

10 Conclusion and Prospects

Figure 10-1 summarises the results of this thesis. Selected morphological apomorphies and estimated minimum ages are indicated together with bootstrap support values from the molecular phylogenies (Figs. 3-10, 5-1, 6-3). Subordinate taxa of Boraginales are indicated.

Boraginales with approximately 2700 species comprise Boraginaceae *s.str.* (1500 species), Hydrophyllaceae *s.str.* (250 species), Heliotropiaceae (500 species), Cordiaceae (300 species), and Ehretiaceae (150 species). A clear morphological apomorphy is still missing for Boraginales, but the monophyly has molecular support from the ITS1 (GOTTSCHLING *et al.* 2001) as well as from other markers such as *rbcL*, 18S, and *atpE* (CHASE *et al.* 1993, OLMSTEAD *et al.* 1993, ALBACH *et al.* 2001, BREMER *et al.* 2002). A reconstruction of the complete phylogeny of Boraginales cannot be provided at this moment in time because both of incomplete knowledge on character evolution and of the uncertainty with regard to the closest living relatives of Boraginales. However, the major topology of the phylogeny provided in this thesis may be helpful to discuss character polarisation.

The relationships of the subordinate taxa of Boraginales can be largely clarified. “Boraginaceae” *sensu* GÜRKE (1893) are polyphyletic when Hydrophyllaceae *s.str.* and parasitic Lennoaceae are excluded (GOTTSCHLING *et al.* 2001, DIANE *et al.* 2002b, GOTTSCHLING & HILGER in prep.), which has been also demonstrated in former molecular studies with a more limited taxon sampling (e.g., CHASE *et al.* 1993, FERGUSON 1999, SMITH *et al.* 2000). The molecular results confirm the systematic concept of THORNE (1983), who recognised the close relationship of the subordinate taxa of “Boraginaceae” *sensu* GÜRKE (1893, i.e., Boraginaceae *s.str.*, Heliotropiaceae, Cordiaceae, Ehretiaceae) with Hydrophyllaceae *s.str.* and Lennoaceae. Conversely, CRONQUIST (1988) was not right in separating Hydrophyllaceae *s.str.* from the remainder of Boraginales.

Boraginaceae *s.str.* are the sistergroup of a clade comprising Hydrophyllaceae *s.str.*, Heliotropiaceae, Cordiaceae, and Ehretiaceae based both on molecular (GOTTSCHLING *et al.* 2001) and on morphological data (DIANE *et al.* 2002b). Furthermore, Heliotropiaceae, Cordiaceae, and Ehretiaceae constitute a monophyletic group (Primarily Woody Boraginales) based on common derived characters (multilayered endocarp, transfer cells in the seed coat: DIANE *et al.* 2002b) supported by molecular data (GOTTSCHLING *et al.* 2001). Within the Primarily Woody Boraginales, Heliotropiaceae, Cordiaceae, and Ehretiaceae are monophyletic, and Cordiaceae (possibly including *Coldenia*) and Ehretiaceae (including the former Lennoaceae, *Lennoa* and *Pholisma*) appear to be sistergroups (GOTTSCHLING *et al.* 2001, DIANE *et al.* 2002a, GOTTSCHLING & HILGER in prep., GOTTSCHLING *et al.* in prep. a).

Molecular and morphological results provided by this thesis suggest a different evolutionary scenario for Boraginales with a different character polarisation than proposed so far (e.g., JOHNSTON 1950, 1951, HEUBL *et al.* 1990, GOTTSCHLING 2001, GOTTSCHLING & HILGER 2001). Cordiaceae and Ehretiaceae do not represent basal lineages, but together they have a derived position in Boraginales. It is therefore plausible to accept the drupaceous fruits, with the multilayered endocarp, as derived in Boraginales and informative about the close relationship of Heliotropiaceae, Cordiaceae, and Ehretiaceae. A multilayered endocarp is occasionally developed in other asterids such as Acanthaceae, Apocynaceae, Oleaceae, Solanaceae, and Verbenaceae (e.g., ROHWER 1996), but homology with that found in the Primarily Woody Boraginales cannot be stated. SVENSSON (1925) investigated the embryology of some representatives of Boraginales. He suggested a close relationship of Hydrophyllaceae *s.str.* with Heliotropiaceae, Cordiaceae, and Ehretiaceae based on the development of endosperm haustoria that are absent in Boraginaceae *s.str.* This hypothesis is also congruent with the molecular data provided by this thesis (GOTTSCHLING *et al.* 2001).

