

2 A systematic analysis of *Heliotropium*, *Tournefortia*, and allied taxa of the Heliotropiaceae (Boraginales) based on ITS1 sequences and morphological data¹

Abstract

The relationships of *Heliotropium*, *Tournefortia*, *Schleidenia*, *Ixorhea*, and *Ceballosia* of the Heliotropiaceae (Boraginaceae in the traditional sense, Boraginales) are investigated using molecular data (ITS1). These genera form a monophyletic group. Five clades can be distinguished on the basis of molecular data, morphological traits, and distribution. In their current circumscription, *Tournefortia* is polyphyletic and *Heliotropium* is paraphyletic. *Tournefortia* section *Cyphocyema* is the sistergroup to all other ingroup taxa. *Heliotropium* section *Orthostachys* including *Schleidenia* sensu lato is the well supported sistergroup of a clade comprising the other species of *Heliotropium* sensu stricto (s. str.), *Tournefortia* section *Tournefortia*, and *Ceballosia*. *Heliotropium* s. str. forms two clades: one clade includes all species of the Old World, and represents the only monophylum of *Heliotropium* s. str. The other clade consists of all *Heliotropium* s. str. species of the New World but also includes *Tournefortia* section *Tournefortia* and *Ceballosia*. The results suggest that taxonomic changes are inevitable.

2.1 Introduction

Traditionally, the representatives of Heliotropiaceae Schrad. were included in Boraginaceae sensu lato (s. l.) Juss. as subfamily Heliotropioideae (Schrad.) Arn. (GÜRKE 1893). Now, the Boraginaceae appear to be a paraphyletic taxon (CHASE *et al.* 1993, BÖHLE & HILGER 1997, FERGUSON 1999) because the Hydrophyllaceae R. Br. ex Edwards appear to be the sistergroup to

¹ Published as DIANE N., FÖRTHNER H. & HILGER H.H. (2002a): A systematic analysis of *Heliotropium*, *Tournefortia*, and allied taxa of the Heliotropiaceae (Boraginales) based on ITS1 sequences and morphological data. - Amer. J. Bot. **89** (2): 287-295.

a Ehretiaceae Mart. ex Lindl./ Heliotropiaceae clade. Apart from the molecular results, some morphological characters, e.g., anatropous ovules (KHALEEL 1985), support this view. This has led us to accept Heliotropiaceae as a separate family.

Morphological and anatomical investigations of the Boraginaceae s. l. have shown similar trends. The common occurrence of long suspensors (ROSANOFF 1866) in combination with endosperm haustoria (SVENSSON 1925, PAL 1963) led SVENSSON (1925) and DIFULVIO (1978) to remove Heliotropioideae (Schrad.) Arn., Cordioideae (R.Br.) Lindl., and Ehretioideae (Mart. ex Lindl.) Arn. from the Boraginaceae s. l. and to include them in the Heliotropiaceae.

The Heliotropiaceae are small trees, lianas, shrubs, subshrubs, or perennial or annual herbs with pentamerous, tetracyclic flowers and actinomorphic corollas. They are characterized by a terminal style and a highly modified conical stigmatic head with a basal stigma and an infertile apex (conical style-stigma complex; GÜRKE 1893, KHALEEL 1978). The fruits are one- or two-seeded mericarpids or drupes.

The nature of the infrafamilial relationships of Heliotropiaceae are controversial. LINNÉ (1753, 1767) described three genera: *Heliotropium* L., *Tournefortia* L. and the monotypic *Messerschmidia* L. based on differences in habit and fruit shape. Subsequent authors segregated additional small, often monotypic genera, which were not widely accepted (e.g., DECANDOLLE 1845, GÜRKE 1893, JOHNSTON 1935). The increasing confusion within the Heliotropiaceae led FÖRTHNER (1998) to complete a badly needed monographic study of *Heliotropium* and the genera closely associated with it.

FÖRTHNER (1998) recognized a total of about 450 species in Heliotropiaceae (as Heliotropioideae [Schrad.] Arn.). Besides *Heliotropium* and *Tournefortia*, he accepted *Argusia* Böhm., *Schleidenia* Endl., and the monotypic genera *Ceballosia* Kunkel, *Ixorhea* Fenzl, as well as *Nogalia* Verdc. In addition, he proposed the new genus *Hilgeria* Förthner, from the West Indies, into which he transferred three former *Heliotropium* species due to their prostrate herbaceous habit, sessile single flowers, and pedicels strongly elongating after pollination. The small segregate genera differ mainly in aberrant fruit morphology and habit, and it is these differences that have caused the controversial discussion of their systematic position.

