

1 Introduction

Boraginales (= Boraginaceae *s.l.*) comprise about 2700 species and are distributed in tropical, subtropical, and temperate regions of the world. Centres of diversity are in America, East Asia, and in Mediterranean habitats of the Old World and the New World (e.g., AL-SHEHBAZ 1990). Based on molecular data, Boraginales have a basal and isolated systematic position in the asterids (e.g., CHASE *et al.* 1993, ALBACH *et al.* 2001, WIKSTRÖM *et al.* 2001, BREMER *et al.* 2002). Economic importance of Boraginales appeared to be low for a long period of time, but it has turned out that several representatives have medicinally valuable compounds (e.g., pyrrolizidine alkaloids: SELVANAYAGAM *et al.* 1995, STEGELMEIER *et al.* 1999, IOSET *et al.* 2000).

GÜRKE (1893) provided the last comprehensive study on Boraginales and recognised four subfamilies of his Boraginaceae: Boraginoideae, Heliotropioideae, Cordioideae, and Ehretioideae. This systematic concept has been accepted by many authors, but some attempts have been made to elevate single, or all of these subfamilies to family rank to underline their distinctiveness (e.g., SVENSSON 1925, DAHLGREN 1983, TAKHTAJAN 1986). Boraginaceae *s.str.*, Heliotropiaceae, and Cordiaceae are recognised as monophyla based on clear apomorphies, but the monophyly of Ehretiaceae is doubtful. Especially the systematic position of *Coldenia* and *Tiquilia* is uncertain (e.g., RICHARDSON 1977, MILLER 1989), probably they represent isolated lineages within Boraginales.

Detailed phylogenetic studies are still scarce in Boraginales. Ivan M. JOHNSTON, who worked on these plants over 30 years, considered Cordiaceae and Ehretiaceae as taxa with many ancestral character states, and Boraginaceae *s.str.* and Heliotropiaceae as derived lineages within Boraginales (e.g., JOHNSTON 1950, 1951). He emphasised fruit morphology and dispersal for his systematic studies. Tropical and subtropical Cordiaceae and Ehretiaceae have drupes, which are dispersed by animals such as birds or little mammals (endozoochory). Such observations have also been made for some Heliotropiaceae, but, additionally, numerous representatives with schizocarps are found dispersed by themselves (autochory). This type of dispersal can be interpreted as license for the colonisation of localities with only few potential seed predators (e.g., temperate regions). This adaptation has been optimised, and specialised, in numerous variations in Boraginaceae *s.str.* All representatives of Boraginaceae *s.str.* have separating fruits ('nutlets', in German called 'Klausen') that are dispersed by themselves (autochory), by wind (anemochory), or – secondarily – by animals (epizoochory). Progression and character polarisation – from Cordiaceae- and Ehretiaceae-like ancestors over Heliotropiaceae-like forms to Boraginaceae *s.str.* – have been indicated by studies on other character traits (e.g., wood anatomy and pollen, HEUBL *et al.* 1990).

During the last years the significance of molecular methods in phylogenetic systematics has increased. These data suggest a completely different evolutionary scenario from the traditional systematics, although only few molecular studies on Boraginales have been published (e.g., BÖHLE *et al.* 1996, FERGUSON 1999, LÅNGSTRÖM & CHASE 2002). It could be shown that mostly New World Hydrophyllaceae *s.str.* are closely allied to a part of Boraginales, namely Cordiaceae, Ehretiaceae, and Heliotropiaceae (e.g., CHASE *et al.* 1993, BÖHLE & HILGER 1997, FERGUSON 1999) leading to a polyphyletic “Boraginaceae” *sensu* GÜRKE (1893). Furthermore, parasitic Lennoaceae appear to be nested in Boraginales (firstly recognised by HALLIER 1923 based on flower and fruit characters), possibly they are closely allied to Ehretiaceae, or part thereof (SMITH & DEPAMPHILIS 1998, SMITH *et al.* 2000, OLMSTEAD & FERGUSON 2001). The close relationship of all three, Hydrophyllaceae *s.str.* and Lennoaceae and the subordinate taxa of “Boraginaceae” *sensu* GÜRKE (1893, i.e., Boraginaceae *s.str.*, Heliotropiaceae, Cordiaceae, and Ehretiaceae), was firstly proposed by THORNE (1983). Detailed molecular studies on Boraginales covering all major clades, with a more or less integral taxon sampling, are still missing as well as discussions on the plausibility of molecular trees (i.e., discussions on character evolution).

