Plant Syst. Evol. 234: 137–153 (2002)
DOI 10.1007/s00606-002-0195-z

Plant Systematics and Evolution
Printed in Austria

Tribes of Boraginoideae (Boraginaceae) and placement of *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*: A phylogenetic analysis based on *atp*B plastid DNA sequence data

E. Långström[1] and **M. W. Chase**[2]

[1]Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden
[2]Jodrell Laboratory, Royal Botanic Gardens, Kew, UK

Received November 27, 2001; accepted March 20, 2002
Published online: November 14, 2002
© Springer-Verlag 2002

Abstract. The genera *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*, which have been difficult to place within the tribes of the subfamily Boraginoideae (Boraginaceae), are analysed using plastid *atp*B sequence data. A selection of Boraginaceae genera was used to obtain a framework for the phylogenetic position of *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*. *Sericostoma* is found to belong within *Echiochilon*. The new tribe Echiochileae, Boragineae and Lithospermeae are monophyletic but the tribes Eritrichieae and Cynoglosseae are paraphyletic. The biogeography of Echiochileae (*Echiochilon* and *Ogastemma* from Africa and western Asia, and *Antiphytum* from America) is discussed.

Key words: *Antiphytum*, *Echiochilon*, *Ogastemma*, *Sericostoma*, Boraginaceae, Echiochileae phylogeny, *atp*B, tribes, biogeography.

Boraginaceae is a family of herbs, shrubs and trees with a cosmopolitan distribution. The family comprises ca 130 genera and 2300 species (Mabberely 1997). Boraginaceae has for a long time provided a set of controversial phylogenetic problems at different taxonomic levels. For example, the sister group relationships for Boraginaceae (in the euasterid I clade) are uncertain, as either Gentianales, Lamiales, Solanales, or various combinations of these orders could be the sister to Boraginaceae (Olmstead et al. 2000, Savolainen et al. 2000, Soltis et al. 2000, Albach et al. 2001).

Hydrophyllaceae is a mainly new world family of herbs and shrubs with ca 18 genera and 270 species (Mabberley 1997), characterised by capsular fruits. Boraginaceae and Hydrophyllaceae have long been considered to be closely related, and recent molecular studies using *rbc*L indicate that Hydrophyllaceae is nested within Boraginaceae s. lat. (e.g. Olmstead et al. 1992, 1993; Chase et al. 1993). This was supported by a more inclusive study of Hydrophyllaceae based on *ndh*F sequence data (Ferguson 1999), and by phylogenetic analysis of the secondary structure of ITS1, (Gottschling et al. 2001), which also supported the inclusion of the new world parasitic family Lennoaceae in Boraginaceae s. lat. Consequently, the Boraginaceae subfamilies Cordioideae, Ehretioideae, Heliotropioideae, Boraginoideae (recognised mainly on gynoecium characters; e.g. Gürke 1897) and Well-

stedioideae (sensu Pilger 1912) are by some authors treated as families (e.g. Hutchinson 1969, Heywood 1993, Gottschling et al. 2001, Diane et al. 2002). In this article Boraginaceae is used for the family in its widest sense.

Boraginoideae, the largest subfamily of Boraginaceae, has variously been divided into 4–c. 20 tribes (e.g. Popov 1953, 12 tribes), even though most authors have accepted only four to seven tribes (Table 1). Some authors have recognised the tribe Echieae (de Candolle 1846, Baillon 1888, Gürke 1897, Popova and Zemskova 1995, Retief and Van Wyk 1997). De Candolle (1846) and Popova and Zemskova (1995) used the monogeneric tribe Cerintheae and de Candolle (1846) did not recognise the tribe Eritrichieae and treated the species usually included in Eritrichieae as Cynoglosseae.

Examples of genera with uncertain positions within Boraginoideae are *Antiphytum* DC. ex Meisn., *Echiochilon* Desf., *Ogastemma* Brummitt and *Sericostoma* Stocks ex Wight. They are here treated together because morphological characters indicate that they might be closely related (Johnston 1957). *Echiochilon*, *Ogastemma* and *Sericostoma* were morphologically revised by Lönn (1999). They grow in dry, sandy, stony or rocky habitats ranging from Fuerteventura of the Canary Islands (only *Ogastemma*) over northwestern Africa, northern Africa, the Horn of Africa, the Arabian Peninsula and along the coast to India (only *Sericostoma*; detailed maps in Lönn 1999).

Echiochilon has been variously allocated to three different tribes, Echieae, Eritrichieae and Lithospermeae. Some authors (e.g. Baillon 1888, Verdcourt 1991) have clearly expressed their doubts regarding the tribal position of *Echiochilon*. Baillon (1888) for instance wrote that *Echiochilon* could artificially be assigned to Echieae, but in some characters (nutlet characters) it shows similarities with the members of Eritrichieae. Johnston (1924) placed *Echiochilon* in Lithospermeae but later (Johnston 1957) stated its relationships to be unclear. Sauvage and Vindt (1954) and Verdcourt (1991) tentatively placed *Echiochilon* in Eritrichieae. Until Johnston (1957), none of the species of *Echiochilon* with actinomorphic flowers were placed in *Echiochilon* but rather in *Sericostoma* s. lat. (Table 1).

Sericostoma was until 1967 mostly placed in Lithospermeae and *Echiochilon* in Echieae, Eritrichieae or Lithospermeae, but Johnston (1957) suggested that *Echiochilon*, *Ogastemma* and *Sericostoma* are closely related to each other and to the New World genus *Antiphytum*. In 1967, Riedl formed the new tribe Trigonotideae (Riedl 1967) in which he included *Sericostoma s. str.* among other genera. He also included *Antiphytum* in his new tribe, but kept *Echiochilon* in Eritrichieae. Riedl did not mention *Ogastemma*, which traditionally has been placed in Eritrichieae, in his early Trigonotideae circumscriptions (Riedl 1967, 1968) but in a later treatment he included *Ogastemma* in Trigonotideae (Riedl 1997).

The gene used in this study, *atp*B, is a plastid gene that codes for the beta subunit of ATP synthase. It has been used for reconstruction of angiosperm phylogeny in several groups and has been useful in shedding light on problems similar to those addressed in this study (Hoot et al. 1995, Hoot et al. 1997, Chase et al. 1999). The gene *atp*B has a similar rate of evolution to that of *rbc*L (Hoot et al. 1995, Savolainen et al. 2000).

The major goals of this paper are to investigate the phylogenetic positions of *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma* within Boraginaceae (including the interrelationships of the genera) by sampling the subfamilies of Boraginaceae, the tribes of Boraginoideae and putative close relatives of *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*.

