5 Phylogenetic Analysis of Cordiaceae (Boraginales) Inferred from ITS1 Sequence Data [4]

Abstract.

The phylogenetic relationships of Cordiaceae (= Boraginaceae subfam. Cordioideae) are investigated. ITS1 sequences were obtained from 50 ingroup taxa and two outgroup taxa. *Cordia s.l.* in its traditional circumscription (i.e., excluding *Auxemma*, *Patagonula*, and *Saccellium*) turns out to be paraphyletic and falls into four major monophyletic assemblages more or less corresponding to established taxonomic units. Tree topology indicates that *Auxemma*, *Patagonula* and *Saccellium* should be included in *Cordia* in a narrow sense, of which *Varronia* (= *Cordia* sect. *Varronia*) is the sistergroup and is a well differentiated taxon deserving generic rank. *Cordia* as here retrieved in turn falls into three subclades: the *Collococcus* subclade (comprising *Cordia* sects. *Pilicordia*, *Physoclada*, and *Superbiflorae*), the *Myxa* subclade (= *Cordia* sect. *Myxa*), and the *Sebestena* subclade (comprising *Cordia* sects. *Cordia*, *Gerascanthus*, and *Rhabdocalyx p.p.*, including *Auxemma*, *Patagonula*, and *Saccellium*). Data from morphology also support the close relationship of *Auxemma*, *Patagonula*, and *Saccellium* with the *C. sebestena* species group (= *Cordia* sect. *Cordia p.p.*). The entire *Sebestena* subclade is not completely resolved, and phylogeny remains somewhat vague. *Cordia* sect. *Rhabdocalyx* is a polyphyletic assemblage of species belonging to the subclades *Sebestena* and *Collococcus*. Some general hypotheses on the evolution of Cordiaceae are supported by molecular data. The group apparently originated in the New World, the Old World representatives of the subclades *Myxa* and *Sebestena* colonised Africa independently probably by long-distance dispersal. A basic pattern of Cordiaceae has been reconstructed.

5.1 Introduction

Cordia s.l. comprising about 300 species is pantropical in distribution with centres of diversity in Africa and tropical America. The group is very well represented in countries such as Mexico,

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Brazil, and Peru, where they are very important elements in the tropical deciduous forests and shrub communities such as the Andean matorral in inneranden valleys and the western slope of the Andes. *Cordia* is also found in permanently wet habitats such as cloud forest and lowland rain forest. The plants are usually erect shrubs, sometimes trees, and scandent shrubs are found in *Cordia* sects. *Varronia* (in Peruvian cloud and mountain rain forests) and *Myxa* (in East African savannahs).

The other taxa traditionally assigned to Cordiaceae (= Cordioideae of "Boraginaceae" *sensu* GÜRKE 1893) are *Auxemma* (Brazil) and *Patagonula* (Brazil, Argentina) with probably two species each. They are readily distinguished from *Cordia* on the basis of calyx characters: The sepals of *Patagonula* develop into long wings (rotor-shaped) in the mature fruit (Fig. **1-2**), and *Auxemma* has an inflated calyx completely enclosing the fruit. Monotypic *Saccellium* (Bolivia, Brazil, Peru, Argentina), traditionally placed in the Ehretiaceae (= Ehretioideae of "Boraginaceae" *sensu* GÜRKE 1893), also belongs to Cordiaceae (BÖHLE & HILGER 1997, GOTTSCHLING *et al.* 2001) and is therefore included in the present analysis.

The monophyly of Cordiaceae is well-supported based both on molecular data (GOTTSCHLING *et al.* 2001) and on morphological apomorphies such as the presence of an undivided endocarp, four stigmatic lobes (**titelpage**, Fig. **1-5**), and plicate cotyledons (Fig. **1-1**). Cordiaceae are closely related to Ehretiaceae, Heliotropiaceae, and Lennoaceae (recently shown by GOTTSCHLING *et al.* 2001, DIANE *et al.* 2002b), which constitute the Primarily Woody Boraginales. The geological origin of Cordiaceae is dated into the Late Cretaceous (GOTTSCHLING *et al.* subm.).