The systematic position of enigmatic taxa is partly resolved. *Pteleocarpa* is a representative of Gentianales and does not belong to Boraginales (GOTTSCHLING & HILGER in prep.). *Hydrolea*, formerly placed in Hydrophyllaceae, is now recognised as nested in Solanales (e.g., FERGUSON 1999). The systematic position of Lennoaceae (*Lennoa* and *Pholisma*, Fig. 1-6) nesting in Ehretiaceae is likely based on molecular studies of this thesis (GOTTSCHLING *et al.* 2001, GOTTSCHLING & HILGER in prep.) together with those of other authors (e.g., SMITH *et al.* 2000). Furthermore, transfer cells in the seed coat (DIANE *et al.* 2002b) and a multilayered endocarp (HALLIER 1923, YATSKIEVYCH *et al.* 1986) found in *Pholisma* indicate a placement of Lennoaceae within the Primarily Woody Boraginales. *Coldenia* is not closely allied to *Tiquilia* as generally accepted for over a century (e.g., GRAY 1862). RICHARDSON (1977) firstly recognised their distinctiveness and reinstated *Tiquilia* as a taxon separate from *Coldenia*. *Tiquilia* is a representative of Ehretiaceae, while *Coldenia* possibly belongs to Cordiaceae (GOTTSCHLING & HILGER in prep.).

The systematic position of *Codon* (formerly placed in Hydrophyllaceae *s.str.*) and *Wellstedia* (Wellstediaceae) cannot yet be resolved. Both have affinities rather to Boraginaceae *s.str.* than to any other taxon of the asterids (unpublished data from the *trnL_{UAA}* intron) as has been previously demonstrated for *Codon* by FERGUSON (1999). On the other hand, *Codon* has the short hairpin II with a conserved motif in the ITS1 transcript (unpublished), which has been considered as common derived character of Hydrophyllaceae *s.str.*, Heliotropiaceae, Cordiaceae, and Ehretiaceae (GOTTSCHLING *et al.* 2001) indicating a position outside Boraginaceae *s.str.* (with a long and variable hairpin II). Further studies are necessary to resolve these problems, an investigation of, e.g., the seed coat is recommended.

The phylogeny of Cordiaceae (GOTTSCHLING *et al.* in prep. a) is partly resolved with *Cordia* and *Varronia* as sistergroups; *Coldenia* possibly also belongs to Cordiaceae (which had been placed

in Ehretiaceae, GOTTSCHLING & HILGER in prep.). *Cordia* segregates into three monophyletic assemblages, the subclades *Collococcus*, *Myxa*, and *Sebestena*. This resolution is a clear advantage in face of numerous systematic concepts in *Cordia* (including *Varronia*) based both on the elevation of well-recognisable species groups with clear apomorphies and on the negligence of the subsequently paraphyletic remainder. This proceeding is best illustrated with *Auxemma*, *Patagonula*, and *Saccellium*, which have been considered as distinct from *Cordia* since their first description. They certainly have specialised fruit morphologies (as do other representatives of *Cordia* such as *Cordia trichotoma* with a persistent corolla), but molecular (ITS1) and morphological data (wood anatomy, pollen surface) indicate a systematic placement of these taxa in the *Sebestena* subclade of *Cordia* (GOTTSCHLING *et al.* in prep a).