Material and methods

DNA extraction. Total genomic DNA was extracted from fresh or silica-gel dried leaves, seeds or herbarium material, using a modification of the 2X CTAB extraction protocol of Doyle and Doyle (1987). The DNA was purified by $CsCl_2$–ethidium

Table 1. Tribal treatment in Boraginoideae by selected authors to show how the genera in Echiochileae have been placed by those authors (small capitals). Genera marked with bold style are the genera selected for the phylogenetic analysis in this paper. All taxa mentioned by these authors are listed, so there are some synonyms in the list and also some genera now belonging to other families. Authors not accepting Echieae have included those species in Lithospermeae. Only de Candolle (1846) recognised Cerintheae as a tribe (not shown here); he also treated the species usually included in Eritrichieae as Cynoglosseae. Takhtajan (1997) only gave a few examples for each tribe

Tribes accepted Author	Boragineae (Borageae/ Anchusae)	Cynoglosseae	Echieae	Eritrichieae	Lithospermeae	Myosotideae	Trigonotideae	Trichodesmeae
De Candolle 1846	***Anchusa***	*Amsinckia*	ECHIOCHILON		*Alkanna*			
	Borago	ANTIPHYTIM	***Echium***		*Arnebia*			
	Caryolopha	*Caccinia*	***Lobostemon***		*Bothriospermum*			
	Lycopsis	*Craniospermum*	*Macrotomia*		*Colsmannia*			
	Moritzia	***Cynoglossum***			***Lithospermum***			
	Nonea	*Diploloma*			*Macromeria*			
	Psilostemon	*Echinospermum*			*Maharanga*			
	Stomotechium	*Eritrichium*			*Meratia*			
	Symphytum	*Gruvelia*			*Mertensia*			
		Heterocaryum			*Moltkia*			
		Krynitzkia			***Myosotis***			
		Mattia			*Onosma*			
		Omphalodes			*Onosmodium*			
		Pectocarya			*Pentalophus*			
		Plagiobothrys			*Pulmonaria*			
		Rindera			*Stenosolenium*			
		Solenanthus						
		Suchtelenia						
		Trichodesma						
Bentham & Hooker 1873	*Alkanna*	*Actinocarya*		*Amsinckia*	*Ancistrocarya*			
	Anchusa	*Caccinia*		*Asperugo*	ANTIPHYTUM			
	Borago	***Cynoglossum***		*Bothriospermum*	*Arnebia*			
	Lycopsis	*Harpagonella*		*Craniospermum*	***Cerinthe***			
	Nonea	*Heliocarya*		*Echidiocarya*	***Echium***			
	Pulmonaria	***Lindelofia***		ECHIOCHILON	***Lithospermum***			
	Symphytum	*Myosotidium*		*Echiochospermum*	***Lobostemon***			

Table 1 (continued)

Tribes accepted Author	Boragineae (Borageae/ Anchusae)	Cynoglosseae	Echieae	Eritrichieae	Lithospermeae	Myosotideae	Trigonotideae	Trichodesmeae
	Trachystemon *Trigonocaryum*	***Omphalodes*** *Paracaryum* *Pectocarya* *Rindera* *Solenanthus* *Suchtelenia* *Thyrocarpus* ***Trichodesma***		*Eritrichium* *Gastrocotyle* *Microula* *Rochelia*	*Macromeria* *Macrotomia* *Megacaryon* *Mertensia* *Moltkia* *Morizia* ***Myosotis*** *Onosma* *Onosmodium* **Sericostoma** ***Trigonotis*** *Zwackhia*			
Baillon 1888	*Alkanna* ***Anchusa*** ***Borago*** *Lycopsis* *Oskampia* *Pulmonaria* *Symphytum* *Trachystemon* *Trigonocaryum*	*Actinocarya* *Caccinia* ***Cynoglossum*** *Heliocarya* *Kuschakewiczia* ***Lindelofia*** *Myosotidium* ***Omphalodes*** *Paracaryum* *Pectocarya* *Rindera* *Selkirkia* *Solenanthus* *Suchtelenia* *Thyrocarpus* ***Trichodesma***	**Echiochilon** ***Echium*** ***Lobostemon*** *Zwackhia*	*Actinocarya* *Allocarya* *Amsinckia* *Asperugo* *Bothriospermum* *Craniospermum* ***Cryptantha*** *Eremocarya* *Eritrichium* *Gastrocotyle* ***Lappula*** *Microula* *Oreocarya* *Piptocalyx* *Plagiobothrys* *Rochelia* *Sonnea* *Tretocarya*	*Ancistrocarya* **Antiphytum** *Arnebia* *Brachybotrys* ***Cerinthe*** ***Cystostemon*** ***Lithospermum*** *Macromeria* *Macrotomia* *Mertensia* *Moltkia* *Moritzia* ***Myosotis*** *Onosma* *Onosmodium* **Sericostoma** ***Trigonotis***			
Gürke 1897	*Alkanna* ***Anchusa***	*Actinocarya* *Brachybotrys*	**Echiochilon** ***Echium***	*Allocarya* *Amsinckia*	*Ancistrocarya* **Antiphytum**			

Table 1 (continued)

	Borago	*Caccinia*	***Lobostemon***	*Asperugo*	*Arnebia*			
	Lycopsis	***Cynoglossum***	*Megacaryon*	*Bothriospermum*	***Cerinthe***			
	Nonea	*Heliocarya*	*Zwackhia*	*Craniospermum*	***Cystostemon***			
	Pulmonaria	*Kuschakewiczia*		***Cryptantha***	***Lithospermum***			
	Symphytum	***Lindelofia***		*Eremocarya*	*Macromeria*			
	Trachystemon	*Myosotidium*		*Eritrichium*	*Macrotomia*			
	Trigonocaryum	***Omphalodes***		*Gastrocotyle*	*Mertensia*			
		Paracaryum		***Lappula***	*Moltkia*			
		Pectocarya		*Microula*	*Moritzia*			
		Rindera		*Oreocarya*	***Myosotis***			
		Selkirkia		*Piptocalyx*	*Onosma*			
		Solenanthus		*Plagiobotrys*	*Onosmodium*			
		Suchtelenia		*Schistocaryum*	SERICOSTOMA			
		Thyrocarpus		*Sonnea*	***Trigonotis***			
		Trichodesma		*Tretocarya*				
		Tysonia						
Al-Shehbaz 1991[1]	***Anchusa***	*Actinocarya*		*Amsinckia*	*Alkanna*	***Myosotis***	*Amphibologyne*	*Caccinia*
	Anchusella	*Afrotysonia*		*Anoplocaryum*	*Ancistocarya*		ANTIPHYTUM	Suchtelenia
	Borago	*Antiotrema*		*Asperugo*	*Arnebia*		*Bothriospermum*	***Trichodesma***
	Brunnera	***Cynoglossum***		*Craniospermum*	***Buglossoides***		*Brachybotrys*	
	Cynoglottis	*Gyrocaryum*		***Cryptantha***	***Cerinthe***		*Decalepidanthus*	
	Elizaldia	***Lindelofia***		ECHIOCHILON	*Choriantha*		*Mairetis*	
	Gastrocotyle	*Mattiastrum*		***Eritrichium***	***Cystostemon***		*Mertensia*	
	Nonea	***Omphalodes***		*Hackelia*	***Echium***		*Moltkiopsis*	
	Pentaglottis	*Paracaryum*		***Lappula***	*Halacsya*		*Moritzia*	
	Pulmonaria	***Pardoglossum***		*Lepechiniella*	*Lasiarrhenum*		*Neatostema*	
	Symphytum	*Pectocarya*		*Microula*	***Lithodora***		OGASTEMMA	
	Trachystemon	*Rindera*		*Myosotidium*	***Lithospermum***		SERICOSTOMA	
		Solenanthus		*Nesocaryum*	***Lobostemon***		*Sinojohnstonia*	
		Thyrocarpus		*Omphalolappula*	*Macromeria*		*Thaumatocaryon*	
		Trachelanthus		*Plagiobotrys*	*Maharanga*		*Trigonocaryum*	
				Pseudomertensia	*Moltkia*		***Trigonotis***	
				Rochelia	*Onosma*			
				Selkirkia	*Onosmodium*			
				Tianschaniella	*Stenosolenium*			
Riedl 1997	***Anchusa***	*Actinocarya*		*Amsinckia*	*Alkanna*	***Myosotis***	*Bothriospermum*	*Caccinia*

Table 1 (continued)

