



Molecular delimitations in the Ehretiaceae (Boraginales)



Marc Gottschling^{a,b,c,*}, Federico Luebert^{b,d,e}, Hartmut H. Hilger^b, James S. Miller^c

^a Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D-80 638 München, Germany¹

^b Freie Universität Berlin, Institut für Biologie-Botanik, Altensteinstraße 6, D-141 95 Berlin, Germany

^c Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, USA

^d Departamento de Silvicultura, Facultad de Ciencias Forestales y Conservación de la Naturaleza, Universidad de Chile, Santiago, Chile

^e Nees Institut für Biodiversität der Pflanzen Rheinische Friedrich-Wilhelms-Universität Bonn, Meckenheimer Allee 170, D-53115 Bonn, Germany¹

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ABSTRACT

Major taxa of Ehretiaceae (including parasitic Lennoaceae) have not all been included in previous molecular phylogenetic analyses. As a result, the generic limits and their circumscriptions have not been satisfactorily resolved, despite its importance for floristic studies. To clarify which monophyletic groups can be recognized within the Ehretiaceae, sequences from one nuclear (ITS) and three plastid loci (*rps16*, *trnL-trnF*, *trnS-trnG*) were obtained from 67 accessions tentatively assigned to the Ehretiaceae (including 91 new GenBank entries) and covering the known diversity of the group. In phylogenetic analyses, Ehretiaceae were monophyletic when Lennoaceae were included and segregated into nine monophyletic lineages that correspond to accepted, morphologically distinct taxonomic units, namely *Bourreria* (s.l., paraphyletic in its current circumscription if not including *Hilsenbergia*), monotypic *Cortesia*, *Ehretia* (s.l., paraphyletic in its current circumscription if not including *Carmona* and *Rotula*), *Halgania*, monotypic *Lennoa*, *Lepidocordia*, *Pholisma*, *Rocheortia*, and *Tiquilia*. *Bourreria* and *Ehretia* have representatives in both the Old World and the New World, but all other taxa are restricted to the tropical and subtropical Americas (*Cortesia*, *Lennoa*, *Lepidocordia*, *Pholisma*, *Rocheortia*, *Tiquilia*) or Australia (*Halgania*). The historical biogeography of Ehretiaceae can be explained by few colonization events. The molecular trees are also discussed with respect to fruit evolution, where the fusion of endocarp parts may have taken place several times independently.

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1. Introduction

The Ehretiaceae Mart. (=Boraginaceae Juss. subfam. Ehretioideae Arn. used by various authors) comprise about 150 species and are pantropical in distribution, with centers of diversity in Central America and the Caribbean, Africa, and East Asia. The plants are consistently woody perennials (subshrubs, shrubs, or trees) with the exception of the parasitic Lennoaceae Solms. The placement of the latter within the Boraginales was first recognized by Hallier (1923) based on flower and fruit characters and later confirmed by molecular sequence data (Gottschling et al., 2001; Nazaire and Hufford, 2012; Weigend et al., in press). The Ehretiaceae thus appear as a morphologically rather heterogeneous group, and exclusive characters (i.e., apomorphies) are not recognized at present. The only shared floral character of all Ehretiaceae is the more or less bifid style (Gürke, 1893) that is rather considered symplesiomorphic (Gottschling, 2004; Gottschling and Hilger, 2001). Nevertheless, the Ehretiaceae are monophyletic based on

molecular data and constitute the sister group of the Cordiaceae R.Br. ex Dumort. lineage within the Primarily Woody Boraginales (PWB: Gottschling, 2003; Gottschling et al., 2001; Nazaire and Hufford, 2012; Weigend et al., in press).

The generic delimitations of the taxa constituting the Ehretiaceae as well as the phylogenetic relationships within the group have remained controversial. Gürke (1893) provided the last comprehensive work on the Ehretiaceae, and a number of taxa included at that time are now placed in Cordiaceae (i.e., *Coldenia* L. and *Saccellium* Humb. & Bonpl.: Gottschling and Miller, 2006; Gottschling et al., 2005; Moore and Jansen, 2006), Gelsemiaceae (i.e., *Pteleocarpa* Oliv., as inferred from a NCBI Blast Search: Refulio-Rodríguez & Olmstead, unpublished sequence data from NCBI GenBank), and Globulariaceae (i.e., *Poskea* Vatke: Oxelman et al., 2005). Nine taxa at the generic level have been accepted in the Ehretiaceae by recent authors, namely *Bourreria* P.Br., *Ehretia* P.Br. (including *Carmona* Cav., *Cortesia* Cav., and *Rotula* Lour.: Gottschling and Hilger, 2001, 2004b), *Halgania* Gaudich., *Hilsenbergia* Tausch ex Meisn., *Lennoa* Lex., *Lepidocordia* Ducke, *Pholisma* Nutt. ex Hook., *Rocheortia* Sw., and *Tiquilia* Pers.

Ivan M. Johnston, the outstanding monographer of the Boraginales in the first half of the 20th century, was largely silent on

* Corresponding author.

E-mail address: gottschling@bio.lmu.de (M. Gottschling).