The infrageneric classification of *Cordia* has been controversial. Some attempts have been made to segregate *Cordia* into separate genera (e.g, BROWNE 1756, BRITTON & WILSON 1925, FRIESEN 1933, BORHIDI *et al.* 1988). Most recent authors based on GÜRKE (1893) treat *Cordia* in a traditional, wider sense (e.g., MILLER 1985, GAVIRIA 1987, WARFA 1988, HEUBL *et al.* 1990, VERDCOURT 1991). Anything from 5 through almost 20 subgenera, sections, and subsections have been proposed for *Cordia*. The distribution of species over the variously recognised categories has been at least as confusing as the subdivisions themselves: Thus *Cordia* sect. *Varronia sensu* GÜRKE (1893, based on CANDOLLE 1845) is a heterogeneous assemblage of not closely related species, while three of four subsections GÜRKE (1893) placed under *Cordia* sect. *Myxa* really belong to *Cordia* sect. *Varronia* as JOHNSTON (1930) already recognised. Similarly, authors re-established, or described, new genera, which are para- or polyphyletic (e.g., *Varronia sensu* FRIESEN 1933, *Lithocardium* of KUNTZE 1891). The taxonomical enterprises were in times far reaching: About 170 new combinations and names were published in *Gerascanthus*, "... a rather varied group ..." with characters "... overlapping over the different taxonomic groups ..." (BORHIDI *et al.* 1988: 394–395), an assemblage, which is entirely unnatural.

The latest and by far most consistent infrageneric classification of *Cordia* was proposed by Ivan M. JOHNSTON, who initially divided the American species of *Cordia s.l.* into seven sections (JOHNSTON 1930) plus the Old World *Cordia* sect. *Myxa*. Later he included *Cordia* sect. *Pilicordia* in *Cordia* sect. *Myxa* (JOHNSTON 1951). This basic classification was widely accepted by subsequent authors. MILLER (2001) lists the following sections (with species numbers): *Cordia* (about 20 species in the New World, 2 in the Old World), *Gerascanthus* (about 20 species in the New World), *Myxa* (more than 100 species in the New World, approximately 55 species in the Old World), *Rhabdocalyx* (4 species in the New World), *Superbiflorae* (7 species in the New World), *Varronia* (approximately 100 species in the New World). This classification is here used as the taxonomic back drop for the phylogenetic study of Cordiaceae.

Classification of *Cordia* is further complicated by the fact that α-taxonomy is complex, and identification of the species is very difficult in many of the more diverse groups. This is partly due to the extraordinarily high intraspecific variability (e.g., GAVIRIA 1987, MILLER 1989), but also to the complete abandon with new taxa described on the basis of very poorly preserved herbarium specimens in the past (e.g., KRAUSE 1906).

In the present study, we aim at clarifying the relationships between the various groups in *Cordia* and its affinities to *Auxemma*, *Patagonula*, and *Saccellium* using sequence data from the First Internal Transcribed Spacer region (ITS1) and the *trn*L$_{\text{UAA}}$ (group I) intron. Furthermore, we want to determine the systematic placement of enigmatic species such as *C. bordasii* and *C. decandra* and the representatives of the heterogeneous *Cordia* sect. *Rhabdocalyx*. A generalised secondary structure of the ITS1 transcript has been proposed for Boraginales (GOTTSCHLING *et al.* 2001), and the additional characters from secondary structure are here used for the evaluation of the sequences. The secondary structure of the ITS1 transcript is conserved in Cordiaceae constituting an A-rich stem-loop with four major helices (GOTTSCHLING *et al.* 2001).