Tribes accepted Author	Boragineae (Borageae/ Anchusae)	Cynoglosseae	Echieae	Eritrichieae	Lithospermeae	Myosotideae	Trigonotideae	Trichodesmeae
	Borago	*Antiotrema*		*Asperugo*	*Arnebia*		*Brachybotrys*	*Suchtelenia*
	Elizaldia	***Cynoglossum***		*Craniospermum*	***Buglossoides***		*Mertensia*	***Trichodesma***
	Gastrocotyle	*Gyrocaryum*		***Cryptantha***	***Cerinthe***		*Moltkiopsis*	
	Nonea	***Lindelofia***		*Eritrichium*	***Cystostemon***		*Neatostema*	
	Pentaglottis	***Omphalodes***		*Hackelia*	ECHIOCHILON		OGASTEMMA	
	Pulmonaria	*Paracaryum*		***Lappula***	***Echium***		*Omphalotrigonotis*	
	Symphytum	***Pardoglossum***		*Microula*	*Lasiarrhenum*		SERICOSTOMA	
	Trachystemon	*Pectocarya*		*Myosotidium*	***Lithodora***		*Sinojohnstonia*	
		Rindera		*Nesocaryum*	***Lithospermum***		***Trigonotis***	
		Solenanthus		*Plagiobotrys*	***Lobostemon***			
		Thyrocarpus		*Rochelia*	*Macromeria*			
				Selkirkia	*Maharanga*			
					Moltkia			
					Onosma			
					Onosmodium			
Takhtajan 1997	***Anchusa***	*Caccinia*		*Amsinckia*	*Alkanna*	***Myosotis***	*Mertensia*	
	Brunnera	***Cynoglossum***		*Asperugo*	Arnebia		***Trigonotis***	
	Elizaldia	*Mattiastrum*		*Eritrichium*	***Buglossoides***		*Zoelleria*	
	Nonea	***Omphalodes***		*Hackelia*	***Cerinthe***		etc.	
	Pentaglottis	*Paracaryum*		***Lappula***	***Echium***			
	Pulmonaria	*Rindera*		*Plagiobothrys*	*Halacsya*			
	Symphytum	*Solenanthus*		*Rochelia*	***Lithodora***			
	Trachystemon	***Trichodesma***		etc.	***Lithospermum***			
	etc.	etc.			***Lobostemon***			
					Moltkia			
					Onosma			
					etc.			

[1]In the listing of Al-Shehbaz (1991) several more genera than those he mentioned for southeastern United States were included according to different sources

bromide gradient centrifugation (1.5 g/ml) followed by dialysis.

Amplification and sequencing. The *atp*B gene was in most cases amplified as one fragment using the primers of Hoot et al. (1995; S2, S1494R), and the amplified product was purified using QIAquick™ columns according to the manufacturer's protocol. Some samples (i.e. *Tiquilia*) had to be amplified in two separate fragments using primer pairs S2, S766R and S611, S1494R (Hoot et al. 1995). The *atp*B gene was sequenced with the same four primers as used for PCR using PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Inc.), and for some samples DYEnamic ET Terminator Cycle sequencing Kit (Amersham Pharmacia Biotech) on a MegaBACE 1000 (Amersham Pharmacia Biotech) according to the manufacturer's protocols. Sequence assembly and editing was made using Sequencher™ (Gene Codes Corporation), and sequence alignment was done by eye (there was no length variation among the taxa selected).

Selection of taxa. The 38 ingroup taxa were selected from each of the seven tribes of the subfamily Boraginoideae following Riedl (1967) and Al-Shehbaz (1991): Boragineae, Cynoglosseae, Eritrichieae, Lithospermeae, Myosotideae, Trigonotideae and Trichodesmeae; a few from each of the Boraginaceae subfamilies Cordioideae, Ehretioideae and Heliotropioideae and some from the Hydrophylloideae tribes Hydrophylleae, Nameae, Phacelieae and Wigandieae were also included. The ingroup taxa are presented in Table 1 together with other taxa from the tribes of Boraginoideae. The outgroup taxa (in all 12 genera) were chosen from Savolainen et al. (2000); members from Solanales, Lamiales and Gentianales were chosen because these have been indicated to be most closely related to Boraginaceae (e.g. Chase et al. 1993, Olmstead et al. 1993, Savolainen et al. 2000, Soltis et al. 2000), and *Garrya* Douglas ex Lindl. and *Oncotheca* Baill. were selected as outgroups according to earlier analyses and classifications (APG 1998, Savolainen et al. 2000, Soltis et al. 2000). Vouchers or source and accession numbers are presented in Table 2. The aligned data matrix is available from the corresponding author on request. The *Antiphytum* species analysed originates from North America.

Phylogenetic analysis. The data were analysed under the maximum parsimony criterion using PAUP* ver. 4.0b4a (Swofford 1998) for Macintosh Power PC. A heuristic search was performed with 1000 replicates of random taxon entries. TBR (tree–bisection–reconnection) and MULPARS (all most–parsimonious trees are kept) were used, with all characters equally weighted and unordered (Fitch parsimony; Fitch 1971). Bootstrap resampling was performed with 10 000 replicates, using TBR swapping with ten random taxon entries per bootstrap replicate and MULPARS off. The following categories are used to describe the bootstrap results: 50–74, weakly supported; 75–84, moderately supported; 85–100, well supported.

Results

The matrix contained 50 taxa and 1469 characters, of which 246 were potentially phylogenetically informative. The heuristic search produced 779 trees of 879 steps with a consistency index (CI) of 0.60 and a retention index (RI) of 0.76. One of the trees (arbitrarily chosen) is shown in Fig. 1, with branch lengths below (ACCTRAN optimisation) and bootstrap percentages (BP) above the branches, and with clades absent from the strict consensus tree indicated by dotted lines. Boraginoideae (clade B) is a well-supported clade (BP 100), and support for Boraginaceae s. lat. (including Hydrophyllaceae), is also high (clade A; BP 98). *Hydrolea* L. is included in Solanales. Within Boraginoideae the clade corresponding to tribe Boragineae (clade C) is well supported (BP 100), the genera of Cynoglosseae and Eritrichieae are mixed together in a moderately supported clade (clade H; BP 84), and the Lithospermeae, including *Echium* L. and *Cerinthe* L. (excluding *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*), form a clade (F) with moderate support (BP 83). *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma* form a moderately supported clade (clade E; BP 84). *Omphalodes* Miller has an isolated position and *Myosotis* L. falls in the combined Eritrichieae/Cynoglosseae clade (clade H). *Trichodesma* R.Br. is the only representative of Trichodesmeae (Riedl 1967, 1968; A1-Shehbaz 1991), and it is the sister to clade H (the

Table 2. Taxonomy, vouchers or source, accession numbers and databank numbers for the species used in the analyses. The subfamilies and tribes of Boraginaceae follow Al-Shehbaz (1991) except for two cases marked with an asterisk (* follow Riedl 1997). The tribes of Hydrophyllaceae (Hydrophylleae, Nameae and Phacelieae) follow Bentham and Hooker (1876)