¹ Present address.

the generic delimitations of the Ehretiaceae. Miller (1989) recognized four probably monophyletic entities within the Ehretiaceae (*sensu stricto* i.e., without New World Lennoaceae). New World *Tiquilia* is clearly distinct from the remainder of the Ehretiaceae, since those desert dwarf plants have developed numerous specialized characters in accord with their habitats. Central American and Northern South American *Lepidocordia* represents another isolated lineage and exhibits similarity in calyx, corolla, and endocarp morphology to the Heliotropiaceae Schrad. (Johnston, 1950). The Argentinean endemic *Cortesia* and the Australian endemic *Halgania* comprise a questionable third group of shrubby plants growing in xeric habitats as possible elements of the Austral floristic realm (Moreira-Muñoz, 2007). The remaining taxa of the Ehretiaceae, namely *Bourreria* (Americas), *Ehretia* (pantropical), *Hilsenbergia* (East Africa, Madagascar, and the Mascarenes), and *Rochefortia* (Caribbean and Central and South America), seem to constitute a morphologically rather uniform fourth group (Gottschling and Hilger, 2001; Klotz, 1979; Miller, 1989).

The Ehretiaceae have typical asterid characters such as tetracyclic, pentamerous flowers with five antesealous stamens and a bicarpellate gynoecium (superior, with four ovules). Differing from this general pattern, *Lennoa* and *Pholisma* are unique (even within the entire Boraginales except the *Zoelleria* Warb. group of *Trigonotis* Steven) in having ovaries with 7–15 locules and two ovules per locule (Yatskievych and Mason, 1986). Another exception is *Halgania cyanea* Lindl., where the ovary is reduced by the loss of one half of each carpel resulting in a biovulate gynoecium (Lawrence, 1937). Many species of the Ehretiaceae have white, small corollas (<15 mm in diameter), and derivations are only found in a few groups and species. Larger flowers (>15 mm in diameter) occur in some species of *Bourreria*, possibly having originated independently several times (Gottschling and Miller, 2007; Miller, 1999). Bluish corollas are found in *Bourreria* (Leon and Alain, 1957), *Halgania* (Black, 1957; Fægri, 1986), *Lennoa* and *Pholisma* (Yatskievych and Mason, 1986), and *Tiquilia*, while *Bourreria rubra* E.J. Lott & J.S. Mill. is unique in having red corollas (Lott, 1986).

The fruit of the Ehretiaceae is mostly a drupe containing usually the four seeds enclosed in a divided (predominant) or undivided endocarp (rare). *Tiquilia* is somewhat derived with nutlet-like dispersal units (Richardson, 1977), *Lennoa* and *Pholisma* have fragmenting fruits shedding seeds enclosed in pyrenes (or endocarps) that derive from the multi-loculed ovaries (Yatskievych and Mason, 1986), and some species of *Bourreria* exhibit schizocarps (Gottschling, 2004; Gottschling and Miller, 2007). A drupe with four single-seeded pyrenes is the predominant type found in the Ehretiaceae. Drupes with two 2-seeded or one 4-seeded pyrene(s) may have resulted from the fusion of single parts in the evolution of the PWB (Gottschling and Hilger, 2001, 2004b; Hilger, 1992). Undivided endocarps are found in *Ehretia latifolia* DC., *E. microphylla* Lam., and both species of *Lepidocordia*, but these taxa are only distantly related (Gottschling and Hilger, 2001; Miller, 1989; Miller and Nowicke, 1990) and the condition is clearly independently derived. Two-parted endocarps (drupes with two 2-seeded pyrenes) occur in *Cortesia* (Gottschling and Hilger, 2004b) and some species of *Ehretia* (Gottschling and Hilger, 2001; Miller, 1989). Occasionally, the pyrenes have some ornamentation such as the lamellae on the abaxial surface of *Bourreria* and *Hilsenbergia* (Gottschling and Hilger, 2001) as well as of the *Ehretia longiflora* Champ. ex Benth. species group (Gottschling and Hilger, 2004a).

The study of systematic relationship, taxonomic delimitation, biogeography, and character evolution in the Ehretiaceae has been hindered by the lack of a taxon-wide phylogeny. Therefore, the major aim of this study is to infer a phylogeny of the Ehretiaceae based on molecular data in order to investigate the limits and relationships of its constituent taxa. To this end, we obtained sequence information from four loci (nuclear and plastid) covering the

known diversity of the Ehretiaceae. As a result, we recognize nine taxa at the generic level, differing slightly from those listed above and accepted by recent authors, which has last but not least importance for floristic studies. We interpret the molecular trees in terms of biogeography and character evolution (primarily of fruit traits) and hope to contribute to a better understanding of taxonomy and evolution in the PWB.

2. Materials and methods

In total, 83 accessions were investigated, 67 of which are assigned to the Ehretiaceae (Table S1). Within the Ehretiaceae, sampling was broad and included representatives of all relevant groups. Outgroup selection was based on previous studies (Gottschling et al., 2001; Luebert and Wen, 2008; Weigend et al., in press) and comprised members of the Cordiaceae and Heliotropiaceae. Plant material was mainly obtained from herbarium specimens, and additional material was available from silica-dried samples collected in the field and/or from cultivated plants. DNA extraction, polymerase chain reaction (PCR), purification, and sequencing followed standard protocols previously reported by the authors (Gottschling and Hilger, 2001; Luebert et al., 2011a). Primer information and PCR settings are provided in Table S2. In total, 91 new sequences have been generated in the course of the present study. GenBank accessions were also downloaded to complete the data set, especially in *Tiquilia* (Moore and Jansen, 2006) and outgroup taxa (Gottschling et al., 2004; Hilger and Diane, 2003; Luebert and Wen, 2008; Luebert et al., 2011a, 2011b; Mansion et al., 2009).

