

Fossil and Extant Western Hemisphere Boragineae, and the Polyphyly of “Trigonotideae” Riedl (Boraginaceae: Boraginoideae)

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Abstract—Boraginaceae tribe Trigonotideae comprises a heterogenous assemblage of taxa, many of which have been shown to belong to widely divergent lineages in Boraginaceae in the recent past, with some taxa now assigned to three of the four currently recognized tribes of the Boraginaceae s. s., namely the Cynoglosseae, Echiochileae, and Lithospermeae. The systematics of *Moritzia* and *Thaumatocaryon*, the only endemic South American genera of Boraginaceae, have been controversially discussed in the past, and their most recent placement was in Trigonotideae. The present study investigates the phylogenetic relationships of “Trigonotideae” based on micromorphology and molecular data (ITS including 5.8S rRNA, and the *trnL-trnF* spacer). Molecular data show that “Trigonotideae” are polyphyletic, and none of its members is at all closely related to *Trigonotis* itself. *Moritzia* and *Thaumatocaryon* are closely allied to each other and are the sister group of the Old World Boragineae. Flowers, pollen, and fruit morphology strongly support this systematic placement. Extant (native) Boragineae are absent from North America and had not previously been reported from South America, whereas members of the Cynoglosseae, Echiochileae, and Lithospermeae have been reported from both continents. *Moritzia* and *Thaumatocaryon* are thus the only native representatives of Boragineae in the Americas and represent an unexpected western Eurasian/South American disjunction. However, several (widespread and abundant) fossil taxa from the Cenozoic of North America (especially species of †*Prolithospermum*) can be confidently placed into Boragineae. Extant *Moritzia*/*Thaumatocaryon* likely go back to a lineage which reached North America from Europe and then migrated into South America, with subsequent extinction in North America.

Keywords—fossils, molecular phylogeny, *Moritzia*, nutlets, palynology, *Thaumatocaryon*.

Boraginaceae s. s. (= Boraginaceae subfam. Boraginoideae; Gürke 1893) comprise some 1600 species in approximately 100 genera and have their centre of diversity in Eurasia, with fewer species in Africa, Australia, and the Americas (Al-Shehbaz 1991; Riedl 1997). Intrafamilial classification has been widely divergent between different authors, with up to 13 tribes and eight subtribes recognized (Popov 1953). The most recent listing of tribes has been provided by Riedl (1997), recognizing the six tribes Boragineae, Cynoglosseae DC., Eritrichieae Benth. & Hook., Lithospermeae Dumort., Myosotideae Reichenb., and Trigonotideae Riedl. Conversely, recent molecular studies (Långström and Chase 2002; Långström and Oxelman 2003) retrieved Eritrichieae, Myosotideae, and Trigonotideae as nested within Cynoglosseae s. l. The authors therefore advocated the reduction of these three tribes under the latter group, reducing the number of tribes to three. Moreover, an additional and distinct lineage comprising several Old World and New World taxa was recognized and formalized as Echiochileae (Riedl) Långström & M. W. Chase (Lönn 1999; Långström and Chase 2002; Långström and Oxelman 2003).

Traditionally, fruit morphology has been used as the most important character complex for the classification of Boraginales (Gürke 1893; Seibert 1978; Hilger 1985; Gottschling and Hilger 2001; Gottschling et al. 2005), but a comprehensive overview is still wanting. The nutlets of Cynoglosseae s. l. (i.e. including Eritrichieae, Myosotideae, Trichodesmeae) are glochidiate and/or dorsiventrally compressed and often marginally winged (Hilger 1985). The fruits of Echiochileae and Lithospermeae are more or less ovoid, frequently keeled, and sometimes slightly dorsiventrally compressed, strongly incurved, and/or grossly rugose (Seibert 1978). Most characteristically, their fruit wall is strongly sclerenchymatous and more or less extensively calcified (Seibert 1978). Fruit morphology of Boragineae has been studied relatively exten-

sively and is characteristic (Gürke 1893; Guşuleac 1923; Hilger 1985): Nutlets are straight to incurved, laterally compressed, and more or less distinctly keeled. The surface is slightly to strongly tessellate and papillose. The base is usually wide with a distinct basal ring, and the nutlets bear a characteristic elaiosome on their attachment scar.

In addition to the extant data on nutlet morphology, there is a rich fossil record of putative Boraginaceae nutlets from the Miocene and Pliocene of North America (Gabel 1987; Gabel et al. 1998; Segal 1966; Thomasson 1977, 1979). The abundance and diversity of fossil nutlets indicate that the family was both abundant and diverse as early as the Cenozoic, and many fossils are characteristic enough to be associated with extant lineages or genera. Some can be assigned to the *Lithospermum*-species group of Lithospermeae (†*L. dakotense*; Gabel 1987; Gabel et al. 1998; Weigend et al. 2009), others to the Cynoglosseae (*Cryptantha* and †*Prolappula*; Segal 1966; Thomasson 1979). Three fossil taxa (†*Biorbia*, †*Eliasiana*, and †*Prolithospermum*) have been tentatively assigned to Boragineae (Thomasson 1979), a group not at present native to North America.

South America has only some 90 species of Boraginaceae, falling into 11 genera (Johnston 1927), which belong to three of the four tribes based on the classification of Långström and Chase (2002) and Långström and Oxelman (2003). *Amsinckia* Lehm., *Cryptantha* Lehm. ex Fisch. & Mey., *Cynoglossum*, *Hackelia*, *Myosotis*, *Pectocarya* DC. ex Meisn., and *Plagiobothrys* fall into the largest tribe, Cynoglosseae s. l. (Gürke 1893; Johnston 1924, 1927), *Antiphytum* belongs to Echiochileae (Långström and Chase 2002), and *Lithospermum* is the type genus of Lithospermeae. They all have their centers of diversity in North America or the Old World. Only *Moritzia* DC. ex Meisn. and *Thaumatocaryon* Baill. (with three species each) are endemic to South America. They are largely restricted to

south-eastern South America (northern Argentina to southern Brazil; Johnston 1927; Smith 1970; Fernandes-Soares 1973; Ariza-Espinar 2006; Keller 2007), with one species of *Moritzia* ranging to the American Cordillera between central Peru and Costa Rica (Gentry 1972; Brako and Zarucchi 1993; Jørgensen and León-Yáñez 1999). Whereas the phylogenetic relationships of the other American Boraginaceae have been satisfactorily clarified, the affinities of these two South American endemics are still unclear.

Moritzia was originally considered as closely allied to *Anchusa* (Boraginaceae, Meisner 1837-1842; De Candolle 1846), then considered as allied to *Myosotis* and placed into Lithospermeae together with that genus (Bentham and Hooker 1876; Gürke 1893; Johnston 1927) and then placed it into the Eritrichieae (now Cynoglosseae p. p., Johnston 1954). *Thaumatocaryon* was explicitly described as closely allied to *Moritzia* by Baillon (1890; Johnston 1927). *Thaumatocaryon* is placed into Lithospermeae in a recent floristic treatment (Ariza-Espinar 2006), but fruit anatomical studies of Seibert (1978) already showed that *Moritzia* and *Thaumatocaryon* have no relationship to that tribe. Riedl (1967, 1968) tried to resolve the systematic placement of several poorly understood taxa of Boraginaceae by the creation of the "Trigonotideae". It comprises morphologically heterogeneous plants such as *Antiphytum*, *Moritzia*, *Sericostoma* Stocks, *Thaumatocaryon*, and *Trigonotis*. This group has not been widely accepted, and critical fruit anatomical (Seibert 1978) and molecular studies (Långström and Chase 2002; Thomas et al. 2008) led to the removal of several of its constituent taxa into other tribes. Most importantly, *Trigonotis* appears to be allied to *Myosotis* and is deeply nested in Cynoglosseae (Långström and Chase 2002), so that the tribe "Trigonotideae" formally falls into the Cynoglosseae. Trigonotideae subtribe Moltkiopsidinae Riedl has been formally removed into Lithospermeae (Seibert 1978; Thomas et al. 2008), and both *Antiphytum* and *Sericostoma* (and therefore Trigonotideae subtribe Antiphytinae Riedl) have been transferred to the Echiochileae on the basis of molecular and morphological data (Långström and Chase 2002): Neither tribe "Trigonotideae" nor subtribe Antiphytinae exists any longer as circumscribed by Riedl (1968), and a novel, alternative placement has not so far been suggested for *Moritzia* and *Thaumatocaryon*.

The present study addresses the systematic position of the two orphaned representatives of "Trigonotideae", *Moritzia* and *Thaumatocaryon*, on the basis of both molecular (*trnL-trnF* spacer and ITS including 5.8S rRNA) and micromorphological data (fruit, pollen). It also aims at clarifying the systematic placement of the remaining genera of "Trigonotideae" and at integrating the North American fossil record into a historical understanding of the origins of *Moritzia* and *Thaumatocaryon*.

MATERIALS AND METHODS

Plant Material and Microscopy—Specimens from the following herbaria were revised: B, BSB, F, M, MSB, MO, and NY. One species, *Moritzia lindenii* Benth. ex Gürke, was collected in the field in Peru (Weigend et al. 5436). Herbarium material for scanning electron microscopy (SEM) was mounted directly (air dried) onto double-sided adhesive tape, without prior treatment, and sputter coated with gold. The liquid-preserved (FAA) material was previously dehydrated in a graded ethanol series, critical point dried following the standard procedure, and then mounted and sputter coated. Pollen was mounted directly and without any previous treatment. The material was subsequently viewed and photographed with a LEO VP 430 SEM. Vouchers of all specimens seen of *Moritzia* and *Thaumatocaryon*, and for the morphological data of allied taxa, are provided in Appendix 2.

