

## **Caryophyllales phylogenetics: disentangling Phytolaccaceae and Molluginaceae and description of Microteaceae as a new isolated family**

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## ***Caryophyllales* phylogenetics: disentangling *Phytolaccaceae* and *Molluginaceae* and description of *Microteaceae* as a new isolated family**

### **Abstract**

Schäferhoff B., Müller K. F. & Borsch T.: *Caryophyllales* phylogenetics: disentangling *Phytolaccaceae* and *Molluginaceae* and description of *Microteaceae* as a new isolated family. – Willdenowia 39: 209–228. – Online ISSN 1868-6397; © 2009 BGBM Berlin-Dahlem.

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The *Caryophyllales* are one of the major lineages of angiosperms, including some 12 000 species and well known families such as *Amaranthaceae*, *Cactaceae*, *Caryophyllaceae*, *Droseraceae*, *Nyctaginaceae* and *Polygonaceae*. Phylogenetic hypotheses based on molecular characters have led to their circumscription and have considerably improved our understanding of interfamilial relationships. For this study, we generated a data set of the non-coding and rapidly evolving chloroplast *petB-petD* region, consisting of a transcribed spacer and a group II intron for 87 taxa of *Caryophyllales* and 22 outgroups. In addition, we analysed a complementary *matK* data set with complete sequences of the coding region. Trees obtained from both markers were well resolved and especially *petD* data yielded a well supported backbone for the *Caryophyllales*. The order is constituted by two sister clades, caryophyllids and polygonids, the latter containing a carnivorous subclade. Both *Molluginaceae* and *Phytolaccaceae* had been considered as polyphyletic, but not as severely as is now evident from this study with improved taxon sampling. As a great surprise, the hitherto unsampled genus *Microtea* is found with high support in an isolated position as the fourth branch in the caryophyllid clade. On the other hand, *Lophiocarpus* as the second genus of the *Phytolaccaceae* subfamily *Microteoideae* is sister to an *Aizoaceae*-*Nyctaginaceae*-*Phytolaccaceae* lineage. In line with their morphological distinctness, *Microteaceae* are described as a new family. Our data further resolve a distinct *Mollugo* clade, whereas *Hypertelis* appears to have affinities with *Limeum*, suggesting an expanded *Limeaceae*.

Additional key words: eudicots, angiosperm classification, molecular phylogeny, *petD*, *matK*, neotropical plant families

### **Introduction**

With about 12 000 species, *Caryophyllales* are one of the largest eudicot orders. Many of them are adapted to dry or saline habitats and a number of economically important plants (such as spinach, quinoa) or ornamentals (such as cacti and carnations) are found in this order. The core of the *Caryophyllales* has long been considered as a natural group based on their basal or free-central placentation, which led to the name *Centrospermae* (Braun 1864; Eichler 1875–78). The circumscription of *Caryophyllales* reflecting the *Centrospermae* was basically upheld until the 1990ies and is also reflected in major pre-phylo-

genetic classification systems of angiosperms (Dahlgren 1980; Takhtajan 1987; Cronquist 1988; Thorne 1992). A detailed presentation of the classification history was provided by Cronquist & Thorne (1994).

These *Caryophyllales* in the strict sense comprise the only betalain families (*Aizoaceae*, *Amaranthaceae*, *Basellaceae*, *Cactaceae*, *Chenopodiaceae*, *Didiereaceae*, *Halophytaceae*, *Hectorellaceae*, *Nyctaginaceae*, *Phytolaccaceae*, *Portulaccaceae* and *Stegnospemaceae*) in angiosperms (Clement & Mabry 1996b). In *Achatocarpaceae*, however, pigments have never been analysed (Clement

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& Mabry 1996b; Behnke pers. comm.). Following Ehrendorfer (1976), this group, the *Chenopodiinae*, was considered to be monophyletic with the idea that their common ancestor lost anthocyanin biosynthesis. Ehrendorfer (1976) laid out a scenario in which the loss of anthocyanin biosynthesis was associated with a shift to anemophily. *Molluginaceae* and *Caryophyllaceae* were known to possess the mutually exclusive anthocyanins (Stafford 1994) as red pigments like other flowering plants (the *Caryophyllineae*). *Caryophyllales* were further shown to have subtype-P3 sieve element plastids (Behnke 1993, 1994) as a characteristic feature.

First molecular phylogenetic studies of angiosperms using *rbcL* indicated the close relationship of *Polygonaceae* and *Plumbaginaceae* to the centrospermous families (Giannasi & al. 1992; Chase & al. 1993). The same was shown for the carnivorous families *Ancistrocladaceae*, *Dioncophyllaceae*, *Droseraceae* and *Nepenthaceae* (Albert & al. 1992; Chase & al. 1993), which were formerly classified as *Droserales* and *Nepenthales* (e.g., Takhtajan 1997). Genera such as *Simmondsia* and *Rhabdodendron* were then positioned in *Caryophyllales* when adding further *rbcL* and also *atpB* sequences (Savolainen & al. 2000a), although their internal positions did not yet receive significant statistical support. The results were substantial for recognising an expanded order *Caryophyllales*. We also refer to this circumscription here.

Although several molecular phylogenetic analyses dealt specifically with the *Caryophyllales* previous to this study (Giannasi & al. 1992; Manhart & Rettig 1994; Downie & Palmer 1994; Downie & al. 1997; Meimberg & al. 2000; Cuénoud & al. 2002) and most recently Brockington & al. (2009), a number of questions on internal relationships of the *Caryophyllales* remain. The parsimony analysis of *rbcL* sequence data by Manhart & Rettig (1994) already indicated that betalains could also have arisen twice, once in *Amaranthaceae-Chenopodiaceae* and once in a clade comprising other *Centrospermae* except *Caryophyllaceae*. However, *Mollugo* appeared within this clade in the *rbcL* tree. Later analyses with better statistical support on *Caryophyllales* relationships confirmed the non-monophyly of *Chenopodiinae*, indicating a more complex pattern of betalain evolution (Cuénoud & al. 2002).

In terms of taxon sampling Cuénoud & al. (2002) provided the so far most comprehensive tree based on a *matK* fragment (127 taxa). This extended taxon sampling surprisingly showed genera such as *Lophiocarpus* (classified as *Phytolaccaceae*; Rohwer 1993) and *Limeum* (classified as *Molluginaceae*; Endress & Bittrich 1993) to be in isolated positions distinct from the core of their respective families. A combined analysis of *atpB+rbcL+matK+nr18S* of a reduced taxon set (Cuénoud & al. 2002) yielded only slightly improved confidence into hypothesised deep nodes within *Caryophyllales*. Moreover, their results rendered families such as *Phytolaccaceae* and *Portulacaceae* as para- or polyphyletic. Evolutionary relationships of

the so-called “portulacaceous cohort” became only better understood recently, using an again extended taxon sampling and sequence data from multiple rapidly evolving and non-coding genomic regions (Nyffeler 2007; Nyffeler & Eggli in press).

The latest phylogenetic analysis of *Caryophyllales* (Brockington & al. 2009) includes only 36 species but many characters (12 000 nt from plastid genes, 5000 nt from nuclear genes and 24 000 nt from the plastid inverted repeat). The improvement over the two and four-gene analyses of Cuénoud & al. (2002) mostly regards to deep nodes. On the other hand, important deep nodes (such as for placement of *Limeum* and *Stegnosperma*) remain unsupported in the maximum parsimony strict consensus tree, while relationships within shallower clades such as the “portulacaceous cohort” and the “raphide clade” are generally not well clarified. Also, most of the putatively polyphyletic or paraphyletic families were undersampled (e.g., *Phytolaccaceae*) and some families were not included at all (*Agdestidaceae*, *Petiveriaceae*).

Non-coding and rapidly evolving cp DNA has recently been shown to be a valuable tool for inferring plant phylogenetic relationships even on levels far deeper than genera (Borsch & al. 2003; Löhne & Borsch 2005; Müller & al. 2006). A comprehensive approach to sequence and analyse the mutational dynamics of non-coding genomic regions from the chloroplast genome for a set of about 500 genera of eudicots has been carried out within the Eudicot project ([www.eudicots.de](http://www.eudicots.de)). Worberg & al. (2007) were able to get high statistical support for the early branches of eudicots with a combined *matK+petD+trnL-F* data set and similar improvement of tree resolution and support was gained for the rosids (Worberg & al. 2009).

In the present study, the chloroplast *petB-petD* region, consisting of a transcribed spacer and a group II intron, and the *matK* coding region, were analysed with the aim to test the phylogenetic utility of these markers and further illuminate evolutionary relationships in *Caryophyllales*. Special attention was paid to a careful and reasonably dense sampling of taxa.

With respect to taxonomy, this study aims at a revised classification of *Microtea*. This genus has usually been treated under *Phytolaccaceae* but doubts as to its position have been expressed for decades. Interestingly, the study of *Microtea* in the Berlin-Dahlem Botanical Garden and Museum has a history of more than 120 years, starting with Urban (1885) and continued by Eckardt (1954, 1964, 1974). Complementing herbarium, morphological and anatomical work with molecular phylogenetics, the recognition of *Microteaceae* provides a nice example of integrating modern phylogenetic approaches and plant classification.

## Material and methods

**Taxon sampling and material.** — In total 87 species from *Caryophyllales*, representing nearly all families, and

22 species from outgroups were sampled in this study. A complete list of taxa with their sources of origin and voucher information is given in Appendix 1. If available, fresh plant material was silica dried, otherwise herbarium samples were used. Fresh material was obtained primarily from the living collections of the Botanic Garden and Botanical Museum Berlin-Dahlem and from Bonn University Botanical Gardens. All *petD* sequences for *Caryophyllales* and of several outgroups were generated for this study; other outgroup sequences were taken from Worberg & al. (2007). For *matK*, 19 complete coding sequences of *Caryophyllales* taxa were generated newly; others were taken from Müller & Borsch (2005) and from the *Caryophyllales* subset of the angiosperm data set published by Hilu & al. (2003). In case own complete sequences were available for a genus only represented by partial *matK* sequences in the latter data set, these partial sequences were ignored.

**DNA extraction, amplification and sequencing.** — Plant material was homogenized using the Mixer Mill (MM 200, Retsch) and was then extracted with CTAB following the protocol described in Borsch & al. (2003) or with the AVEGene Plant DNA extraction kit (Avegene, Korea).