Phylogenetic analyses were conducted using a data matrix with one nuclear (ITS, comprising the two spacers and the 5.8S rRNA or only ITS1 alone) and three plastid loci (*rps16*, *trnL-trnF*, and *trnS-trnG*). All loci were already used in previous molecular analyses of the Boraginales (Hilger and Diane, 2003; Luebert and Wen, 2008; Luebert et al., 2011a, 2011b; Weigend et al., in press) and were identified as suitable for the taxonomic level investigated here. For 90 of the 332 cells in Table S1 (27%), no sequence information was available. The data set was partitioned into four parts, and the nucleotide sequences were separately aligned using MAFFT v6.624b (Katoh et al., 2005; freely available at <http://align.bmr.kyushuu.ac.jp/mafft/software/>) with the `-auto` option and considering the secondary structure of the molecules (i.e., the 'QINSI' option). The sequences were concatenated afterwards, and the final data matrix is available as NEXUS file upon request.

In order to test for potential incongruence between nuclear and plastid loci, an Incongruence Length Difference (ILD) test (Farris et al., 1994) was conducted as implemented in PAUP* v.4.0b10 (Swofford et al., 2003). Nuclear (ITS) and plastid (*rps16*, *trnL-trnF*, and *trnS-trnG*) partitions were defined, and the test was carried out building a confidence interval with 1000 replicates. Maxtrees was set to 1000, and heuristic searches were performed with 10 random-addition-sequence replicates, saving a maximum of 100 trees in each random-addition-sequence replicate. For purposes of the ILD-test, the taxon set was reduced to the samples, for which all four loci were available, and the outgroup representation was reduced to two Heliotropiaceae and one Cordiaceae in order to avoid potential incongruence caused by outgroup taxa, where incongruence has been reported (Luebert et al., 2011a).

Phylogenetic analyses of single loci as well as concatenated sequences were then carried out using the resources available from the CIPRES Science Gateway (Miller et al., 2010) with maximum likelihood (ML) and Bayesian Inference methods. Previous to the phylogenetic analyses, optimal substitution models separately for each locus were selected using MrModelTest v2 (Nylander, 2004), which identified the GTR + Γ as the best-fit model for all four loci.

For ML calculations, RAxML v7.2.6 (Stamatakis, 2006; freely available at <http://www.kramer.in.tum.de/exelixis/software.html>) was applied. To determine best fitted ML-trees, we executed 10-tree searches from distinct random stepwise addition sequence maximum parsimony starting trees and 1000 non-parametric bootstrap replicates. Bayesian analyses was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003; freely available at <http://mrbayes.csit.fsu.edu/download.php>), under the random-addition-sequence method with 10 replicates. We ran two independent analyses of four chains (one cold and three heated) under the partition data mode with 15,000,000 cycles, sampled every 1000th cycle, with an appropriate burn-in (10%) as inferred from the evaluation of the trace files using Tracer v1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>). Statistical support values (LBS: ML bootstrap support, BPP: Bayesian posterior probabilities) were drawn on the resulting, best-scoring ML trees.

3. Results

The alignment (in order ITS, *rps16*, *trnL-trnF*, *trnS-trnG*) was 824 + 1048 + 1028 + 1296 = 4196 bp long and comprised 351 + 188 + 153 + 195 = 887 parsimony informative sites (21%, mean of 10.69 per terminal taxon) as well as 2199 distinct RAxML alignment patterns. The results of the ILD test indicated that the hypothesis of ‘congruence between nuclear and plastid datasets’ could not be rejected ($p = 0.067$). No conflict between topologies of trees calculated with ITS and plastid loci separately (>75 LBS) was detected (Fig. S1). Rather, most of the major lineages were retrieved independently of a particular locus under investigation (Fig. S1, data for all separate analyses not shown), but they are less resolved than the analysis based on the concatenated data set. We therefore base our discussion on the results of the analyses using concatenated nuclear + plastid sequences. Tree topologies were largely congruent, independently of whether the Bayesian or the ML algorithm was applied. Many nodes showed high if not maximal statistical support values. Fig. 1 shows the best-scoring ML tree ($-\ln = 25,305.96$), with the 67 members of the Ehretiaceae retrieved as monophyletic (100 LBS, 1.00 BPP) and as sister group of the Cordiaceae (100 LBS, 1.00 BPP).

The relationships within the Ehretiaceae were not fully resolved, but they segregated into nine mostly highly supported lineages: *Bouyeria* (including *Hilsenbergia*: 100 LBS, 1.00 BPP), monotypic *Cortesia* (3 accessions: 100 LBS, 1.00 BPP), *Ehretia* (including *Carmona* and *Rotula*: 93 LBS, 1.00 BPP), *Halgania* (99 LBS, .97 BPP), monotypic *Lennoa* (1 accession), *Lepidocordia* (100 LBS, 1.00 BPP), *Pholisma* (99 LBS, .99 BPP), *Rochefortia* (74 LBS, .99 BPP), and *Tiquilia* (100 LBS, 1.00 BPP). Both dioecious *Lepidocordia* and *Rochefortia* formed a monophyletic group (100 LBS, 1.00 BPP) and constituted the sister group of *Bouyeria* (99 LBS, 1.00 BPP). Together, they appeared monophyletic with *Tiquilia*, but this was weakly supported in the ML analysis only (70 LBS).