5.2 Materials and Methods

50 species of Cordiaceae were been investigated (Table **1**, appendix). Representatives of Ehretiaceae and Heliotropiaceae were used for the user specified outgroup comparison. DNA was obtained from silica dried leaves from our own field trips (Mexico, Panama: MILLER *et al.* 1983, 1987, 1991; Cuba: HILGER 1999, GOTTSCHLING 1999; Peru: WEIGEND *et al.* 2000, 2001) and from herbarium specimens of the herbaria B, KUN, M, and MO. DNA extraction, PCR, purification, and sequencing followed standard protocols, which are described in detail in GOTTSCHLING & HILGER (2001). The sequences were manually aligned using Se-Al v2.0a72 (RAMBAUT 2001). On the basis of a framework dictated by the secondary structure of the ITS1 transcript (GOTTSCHLING *et al.* 2001) another alignment (bi-directional, complementary) was investigated. The complete data matrices are available on request in NEXUS format. Furthermore, *trn*L sequences have been checked for deletions and insertions in the alignment editor. Parsimony analyses were performed using NONA 2.0 (GOLOBOFF 1993) and PAUP* 4.0b1 for Power Macintosh PC (SWOFFORD 1998). A distance procedure (neighbour-joining with 2000 replicates, KIMURA 1980) was calculated with TREECON (PEER & WACHTER 1993). Searches for best trees and bootstrap analyses

were run as in GOTTSCHLING & HILGER (2001). Gaps were considered as informative and calculated as fifth base (since otherwise the information from secondary structure is lost).

5.3 Results

All programs yielded trees with the same major topology and similar bootstrap support values (BS) with both parsimony searches and neighbour-joining. Using parsimony searches (PAUP) we obtained 64 shortest trees with L = 631 (CI: 0.556, RI: 0.801) using a usual alignment and 9853 shortest trees with L = 707 (CI: 0.536, RI: 0.798) using the alignment based on the secondary structure of the ITS1 transcript (not shown). Figure **5-1** shows a distance tree (TREECON) of *Cordia* and its allies. All representatives under investigation constitute a monophylum compared to the outgroup (99/91 BS). Within this monophylum we recognise four monophyletic groups (bootstrap support values from neighbour-joining: regular alignment / secondary structure alignment) with *Auxemma*, *Patagonula*, and *Saccellium* nested in *Cordia*:

1. The *Varronia* subclade (= *Cordia* sect. *Varronia*) with an exclusive New World distribution is monophyletic (100/100 BS) and is poorly resolved. Only the three species with 'spicate-syndesmic' inflorescences (*V. curassavica*, *V. lenis*, *V. stenophylla*) are clearly retrieved as a monophylum (98/99 BS).
2. The *Collococcus* subclade (100/100 BS) with a primarily New World distribution comprises *Cordia* sects. *Pilicordia*, *Physoclada* (monotypic: *C. nodosa* with characteristical ant domatia), and *Superbiflorae*. *C. nodosa* is nested in the *Collococcus* subclade. Furthermore, *C. varronifolia* (*Cordia* sect. *Rhabdocalyx*) is nested in the strongly supported monophylum representing *Cordia* sect. *Superbiflorae* (96/95 BS).
3. The *Myxa* subclade (100/100 BS) with a largely Old World distribution comprises *Cordia* sect. *Myxa* and some enigmatic New World species (i.e., *C. lutea* and *C. dentata*). All Old World taxa constitute a moderately supported monophylum (62/59 BS), respectively a well-supported monophylum after the exclusion of the two basal taxa *C. africana* and *C. mairei* (99/98 BS).
4. The *Sebestena* subclade (62/77 BS) with a mostly New World distribution comprises *Cordia* sects. *Cordia*, *Gerascanthus*, *Rhabdocalyx p.p.*, *C. decandra*, and the three formerly segregated taxa *Auxemma*, *Patagonula*, and *Saccellium*. The *Sebestena* subclade itself is not well resolved. Neither *Cordia* sects. *Gerascanthus* nor *Rhabdocalyx* (excluding *C. varronifolia* that is nested in *Cordia* sect. *Superbiflorae*) seem to be monophyletic assemblages, only the *C. sebestena* species group (= *Cordia* sect. *Cordia*, excluding *C. bordasii*) is possibly monophyletic (but BS < 50). Compared to the other subclades the branches found in the *Sebestena* subclade are very long. A close relationship of

Patagonula and *Saccellium* (BS < 50 in the ITS1 data set) can be inferred from the *trn*L_{UAA} intron data set (Fig. **5-2**): Only these taxa share a unique 5 bp insertion.