It has turned out that investigations of the secondary structure facilitate the understanding of molecular evolution and is therefore a tool for optimising alignments (e.g., COLEMAN & MAI 1997, COLEMAN *et al.* 1998, AN *et al.* 1999, WOLF *et al.* 2002). In many cases the secondary structure is conserved at higher taxonomical levels, while the primary sequence is highly variable. Studies on the secondary structure may increase the number of structural characters, but they are nearly absent in molecular systematics of angiosperm.

Molecular methods are also used to investigate time estimates of separation events as a tool in historical biogeography (e.g., RENNER *et al.* 2000, MALCOMBER 2002, SOLTIS *et al.* 2002) to replace conjecture. Therefore, fossils and/or geological events (e.g., separation of continents) are used to calibrate a ‘molecular clock’. It is possible to develop hypotheses on historical biogeography based both on a well-resolved phylogeny and on time estimates inferred from a molecular clock. A high geological age of a clade indicates, e.g., the possibility of plate tectonics as separation event responsible for South America/Africa-disjunctions, while lower estimates suggest other explanations (e.g., migrations, long-distance dispersal). Boraginales provides the possibility of several case studies in disjunct taxa such as *Bourreria*, *Ehretia* (both Ehretiaceae), *Cordia* (Cordiaceae), and *Tournefortia* (Heliotropiaceae). Time estimates as interdisciplinary research field combining biological, molecular, and geological aspects are still very young. Data on tropical, woody plants in general and Boraginales in particular are very scarce. WIKSTRÖM *et al.* (2001) dates the origin of Boraginales into the Upper Cretaceous (approximately 80 Ma).

In this thesis, I want to elucidate phylogenetic aspects of representatives of the Boraginales (i.e., in their current circumscription: Boraginaceae *s.str.*, Cordiaceae, Ehretiaceae, Heliotropiaceae, Hydrophyllaceae *s.str.*, and Lennoaceae). My major interest lies in Cordiaceae, Ehretiaceae, and taxa assigned to them, since Boraginaceae *s.str.*, Hydrophyllaceae *s.str.*, and Heliotropiaceae

have already been studied by other authors (e.g., BÖHLE *et al.* 1996, FERGUSON 1999, DIANE *et al.* 2002a, LÅNGSTRÖM & CHASE 2001). The primary aims of the studies conducted for this thesis were:

- Which major subordinate taxa can be recognised in Boraginales, and how are they related? Which basic pattern can be reconstructed for Boraginales?
- What is the systematic position of enigmatic taxa (i.e., taxa without a clear apomorphy shared with any other subordinate taxon of Boraginales) such as *Codon*, *Coldenia*, *Hydrolea*, Lennoaceae (*Lennoa* and *Pholisma*), *Pteleocarpa*, *Tiquilia*, and *Wellstedtia*?
- Which taxa belong to a monophyletic taxon Ehretiaceae (i.e., *Ehretia* and its relatives), and how are the relationships of these taxa?
- Which major subordinate taxa can be recognised in Cordiaceae, and how are they related? Which basic pattern can be reconstructed for Cordiaceae?
- Which common structural elements in the secondary structure of the phylogenetic markers used (nuclear First Internal Transcribed Spacer: ITS1, chloroplast intron *trnL_{UAA}*) can be found? Are they useful for optimising alignments and understanding molecular evolution?
- Which geological minimum ages can be estimated for disjunct taxa in Boraginales, and which conclusions on historical biogeography can be made?

The ambition of this thesis is to come to solid conclusions based on an interdisciplinary approach (plant geography, phylogenetic and molecular systematics, palaeontology, geology) and the use of different experimental techniques (sequencing, molecular calculations, molecular clock calibrations, light and electron microscopy). Furthermore, a detailed knowledge of the plants under investigation based on observing and collecting plants in the field (own field trips to Cuba: GOTTSCHLING 1999, and Peru: WEIGEND *et al.* 2001) is as necessary as the study of herbarium specimen (herbaria visited: B, BSB, F, MO, USM, W, WIS). Some results of this thesis are submitted to, or published in, international journals (see footnotes) and/or have been presented on international symposia (Deep Morphology: Vienna 2001; Biodiversität und Evolutionsbiologie: Bochum 2001; Botany 2002: Madison 2002, see literature cited).