Family	Subfamily/tribe for Bor. and Hydr.	Species	Voucher/source	EMBL/Genbank
Antirrhinaceae		*Antirrhinum majus* L.	Savolainen et al. 2000	AJ235395
Apocynaceae		*Dischidia lanceolata* Decne.	Savolainen et al. 2000	AJ235458
Bignoniaceae		*Catalpa bignonioides* Walt.	Savolainen et al. 2000	AJ235428
Boraginaceae	Borageneae	*Anchusa officinalis* L.	*Chase 6054* (K)	AJ504808
Boraginaceae	Borageneae	*Borago officinalis* L.	*Chase 2746* (K)	AJ504810
Boraginaceae	Borageneae	*Nonea versicolor* Sweet	*Lönn 201*(UPS)	AJ504831
Boraginaceae	Borageneae	*Pentaglottis sempervirens* (L.) L. H. Bailey	*Chase 6058* (K)	AJ504836
Boraginaceae	Cordioideae	*Cordia macrostachya* Roem. & Schult.	*Chase 6059* (K)	AJ504813
Boraginaceae	Cordioideae	*Patagonula americana* L.	*Hilger*, *Medan* and *Roitman ARG 95/1* (BSB)	AJ504835
Boraginaceae	Cynoglosseae	*Cynoglossum officinale* L.	*Sennblad sn.* (K)	AJ504815
Boraginaceae	Cynoglosseae	*Lindelofia longiflora* Baill.	*Chase 6062* (K)	AJ504825
Boraginaceae	Cynoglosseae	*Omphalodes verna* Moench	*Chase 6064* (K)	AJ504833
Boraginaceae	Cynoglosseae	*Pardoglossum cheirifolium* (L.) Barbier & Mathez	*Chase 6065* (K)	AJ504834
Boraginaceae	Cynoglosseae/ Trichodesmeae*	*Trichodesma scottii* Balf.f.	*Chase 2912* (K)	AJ504842
Boraginaceae	Ehretioideae	*Ehretia cymosa* Thonn.	*Chase 6162* (K)	AJ504820
Boraginaceae	Ehretioideae	*Saccellium lanceolatum* Humb. & Bonpl.	*Hilger, Medan* and *Roitman ARG 95/39* (BSB)	AJ504838
Boraginaceae	Ehretioideae	*Tiquilia plicata* (Torr.) A. T. Richardson	*Hilger 19/7/1994* (BSB)	AJ504840
Boraginaceae	Eritrichieae	*Cryptantha virgata* Payson	*Chase 6092* (K)	AJ504814
Boraginaceae	Eritrichieae	*Lappula squarrosa* Dum.	*Chase 6549* (K)	AJ504824
Boraginaceae	Eritrichieae/ Trigonotideae*	*Ogastemma pusillum* (Coss. & Durieu ex Bonnet & Barratte) Brummitt	*Boulos and cope 17614* (K)	AJ504832
Boraginaceae	Heliotropioideae	*Heliotropium lasiocarpum* Fisch. & Mey.	*Chase 6091* (K)	AJ504822

Table 2 (continued)

Boraginaceae	Heliotropioideae	*Heliotropium messerschmidioides* Gürke	*Chase 6025* (K)	AJ504841
Boraginaceae	Hydrophylleae	*Hydrophyllum canadense* L.	*Chase 2548* (K)	AJ504823
Boraginaceae	Hydrophylleae	*Nemophila insignis* Benth.	*Chase 6550* (K)	AJ504830
Boraginaceae	Lithospermeae	*Buglossoides purpureo-caeruleum* (L.) I. M. Johnst.	*Chase 6055* (K)	AJ504811
Boraginaceae	Lithospermeae	*Cerinthe major* L.	*Chase 6056* (K)	AJ504812
Boraginaceae	Lithospermeae	*Cystostemon heliocaris* (S. Moore) A. G. Miller & H. Riedl	Thulin, Dahir and *Osman 9427* (UPS)	AJ504816
Boraginaceae	Lithospermeae	*Echium vulgare* L.	*Chase 6061* (K)	AJ504819
Boraginaceae	Lithospermeae	*Lithodora diffusa* I. M. Johnst.	*Chase 6063* (K)	AJ504826
Boraginaceae	Lithospermeae	*Lithospermum arvense* L.	*Lönn 200* (UPS)	AJ504827
Boraginaceae	Lithospermeae	*Lobostemon fruticosus* Buek	*Chase 6090* (K)	AJ504828
Boraginaceae	Myosotideae	*Myosotis arvensis* (L.) Hill	*Chase 6057* (K)	AJ504829
Boraginaceae	Nameae	*Wigandia caracasana* Kunth	*Chase 6060* (K)	AJ504843
Boraginaceae	Phacelieae	*Emmenanthe penduliflora* Benth.	*Chase 6093* (K)	AJ504821
Boraginaceae	Phacelieae	*Phacelia grandiflora* A. Gray	*Chase 6551* (K)	AJ504837
Boraginaceae	See Table 1	*Echiochilon collenettei* I. M. Johnst.	*Thulin* and *Warfa 6199* (UPS)	AJ504817
Boraginaceae	See Table 1	*Echiochilon kotschyi* (Boiss. & Hohen.) I. M. Johnst.	*Thulin, Eriksson, Gifri* and *Långström 8231* (UPS)	AJ504818
Boraginaceae	Trigonotideae	*Antiphytum hintoniorum* L. C. Higgins & B. L. Turner	*Patterson 7475* (NY)	AJ504809
Boraginaceae	Trigonotideae	*Sericostoma pauciflorum* Stocks ex Wight	*Rechinger 28501* (S)	AJ504839
Boraginaceae	Trigonotideae	*Trigonotis brevipes* Maxim.	*Fukoka 77* (WU)	AJ504845
Boraginaceae	Trigonotideae	*Trigonotis peduncularis* (Trevir.) Benth.	*Tyson 4900* (WU)	AJ504844
Convolvulaceae		*Ipomoea mauritiana* Jacq.	Savolainen et al. 2000	AJ235505
Garryaceae		*Garrya elliptica* Douglas	Savolainen et al. 2000	AJ235479
Hydroleaceae		*Hydrolea sp.*	*Chase 3245* (K)	AJ504846
Oleaceae		*Jasminum polyanthum* Franch.	Savolainen et al. 2000	AJ235508
Oncothecaceae		*Oncotheca balansae* Baill.	Savolainen et al. 2000	AJ235549
Rubiaceae		*Cinchona pubescens* Vahl.	Savolainen et al. 2000	AJ235434
Solanaceae		*Nicotiana tabacum* L.	Deno et al. 1983	V00162

Table 2 (continued)

Family	Subfamily/tribe for Bor. and Hydr.	Species	Voucher/source	EMBL/Genbank
Solanaceae		*Solanum nodiflorum* Desv. ex Dun.	Savolainen et al. 2000	AJ235604
Verbenaceae		*Verbena scabrido-glandulosa* Turrill	Savolainen et al. 2000	AJ235639