Sampling—Sequences of two species of *Thaumatocaryon* and one species of *Moritzia* were included in the molecular data matrix, covering most of the taxa considered as allied to *Thaumatocaryon* and *Moritzia* by Riedl (1967, 1968), such as *Trigonotis* and *Mertensia* Roth, and representatives of the four subordinate taxa of the Boraginaceae s. s. currently recognized (Långström and Chase 2002; Weigend et al. 2009). The final data matrices thus comprised 56 (*trnL-trnF*) and 32 sequences (combined marker analysis) of Boraginaceae. New World groups in Cynoglosseae s. l. were also represented. Sequences of Cordiaceae, Heliotropiaceae, and Hydrophyllaceae were used as outgroup representatives. Based on the results of the single-marker analysis (*trnL-trnF*) sampling was reduced for the combined marker analysis, and more distantly allied taxa were excluded. This data matrix had members of Echiochileae as outgroup and included a reduced sampling of Lithospermeae and an extensive sampling of Boraginaceae Voucher information and GenBank accession numbers are provided in Appendix 1.

DNA Extraction, Amplification, and Sequencing—DNA extraction, PCR, purification, and sequencing followed standard protocols as described in detail in Gottschling and Hilger (2001). The same primers were used for amplification and sequencing. The *trnL-trnF* spacer was amplified with primers c and f of Taberlet et al. (1991), the primers P5 and P4 of White et al. (1990) were used for ITS. Cycle sequencing was carried out with the BigDye® Terminator v1.1 cycle sequencing kit (Perkin Elmer, Foster City, California), with a total reaction volume of 5 µl. Initial denaturation (96°C for 1 min) was followed by 30 cycles of denaturation (96°C for 30 s), annealing (52°C for 20 s), and elongation (60°C for 4 min). Samples were sequenced on an Applied Biosystems 3130xl genetic analyzer.

Alignment and Comparative Sequence Analysis—The initial sequence data were edited using ChromasPro V. 1.33 (Technelysium Pty. Ltd. 2003–2005). Aligning was performed by hand (Align Ver.07/04, Hepperle 2004), and the final matrices are deposited in TreeBASE (study number S2483). Maximum likelihood (ML) based phylogenetic analyses were conducted using RAxML (Stamatakis 2006; Stamatakis et al. 2008), applying a GTR substitution matrix with estimating the proportion of invariable sites and per gene branch length optimization. We executed 10-tree searches from distinct random stepwise addition sequence maximum parsimony (MP) starting trees and 1,000 nonparametric bootstraps with RAxML under the partition data mode. The bootstrap support values were drawn on the best-scoring ML tree.

Bayesian phylogenetic analyses were performed with BEAST (v1.4.7; Drummond, and Rambaut 2007) on a Linux-cluster at the North German High Performance Computer, consisting of 180 cores with 32 processors and 64 GB of memory each. The settings were those recommended for interspecific data that might or might not satisfy the molecular clock. A Yule branching process (appropriate for divergent rather than reticulate relationships) with a uniform prior was adopted. For the (best fitting) GTR + Γ + i nt substitution model with four discrete categories, we applied a relaxed clock with rate variation across branches modeled using a lognormal distribution and initially assumed to be uncorrelated. The unweighted pair group method with arithmetic mean was used to construct a starting tree for BEAST analyses, and the final topology was estimated by combining three independent chains each of 20,000,000 generations, sampling every 1,000th iteration. Tracer 1.4 (Rambaut and Drummond 2007) was used to confirm adequate combining of the MCMC chains and appropriate burn-in (10%).

RESULTS

Micromorphology—**FLORAL MORPHOLOGY**—The flowers of *Moritzia* and *Thaumatocaryon* are radially symmetrical and pentamerous, with a largely united calyx (one half to two thirds of its length in flower; Figs. 1 B, D, F, H, J). In *Moritzia*, the calyx lobes have hooked (uncinate) trichomes on the outside (Figs. 1 H, J, L, 2A). The corolla is usually hypocrateriform, with a well-developed cylindrical tube and five spreading, rarely erect corolla lobes. Faucal scales are well developed (*M. ciliata* DC. ex Meisn.) or indistinct (other species) and present mainly in the form of long trichomes with a conspicuously papillose surface (Figs. 1 C, E, G, I, K, 2B). The corolla lobes are ad- and abaxially densely papillose and pubescent (Fig. 2C). The basal scales (annulus) are very weakly developed and present in the form of an indistinct thickening near the base of the corolla tube. A distinct nectary ring with secretory ducts

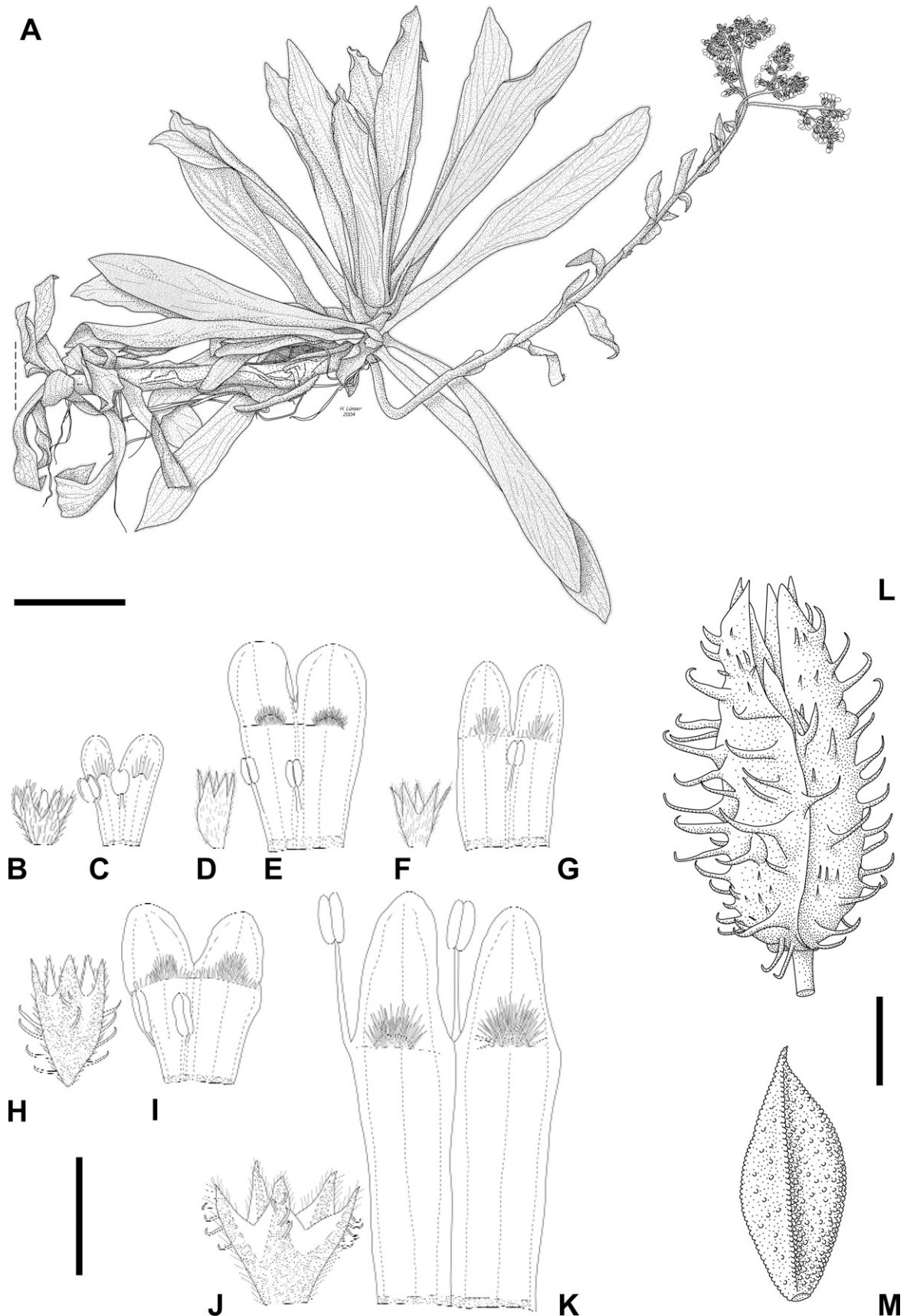


FIG. 1. Habit, flower and fruits of *Moritzia* and *Thaumtocaryon*. A–E. *Moritzia lindenii* (Weigend et al. 5346). A. habit. B. Calyx. C. Adaxial side of corolla (partial). D. Fruiting calyx. E. Nutlet, ventral view. F–G. *M. dusenii* (Hatschbach 30567). F. Calyx. G. Adaxial side of corolla (partial). H–I. *Thaumtocaryon dasyanthum* (Hatschbach 15402). H. Calyx. I. Adaxial side of corolla (partial). J–K. *T. tetraquetrum* (Hatschbach 23011). J. Calyx. K. Adaxial side of corolla (partial). L–M. *T. sellowianum* (Gibbs et al. 3417). L. Calyx. M. Adaxial side of corolla (partial).