The remaining five taxa of the Ehretiaceae (i.e., *Cortesia*, *Ehretia*, *Halgania*, *Lennoa*, *Pholisma*) constituted a second monophyletic group although with low branch support (64 LBS). Parasitic *Lennoa* and *Pholisma* (i.e., the Lennoaceae) were monophyletic (100 LBS, 1.00 BPP) and the sister group of *Cortesia*, *Ehretia*, and *Halgania* (62 LBS, .91 BPP). The latter two appeared closely related, although the support value was high in the Bayesian analysis only (59 LBS, .98 BPP). Within *Ehretia*, four lineages could be distinguished (Fig. 2): (1) a clade of both Old and New World (ca eight) species, including the type species, *E. tinifolia* L. (95 LBS, 1.00 BPP), (2) a clade of Old World (ca 30) species containing *Ehretia aquatica* Lour. (former *Rotula*) (100 LBS, 1.00 BPP), (3) *E. longiflora* (as the sole representative of a small Asian group of ca 3 species), and (4) *E. microphylla* (=monotypic *Carmona*). *Ehretia microphylla* and the

Old World clade including *Rotula* appeared closely related (64 LBS, .99 BPP).

Some biogeographic correlations can be inferred from this analysis of molecular sequence data in the Ehretiaceae. Most of the major lineages were either distributed in the Old World (i.e., Australian *Halgania*) or in the New World (i.e., *Cortesia*, *Lennoa*, *Lepidocordia*, *Pholisma*, *Rochefortia*, *Tiquilia*), but the New World taxa did not form a monophyletic group in the molecular trees. Only *Bouyeria* and *Ehretia* (in their broad circumscriptions outlined here) contained both New World and Old World species. Within *Bouyeria*, Old World species (i.e., those recently assigned to *Hilsenbergia*) were monophyletic (87 LBS, 1.00 BPP), but the remaining species did not constitute a New World sister group. In turn, all three New World species of *Ehretia* were embedded in a paraphyletic group of Old World species, as *E. acuminata* R.Br. and *E. macrophylla* Wall. were more closely related to the New World species (95 LBS, 1.00 BPP) than to other Old World species such as *E. laevis* Roxb. and *E. microphylla*. However, the New World species did not constitute an unambiguous monophyletic group because of the poor statistical support within the clade containing the type species, *E. tinifolia*.

4. Discussion

The application of molecular sequence data has greatly improved the classification of the angiosperms in general and also of the Boraginales (Gottschling et al., 2001; Weigend et al., in press). Some taxa previously considered members of the Ehretiaceae (Gürke, 1893) have already been shown to belong to other lineages (see Introduction). In a previous molecular analysis using four plastid loci (Weigend et al., in press), the Ehretiaceae (including the parasitic Lennoaceae) have been reliably identified as one of the major lineages of the Boraginales. This monophyly is confirmed also in the present study partly using alternative loci and a much broader taxon sampling with respect to the Ehretiaceae. The molecular results have particular importance, because the Ehretiaceae are the only lineage of the PWB, of which a morphological apomorphy is not yet known.

Our molecular results indicate nine monophyletic taxa in the Ehretiaceae corresponding to accepted taxonomic units and to be recognized at the generic level, namely *Bouyeria* (including *Hilsenbergia*), *Cortesia*, *Ehretia* (including *Carmona* and *Rotula*), *Halgania*, *Lennoa*, *Lepidocordia*, *Pholisma*, *Rochefortia*, and *Tiquilia*. With the exception of *Ehretia*, of which exclusive characters remain elusive at present, all these taxa are monophyletic also based on morphological apomorphies (Fig. 2) such as the spiny habit of *Rochefortia* (Klotz, 1979) or the solanoid flower of *Halgania* (Fægri, 1986). Unfortunately, not all of the deeper nodes are highly supported in the molecular trees, and morphological data from the literature do not contribute to better resolved relationships within the Ehretiaceae. However, the identification of the close relationship between *Lepidocordia* and *Rochefortia* as inferred from the molecular data is at least notable, as they are the only dioecious members of the Ehretiaceae (Miller and Nowicke, 1990), which can be interpreted as a synapomorphy.

The generic delimitations within the Ehretiaceae supported by this study are largely congruent to the traditional classification with a few exceptions. Firstly, Argentinean *Cortesia* is not embedded in *Ehretia*, as it has been inferred from a molecular ITS1 analysis (Gottschling and Hilger, 2004b) using limited locus and taxon sampling. Instead, it appears to be the sister species to Australian *Halgania*, representing a not uncommon disjunction and confirming previously proposed phylogenetic relationships based on morphology (Miller, 1989). Secondly, the taxonomic distinction between New World *Bouyeria* and Old World *Hilsenbergia* (Miller,