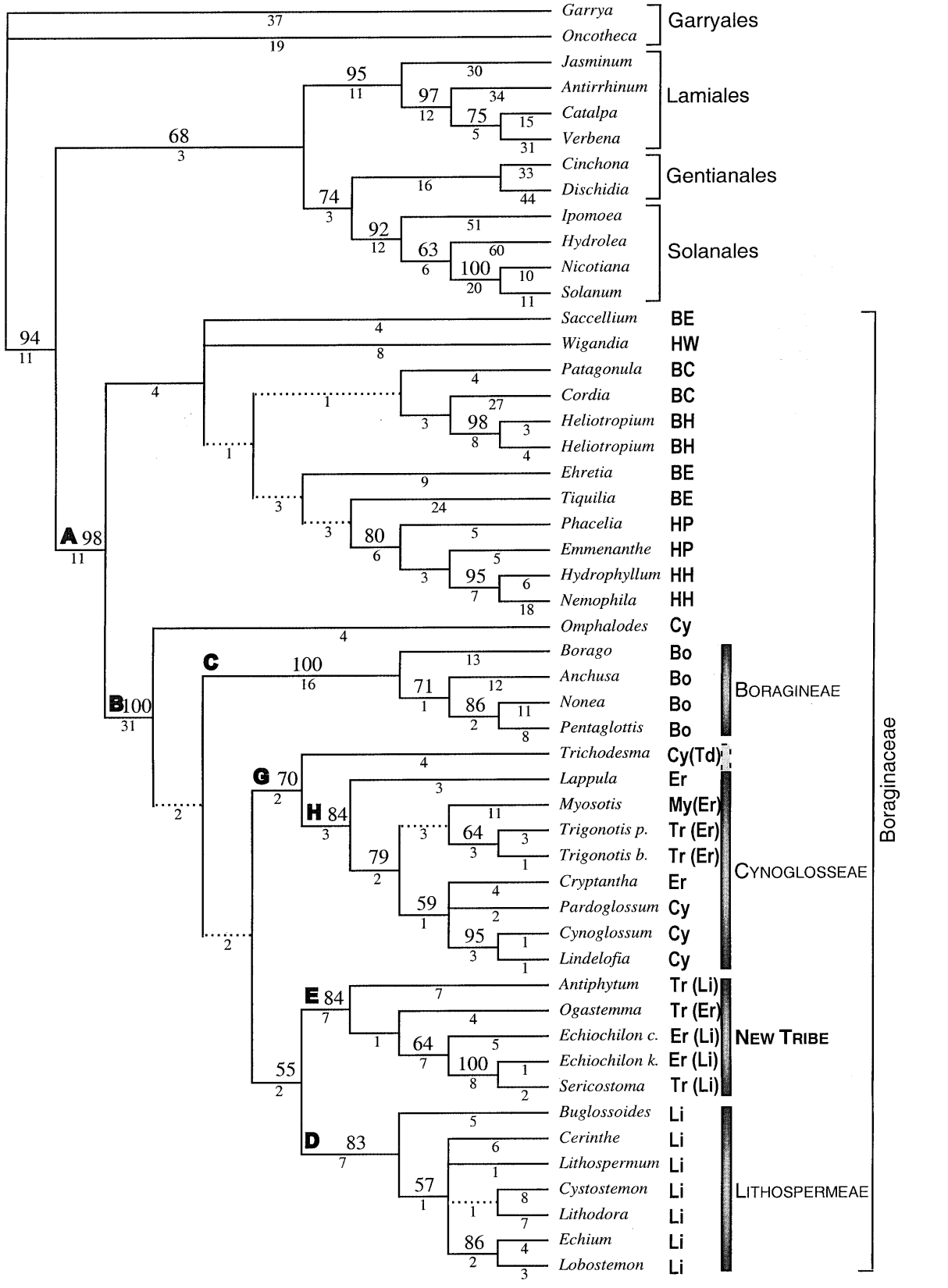
combined Eritrichieae/Cynoglosseae), forming a weakly supported clade (clade G; BP 70) together with clade H.

Discussion

Four main conclusions can be drawn from this study. 1: The family Boraginaceae and Boraginoideae are well supported, as are the component tribes Boragineae and Lithospermeae (the latter only moderately supported). 2: Cynoglosseae and Eritrichieae form a clade with the genera interdigitated. 3: The genera *Antiphytum*, *Echiochilon*, *Ogastemma*, and *Sericostoma* form a clade (BP 84) with uncertain relationships to the other tribes, hence a new tribe is formed. 4: The monotypic genus *Sericostoma* is sunk into *Echiochilon*, and a new combination needs to be made.

Boraginaceae and Boraginoideae. The cladistic analysis of *atp*B gene sequences of Boraginaceae gives strong support for Boraginaceae s. lat. and for Boraginoideae. Boraginoideae is marked by a long branch (31 steps) and strong bootstap support (BP 100). The reason for this branch being so long might be that the subfamily has experienced rapid evolution or that the split occurred a long time

Fig. 1. One of the 779 most parsimonious trees (879 steps, CI = 0.60, RI = 0.76) from analysis of the *atp*B data from 50 taxa. Node B corresponds to Boraginoideae. Numbers above the branches are bootstrap percentages, the nodes lacking numbers received less that 50% bootstrap support. Numbers below the branches are estimated numbers of substitutions (ACCTRAN optimisation). Groups not present in the strict consensus tree are indicated with dotted lines. Classification according to Al-Shehbaz (1991) and Riedl (1997) in Table 1 abbreviated as follows: the tribes of Boraginoideae: **Bo** Boraginae, **Cy** Cynoglosseae, **Er** Eritrichieae, **Li** Lithospermeae, **My** Myosotideae, **Td** Trichodesmeae, **Tr** Trigonotideae; the subfamilies of Boraginaceae except Boraginoideae: **BC** Cordioideae, **BE** Ehretioideae, **BH** Heliotropioideae; **HH** Hydrophylleae, **HN** Nameae, **HP** Phacelieae, **HW** Wiganideae. Alternative or earlier classifications are written within parenthesis


Garrya
Oncotheca
Garryales
Jasminum
Antirrhinum
Catalpa
Verbena
Lamiales
Cinchona
Dischidia
Gentianales
Ipomoea
Hydrolea
Nicotiana
Solanum
Solanales
Saccellium BE
Wigandia HW
Patagonula BC
Cordia BC
Heliotropium BH
Heliotropium BH
Ehretia BE
Tiquilia BE
Phacelia HP
Emmenanthe HP
Hydrophyllum HH
Nemophila HH
Omphalodes Cy
Borago Bo
Anchusa Bo
Nonea Bo
Pentaglottis Bo
BORAGINEAE
Trichodesma Cy(Td)
Lappula Er
Myosotis My(Er)
Trigonotis p. Tr (Er)
Trigonotis b. Tr (Er)
Cryptantha Er
Pardoglossum Cy
Cynoglossum Cy
Lindelofia Cy
CYNOGLOSSEAE
Antiphytum Tr (Li)
Ogastemma Tr (Er)
Echiochilon c. Er (Li)
Echiochilon k. Er (Li)
Sericostoma Tr (Li)
NEW TRIBE
Buglossoides Li
Cerinthe Li
Lithospermum Li
Cystostemon Li
Lithodora Li
Echium Li
Lobostemon Li
LITHOSPERMEAE
Boraginaceae
A 98
B 100
C
D 83
E 84
G 70
H 84

ago. Morphologically the subfamily is distinct with a deeply four-lobed ovary maturing to four nutlets and a basifixed style sometimes leaving a conical or subulate structure (gynobase) bearing the style in the centre between the seperating nutlets. The term gynobase is also often used for the area where the style and the nutlets are attached whether it is protruding or not.

Borageineae have BP 100 and is characterised by usually well-developed faucal appendages, a simple style with one or two stigmas, nutlets with a basal attachment scar often with an annular rim around it and a flat gynobase. Lithospermeae is moderately supported (BP 83), and the analysis of *atp*B supports the classical delimitation of the tribe with just a few genera excluded (e.g. *Echiochilon* and *Antiphytum*). Lithospermeae has the style usually divided at the apex with two to four stigmas, combined with nutlets with a broad basal attachment scar and a flat gynobase.

Cynoglosseae and Eritrichieae. Cynoglosseae and Eritrichieae form a moderately supported clade together, with their respective genera interdigitated. These two tribes share many morphological characters (Riedl 1997) and have in several investigations (e.g. Hilger 1985) been treated as sister taxa. Characters used for separating them were e.g. the usually well developed faucal appendages (scales or swellings partly closing the throat) and nutlets with an apical or subapical attachment of Cynoglosseae, compared to the members of Eritrichieae sometimes lacking faucal appendages and having dorsally keeled nutlets with a submedial attachment. Both have an undivided style with one stigma and usually striking appendages or wings on the nutlets. They also have heterocolpate pollen, unlike Lithospermeae and Borageineae which have colporate pollen. In this analysis, the genera of the two tribes are mixed. Further studies are needed to determine the position of *Eritrichium* Schrad. Cynoglosseae (W.D.J. Koch, Syn. Fl. Germ. Helv.: 496; 1837), which has priority over Eritrichieae (Gürke in Engler. and Prantl., Die natürlichen pflanzenfamilien 4 (3a): 81; 1893) will be used for clade H in the further text.