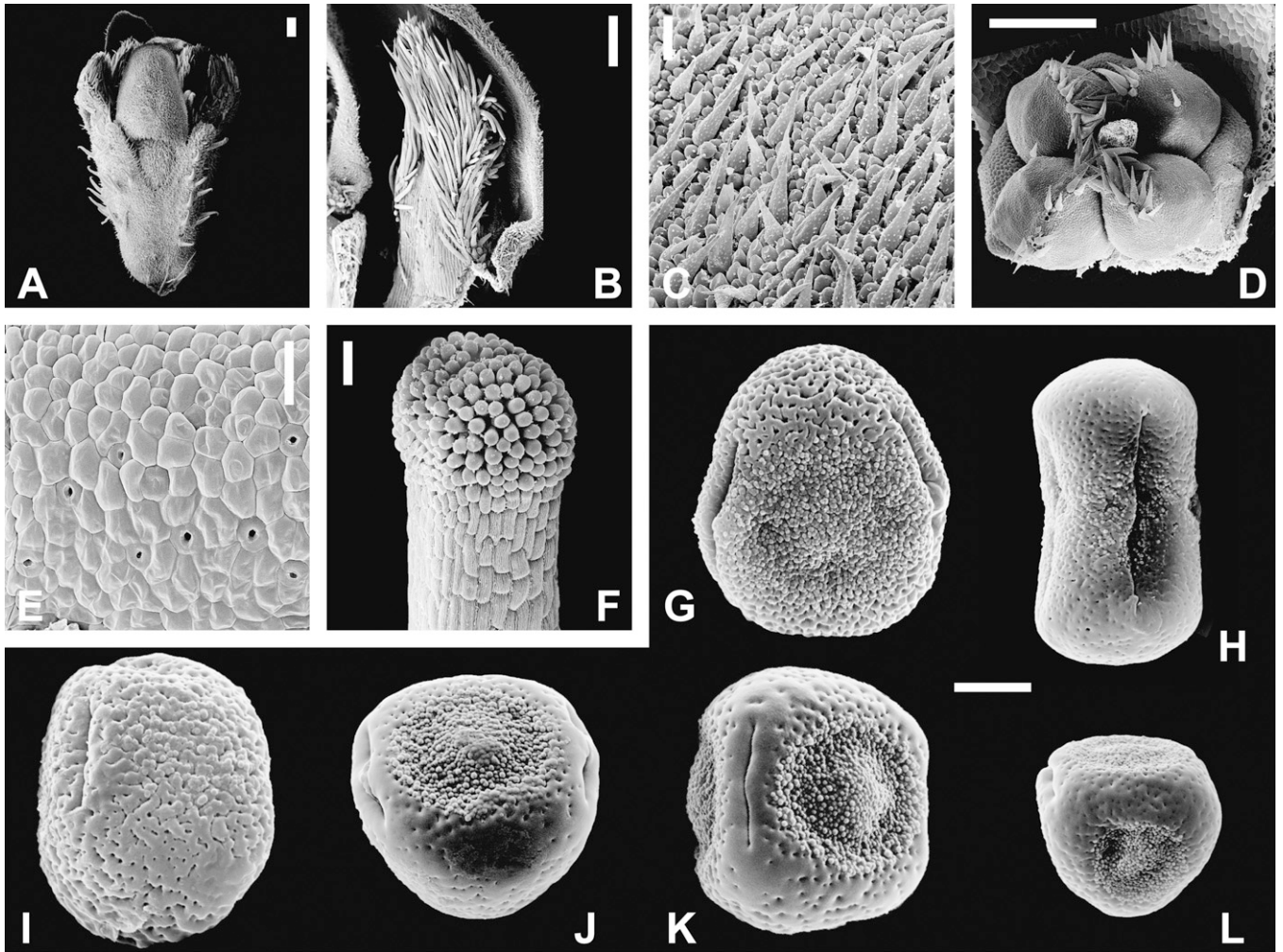


FIG. 2. Flower and pollen morphology of *Moritzia* and *Thaumatacaryon*. A–F. *Moritzia ciliata* (Forstreuter s. n.). A. Flower bud, note uncinata trichomes on the calyx. B. Penicillate (or hairy) faucal scale. C. Adaxially papillose and pubescent petal surface. D. Gynoecium with four mericarpids and style base in the centre. E. Disc nectary at the base of gynoecium with secretory stomata. F. Capitata stigma with receptive surface formed by simple papillae of the *Borago-Symphytum* type. G. *Moritzia lindenii*, pollen grain, equatorial view (Eriksen 58018). H. *Moritzia dusenii*, pollen grain, equatorial view (G. L. Smith 1107). I. *M. ciliata*, pollen grain, oblique equatorial view (Eugenio 275). J–K. *T. tetraquetrum*, pollen grains, oblique equatorial view with distinct mesocolpial fields (Hatschbach 23011). L. *T. sellowianum*, pollen grain, oblique polar view with distinct meso- and apocolpial fields (Gibbs et al. 3417; scale bars: 500 µm, Figs. C–D, G; 50 µm, Figs. F, H–I; 5 µm, Figs. J–L).

is found (Fig. 2E) between the base of the corolla tube and the basis of the four mericarpids (Fig. 2D). The style is filiform and gynobasic and inserted between the four mericarpids (Fig. 2D). The stigma is obscurely bilobed or capitata in *M. ciliata* (Fig. 2F). The receptive surface of the stigma consists of unicellular papillae without a lobed plate-like cap at the tip. The usually short filaments are epipetalous and usually included in the corolla tube, but long exserted in *M. dusenii* I. M. Johnst.

POLLEN—*Moritzia* pollen is prolate and ca. 20 µm long (polar length) and 10 µm wide (equatorial length, P/E = ca. 2). It is subcircular in the equatorial plane, sometimes with a slight equatorial constriction in *M. dusenii* (Fig. 2H). Grains are three-colpate, with narrowly fusiform apertures 12 µm long. The tectum is microreticulate in *M. dusenii* and *M. ciliata* and perforate in *M. lindenii*. The mesocolpial region is either finely rugose from granular thickenings (*M. lindenii*, Fig. 2G) or small, indistinctly rugose (*M. ciliata*, Fig. 2I) or large, distinctly rugose (*M. dusenii*, Fig. 2H). *Thaumatacaryon* is palynologically distinct from *Moritzia*. Grains are tetrahedral or

hexahedral in shape and ca. 10 µm wide. They are triangular in the equatorial plane (Fig. 2J–K) and have a perforate tectum. Grains are three-colpate and have distinct verrucose mesocolpial fields. Pollen grains may additionally have distinct verrucose apocolpial field at one or both poles (Fig. 2L). Sculpturing of the apocolpial fields is identical to that of the mesocolpial fields.

FRUIT—The fruits of *Moritzia* and *Thaumatacaryon* are always one-seeded and consist of a single nutlet, the other mericarpids are abortive (Fig. 3E). Nutlets are 2.5 × 1.5–3 × 2 mm in size and remain firmly fixed in the accrescent calyx at maturity. The nutlets of *Thaumatacaryon* are longer than the calyx and their tip protrudes from the apex of the calyx for ca. ¼ of their length (Fig. 3A), the nutlets of *Moritzia* are completely enclosed (Fig. 1L). Nutlets are laterally compressed, narrowly elliptical in dorsal view, and oblique ovoid-triangular in lateral view (Figs. 1M, 3B, F, G). The nutlet surface is indistinctly tessellate in *Moritzia* (Fig. 3G), but not in *Thaumatacaryon* (Fig. 3B). Epidermis cells are protracted into papillae (Fig. 3H), and the nutlets are distinctly stalked (Figs. 3B, F, G). The stalk is ca.

