6 A systematic analysis of Heliotropiaceae (Boraginales) based on *trn*L and ITS1 sequence data[7]

Summary

The infrafamilial relationships of Heliotropiaceae (Boraginaceae subfam. Heliotropioideae according to classical systems) are reevaluated using molecular data of nuclear ITS1 (86 species) and plastidal *trn*L_{UAA} intron (66 species) sequences. The results obtained from our investigations show that traditional generic limits warrant adjustment. Heliotropiaceae fall into two large clades. The first clade includes, in basal position, the genus *Ixorhea*. The genus *Myriopus* (formerly *Tournefortia* sect. *Cyphocyema*) is sister to *Euploca* (formerly *Hilgeria*, *Schleidenia, Heliotropium* sect. *Orthostachys*). The remaining sections of *Heliotropium*, *Tournefortia* sect. *Tournefortia* and the three small genera *Argusia*, *Ceballosia*, and *Nogalia*, segregated from *Heliotropium*, constitute the second large clade. *Argusia*, *Ceballosia*, and *Nogalia* cluster within clades of *Heliotropium* and therefore are reincluded into this genus. Within *Heliotropium* the species of former *Tournefortia* sect. *Tournefortia* represent a lineage of tropical New World *Heliotropium* species, growing in humid environments, whereas all other *Heliotropium* species are found in semi-arid habitats. Before new combinations in the genus *Heliotropium* are made for "*Tournefortia*", the exact relationship within New World *Heliotropium* needs to be resolved, and a revision of "*Tournefortia*" is inevitable. We advocate maintain the genus *Tournefortia,* which is easily to define and we conclude that under this definition the genus *Heliotropium* is paraphyletic. Five genera are thus accepted; 22 new combinations within Heliotropiaceae are presented.

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6.1 Introduction

In this study expands upon previous investigations (DIANE *et al*. 2002) in evaluating infrafamilial relationships of Heliotropiaceae, based upon molecular and morphological data. In Heliotropiaceae generic limits have fluctuated dramatically. Problems in classifying Heliotropiaceae originated, at least in part, from LINNÉ (1753), with generic definitions being largely to exclusively based on fruit morphology. The two large core-genera have always been maintained, namely *Heliotropium* and *Tournefortia*, but various segregates have been discussed controversial (DECANDOLLE 1845, GÜRKE 1893, JOHNSTON 1935). FÖRTHER (1998) recognized a total of ~450 species in Heliotropiaceae (as Heliotropioideae [Schrad.] Arn.). Besides *Heliotropium* L. and *Tournefortia* L., he accepted *Argusia* Böhm., *Schleidenia* Endl. and the monotypic genera *Ceballosia* (L.f.) Kunkel *ex* Förther, *Ixorhea* Fenzl, as well as *Nogalia* Verdc. Furthermore, he erected the new genus *Hilgeria* Förther, comprising three aberrant species from the West Indies, formerly included into *Heliotropium*. The small segregate genera differ mainly in fruit morphology (mostly dry vs. drupaceous) and habit from the two large genera, which otherwise comprise an enormous range of morphological characters.

DIANE *et al.* (2002a) described the relationship among *Heliotropium*, *Tournefortia*, *Schleidenia*, *Ixorhea*, and *Ceballosia,* and pointed out that *Tournefortia* and *Heliotropium* are not monophyletic.

This study includes data from additional taxa within the genus *Hilgeria*, as well as *Argusia* (two Asian species) and the monotypic African genus *Nogalia*. Furthermore, we include further representatives of *Heliotropium* and *Tournefortia,* covering the entire geographical and ecological range.

For sequence analysis we use the nuclear internal transcribed spacer region (ITS1) and the plastidal *trn*L_{UAA} intron.

6.2 Materials and Methods

Sampling—We sequenced the plastidal *trn*L_{UAA} intron of 66 species of Heliotropiaceae. 49 new nuclear ITS1 sequences of Heliotropiaceae were added to 37 ITS1 sequences already published by DIANE *et al*. (2002a). Samples used for molecular analyses were obtained from either silica dried material, fresh material, or herbarium material. We used one species each of Hydrophyllaceae and Ehretiaceae for outgroup comparison. Sources of plant material, used in this analysis are shown in Table **6-1** (appendix) together with GenBank accession numbers.

DNA extraction, amplification, and sequencing—DNA was isolated using a modified CTAB (cetyltrimethylammonium bromide) extraction protocol from DOYLE & DOYLE (1990), amplificated and sequenced as described in DIANE *et al*. (2002a). The PCR primers for the chloroplast DNA (cpDNA) *trn*L$_{\mathrm{UAA}}$ intron correspond to TABERLET et al. (1991), and the ITS1 primers were those used by BALDWIN (1992).

Phylogenetic analysis—Sequences were edited and maually aligned with the Alignment-Editor Align 32 (HEPPERLE 1997). The alignment of the ITS1 region was improved by using the secondary structure according GOTTSCHLING *et al*. (2001). “Hairpin” and “stem loop” regions were identified and separately aligned. Indels were coded as “missing characters”; parsimony informative indels were coded separately as present/absent following the “simple gap coding” method of SIMMONS & OCHOTERENA (2000). Indels which are present or absent only in different accessions of the same species were not coded. Indels of doubtful homology were not coded (especially in overlapping gap regions, which occur preferable in helix I). All alignments are available from the authors on request.