Narrowly endemic *Ixorhea* only occurs in the province of Salta in Argentina. It is a resinous shrub with four unusually large (up to 10 mm long), winged mericarpids. The systematic

relationships of *Ixorhea* within the Heliotropiaceae are unknown. It is the only taxon in Heliotropiaceae that has never been associated with either *Heliotropium* or *Tournefortia* (FENZL 1886, SPEGAZZINI 1901, HAUMAN 1922, DIFULVIO 1978). *Ceballosia* is a shrub of the Macaronesian Islands with two-seeded mericarps that have striking surface protuberances. Its systematic position was also unresolved prior to this study. Previous authors either included *Ceballosia* in *Tournefortia* (ROEMER & SCHULTES 1819), *Heliotropium* (KUNTZE 1891), or remained uncertain about its position (JOHNSTON 1935). HILGER (1989) and FÖRTHNER (1998) considered *Ceballosia* as a possibly relict link between *Tournefortia* and *Heliotropium*. Pantropical *Schleidenia* is distinguished by herbaceous habit, pedicellate, apparently solitary flowers, and drupaceous fruits. While GÜRKE (1893) and JOHNSTON (1928) reduced it to synonymy under *Heliotropium*, FÖRTHNER (1998) reestablished its generic rank. The species of Central Asian *Argusia* are characterized by perennial herbaceous habit and two-seeded, trichomatose mericarps with a corky exocarp. JOHNSTON (1935) redefined the generic name *Messerschmidia* L. for this taxon and transferred three species of *Tournefortia* to it, based on their exocarp characters. This name was still used by RIEDL (1967) in his *Flora Iranica*, but DANDY (1972), HEINE (1976), and CZEREPANOV (1981) step by step transferred each *Messerschmidia* species to *Argusia* as the appropriate name. Another benefit of this transfer was the elimination of the generic name *Messerschmidia* (also spelled *Messersmidia* and *Messerschmidtia*), which lead to much of confusion (see JOHNSTON [1935] for a detailed discussion). VERDCOURT (1987) renamed *Heliotropium drepanophyllum* Baker and created a new genus to accommodate *Nogalia drepanophylla* (Baker) Verdc. because of the shape of the fruit and the structure of the endocarp. Monotypic *Nogalia* from Somalia and Southwestern Arabia is a weakly succulent herb or subshrub with trichomatose drupaceous fruits.

The species of *Heliotropium* are nearly cosmopolitan or pantropical. They are herbs, subshrubs, or, very rarely, shrubs and are characterized by dry fruits, which divide into four or two mericarps. Pantropical *Tournefortia*, on the other hand, consists of small trees or lianas with drupaceous fruits, which never divide into mericarps.

The infrageneric classification of *Heliotropium* into sections has been a controversial subject. DECANDOLLE (1845) subdivided *Heliotropium* into four sections and excluded the genus *Heliophytum* DC. GÜRKE (1893) recognized seven sections and excluded the genus *Cochranea* Miers. FÖRTHNER (1998) split the genus into 19 sections (nine Old World, seven New World and

three cosmopolitan sections), once again including both *Heliophytum* and *Cochranea* in *Heliotropium*.

The taxonomy of *Tournefortia* is also problematical. DECANDOLLE (1845) recognized five sections. Three of these sections are currently referred to other genera: species of *Mallota* A.DC. and *Argusia* (Amman) DC. (sic) now constitute the genus *Argusia* Böhm. GÜRKE (1893) placed section *Messerschmidia* sensu DC. under *Heliotropium*. The two remaining sections, *Pittonia* HBK. and *Tetrandra* DC., were renamed by JOHNSTON (1930) as the two sections *Tournefortia* (*Eutournefortia* I.M.Johnst., nomen illegitimum) and *Cyphocyema* I.M.Johnst. Currently the division of JOHNSTON (1930) is accepted (AL-SHEBAZ 1991, FÖRTHNER 1998) but a critical evaluation of this division is still missing.