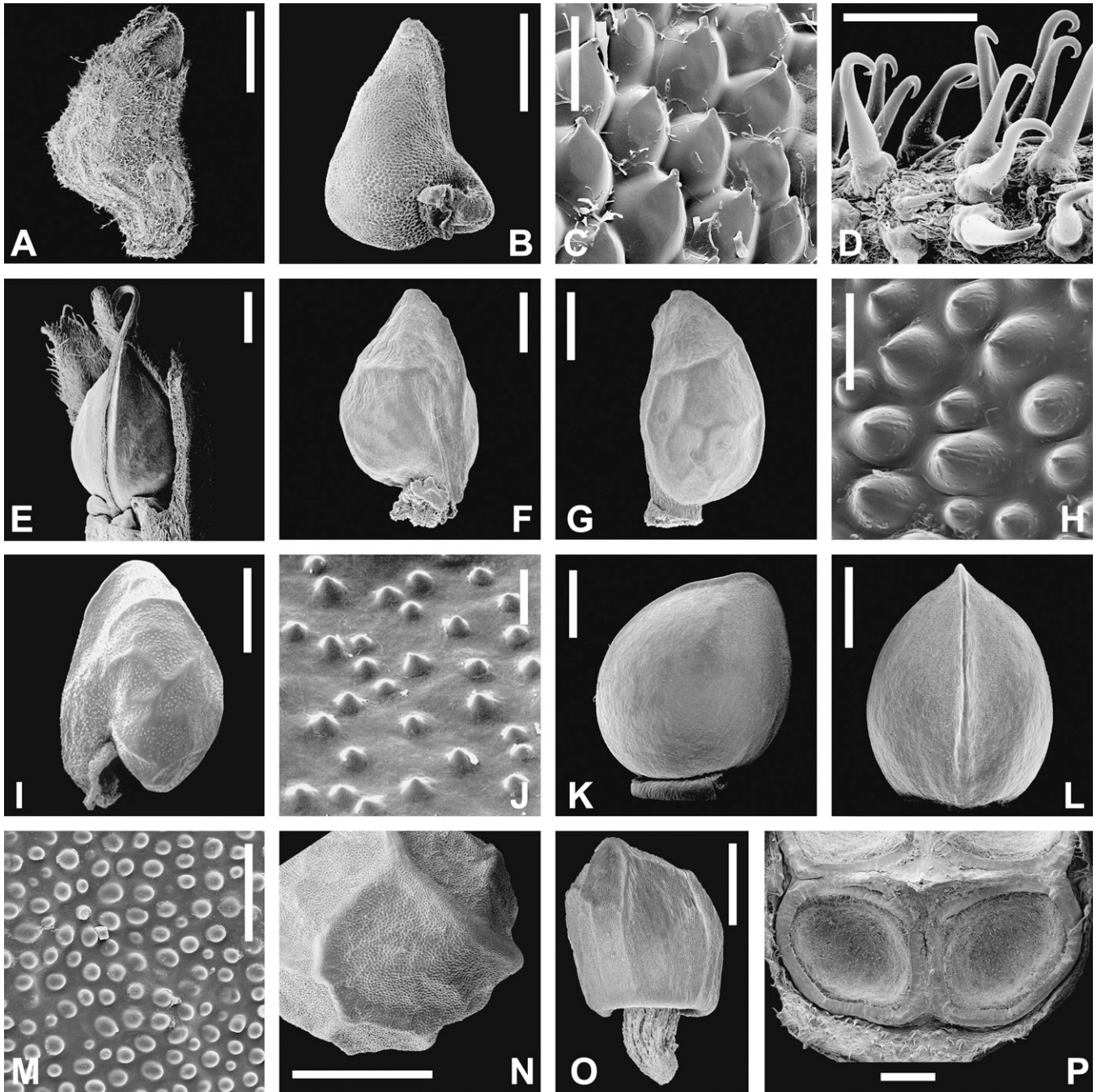


FIG. 3. Fruit morphology of *Borago*, *Moritzia*, *Pentaglottis*, *Pulmonaria*, and *Thaumtocaryon*. A–C. *T. dasyanthum* (Pedersen 15946). A. mature fruit enclosed in pubescent calyx. B. mature fruit liberated from calyx showing thin basal stalk. C. detail of papillose fruit epidermis. D. *M. lindenii*, uncinata trichomes on the outside of the calyx (Whitmore 86). E–H. *M. ciliata* (Forstreuter s. n.). E. single fully developed nutlet with un or underdeveloped (abortive) other mericarpids in partially open calyx. F. mature nutlets, oblique ventral view showing distinct ventral keel and obscurely tessellate surface. G. mature nutlet, lateral view, showing the short, broad stalk and the obscurely tessellate surface. H. *M. lindenii*, detail of nutlet epidermis (Weberbauer 8081). I–J. *Pentaglottis sempervirens*, mature nutlet, lateral view (Weigend 9065). I. mature nutlet, lateral view. J. detail of papillose epidermis. K–L. *Pulmonaria mollissima* (Schultka s. n.). K. mature nutlet, lateral view, showing the typical collar-like basal ring below constriction. L. mature nutlet, ventral view, showing distinct keel. M. detail of papillose nutlet epidermis. N–P. *B. pygmaea* (Weigend 9076). N. mature nutlet apical view, showing the tessellate and papillose nutlet surface. O. mature nutlet, lateral view, showing broad base and protruding elaiosome. P. *B. officinalis*, deeply concave scars left by shed nutlets (Ackermann 740; scale bars: 1 mm, Figs. B–C, F–H, J, L–M, O–P; 500 μ m, Fig. G; 50 μ m, Figs. D, I, K, N).

0.5 mm long and 0.2 mm wide in *Thaumtocaryon* and ca. 0.2 mm long and 0.5 mm wide in *Moritzia*. The nutlet remains firmly attached to the gynobase and enclosed in the calyx at maturity, an elaiosome is absent. Fruits break off at the pedicel for dispersal.

The fruits of other Boraginaceae are usually broadly sessile [e.g. *Borago pygmaea* (DC.) Chater and Greuter, Fig. 3O],

sometimes with a constriction near the base, and then abruptly widened in a collar-like basal ring (*Pulmonaria* L., Figs. 3K, L). Nutlet surface is typically papillose (Figs. 3J, M), often with an irregularly tessellate structure (Figs. 3I, N). The fruits of western European *Pentaglottis* Tausch (Figs. 3I) are similar to those encountered in *Moritzia* and *Thaumtocaryon*. They are provided with a distinct, albeit curved stalk, have

no basal dilation ("ring"), but differ in having a small elaisome on the cicatrix. The body of the fruit is laterally compressed, obscurely tessellate, and densely papillose. In *Pulmonaria*, nutlets are laterally compressed, ovoidal in dorsal view, ventrally and dorsally keeled, and slightly oblique, also showing similarity to the nutlets of *Moritzia* and *Thaumatocaryon*. However, all Boragineae, including *Pentaglottis*, have the typical elaisome, formed by a part of the receptacle, breaking off with the fruit (Fig. 3O), and leaving a concave scar in the receptacle (Fig. 3P). Also, all nutlets of Eurasian Boragineae become readily detached from the receptacle when barely mature and are dispersed individually from the widely open calyx.

MOLECULAR DATA—Two datasets were investigated: *trnL-trnF* alone and ITS-*trnL-trnF*. Information on the alignments is provided in Table 1. Highly supported, contradicting tree topologies were not observed in single gene analyses, indicating parallel evolution of the two loci. The most comprehensive sampling was used for the *trnL-trnF* analysis (Fig. 4). Boragineae were monophyletic and fell into four well-supported clades: Echiochileae (100 ML bootstrap support value: LBS, 1.00 Bayesian posterior probability: BPP), Cynoglosseae s. l. (100 LBS, 0.94 BPP), Boragineae (99 LBS, 0.96 BPP), and Lithospermeae (97 LBS, 0.99 BPP). Boragineae and Lithospermeae constituted sister groups (85 LBS), whose closest relatives were Cynoglosseae (80 LBS). *Trichodesmeae* (i.e. *Trichodesma* R. Br. and *Caccinia* Savi, 99 LBS, 1.00 BPP) was retrieved as sister group to the remainder of Cynoglosseae (80 LBS, 0.91 BPP).

Taxa formerly assigned to "Trigonotideae" were retrieved at six different positions in the DNA tree, covering three tribes: (i) *Bothriospermum* Bunge, *Mertensia*, and *Trigonotis* were deeply nested as distinct lineages within the Cynoglosseae. *Bothriospermum* was closely allied to Eurasian *Cynoglossum* s. s. (79 LBS, 0.98 BPP), with American Cynoglosseae (i.e. *Amsinckia*, *Cryptantha*, and *Plagiobothrys*) as its sister group (100 LBS, 1.00 BPP). *Mertensia* was the sister group of Eritricheae (64 LBS), and East Asian *Trigonotis* was closely allied to cosmopolitan *Myosotis* (99 LBS, 1.00 BPP). (ii) *Mairetis* I. M. Johnst. and *Neatostema* I. M. Johnst. were closely related to each other (97 LBS, 1.00 BPP) and were retrieved deeply nested in Lithospermeae, with *Cerintho* L. as sister group (66 LBS). (iii) *Moritzia* and *Thaumatocaryon* were retrieved on a single, well-supported branch (100 LBS, 1.00 BPP), as closest relatives of Boragineae s. s. (94 LBP, 0.99 BPP). This sister group relationship was well supported (99 LBP, 0.96 BPP).

The combined data set ITS-*trnL-trnF*, using Echiochileae as outgroup (Fig. 5), rendered basically the same results and retrieved Lithospermeae (100 LBS, 0.94 BPP) and Boragineae (100 LBS) as sister groups. Subclades within Boragineae were not clearly resolved in their position. However, *Moritzia* and *Thaumatocaryon* were again closely allied (100 LBS, 1.00 BPP) and constituted the sistergroup of the Eurasian Boragineae.

TABLE 1. Primary sequence analysis and optimal model parameters of the different alignment partitions.

Alignment	# taxa	# distinct alignment patterns (per taxon)	alpha (RAxML)		-ln L
			<i>trnL-trnF</i>	ITS	
<i>trnL-trnF</i>	56	563 (10.05)	1.542668	—	5,465.74
ITS- <i>trnL-trnF</i>	32	649 (20.28)	1.023296	0.430089	8,345.74

DISCUSSION

The Four Tribes of Boragineae—The *trnL-trnF*-data confirm previous studies (Långström and Chase 2002) and retrieve four clades of Boragineae s. s. at the highest taxonomic level, corresponding to Boragineae s. l., Cynoglosseae s. l., Echiochileae, and Lithospermeae s. l. The sampling is here considerably expanded and allows for a better resolution with regard to the phylogenetic relationships of a broad range of genera. The tribe *Trichodesmeae* is retrieved as sister group to Cynoglosseae s. s. and may deserve further attention. The recognition of additional tribes will require careful and broadly based studies in both molecular phylogeny and morphology.

Polyphyletic "Trigonotideae"—Molecular and morphological data clearly indicate that "Trigonotideae" (Riedl 1968) are highly polyphyletic, with its genera distributed over all four subordinate taxa of Boragineae. It had been clarified previously that *Antiphytum* and *Sericostoma* (Trigonotideae subtribe Antiphytinae) are closely allied to each other and to *Echiochilon* (Långström and Chase 2002). This tribe was therefore represented by two genera in the present analysis. Moreover, *Mairetis*, *Moltkiopsis* I. M. Johnst., and *Neatostema* are firmly placed in Lithospermeae s. l. (Seibert 1978; Thomas et al. 2008). Riedl (1997), in his most recent treatment, placed both *Myosotis* and *Trigonotis* in monotypic tribes, but our (and previous: Långström and Chase 2002) data suggest that they are sister groups and deeply nested within the Cynoglosseae. *Bothriospermum*, *Trigonotis*, and *Mertensia* were originally placed in Lithospermeae (De Candolle 1846; Bentham and Hooker 1876; Gürke 1893; Johnston 1927) and subsequently removed to "Trigonotideae" (Riedl 1968). *Bothriospermum* [Trigonotideae subtribe Bothriosperminae (M. Popov) Riedl = Lithospermeae subtribe Bothriosperminae M. Popov], *Mertensia*, and *Trigonotis* [both Trigonotideae subtribe Trigonotidinae (M. Popov) Riedl = Lithospermeae subtribe Trigonotidinae M. Popov] are here firmly placed in Cynoglosseae. But in this clade no two of these three genera are in any way closely related to each other, and "Trigonotideae" (Riedl 1968) thus represents a polyphyletic assemblage of taxa in every respect.

