, —, —, —, —. *Nicotiana tabacum*, Clarkson et al. (2004), —, —, —, —, —, —, —, —, —. *Nicotiana tabacum*, Marshall et al. (2001), —, —, —, —, —, —, —, —, —. *Solanum dulcamara* L., James et al. (unpublished), FJ395444, —, FJ395606, —, —, —. *Solanum dulcamara*, Bohs and Olmstead (1997), —, —, —, —, —, —, —, —, —. *Solanum dulcamara*, Poczei et al. (unpublished), —, —, —, —, —, —, —, —, —. *Solanum dulcamara*, Spooner et al. (unpublished), —, —, —, —, —, —, —, —, —. *Solanum dulcamara*, Spooner et al. (unpublished), —, —, —, —, —, —, —, —, —. VAHLIACEAE: *Vahlia capensis* (L. f.) Thunb., Bremer et al. (2002), AJ429316, AJ429312, —, —, —, —, —, —, —, —, —. *Vahlia capensis*, Morgan and Soltis (1993), —, —, —, —, —, —, —, —, —.

Voucher information and GenBank accessions for sequence data generated in this study. Order for taxa is listed as follows: Taxon, collection locality, collector, collection number, herbarium where voucher is deposited (abbreviations follow Index Herbariorum), and GenBank accession numbers *matK*, *ndhF*, *trnL-trnF* (including the *trnL* intron), ITS. Missing sequence data are indicated by —.

Amblynotus rupestris (Pall. ex Georgi) Popov ex Serg., Russia, East Siberia, Rolfmeier 1204 (KSC), JQ388520, JQ388548, JQ388575, JQ388494. *Amsinckia lycopsoides* Lehm., Washington, Kittitas Co., Legler 3708 (WS), JQ388521, JQ388549, JQ388576, JQ388495. *Asperugo procumbens* L., Sweden, Uppsala City, Alm 1283 (WS), JQ388522, JQ388550, JQ388577, JQ388496. *Asperugo procumbens*, Washington, Kittitas Co., Knoke 292 (WS), JQ388523, JQ388551, JQ388578, JQ388497. *Brachybotrys paridiformis* Maxim. ex Oliv., China, Jilin Province, Wei Cao s. n. (WS), JQ388524, JQ388552, —, JQ388498. *Eritrichium aretioides* (Cham.) DC., Alaska, Noatak Quad, Parker, Elven, & Solstad 14806 (O), JQ388525, JQ388553, JQ388579, —. *Eritrichium chamissonis* DC., Canada, Yukon Territory, Solstad & Elven 03/0601A (O), JQ388526, JQ388554, JQ388580, —. *Eritrichium heterocarpum* Y. S. Lian & J. Q. Wang, China, Qinghai Province, Ho, Bartholomew, & Gilbert 64 (PE), JQ388527, JQ388555, —, —. *Eritrichium nanum* Schrad., Colorado, El Paso Co., Nazaire 1809 (WS), JQ388528, JQ388556, JQ388581, JQ388499. *Eritrichium sericeum* (Benth.) A. DC. ssp. *sericeum*, Russia, West Chukotka, Petrovsky & Plieva s. n. (O), JQ388529, JQ388557, —, —, —, —. *Eritrichium splendens* Kearney ex W. Wight, Alaska, Noatak Quad, Solstad & Elven 03/1216 (O), JQ388530, JQ388558, JQ388582, JQ388501. *Eritrichium villosum* (Ledeb.) Bunge, Russia, Chukotka, Solstad & Elven 05/0201 (O), JQ388531, JQ388559, —, —, —, —. *Hackelia diffusa* (Lehm.) I. M. Johnst., Washington, Kittitas Co., Lopushinsky 07-6 (WS), JQ388532, JQ388560, JQ388583, JQ388503. *Hackelia micrantha* (Eastw.) J. L. Gentry, Oregon, Grant Co., Hincliff 869 (WS), JQ388533, JQ388561, JQ388584, JQ388504. *Lappula anocarpa* Chang J. Wang, China, Xinjiang Province, Juan Qiu 08-0007 (XJA), JQ388534, JQ388562, JQ388585, JQ388505. *Lappula squarrosa* (Retz.) Dumort., Russia, Western Siberia, Altai, Rolfmeier 1197 (KSC), JQ388535, JQ388563, JQ388586, JQ388506. *Mertensia alpina* (Torr.) G. Don, Colorado, El Paso Co., Nazaire 1810 (WS), JQ388536, JQ388564, JQ388587, JQ388507. *Mertensia bella* Piper, Idaho, Idaho Co., Nazaire 1781 (WS), JQ388537, JQ388565, JQ388588, JQ388508. *Mertensia davurica* (Sims) G. Don, China, Hebei Province, Nazaire 1889 (WS), JQ388538, JQ388566, JQ388589, JQ388509. *Mertensia maritima* (L.) Gray, Canada, Nunavut, Arctic Bay, Kines s. n. (WS), JQ388539, JQ388567, JQ388590, JQ388510. *Mertensia oblongifolia* (Nutt.) G. Don var. *nevadensis* (A. Nelson) L. O. Williams, Oregon, Harney Co., Nazaire & Bunch 1748 (WS), JQ388540, JQ388568, JQ388591, JQ388511. *Mertensia paniculata* (Aiton) G. Don var. *borealis* (J. F. Macbr.) L. O. Williams, Oregon, Union Co., Nazaire & Bunch 1752 (WS), JQ388541, JQ388569, JQ388592, JQ388512. *Mertensia sibirica* (L.) G. Don, China, Shanxi Province, Nazaire 1892 (WS), JQ388542, JQ388570, JQ388593, JQ388513. *Mertensia virginica* L., Kentucky, Jefferson Co., Collins ch3 (WS), JQ388543, JQ388571, JQ388594, JQ388514. *Myosotis krylovii* Serg., Russia, Western Siberia, Altai, Rolfmeier 1199 (KSC), JQ388544, JQ388572, —, —, —, —. *Omphalodes* sp., China, Xinjiang Province, collector unknown 090 (XJA), JQ388545, —, —, —, —. *Pseudomertensia echioides* Riedl, India, Khyalung, Koelz 5183 (UC), JQ388546, —, —, —, —. *Pseudomertensia malkioides* (Royle ex Benth.) Kazmi var. *malkioides*, Pakistan, Baltistan, Webster 6241 (UC), JQ388547, JQ388573, —, —, —, —. *Trigonotis formosana* Hayata, Taiwan, Chiayi Hsien, Bartholomew & Boufford 6160 (US), —, —, —, —, —, —.