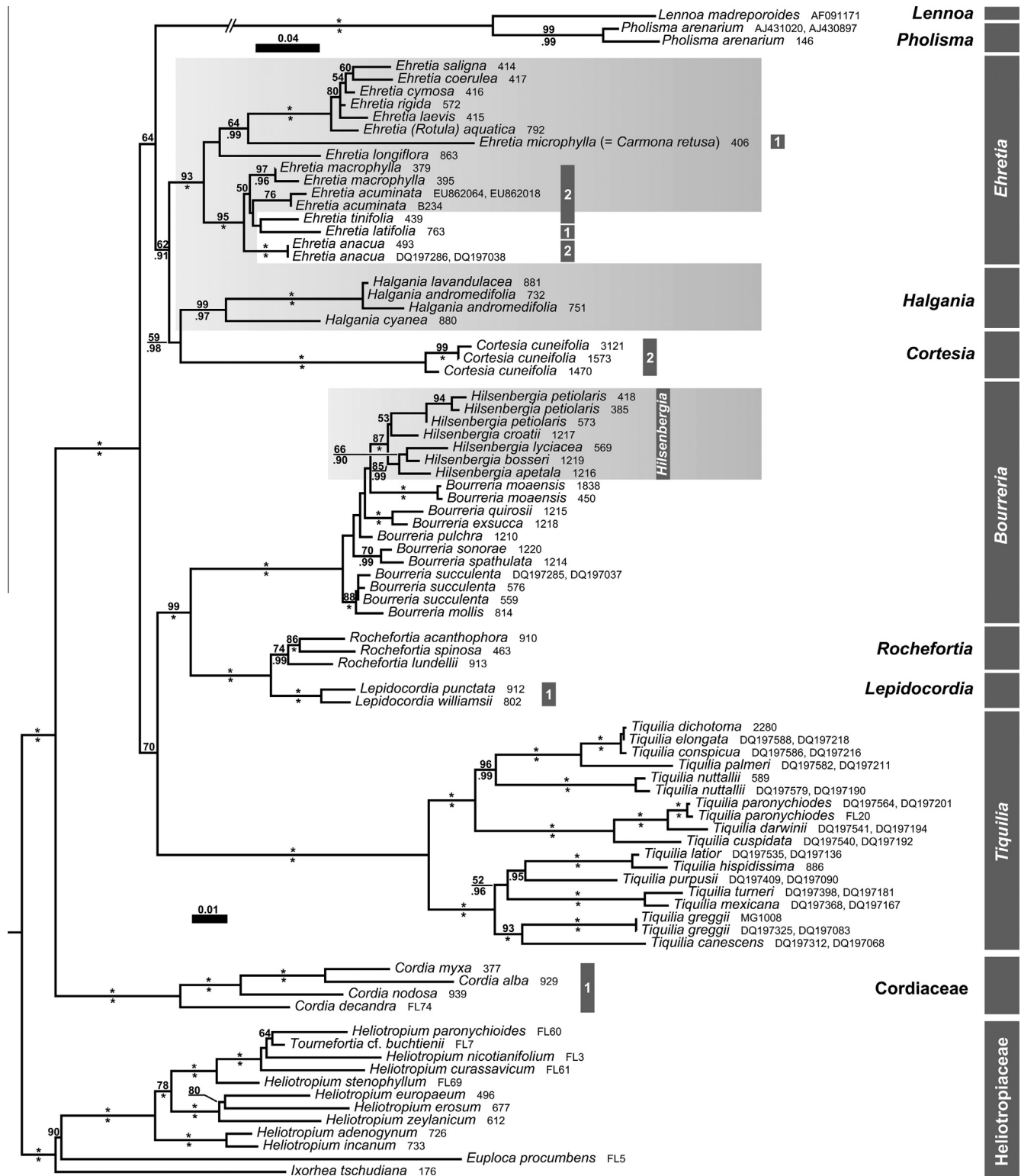


Fig. 1. Monophyletic Ehretiaceae with nine lineages at the generic level. Maximum Likelihood (ML) tree ($-\ln = 25,305.96$) obtained from the analysis in RAxML. Numbers on branches indicate ML bootstrap values (≥ 50 , above) and Bayesian posterior probabilities ($\geq .90$, below). Asterisks indicate maximal statistical support. The tree is rooted with Cordiaceae and Heliotropiaceae. Relevant clades of the Ehretiaceae are indicated, and Old World taxa are highlighted by grey shading. Taxa showing fused pyrenes (symericarpy: [Gottschling and Hilger, 2001, 2004b](#); [Hilger, 1992](#)) are indicated, and the numbers of endocarp parts is specified (all other species have four pyrenes or nutlets). Numbers behind species names follow an internal numbering (for other accessions, GenBank numbers are provided).

2003) does not appear justified if not strictly expressed as paraphyletic. Neither *Bourreria sensu stricto* is clearly retrieved as monophyletic, nor is the molecular divergence (as inferred from branch lengths in a phylogram) between the New World and the

Old World species as high as in, for example, *Ehretia*, *Halgania*, or *Tiquilia*. Thus, the monophyly of *Bourreria sensu lato* is definite if *Hilsenbergia* is included based on molecular data provided by this and previous studies ([Gottschling and Hilger, 2001](#)) as well as by