Phylogeny analyses were performed by PAUP* 4.0b1 (PC version) and TREECON for Windows (version 1.3b). Parsimony analyses (PAUP: SWOFFORD 2002) were performed using a heuristic search. The starting trees were obtained by random stepwise addition to the taxa with 1000 replicates, tree-bisection-reconnection (TBR) branch swapping, saving all parsimonious trees (“MulTrees” on), and MAXTREES set to “autoincrease”. First, starting trees were obtained (addition sequence random, 1000 replicates, TBR branch swapping, “MulTrees” off, “steepest descent” on). The resulting shortest starting trees were then subjected to TBR branch swapping (“MulTrees” on) to the limit of computer capacity. All characters were weighted equally, and character state transitions were treated as unordered. Bootstrap resampling (FELSENSTEIN 1985) was performed with 1000 replicates and a heuristic search, with random addition of taxa (10 addition sequence replicates), with a limit of 100 trees kept at each step. In addition, Neighbor-Joining analyses (SAITOU & NEI 1987) were performed using a heuristic search run in TREECON (VAN DE PEER & DE WACHTER, 1994). Gaps were treated as missing data instead of "gap coding". Sequence divergence values were calculated by Kimura's two–parameter distance models (KIMURA 1980) and a bootstrap analysis with 1000 replicates.

The *trn*L dataset was separately analyzed, followed by a combined *trn*L and ITS1 dataset to find the robust main clades. Each main clade was then individually analyzed using ITS1 sequences. With regard to the analysis of the HELIOTROPIUM II clade we combined the *trn*L and ITS1 datasets, because in this case the resolution increased. The number of outgroup species was reduced in order to minimize the homoplasy content. The names of the main clades identified in this study are indicated in capital letters.

6.3 Results

1—Complete DNA sequence analysis

trnL dataset—Analysis of the *trn*L dataset, including 20 separated coding indels (of a total of 568 characters 106 are parsimony informative), resulted in 40,100 most parsimonious trees (l=207 steps, CI=0.899, RI=0.975, HI=0.101; strict consensus tree see Fig. **6-1**). With respect to the outgroup, all ingroup taxa constitute a monophylum (99% bootstrap support). Within the strict consensus tree, two main clades with high bootstrap values can be distinguished. In the first large clade (77% bootrtrap support), IXORHEA (*Ixorhea*) is sister (99% bootstrap support) to MYRIOPUS (*Tournefortia* section *Cyphocyema* I.M. Johnst.) and EUPLOCA (*Heliotropium* section *Orthostachys* R.Br., *Schleidenia*, *Hilgeria*), the latter supported by 100% each. The second large clade (100% bootstrap support) comprises all remaining species of Heliotropiaceae and is subdivided into three subclades. Of these, HELIOTHAMNUS (*Heliotropium* section *Heliothamnus* I.M. Johnst.) forms the unsupported sister group of the remaining species (with 66% bootstrap value weakly supported). A second, well supported subclade (80% bootstrap support), comprises the *Heliotropium* species of the Old World, including *Ceballosia*, *Nogalia*, and *Argusia sogdiana.* Together with the unsupported *Argusia sibirica* we call it HELIOTROPIUM II. All *Heliotropium* species of the New World and *Tournefortia* section *Tournefortia* I.M. Johnst. remain unresolved and form the third subclade, HELIOTROPIUM I.

The corresponding Kimura-2-parameter based neighbor-joining (NJ) tree (Fig. **6-2**) is highly congruent with the parsimony strict consensus tree (Fig. **6-1**). It shares nearly the same topology, and revealed identical clades. On clade one IXORHEA, MYRIOPUS, and EUPLOCA are less well supported (58% bootstrap support). On clade two HELIOTHAMNUS is also separated. HELIOTROPIUM I (59% bootstrap support) constitutes the sister group of HELIOTROPIUM II (62% bootstrap support).

Combined trnL-ITS1 dataset—Analysis of the combined *trn*L-ITS1 dataset, including 20 separated indels of the *trn*L dataset whereas ITS1 remains uncoded, did not change the well supported clades of the single analysis of the *trn*L dataset. The combined analysis (of a total of 912 characters 251 are parsimony informative) resulted in 2,604 most parsimonious trees (l=950 steps, CI=0.622, RI=0.832, HI=0.378; strict consensus tree see Fig. **6-3**).

The ITS1 dataset within Heliotopiaceae is very heterogenous. Nevertheless, bootstrap values of the combined analysis partly increases in the main clades which are retrieved in the single marker analysis. IXORHEA is isolated in combined analysis, due to problematic positions in the ITS1 alignment. This leads to a lower bootstrap support (75%) for the remaining species of Heliotropiaceae. However, the first large clade (83% bootstrap support) of combined analysis contains MYRIOPUS and the large EUPLOCA crown clade as sisters with 100% bootstrap support each. The second large clade with all other species of Heliotropiaceae is still supported by 100%. HELIOTHAMNUS (99% bootstrap support) is basal situated to HELIOTROPIUM I and II (72% bootstrap support). HELIOTROPIUM I is supported by 66%, whereas HELIOTROPIUM II is partly unresolved.