The fragment amplified contains the *petB-petD* intergenic spacer, the *petD* 5'-exon and the *petD* group II intron. Both for PCR amplification and sequencing, the primers pipetB1411F and pipetD738R (Löhne & Borsch 2005) were used. If amplification of the entire fragment did not succeed, the region was amplified in two overlapping halves using the primers CApetD324R (5'-ATC CCY TGT TTC ACT CCG ATA G-3') and CApetD194F (5'-CAG GCT CCG TAA RAT CCA G-3') in combination with one of the primers mentioned above. PCR reaction was performed in 50 µl volume containing 5 units of SAWADY Taq polymerase (PeqLab), 8 µl dNTPs (each 1.25 mM), 5 µl 5× Taq buffer (PeqLab), 2 µl of each forward and reverse primer, 4 µl genomic DNA and H<sub>2</sub>O to 50 µl. For *matK* a broad spectrum of mostly family-specific internal amplification primers was used to amplify the whole *trnK* intron including the CDS (coding sequence) in two overlapping halves. Primer trnKF-bryo (Wicke & Quandt 2009) served as forward primer for the upstream fragment, with the reverse primers ACmatK1401R (this study; 5'-ATG GAT TCG TAT TCA CAT AC-3') for *Aizoaceae*, *Cactaceae*, *Didiereaceae*, *Nyctaginaceae*, *Portulacaceae*; ACmatK1400R (Müller & Borsch 2005) for *Amaranthaceae* and *Basellaceae*; CARYmatK1440R (this study; 5'-AKC GTA AAT GAG AGG ATT G-3') for *Caryophyllaceae*; PLUMmatK1401R (this study, 5'-ATG GAT TGA TAT TCA CAC AC-3') for *Plumbaginaceae* and POLYmatK1401R (this study, 5'-ATG GAT TCG TAT TCA CAC AC-3') for *Polygonaceae*. Thermal cycling was performed on a T3 thermocycler (Biometra, Göttingen) with an initial denaturation step (90 s) at 96 °C followed by 34 cycles

of 30 s at 95 °C, 60 s at 50 °C, 90 s at 72 °C, and a final extension step of 20 min at 72 °C. Fragments were visualised using the Flu-o-blu system (Biozym, Hamburg, Germany) and excised from a 1.5 % agarose gel (NEEO-Agarose, Roth, Germany). The DNA was then purified using the AVEGene Gel Extraction Kit (Avegene, Korea) according to the manufacturer's protocol. PCR products were directly sequenced using the DCTS Quick Start Kit (Beckman Coulter). The reaction mix contained 3 µl DCTS Quick Start Kit (Beckman Coulter), 0.5 µl primer (20 pm/µl), 0.5–6.5 µl DNA template and ultrapure water to obtain a total volume of 10 µl. The cycle sequencing temperature profile consisted of 30 cycles of 96 °C for 20 s, 50 °C for 20 s and 60 °C for 4 min, on the thermocycler mentioned above. Samples were run on an automated capillary sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter). Alternatively, cleaned fragments were sequenced via Macrogen Inc. (Seoul, South Korea; all new *matK* and *petD* sequences from BGBM). Pherograms of the latter source (ABI 3730 capillary sequencer) were usually clean and well readable until 850 nt, allowing to sequence the whole *trnK* intron with four primers. Pherograms were edited manually using the software PhyDE v0.995 (Müller & al. 2005+).

**Sequence alignment.** — Beside substitutions, non-coding chloroplast DNA shows a high number of length mutational events. Correct homology assessment and gap placing has to take into account the different kinds of length mutations. Alignment followed rules described in detail in Löhne & Borsch (2005). Where detected, inversions were reverse-complemented and aligned to the rest and treated as homologous.

**Parsimony tree search.** — Parsimony ratchet analysis using PAUP\* (Swofford 1998) and PRAP (Müller 2004) was carried out using ten random addition cycles of 200 ratchet iterations with 25 % of the positions being reweighted. A strict consensus was computed from the shortest trees found. Tree evaluation was done via bootstrapping with 10 000 replicates with keeping only one tree in memory. For Maximum Parsimony (MP) analysis, length mutations were coded according to a modified simple indel coding method (Müller 2006), which resulted in a matrix of 100 indel characters.

**Bayesian Inference.** — Bayesian Inference of phylogeny was conducted using MrBayes (Ronquist & Huelsenbeck 2003) employing the GTR+Γ+I model. Two runs of four MCMC chains were run simultaneously for two million generations, sampling the chains every 100th generation. Trees were summarised with the burn-in conservatively set to the first 25 % of generations.

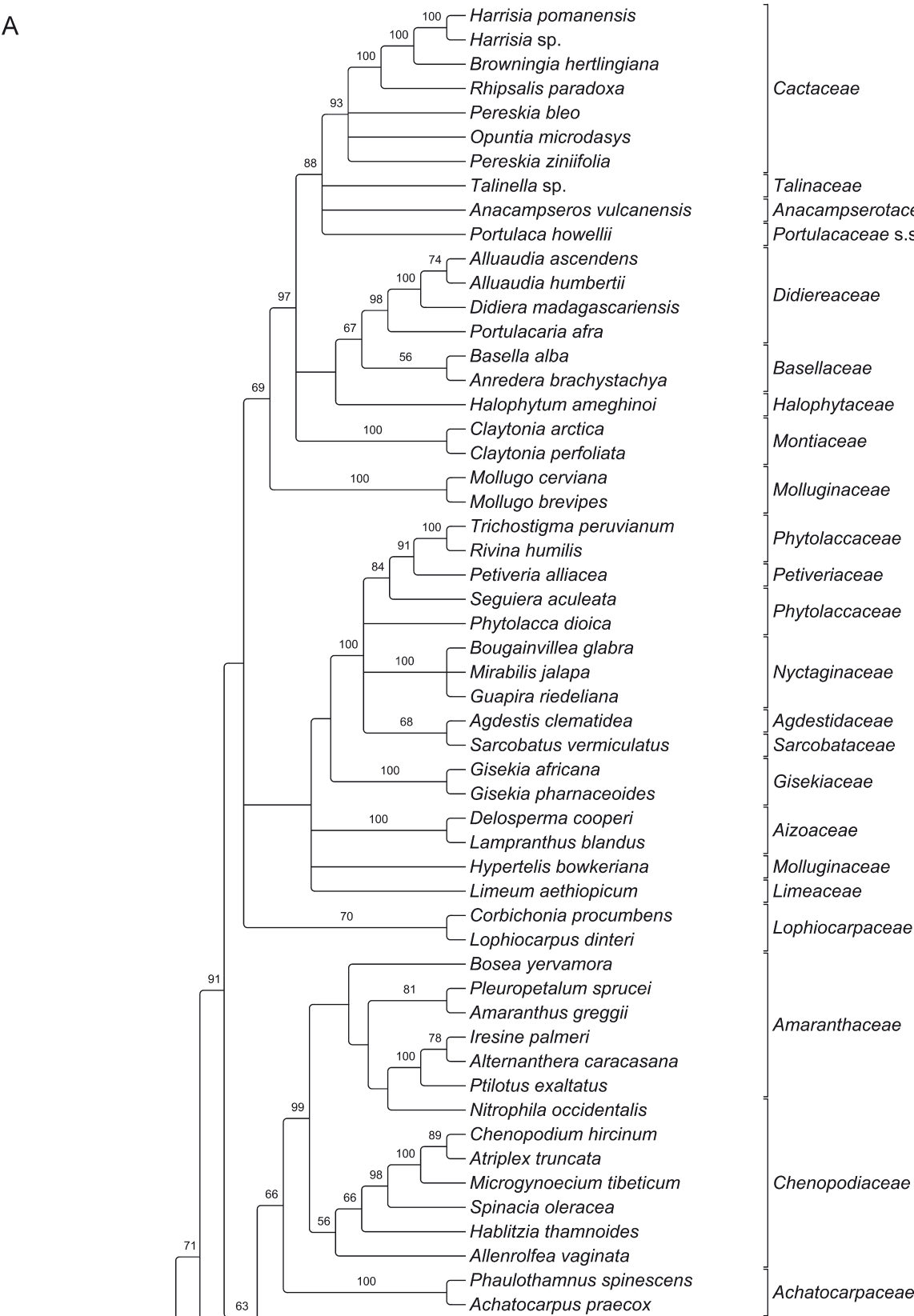
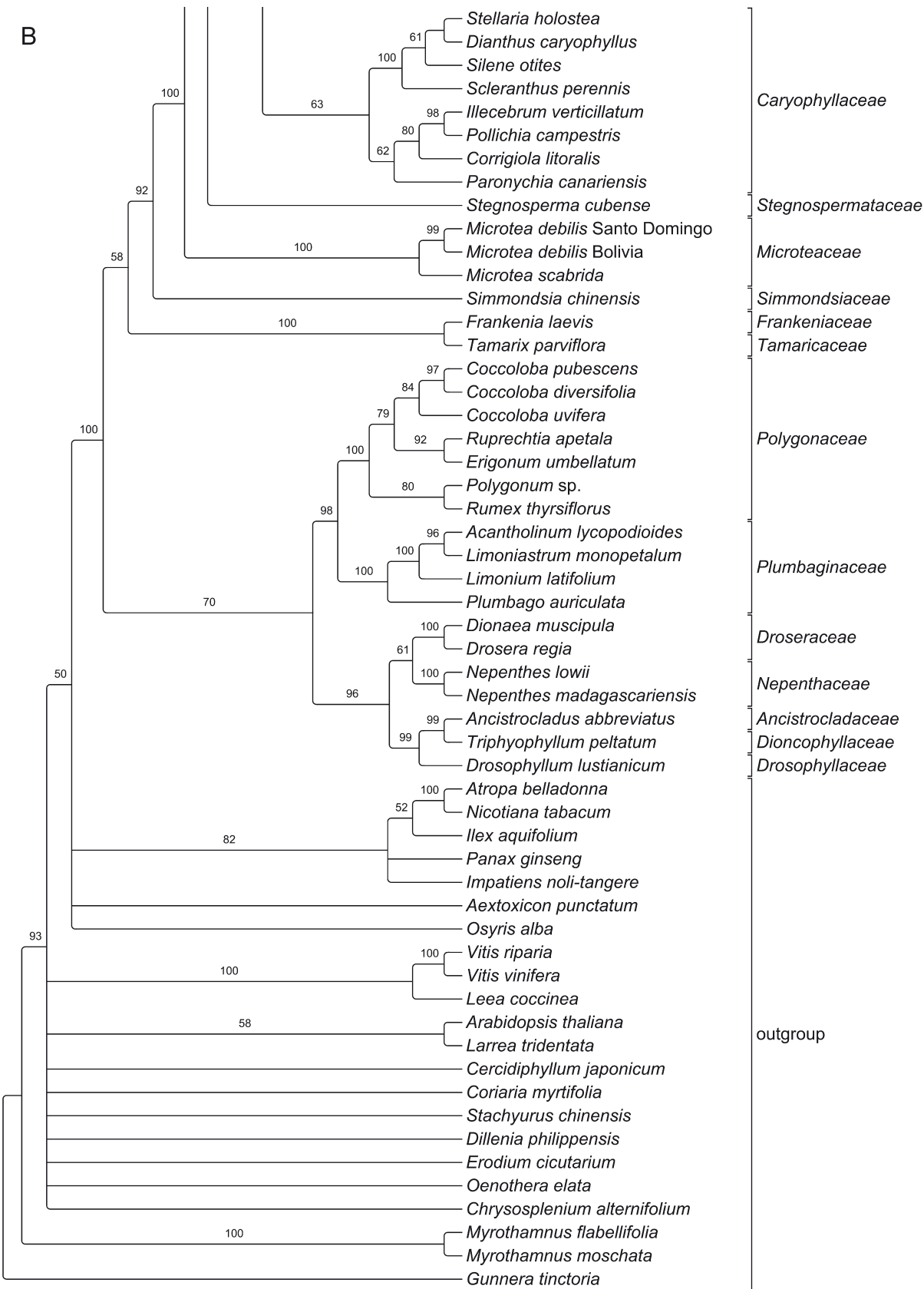


Fig. 1A–B. Maximum Parsimony tree (strict consensus) of *Caryophyllales* based on the *petD* region including a matrix of coded microstructural changes. – Values above branches are bootstrap percentages.





