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MOLECULAR PHYLOGENY OF THE CARYOPHYLLACEAE (CARYOPHYLLALES) INFERRED FROM CHLOROPLAST *MATK* AND NUCLEAR RDNA ITS SEQUENCES[1]

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Caryophyllaceae is a principally holarctic family including around 2200 species often classified into the three subfamilies Alsinoideae, Caryophylloideae, and Paronychioideae. Complex and possibly homoplasious morphological characters within the family make taxa difficult to delimit and diagnose. To explore part of the morphological evolution within the family, we investigated the phylogeny of the Caryophyllaceae by means of analyzing plastid and nuclear sequence data with parsimony and Bayesian methods. We describe a mode of tracing a stable phylogenetic signal in ITS sequences, and a significant common signal is shared with the plastid data. Parsimony and Bayesian analyses yield some differences in tree resolution. None of the subfamilies appear monophyletic, but the monophyly of the Caryophylloideae is not contradicted. Alsinoideae are paraphyletic, with *Arenaria* subg. *Eremogone* and *Minuartia* subg. *Spergella* more closely related to the Caryophylloideae. There is strong support for the inclusion of *Spergula-Spergularia* in an Alsinoideae-Caryophylloideae clade. Putative synapomorphies for these groupings are twice as many stamens as number of sepals and a caryophyllad-type of embryogeny. Paronychioideae form a basal grade, where tribe Corrigioleae are sister to the rest of the family. Free styles and capsules with simple teeth are possibly plesiomorphic for the family.

Key words: Alsinoideae; Caryophyllaceae; Caryophylloideae; ITS; *matK*; Paronychioideae; phylogeny.

Caryophyllaceae is a large family of 86 genera of annual or perennial herbs (rarely shrubs) distributed across the globe predominantly in the holarctic, but concentrated in the Mediterranean and Irano-Turanean region. The majority of the approximately 2200 species of the family are heliophytes occurring in dry, open habitats and totally absent from the lowland rain forests; some of its members are restricted to mountainous regions, among which some of the highest occurring seed plants can be found (Bittrich, 1993).

Despite the size of the family, its wide range of habitats and global ubiquity, the systematics of the Caryophyllaceae still relies on overall similarities of morphological characters used for recognizing taxa. Even though meticulous morphological studies have been conducted to establish reasonably well-diagnosed taxa (e.g., McNeill, 1962; Bittrich, 1993), Caryophyllaceae systematics and classification have never been tested in detail under a phylogenetic framework (Bittrich, 1993). Thus, there is great need of a phylogenetic hypothesis based on molecular data that would enable current knowledge on morphology to be tested and provide an opportunity to interpret the supposedly complex pattern of morphological evolution within the family.

The understanding of phylogenetic relationships among angiosperms has greatly increased in the last few years, in particular as a result of analyses of molecular data accumulated from multiple regions. Among new discoveries is the repositioning of the Caryophyllaceae within the Caryophyllales as sister to the Amaranthaceae in a clade in turn sister to the core Caryophyllales (Soltis et al., 2000; Cuénod et al., 2002). This is in contrast to previous hypotheses placing the Caryophyllaceae as one of the most recent lineages within the order (Eckardt, 1964), and it discredits the alleged close relationship of the family with the Molluginaceae based on their common production of anthocyanins instead of betalains as the rest of the Caryophyllales (Bittrich, 1993; Judd and Olmstead, 2004). The monophyly of Caryophyllaceae can be recognized by the consistent morphological characters and is well supported by recent molecular results (Cronquist, 1981; Cuénod et al., 2002; Judd et al., 2002). Examples of alleged synapomorphic morphological characters are the ontogenetic reduction of the septa in the ovary and the type of sieve element plastids (Bittrich, 1993). Even though the Molluginaceae and Caryophyllaceae are no longer considered as close relatives (Soltis et al., 2000; Cuénod et al., 2002), Gilbert (1987) and more recently Smissen et al. (2002) discuss the position of the two Caryophyllaceae genera *Telephium* and *Corrigiola* (Paronychioideae-Corrigioleae), especially in relation to the fact that these genera might belong to the Molluginaceae (but see Downie et al., 1997).