The corresponding Kimura NJ tree (Fig. **6-4**) shares the same topology regarding the position of IXORHEA, and the two large and well supported main clades (92% respectively 97% bootstrap support). Within the large HELIOTROPIUM and HELIOTHAMNUS clades tree resolution collapses, due to increasing homoplasy content of the ITS1 dataset within the entire family, and due to the lack of “gap coding” which is in the programs used for the Neighbor-Joining analysis impossible.

2—ITS1 trees of main clades identified in 1

The ITS1 dataset of the main clades EUPLOCA-MYRIOPUS, HELIOTROPIUM I, and HELIOTROPIUM II were separately analyzed, in order to obtain trees with increased resolution. Additional species were added.

MYRIOPUS *and* EUPLOCA—Analysis of the ITS1 dataset of the MYRIOPUS and EUPLOCA clade, including 7 separated coding indels (of a total of 309 characters 107 are parsimony informative), resulted in 112 most parsimonious trees (l=332 steps; CI=0.723, RI=0.803, HI=0.277; majority–rule consensus tree see Fig. **6-5a**). Well-supported clades are MYRIOPUS (100% bootstrap support) and EUPLOCA (96% bootstrap support). They occur as a sister group relationship, whereas IXORHEA does not cluster and fall into the outgroup. Within EUPLOCA, the South American species *H. chrysanthum* and *H. mendocinum,* characterized by underground tubers, form a strongly supported clade (99% bootstrap support, "tuber" clade) and are, weakly supported (69% bootstrap support), sister to the remaining species of EUPLOCA. The latter fall into five subclades, three of them are well-supported. The relationships between these subclades are unresolved. The first subclade (90% bootstrap support) corresponds to *Heliotropium* sect. *Orthostachys* subsect. *Ebracteata* I.M. Johnst. (South American *H. campestre* and *H. procumbens*, African *H. ovalifolium*). North American *H. convolvulaceum* is unsupported in sister group relationship to an unresolved subclade comprising: The Caribbean clade (Caribbean species *H. humifusum*, *H. bursiferum*, and *Hilgeria*), the *Schleidenia* clade (New and Old World species of the genus *Schleidenia*), and the African-Australian species. In this subclade, the Caribbean clade (including the genus *Hilgeria*) is strongly supported (90% bootstrap support) as well as the African-Australian subclade (95% bootstrap support). The *Schleidenia* subclade remains unsupported.

The Neigbor-Joining analysis (Fig. **6-5b**) shows nearly the identical topology. Long distances and high bootstrap percentages characterize the main clades and subclades: MYRIOPUS, EUPLOCA, "*Ebracteata"* clade, "tuber" clade, "Caribbean" clade, and "African-Australian" clade. The major deviation from the parsimony analysis is the position of the *Ebracteata* clade (97% bootstrap support), which constitute in the NJ analysis unsupported sister to the remaining species of EUPLOCA. The three sequenced species of the genus *Schleidenia* (*H. antillanum, S. baclei, S. lagoensis*) are in unresolved position between the Caribbean and African species.

HELIOTROPIUM I—Analysis of the ITS1 dataset of the HELIOTROPIUM I clade, including 6 separated coding indels (of a total of 317 characters 99 are parsimony informative), resulted in 4,317 most parsimonious trees (l=460 steps; CI=0.724, RI=0.743, HI=0.276; majority–rule consensus tree see Fig. **6-6a**). The Old World representatives (HELIOTROPIUM II, excluding *Ceballosia*) constitute, weakly supported (64% bootstrap support), a sister group to HELIOTROPIUM I. *Ceballosia* is related to HELIOTROPIUM I, but unsupported. Within HELITROPIUM I nine subclades are distinguishable, but mostly in unsupported relationships. Within HELITROPIUM I, section *Cochranea* (Miers) Kuntze (84% bootstrap support) is undoubtedly basal to the remaining species of HELIOTROPIUM I (73% bootstrap support). These remaining species cluster in various subclades of the sections *Plagiomeris* I.M. Johnst. (100% bootstrap support), *Tiaridium* (Lehm.) Griseb. (99% bootstrap support), *Heliotrophytum* G. Don. (100% bootstrap support), *Hypsogenia* I.M. Johnst., *Coeloma* (DC.) I.M. Johnst. (96% bootstrap support), and *Schobera* (Scop.) I.M. Johnst. One unsupported subclade comprises the “halophytes” [species of section *Platygyne* Benth. (99% bootstrap support), including *Tournefortia argentea* and *T. gnaphalodes*]. Another unsupported subclade comprises the remaining species of *Tournefortia* section *Tournefortia* (“*Tournefortia*” clade).

The Neighbor-Joining analysis (Fig. **6-6b**) result, with respect to the main clades, in the same topology of the tree like the parsimony analysis. HELIOTROPIUM I (56% bootstrap support), including *Ceballosia*, is in sister group relationship to HELIOTROPIUM II (69% bootstrap support). Section *Cochranea* (98% bootstrap support) is the sister group of the remaining HELIOTROPIUM I (74% bootstrap support) species, which cluster in the same subclades as in the parsimony analysis. The exceptions are *Tournefortia argentea* and *T. gnaphalodes,* both clustering randomly. The relationships between the subclades remain unsupported. They do not agree with the results obtained from the majority-rule consensus tree (Fig. **6-6a**).