Results

*PetD* data set and trees

For the *Caryophyllales* and outgroup taxa sequenced in this study, the *petB-petD* region ranged from 711 to 1281 nucleotides in length and resulted in an aligned matrix of 2787 characters. Several mutational hotspots both in the *petB-petD* spacer and the *petD* intron were excluded from phylogenetic analyses due to ambiguous homology assessment. In total, 880 characters had to be marked as mutational hotspot. Alignment and matrix are available from the eudicot website ([www.eudicots.de](http://www.eudicots.de)) and from the authors. For *petD*, parsimony ratchet search calculated a strict consensus of 972 shortest trees (length: 4735 steps, CI: 0.413, RI: 0.673, RC: 0.278). Bayesian Inference (BI) of phylogeny (considering only substitutions) resulted in a tree topology largely congruent to that from MP analysis. If differences were observed, they were soft incongruities (i.e., inconsistencies), lacking convincing support. However, resolution and support values are higher in BI compared to MP, and interestingly BI finds evidence for monophyly of *Caryophyllales* II (polygonids).

*MatK* data set and trees

Only characters from the *matK* CDS were used in this study, although the flanking *trnK* intron sequences were also generated here and will be analysed and published elsewhere. The *matK* data set consisted of 1718 characters; the sequence length range was between 540 and 1524 nucleotides due to incomplete sequences. There were no characters excluded from phylogenetic analyses. Modified simple indel coding resulted in 253 coded indels. Complete sequence statistics are given in Table 1. For the *matK* data set, 32 equally short trees were found (5348 steps, CI: 0.411, RI: 0.596, RC: 0.245); a strict consensus tree was calculated (not shown). Bayesian analysis of the *matK* data set yielded a tree topology mostly congruent to those from MP. Some nodes were significantly better supported or even resolved inconsistently in BI than with MP as is indicated in Fig. 3. Like in *petD*, resolution and support values for crucial nodes are higher in BI (considered as well supported if posterior probability > 0.95).

Combined data set and trees

Results from MP tree searches for each *petD* and *matK* with coded indels are shown in Fig. 1 and 2, respectively. Analyzing a data set that combined nucleotide data of

those taxa for which both *petD* and *matK* were available with the MP approach resulted in only 8 shortest trees (7421 steps, CI: 0.440, RI: 0.624, RC: 0.275); the strict consensus tree of those trees is shown in Fig. 4.

Discussion

Two major clades within monophyletic *Caryophyllales*

The monophyly of *Caryophyllales* found in previous studies is confirmed with maximum confidence by both *petD* and complete *matK* sequences (Fig. 1–3), although the *matK* gene tree inferred with MP has only moderate support for this (not shown; 79 % JK). Partial *matK* sequences in Cuénoud & al. (2002) and Hilu & al. (2003) had no or weaker support, indicating the significantly enhanced usefulness of complete *matK* data sets. Multi-gene analyses (Cuénoud & al. 2002; Brockington & al. 2009) converge with *petD* group II intron (and *petD+matK* combined) analyses on maximum support for the *Caryophyllales* in a broad sense.

The *Caryophyllales* consist of two major clades. They are congruently depicted by this study (non-coding and rapidly evolving plastid regions) and the trees in Brockington & al. (2009). The four-gene (18S, *rbcL*, *atpB* and partial *matK*) and two-gene (*rbcL*+partial *matK*) analyses by Cuénoud & al. (2002) were inconsistent in placing *Rhabdodendron* as sister to the remainder of all *Caryophyllales*. BI (Fig. 3) of *matK* also shows improved confidence over MP (tree not shown, Cuénoud & al. 2002, Fig. 3) for *Rhabdodendron* and *Simmondsia* to be members of the core *Caryophyllales*. This “core” of the *Caryophyllales* clade (sensu Cuénoud & al. 2002) includes the *Centrospermae* and corresponds to the *Caryophyllales* I in Hilu & al. (2003). Considering the emerging overall agreement for the existence of this clade, we call it the caryophyllid clade. It can be easily distinguished from the polygonid clade within *Caryophyllales* (Fig. 2, 4). The latter corresponds to the so-called “non-core *Caryophyllales*” (Cuénoud & al. 2002; Brockington & al. 2009) or *Caryophyllales* II (Hilu & al. 2003).

For the circumscription of the polygonids, the position of the *Frankeniaceae-Tamaricaceae* clade requires some discussion. Support for the monophyly of polygonids is weak in *petD* data alone using the parsimony approach. The lineage is placed inconsistently in various positions depending on taxon sampling. Using Bayesian Inference, polygonids reach a posterior probability of 0.86, and *Frankeniaceae* and *Tamaricaceae* together

Table 1. Sequence statistics of *petD* and *matK* datasets of *Caryophyllales* analysed in this study. For the *petD* matrix, parts of the sequences (hotspots) could not be included due to uncertain homology. Values calculated from the matrix (underlined) are thus not based on all nucleotides.

Character set	# characters	length range	mean	% divergence	ti/tv	% variable	% informative	% GC	% A	% C	% G	% T
<i>petD</i> data set	1907	711–1281	1009.39	<u>13.8</u>	<u>1.154</u>	<u>42.108</u>	<u>30.309</u>	33.746	32.64	15.565	18.18	33.614
<i>matK</i> data set	1718	540–1524	1253.42	15.058	1.291	69.034	52.619	32.705	30.406	16.49	16.215	36.888

are sister to the remainder of polygonids. *Frankeniaceae* and *Tamaricaceae* generally appear in a clade with high support. It also has been found using coding markers as *rbcL* or *matK* (Nandi & al. 1998; Meimberg & al. 2000; Savolainen & al. 2000b; Soltis & al. 2000; Cuénoud & al. 2002; Hilu & al. 2003; Brockington & al. 2009). As an alternative hypothesis derived from *matK* and *trnK/matK*, respectively, a clade comprising the *Frankeniaceae-Tamaricaceae* lineage and *Plumbaginaceae* plus *Polygonaceae* was found (Meimberg & al. 2000; Cuénoud & al. 2002; Hilu & al. 2003) and is also evident from Bayesian Inference of complete *matK* sequences in this study (Fig. 3). Brockington & al. (2009) resolve the *Frankenia-Tamarix* clade sister to *Plumbaginaceae-Polygonaceae* within polygonids.

### Relationships within polygonids

*Plumbaginaceae* and *Polygonaceae* are both monophyletic and sister groups. Both *Plumbaginaceae* and *Polygonaceae* are recovered with *petD* sequences, and also their sister group relationship, which received high statistical support elsewhere (Nandi & al. 1998; Meimberg & al. 2000; Soltis & al. 2000; Cuénoud & al. 2002; Hilu & al. 2003; Brockington & al. 2009). A sister group relationship between the *Frankeniaceae-Tamaricaceae* clade and the *Plumbaginaceae-Polygonaceae* clade, as suggested earlier (Meimberg & al. 2000; Cuénoud & al. 2002; Hilu & al. 2003; Brockington & al. 2009), is not supported by *petD* data.

The carnivorous clade with the families *Ancistrocladaceae*, *Dioncophyllaceae*, *Droseraceae*, *Drosophyllaceae* and *Nepenthaceae* is recovered by *petD* sequences plus coded indels with bootstrap support comparable to previous studies (Meimberg & al. 2000; Cuénoud & al. 2002; Brockington & al. 2009). Within this clade, *Ancistrocladaceae* and *Dioncophyllaceae* are sisters with maximum support as depicted earlier (Meimberg & al. 2000; Cuénoud & al. 2002; Hilu & al. 2003). *Drosophyllum lusitanicum* as sister to the former clade is supported in the present study comparable to Meimberg & al. (2000), Cuénoud & al. (2002) and Hilu & al. (2003). Monophyly of both *Droseraceae* and *Nepenthaceae* are recovered in the *petD* data set, but their placement to each other and to the clade of *Ancistrocladaceae*, *Dioncophyllaceae* and *Drosophyllaceae* is not resolved. Even far larger character sets (Brockington & al. 2009) could not clarify this question.

### Relationships within caryophyllids

The caryophyllid clade was discovered by Cuénoud & al. (2002) and Hilu & al. (2003) using *matK* sequence data. In the most recent study on *Caryophyllales* phylogeny, Brockington & al. (2009) could substantiate the inclusion of *Simmondsia* and *Rhabdodendron* as the first branching taxa into this clade with a large number of sequence data from coding genes. Based on *petD* data alone, the sister group relationship between *Simmondsia chinensis* and

the remaining caryophyllids is supported with 100 % bootstrap (BS), comparable to values from much larger data sets (Brockington & al. 2009). Previous data sets (Cuénoud & al. 2002; Brockington & al. 2009), including *Asteropeia* and *Physena*, found these two genera to constitute a monophylum branching after *Simmondsia* in *Caryophyllales* I. This lineage is also evident in our *matK* tree (Fig. 3).

A surprising new result from the present study is the position of *Microtea* (*Phytolaccaceae* subfam. *Microteoideae*) that is found as a successive sister to all other caryophyllids after the grade of *Simmondsia*, *Rhabdodendron* and *Asteropeia-Physena*. Thus, *Microtea* is not even closely related to other *Phytolaccaceae* and rather represents an isolated lineage in *Caryophyllales* (see below).