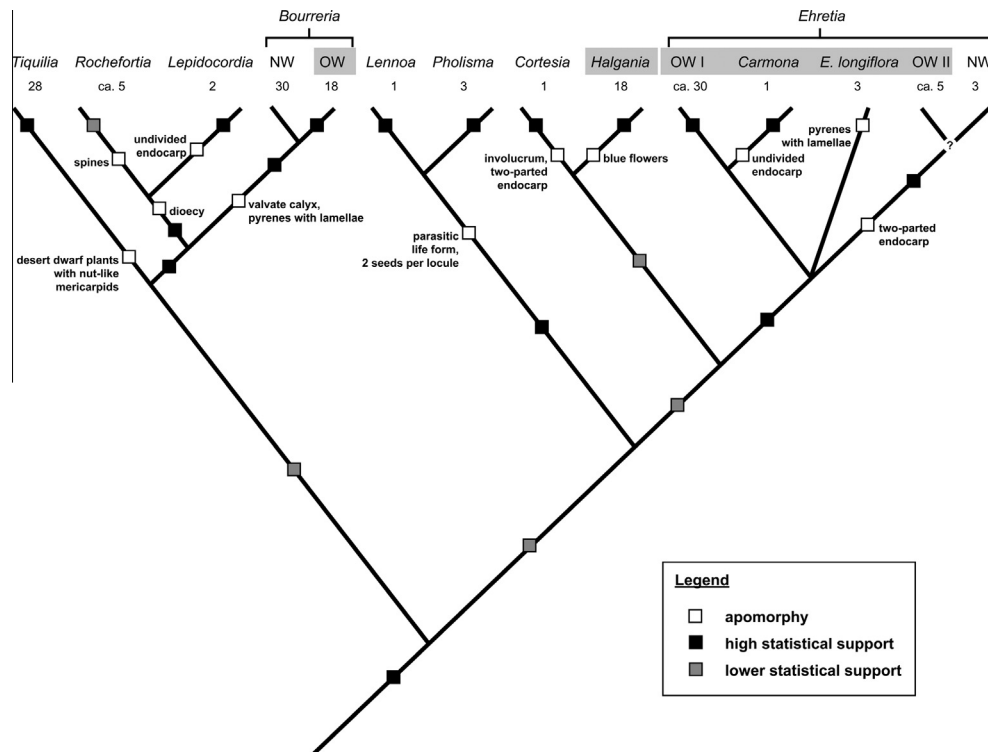


Fig. 2. The nine lineages of the Ehretiaceae at the generic level and their phylogenetic relationships. Annotated cladogram summarizing the results of the study (symbols see Legend; statistical support is considered high, if LBS > 85 and BPP > .95 or taxa such as *Carmona* and *Cortesia* are monotypic; numbers below taxon names indicate estimated total number of species).

clear apomorphies such as the valvate calyx and pyrenes with lamellae on their abaxial surface (Gottschling, 2004; Gottschling and Hilger, 2001; see also the Graphical Abstract).

Fruit anatomy yields important characters for inferring evolution in the Ehretiaceae (Al-Shehbaz, 1991; Gottschling and Hilger, 2001; Miller, 1989). The four-parted endocarp might be considered the ancestral condition in the group, as it is the predominant type throughout all Boraginales (Weigend et al., in press). Moreover, all species or groups of the Ehretiaceae showing fusion of pyrenes (syn-mericarpy: Gottschling and Hilger, 2001, 2004b; Hilger, 1992) have derived phylogenetic positions in molecular trees (i.e., are polyphyletic). However, some previous homology hypotheses must be revised in the light of the present study. Both *Cortesia cuneifolia* Cav. and *E. microphylla* have been recognized as close relatives of the clade containing the type species of *Ehretia*, *E. tinifolia*, all showing syn-mericarpy (Gottschling and Hilger, 2001, 2004b). The broader taxon sample and the more extensive genetic data now demonstrate that *Cortesia* is distinct from *Ehretia* and that *E. microphylla* is more closely related to a group of Old World *Ehretia* species (that include the former segregate *Rotula*) than to the clade including *E. tinifolia*. As a consequence, syn-mericarpy in *Cortesia*, *E. microphylla*, and *E. tinifolia* with its relatives appears to be independent evolutionary derivations. The fusion of pyrenes within Ehretiaceae has thus evolved multiple times and more frequently than assumed before (similar assumptions can be stated for the Heliotropiaceae, see Hilger, 1992).

Biogeographic patterns in the Ehretiaceae also deserve some discussion. Basically, the Ehretiaceae are pantropical in distribution (Gottschling et al., 2004), but most of the major lineages outlined here have distributions restricted to either the New or Old World. Only *Bourreria* with a Neotropical/East African disjunction, and *Ehretia* with a pantropical distribution, bridge the two hemispheres. An Old World rather than a New World origin of *Ehretia* is inferred (whether Africa or Asia cannot be ascertained by the

data presented here), as the Old World species group is paraphyletic with the three New World species (revised in Miller, 1989) embedded at highly derived phylogenetic positions in the tree (Fig. 2). At least one (and maximally two) secondary colonization event(s) to the New World (likely via Beringia: Gottschling et al., 2004) appears therefore plausible (i.e., more steps are needed to explain a New World origin of *Ehretia*). The Old World origin of *Ehretia* is also corroborated because its oldest reliable fossils come from the European Eocene (Chandler, 1964; Gottschling and Hilger, 2003; Gottschling et al., 2002).

Using the *Ehretia* fossils as calibrator for a chronogram of some Ehretiaceae, Gottschling et al. (2004) showed that the *Bourreria* split is dated to the Eocene. Therefore, any separation event leading to disjunct distribution areas at this taxonomic level appears geologically too young in the Ehretiaceae so that vicariance could have played a role in diversification. Rather, disjunct distribution areas in the Ehretiaceae probably result from inter-plate dispersal (Morley, 2003). The Eastern African members of *Bourreria* (accepted and segregated again as *Hilsenbergia*: Miller, 2003) should be considered independently from other members of the Ehretiaceae arrived in the Old World via long distance dispersal by yet unknown vector (Gottschling et al., 2004) or because of migration and extinction elsewhere.