Combined trnL - ITS1 dataset of **HELIOTROPIUM II**—Analysis of the combined *trn*L and ITS1 datasets of the HELIOTROPIUM II clade, including 7 separated coding indels (of a total of 859 characters 100 are parsimony informative), resulted in 84 most parsimonious trees (l=373 steps; CI=0.751, RI=0.748, HI=0.249; majority–rule consensus tree see Fig. **6-7a**). Supported by 70% bootstrap value, the HELIOTROPIUM I representatives, including *Ceballosia*, constitute an unsupported sister group to HELIOTROPIUM II (excluding *Ceballosia*). Within HELIOTROPIUM II five subclades are identified, three of them are supported by high bootstrap values. A well supported (95% bootstrap support) large crown clade comprises species of the sections *Heliotropium* (≡ *Heliotropium* L. sects. *Agoraea* Bunge, *Gyrostachys* G. Don), *Pleurolasia* Bunge, *Odontotropium* Griseb., and *Chamaetropium* Griseb. *H. supinum* (sect. *Chamaetropium*) cluster well with *Nogalia drepanophyllum*. The sister group (99% bootstrap support) to this crown clade constitute the species of section *Pterotropium* (DC.) Bunge. The remaining species of HELIOTROPIUM II belong to the sections *Rutidotheca* (A.DC.) Verdc., *Zeylanica* Förther, *Pseudocoeloma* Förther the genus *Argusia*, and they remain all in unsupported basal position of the total HELIOTROPIUM II clade.

A higher resolution of the dataset is found in the Neighbor-Joining Analysis (Fig. **6-7b**). General topology is identical to the parsimony analysis. The *Heliotropium supinum*/ *Nogalia drepanophyllum* clade constitutes the well supported (74% bootstrap support) sister group of the *Heliotropium*/ *Pleurolasia*/ *Odototropium* crown clade. The basal clades (sections *Rutidotheca*, *Zeylanica*, *Pseudocoeloma*, and genus *Argusia*) of HELIOTROPIUM II show short distances between each other and long distances within the taxa of each clade.

ITS1 in the region of helix 1—The main characteristic of all HELIOTROPIUM II species, except the basally situated *Argusia sibirica* and *Ceballosia fruticosa*, is a radical abridgement of helix I (about secondary structure of Heliotropiaceae see Figs. **3-6** and **3-8** in GOTTSCHLING *et al.* 2001), which results in a large deletion in the alignment (Fig. **6-8**). All species of Heliotropiaceae, except HELIOTROPIUM II species (exclude *Ceballosia fruticosa* and *Argusia sibirica*), are characterized by a long helix I of pairing regions, about up to 25 bp (Fig. **6-9a**, **b**, **d**; the homologous regions are labelled I to III), or up to 13 bp long (Fig. **6-9c**). A partial loss of the apical region of helix I (Fig. **6-9c**; loss of homologous region III) was found in EUPLOCA, HELIOTROPIUM I and is characteristic for HELIOTHAMNUS (Fig. **6-8**). A nearly complete abridgement (Fig. **6-9e**; loss of homologous regions II, and III) found only in HELIOTROPIUM II for the most species (Fig. **6-8**). A total abridgement of helix I found within HELIOTROPIUM II for *Argusia sibirica*, *Heliotropium nelsonii*, and *H. zeylanicum* (Fig. **6-9f**).

The region of helix I is very informative, but it is difficult to implement a “gap coding” here. Due to completely overlapping gaps, it is not possible to detect whether a subset of bases, lost before the indels, is responsible for the longer gap. In the region of helix I we therefore prefer not to use “gap coding”. This method results in a decrease of bootstrap support, because large informative regions of ITS1 sequences were not used for the phylogenetic sequence analysis.

6.4 Discussion

Intrafamilial relationships—These molecular studies of Heliotropiaceae strongly support the results of DIANE *et al*. (2002a), which contradicted traditional taxonomic circumscription particularly with regard to the large core-genera *Heliotropium* and *Tournefortia* (DECANDOLLE 1845, GÜRKE 1893, JOHNSTON 1928, 1930, 1935, FÖRTHER 1998). The inclusion of a second marker (the *trn*L$_{UAA}$ intron) besides the ITS1 region in our analyses, led to a better differentiation of main clades and higher resolution within each of them.

A summary of the results including morphological-anatomical traits is shown in Figure **6-10**.

Our results show that each of the two clades, IXORHEA, MYRIOPUS and EUPLOCA on the one hand and HELIOTHAMNUS, HELIOTROPIUM I, and HELIOTROPIUM II on the other hand, constitute a monophyletic group. Both clades are well-supported by both the *trn*L and the combined *trn*L/ITS1 dataset. In the combined *trn*L/ ITS1 dataset only the position of IXORHEA remains unresolved.

In the following, each main clade will be specified:

IXORHEA—Analysis of ITS1 sequences could not unambiguously resolve the exact systematic position of monotypic *Ixorhea* (DIANE *et al*. 2002a). In contrast, the more conservative sequences of *trn*L clarify its sister group relationship to the MYRIOPUS/ EUPLOCA clade.