The placement of *Stegnosperma cubense* as inferred from *petD* sequences differs from other analyses. *Stegnosperma* is the next branch following *Microtea* in the *petD* MP tree (Fig. 1) or is unresolved between the CACA clade (*Caryophyllaceae*, *Achatocarpaceae*, *Chenopodiaceae*, *Amaranthaceae*) and the remainder of *Caryophyllales* (BI, Fig. 2). Based on *rbcL*, *Stegnosperma* appeared together with *Cactaceae*, *Didiereaceae*, *Basellaceae*, *Molluginaceae*, *Phytolaccaceae*, *Nyctaginaceae*, *Gisekiaceae* and *Aizoaceae* (Manhart & Rettig 1993) and *Cactaceae*, *Didiereaceae* and *Basellaceae* (Rettig & al. 1992), respectively, but both topologies were lacking reliable support. The by now largest data set in terms of sequence characters (Brockington & al. 2009) places *Stegnosperma* sister to a so-called “globular inclusion clade”. In the two-gene analysis of Cuénoud & al. (2002) it is in the same position, although no *matK* sequence seems to exist (*Stegnosperma* is missing from Fig. 3 of Cuénoud & al. 2002; and no entry is in GenBank). Branches among *Stegnosperma*, *Limeum*, the CACA clade, the “raphide clade” and the “portulacaceous cohort” are extremely short in all studies existing so far. The respective nodes are completely unsupported in the MP tree of the total evidence data set of Brockington & al. (2009) and their maximum likelihood (ML) tree only provides support for a *Stegnosperma-Limeum* grade.

In line with previous studies employing different DNA markers (Manhart & Rettig 1993; Müller & Borsch 2005), monophyly of a group comprising *Amaranthaceae*, *Chenopodiaceae*, *Achatocarpaceae* and *Caryophyllaceae* is moderately to highly supported in this study. Using partial *matK* (Cuénoud & al. 2002), *Caryophyllaceae* were found branching after *Simmondsia/Rhabdodendron* and *Asteropeia*, albeit without any bootstrap support. Signal of complete *matK* (Fig. 3) is in line with *petD* (Fig. 1, 2) and multi-gene data sets (Brockington & al. 2009). Within this clade, *Amaranthaceae* and *Chenopodiaceae* together form a well supported monophylum, which is in line with all previous studies (Giannasi & al. 1992; Rettig & al. 1992; Manhart & Rettig 1993; Downie & Palmer 1994; Downie & al. 1997; Cuénoud & al. 2002; Hilu &



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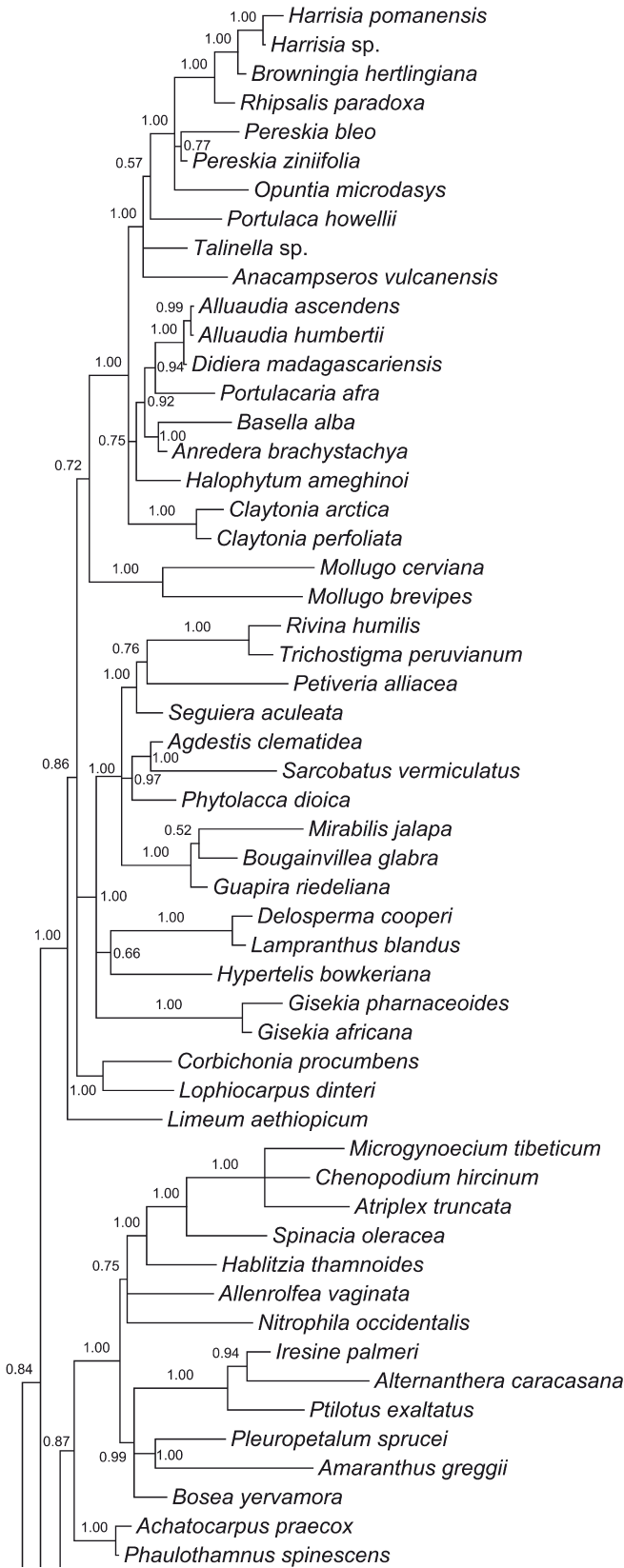
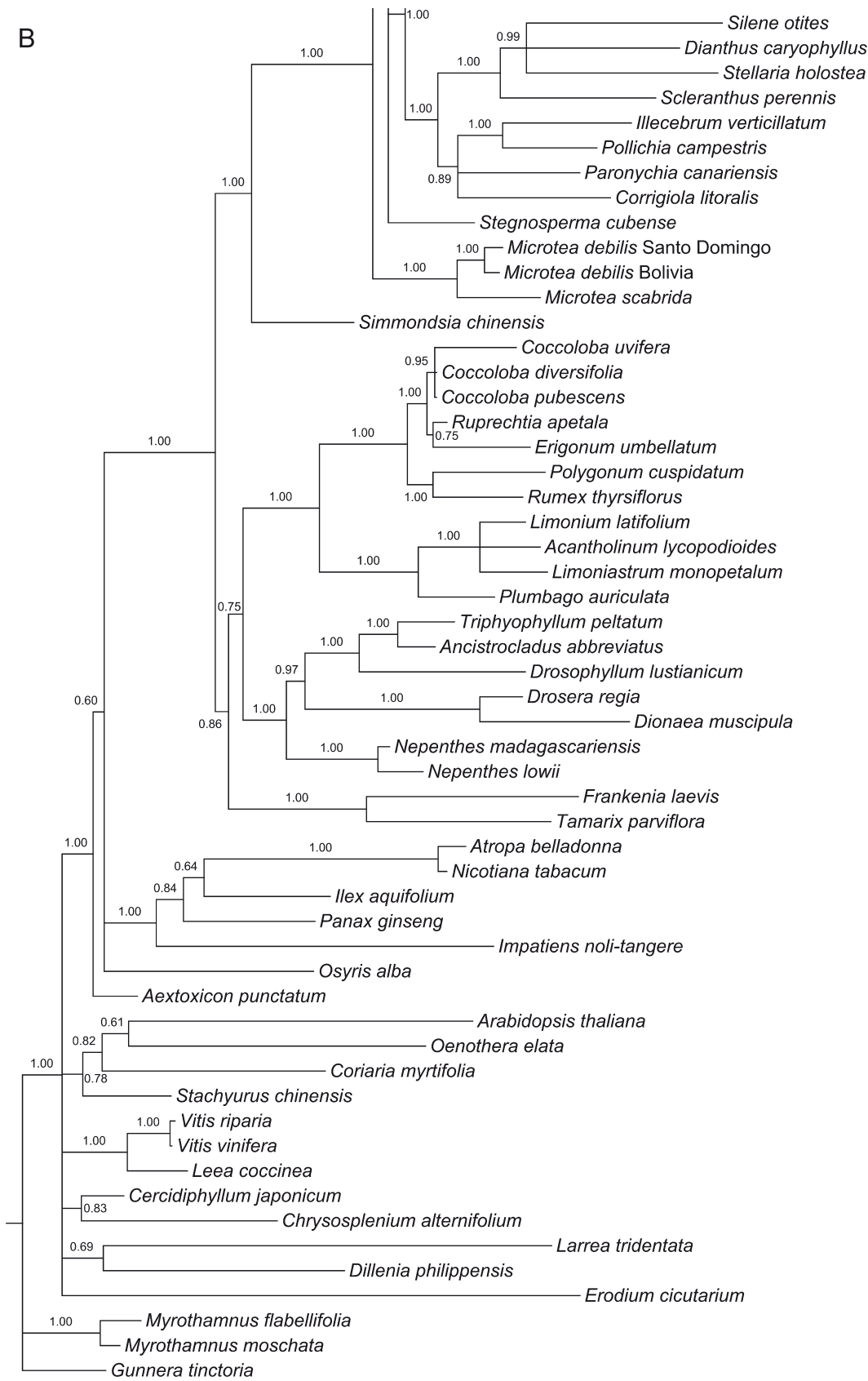
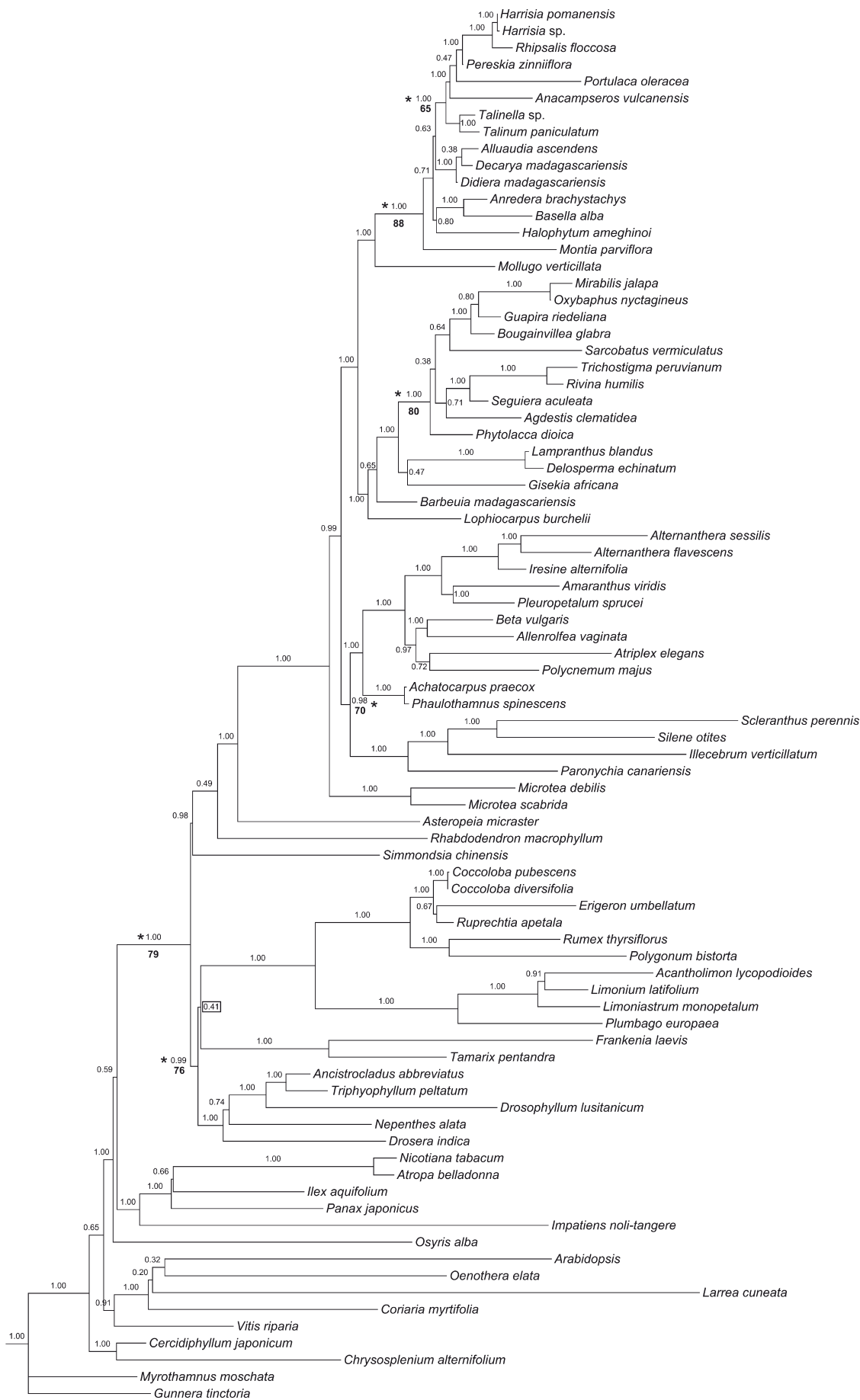