The main aim of this study was to evaluate the infrafamilial relationships of Heliotropiaceae with special reference to *Heliotropium* and *Tournefortia* by sequence analysis of the nuclear ribosomal internal transcribed spacer region (ITS1) and morphological data.

2.2 Materials and Methods

Sampling—For the analysis of intrafamilial relationships, we sequenced the ITS1-region from 40 species of Heliotropiaceae. Either fresh material, silica dried material, or herbarium specimens were used for the molecular studies. We used two species of *Ehretia* P.Br. for the outgroup comparison. Sources of plant material and vouchers used in this analysis are listed in Table 2-1 (appendix).

DNA extraction, amplification and sequencing—The ITS1-primers were those used by BALDWIN (1992). The DNA-segment was amplified in one step, using the primers P1 (5'-TTC AAC GAG GAA TTC CTA GT-3') and P2 (5'-TAC GTT CTT CAT CGA TGC GA-3').

Genomic DNA was extracted using a modified CTAB (cetyltrimethylammonium bromide) extraction protocol from DOYLE & DOYLE (1990; tissue ground in sea-sand, 70% [v/v] isopropanol substituted for the RNase step). Approximately 40 mg of leaf tissue were used for each extraction. The DNA was amplified with Taq PCR (polymerase chain reaction) kits (Quiagen, Hilden, North Rhine-Westphalia, Germany). PCR products were cleaned with QIAquick PCR purification columns (Quiagen, Hilden, North Rhine-Westphalia, Germany),

quantified with a 100-bp (base pair) DNA ladder (MBI-Fermentas, Sankt Leon-Rot, Baden-Württemberg, Germany), and cycle-sequenced with a GeneAmp PCRSystem 2400 (Perkin Elmer, Weiterstadt, Hesse, Germany). A SequiThermExcel II sequencing kit (Epicentre Technologies, Madison, Wisconsin, USA) was used with a stop-/loading-solution for terminating. Sequences were run on a GATC model 1500 (GATC, Konstanz, Baden-Württemberg, Germany). Polyacrylamide gels were prepared using SequaGel-6 (National Diagnostics, Atlanta, Georgia, USA). The biotinylized PCR products were transferred onto a Biodyne A nylon membrane (Pall Filtron, Dreieich, Hesse, Germany) and visualized by a reaction using basic phosphatase.

Phylogenetic analyses—Sequences were edited with the Alignment-Editor Align32 (HEPPERLE 1997) and manually aligned (Table 2-2, appendix). Phylogenetic analyses were performed by PAUP* 4.0b1. Parsimony analysis (SWOFFORD 1998) was performed using a heuristic search. The starting trees were obtained by random stepwise addition to the taxa with 100 replicates, TBR (tree-bisection-reconnection) branch swapping, saving all parsimonious trees, and MAXTREES set to “autoincrease“. All characters were weighted equally, and character state transitions were treated as unordered. Gaps were treated as missing data, because of the large deletions. We added an additional set of characters to the data matrix to signify the presence or absence of seven characteristic informative deletions (Table 2-2, appendix). These additional characters were also unweighted. Bootstrap resampling (FELSENSTEIN 1985) was performed with 1000 replicates and a heuristic search. The starting trees were obtained by random addition, with 100 random addition replicates, the TBR (tree-bisection-reconnection) branch swapping option and the MULTREE option were in effect. MAXTREES was set to 100 for each bootstrap replicate. Gaps were treated as missing data.

Neighbor-joining analyses (SAITOU & NEI 1987) were performed using a heuristic search run in PAUP. Sequence divergence values were calculated by Kimura’s two-parameter method (KIMURA 1980) with the settings: ADDSEQ = random, NREPS = 100, and TBR branch swapping. Bootstrap analysis with neighbor-joining search (Kimura’s two-parameter method) was performed with 1000 replicates.

2.3 Results

Analysis of ITS1-region data—279 bp of aligned sequence data and 7 potential phylogenetically informative larger deletions were used in the infrafamilial analysis (Table 2-2, appendix). Of the total of 286 sites, 133 (46.5%) were parsimony-informative, 36 (12.6%) were uninformative, and 117 (40.9%) sites were constant. The heuristic search found 426 most parsimonious trees, for which a strict consensus tree was computed (Fig. 2-1, L [tree length] = 460, CI [consistency index] = 0.60, RI [retention index] = 0.75). Figure 2-2 shows the corresponding neighbor-joining tree.