Ixorhea is chacaterized mainly by autapomorphic morphological traits: Elongated and winged mericarpids enclosing a straight embryo, and the whole plant being completely resinous (DIFULVIO 1978). However, independent of its exact systematic position, *Ixorhea* does not cluster inside any clade. Therefore, with regard to nomenclature, no changes are suggested.

MYRIOPUS—Without doubts the species of *Tournefortia* sect. *Cyphocyema*, clustering in the first main clade, are not closely related to *Tournefortia* sect. *Tournefortia*, appearing in the second main clade. Traditionally *Tournefortia* species are defined mainly by obviously convergent traits such as liana or subscandent habit, or the presence of drupaceous fruits. The assumption of DIANE *et al*. (2002a) that MYRIOPUS species take a basal position within Heliotropiaceae must be revised. All available molecular data speak for the well-supported sister group relationship of MYRIOPUS and EUPLOCA. Such a close relationship had already been assumed by JOHNSTON (1930), but without explanation. The following morphological-anatomical apomorphies support this assumption: a curved embryo, corolla lobes with involute margins (DIANE *et al.* in press a, Figs. **7-1B**, **I**), and the complete lack of calcium oxalate druses in leaf mesophyll. Instead of crystal druses, bundles of calcium oxalate needles are present in the epidermis in some species of MYRIOPUS and EUPLOCA (DIANE *et al.* in press b). The characteristic papillose to pubescent apex of the connate anthers, which close the corolla tube above the style-stigma-complex, seems to be plesiomorphic within Heliotropiaceae. Beside MYRIOPUS and EUPLOCA these characters also occur within *Ixorhea* and the HELIOTHAMNUS clade. However, in *Ixorhea* the anthers are merely long protracted and apically compressed, and in HELIOTHAMNUS the pubescent apices of the anthers are only sometimes connected.

The monophyly of the MYRIOPUS clade, as well as the clear morphological circumscription justify the general acceptance of the genus *Myriopus* Small (SMALL 1933). The species of MYRIOPUS are charactericed by the following traits: lianas with drupaceous, deeply 4-lobed, fruits with one-seeded and 4-layered endocarpids (DIANE *et al.* in press a, Fig. **6-1D**), a thick tissue of transfer cells in the placenta region (DIANE *et al.* 2002a, Fig. **4-14** to **16**), characteristic flowers with subulate corolla lobes, inflated bases of the corolla tubes, and involute corolla margins in the buds.

The necessary combinations, of the species investigated, from *Tournefortia* to *Myriopus* are presented in Appendix **6-1**.

EUPLOCA—The species of *Heliotropium* section *Orthostachys* are distinctly separated from the remaining species of *Heliotropium* and cluster together with *Hilgeria* and *Schleidenia*. The EUPLOCA clade shows a higher affinity to the genus *Myriopus* than to the remaining species of *Heliotropium*. This relationship is also well-supported by morphological-anatomical data.

All species of EUPLOCA show mericarpid or endocarpid structures with surface sculpturings described as “pits” (precise description in DIANE *et al.* 2002a), kranz-chlorenchyma organisation in leaves of almost all species (exceptions found only in *Heliotropium* sect. *Ebracteata*, DIANE *et al.* in press b), and the exclusive occurrence of characteristic trichomes on a pedestal of distinctly enlarged foliar epidermis cells (trichome type 3 in DIANE *et al.* in press b).

Within the EUPLOCA clade we identified several subclades. One well-supported subclade comprises South American species (*H. chrysanthum*, *H. mendocinum*) with underground tubers as a particular adaptation to semi-arid habitats with seasonal or erratic dry periods. *Heliotropium* subsect. *Ebracteata* (as *Ebracteata*-clade), appears to be monophyletic. The species are characterized by completely bractless inflorescences, whereas the remaining species of EUPLOCA are bracteate. The *Ebracteata*-clade comprises Old World (*H. ovalifolium*) and New World (*H. procumbens*, *H. campestre*) species as well as few species with kranz-chlorenchyma organisation (*H. campestre*). However, most species are lack kranz-chlorenchyma organisation (*H. procumbens*, *H. ovalifolium*) in the leaves (DIANE *et al*. in press b, FROHLICH 1978). Due to the contradicting position in parsimony or NJ trees, the exact phylogenetic position of the *Ebracteata* clade within EUPLOCA remains unclear. Furthermore, we identified a Caribbean clade, which comprises dwarf-shrubs with multiflowered inflorescences (*H. bursiferum*), procumbent subshrubs with solitary flowers *(H. humifusum*), and procumbent herbs with solitary flowers and postflorally elongating pedicels (defined as the genus *Hilgeria*).

The species of the pantropical genus *Schleidenia* clustered unsupported between the Caribbean and African-Australian clade. Nevertheless, morphological traits, in particular the characteristic drupaceous fruits (FÖRTHER 1998), indicate this group as a monophylum.

The African-Australian clade comprises in basal positions the species *H. rariflorum* (distributed from Southeastern Africa, the Arabian Peninsula and southern Iran to southern Pakistan) and *H. strigosum* (from West Africa, the Arabian Peninsula to India, Pakistan, and Australia) and in crowen position a large number of Australian species. *H. strigosum* is sister to an apparently evolutionary young Australian group which undertook a rapid radiation while colonizing this continent (CRAVEN 1996).