Myosotis has in recent works been accepted as forming its own tribe, Myosotideae (Popov 1953, Riedl 1967, Al-Shehbaz 1991), based on having the corolla lobes contorted in bud, rare character in Boraginoideae, smooth and often laterally compressed nutlets with a basal attachment, and a flat gynobase. In our analysis *Myosotis* fell within Cynoglosseae. *Myosotis* has heterocolpate pollen as in the rest of Cynoglosseae and also an undivided style with one stigma.

The position of *Omphalodes*, which has been referred to Eritrichieae, is unresolved within Boraginoideae.

New tribe. The genera in clade E were earlier placed in either Lithospermeae or Eritrichieae, but recently three of them were placed in Trigonotideae (Riedl 1967, 1968, 1997). Riedl (1967) formed Trigonotideae by bringing together anomalous genera with similar gynoecial development from Lithospermeae and Eritrichieae. Characters for Trigonotideae are smooth or tuberculate nutlets with an angular ventral (inner) side, an attachment scar basal or submedial and a flat gynobase. Trigonotideae is not supported by this analysis since *Trigonotis* is nested within Cynoglosseae, and *Antiphytum*, *Ogastemma*, and *Sericostoma* belong to the *Echiochilon* clade (E).

A derived character for the *Echiochilon* clade is the more or less spherical or square pollen, compared to the heteropolar or prolate pollen of Lithospermeae. Other diagnostic characters are for example a bifid style with two stigmas, a basal or submedial attachment scar (in some cases in *Antiphytum* and *Echiochilon* with a short stipe-like prolongation) and a flat to pyramidal gynobase. Both opposite and alternate leaves are found in *Antiphytum* and *Echiochilon*. *Antiphytum* (Johnston 1966) and *Ogastemma* have the basal leaves opposite and the upper alternate. Well developed faucal appendages are only present in *Antiphytum*. *Ogastemma* has five weak invaginations forming a ring below the limb, and the

Echiochilon species completely lack faucal appendages. *Antiphytum* and *Ogastemma* bear the stigmas terminal on the style, whereas in *Echiochilon* the stigmas are positioned just below or around the bifid sterile tip of the style. The pollen of *Antiphytum* (Johnston 1966) and *Ogastemma* (Lönn 1999) are three-aperturate (colpate or colporate), nearly circular in equatorial view and three-sided in polar view with the colpi on the truncate corners. The *Echiochilon* pollen are two or three-aperturate (colpate or colporate), square in equatorial view and rectangular-rounded to three-sided in polar view with the apertures situated on the sides (not on the corners; Lönn 1999).

Echiochilon has always had an uncertain position in either Eritrichieae or Lithospermeae but here forms a clade together with *Sericostoma* (from Lithospermeae ot Trigonotideae), *Ogastemma* (from Eritrichieae), and *Antiphytum* (from Lithospermeae or Trigonotideae; Cynoglosseae, de Candolle 1846), as earlier suggested by Johnston (1957). Also Seibert (1974) suggested in his study of fruit morphology in Lithospermeae that *Antiphytum*, *Echiochilon* and *Ogastemma* are closely related to each other, and that they belong in Lithospermeae. The sister group relation of the *Echiochilon* clade (clade E) to Lithospermeae (clade D) is weakly supoported by *atp*B data. The new tribe is named after *Echiochilon* which already had named a subtribe, and is the most species–rich of the taxa included at this node.

Echiochileae (H. Riedl) Långström & M.W. Chase, In: Långström E, Systematics of *Echiochilon* and *Ogastemma* (Boraginaceae), and the phylogeny of Boraginoideae, paper II, PhD - thesis, Uppsala University, Uppsala, Sweden (2002).

Basionym: Echiochilinae H. Riedl, In: Rechinger KH (Ed.) Fl. Iranica 48: 57 (1967).

Sericostoma. In the *Echiochilon* clade (clade E), there is support for including monotypic *Sericostoma* in *Echiochilon*. When *Echiochilon* was described in 1800, it was treated as a monotypic genus related to *Echium* but with bilabiate corollas. *Sericostoma* was described (in 1852) as having small actinomorphic flowers, and all *Echiochilon* species with small actinomorphic flowers ended up in *Sericostoma* whereas species with larger, zygomorphic flowers were added to *Echiochilon*. Johnston (1957) later realised that the actinomorphic-flowered species of *Sericostoma* are closely related to the species of *Echiochilon* and transferred all but one species to *Echiochilon*. *Sericostoma pauciflorum* Stocks ex Wight is a distinct species, and it ended up alone in the genus. Earlier morphological investigations of *Echiochilon* and *Sericostoma* (Lönn 1999) could not provide enough evidence for a union of the two genera, but the results of this analysis clearly show that *S. pauciflorum* is nested inside *Echiochilon*. This is corroborated by data from nuclear ribosomal ITS (internal transcribed spacer; unpubl. data) DNA sequences. Hence we transfer *Sericostoma pauciflorum* to *Echiochilon*.

Echiochilon pauciflorum (Stocks ex Wight) Långström & M.W. Chase In: Långström E., Systematics of *Echiochilon* and *Ogastemma* (Boraginaceae), and the phylogeny of Boraginoideae, paper II, PhD – thesis, Uppsala University, Uppsala, Sweden (2002). Basionym: *Sericostoma pauciflorum* Stocks ex Wight, Icon. Pl. Ind. Orient. (Wight) 4 (2): 15, t. 1377 (1848). Type: Lectotype selected by Lönn (1999): [Icon] Wight, Icon. Pl. Ind. Orient. (Wight) 4 (2): t. 1377 (1848).

Sericostoma pauciflorum var. *qeshmensis* A. Ghahreman & F. Attar (Ghahreman and Attar 1996) is a synonym to *Echiochilon kotschyi* (Boiss. & Hohen.) I.M. Johnst., which is known to occur on Queshm island in Iran (Lönn 1999).

Biogeography. The distribution of Echiochileae (clade E in Fig. 1) with an American to northern African/western Asian trans-Atlantic disjunction is shown in Fig. 2. Trans-Atlantic disjunctions between the Old World and the New World have been discussed recently (e.g. Macey et al. 1999, Thulin 1999, Sanmartín et al. 2001, Tiffney and Manchester 2001) and Lavin et al. (2000)