In summary, the molecular trees indicate that the initial diversification of the Ehretiaceae might have taken place in the New World and that *Bourreria*, *Lennoa*, *Lepidocordia*, *Pholisma*, *Rochefortia*, and *Tiquilia* are native lineages to the Neotropics. Whether the morphologically and geographically isolated Argentinean *Cortesia* represents a native New World lineage or derived together with *Ehretia* and *Halgania* from an ancestor formerly present in Africa, Asia, and/or Australia, remains an interesting question for future research. For these possible elements of the Austral floristic realm (Moreira-Muñoz, 2007), Antarctica may have acted as a stepping-stone for dispersal from South America to New Zealand

(or *vice versa*) that has been demonstrated for *Myosotis* from the Boraginaceae (Winkworth et al., 2002) and also for other plant groups such as Atherospermataceae (Renner et al., 2000), *Abrotanella* (Asteraceae: Wagstaff et al., 2006), and Alstroemeriaceae (Chacón et al., 2012).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.12.005>.

References

- Al-Shehbaz, I.A., 1991. The genera of Boraginaceae in the Southeastern United States. *J. Arnold Arb. Suppl. Ser. 1*, 1–64.
- Black, J.M., 1957. Family 96.—Boraginaceae. Oleaceae—Compositae. Stevenson, Adelaide, pp. 706–711.
- Chacón, J., de Assis, M.C., Meerow, Alan W., Renner, S.S., 2012. From East Gondwana to Central America: historical biogeography of the Alstroemeriaceae. *J. Biogeogr.* 39, 1806–1818.
- Chandler, M.E.J., 1964. The Lower Tertiary floras of Southern England 4. British Museum, London.
- Fægri, K., 1986. The solanoid flower. *Trans. Bot. Soc. Edinburgh* 45 (Suppl 1), 51–59.
- Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. *Cladistics* 10, 315–319.
- Gottschling, M., 2003. Phylogenetic analysis of selected Boraginales. Systematische Botanik und Pflanzengeographie. Freie Universität Berlin (PhD thesis), Berlin. http://www.diss.fu-berlin.de/diss/receive/FUDISS_thesis_00000001181.
- Gottschling, M., 2004. Floral ontogeny in *Bourreria* (Ehretiaceae, Boraginales). *Flora* 199, 409–423.
- Gottschling, M., Hilger, H.H., 2001. Phylogenetic analysis and character evolution of *Ehretia* and *Bourreria* (Ehretiaceae, Boraginales) and their allies based on ITS1 sequences. *Bot. Jahrb. Syst.* 123, 249–268.
- Gottschling, M., Hilger, H.H., 2003. First fossil record of transfer cells in angiosperms. *Am. J. Bot.* 90, 957–959.
- Gottschling, M., Hilger, H.H., 2004a. Characterisation of a novel fruit type found in *Ehretia* (Ehretiaceae, Boraginales). *Blumea* 49, 145–153.
- Gottschling, M., Hilger, H.H., 2004b. The systematic position of *Ehretia cortesia* nom. nov. (≡ *Cortesia cuneifolia*: Ehretiaceae, Boraginales) inferred from molecular and morphological data. *Taxon* 53, 919–923.
- Gottschling, M., Miller, J.S., 2006. Clarification of the taxonomic position of *Auxemma*, *Patagonula*, and *Saccellium* (Cordiaceae, Boraginales). *Syst. Bot.* 31, 361–367.
- Gottschling, M., Miller, J.S., 2007. A revision of *Bourreria* (Boraginales, Ehretiaceae) in South America. *Ann. MO Bot. Gard.* 94, 734–744.
- Gottschling, M., Hilger, H.H., Wolf, M., Diane, N., 2001. Secondary structure of the ITS1 transcript and its application in a reconstruction of the phylogeny of Boraginales. *Plant Biol.* 3, 629–636.
- Gottschling, M., Mai, D.H., Hilger, H.H., 2002. The systematic position of *Ehretia* fossils (Ehretiaceae, Boraginales) from the European Tertiary and implications for character evolution. *Rev. Palaeobot. Palynol.* 121, 149–156.
- Gottschling, M., Diane, N., Hilger, H.H., Weigend, M., 2004. Testing hypotheses on disjunctions present in the Primarily Woody Boraginales: Ehretiaceae, Cordiaceae, and Heliotropiaceae, inferred from ITS1 sequence data. *Int. J. Plant Sci.* 165, S123–S135.
- Gottschling, M., Miller, J.S., Weigend, M., Hilger, H.H., 2005. Congruence of a phylogeny of Cordiaceae (Boraginales) inferred from ITS1 sequence data with morphology, ecology, and biogeography. *Ann. MO Bot. Gard.* 92, 425–437.
- Gürke, M., 1893. Boraginaceae (Parts I–III). In: Engler, A., Prantl, K. (Eds.), *Die natürlichen Pflanzenfamilien IV* (3a). Engelmann, Leipzig, pp. 71–97.
- Hallier, H., 1923. Über die Lennoeen, eine zu Linné's Bicornes verirrte Sippe der Boraginaceen. *Beih. Bot. Centralbl.* 40 (2. Abteilung), pp. 1–19.