Summarizing all urges a formal taxonomic recombination of EUPLOCA, including all species of *Heliotropium* section *Orthostachys*, *Schleidenia*, and *Hilgeria* into the genus *Euploca* Nutt. (NUTTALL 1837) the oldest available generic name in this group. *Schleidenia* Endl. (ENDLICHER 1839) as formerly proposed (DIANE *et al*. 2002a) is younger. Combinations are presented in Appendix **6-1**.

HELIOTHAMNUS, HELIOTROPIUM I and II—These clades constitute a monophylum, well-supported by both molecular and morphological (presence of always straight embryos) data. Within this large clade, comprising species of *Heliotropium, Tournefortia* sect. *Tournefortia*, *Ceballosia*, *Argusia* and *Nogalia* not all systematic relationships between the clades are undoubtedly clarified.

HELIOTHAMNUS—The species of the Andean *Heliotropium* sect. *Heliothamnus* are excluded as a well-supported clade from HELIOTROPIUM I and II. This clade, probably segregated with the upfolding of the Andes, is characterized by plesiomorphic traits such as one-seeded mericarpids and anthers with pubescent and sometimes connected apices.

HELIOTROPIUM I *and* II—HELIOTROPIUM I and HELIOTROPIUM II are well-supported sister clades. They differ morphologically from the remaining species of Heliotropiaceae by the lack of pubescent or papillose apices of the anthers, which are never connate. Two-seeded endocarpids, and "empty chambers" are present only in these two groups. Obviously air-filled empty chambers next to the locules developed several times independently. They are of different shape and localisation and are present in species of different clades [see Figures of e.g. *Tournefortia usambarensis* ("Tournefortia" clade of HELIOTROPIUM I) in VERDCOURT 1991, p. 49 Fig. 10; *Ceballosia fruticosa* (HELIOTROPIUM II) in HILGER 1989 p. 127 Fig. 6d; *Heliotropium indicum* (*Tiaridium* clade of HELIOTROPIUM I) in ROSANOFF 1866, Plate VI, Fig. 15, 18, 19].

Within HELIOTROPIUM I empty chambers are present in all two-seeded "*Tournefortia"* species, within sect. *Heliotrophytum* only in *H. nicotianaefolium*, and within sect. *Tiaridium* only in *H. indicum*.

HELIOTROPIUM I—This clade comprises *Heliotropium* species distributed in the New World and the species of *Tournefortia* sect. *Tournefortia* as a part of *Heliotropium*.

With respect to the sectional relationships of *Heliotropium* species in HELIOTROPIUM I they cluster in by FÖRTHER (1998) accepted sections, but with unresolved relationships among another. The only exceptions are:

a) the species of sect. *Cochranea*. They are well-supported in a basal position within HELIOTROPIUM I.
b) all halophytic species. They comprise species of sect. *Platygyne*, *Tournefortia gnaphalodes* and *T. argentea* and cluster together. The systematic position of both *Tournefortia* species has been controversially discussed till now. In Table **6-2** we show the nomenclatural tangle of these (and other Heliotropiaceae) species.

JOHNSTON (1930, 1935, 1949, 1951), placed these *Tournefortia* species several times either *Messerschmidia* or *Tournefortia*, and pointed out that these species are closer related to

Heliotropium than to *Tournefortia*. Our results demonstrate that the halophytes constitute a distinct subclade of HELIOTROPIUM I.

The most important and surprising result, pointed out for the first time in DIANE *et al*. (2002a), is the unrecognized fact that the species of *Tournefortia* section *Tournefortia* and the *Heliotropium* species of the New World are very close related. "*Tournefortia*" represent a lineage of tropical New World *Heliotropium* species from humid environments (except halophytic *T. argentea*, *T. gnaphalodes*), whereas *Heliotropium* species itself prefer semi-arid habitats.

The radiation into humid-tropical conditions caused a change of growth forms and fruit types, and resulted in liana- or subscandent-species with drupaceous fruits. Beside molecular data, a close relationship of "*Tournefortia*" and New World *Heliotropium* species is also reflected in leaf anatomy, described in detail by DIANE *et al*. (in press b). Species of both groups are characterized by nearly identical leaf anatomy. They share important characters such as the lack of calcium oxalate tubes in the mesophyll or characteristic unicellular lithocysts with reduced trichome tips, which are not found in the species of the MYRIOPUS or EUPLOCA clades.

Thus, the species of *Tournefortia* section *Tournefortia* warrant incorporation into the genus *Heliotropium*. However, before new combinations in the genus *Heliotropium* are made for "*Tournefortia*", the exact relationship within New World *Heliotropium* needs to be resolved, and a revision of "*Tournefortia*" is inevitable. Currently, we advocate maintaining the genus *Tournefortia,* which is easily defined; we conclude that under this definition the genus *Heliotropium* is paraphyletic.

Actually, the only exceptions are halophytic *T. gnaphalodes* fall in synonymy of *Heliotropium* (Tab. **6-2**). For *T. argentea* we propose a new combination presented in Appendix **6-1** (see also Tab. **6-2**).

HELIOTROPIUM II—This clade comprises *Heliotropium* species distributed in the Old World plus *Argusia sibirica*, *A. sogdiana*, *Ceballosia fruticosa*, and *Nogalia drepanophyllum*, which should be maintained in *Heliotropium*, the genus under which they were described initially (Tab. **6-2**).