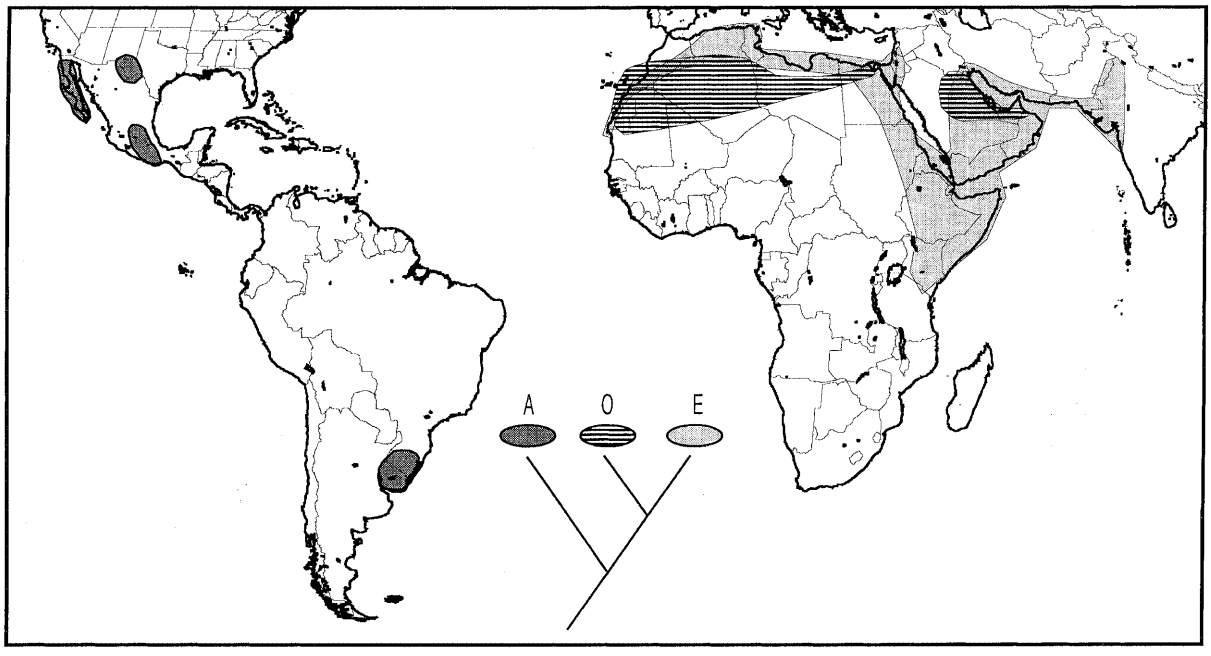


Fig. 2. Distributions of *Antiphytum*, *Ogastemma* and *Echiochilon* labelled with A, O and E in the cladogram that shows their relationships and respective pattern

predict the trans-Atlantic vicariant pattern to be common within plant families that diversified during the Tertiary in xeric and seasonally dry vegetation.

According to Raven and Axelrod (1974) the Boraginaceae are thought to have a mainly Eurasian origin with repeated introductions to the southern land masses. At least for Boraginoideae this is supported by the fact that all tribes have their main distribution in the Old World (Boragineae – almost exclusively Eurasian, Cynoglosseae s. lat. – ca 2/3 Old World, Lithospermeae – more than 2/3 Old World, Trichodesmeae – all Old World; A1-Shehbaz 1991). The old vicariance pattern between Africa and the American continent (the break-up of Gondwanaland ca 100 Myr ago) is not possible here because Echiochileae can be assumed to be much younger than 100 Myr. Wikström et al. (2001) estimated the split between Lamiales and Boraginales to be ca 77–81 Myr, and dated the split between *Borago* and *Hydrophyllum* (corresponds to clade A, Fig. 2) to ca 56–59 Myr.

A scenario for how the present distribution pattern of Echiochileae has arisen, following the hypothesis of Raven and Axelrod (1974), could be that Echiochileae originated in Eurasia, *Antiphytum* spread to North America via the Thulean Bridge which connected southern Europe to eastern North America through southern Greenland ca 70–45 Myr (Tiffney 1985, Sanmartín et al. 2001), and *Echiochilon* and *Ogastemma* (or their precursor) spread southwards because of the stepwise climatic cooling starting in the Early Eocene (Tiffney and Manchester 2001), which allowed thermophilic plants to migrate of the south.

Echiochilon and *Ogastemma* (or their precursor) could have entered Africa either during the Palaeocene (63 Myr) when Europe and Africa became connected or later in the Miocene (20–17 Myr) when the Mediterranean Basin was formed. The latter theory is the most probable because *Echiochilon* and *Ogastemma*, judging from the dating of Wikström et al. (2001), had not originated on the first

occasion. *Ogastemma* is now distributed on Fuerteventura, in the Sahara Desert, and in the northern part of Arabia to the west of the Persian Gulf (Fig. 2). The Sahara Desert was formed during the Late Miocene (10 Myr), whereas Fuerteventura, the oldest of the Canary Islands, uplifted in the Early Miocene, ca 23–20 Myr.

Future research. Further sampling and preferably also data from other regions are needed to be able to resolve the affinites of *Trichodesma* and *Omphalodes*. It would also be interesting to analyse the other members of Trichodesmeae to see if there is molecular support for the tribe suggested by Riedl (1957). A phylogenetic study of the species of *Echiochilon* is in progress and will be the basis for a biogeographic study of that group.

This work was supported by grants to Elisabeth Långström from Helge Ax:son Johnsons stiftelse and The Royal Swedish Academy of Sciences (KVA). We are grateful to Kåre Bremer, Bengt Oxelman and Mats Thulin for valuable comments on the manuscript and to Isabel Sanmartín for valuable comments on the biogeography part. Hartmut Hilger kindly contributed DNA from *Patagonula* and herbarium material of *Saccellium* and *Tiquilia*, he has also given many helpful comments on the manuscript. Nahid Heidari has been of great help in the lab. Robyn Cowan and Jeffrey Joseph are thanked for giving good advice on the lab work. The following herbaria have kindly provided herbarium material for the study: G, K, S, UPS, W and WU. Arne Anderberg, Bruno Wallnöfer and Ghebrehiwet Medhanie are gratefully acknowledged for sending material.

References

Al-Shehbaz I. (1991) The genera of Boraginaceae in the southeastern United States. J. Arnold Arbor. Suppl. Ser. 1: 1–169.

Albach D. C., Soltis P. S., Soltis D. E., Olmstead R. G. (2001) Phylogenetic analysis of asterids based on sequences of four genes. Ann. Missouri Bot. Gard. 88: 163–212.

APG (1998) An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85: 531–553.

Baillon H. (1888) Boraginacées. In: Baillon H. (ed.) Hist. Pl. (Baillon). Librairie Hachette & Cie, Paris, pp. 343–402.

Bentham G., Hooker J. D. (1873) Genera Plantarum (Bentham & Hooker). Lovell Reeve & Co, Williams & Norgate, Covent Garden, London, vol. 2.

Candolle A. de. (1846) Borrage. In: de Candolle A. (ed.) Prodr. (DC.). Fortin, Masson et sociorum, Paris, vol. 10: 1–178.

Chase M. W., Soltis D. E., Olmstead R. G., Morgan D., Les D. H., Mishler B. D., Duvall M. R., Price R. A., Hillis H. G., Qiu Y. L., Kron K. A., Rettig J. H., Conti E., Palmer J. D., Manhart J. R., Sytsma K. J., Michaels H. J., Kress W. J., Karol K. G., Clark W. D., Hedrén M., Gaut B. S., Jansen R. K., Kim K. J., Wimpee C. F., Smith J. F., Furnier G. R., Strauss S. H., Xiang Q. Y., Plunkett G. M., Soltis P. S., Swensen S. M., Williams S. E., Gadek P. A., Quinn C. J., Eguiarte L. E., Goldenberg E., Learn G. H., Graham S. W., Barrett S. C. H., Dayanandan S., Albert V. A. (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.

Chase M. W., Morton C. M., Kallunki J. A. (1999) Phylogenetic relationships of Rutaceae: A cladistic analysis of the subfamilies using evidence from *rbc*L and *atp*B sequence variation. Amer. J. Bot. 86: 1191–1199.

Diane N., Förther H., Hilger H. H. (2002) A systematic analysis of *Heliotropium*, *Tournefortia*, and allied taxa of the Heliotropiaceae (Boraginales) based on ITS1 sequences and morphological data. Amer. J. Bot. 89: 287–295.

Doyle J., Doyle J. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Ferguson D. M. (1999) Phylogenetic analysis and relationships in Hydrophyllaceae based on *ndh*F sequence data. Syst. Bot. 23: 253–268.

Fitch W. M. (1971) Toward defining the course of evolution: minimal change for a specific tree topology. Syst. Zool. 20: 406–416.