***Moritzia* and *Thaumatocaryon* are Closely Related to Boragineae**—*Moritzia* and *Thaumatocaryon* are retrieved as the sister group of Boragineae in both molecular data sets, macro- and micromorphological data are in agreement with this systematic placement. The fruits of *Moritzia* and *Thaumatocaryon* share a set of exclusive, and phylogenetically probably derived, characters with Boragineae (nutlets laterally compressed, distinctly stalked, neither glochidiate nor winged, surface papillose and tessellate). Nutlets of *Moritzia* and *Thaumatocaryon* only differ from all other extant Boragineae in the absence of an elaisome, the strict reduction to a single mature nutlet, and the firm inclusion of the nutlet in the calyx. In Boragineae, dispersal of the individual nutlets as diaspores has been optimized (early shedding, elaisome indicates ant dispersal), whereas *Moritzia* and *Thaumatocaryon* have nutlets enclosed in the calyx with epizoochorous dispersal (Melcher et al. 2000).

Pollen of Boragineae has been studied intensively in the past decades (Clarke 1977; Sahay 1979; Ning et al. 1992; Díez 1994; Bigazzi and Selvi 1998; Retief and Van Wyk 1999). *Anchusa*, *Brunnera* Steven, *Nonea* Medik, *Pentaglottis*, *Pulmonaria*, and *Symphytum* L. typically have pollen closely resembling that of

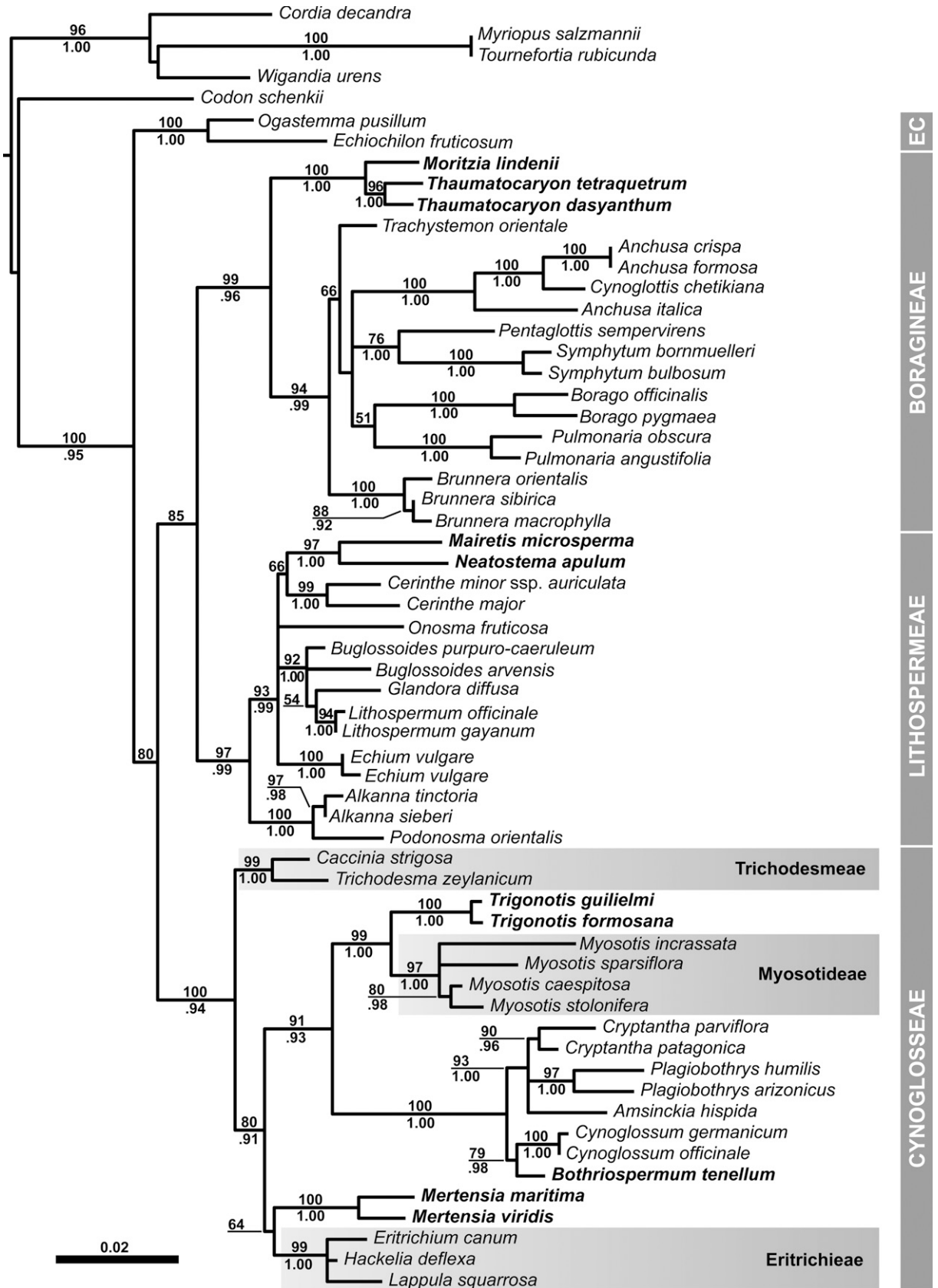


FIG. 4. Maximum likelihood phylogram of Boraginaceae based on the *trnL-trnF* dataset, with *Cordia* (Cordiaceae), *Myriopus* and *Tournefortia* (Heliotropiaceae), and *Codon* and *Wigandia* (Hydrophyllaceae) as outgroups. Numbers above branches: Maximum likelihood bootstrap support values, numbers below branches: Bayesian posterior probabilities. Branch lengths are to scale. Species names in bold represent members of "Trigonotideae". The four tribes (EC, Echiochileae) are shown in the broad sense (i.e. including the members of the former "Trigonotideae").

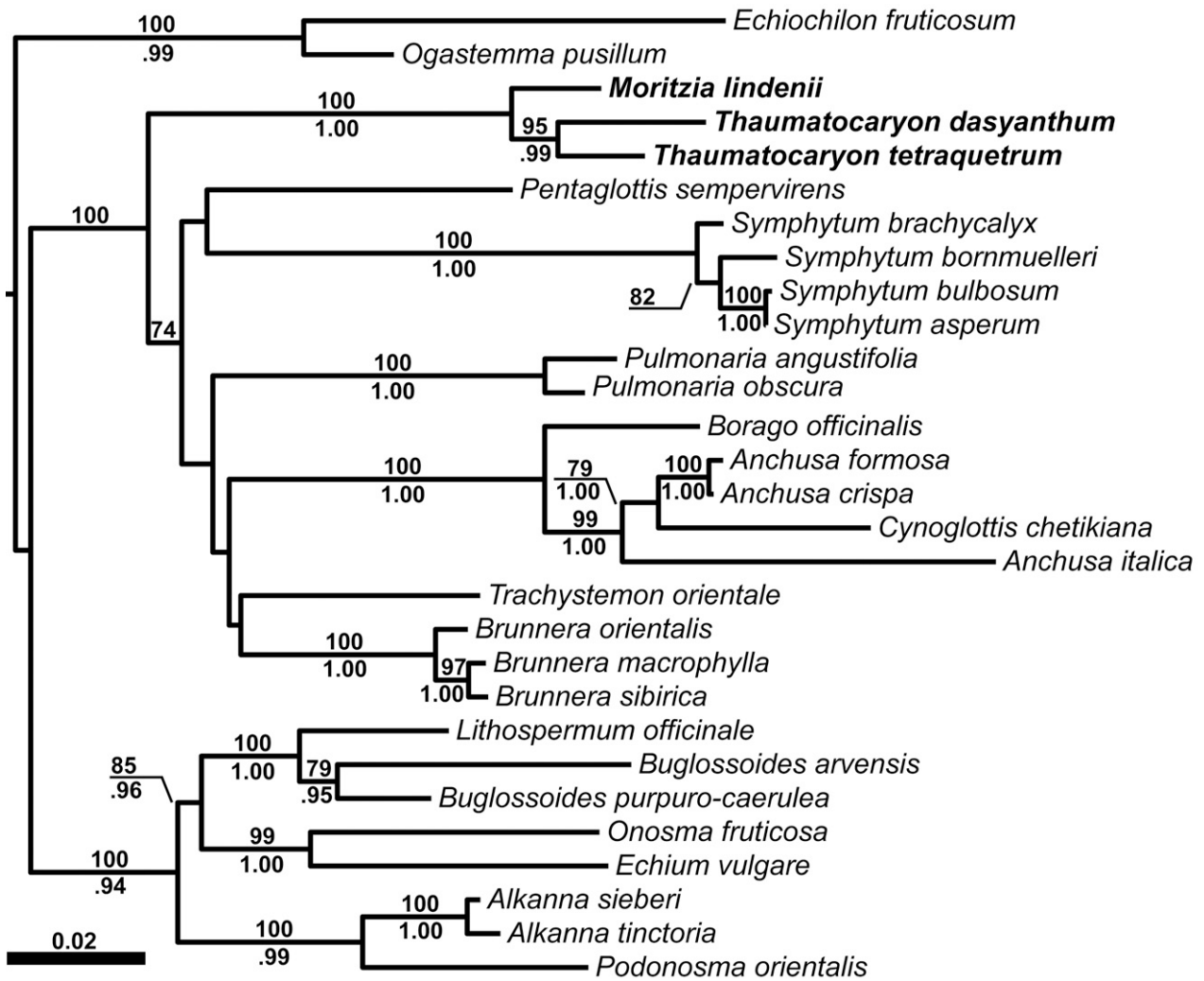


FIG. 5. Maximum Likelihood phylogram of Boragineae and Lithospermeae based on the combined ITS-*trnL-trnF* dataset, with *Echiochilon* and *Ogastemma* (EC, Echiochileae) as outgroups. Numbers above branches: Maximum likelihood bootstrap support values, numbers below branches: Bayesian posterior probabilities. Branch lengths are to scale. Species names in bold represent members of "Trigonotideae".