Traditionally, Caryophyllaceae are divided into the three subfamilies Alsinoideae, Caryophylloideae, and Paronychioideae (Pax and Hoffman, 1934; Bittrich, 1993; Rabeler and Bittrich, 1993). The family rank has been suggested for all of these, but only Paronychioideae, or part of it, have sometimes appeared in the literature as a distinct family (Illecebraceae),

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though with differing circumscription (e.g., Hutchinson, 1974). The Paronychioideae are defined only by the Solanad-type of embryogeny and the stipulate leaves, with this latter often described as plesiomorphic within the Caryophyllaceae (Judd et al., 2002). Historically, apetalous flowers and indehiscent fruits were considered as characters diagnosing the Paronychioideae, in contrast with the allegedly "primitive" petalous flowers and capsules of the rest of the family (Bittrich, 1993). Bittrich (1993) claims that it is often difficult to distinguish clearly between proper petals and staminodes in some genera, raising doubts on the homology of the structures (e.g., in *Geocarpon* and *Polycarpea* spp.). On the other hand, Petrusson and Thulin (1996) interpret the petal-like appendages of some members of the Paronychioideae as staminodes that replace the inner whorl of stamens. Further, some genera of the Paronychioideae traditionally included in the tribe Polycarpeae have capsules, whereas isolated cases of indehiscent fruits are recorded also within the Alsinoideae (e.g., *Scleranthus*), therefore making the character homoplastic if "optimized" on the current classification (Bittrich, 1993; Smissen et al., 2002).

The Caryophyllad-type of embryogeny of Alsinoideae and Caryophylloideae was claimed by Bittrich (1993) to represent a possible synapomorphy for the two taxa. Furthermore, Bittrich (1993) also points out that the development of diverticles in the embryo sac and the decussate leaves might constitute yet another two synapomorphies for the group. Bittrich (1993) also argues on the evolution of chromosome numbers within the group, suggesting a possible origin of Caryophylloideae from "derived" Alsinoideae (based on Fernandes and Leitão, 1971). Under such a scenario, the Alsinoideae form a paraphyletic group and its circumscription should be reconsidered. However, until further knowledge on these aspects, Bittrich (1993) decided to maintain a subfamily Alsinoideae diagnosed by nectary glands at the base of the episepalous stamens. Subfamily Caryophylloideae is widely recognized as well supported by morphological characters: the formation of a calyx tube and the closed or semiclosed petal venation seem to be consistent within the group (Bittrich, 1993; Judd et al., 2002). Clawed petals, corolla scales, and the formation of an anthophore are sometimes reported as possible synapomorphies for the subfamily, but their consistency needs confirmation (Bittrich, 1993).

According to Bittrich (1993), further division of the subfamilies into tribes is mainly based on arbitrary interpretations on morphology, rather than on phylogenetic relationships, and only a few groups within the Caryophyllaceae have recently been revised with modern methods (Smissen, 1999; Oxelman et al., 1997, 2001, 2002). Nevertheless, Bittrich (1993) justified the maintenance of a number of suprageneric taxa because they are diagnosed on some particularly "reliable" morphological characters. However, there are indications of a complex pattern of morphological evolution within the family, and this complexity obscures attempts to obtain clearly delimited taxa, which often contrast to the few molecular–phylogenetic results produced so far (e.g., Oxelman and Liden, 1995; Nepokroeff et al., 2001, 2002). Similarly, generic delimitation is notoriously difficult in the Caryophyllaceae, supposedly due to highly homoplastic characters displaying repeated events of reversals and parallelisms (Kurtto, 2001; Oxelman et al., 2001). Thus, large, morphologically heterogeneous genera (e.g., *Silene*, *Arenaria*, *Minuartia*, *Paronychia*, and *Herniaria*) are inadequately circumscribed and probably not monophyletic (McNeill, 1962; Bittrich, 1993; Oxelman et al., 2001).