The relationships between these four monophyletic groups can be largely clarified. The *Varronia* clade is the sistergroup of the remainder of all other ingroup taxa (89/98 BS). The *Sebestena* subclade appears to be the sistergroup of the subclades *Collococcus* and *Myxa*, the close relationship of the latter is well-supported (73/84 BS).

Hairpin I and IV of the secondary structure of the ITS1 transcript differ slightly in length, especially hairpin I is shorter in the Old World representatives of the *Myxa* subclade (excluding *C. africana* and *C. mairei*) as compared to all other taxa (from about 17 through 13, not shown). Furthermore, the conservative hairpin III is 5 bp long in the subclades *Sebestena* and *Varronia*, 6 bp long in the subclades *Collococcus* and *Myxa*. While the major topologies do not differ between different programs and methods, the bootstrap support values vary slightly using different alignments.

5.4 Discussion

The molecular tree. Cordiaceae (= Cordioideae of "Boraginaceae" *sensu* GÜRKE 1893) as here identified constitute a monophylum. This hypothesis is supported by molecular data (99/91 BS) and was never seriously doubted. JOHNSTON (e.g., 1930, 1951), who worked on *Cordia* and its allies for over 25 years, already emphasised the naturalness of the group with the only exception of *Saccellium*, which he erroneously placed in Ehretiaceae (= Ehretioideae of "Boraginaceae" *sensu* GÜRKE 1893).

JOHNSTON (1930) proposed the first comprehensive and modern classification for American species of *Cordia*, and his classification is rather close to the molecular results here presented. He primarily distinguished seven infrageneric taxa, later he reduced this initially proposed *Cordia* sects. *Pilicordia* under the consequently pantropical *Cordia* sect. *Myxa* (JOHNSTON 1951).

On the basis of our molecular data we recognise four major clades in *Cordia s.l.*: 1. the *Varronia* subclade (= *Cordia* sect. *Varronia*; 100/100 BS) with *Varronia bullata* L. as type species; 2. the *Collococcus* subclade (comprising *Cordia* sects. *Pilicordia*, *Physoclada*, and *Superbiflorae*; 100/100 BS) with *Cordia collococca* L. as type species; 3. the *Myxa* subclade (= *Cordia* sect. *Myxa*, plus *C. dentata* and *C. lutea*; 100/100 BS) with *Cordia myxa* L. as type species; 4. the *Sebestena* subclade (comprising *Cordia* sects. *Cordia*, *Gerascanthus*, *Rhabdocalyx p.p.*, *C. decandra*, *Auxemma*, *Patagonula*, and *Saccellium*; 62/77 BS) with *Cordia sebestena* L. (**titelpage**, Fig. **1-3**) as type species.

Phylogenetic relationships of these taxa has not been discussed extensively. The distinctness of *Varronia* has been previously emphasised by many authors (e.g., JOHNSTON 1949, TARODA &

GIBBS 1986, BORHIDI *et al.* 1988, NOWICKE & MILLER 1990). Within the remainder of *Cordia* the distance tree suggests a sistergroup relationship between the subclades *Collococcus* and *Myxa* (73/84 BS), which has already been hinted at by JOHNSTON (1951) when he included exclusively New World *Cordia* sect. *Pilicordia* in *Cordia* sect. *Myxa*. Together they are the sistergroup of the *Sebestena* subclade (89/98 BS).

Relationships within the *Sebestena* subclade are poorly resolved, and both, *Cordia* sects. *Rhabdocalyx* (excluding *C. varronifolia* that is nested in *Cordia* sect. *Superbiflorae*) and *Gerascanthus*, are not monophyletic. The poor resolution within the *Sebestena* subclade is probably based on 'long-branch attractions' (e.g., WÄGELE 2000). Many representatives (especially, e.g., *C. parvifolia* and *Saccellium*) have long branches in the ITS1 distance tree, which may produce random clusters ('plesiomorphy trap' or – better – in this case: 'convergent trap'). However, at least the close relationship of *Patagonula* and *Saccellium* is based on an apomorphic insertion in the *trn*L_{UAA} intron (Fig. **5-2**).