Moritzia, which is prolate, 3–4 (–5) colpate, with a microreticulate to perforate exine, and, importantly, with a distinct rugulose mesocolpial ring. Some Boragineae have more derived pollen types in some (e.g. *Anchusa*, *Nonea*, *Symphytum*) or all of their species (*Borago*), but the basic pattern appears to be nearly identical to that of *Moritzia lindenii*. This pollen morphology is not found elsewhere in Boraginaceae and can be considered apomorphic and a good indicator of the close relationship between Boragineae and *Moritzia/Thaumatacaryon*. The more aberrant pollen types of *Moritzia ciliata*, *M. dusenii*, and above all, *Thaumatacaryon* can be considered derived from the *Moritzia lindenii*-type by a widening of the rugose mesocolpial band into distinct, circular areas. *Thaumatacaryon*, with its hexagonal pollen grains and clearly delimited mesocolpial fields plus (in *T. selowianum*) additional apocolpial fields, seems to represent the derived condition. Moreover, the receptive surface of the *Moritzia* stigma consists of unicellular papillae without lobed plate-like cap at the tip, and perfectly fits into the *Borago-Symphytum* type described by Bigazzi and Selvi (2000). Pollen, flower, and fruit morphology thus provide strong evidence for the close relationship of *Moritzia* and *Thaumatacaryon* to Boragineae.

Fossil Record and Phytogeography—The discovery of a sister group relationship between *Moritzia/Thaumatacaryon* and the Boragineae is surprising, since it represents an unexpected and exclusive western Eurasian/South American disjunction in Boraginaceae (Fig. 6). Boragineae are nowadays largely restricted to the Mediterranean basin and western Asia, with a main centre of diversity in the Eastern Mediterranean and the western Irano-Turanian region (Tutin et al. 1972; Selvi and Bigazzi 1998, 2003; Hilger et al. 2004; Selvi et al. 2004), only a handful of species reach east Asia (*Pulmonaria*; Zhu et al. 1995), or Africa (*Anchusa*; Hilger et al. 2004). Extant native species are absent from the Americas (Johnston 1924, 1927; Kartesz 1994), but several fossil nutlets of Boragineae have been reported from North America. Thomasson (1979) suggests an affinity of †*Biorbia*, †*Eliasiana*, and †*Prolithospermum* to *Pentaglottis sempervirens* (Boragineae).

†*Biorbia fossilia* and †*Prolithospermum johnstonii* are the most characteristic and abundant fossil taxa in the Ogallala formation in Kansas (Late Miocene – early Pliocene; Thomasson 1979) and have also been reported from South Dakota and Nebraska, †*Prolithospermum* also from Texas (Thomasson 1977; Gabel et al. 1998). The nutlets of †*Prolithospermum johnstonii*

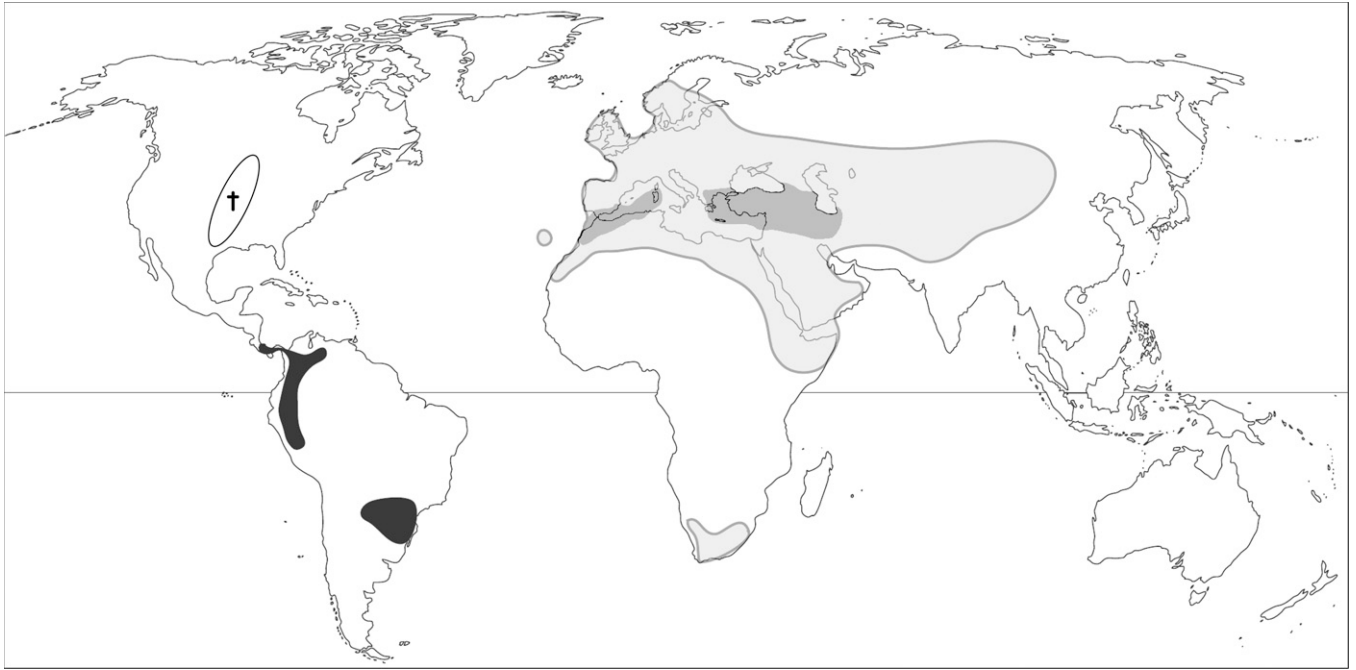


FIG. 6. Distribution map of extant and fossil Boragineae including *Moritzia* and *Thaumatocaryon*. Grey: Eurasian Boragineae, darker shade: centres of diversity, † fossil Boragineae-nutlets, black: *Moritzia* and *Thaumatocaryon*.

are virtually indistinguishable in shape and size from those of western European *Pentaglottis* (Thomasson 1979), and †*Biorbia* closely conforms to the morphology found, for example, in extant *Pulmonaria* (compare Gabel et al. 1998, Fig. 4.6). The cicatrices of †*Biorbia* and †*Prolithospermum* are distinctly concave, indicating the presence of an elaiosome, lost during fossilization, similar in shape and size to that of extant Eurasian Boragineae. All three fossil taxa share the strong development of the abaxial side of the nutlet with *Moritzia* and *Thaumatocaryon* (present only in *Pentaglottis* amongst Eurasian Boragineae). †*Biobia*, †*Eliasiana*, and †*Prolithospermum* thus indeed conform most closely to Boragineae amongst extant Boragineae.

The abundance and wide distribution of the fossils argues that Boragineae were widespread, diverse, and abundant in the late Miocene and early Pliocene of North America. Fossil taxa likely represent an extinct North American lineage, descendants of which may have reached South America. The most plausible hypothesis is therefore that South American *Moritzia* and *Thaumatocaryon* go back to dispersal of Boragineae from Europe to North America, followed by migration to South America, and subsequent extinction in North America.

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APPENDIX 1. Voucher information for the molecular data. Plant name, country of origin (or where cultivated), collector of voucher and voucher number, herbarium of voucher, GenBank accession number *trnL-trnE*, ITS.