The use of molecular data in the investigation of relationships within morphologically complex families has been a powerful approach for achieving well-delimited taxa (e.g., Ericaceae: Kron et al., 2002; Asteraceae: Kim and Jansen, 1995). Ideally, DNA-based cladistic analyses can provide an independent source of information capable of resolving relationships among morphologically intractable groups. With sufficient taxon sampling that includes representatives from all major subfamilial taxa, DNA-based topologies can be the starting point for reinterpretations on the development and evolution of intricate morphological characters. As a first step, morphological features may simply be optimized on molecular trees, with extensive studies on morphology as an inevitable second step (Schönenberger and Conti, 2003).

The use of ITS data in phylogenetic reconstruction is very common but not without controversy. Álvarez and Wendel (2003) surveyed a number of systematic papers that contained phylogenetic analyses based on ITS sequences and concluded that, while such data is routinously utilized, very few papers touch the various problems connected to either the structure of this particular nuclear region or to the practical procedures used in the analyses (e.g., alignment). On the other hand, different authors have described ways to extract stable phylogenetic information from hypervariable ITS sequences at high taxonomic level. For example, in a study on the phylogeny of the Portulacaceae, Hershkovitz and Zimmer (1999) tested whether the alignment decisions of ambiguous regions of ITS sequences would potentially bias the phylogenetic results of groups otherwise supported by unambiguously aligned regions alone. Soltis et al. (2001) described a way to test the reliability of the phylogenetic information within the Saxifragaceae from the ITS region through a series of alignments in which well-supported clades were found repeatedly despite the application of very unequal gap opening penalty costs. In fact, it is common that alignment procedures are presented very briefly and as being without any whatsoever problems and the following almost standardized phrase could easily be pasted into many systematic papers: "The sequences were aligned in ClustalX (Thompson et al., 1994) and subsequently adjusted manually in BioEdit 5.0.9 (Hall, 1999)." However, as sequence divergence increases with the taxonomic level of the study, alignment procedures of variable regions such as ITS might require extensive work before extracting a reliable phylogenetic signal because the great genetic distance between one or more groups might negatively affect the phylogenetic reconstruction. As Simmons and Freudenstein (2003) put it, great sequence divergence may cause errors in the homology hypotheses of the alignment as well as in the homology assessments of character states. Simmons and Freudenstein (2003) also suggest that increasing taxon sampling is a proper method to provide intermediate character states and decrease genetic distances between the groups, thus breaking up long branches that can affect both alignment and phylogenetic inference.

In this study, we utilize ITS sequence data, even though we are aware of its pitfalls, in combination with the widely used chloroplast marker *matK*. Apart from applying the aligning procedures suggested by the authors mentioned earlier, we describe a mode of alignment of the ITS region that was previously applied by Fior et al. (2003) for taxa within the Ericales. We emphasize the importance of including unlinked sequence regions in order to disentangle the evolutionary history of a group that is not old enough to be phylogenetically analyzed properly by a single cellular component (i.e., the plastid).

As a first attempt to place the subfamilial relationships within the Caryophyllaceae in a phylogenetic context, Smissen et al. (2002) included 15 genera, each represented by a single species, in a study based on the plastid region *ndhF*. According to the results, the current classification is inadequate, and neither the subfamilies nor the tribes seem to be monophyletic. However, as the authors point out, the restricted taxon sampling and the inference from a single chloroplast DNA region can be insufficient to provide resolved and robust phylogenies for large groups, such as the Caryophyllaceae.

In this study, we investigate the phylogeny of the Caryophyllaceae further by widening the taxon sampling and by using data from the chloroplast (*matK*) and nuclear (ITS) regions. We aim to give a new insight into the evolution of the family and possibly shed some light on the complex patterns of morphological variation. Other aims are to identify major monophyletic groups and test the traditional classification of the Caryophyllaceae (Bittrich, 1993), as well as to investigate the limit against the Molluginaceae.