Fig. 2A–B. Phylogram of the Bayesian analysis of *Caryophyllales* based on the *petD* region. – Values above branches are posterior probabilities.





al. 2003; Kadereit & al. 2003; Müller & Borsch 2005). However, whereas the monophyly of *Amaranthaceae* has been shown with high confidence (e.g., Müller & Borsch 2005) the monophyly of *Chenopodiaceae* is still under debate. Hitherto existing multi-gene analyses only include *Celosia* and *Spinacia* (Cuénoud & al. 2002; Brockington & al. 2009), limiting insights into the monophyly of the respective families. Most interestingly, *Amaranthaceae* and *Chenopodiaceae*, respectively, appear monophyletic in gene trees of *petD* (Fig. 1, 2) and *matK* (Fig. 3) with the exception of an inconsistent position of *Polycnemoideae* (*Nitrophila*, *Polycnemum*). Earlier, using *trnK/matK* the monophyly of *Amaranthaceae* was affirmed (100 % BS, Müller & Borsch 2005); *Chenopodiaceae* were found to be paraphyletic, with *Chenopodiaceae* subfam. *Polycnemoideae* being the sister of *Amaranthaceae*. Relationships of *Amaranthaceae* and *Chenopodiaceae* need further investigation, especially to test the circumscription of *Chenopodiaceae*. *Achatocarpaceae* as sister to the *Amaranthaceae-Chenopodiaceae* alliance is strongly supported here (Fig. 4). This relationship has been suggested by various studies before (Manhart & Rettig 1993; Cuénoud & al. 2002; Kadereit & al. 2003; Müller & Borsch 2005).

The clade consisting of *Cactaceae*, *Portulacaceae*, *Basellaceae*, *Didiereaceae*, *Halophytaceae*, *Lophiocarpus* and *Mollugo* corresponds to the “higher core II” clade (Hilu & al. 2003). Resolution and support are, compared to that of *matK* data (Cuénoud & al. 2002; Hilu & al. 2003), relatively weak within this clade. *Cactaceae* (58 % BS, pp = 1.00) were found in a clade (pp = 1.00) together with *Talinella*, *Anacampseros* and *Portulaca*, which is in line with earlier findings (Applequist & Wallace 2001; Applequist & al. 2006; Nyffeler 2007; Nyffeler & Eggli in press) using *ndhF* sequences. Combining the chloroplast genes *ndhF*, *matK* and the mitochondrial *nad1*, *Anacampseros* s.l. was depicted sister to *Cactaceae* (78 % BS and 0.72 pp in Bayesian Inference; Nyffeler 2007). However, it is noteworthy that phylogenetic hypotheses contradict each other depending on the genome analysed. In mitochondrial data, parts of *Portulaca* are sister to *Cactaceae*, while in chloroplast data, *Anacampseros* s.l. is found sister to *Cactaceae* with a Bayesian approach. Relationships among *Cactaceae*, *Anacampseros* s.l. and *Portulaca* remain unclear (Fig. 4) with *matK* and *petD* alone. Nevertheless, we follow the more extensive analyses of Nyffeler & Eggli (in press) in recognising *Anacampserotaceae* and *Talinaceae* separate from *Portulacaceae* (here only represented by *Portulaca*).

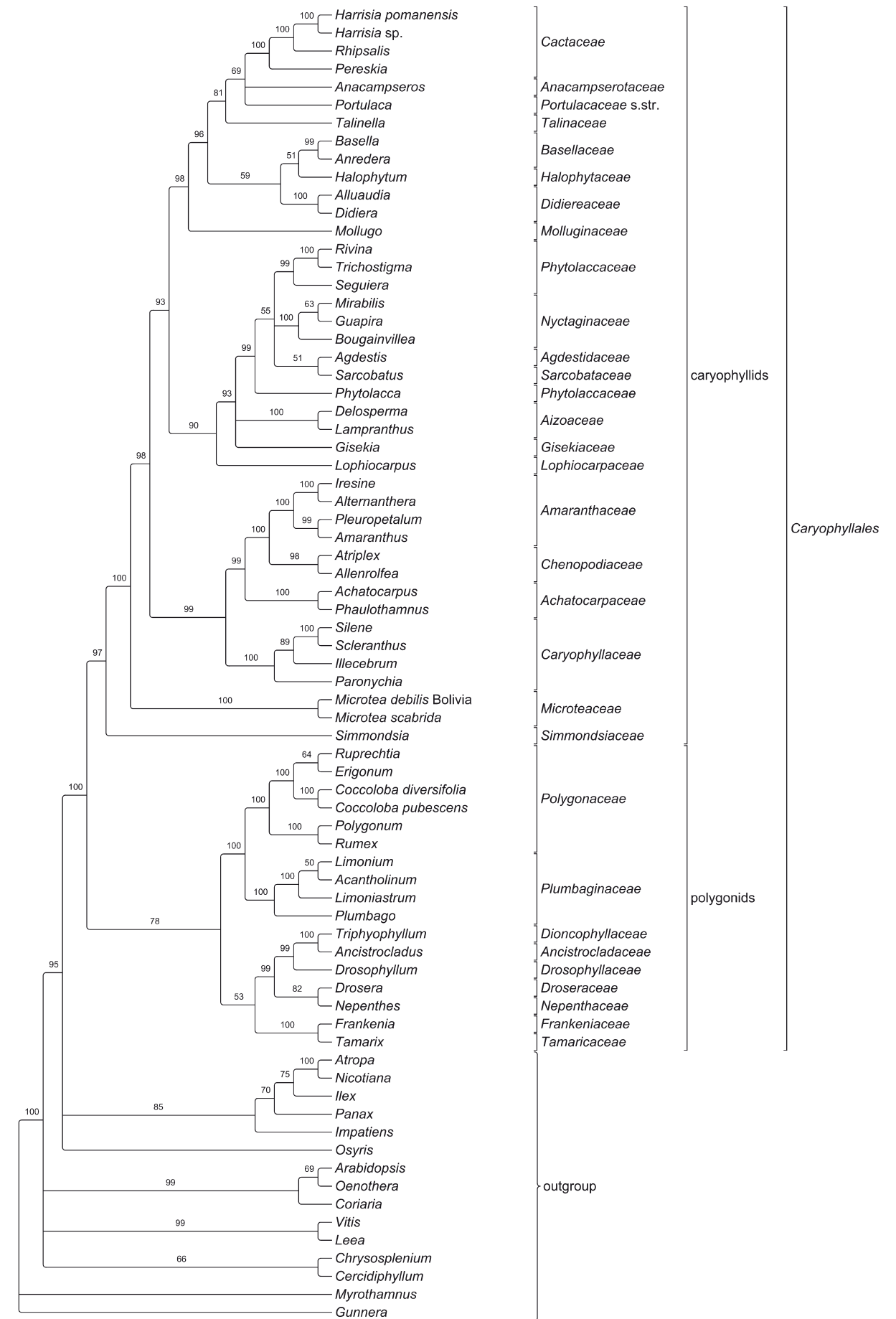
Both *Claytonia* species do not cluster with the other *Portulacaceae* in the current study (Fig. 1, 2), support-

ing a classification under a separate family *Montiaceae*. As reported previously (Applequist & Wallace 2001; Applequist & al. 2006; Nyffeler 2007). The circumscription of *Didiereaceae* has been revised based on molecular data (Applequist & Wallace 2003) including now the genera *Portulacaria*, *Ceraria* and *Calyptrotheca* (all formerly *Portulacaceae*). In our *petD* data set, a clade comprising *Didiereaceae* and *Portulacaria* was found with support, too. Usage of *ndhF* (Applequist & Wallace 2001) could not resolve the relationship between those two families. Combining three genes representing all three genomes (Nyffeler 2007), *Basellaceae* were found sister to a clade including *Didiereaceae*, *Portulacaceae* and *Cactaceae*, but this topology yielded only 65 % BS and 0.56 pp. Positions of *Basellaceae* and *Didiereaceae* within higher core II remained unclear. The position of *Halophytum ameghinoi* (*Halophytaceae*) as sister to a *Basellaceae* plus *Didiereaceae* clade was not supported in Brockington & al. (2009). However it is resolved congruently in this study (Fig. 2). *Halophytum* was not included in most studies dealing with *Cactaceae* and their nearest relatives (Applequist & Wallace 2001; 2003; Applequist & al. 2006; Nyffeler 2007). *Halophytaceae* belong to the “portulacaceous cohort”, but understanding their position requires further work.