HELIOTROPIUM II is well-supported by the *trn*L dataset, and by the loss of nearly the whole helix I in the ITS1 secondary structure. Their very characteristic spear-like trichomes on the leaves (trichome type 5, detailed described in DIANE *et al*. in press b), support the monophyly of this group of Old World species. Exceptions are *Argusia sibirica* and *Ceballosia fruticosa*. Both species take an uncertain intermediate positions between the clades HELIOTROPIUM I and II, supported also by comparative leaf anatomy (DIANE *et al*. in press b).

Within HELIOTROPIUM II three major subclades have been identified. A large subclade is strongly indicated, comprising species of the sections *Heliotropium*, *Pleurolasia*, and *Odontotropium* as accepted by FÖRTHER (1998). FÖRTHER (1998) regarded them as closely related because of the shape of the style-stigma-complex and flower indument. However, our molecular data show that none of these tree sections is monophyletic. Current morphological circumscriptions do not reflect the phylogenetic relationships; *H. arbainense* and *H. hirsutissimum,* two species of sect. *Odontotropium,* serve as a good example. Section *Odontotropium* is defined by the occurrence of compact fornices-like intercalary teeth in epipetalous position inside the corolla tube. According to the molecular findings, these traits have to be regarded as convergent, because both species do not cluster together. *H. hirsutissimum* cluster with *H. suaveolens* (sect. *Heliotropium*). Indeed, FÖRTHER (1998) pointed out that *H. hirsutissimum* and *H. suaveolens* are nearly similar with the only exception of the lack or presence of intercalary teeth. Therefore, further investigations are necessary to find exact morphological definitions for sectional disposition.

In sister group relationship to above mentioned clade is the monotypic section *Chamaetropium* (*H. supinum*) which clusters with *H. drepanophylla* (Tab. **6-2**). The latter was separated as *Nogalia drepanophyllum* by VERDCOURT (1987). Apart from the molecular data, the close relationship of these two species is also supported by morphological traits. Both species exclusively share distinctly urceolate, inflated calyces which enclose the fruits at maturity and act as a dispersing unit.

The species of section *Pterotropium* constitute a natural group which is the sister of the subclades described above. They are characterized by lateral winged or bulging two- or one-seeded fruits (FÖRTHER 1998).

The remaining species of HELIOTROPIUM II, *H. zeylanicum, H. lineare, H. ciliatum, H. nelsonii*, and also *Argusia sogdiana* are unsupported in basal position of this clade. The aberrant morphology (FÖRTHER 1998) probably indicates these species as relicts of phylogenetically old lineages. Further investigations are necessary to resolve exact relationships.

With regard to both species of the genus *Argusia,* they do not constitute a natural group, this supported by molecular data and leaf anatomy (DIANE *et al*., in press b).

Appendix 6-1: Taxonomic recombinations within HELIOTROPIUM I, MYRIOPUS, and EUPLOCA

The current available molecular and morphological-anatomical results lead to nomenclatural recombination within the clades HELIOTROPIUM I, MYRIOPUS, and EUPLOCA which are here proposed:

HELIOTROPIUM I

Heliotropium foertheri Diane & Hilger ***nom. nov.***
pro *Tournefortia argentea* L.f., Suppl. Pl.: 133 (1781).
non *Heliotropium argenteum* Lehm. , Pl. asperif. nucif.: 73 (1818), sect. *Heliothamnus* (FÖRTHER 1998, p. 80). The name is in honor of Harald Förther, the important student of Heliotropiaceae.
For synonymies see Tab. **6-2**.

MYRIOPUS

Myriopus Small, in Man. S. E. Fl. 1131 (1933).
≡ *Tournefortia* L. sect. *Cyphocyema* I.M. Johnst., Contr. Gray Herb. 92: 72 (1930).
≡ *Messerschmidtia* [R. & Sch. (non. Linn.)] Miers, Ann. Mag. Nat. Hist. ser. 4, 2: 203 (1868) *nomen confusum*.
For further synonymies see JOHNSTON (1930).

Typus species: *Myriopus volubilis* (L.) Small, Man. S. E. Fl.: 1131 (1933).
≡ *Tournefortia volubilis* L., Sp. Pl.: 140 (1753). (Basionym)
For further synonymies see JOHNSTON (1935b, 1949, 1953, 1964).

Myriopus psilostachya (Kunth) Diane& Hilger **comb. nov**.
≡ *Tournefortia psilostachya* Kunth, Nov. Gen. et Sp. 3: 7 (1818). (Basionym)

Myriopus salzmannii (DC.) Diane & Hilger **comb. nov**.
≡ *Tournefortia salzmannii* DC., Prodr. 9: 524 (1845). (Basionym)
For further synonymies see JOHNSTON (1930, 1935b).

EUPLOCA

Euploca Nutt., Trans. Amer. Phil. Soc., n.s. 5: 189 (1837).
≡ *Heliotropium* L. sect. *Euploca* (Nutt.) A. Gray, Proc. Amer. Acad. Arts 10: 49 (1874).
= *Schleidenia* Endl., Gen. pl.: 646 (1839).
= *Heliotropium* L. sect. *Orthostachys* R.Br. subsect. *Axillaria* I.M. Johnst., Contr. Gray Herb. 81: 47 (1928).
= *Hilgeria* Förther, Sendtnera 5: 132 (1989).
For further synonymies see FÖRTHER (1998).