The distinctness of *Auxemma*, *Patagonula*, and especially *Saccellium* had never been doubted, and they have invariable been kept as taxa separate from *Cordia*. Only MIERS (1875) in his treatment "on the Auxemmeæ" associated *Auxemma*, *Patagonula*, and *Saccellium* with other newly described genera representing species of the *C.* s*ebestena* species group without giving any details about his view of the relationships. A close examination of the morphological characters underscores the plausibility of the placement of *Auxemma*, *Patagonula*, and *Saccellium* within *Cordia* (see below).

Since the representatives of *Cordia* sect. *Gerascanthus* do not constitute a monophylum with the subclades *Collococcus* and *Myxa*, a segregation of *Cordia* into three entities *Cordia*, *Gerascanthus*, and *Varronia* (BORHIDI *et al.* 1988, incited by NOWICKE & RIDGEWAY 1973) is not justified.

Information from secondary structure. It could be shown that investigation on the secondary structure is a useful tool for recognising homologous base pairings (GOTTSCHLING *et al.* 2001). Length variation of hairpins is especially known from Heliotropiaceae (GOTTSCHLING *et al.* 2001, DIANE *et al.* 2002a). In Cordiaceae differences in the secondary structure are found in hairpin IV, III, and especially hairpin I, while hairpin II is conserved. The usual length of hairpin I is about 17 bp (plesiomorphic condition, found in most sequences investigated), while in the Old World species of the *Myxa* subclade (excluding *C. africana* and *C. mairei*) it is reduced to about 13 bp (apomorphic). The shortened hairpin I (when gaps are calculated as fifth base) is a reason for the high bootstrap supports (99/98 BS) of the *Myxa* subclade (excluding New World species and *C. africana* and *C. mairei*). Furthermore, using secondary structure for the alignment improves bootstrap support for, e.g., the sistergroup relationship between the Subclades *Collococcus* and *Myxa* (from 73 to 84 BS) and the monophyly of the *Sebestena* subclade (from 62 to 77 BS).

Plausibility of the tree: Morphological characters. The systematic importance of fruit anatomy has been demonstrated for Ehretiaceae (e.g., GÜRKE 1893, MILLER 1989, GOTTSCHLING 2001, GOTTSCHLING & HILGER 2001, GOTTSCHLING *et al.* 2002). In Cordiaceae the drupe consists of a very hard, undivided endocarp enclosing four or (by abortion) fewer seeds, a feature only sporadically found in other Boraginales (e.g., *Ehretia microphylla* of Ehretiaceae, *Heliotropium supinum* of Heliotropiaceae, species of *Rochelia* of Boraginaceae *s.str.*). An undivided endocarp (Fig. **5-3**) is the most striking apomorphy of all representatives of Cordiaceae as retrieved by molecular data (99/91 BS) apart from the twice bifid style (**titelpage**, Fig. **5-4**). It is derived from the four-parted endocarp found in most other representatives of Boraginales. Plicate cotyledons (Figs. **1-1**, **2-7**, **5-5**) are another unique character for Boraginales, which are doubtless an aut-apomorphy for Cordiaceae. This feature may be causally related to the loss of endosperm.

Traditionally, *Saccellium* has been placed in Ehretiaceae because of its bifid style (not twice bifid as in Cordiaceae). Molecular data indicate a placement of *Saccellium* within Cordiaceae (already shown in GOTTSCHLING *et al.* 2001). Since it shares an undivided endocarp and plicate cotyledons (already observed by CANDOLLE 1845 and MIERS 1875), which are here considered as apomorphic for Cordiaceae, this placement does not come as a surprise. Conversely, the bifid style cannot be used to identify primarily relationships in Boraginales since it represents the plesiomorphic condition and is thus possibly a simple reversion in *Saccellium*.