Alkanna sieberi A. DC., GREECE, Crete, A. Kagiampaki 2000/10 (BSB), BSB DNA# 0643; J763263; FJ763199; *Alkanna tinctoria* (L.) DC.; SPAIN, H. H. Hilger s. n. 05.06.2006 (BSB); BSB DNA# 2123; FJ763304; FJ763250; *Amsinckia calycina* (Moris) Chater; PERU, M. Weigend & Ch. Schwarzer 8031 (BSB); BSB DNA# 1918; GQ285246;—; *Anchusa crispata* Viv.; FRANCE, Corse, F. Selvi & M. Bigazzi 99.005 (FI); BSB DNA# 0791; GQ285252; GQ285227; *Anchusa formosa* Selvi, Bigazzi & Bacchetta; ITALY, M. Bigazzi & F. Selvi 97.006 (FI); BSB DNA# 0781; GQ285251; GQ285226; *Anchusa italica* Retz; SPAIN, H. H. Hilger s. n. 03.06.2006 (BSB); BSB DNA# 2127; GQ285268; GQ285233; *Borago officinalis* L.; Cult. Syst. Bot. Berlin, O. Mohr 600 (BSB); BSB DNA# 2070; FJ763302; FJ763248; *Borago pygmaea* (DC.) Chater & Greuter; Cult. Syst. Bot. Berlin, M. Weigend 9076 (BSB); BSB DNA# 2630; GQ285274;—; *Bothriospermum tenellum* (Hornem.) Fisch. & C. A. Mey.; CHINA, Sichuan, Jinfushan, July 2007, Peng Li ZC-金-2 (BSB); BSB DNA# 2614; GQ285272;—; *Brunnera macrophylla* I. M. Johnst.; Cult. Syst. Bot. Berlin, H. H. Hilger s. n. 25.04.2000 (BSB); BSB DNA# 0628; GQ285247; GQ285223; *Brunnera orientalis* I. M. Johnst.; TURKEY, F. Selvi & M. Bigazzi 00.28 (BSB, FI), BSB DNA# 0829; GQ285253; AF531087; *Brunnera sibirica* Steven; Cultivated, M. Weigend 9066 (BSB); BSB DNA# 2624; GQ285273; GQ285234; *Buglossoides arvensis* (L.) I. M. Johnst.; GERMANY, H. H. Hilger & A. Werres s. n. 27.05.1999 (BSB); BSB DNA# 0473; FJ763256; FJ763192; *Buglossoides purpureo-caerulea* (L.) I. M. Johnst.; Cult. Institut für Biologie – Systematische Botanik und Pflanzengeographie, FU Berlin, M. Weigend 8136 (BSB); BSB DNA# 2351; FJ763308; AJ555897; *Caccinia strigosa* Boiss.; Cult. Institut für Biologie – Systematische Botanik und Pflanzengeographie, FU Berlin, O. Mohr M592 (BSB); BSB DNA# 0168; GQ285241;—; *Cerinth major* L.; ITALY, M. Bigazzi & F. Selvi 04.22 (BSB); BSB DNA# 1702; FJ763298;—; *Cerinth minor* (L.) ssp. *auriculata* (Ten.) Domac; ITALY, M. Bigazzi & F. Selvi 03.06 (BSB); BSB DNA# 1521; FJ763281;—; *Codon schenkii* Schinz; NAMIBIA, H. & E. Walter 118 (B); BSB DNA# 2375; GQ285270;—; *Cordia decandra* Hook. & Arn. EF688851; EF688903.1; *Cryptantha parviflora* Reiche; PERU, M. Weigend & Ch. Schwarzer 7813 (BSB); BSB DNA# 1911; GQ285263;—; *Cryptantha patagonica* (Speg.) I. M. Johnst.; ARGENTINA, M. Weigend et al. 5957 (BSB); BSB DNA# 1061; GQ285256;—; *Cynoglossum germanicum* Jacq;

Cult. Botanischer Garten Berlin-Dahlem, H. H. Hilger s. n. 22.06.1999 (BSB); BSB DNA# 0487; GQ285245;—; *Cynoglossum officinale* L.; Cult. Botanical Garden Berlin-Dahlem, May 2000, H. H. Hilger s. n. (BSB); BSB DNA# 0646; GQ285248;—; *Cynoglossum chetikiense* Vural & Kit Tan ssp. *paphlagonica* (Bornm.) Vural & Kit Tan; TURKEY, F. Seloi & M. Bigazzi 98.008 (BSB, FI); BSB DNA# 0830; GQ285254; GQ285228; *Echiochilon fruticosum* Desf.; ISRAEL, A. Danin et al. Rec. It. 04.024 (TUEB); BSB DNA# 2370; FJ763310; AJ555908; *Echium creticum* L.; Cult. Botanischer Garten München-Nymphenburg, H. Förther s. n. 17.09.1996 (BSB); BSB DNA# 2074; FJ763303; FJ763249; *Echium vulgare* L.; Cult. Institut für Biologie – Systematische Botanik und Pflanzengeographie, FU Berlin, O. Mohr 597 (BSB); BSB DNA# 2063; FJ763301; FJ763247; *Eritrichium canum* (Benth.) Kitam.; Cult. Botanischer Garten München-Nymphenburg, H. Förther s. n. (M); BSB DNA# 0343; GQ285242;—; *Glandora diffusa* (Lag.) D. C. Thoiass; SPAIN, H. Scholz & P. Hiepko 947 (B); BSB DNA# 1781; FJ763300;—; *Hackelia deflexa* Opiz; Italy, W. Frey s. n. (BSB); BSB DNA# 0480; GQ285244;—; *Lappula squarrosa* (Retz.) Dumort.; Cult. Syst. Bot. Berlin, O. Mohr 591 (BSB); BSB DNA# 2059; GQ285265;—; *Lithospermum gayanum* (Weddell) I. M. Johnston.; PERU, M. Weigend & Ch. Schwarzer 8060 (BSB); BSB DNA# 1684; FJ763297;—; *Lithospermum officinale* L.; GERMANY, A. Werres & M. Ristow s. n. 15.7.1998 (BSB); BSB DNA# 0409; FJ763254; FJ763189; *Mairetia microsperma* (Boiss.) I. M. Johnston.; MOROCCO, D. Podlech 48277 (MSB); BSB DNA# 0512; FJ763257;—; *Mertensia maritima* (L.) Gray; UNITED KINGDOM, Shetlands, H. H. Hilger s. n. anno 2002 (BSB); BSB DNA# 1230; GQ285259;—; *Mertensia virginica* (L.) Pers. ex Link; cult. Institut für Biologie – Systematische Botanik und Pflanzengeographie, FU Berlin, M. Weigend 8134 (BSB); BSB DNA# 2088; GQ285267;—; *Moritzia lindenii* Benth. ex Gürke; PERU, M. Weigend et al. 5436 (BSB); BSB DNA# 0977; GQ285255; GQ285231; *Myosotis caespitosa* DC.; FINLAND, H. H. Hilger 1575 (BSB); BSB DNA# 1902; GQ285262;—; *Myosotis incrassata* Guss.; GREECE, Crete, H. H. Hilger Kreta 1998/5 (BSB); BSB DNA# 0378; GQ285243;—; *Myosotis sparsiflora* Mikan; Cult. Syst. Bot. Berlin, M. Weigend 8138 (BSB); BSB DNA# 0002; GQ285239;—; *Myosotis stolonifera* J. Gray; UNITED KINGDOM, Shetlands, Hilger s. n. 09.08.2002 (BSB); BSB DNA# 1229; GQ285258;—; *Myriopus salzmannii* (DC.) Diane & Hilger; EF688800.1; EF688853.1; *Neatospermum apulum* (L.) I. M. Johnston.; GREECE, Crete, A. Kagiampaki 2000/11 (BSB); BSB DNA# 0639; FJ763262;—; *Ogastemma pusillum* (Coss. & Durieu ex Bonnet & Barratte) Brummitt; SAUDI ARABIA, R. Fitzgerald 73c (READING); BSB DNA# 0754; FJ763265; FJ763201; *Onosma fructifera* Sibth.; CYPRUS, H. H. Hilger 2000/10 (BSB); BSB DNA# 0620; FJ763260; FJ763196; *Pentaglottis sempervirens* (L.) Tausch; Cultivated, M. Weigend 9065 (BSB); BSB DNA# 0668; GQ285250; GQ285225; *Plagiobothrys arizonicus* (A. Gray) Greene ex A. Gray; U. S. A., H. H. Hilger H6/1998 (BSB); BSB DNA# 1914; GQ285264;—; *Plagiobothrys humilis* (Ruiz & Pav.) I. M. Johnston.; PERU, Weigend & Schwarzer 8075 (BSB); BSB DNA# 2178; GQ285269;—; *Podonosma orientalis* (L.) N. Feinbrun var. *palmyrena* Mout.; SYRIA, F. Seloi et al. 07/16 (BSB); BSB DNA# 2309; FJ763307; FJ763253; *Pulmonaria angustifolia* L.; ITALY, M. & K. Weigend 1999/45 (BSB); BSB DNA# 2086; GQ285266; GQ285232; *Pulmonaria obscura* Dumort.; GERMANY, H. H. Hilger s. n. 17.07.2000 (BSB); BSB DNA# 0681; FJ763264; FJ763200; *Symphytum asperum* Lepech. var. *armenicum* (Buckn.) A. Kurtto; TURKEY, B. Tarykalya 2540 (BSB); BSB DNA# BT2540;—; GQ285238; *Symphytum bornmuelleri* Bucknall.; TURKEY, B. Tarykalya 2495 (B); BSB DNA# BT2495; GQ285276; GQ285237; *Symphytum brachycalyx* Boiss.; TURKEY, B. Tarykalya 2415 (BSB); BSB DNA# BT2415;—; GQ285236; *Symphytum bulbosum* Schimp.; TURKEY, B. Tarykalya 2369 (B); BSB DNA# BT2369; GQ285275; GQ285235; *Thaumatocaryum dasyanthum* (Cham.) Fresen.; BRAZIL, R. C. Molon et al. 12177 (NY); BSB DNA# 1652; GQ285271; GQ285230; *Thaumatocaryum tetraquetrum* (Cham.) I. M. Johnston.; BRAZIL, *Hatschbach* s. n. (M); BSB DNA# 1649; GQ285260; GQ285229; *Tournefortia rubicunda* Salzm.; EF688799; EF688852.1; *Trachystemon orientale* (L.) G. Don; Botanischer Garten Gießen, H. H. Hilger s. n. anno 2000 (BSB); BSB DNA# 0666; GQ285249; GQ285224; *Trichodesma zeylanicum* (Burm. f.) R. Br.; KENYA, W. Schultka 12 (BSB); BSB DNA# 0163; GQ285240;—; *Trigonotis formosana* Hayata var. *elevatovonosa* (Hayata) S. D. Shen & J. C. Wang; TAIWAN, M. Weigend 8128 (BSB); BSB DNA# 1895; GQ285261;—; *Trigonotis guilielmi* A. Gray ex Gürke; JAPAN, T. Azuma 2001 (BSB); BSB DNA# 1124; GQ285257;—; *Wigandia urens* Humb., Bonpl. & Kunth; EF688846; AF091212.