### Polyphyly of *Molluginaceae* and complex evolution of betalain families

In line with earlier findings (Cuénoud & al. 2002), *Limeum* (*Limeaceae*) is inconsistently placed apart from remaining *Molluginaceae*. A statistically well supported hypothesis of the systematic position of *Limeum* is still missing. Even a data set with over 42 000 nucleotides did not result in a reliable placement of that taxon (Brockington & al. 2009). *Limeaceae* were validated by Shipunov ex Reveal in 2005. *Corbichonia* was already shown to be distant from *Mollugo* (Cuénoud & al. 2002), rendering *Molluginaceae* polyphyletic. It was recently included into *Lophiocarpaceae* along with *Lophiocarpus* (formerly *Phytolaccaceae*) (Doweld & Reveal 2008). Using *matK* (Cuénoud & al. 2002), the *Molluginaceae* genera *Pharnaceum*, *Suessenguthiella*, *Adenogramma*, *Glischrothamnus* and *Glinus* formed a moderately supported group sister to the portulacaceous cohort but *Mollugo* was not sampled. In our study *Mollugo* is resolved in the same position with high confidence (Fig. 1–4). Furthermore, the genera *Coelanthum*, *Macarthuria*, *Polypoda*, *Psammotropha* and *Telephium* have so far not been sampled in any molecular phylogenetic study. For a better understanding of the evolution of pigments, a complete sampling of *Molluginaceae* in combination with pigment

Fig. 3. Phylogram of the Bayesian analysis of *Caryophyllales* based on the *matK* CDS. – Values above branches are posterior probabilities. Some nodes that gained significantly less support in the MP analysis (strict consensus not shown here) are marked with an asterisk. Respective bootstrap values are provided below branches. Branches inconsistently resolved in MP include *Drosera* (sister to *Nepenthes*), the *Frankenia-Plumbago* lineage (sister to the carnivorous clade), *Simmondsia* and *Rhabdodendron* (in a polytomy), *Nyctaginaceae* and *Sarcobatus* (in a polytomy), *Basellaceae* and *Halophytum* (in a polytomy).





data and insights into possible deviations of biosynthetic pathways are needed.

The clade including *Aizoaceae*, *Gisekiaceae*, *Hypertelis bowkeriana* (*Molluginaceae*), *Nyctaginaceae*, *Petiveriaceae* and *Phytolaccaceae* corresponds to higher core I *Caryophyllales* (Hilu & al. 2003) or the raphide clade (Brockington & al. 2009). Surprisingly, *Hypertelis* is placed sister to *Gisekiaceae* based on *petD* data, but this position lacks convincing support. *Hypertelis* was not included in earlier studies, underscoring that a dense taxon sampling among *Molluginaceae* is needed to clarify if there are separate lineages within this family and how they are composed. In any case, betalain evolution in *Caryophyllales* is much more complex than previously thought. Rather than assuming a single origin of betalain biosynthesis in a common ancestor of all betalain taxa, multiple shifts between anthocyanin and betalain pathways may have to be considered in light of the current phylogenetic trees. Although their biosynthesis is mutually exclusive to anthocyanins (Stafford 1994), the anthocyan taxa in *Caryophyllaceae*, *Lophiocarpaceae*, *Limeaceae* and *Molluginaceae* are scattered over a betalain producing radiation.

### Disentangling *Phytolaccaceae*

The taxonomic history of *Phytolaccaceae* shows us how the family was disentangled step by step over the last decades. Whereas Heimerl (1934) included *Agdestis*, *Barbeuia* and *Stegnosperma*, the latter was raised to family level by Nakai (1942), a treatment supported by a morphological study of Bedell (1980). The first *rbcL* data (Manhart & Rettig 1994) did not support a placement of *Stegnosperma* within *Phytolaccaceae*. Further molecular phylogenetic analyses (Cuénoud & al. 2002) provided robust evidence of *Stegnosperma* being distant from other *Phytolaccaceae* in core *Caryophyllales* (Cuénoud & al. 2002), although the deep nodes in their analyses remained unsupported (however, there seems to be no *matK* fragment available in GenBank from Cuénoud & al. 2002). The multi-gene analysis of Brockington & al. (2009) and the *petD* data of this study clarified the isolated position of *Stegnosperma* in *Caryophyllales*, supporting its classification in a family of its own.

The three respective families (*Agdestidaceae*, *Barbeuiaceae*, *Stegnospermaceae*) are now generally recognised (e.g., APG III). *Lophiocarpaceae* were published recently by Doweld & Reveal (2008) based on the results of Cuénoud & al. (2002) and contain two genera, *Corbichonia* and *Lophiocarpus*. They represent the most distant lineage as depicted in all molecular phylogenetic analyses (Cuénoud & al. 2002; Brockington & al. 2009; this study, Fig. 1–4).

Close relatives of *Phytolaccaceae* are in the so-called “raphide clade” (Brockington & al. 2009) that also has

been recovered by most previous analyses (Cuénoud & al. 2002) and is corroborated here (Fig. 4). The presence of raphid crystals in vegetative tissues of members of this group of taxa was initially described by Rodman (1994). Families within this clade are *Agdestidaceae* (not sampled by Brockington & al. 2009), *Aizoaceae*, *Gisekiaceae*, *Nyctaginaceae*, *Petiveriaceae* (not sampled by Brockington & al. 2009), *Phytolaccaceae* and *Sarcobataceae*. The last family was separated by Behnke (1997) from *Chenopodiaceae* considering form and size of sieve-element plastids and first *rbcL* data (Clement & Mabry 1996a).

*Nyctaginaceae* are recovered in the present study with maximum support in both MP and BI trees, which is slightly higher than earlier [85 % BS and 96 % JK respectively (Cuénoud & al. 2002; Hilu & al. 2003)]. *Petiveria* as nested within *Phytolaccaceae* subfam. *Rivinoideae* was found previously with only medium confidence (Cuénoud & al. 2002) but is recovered here also with *petD* data (no complete *matK* CDS for *Petiveria* is yet available). With *Phytolacca* being sister to a clade of *Sarcobatus* and *Agdestis*, it is obvious that the familial circumscription of *Phytolaccaceae* even only including *Agdestidoideae*, *Phytolacchoideae* and *Rivinoideae* currently does not represent a monophyletic group. It will not only be necessary to broaden the taxon sampling among *Phytolaccaceae* s.str, but also to carry out a thorough study of both molecular and morphological characters. Based on *matK* (Cuénoud & al. 2002) data, *Sarcobatus* was found sister to *Nyctaginaceae* (unsupported) and *Agdestis* sister to *Phytolaccaceae* subfam. *Rivinoideae*. In the same study, after combining sequences from *rbcL* and *matK*, *Agdestis* and *Sarcobatus* appear as sisters with 77 % BS support, congruent to findings in Hilu & al. (2003). *PetD* trees of this study give another hint for the close relationship of these two families (Fig. 1, 2).

### *Microtea* as isolated lineage and description of *Microteaceae* as a new family

The isolated phylogenetic position of *Microtea* is highly supported statistically in all our trees (Fig. 1–4). The same regards to a very distant relationship of *Microtea* and *Lophiocarpus*. The latter is sister to *Corbichonia* (formerly classified within *Molluginaceae*; Endress & Bittrich 1993) in an early-branching position of the so-called “globular inclusion clade”. *Microtea* and *Lophiocarpus* have long been suspected as being deviant members of different families. Heimerl (1934) thought the two genera share characters of *Chenopodiaceae* and *Phytolaccaceae*. The organisation of the ovary was considered more similar to *Amaranthaceae/Chenopodiaceae* than to *Phytolaccaceae* (Eckardt 1954, 1964, 1974). The subfamily *Microteoideae* of *Phytolaccaceae* was formally recognised by Nowicke (1968). Although this

Fig. 4. Maximum parsimony tree (strict consensus) of *Caryophyllales* based on the *petD* + *matK* data sets combined including a matrix of coded microstructural changes, values above branches are bootstrap percentages.



Fig. 5. *Microtea portoricensis* Urb. – holotype in the herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem (B).

treatment was accepted by Rohwer (1993), he mentioned that *Microteoideae* may eventually be better classified within *Chenopodiaceae*. Behnke (1993) also considered *Microtea* to have an unclear familial position. Previous molecular phylogenetic studies of *Caryophyllales* (e.g., Cuénoud & al. 2002) or *Chenopodiaceae* (e.g., Kadereit & al. 2003) did not sample *Microtea*. Its inclusion in the current study thus yields clear data for an unequivocal re-classification.

*Microtea* was described by Swartz in 1788. Unique to this genus are the muricate to spiny achenes (Urban 1885), whereas the single-ovuled ovaries are shared with *Lophiocarpus* (Urban 1885; Eckardt 1964; Nowicke 1968). The latter character was also the reason to describe *Phytolaccaceae* subfam. *Microteoideae* Eckardt ex Nowicke (1968), comprising those two genera. All species of *Microtea* are annual herbs with small flowers in racemiform thyrsoïd inflorescences (see Fig. 5). Several species were described in this genus by Moquin (1849), then by Urban (1885) from the Antilles, but also under *Lophiocarpus* [e.g., *M. burchellii* (Hook.f.) N.E.Br.], and more recently by Marchioreto & deSiqueira (1998) from Brazil. There are 21 species names and six additional names of infra-specific taxa. In the absence of a modern monograph, the diversity of *Microtea* and the *Microteaceae* may be estimated to encompass a dozen species. *Microteaceae* has a distribution ranging from Central America and the Antilles throughout South America and is thus one of the families restricted to the Neotropics.

#### ***Microteaceae* Schäferhoff & Borsch, fam. nov.**

Type: *Microtea* Swartz, Prodr. Ind. Occ. 4: 53. 1788.

Herbae annuae, raro basi suffrutescentes; caulibus erectis vel ascendentibus, ramosis, usque ad 25(–60) cm altis; foliis alternis, sessilibus, lanceolatis vel ovato-ellipticis, glabris; inflorescentia spiculata vel racemosa; floribus hermaphroditis, actinomorpha, pedicellatis aut sessilibus; bractea floralis basi pedicelli unica, elliptica, membranacea, glabra, persistente; bracteolis 2 vel nullis, lanceolatis, membranaceis, flore aequilongis vel brevioribus; tepalis 5 (vel 4), aequalibus, ellipticis vel lineari-ellipticis, uninerviis; staminibus 5–8, filamentis filiformibus subhypogynis imoque calycis insertis, 5 exterioribus cum ejusdem laciniis alternis; pollinis graminibus pantoporatis et aequae microspinulosis; ovario simplice, uniloculari; ovula campylotropa; stylis 3–5, distinctis; stigmatibus papilloso-penicilliformibus; acheniis laevibus, subreticulato-tuberculatis vel projecturis spiniformibus dense tectis; seminibus lenticularibus vel subglobosis, testa nigra, crustacea.

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## Appendix 1.