Stigma shape. In most representatives of Cordiaceae as well as in the outgroup capitate or clavate stigmas are found (Fig. **5-8**, plesiomorphic condition). Linear stigmas on filiform stylar branches occur only sporadically (e.g., in *V. bullata*), and in Old World species of the *Myxa* subclade (excluding *C. africana* and *C. mairei*, Figs. **1-5**, **5-9**) the linear stigmas are often linked to dioecy. Linear stigmas have to be considered as apomorphy of the corresponding species group within the *Myxa* subclade, which is supported as a monophylum by molecular data (99/98 BS).

Pollen morphology. Pollen morphology yields some important characters in Cordiaceae (e.g., SAHAY 1979, BORHIDI *et al.* 1988, HEUBL *et al.* 1990, NOWICKE & MILLER 1990). All pollen types found in Cordiaceae lack pseudocolpi (probably apomorphic), while pseudocolpi are present in the other Primarily Woody Boraginales (probably plesiomorphic). At least three different pollen types can be distinguished:

3-colporate pollen, with a variously spinulose exine, is found in the subclades *Collococcus*, *Myxa*, and *Sebestena* (excluding the *C. sebestena* species group and *Auxemma*, *Patagonula*, and *Saccellium*), the spines are often very prominent in the *Collococcus* subclade. Since taxa with the 3-colporate type, with spinulose exine, are paraphyletic, it is most parsimonious to assume this type as plesiomorphic condition in Cordiaceae. Both other types seems to base monophyla: The 3-porate type (with spinulose exine, occasionally with reticulate muri) of *Varronia* is unique among Boraginales and doubtless an apomorphy (which has 100/100 BS in the molecular analysis). 3-colporate pollen, with reticulate and/or striate ornamentation, is only found in the *C. sebestena* species group and, furthermore, in *Auxemma*, *Patagonula*, and *Saccellium.* This does thus not confirm the "... separate generic status ..." of *Auxemma* and *Patagonula* (HEUBL *et al.* 1990: 129), but rather lend further support to the at first glance heterogeneous clade comprising the *C. sebestena* species group and *Auxemma*, *Patagonula*, and *Saccellium* (but BS < 50). *C. bordasii* has a rugulate ornamation, and MILLER & NOWICKE (1989) suggested therefore a close relationship with the *C. sebestena* species group. Unfortunately, this plausible hypothesis is not supported by ITS1 data (*C. bordasii* is nested in *Cordia* sect. *Gerascanthus p.p.*).

Crystal types. HEUBL *et al.* (1990) distinguished several crystal-types in the wood of Cordiaceae. The presence of crystal sand in all taxa investigated indicates it as plesiomorphic condition. Additionally, prismatic crystals are found in the *Sebestena* subclade (excluding the *C. sebestena* species group), and columnar crystals are present in the *C. sebestena* species group. These crystal types are currently only known from this monophylum in the Boraginales. We consider both crystal types as homologous and as lending additional support to the monophyly of the *Sebestena* subclade (62/77 BS). The columnar crystals in the *C. sebestena* species group appear to be derived from the less complex prismatic crystals found today in the remainder of the *Sebestena* subclade.

Inflorescence morphology. Most representatives of Cordiaceae have thyrsoids with mono- or dichasial paracladia that are also found in the outgroup (most Boraginales; plesiomorphic condition). However, in *Varronia* syndesmia (UHLARZ & WEBERLING 1977) have arisen from thyrsoids *via* complex growth processes, leading to a doubling back of the inflorescence apex and the characteristical 'disorderly' appearance of most *Varronia* inflorescences (older flowers are interspersed between young buds, flowering sequence is often acropetalous). Two major types of syndesmia (distinguishable in early ontogeny) have been proposed: 'cephaloid' and 'spicate' (e.g., GAVIRIA 1987, Figs. **1-4**, **5-6** and **5-7**). UHLARZ & WEBERLING (1977) demonstrated that the 'spicate-syndesmic' inflorescence can be derived from the 'cephaloid-syndesmic' inflorescence, this transition is also retrieved by our molecular data (98/99 BS). Thus the 'cephaloid-syndesmic' inflorescence of the majority of representatives of *Varronia* can be regarded as an apomorphy for the entire subclade (which has 100/100 BS). Conversely, the glomerulate inflorescences found in *Cordia* sect. *Gerascanthus* originate from simple congestion and are not homologous to the syndesmia of the *Varronia* subclade.