MATERIALS AND METHODS

Terminal taxa were chosen to cover the most recent classification of the Caryophyllaceae in Bittrich (1993), and a significant number of representatives of the three subfamilies and all but two monogeneric tribes (i.e., Geocarpeae and Habrosieae: subfamily Alsinoideae) were hence included as listed in Table 1. Comparatively, many species were selected from the suspected non-monophyletic genera (e.g., *Minuartia*, *Arenaria*) in order to cover their morphological diversity and for testing the nature of these taxa. Infrageneric nomenclature in these genera follows McNeill (1962). Plant species included in this study, their classification, and voucher details are reported in the Appendix. Previous authors have used Molluginaceae as an outgroup in their phylogenetic analyses of the Caryophyllaceae (Smissen et al., 2002). However, we included terminals from the Molluginaceae in the ingroup in order to examine the uncertain affinities with the genera *Corrigiola* and *Telephium*, and representatives of the Amaranthaceae were used as outgroups in accordance with recent results inferred from different DNA regions (Downie et al., 1997; Soltis et al., 2000; Cuénod et al., 2002).

Total DNA was extracted from fresh, silica-gel-dried or herbarium material. Leaves were ground using a Bead Beater (Biospec Products, Techtum Lab, Umeå, Sweden) and subsequently treated with the DNEasy Plant DNA Extraction kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol.

The plastid region *matK* was amplified using primers 390F (5′-CGATCTATTCATTCAATATTTC-3′) and 1440R (5′-GTGTTTACGAG CYAAAGTTC-3′) by polymerase chain reaction (PCR, 26 cycles, 1-min denaturation at 94°C, 30-s annealing at 48°C, 1-min extension at 72°C, 8-min final extension), which amplify ~940 base pairs between position 429 and 1327 of the *matK* sequence of Schmitz-Linneweber et al. (2001) for *Spinacia oleracea* L. The nuclear regions ITS1 and ITS2 were separately amplified by PCR using the pairs of universal primers ITS-1F (5′-TCCGTAGGT GAACCTGCGGAAGGATCATTG-3′) with ITS-2R (5′-GCTACGTTCTT CATCGATGC-3′) and ITS-3F (5′-GCATCGATGAAGAACGTAGC-3′) with ITS-4R (5′-TCCTCCGCTTATTGATATGC-3′), respectively (35 cycles, 30-s denaturation at 95°C, 30-s annealing at 50°C, 1-min 30-s extension at 72°C, 8-min final extension). All PCRs contained 10 μmol/L primers in 25-μL reactions by using Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Uppsala, Sweden) and following the manufacturer's standard protocol. For cases in which the amplifications were weak, a second round of PCR was performed maintaining the thermal cycling profile of the first but decreasing the number of cycles depending on the amount of successfully amplified products. This was empirically estimated by eye after electrophoresis of the fragments marked with 1-μL ethidium-bromide in a 1% agarose gel. This precaution was of primary importance for ITS, where the lower number of cycles were combined with the raising of the annealing temperature to 54°C to avoid the formation of nonspecific amplification products. Amplification products were then purified using QIAquick PCR purification kit (Qiagen).

TABLE 1. Number of genera per family in Caryophyllaceae (from Bittrich, 1993) and number of genera sampled in this study.

Subfamily	Tribe	Total genera	No. of genera sampled
Paronychioideae	Polycarpeae	16	6
	Paronychieae	15	7
	Corrigioleae	2	2
Alsinoideae	Alsineae	23	9
	Pycnophylleae	1	1
	Sclerantheae	2	1
	Geocarpeae	1	0
	Habrosieae	1	0
Caryophylloideae	Caryophylleae	17	7
	Drypideae	1	1
	Sileneae	6	4

Cycle sequencing (25 cycles, 10-s denaturation at 96°C, 5-s annealing at 56°C, 4-min extension at 60°C) with dye terminators (BigDye Terminator v3.1 Cycle Sequencing kit from Applied Biosystems, Warrington, Cheshire, UK) was performed in 10-μL volumes, and the products were then purified by ethanol precipitation. The redissolved samples were run on an Applied Biosystems 3100 Genetic Analyzer automated DNA sequencer following the manufacturer's protocols.