Ehretiaceae had been the only taxon of Boraginales, for which monophyly is doubtful in the absence of apomorphic characters. Furthermore, high variation in the ITS1 sequences provided difficulties with the alignment and, therefore, rendered a well-resolved molecular phylogeny of Ehretiaceae problematical. However, sequences of the less variable *trnL_{UAA}* intron indicate the following monophyletic taxa as representatives of a monophyletic taxon Ehretiaceae: *Bourreria*, *Ehretia* (including *Carmona*, *Rotula*, and probably also *Cortesia* based on fruit anatomy), *Halgania*, *Lennoa*, *Lepidocordia*, *Pholisma*, *Rochefortia*, and *Tiquilia* (GOTTSCHLING & HILGER in prep.). The relationships of these taxa are not completely resolved at this moment.

Analysing the secondary structure has turned out to be a useful tool for understanding molecular evolution (e.g., COLEMAN & MAI 1997, COLEMAN *et al.* 1998, GOTTSCHLING *et al.* 2001, WOLF *et al.* 2002, GOTTSCHLING & HILGER in prep.). At least two independent abridgements of hairpins in the ITS1 transcript could be found in representatives of *Heliotropium* and *Cordia* (GOTTSCHLING *et al.* 2001, DIANE *et al.* 2002a, GOTTSCHLING *et al.* in prep. a), increasing the number of structural characters. The identification of homologous regions in the ITS1 transcript helps to optimise the alignment and, therefore, renders a molecular analysis at higher taxonomical levels possible (GOTTSCHLING *et al.* 2001). Additionally, the conservation of the secondary structure of the *trnL_{UAA}* intron and therefore the homology of this molecule in cyanobacteria, green algae, and angiosperms (CECH 1988, KUHSEL *et al.* 1990) could be supported (GOTTSCHLING & HILGER in prep.). These applications of sequence data should also be advanced for other plant groups and other markers to optimise data processing in molecular studies.

The time estimates suggest an origin of all major taxa of Boraginales in the mid Cretaceous (GOTTSCHLING *et al.*, *subm.*), which is older than supposed so far (WIKSTRÖM *et al.* 2001). Yet, plate tectonics as separations event seem to play a minor role in the historical biogeography of Heliotropiaceae, Cordiaceae, and Ehretiaceae as has also been demonstrated for many other angiosperm taxa (e.g., SUH *et al.* 1993, XIANG *et al.* 2000, RENNER *et al.* 2000). However, the investigations of the Primarily Woody Boraginales are significant because studies on tropical, woody plant groups are still scarce (e.g., RENNER & MEYER 2001, MALCOMBER 2002). Most

disjunctions in the Primarily Woody Boraginales are best explained by long-distance dispersal based on the abundant occurrence of drupaceous fruits with their potential for endozoochory (Fig. 1-12). Three major problems are involved in time estimates based on molecular data: inconstancy of substitution rates, doubtful systematic placement of fossils, and inaccuracy of absolute geological dating. However, the precision of time estimates will increase with the knowledge on molecular evolution (different genomes, different markers) that is still very poor.

During the last years the application of molecular methods in the working group of Hartmut H. HILGER (Freie Universität Berlin) yielded many new insights in the evolution of Boraginales (e.g., BÖHLE *et al.* 1996, BÖHLE & HILGER 1997, GOTTSCHLING *et al.* 2001, DIANE *et al.* 2002a, GOTTSCHLING & HILGER in prep.). However, a comprehensively phylogenetic analysis based on morphological data is still lacking in Boraginales. Many detailed data of flower and fruit morphology (especially on ontogeny, HILGER 1985) have been accumulated for Boraginaceae *s.str.*, Heliotropiaceae, and Hydrophyllaceae *s.str.* (e.g., SEIBERT 1978, HILGER 1985, 1987, 1992, HOFMANN 1994, 1999). Data from the literature (e.g., PITOT 1939a, b, FULVIO 1965, YATSKIEVYCH *et al.* 1986), preliminary (GOTTSCHLING 2001, GOTTSCHLING & HILGER 2001), and unpublished investigations of flower and fruit anatomy indicate a large variation of different types of generative organs in Cordiaceae and Ehretiaceae. As a next step, these morphological data should be compiled and phylogenetically evaluated in direct reference to the molecular data. I hope that the present thesis proves one fruitful step towards the continued study of the phylogeny of Boraginales at the Institut für Biologie – Systematische Botanik und Pflanzengeographie.