All ingroup taxa constitute a monophyletic group with respect to the outgroup (Fig. 2-1). The ITS1 strict consensus tree indicates that neither *Tournefortia* nor *Heliotropium* are monophyletic. Within the parsimony tree, five larger clades were found with high bootstrap support. *Tournefortia* section *Cyphocyema* (100% bootstrap support [BS]) constitutes the sister group of all other ingroup taxa. *Heliotropium* section *Orthostachys* including *Schleidenia* (95% BS) is the sister group of a large clade including all other *Heliotropium* species (*Heliotropium* sensu stricto [s. str.] in the following), *Ceballosia*, and *Tournefortia* section *Tournefortia* (72% BS). This latter clade consists of three subclades, one of which represents the *Heliotropium* s. str. species of the Old World (86% BS). The alignment (Table 2-2) shows a single characteristic deletion between positions 61 and 111, which supports the monophyly of the Old World *Heliotropium* s. str. species. The second subclade with very good bootstrap support (99% BS) is *Heliotropium* s. str. section *Heliothamnus* (*H. arborescens*, *H. mandonii*) from the South American Andes. The third subclade is more weakly supported (51% BS) and includes the *Heliotropium* s. str. species of the New World together with *Ceballosia* and *Tournefortia* section *Tournefortia*. *Ixorhea* constitutes the sister group (57% BS) to all ingroup taxa, with the exception of *Tournefortia* section *Cyphocyema*.

One tree was obtained from the neighbor-joining analysis (Fig. 2-2). The monophyly of Heliotropiaceae was strongly supported (100% BS). The neighbor-joining tree shares nearly the same topology as the strict consensus tree of the parsimony analysis. It also indicates that *Heliotropium* and *Tournefortia* are not monophyletic and underlines the large phylogenetic distance between the two *Tournefortia* sections. In the neighbor-joining tree, the same five larger clades are found with high bootstrap support: *Tournefortia* section *Cyphocyema* (100% BS); *Heliotropium* section *Orthostachys* including *Schleidenia* (92% BS); the large clade of all other

investigated *Heliotropium* s. str. species, *Ceballosia*, and *Tournefortia* section *Tournefortia* (71% BS); the subclade of the *Heliotropium* s. str. species of the Old World (79% BS); the subclade of *Heliotropium* s. str. section *Heliothamnus* (100% BS); the last subclade includes species of *Heliotropium* s. str. of the New World, *Ceballosia*, and *Tournefortia* section *Tournefortia* (not supported). The neighbor-joining tree and the strict consensus tree of the maximum parsimony analysis only differ in the position of *Ixorhea*. The neighbor-joining tree indicates a sister group relationship of *Ixorhea* to the large clade of *Heliotropium* s. str., *Tournefortia* section *Tournefortia*, and *Ceballosia* (71% BS).

2.4 Discussion

Intrafamilial relationships—Our molecular analyses contradict the traditional taxonomic circumscription of *Heliotropium* and *Tournefortia* (DECANDOLLE 1845, GÜRKE 1893, JOHNSTON 1928, 1930, 1935, FÖRTHNER 1998). *Tournefortia* species (Figs. 2-1 and 2-2) occur in two distinct clades. *Tournefortia* section *Cyphocyema* appears monophyletic and distinct, while the relationships and phylogeny of *Tournefortia* section *Tournefortia* remain unresolved. The ITS1 data suggest that *Tournefortia*, as traditionally defined by (convergent) traits such as drupaceous fruits and ligneous habit, is polyphyletic. *Tournefortia* section *Cyphocyema* is the monophyletic sister group of all other taxa under investigation. The species of *Tournefortia* section *Tournefortia* are all nested (but not together) in the well-supported New World *Heliotropium* s. str. clade (excluding section *Orthostachys*).