Double readings were made for both genes. Sequences were assembled with the Staden Package (Staden et al., 1998). The *matK* sequences generally overlapped for ~70% of their overall length, whereas full overlapping was obtained for both ITS fragments. For *Arenaria musciformis* Triana & Planch., *A. fridericae* Hand.-Mzz., and *A. pogonantha* W.W. Sm., no sequences for ITS2 were obtained.

To expand the taxonomic diversity covered in the study, a number of sequences available from GenBank were downloaded and added to those obtained in the laboratory. Some of these nuclear sequences (for *Drymaria laxiflora* Benth., *Spergularia marina* (L.) Griseb., *Arenaria benthamii* Fenzl ex Torr. & Gray and *Pycnophyllum bryoides* (Phil.) Rohrb.) only comprised data from ITS2. Sequences for outgroup taxa *Amaranthus* and *Chenopodium* were used in the combined analysis regardless of the fact that they come from different species. Hence, the species names are missing in the combined tree.

Alignments were made with ClustalX (Thompson et al., 1997). Alignment of *matK* was straightforward (Appendix S1, see Supplemental Data accompanying online version of this article), whereas ITS was more problematic. As mentioned, the use of noncoding sequences in phylogenetic analyses should be used very cautiously at familial taxonomic levels. The identification of homologous sites to use as informative characters can indeed be problematic and should not be underestimated (e.g., Simmons and Freudenstein, 2003). As mentioned, we adopted three approaches in order to explore the ITS data: one used by Fior et al. (2003), which is very similar to the procedures applied by Soltis et al. (2001), one used by Hershkovitz and Zimmer (1999), and one used by Simmons and Freudenstein (2003). Hence, we tested the tree topologies that could result from different alignments implemented by the variation of the parameters in ClustalX. A large number of trials were performed in which different pairwise and multiple alignment parameters were applied in a series of analyses to test ~50 possible combinations. The gap-opening and -extension penalty costs were reset in every trial for either parameters to increasing values, and all final alignments run in PAUP* version 4.0b10 (Swofford, 2003) in 1000-replicate bootstrap analyses. By comparing the results, we concluded that the basic topology of the tree was conserved in all alignments, with the well-supported groups always having consistently high bootstrap values. What could differ to some extent in some trials was the resolution of the poorly supported branches at the base of the tree, which could garner just enough support to appear in the 50% majority-rule consensus tree. Adopting a rather conservative approach, we concluded that collapsing all branches that received bootstrap support lower than 70% would produce a consensus tree where, most importantly, bootstrap values were also compatible among all alignments. In conclusion, the final alignment used in the analyses presented here was the one produced with the standard settings for ClustalX (Appendix S2, see Supplemental Data accompanying online version of this article).

In a second step, we explored the ITS sequences applying the procedures suggested by Hershkovitz and Zimmer (1999) in their study of the Portulacaceae. Hence, ambiguously aligned regions were deleted and the alignment reexamined in order to test how alignment decisions would affect the phylogenetic results. In a similar way to the Portulacaceae, ITS2 is reasonably well conserved within the Caryophyllaceae, and the emphasis was therefore put

into the G-C rich hypervariable region between the two ITS1 motifs broadly conserved in angiosperms (Hershkovitz et al., 1999).

Ultimately, we adopted the Simmons and Freudenstein (2001) approach to test homology assessments in the alignments, and hard-to-align sequences were excluded from the data set. The subset of terminals was then re-analyzed using both the same alignment prior to deletion of problematic taxa and after re-alignment. The resulting trees were then compared to the tree constructed using all terminals.