Corolla size. As in most groups of angiosperms corolla size is highly homoplasious: Species with large corollas (more than 20 mm in diameter, apomorphic condition) are found in all four subclades (e.g., *Varronia lauta*, *C. mairei* from the *Myxa* subclade, the latter with flowers up to 10 cm long), while most Cordiaceae (and outgroup representatives) have relatively small corollas (not exceeding 15 mm in diameter, plesiomorphic condition). There are only two clades with exclusively large corollas (MILLER & NOWICKE 1989), the *C. sebestena* species group (in the *Sebestena* subclade, **titelpage**, Fig. **1-3**) and *Cordia* sect. *Superbiflora* (in the *Collococcus* subclade). The molecular data suggest the convergent development of large corollas that base the monophyly of each, the *Cordia* sect. *Superbiflora* (96/95 BS) and the *C. sebestena* species group (BS < 50).

Fruit morphology. After pollination the young drupe is protected by the calyx in all species of Cordiaceae, often an accrescent calyx completely encloses the mature drupe (e.g., in many species of the *Sebestena* subclade: possibly apomorphic; 62/77 BS). The carnose calyx found in the *C. sebestena* species group (Fig. **5-3**) has an attractive function in fruit dispersal for animals such as birds and mammals and is unique in Boraginales and argues for the monophyly of the corresponding group (BS < 50). The calyx is sporadically also inflated (e.g., in some species of *Varronia*). The drupes of *Auxemma* and *Saccellium* are completely enclosed by a strongly inflated, papery calyx, and superficially look very similar (Figs. **2-4**, **5-10**). They are different from all other Cordiaceae fruits, and thus it was the reason for their generic segregation. This similarity seems to be due to convergence since these taxa are not retrieved as sister taxa: *Saccellium* is sister to *Patagonula*, with spreading, rotor-shaped calyx lobes on the drupe (Fig. **1-2**), as inferred from the 5 bp insertion found in the *trn*$\mathrm{L}_{\mathrm{UAA}}$ intron (Fig. **5-2**).

In some, but not all species of the *Collococcus* subclade (represented by, e.g., *C. collococca*, *C. elliptica*, and *C. nodosa*) the fruit becomes asymmetrically oblique during ontogeny (Figs. **5-11** and **5-12**). This feature is occasionally found in other Boraginales (e.g., *Caccinia* and *Trichodesma* of Boraginaceae *s.str.*), but unique in Cordiaceae and can be regarded as apomorphy for a species group within the *Collococcus* subclade (71/78 BS).

Conclusion: Basic pattern of Cordiaceae. Figure **5-13** summarises the results of this study. Morphological apomorphies, geographical separation and other events are indicated together with high bootstrap support values from the molecular phylogeny (Fig. **5-1**). Subordinate taxa of *Cordia s.l.* are indicated.

The last common ancestor of Cordiaceae appears to have lived in tropical climates in the Upper Cretaceous in the Western part of the Gondwana continent (GOTTSCHLING *et al.* subm.). The ancestral character states as deduced from the cladogram are: Woody habit, small trees or shrubs; phyllotaxis alternate; leaves undivided, obovate, estipulate, with an entire margin; flowers bisexual, calyx with valvate aestivation, enclosing the drupe at least in early ontogeny, and persisting in mature fruits, corolla sympetalous, with short tube and spreading lobes, stamens included, style with four stigmas (apomorphic for Cordiaceae); pollen 3-colporate with a spinulose exine; fruits with undivided endocarp (apomorphic for Cordiaceae) with some irregular ridges on the outer surface and with a brightly coloured exocarp (endozoochoria); cotyledons plicate (apomorphic for Cordiaceae).