The phylogeny of *Heliotropium* (Fig. 2-1) inferred from molecular data does not contradict other molecular analyses of this taxon (BÖHLE & HILGER 1997). *Heliotropium* is paraphyletic, identified as such on the basis of plesiomorphic traits like free mericarpids. Four well supported clades can be distinguished by molecular analyses. Pantropical *Heliotropium* section *Orthostachys*, including *Schleidenia*, is the sistergroup of all other *Heliotropium* s. str. species. This grouping is supported by flower and fruit morphological traits (see below). The second highly supported clade includes the *Heliotropium* s. str. species of section *Heliothamnus*, a well-characterized group of *Heliotropium* s. str. from the South American Andes. The third strongly supported clade comprises the *Heliotropium* s. str. species of the Old World. The fourth clade includes the unresolved New World clade of *Heliotropium* s. str. plus *Ceballosia* and

Tournefortia section *Tournefortia*. *Heliotropium* s. str. species of the New World are much more closely related to *Tournefortia* section *Tournefortia*, than to *Heliotropium* section *Orthostachys*.

Our molecular results cannot resolve the exact position of the morphologically aberrant taxon *Ixorhea* (Figs. 2-1 and 2-2). The strict consensus tree indicates *Ixorhea* as one of the basal clades between *Tournefortia* section *Cyphocyema* and the clade of *Heliotropium*, *Schleidenia*, *Ceballosia*, and *Tournefortia* section *Tournefortia*. On the other hand, the neighbor-joining tree demonstrates an affinity of this taxon to the clade of the *Heliotropium* s. str. species of the New and Old World, *Tournefortia* section *Tournefortia*, and *Ceballosia* and a large genetic distance to *Tournefortia* section *Cyphocyema* and *Heliotropium* section *Orthostachys* including *Schleidenia*.

Tournefortia* section *Cyphocyema—Our results indicate that *Tournefortia* section *Cyphocyema* is a monophyletic group and has no close relationships to *Tournefortia* section *Tournefortia*. DECANDOLLE (1845), GÜRKE (1893), and JOHNSTON (1930) united all the woody Heliotropiaceae (mostly climbers or small trees) with drupaceous fruits and four or two endocarpids in *Tournefortia*. JOHNSTON (1930) renamed two very distinct sections *Cyphocyema* and *Tournefortia* in *Tournefortia*. He emphasized his doubts with regards to the real relationship between these sections (JOHNSTON 1930). “Drupaceous fruits“ and “woody habit“ are convergent characters, and are not appropriate for defining a monophyletic taxon (Fig. 2-3). The tropical American section *Cyphocyema* is characterized by the following traits (Fig. 2-3): (1) apex of the anthers is always hairy; (2) anthers connate; (3) fruits drupaceous, distinctly lobed, and never dividing into mericarpid; (4) four one-seeded endocarpids, each strongly curved with curved embryos (endocarpid type I in Fig. 2-4d) (MIERS 1868, JOHNSTON 1930); (5) corolla lobes elongate, very narrow with involute margins (Fig. 2-4a; MIERS 1868, JOHNSTON 1930); (6) habit climbing or subscandent (MIERS 1868, JOHNSTON 1930); and (7) tetrahedral crystals in the wood of some species (HEUBL *et al.* 1990).

The genus *Tournefortia* in its current circumscription is polyphyletic. A comparison between the morphological traits of *Tournefortia* section *Cyphocyema* with section *Tournefortia* (Fig. 2-3) shows that there are, except for drupaceous fruit and woody habit, no shared characters between these *Tournefortia* sections. The curved embryo is a plesiomorphic trait also found in Ehretiaceae (GOTTSCHLING & HILGER 2001). The hairy apex of the connate anthers occurs within *Heliotropium* section *Orthostachys* and *Heliotropium* s. str. section *Heliothamnus*, and it also seems to be a plesiomorphic trait within the Heliotropiaceae. All these morphological

features strongly support a basal position of *Tournefortia* section *Cyphocyema* within Heliotropiaceae.

Ixorhea—The molecular results show that *Ixorhea* is, without doubt, a member of Heliotropiaceae. The exact position of the morphologically aberrant taxon is still unresolved. It is not nested in one of the subclades. *Ixorhea* is morphologically characterized by the following three traits (Fig. 2-3): (1) fruits dry, always dividing into winged mericarpid; (2) four one-seeded endocarpid, embryo straight (DIFULVIO 1978); and (3) they are resinous shrubs.