### Plant material used in this study

**Appendix 1. Plant material used in this study.** Collections made in the field first list country and locality in the case of both herbarium specimens and silica gel dried samples. Samples obtained from the living collections of the Botanic Garden Berlin-Dahlem and Bonn University Botanical Gardens first list the garden accession number and then country and locality data in square brackets. Collector and collection number are given in italics, the herbarium abbreviation in parentheses. Information on the specific history of the plant individual sampled can be obtained from the label that goes with the herbarium voucher. A unique identifier is given for every DNA isolate; it follows the specimen data and precedes the EMBL/GenBank accession number. Sequences of outgroups are completely from the matrix of Worberg & al. (2007). For sequences generated from other material than the isolates listed here, the respective publication is indicated (see below).

**Outgroups:** *Aextoxicaceae*: *Aextoxicon punctatum* Ruiz & Pav., Löhne & Borsch (2005), *petD* AY590831; Müller & al. 2006, *matK* DQ182342. *Aquifoliaceae*: *Ilex aquifolium* L., *petD*: AM396557, *matK*: AF542607. *Araliaceae*: *Panax ginseng* C.A. Mey., Kim & Lee (2004), *petD* AY582139; Kim & Lee (2004) *matK* AY582139. *Balsaminaceae*: *Impatiens noli-tangere* L., *petD*: AM396556, *matK*: AF542608. *Brassicaceae*: *Arabidopsis thaliana* (L.) Heynh., Sato & al. (1999), *petD*: NC000932, Sato & al. (1999), *matK*: NC000932. *Cercidiphyllaceae*: *Cercidiphyllum japonicum* Siebold & Zucc., *petD*: AM396545, *matK*: AM396508. *Coriariaceae*: *Coriaria myrtifolia* L., *petD*: AM396553, *matK*: AF542600. *Dilleniaceae*: *Dillenia philippinensis* Rolfe, *petD*: AM396549. *Geranaceae*: *Erodium cicutarium* (L.) L'Hér, *petD*: AM396552. *Gunneraceae*: *Gunnera tinctoria* (Molina) Mirb., *petD*: AM396542, *matK*: AM396506. *Leeaceae*: *Leea coccinea* Planch., *petD*: AM396548. *Myrothamnaceae*: *Myrothamnus flabellifolia* Welw., *petD*: AM396543; *M. moschata* Baill., *petD*: AM396544, *matK*: AF542591. *Onagraceae*: *Oenothera elata* Kunth, Hupfer & al. (2000), *petD* NC002693, Hupfer & al. (2000) *matK* NC002693. *Saxifragaceae*: *Chrysosplenium alternifolium* L., *petD*: AM396546, *matK*: AM396496. *Solanaceae*: *Atropa belladonna* L., Schmitz-Linneweber & al. (2002), *petD*: NC004561, Schmitz-Linneweber & al. (2002), *matK*: NC004561. *Nicotiana tabacum* L., Shinozaki & al. (1986), *petD*: NC001879, Shinozaki & al. (1986), *matK*: NC001879. *Stachyuraceae*: *Stachyurus chinensis* Franch., *petD*: AM396555. *Vitaceae*: *Vitis riparia* A. Gray, *petD*: AM396547, *matK*: AF542593. *Zygophyllaceae*: *Larrea tridentata* Coult., *petD*: AM396554.

*Caryophyllales*: *Achatocarpaceae*: *Achatocarpus praecox* Grieseb., BGBM 142-78-94-10 [Argentina], *Leuenberger, Arroya-Leuenberger & Eggli 4345* (B), AC073, *petD* FN598616 (this study), *matK* AY514845. *Phaulothamnus spinescens* A.

Gray, USA, Texas, *Borsch, Müller & Pratt 3446* (B, ISC), AC060, *petD* FN598617 (this study), *matK* AY514846. *Agdestidaceae*: *Agdestis clematidea* Moc. & Sessé, Mexico, *Saynes V. 5583* (MEXU), AC418, *petD* FN598638 (this study); Cuénoud & al. (2002), *matK* AY042538. *Aizoaceae*: *Delosperma cooperi* L. Bolus, BG Bonn 3632 [without locality data], *Schäferhoff s.n.* (BONN), AC331, *petD* FN598633 (this study). *D. echinatum* Schwantes, Cuénoud & al. (2002), *matK* AY042575. *Lampranthus blandus* Schwantes, BGBM 016-19-82-70 [South Africa], *Cubr 28489* (B-Gartenherbar), AC642, *petD* FN598634 (this study), *matK* FN597631 (this study). *Amaranthaceae*: *Alternanthera caracasana* Kunth, USA, Texas, *Borsch, Müller & Pratt 3433* (B, ISC), AC058, *petD* FN598619 (this study). *A. flavescent* H.B.K., Sage & al. (2007), *matK* AM887484. *A. sessilis* R.Br., Müller & Borsch (2005), *matK* AY514796. *Amaranthus greggii* S. Watson, USA, Texas, *Pratt, Müller, Borsch 207* (B, ISC), AC059, *petD* FN598622 (this study). *A. viridis* L., Sage & al. (2007), *matK* AM887488. *Bosea yervamora* L., BG Meise 75-2966 [without locality data], no voucher, AC029, *petD* FN598623 (this study). *Iresine palmeri* Standl., USA, Texas, *Borsch, Müller & Pratt 3445* (B, ISC), AC054, *petD* FN598618 (this study). *I. alternifolia* (Uline & W. L. Bray) S. Watson, Borsch & al. (unpubl. data), *matK* AM887490. *Pleuropetalum sprucei* Standl., BG Bonn 16484 [Venezuela], *Borsch 3996* (B), AC020, *petD* FN598621 (this study), *matK* AF542596. *Ptilotus exaltatus* Nees, BGBM 231-15-74-80 [Australia]: *Cubr 46421* (B-Gartenherbar), AC644, *petD* FN598620 (this study). *Anacampse-rotaceae*: *Anacampseros vulcanensis* Añon., BG Bonn, *Borsch 3595* (BONN), AC049, *petD* FN598660 (this study), *matK* AF542597. *Ancistrocladaceae*: *Ancistrocladus abbreviatus* Airy Shaw [without locality data], BG Bonn 16430, *Schäferhoff 5* (BONN), AC291, *petD* FN598602 (this study); Meimberg & al. (2001), *matK* AF315939. *Asteropeiaceae*: *Asteropeia micraster* Hallier f., Cuénoud & al. (2002), *matK* AY042549. *Barbeuiaceae*: *Barbeuia madagascariensis* Steud., Cuénoud & al. (2002), *matK* AY042552. *Basellaceae*: *Anredera brachystachys* (Moq.) Sperling, BGBM 185-03-98-33 [Ecuador], *Cubr 46782*, AC646, *petD* FN598654 (this study), *matK* FN597626 (this study). *Basella alba* L., BG Bonn 03671 [without locality data]: *Schäferhoff 8* (BONN), AC299, *petD* FN598653 (this study); Cuénoud & al. (2002), *matK* FN598653. *Cactaceae*: *Browningia hertlingiana* (Backeb.) Buxb., BG Bonn 2416, [no voucher yet], *petD* FN598666 (this study). *Harrisia pomanensis* (F.A.C. Weber ex K. Schum.) Britton & Rose, 160-37-86-10 [Argentina, La Rioja], *Leuenberger 3608* (B), AC632, *petD* FN598664, *matK* FN597629 (this study). *Harrisia* sp., BGBM 304-08-99-10 [Dominican Republic, Pedernales], *Cubr 46781* (B), AC639, *petD* FN598665 (this study), *matK* FN597627 (this study). *Opuntia microdasys* (Lehm.) Pfeiff., without locality data, *Schäferhoff s.n.* (BONN), AC312, *petD* FN598667 (this study). *Pereskia bleo* DC., BG Bonn 22, [Guadeloupe], *Schäferhoff s.n.* (BONN), AC300, *petD* FN598662 (this study). *P. ziniiflora* DC., BGBM 261-19-93-60/1 [Cuba], *Cubr 40527* (B-Gartenherbar), AC645, *petD* FN598663 (this study), *matK* FN597625 (this study). *Rhipsalis paradoxa* Salm-Dyck, Worberg & al. (2007), *petD* AM396555;