These character states can now be used to investigate the precise phylogenetic relationships of Cordiaceae within Boraginales.

5.5 Formal taxonomy

Our analysis retrieves a basal dichotomy in Cordiaceae, namely between *Varronia* and the remainder of *Cordia* in its traditional circumscription including *Auxemma*, *Patagonula*, and *Saccellium*. This underscores the correctness of the proposal by multiple authors (e.g., JOHNSTON 1949, TARODA & GIBBS 1986, NOWICKE & MILLER 1990) to segregating *Varronia* from *Cordia* and re-establishing the genus *Varronia* P.BR. The correct combinations of the corresponding species names have already largely been provided (BORHIDI *et al.* 1988). On the other hand, *Auxemma*, *Patagonula*, and *Saccellium* cannot be maintained as independent genera and have to be included in a more narrowly circumscribed genus *Cordia* L. (i.e., excluding *Varronia*) to avoid paraphyly. To conserve generic status for these segregates, without recognising paraphyletic entities, a total of at least eight genera would have to be established. This would mean segregating very similar groups such as *Collococcus* P.BR., *Cordia* L. (or *Sebestena* BOEHM. when *C. myxa* is chosen as type of *Cordia*), *Gerascanthus* P.BR., and *Myxa* (ENDL.) FRIESEN (when *C. sebestena* is chosen as type of *Cordia*) into distinct genera, which seems highly undesirable (at least for non-specialists and field-workers). It would also require the creation of a large number of new combinations and names and would thus run counter to the desirable aim of taxonomic stability.

I. *Varronia* P.BR.

Most new combinations have already been provided by BORHIDI *et al.* (1988). However, two species still requires a new name:

1. *Varronia lauta* (I.M.JOHNST.) GOTTSCHLING *comb. nov.*, ≡ *Cordia lauta* I.M.JOHNST., J. Arnold Arbor. **37**: 288 (1956), basionym.

2. *Varronia leucocephala* (MORIC.) GOTTSCHLING *comb. nov.*, ≡ *Cordia leucocephala* MORIC., Pl. nouv. Amér.: 148, tab. 88 (1847), basionym.

II. *Auxemma* MIERS

3. *Cordia gardneriana* (MIERS) GOTTSCHLING *comb. nov.*, ≡ *Auxemma gardneriana* MIERS, Trans. Linn. Soc. (Ser. 2) **1**: 23, tab. 5 (1875), basionym.

4. *Cordia glazioviana* (TAUB.) GOTTSCHLING *comb. nov.*, ≡ *Auxemma glazioviana* TAUB., Bot. Jahrb. Syst. **15**, Beibl. **34**: 11 (1892), basionym.

5. *Cordia oncocalyx* ALLEMÃO, Trab. Comm. Sci. Expl. Bot. **1**: 11 (1862), ≡ *Auxemma oncocalyx* (ALLEMÃO) BAILL., Hist. pl. **10**: 396 (1890).

III. *Patagonula* L.

6. *Cordia americana* (L.) STEUD. ex GOTTSCHLING *comb. nov.* [≡ *Cordia americana* STEUD., Nomencl. bot. **2** (ed. 2): 309 (1841), *in syn.*], ≡ *Patagonula americana* L., Sp. pl.: 149 (1753), basionym.

7. *Cordia incognita* GOTTSCHLING *nom. nov. pro* ≡ *Patagonula bahiensis* MORIC., Pl. nouv. Amér.: 144, tab. 86 (1847), *non* ≡ *Cordia bahiensis* DC. in A.DC., Prod. **9**: 489 (1845).

IV. *Saccellium* HUMB. & BONPL.

8. *Cordia saccellia* GOTTSCHLING *nom. nov. pro* ≡ *Saccellium lanceolatum* HUMB. & BONPL., Pl. aequinoct. **1**: 47, tab. 13 (1808), *non* ≡ *Cordia lanceolata* (DESV.) HUMB., BONPL. & KUNTH, Nov. gen. sp. **3**: 75 (1818).