For *Ixorhea*, the molecular results currently available indicate two possibilities for its phylogenetic relationships in Heliotropiaceae. On one hand, *Ixorhea* might be a sistergroup of the large clade of *Heliotropium* (including *Heliotropium* section *Orthostachys* and *Schleidenia*) and *Tournefortia* section *Tournefortia* (strict consensus tree, 57% BS). On the other hand, *Ixorhea* might be closely related to the clade of the *Heliotropium* species of the New and Old World, *Tournefortia* section *Tournefortia*, and *Ceballosia* (neighbor-joining tree). Morphological traits (Fig. 2-3) do not contradict these placements, and both possibilities are conceivable and in agreement with the results of DIFULVIO (1978). The morphology of this taxon is so aberrant that future investigations will have to clarify its precise relationships within the Heliotropiaceae.

Heliotropium* section *Orthostachys* and *Schleidenia—The monophyletic group *Heliotropium* section *Orthostachys* including *Schleidenia* is well supported based on molecular results. JOHNSTON (1928) treated *Schleidenia* as subsection *Axillaria* of section *Orthostachys*, the largest and probably the most difficult group of *Heliotropium*, and the molecular data confirm this placement. *Heliotropium* section *Orthostachys* including *Schleidenia* is defined by the following four traits: (1) apex of the anthers is always hairy; (2) anthers mostly connate (compare Fig. 2-4b with Fig. 2-4c); (3) four one-seeded endocarpid with characteristic surface sculpturing (described below), with curved embryos (endocarpid type II in Figs. 2-4e, f); and (4) Kranz chlorenchyma organization in leaves of some species (FROHLICH 1978).

The four mericarpid of *Orthostachys* share a characteristic surface sculpturing, described by different authors as “Male“ (which means “markings” in German, FÖRTHNER 1998) or “pits“ (Fig. 2-4e, JOHNSTON 1928, FROHLICH 1978). *Schleidenia* shares a similar structure, which is not immediately visible because of the more or less fleshy mesocarp (Fig. 2-4f). Both taxa are characterized by the occurrence of Kranz chlorenchyma organization in leaves of some species. These autapomorphic morphological-anatomical traits and the molecular results strongly support

an isolated position. Plesiomorphic morphological traits like the connate anthers with hairy apices and the curved embryo do not occur in the large clade of New and Old World *Heliotropium* s. str. species, *Tournefortia* section *Tournefortia*, and *Ceballosia* (for an exception see under *Heliotropium* s. str. section *Heliothamnus* below).

The molecular results confirm that *Schleidenia* and *Heliotropium* section *Orthostachys* are closely related, which has already been postulated by JOHNSTON (1928) and FÖRTHNER (1998), on the basis of morphological traits. Section *Orthostachys* seems to be paraphyletic, with *Schleidenia* forming a weakly supported (58% BS) clade within *Orthostachys*. Further investigations are necessary to demonstrate their precise relationships. Nevertheless, both taxa are characterized by separate fruit characters and are provisionally treated as sistergroups in Fig. 2-3. Drupaceous fruits, pedicellate single flowers, and exclusively herbaceous habit identify *Schleidenia*. *Heliotropium* section *Orthostachys*, on the other hand, is characterized by four one-seeded mericarpids, many-flowered inflorescences and shrubby or herbaceous habit.

Heliotropium* s. str. section *Heliothamnus—The New World clade of *Heliotropium* s. str., *Tournefortia* section *Tournefortia*, and *Ceballosia* excludes a well-supported clade (99% BS) with Andean species of *Heliotropium* s. str. section *Heliothamnus* (*H. mandonii*, *H. arborescens*). This latter group is morphologically characterized by the following four traits (Fig. 2-3): (1) apex of the anthers is always hairy; (2) anthers mostly connate (compare Fig. 2-4b with Fig. 2-4c); (3) four one-seeded endocarpids, embryo straight (JOHNSTON 1930, MIERS 1868); and (4) they are shrubs.

The species of section *Heliothamnus* have connate anthers with hairy apex and four endocarpids like those of *Heliotropium* section *Orthostachys* and *Tournefortia* section *Cyphocyema*. Possibly this plesiomorphic trait indicates a basal position within the large clade of *Heliotropium* s. str., *Tournefortia* section *Tournefortia*, and *Ceballosia*. Further investigations are necessary to substantiate this hypothesis.