Typus species: *Euploca convolvulacea* Nutt., Trans. Amer. Philos. Soc., n.s. 5: 190 (1837).
≡ *Heliotropium convolvulaceum* (Nutt.) A.Gray, Mem. Amer. Acad. Arts, n.s. 6: 403 (1859).
For further synonymies see FÖRTHER (1998).

Euploca antillana (Urb.) Diane & Hilger **comb. nov.**
≡ *Heliotropium antillanum* Urb., Symb. antill. 4(3): 528 (1910). (Basionym)

Euploca baclei (DC.) Diane & Hilger **comb. nov.**
≡ *Heliotropium baclei* DC., Prodr. 9: 546 (1845). (Basionym)

Euploca ballii (Domin) Diane & Hilger **comb. nov.**
≡ *Heliotropium ballii* Domin, Biblioth. Bot. 22(89): 1098 (1928). (Basionym)

Euploca bursifera (C. Wright) Diane & Hilger **comb. nov.**
≡ *Heliotropium bursiferum* C. Wright, Griseb., Cat. pl. cub.: 211 (1866). (Basionym)

Euploca campestris (Griseb.) Diane & Hilger **comb. nov.**
≡ *Heliotropium campestre* Griseb., Abh. Königl. Ges. Wiss. Göttingen 19: 186 (1874). (Basionym)
For further synonymies see FÖRTHER (1998).

Euploca chrysantha (Phil.) Diane & Hilger **comb. nov.**
≡ *Heliotropium chrysanthum* Phil., Anales Univ. Chile 21: 401 (1862). (Basionym)

Euploca cupressina (Craven) Diane & Hilger **comb. nov.**
≡ *Heliotropium cupressinum* Craven, Austral. Syst. Bot. 9: 570 (1996). (Basionym)

Euploca humifusa (Kunth) Diane & Hilger **comb. nov.**
≡ *Heliotropium humifusum* Kunth, Nov. gen. sp. 3: 85 (1818). (Basionym)
For further synonymies see FÖRTHER (1998).

Euploca hypogaea (Urb. & Ekman) Diane & Hilger **comb. nov.**
≡ *Heliotropium hypogaeum* Urb. & Ekman, Ark. Bot. 22 A(10): 105 (1929). (Basionym)
≡ *Hilgeria hypogaea* (Urb. & Ekman) Förther, Sendtnera 5: 133 (1998).

Euploca lagoënsis (Warm.) Diane & Hilger **comb. nov.**
≡ *Heliotropium lagoënse* (Warm.) Gürke, Engl. & Prantl, Nat. Pflanzenfam. 4(3a): 97 (1893).
≡ *Schleidenia lagoënsis* Warm., Vidensk. Meddel. Dansk Naturhist. Foren. Kjobenhavn 1867: 15 (1867). (Basionym)
For further synonymies see FÖRTHER (1998).

Euploca mendocina (Phil.) Diane & Hilger **comb. nov.**
≡ *Heliotropium mendocinum* Phil., Anales Univ. Chile 21: 400 (1862). (Basionym)

Euploca ovalifolia (Forssk.) Diane & Hilger **comb. nov.**
≡ *Heliotropium ovalifolium* Forssk., Fl. aegypt.-arab.: 38 (1775). (Basionym)

Euploca procumbens (Mill.) Diane & Hilger **comb. nov.**
≡ *Heliotropium procumbens* Mill., Gard. dict. ed. 8: no. 10 (1768). (Basionym)
For further synonymies see FÖRTHER (1998).

Euploca pulvina (Craven) Diane & Hilger **comb. nov.**
≡ *Heliotropium pulvinum* Craven, Austral. Syst. Bot. 9: 577 (1996). (Basionym)

Euploca rariflora (Stocks) Diane & Hilger **comb. nov.** subsp. *hereroensis* (Schinz)
≡ *Heliotropium hereroense* Schinz, Vierteljahresschr. Naturf. Ges. Zürich 60: 404 (1915). (Basionym)
For further synonymies see FÖRTHER (1998).

Euploca serpylloides (Griseb.) Diane & Hilger **comb. nov.**
≡ *Heliotropium serpylloides* Griseb., Cat. pl. cub.: 212 (1866). (Basionym)
≡ *Hilgeria serpylloides* (Griseb.) Förther, Sendtnera 5: 133 (1998).

Euploca strigosa (Willd.) Diane & Hilger **comb. nov.**
≡ *Heliotropium strigosum* Willd., Sp. pl. 1(2): 743 (1798). (Basionym)
For further synonymies see FÖRTHER (1998).

Euploca styotricha (Craven) Diane & Hilger **comb. nov.**
≡ *Heliotropium styotrichum* Craven, Austral. Syst. Bot. 9: 580 (1996). (Basionym)

Euploca tenuifolia (R.Br.) Diane & Hilger **comb. nov.**
≡ *Heliotropium tenuifolium* R.Br., Prodr.: 494 (1810) (Basionym)
For further synonymies see FÖRTHER (1998).