Both using the Hershkovitz and Zimmer's (1999) and the Simmons and Freudenstein's (2001) approach, the basic topology of the tree was retained in all trials, and altering the alignment only affected the resolution of inadequately supported nodes. Ambiguously aligned regions and "hard-to-align" terminals obviously have a negligible effect on the tree topology, and consequently they were kept in the data matrix.

In conclusion, applying widely different settings in ClustalX as well as inclusion/deletion of equivocal sites and problematic terminals had little or no effect on well-supported parts of our ITS topology. So, we maintain that our ITS data in fact contains a fully traceable phylogenetic signal.

The final *matK* data set comprises 60 terminals and 734 characters, 390 of which are informative, and the final ITS data set comprises 72 terminals and 606 characters, 397 of which are informative. The combined data set includes terminals for which both gene sequences were available. It comprises 46 terminals and 1303 characters, 736 of which are informative. The data sets were analyzed separately and in combination.

Phylogenetic analyses were performed using parsimony (PA) and Bayesian inference (BI) methods.

Parsimony analyses were performed using PAUP* 4.0b10 (Swofford, 1999). Most parsimonious trees were obtained using 10 000 replicates of random addition sequence with equal weights and tree-bisection-reconnection (TBR) branch swapping. Using the *matK* matrix and the combined data set, only 100 trees were held at each replicate to complete the search in a reasonable time (overnight). Given the basal polytomy of the trees, these settings were preferred to enable the program to look for shortest trees in a greater number of tree spaces rather than spending time in swapping non-optimal tree lengths. Internal support was assessed using 10 000 bootstrap replicates with TBR swapping and random addition of taxa, with a limit of 10 trees kept in each replicate. Gaps were treated as missing data, and the ingroup was not forced to be monophyletic.

Bayesian inference analyses were performed using MrBayes3.0b4 (Huelsenbeck and Ronquist, 2001). Evolutionary models were chosen with MrModeltest version 1.0b (Nylander, 2002) in combination with PAUP* 4.0b10 (Swofford, 2003). This program is a simplified version of Modeltest (Posada and Crandall, 1998) that tests only the models of evolution that are implemented both in PAUP* 4.0b10 and MrBayes version 3.0 (24 of the 56 models in Modeltest). The hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) both selected for *matK* the general time reversible (GTR) nucleotide substitution model (Lanave et al., 1984; Tavaré, 1986; Rodríguez et al., 1990) with a gamma distribution of substitution rates among sites (Γ). Initial substitution rates for *matK* were set to $r(a - c) = 1.4753$, $r(a - g) = 1.4919$, $r(a - t) = 0.2724$, $r(c - g) = 0.8117$, $r(c - t) = 1.9464$, $r(g - t) = 1.0000$, and a Γ shape parameter of 1.2000. Base frequencies were estimated. Settings for Bayesian inference analyses of ITS were selected by both hLRT and AIC in GTR + Γ with a proportion of invariant sites (I). Initial substitution rates for ITS were set to $r(a - c) = 1.1256$, $r(a - g) = 2.1621$, $r(a - t) = 2.2375$, $r(c - g) = 0.4135$, $r(c - t) = 3.4116$, $r(g - t) = 1.0000$, and a Γ shape parameter of 1.4232. Base frequencies were estimated. Moreover, because *matK* is a protein-coding gene, we performed an analysis allowing for different rates of codon site substitution and compared the results with the overall gamma distribution analysis. The combined data set was analyzed in two ways: (1) allowing for both different distribution rates for *matK* and ITS and (2) four separate gamma distributions for the three codons of *matK* plus the noncoding ITS. Bayesian inference was estimated running four simultaneous chains of which three were heated (Metropolis-coupled Markov chain Monte Carlo) for 1 000 000 generations, sampling one tree every 10th generation. Three separate BI analyses were performed to make sure that the Markov chain did not fail to find an optimal tree island. "Burn-in" was identified for each analysis by examining the output files. Trees from the first 10 000 to 20 000 generations were excluded from consensus tree calculations. Majority-rule consensus of trees sampled after the burn-in was calculated in PAUP*.