Heliotropium* s. str. species of the New World, *Ceballosia*, and *Tournefortia* section *Tournefortia—This large clade, including New and Old World *Heliotropium* s. str. species, *Ceballosia*, and *Tournefortia* section *Tournefortia* is less well supported (72% BS) than the other clades and falls into three subclades. The subclade of the New World *Heliotropium* s. str. species including *Tournefortia* section *Tournefortia* and *Ceballosia* is still unresolved (51% BS) but indicates a close relationship between these taxa. It is, however, characterized by the following

three morphological traits that suggest it may be as a likely monophylum (Fig. 2-3): (1) embryos straight (JOHNSTON 1930, MIERS 1868); (2) endocarpids are two-seeded in many species (JOHNSTON 1930, FÖRTHNER 1998); and (3) empty chambers (cavities) border on the locules of some species (JOHNSTON 1930, FÖRTHNER 1998).

Autapomorphic morphological traits like straight embryos, occurrence of two-seeded endocarpids, and empty chambers support the molecular results. To clarify the exact relationships between these taxa further molecular analyses with more highly resolving markers are necessary. At this stage of investigation, it is not possible to assume any sistergroup relationships.

On the other hand, morphological traits separate *Tournefortia* section *Tournefortia* from section *Cyphocyema* and confirm the assumption of JOHNSTON (1930) that the *Tournefortia* sections are not closely related. The interesting fact that the woody species of *Tournefortia* section *Tournefortia* seem to be derived from herbaceous or shrubby *Heliotropium* s. str. species of the New World has not been predicted. Investigations on wood anatomy (RECORD & HESS 1941) of some *Tournefortia* species of section *Tournefortia* show decidedly heterogeneous rays, with most of the cells upright or square. Predominance of upright ray cells indicates secondary woodiness, which in turn indicates herbaceous ancestry, e.g., within the Asteraceae (CARLQUIST 1992).

***Heliotropium* s. str. species of the Old World**—The molecular results demonstrate strong support for a monophyly of the Old World *Heliotropium* s. str. species, but they have no clear morphological characters separating them from their sister clade (*Heliotropium* s. str. of the New World, *Ceballosia*, and *Tournefortia* section *Tournefortia*) (Figs. 2-2 and 2-3). On the other hand, the alignment of ITS1 shows a single characteristic long deletion between positions 61 and 111, which separates and defines the *Heliotropium* s. str. species of the Old World.

The large deletion can be regarded as an autapomorphic character of that group based on a single deletion event. Monophyly indicates that this clade of *Heliotropium* s. str. species seems to go back to a single colonization event from the New World. Other *Heliotropium* s. str. species, e.g., cosmopolitan *H. curassavicum*, colonized the Old World later, and was perhaps introduced by man, but the ancestors of this species will be found in the New World.

Taxonomical consequences—*Heliotropium* and *Tournefortia* are not monophyletic on the basis of molecular and morphological data. Classification should reflect phylogeny, and nomenclatural changes are therefore necessary. *Tournefortia* section *Cyphocyema* constitutes a separate lineage based on both molecular results and complex morphological traits. The only generic name available for this group is *Myriopus* Small (SMALL 1933).

A clade including *Heliotropium* section *Orthostachys* and *Schleidenia* is also well supported and should be removed from *Heliotropium* s. str. The species of this clade can be accommodated in a more broadly defined genus *Schleidenia* Endl. The taxonomic recombination for the investigated taxa will be made next.

Currently, we advocate a conservative approach for *Heliotropium* s. str., *Ceballosia*, and *Tournefortia* section *Tournefortia*. The exact relationships are still unclear and a reduction of all taxa under one genus name would be premature. The exact position of *Ixorhea* within the Heliotropiaceae is still unresolved.

In summary we conclude, that the superficial morphological resemblance of the genera *Schleidenia* and *Heliotropium* s. str. on the one hand, and *Myriopus* and *Tournefortia* on the other, has consistently confused previous authors and has obscured the phylogenetic relationships within the Heliotropiaceae. We expect that similar patterns will emerge once other major taxa of Boraginales are studied in detail.