- Hilger, H.H., 1992. Morphology of *Heliotropium* (Boraginaceae) dispersal units. *Bot. Acta* 105, 387–393.
- Hilger, H.H., Diane, N., 2003. A systematic analysis of Heliotropiaceae (Boraginales) based on *trnL* and ITS1 sequence data. *Bot. Jahrb. Syst.* 125, 19–51.
- Johnston, I.M., 1950. Studies in the Boraginaceae XIX. *J. Arnold Arb.* 31, 172–187.
- Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* 33, 511–518.
- Klotz, G., 1979. Die Boraginaceen-Gattung *Rochefortia* Swartz in Cuba. *Wiss. Ztschr. Friedrich-Schiller-Univ. Jena, Math.-Nat. R.* 28, 643–647.
- Lawrence, J.R., 1937. A correlation of the taxonomy and the floral anatomy of certain of the Boraginaceae. *Amer. J. Bot.* 24, 433–444.
- Leon, H., Alain, H., 1957. Familia 3.—Boraginaceae. *Flora de Cuba, La Habana*, pp. 252–278.
- Lott, E.J., 1986. *Bourreria rubra* (Boraginaceae), a new species from coastal Jalisco, Mexico. *Ann. MO Bot. Gard.* 73, 216–218.
- Luebert, F., Wen, J., 2008. Phylogenetic analysis and evolutionary diversification of *Heliotropium* sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Syst. Bot.* 33, 390–402.
- Luebert, F., Brokamp, G., Wen, J., Weigend, M., Hilger, H.H., 2011a. Phylogenetic relationships and morphological diversity in Neotropical *Heliotropium* (Heliotropiaceae). *Taxon* 60, 663–680.
- Luebert, F., Hilger, H.H., Weigend, M., 2011b. Diversification in the Andes: age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). *Mol. Phylogenet. Evol.* 61, 90–102.
- Mansion, G., Selvi, F., Guggisberg, A., Conti, E., 2009. Origin of Mediterranean insular endemics in the Boraginales: integrative evidence from molecular dating and ancestral area reconstruction. *J. Biogeogr.* 36, 1282–1296.
- Miller, J.S., 1989. A revision of the New World species of *Ehretia* (Boraginaceae). *Ann. MO Bot. Gard.* 76, 1050–1076.
- Miller, J.S., 1999. New Boraginaceae from tropical America 1: new species of *Bourreria* and *Tournefortia* from Costa Rica and a note on the publication of *Cordia collococca*. *Novon* 9, 230–235.
- Miller, J.S., 2003. Classification of Boraginaceae subfam. Ehretioideae: resurrection of the genus *Hilsenbergia* Tausch ex Meisn. *Adansonia*, ser. 3 25, 151–189.
- Miller, J.S., Nowicke, J.W., 1990. Dioecy and a reevaluation of *Lepidocordia* and *Antrophora* (Boraginaceae: Ehretioideae). *Amer. J. Bot.* 77, 543–551.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA, pp. 1–8.
- Moore, M.J., Jansen, R.K., 2006. Molecular evidence for the age, origin, and evolutionary history of the American desert plant genus *Tiquilia* (Boraginaceae). *Mol. Phylogenet. Evol.* 39, 668–687.
- Moreira-Muñoz, A., 2007. The Austral floristic realm revisited. *J. Biogeogr.* 34, 1649–1660.
- Morley, R.J., 2003. Interplate dispersal paths for megathermal angiosperms. *Perspect. Plant Ecol. Evol. Syst.* 6, 5–20.
- Nazaire, M., Hufford, L., 2012. A broad phylogenetic analysis of Boraginaceae: Implications for the relationships of *Mertensia*. *Syst. Bot.* 37, 758–783.
- Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Oxelman, B., Kornhall, P., Olmstead, R.C., Bremer, B., 2005. Further disintegration of Scrophulariaceae. *Taxon* 54, 411–425.
- Renner, S.S., Foreman, D.B., Murray, D., 2000. Timing transantarctic disjunctions in the Atherospermataceae (Laurales): evidence from coding and noncoding chloroplast sequences. *Syst. Biol.* 49, 579–591.
- Richardson, A.T., 1977. Monograph of the genus *Tiquilia* (*Coldenia, sensu lato*), Boraginaceae: Ehretioideae. *Rhodora* 79, 467–572.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Stamatakis, A., 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Swofford, D.L., 2003. PAUP* Phylogenetic analysis using parsimony (*and other methods), v. 4.0 beta 10. Sinauer Associates, Sunderland (MA).
- Wagstaff, S.J., Breitwieser, I., Swenson, U., 2006. Origin and relationships of the austral genus *Abrotanella* (Asteraceae) inferred from DNA sequences. *Taxon* 55, 95–106.
- Weigend, M., Luebert, F., Gottschling, M., Couvreur, T.L.P., Hilger, H.H., Miller, J.S., in press. From capsules to nutlets – phylogenetic relationships in the Boraginales. *Cladistics*. <http://onlinelibrary.wiley.com/doi/10.1111/cla.12061/pdf>.
- Winkworth, R.C., Wagstaff, S.J., Glenny, D., Lockhart, P.J., 2002. Plant dispersal N.E.W.S. from New Zealand. *Trends Ecol. Evol.* 17, 514–520.
- Yatskievych, G., Mason Jr., C.T., 1986. A revision of the Lennoaceae. *Syst. Bot.* 11, 531–548.