Ghahreman G., Attaar F. (1996) A floristic study report concerning Qeshm island. Iranian J. Bot. 7: 57–62.

Gürke M. (1897) Boraginaceae. In: Engler A., Prantl K. (eds.) Die natürlichen pflanzenfamilien Engelmann, Leipzig, vol. 4: 71–131.

Gottschling M., Hilger H. H., Wolf M., Diane N. (2001) Secondary structure of the ITS1 transcript and its application in a reconstruction of the phylogeny of Boraginales. Plant Biol. 3: 629–636.

Heywood V. (1993) Flowering plants of the world. B. T. Batsford, London.

Hilger H. H. (1985) Ontogenie, Morphologie und systematische Bedeutung geflügelter und glochidientragender Cynoglosseae- und Eritrichieae-Früchte (Boraginaceae). Bot. Jahrb. Syst. 105: 323–378.

Hoot S. B., Culham A., Crane P. R. (1995) The utility of *atp*B gene sequences in resolving phylogenetic relationships: comparison with *rbc*L and 18S ribosomal DNA sequences in the Lardizabalaceae. Ann. Missouri Bot. Gard. 82: 194–207.

Hoot S. B., Kadereit J. W., Blattner F. R., Jork K. B., Schwarzbach A. E., Crane P. R. (1997) Data congruence and phylogeny of the Papaveraceae s. l. based on four data sets: *atp*B and *rbc*L sequences, *trn*K restriction sites, and morphological characters. Syst. Bot. 22: 575–590.

Hutchinson J. (1969) Evolution and phylogeny of flowering plants. Academic Press, London & New York.

Johnston I. M. (1924) Studies in the Boraginaceae - III. Contr. Gray Herb. 73: 42–78.

Johnston I. M. (1957) Studies in the Boraginaceae, XXIX. *Echiochilon* and related genera. J. Arnold Arbor. 38: 255–293.

Johnston I. M. (1966) Boraginaceae. In: Lundell C. L. (ed.) Flora of Texas. Texas Research Foundation, Renner, Texas, 1: 123–221.

Lavin M., Thulin M., Labat J.-N., Pennington R. T. (2000) Africa, the odd man out: molecular biogeography of Dahlbergioid legumes (Fabaceae) suggest otherwise. Syst. Bot. 25: 449–467.

Lönn E. (1999) Revision of the three Boraginaceae genera *Echiochilon*, *Ogastemma* and *Sericostoma*. Bot. J. Linn. Soc. 130: 185–259.

Mabberley D. J. (1997) The Plant-Book. Cambridge University Press, Cambridge.

Macey J. R., Schulte J. A. II, Larsson A., Tuniyev B. S., Orlov N., Papenfuss T. J. (1999) Molecular phylogenetics, tRNA Evolution, and historical biogeography in Anguid lizards and related taxonomic families. Molec. Phylogenet. Evol. 12: 250–272.

Noteboom H. P., de wilde W. J., Kirkup D. W., Stevens P. F.

Olmstead R. G., Michales H. J., Scott K. M., Palmer J. D. (1992) Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbc*L. Ann. Missouri Bot. Gard. 79: 249–265.

Olmstead R. G., Bremer B., Scott K. M., Palmer J. D. (1993) A parsimony analysis of the Asteridae sensu lato based on *rbc*L Sequences. Ann. Missouri Bot. Gard. 80: 700–722.

Olmstead R. G., Kim K.-J., Jansen R. K., Wagstaff S. J. (2000) The phylogeny of the Asteridae sensu lato based on chloroplast *ndh*F gene sequences. Molec. Phylogenet. Evol. 16: 96–112.

Pilger R. (1912) Die Gattung *Wellstedia* in Südwestafrika. Bot. Jahrb. Syst. 46: 558–561.

Popov M. G. (1953) Boraginaceae. In: Shishkin B. K. (ed.) Flora SSSR. Izdatel'stvo Akademii Nauk SSSR, Moskva-Leningrad, vol. 19: 97–691. Translation by: R. Lavoott (1974). Keter Publishing House Jerusalem Ltd., Jerusalem, vol. 19: 73–507.

Popova T. N., Zemskova E. A. (1995) Palynomorphology study of some species of Boraginaceae (subfamily Boraginoideae). Bot. Zhurn. (St. Petersburg) 80: 1–13.

Raven P. H., Axelrod D. I. (1974) Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 61: 539–673.

Retief E., Van Wyk A. E. (1997) Palynology of southern African Boraginaceae: The genera *Lobostemon*, *Echiostachys* and *Echium*. Grana 36: 271–278.

Riedl H. (1967) Boraginaceae. In: Rechinger K. H. (ed.) Flora Iranica. Akademische Druck-u. Verlagsanstalt, Graz, Austria, vol. 48: 1–281.

Riedl H. (1968) Der neue Tribus Trigonotideae und das System der Boraginoideae. Oesterr. Bot. Z. 115: 291–321.

Riedl H. (1997) Boraginaceae. In: Kalkman C., Noteboom H. P., de Wilde W. J., Kirkup D. W., Stevens P. F. (eds.) Flora Malesiana, Ser. 1, Spermatophyta Publications Department, Rijksherbarium, Leiden, 13: 43–168.

Sanmartín I., Enghoff H., Ronquist F. (2001) Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc. 73: 345–390.

Sauvage C., Vindt J. (1954) Flore du Maroc. Trav. Inst. Sci. Cherifien, Ser. Bot.: 3, vol 2, Editions Internationales, Tanger.

Savolainen V., Chase M. W., Hoot S. B., Morton C. M., Soltis D. E., Bayer C., Fay M. F., Bruijn A. Y. D., Sullivan S., Qiu Y.-L. (2000) phylogenetics of flowering plants based on Combined analysis of plastid *atp*B and *rbc*L gene sequences. Syst. Biol. 49: 306–362.

Seibert J. (1974) Fruchtanatomische Untersuchungen an Lithospermeae (Boraginaceae). Diss. Bot. 44.

Soltis D. E., Soltis P. S., Chase M. W., Mort M. E., Albach D. C., Zanis M., Savolainen V., Hahn W. H., Hoot S. B., Fay M. F., Axtell M., Swensen S. M., Prince L. M., Kress W. J., Nixon K. C., Farris J. S. (2000) Angiosperm phylogeny inferred from 18S rDNA, *rbc*L, and *atp*B sequences. Bot. J. Linn. Soc. 133: 381–461.

Swofford D. L. (1998) Phylogenetic analysis using parsimony, 4.0. Sinauer, Sunderland, Massachusetts.

Takhtajan A. L. (1997) Diversity and classification of flowering plants. Columbia University Press, New York.

Thulin M. (1999) Chapmannia (Leguminosae-Stylosanthinae) extended. Nord. J. Bot. 19: 597–607.

Tiffney B. H. (1985) The Eocene North Atlantic land bridge: its importance in tertiary and modern phytogeography of the Northern Hemisphere. J. Arnold Arbor. 66: 243–273.

Tiffney B. H., Manchester S. R. (2001) The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere tertiary. Int. J. Plant Sci. 162 (6 Suppl.): S3–S17.

Verdcourt B. (1991) Boraginaceae. In: Polhill R. M. (ed.) Flora of Tropical East Africa. Brookfield, Balkema, A. A., Rotterdam.

Wikström N., Savolainen V., Chase M. W. (2001) Evolution of the angiosperms: calibrating the family tree. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 268: 2211–2220.

Addresses of the authors: Elisabeth Långström (e-mail: elisabeth.langstrom@ebc.uu.se), Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden. Mark W. Chase, Jodrell Laboratory, Royal Botanic Gardens, Kew, TW9 3DS, UK.