(MO). Rambo 43573 (B). Rambo 57066 (B). Rambo 54452 (B). Jürgens 124 (B). Rambo 44040 (B). *Sehnem* 9429 (NY). *Sehnem* 8949 (NY). J. *Eugenio* 275 (NY). Jürgens 264 (B). Matzenbacher 22 (F). Bueno 2534 (F). - **Moritzia dusenii** I. M. Johnston. - TYPE: **Brazil. Paraná.**: Curitiba, 900 m. alt., 30.10.1915, P. Dusén 17273 (holotype: GH; isotype: MO!). - **Brazil. Paraná.** Pereira 8054 (B). *Hatschbach* 30567 (NY). Lindeman & de Haas 2464 (NY). Dusén 8780 (NY). Tsugaru et al. B-2374 (NY). Kozera & de O. Dittrich 315 (NY). *Hatschbach* 22141 (NY). *Hatschbach* 10225 (B). *Hatschbach* 47864 (MO). Budziak & Cordeiro 5 (MO). G. L. Smith et al. 1107 (MO). Forstreuter s. n. (TUEB). **Santa Catarina.** L. B. Smith & Klein 13306 (MO). Reitz & Klein 13344 (B). **Rio Grande do Sul.** Rambo 53057 (B). Camargo 2204 (B). - **Moritzia lindenii** (A. DC.) Benth. ex Gürke (= *Meratia lindenii* A.D.C.) - TYPE: **Venezuela [Trujillo or Mérida]**, Hautes Andes de Trujillo & de Mérida, depuis 4,000 jusqu'à 14,500 pieds de hauteur, 1842, M. J. Linden 444 (G-DEL, fragment & photographs Field Museum 27078 !). - **Costa Rica. San José.** Davidse & Pohl 1140 (F, NY); Burger 7944 (F, MO, NY); Whitmore 86 (F, MO); Whitmore 86 (F); Weston 6177 (F, MO); Burger 8273 (F). **Cartago.** J. Gentry & Burger 2674 (F); Heithaus 259 (F, MO); Skutch & Barrantes 5171 (F, NY); Davidse 24781 (MO); Webster et al. 12306 (F). **Limón.** Davidse & Herrera 29352 (MO); Herrera 3728 (F, MO). **Bocas del Toro:** (MO). **Colombia. Cundinamarca.** Fosberg 20707 (NY); Uribe Uribe 4939 (NY); Barclay & Juajibioy 7170 (MO); Schultes 20183 (MO); Penell 2052 (G, MO, NY, US). **Boyacá.** Melampy 1008 (MO); Melampy 647 (MO). **Cauca.** von Sneijders 1892 (F, NY); A. Gentry et al. 47741 (MO); A. Gentry et al. 47725^a (MO); Lehmann 5580 (F); Cuatrecasas 23444 (F). **Huila.** D'Arcy et al. 15604 (MO). **Nariño.** Ramírez P. 4992 (MO, PSO). **Quindío.** Stein 3473 (MO). **Putumayo:** Schultes & Cabrera 18888 (MO); Cuatrecasas 11757 (F). **Santander.** Cuatrecasas & García Barriga 10030 (F). **Venezuela. Lara.** Liesner et al. 8088 (MO). **Mérida.** Bernardi 599 (NY); Bernardi 583 (NY); Humbert 26383 (NY); Steyermark 56281 (F); Hanselman 25 (MO). **Trujillo.** Aristeguieta 3547 (NY); Gehrig 175 (F, MO). **Ecuador: Chimborazo:** Acosta Solis 7224 (F). **Napo-Pastaza.** Prescott 556 (NY); Holm-Nielsen & Øllgard 24391 (MO, NY); Øllgard et al. 34451 (AAU, QCNE, QCA, MO); Øllgard et al. 34479 (AAU, QCNE, QCA, MO); Øllgard et al. 34264 (AAU, QCNE, QCA, MO). **Azuay.** Jaramillo 7154 (MO, QCA); Dorr & Barnett 5963 (MO); Acosta Solis 5092 (F). **Pichincha.** Cuamacás & Guiño 483 (MO); Holm-Nielsen & Øllgard 24380 (MO). **Loja.** Luteyn & Romolero 14513 (MO, NY); Madsen 85565 (MO, AAU); Laegard et al. 58193b (AAU, MO); Palacios 12874 (MO); Jørgensen et al. 787 (MO, QCNE). **Morona-Santiago.** Holm-Nielsen et al. 29446 (F, MO, NY); Holm-Nielsen et al. 29404 (AAU, QCA, QCNE, MO, NY); Dorr & Barnett 5963 (NY); Øllgard et al. 38303 (AAU, QCNE, MO); Barclay & Juajibioy 8628 (MO). **Carchi.** Prescott 710 (NY); Eriksen 59018 (AAU, MO); Palacios 11666 (MO); Holm-Nielsen et al. 5358 (AAU, MO); Penland & Summers 867 (F). **Santiago-Zamora.** Steyermark 54332 (F); Steyermark 53490 (F). **Peru. Cajamarca.** Weberbauer 6081 (F); Campos et al. 5124 (MO). **Amazonas.** Barbour 3378 (MO). **Huánuco.** Weigend et al. 5436 (BSB, USM). - *Pentaglottis sempervirens* (L.) Tausch, cult. Syst. Bot. Berlin, Weigend 9065 (BSB). - *Pulmonaria mollissima* Kern., Kazakhstan, Schultka s. n. (BSB). - *Thaumatocaryon dasyanthum* (Cham.) I. M. Johnston. [= *Anchusa dasyantha* Cham. = *Moritzia dasyantha* Fresen.] - TYPE: **Brazil:** without detailed locality, Sellow 7964 (holotype B†; isotype US!). - **Brazil. Paraná.** *Hatschbach* 30705 (NY); *Hatschbach* 15402 (MO, NY); *Hatschbach* 22431 (MO); Pedersen 15946 (F, NY); *Hatschbach* 16933 (B). **Santa Catarina.** L. B. Smith & Reitz 12483 (MO); Reitz & Klein 11318 (NY); Reitz & Klein 16467 (B); L. B. Smith & Klein 13046 (B, F). - *Thaumatocaryon sellowianum* (Cham.) I. M. Johnston. [= *Anchusa sellowiana* Cham. = *Moritzia sellowiana* Fresen. = *Moritzia dasyantha* (Cham.) I. M. Johnston. var. *sellowiana* (Cham.) Brand = *Thaumatocaryon dasyanthum* (Cham.) I. M. Johnston. var. *sellowianum* (Cham.) I. M. Johnston.]. - TYPE: **Brazil,** without detailed locality, Sellow s. n. (holotype B†; isotype US!). - **Brazil. Paraná:** Reitz & Klein 17809 (B, NY); Dusén 11091 (F). **Rio Grande do Sul.** Molon et al. 12177 (MO, NY); *Hatschbach* & Ribas 61285 (NY). **Santa Catarina.** Krapovickas & Vanni 36932 (F); Sobral et al. 4782 (F); Reitz & Klein 5470 (B, NY); L. B. Smith & Reitz (NY); L. B. Smith et al. 7708 (MO); Reitz & Klein 14022 (B); Reitz & Klein 14160 (B). **São Paulo:** Gibbs et al. 3417 (NY); Kuhlmann 32449 (F). - *Thaumatocaryon tetraquetrum* (Cham.) I. M. Johnston. [= *Moritzia tetraquetra* (Cham.) Brand = *Antiphyllum tetraquetrum* (Cham.) DC. = *Anchusa tetraquetra* Cham. - TYPE: **Brazil,** without detailed locality, Sellow s. n. (holotype B†, isotypes GH!, US!); = *Thaumatocaryon hilarii* Baill., = *Moritzia tetraquetra* Cham. subvar. *asperior* Brand = *Antiphyllum bornmuelleri* Pilger var. *asperior* Pilger, = *Antiphyllum bornmuelleri* Pilger = *Moritzia tetraquetra* Brand var. *bornmuelleri* (Pilger) Brand]. - **Brazil. Paraná.** *Hatschbach* 23011 (MO). *Cervi* & Dunaiski 2907 (NY); *Hatschbach* 15230 (F). **Santa Catarina.** Hoehne 23199 (F); L. B. Smith & Klein 10868 (F); L. B. Smith & Klein 13503 (B, MO); L. B. Smith & Klein 13694 (NY); Reitz & Klein 14129 (B). **Rio Grande do Sul:** Sobral et al. 5337 (NY). **Paraguay.** Hassler 5231 (G, MO!); Fiebrig 5885; Fiebrig 6131 (G); Bernardi 18617 (NY). **Argentina. Misiones.** Guglianone et al. 2945 (MO).

APPENDIX 2. Specimens studied and morphology vouchers.

Borago officinalis L., cultivated at Berlin, Ackermann 740 (BSB). - *Borago pygmaea* (DC.) Chater & Greuter, cultivated at Berlin, Weigend 9076 (BSB). - *Moritzia ciliata* (Cham.) DC. ex Meisn. [= *Anchusa ciliata* Cham.] - TYPE: **Brazil,** without detailed locality, Sellow 672 (holotype B†; isotype US!). - **Brazil. Rio Grande do Sul.** Sobral 3471 (F). Reineck (G). Wasum et al. 6312