- Nyffeler (2002) *matK* AM396551. **Caryophyllaceae: *Corrigiola litoralis*** L., BG Bonn 03472 [without locality data] *Schäferhoff 1* (B), AC311, *petD* FN598615 (this study). **Dianthus caryophyllus** L., BG Bonn 08031 [without locality data], *Schäferhoff s.n.*, AC334, *petD* FN598610 (this study). **Illecebrum verticillatum** L., Germany, *Borsch & Müller 3541* (B), AC063, *petD* FN598612 (this study), *matK* AY514849. **Paronychia canariensis** Juss., BGBM 041-36-00-10/2 Spain, Teneriffa, *Dürbye 1126* (B), AC641, *petD* FN598614 (this study), *matK* FN597636 (this study). **Pollichia campestris** Aiton, South Africa, *Müller 850* (B), AC353, *petD* FN598613 (this study). **Scleranthus perennis** L., Germany, North Rhine Westfalia, Eifel, *Borsch 3389* (B), AC034, *petD* FN598609 (this study), *matK* AY514847. **Silene otites** Sm., Netherlands, *Borsch 3495* (B), AC071, *petD* FN598608 (this study), *matK* AY514848. **Stellaria holostea** L., BG Bonn 14501, Germany, Eifel, *Krämer & s.n.* (BONN), AC293, *petD* FN598611 (this study). **Chenopodiaceae: *Allenrolfea vaginata*** Kuntze, BG Bonn 2488 [Argentina], *Borsch 3994* (B, BONN), AC017, *petD* FN598625, *matK* AY514828. *Atriplex elegans* (Moq.) D. Dietr., USA, *Borsch, Pratt & Müller 3425* (B, KAS, ISC), *petD* FN598628 (this study), *matK* AY514830. **Chenopodium hircinum** Schrad., Argentina, *Tolaba 2951* (B, JUA, LPB), AC657, *petD* FN598627. **Hablitzia tamnoides** M. Bieb., BG Bonn 03609-90 [without locality data], *Borsch 3546* (B), AC018, *petD* FN598624. **Microgynoeicum tibeticum** Hook.f., China, Quinghai, Tibet, *Dickoré 4284* (B, KAS), AC656, *petD* FN598626. **Nitrophila occidentalis** S. Watson, USA, Utah, *Pratt 204*, AC089, *petD* FN598629. **Spinacia oleracea** L., Schmitz-Linneweber & al. (2001), *petD* NC\_002202, *matK* NC\_002202. **Didiereaceae: *Alluaudia ascendens*** Drake, BG Bonn 15807 [Madagascar] *Schäferhoff 1* (BONN; Foto), AC294, *petD* FN598656 (this study); Cuénoud & al. (2002), *matK* AY042541. **A. humbertii** Choux, BGBM 027-79-74-80 [Madagascar], *Rauh 1108* (HEID), *Schwerdtfeger 18835* (B-Gartenherbar), AC637, *petD* FN598658 (this study). **Decarya madagascariensis** Choux, Cuénoud & al. (2002), *matK* AY042574. **Didierea madagascariensis** Baillon BGBM 014-79-74-80 [Madagascar], *Rauh 7365* (HEID), *Schwerdtfeger 16739* (B-Gartenherbar), AC634, *petD* FN598657, *matK* FN597628 (this study). **Portulacaria afra** Jacq., BG Meise 19750159, no voucher, AC355, *petD* FN598655 (this study). **Dionocphyllaceae: *Triphiophyllum peltatum*** (Hutchinson & Dalziel) Airy Shaw, BG Bonn 10713 [without locality data], *Schäferhoff 61* (BONN), AC301, *petD* FN598601 (this study); Meimberg & al. (2001), *matK* AF315940. **Droseraceae: *Dionaea muscipula*** J. Ellis, BG Bonn 14527 [USA, N-Carolina, New Hanover County, Wilmington], *Rehder s.n.*, AC336, *petD* FN598597 (this study). **Drosera indica** L., Meimberg & al. (2000), *matK* AF204848. **D. regia** Stephens, BG Bonn 1851, (no voucher yet), N557, *petD* FN598596 (this study). **Drosophyllaceae: *Drosophyllum lusitanicum*** Link, BG Bonn 9015 [Spain]: *Schäferhoff 63* (BONN), AC024, *petD* FN598600 (this study), *matK* AY514860. **Frankeniaceae: *Frankenia laevis*** L., BG Bonn s.n. [without locality data]: *Müller 884* (BONN), AC128, *petD* FN598583 (this study), *matK* AY514853. **Gisekiaceae: *Gisekia africana*** Kuntze, Ethiopia, *Wondafrash 2164* (B, ETH), AC303, *petD* FN598637 (this study); Cuénoud & al. (2002), *matK* AY042591. **G. pharnaceoides** L., Ethiopia, *Wondafrash 2148* (B, ETH), AC304, *petD* FN598636 (this study). **Halophytaceae: *Halophytum ameghinii*** Speg., Sukkulentsammlung Zürich s.n.: (Foto B), AC048, *petD* FN598652 (this study), *matK* AY514852. **Limeaceae: *Limeum aethiopicum*** Burm.f., *Hilliard & Burret 10671* (S), ED318, *petD* FN598630 (this study). **Lophiocarpaceae: *Corbichonia decumbens*** (Forsk.) Exell, South Africa, *Merxmüller & Giess 30613* (M), AC502, *petD* FN598631 (this study). **Lophiocarpus burchellii** Hook.f., Cuénoud & al. (2002), *matK* AY042611. **L. dinteri** Engl., *Wanntorp 267* (S), ED317, *petD* FN598632 (this study). **Microteaceae: *Microtea debilis*** Sw., Hispaniola, Dominican Republic, *Clase & de la Cruz 2701* (JBSD), AC308, *petD* FN598604 (this study); Bolivia, *Beck 17025* (B, LPB), AC662, *petD* FN598605 (this study), *matK* FN597632 (this study). **M. scabrida** Urb., Bolivia, *Beck 28296* (B, LPB), AC660, *petD* FN598606 (this study), *matK* FN597533. **Molluginaceae: *Hypertelis bowkeriana*** Sond., BG Bonn 9964 [without locality data], *Schäferhoff 54* (BONN), AC314, *petD* FN598635 (this study). **Mollugo brevipes** Urb., Cuba, *Borsch, Rankin, Urquiola & Acosta 3947* (B, HAJB, HPPR), AC628, *petD* FN598649 (this study). **M. cerviana** (L.) Ser., South Africa, *Müller 847* (B, PRE), AC352, *petD* FN598648 (this study). **M. verticillata** L., Fior & al. (2006), *matK* AY936330. **Montiaceae: *Claytonia arctica*** Adam, Russia, Verchowye reki Opi Opskoje plato Scheben, *Andrianova s.n.*, AC332, *petD* FN598650 (this study). **C. perfoliata** Donn. ex Willd., Germany, Bochum, *Schäferhoff s.n.* (BONN), AC333, *petD* FN598651 (this study). **Montia parvifolia** (Moq. ex DC.) Greene, Cuénoud & al. (2002), *matK* AY042616. **Nepenthaceae: *Nepenthes lowii*** Hook. f., BG Bonn 09957 [without locality data], *Schäferhoff s.n.* (BONN, Foto), AC302, *petD* FN598599 (this study). **N. madagascariensis** Poir., BG Bonn 25236 [Madagascar]: *Barthlott s.n.* (BONN), AC335, *petD* FN598598 (this study). **Nyctaginaceae: *Bougainvillea glabra*** Choisy, BG Bonn 18196 [without locality data], *Schäferhoff 2* (BONN), AC295, *petD* FN598646 (this study); Cuénoud et al. (2002), *matK* AY042560. **Guapira riedeliana** (Fisch.) Lundell, BGBM 060-95-74-8013 [without locality data], *Schwerdtfeger 25465* (B), AC630, *petD* FN598647 (this study), *matK* FN597630 (this study). **Mirabilis jalapa** L., BG Bonn 03624 [without locality data], *Schäferhoff* (BONN), AC305, *petD* FN598645 (this study); Cuénoud & al. (2002), *matK* AY042614. **Oxybaphus nyctagineus** Sweet, Cuénoud & al. (2002), *matK* AY042624. **Petiveriaceae: *Petiveria alliacea*** L., BG Bonn 8687 [without locality data], *Schäferhoff 6* (BONN), AC309, *petD* FN598644 (this study). **Phytolaccaceae: *Phytolacca dioica*** L., BG Bonn 17776 [Spain, Teneriffa], *Seehawer s.n.* (BONN), AC296, *petD* FN598640 (this study); Cuénoud & al. (2002), *matK* AY042631. **Rivina humilis** L., BG Bonn 9865 [without locality data], *Borsch 3542* (B, BONN), AC023, *petD* FN598642 (this study), *matK* AY514850. **Seguiera aculeata** Jacq., BGBM 05-43-74-83-00, *Cubr 20984* (B-Gartenherbar), AC659, *petD* FN598641 (this study), *matK* FN597634. **Trichostigma peruvianum** (Moq.) H. Walter, BGBM 136-79-74-83/3 (B-Gartenherbar), AC661, *petD* FN598643 (this study), *matK* FN597635 (this study). **Plum-**

**baginaceae:** *Acantholimon lycopodioides* (Girard) Boiss., BGBM 009-25-84-13/4 [Pakistan, Gilgit], *Cubr* 27249 (B-Gartenherbar), AC640, *petD* FN598587 (this study), *matK* FN597642 (this study). ***Limoniastrum monopetalum*** Boiss., BGBM 109-08-01-10/2 [France, Aude], *Cubr* 43356 (B-Gartenherbar), AC643, *petD* FN598588 (this study), *matK* FN597641 (this study). ***Limonium latifolium*** (Sm.) O. Ktze., BG Bonn 03678 [without locality data], *Müller* 883 (BONN), AC127, *petD* FN598585 (this study), *matK* AY514861. ***Plumbago auriculata*** Blume, BG Bonn 16603 [without locality data], *Schäferhoff* 3 (BONN), AC297, *petD* FN598586 (this study). ***P. europaea*** L., Cuénoud & al. (2002), *matK* AY042634. ***Polygonaceae:*** ***Coccoloba diversifolia*** Jacq., *BGMK* 260-44-93-10/1 [Cuba, Granma, Sierra Maestra], *Stohr s.n.* (B), AC635, *petD* FN598593 (this study), *matK* FN597640. ***C. pubescens*** L., BGBM 146-35-74-80 [without locality data], *Schwerdtfeger* 6261 (B), AC629, *petD* FN598594 (this study), *matK* FN597639 (this study). ***C. uvifera*** L., BG Bonn 18414 [without locality data]: *Schäferhoff* 7 (BONN), AC310, *petD* FN598592 (this study). ***Erigonum umbellatum*** Torr., BGBM 071-06-86-10 [USA, Colorado], *Cubr* 27052 (B-Gartenherbar), AC638, *petD* FN598590 (this study), *matK* FN597643 (this study). ***Polygonum bistorta*** L., Meimberg & al. (2000), *matK* AF204859. ***P. cuspidatum*** Siebold & Zucc., BG Bonn, [Germany, Melbweiher, Bonn], *Schäferhoff* (BONN), AC306, *petD*: FN598591 (this study). ***Rumex thyrsiflorus*** L., Germany, Berlin, *Borsch* 3995 (B), AC636, *petD* FN598595 (this study), *matK* FN597638 (this study). ***Ruprechtia apetala*** Wedd., BGBM 007-14-011-10 [Argentina, Salta], *Leuenberger & Eggli* 4859 (B), AC631, *petD* FN598589 (this study), *matK* FN597637 (this study). ***Portulacaceae:*** ***Portulaca howellii*** (D. Legrand) Eliasson, BG Bonn, AC050, *petD* FN598661 (this study). ***P. oleracea*** L., Edwards & al. (2005), *matK* AY875349. ***Rhabdodendraceae:*** ***Rhabdodendron macrophyllum*** (Spruce ex Benth.) Huber, Cuénoud & al. (2002), *matK* AY042642. ***Sarcobataceae:*** ***Sarcobatus vermiculatus*** Torr., USA, South Dakota, *Müller* 88/233 (B), AC354, *petD* FN598639 (this study); Cuénoud & al. (2002), *matK* AY042652. ***Simmondsiaceae:*** ***Simmondsia chinensis*** (Link) C. K. Schneid., BG Bonn 19090 [without locality data], *Borsch* 3596 (B, BONN) AC037, *petD* FN598603 (this study), *matK* AY514854. ***Stegnospermataceae:*** ***Stegnosperma cubense*** A. Rich., Dominican Republic, *Clase, Veloz & Florián* 3031-C (JBSD), AC307, *petD* FN598607 (this study). ***Talinaceae:*** ***Talinella*** sp., BG Bonn [Madagascar], *Röösli s.n.*, AC045, *petD* FN598659 (this study), *matK* AY514859. ***Talinum paniculatum*** (Jacq.) Gaertn. ***Tamaricaceae:*** ***Tamarix parviflora*** DC., BG Bonn 9340 [without locality data], *Schäferhoff* 4 (BONN), AC298, *petD* FN598584 (this study). ***T. pentandra*** Hampe. ex Bunge, Cuénoud & al. (2002), *matK* AY042663.

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