In the bootstrap analyses, only branches with a value $\geq$70% were retained, and in the Bayesian inference, the corresponding value was a posterior probability of $\geq$0.95.

RESULTS

In all three data sets, the trees resulting from parsimony (PA) and Bayesian (BI) analyses were largely congruent in their topologies. The three Bayesian inference analyses run for each data set always produced the same tree topology. The two models chosen for the *matK* data set resulted in consensus trees with identical topologies; the support values presented here are those derived from the GTR + Γ model with each codon position treated separately.

Combined analysis—Both PA and BI recognize a number of minor clades showing high support, but the trees differ significantly in the support that the two methods yield for some nodes (Fig. 1). More precisely, BI reveals a number of clades that find no support in the bootstrap analysis. Representatives of Alsinoideae and Caryophylloideae form a monophyletic group (0.97 PP), where two major clades are resolved. One (group 1; 0.98 PP) includes current members of the Alsinoideae tribe Alsineae except *Arenaria* subg. *Eremogone* and *Minuartia* subg. *Spergella* (100% BP, 1.00 PP), which group in the second clade. This (group 2; 0.96 PP) is composed of a basal trichotomy comprising the aforementioned alsinoid members along with the two tribes of subfamily Caryophylloideae, viz., Sileneae and Caryophylleae, both receiving full support by bootstrap and BI (100% BP, 1.00 PP). The third tribe of the Caryophylloideae, Drypideae, is monogeneric and is resolved by BI among representatives of subfamily Alsinoideae in Group1 (1.00 PP). Within this group, high posterior probability is received by two main lineages, one (0.97 PP) resolving *Cerastium*, *Stellaria*, *Holosteum*, and *Arenaria* subg. *Odontostemma* in a clade (group B; 100% BP, 1.00 PP) sister to *Arenaria* subg. *Arenaria*, *Arenaria* subg. *Leiosperma* and *Moehringia* (group C; 100% BP, 1.00 PP), and the other including *Sagina*, *Bufonia*, *Minuartia*, and *Drypis* (group D; 1.00 PP). This result suggests that some of the largest genera in the family, such as *Arenaria*, *Moehringia*, and *Minuartia*, do not constitute monophyletic groups.

At the base of the tree, subfamily Paronychioideae is not monophyletic, and a number of minor clades are recognized, which jointly form a grade. *Corrigiola* (tribe Corrigioleae) is placed by both PA and BI as sister to the rest of the family (99% BP, 1.00 PP), and it does not group with *Mollugo*. The next branch in the grade comprises members belonging to tribes Polycarpeae (100% BP, 1.00 PP) and Paronychieae (76% BP, 1.00 PP), forming a clade (group E; 0.95 PP). *Spergularia* (tribe Polycarpeae) is located in the clade including Caryophylloideae and Alsinoideae (100% BP, 1.00 PP), and further resolution is suggested by BI that supports a sister relationship to the remainder of the group (0.97 PP).

matK data set—The tree (Fig. 2) is fully compatible with the combined analyses, but the overall resolution is slightly lower. Within the Alsinoideae + Caryophylloideae clade (100% BP, 1.00 PP), *matK* brings Bayesian support to group 2 (0.95 PP), with internal resolution consistent with the combined analysis. Tribe Sileneae (76% BP, 1.00 PP), Caryophylleae (100% BP, 1.00 PP) and the alsinoid group A (100% BP, 1.00 BP) are recovered by both analytical methods. Group 1 remains collapsed in a basal polytomy composed of major alsinoid clades supported only by BI, more precisely group B (100% BP, 1.00 PP), C (100% BP, 1.00 PP), and D (1.00 PP), with groups B and C forming a clade (0.99 PP). Another branch

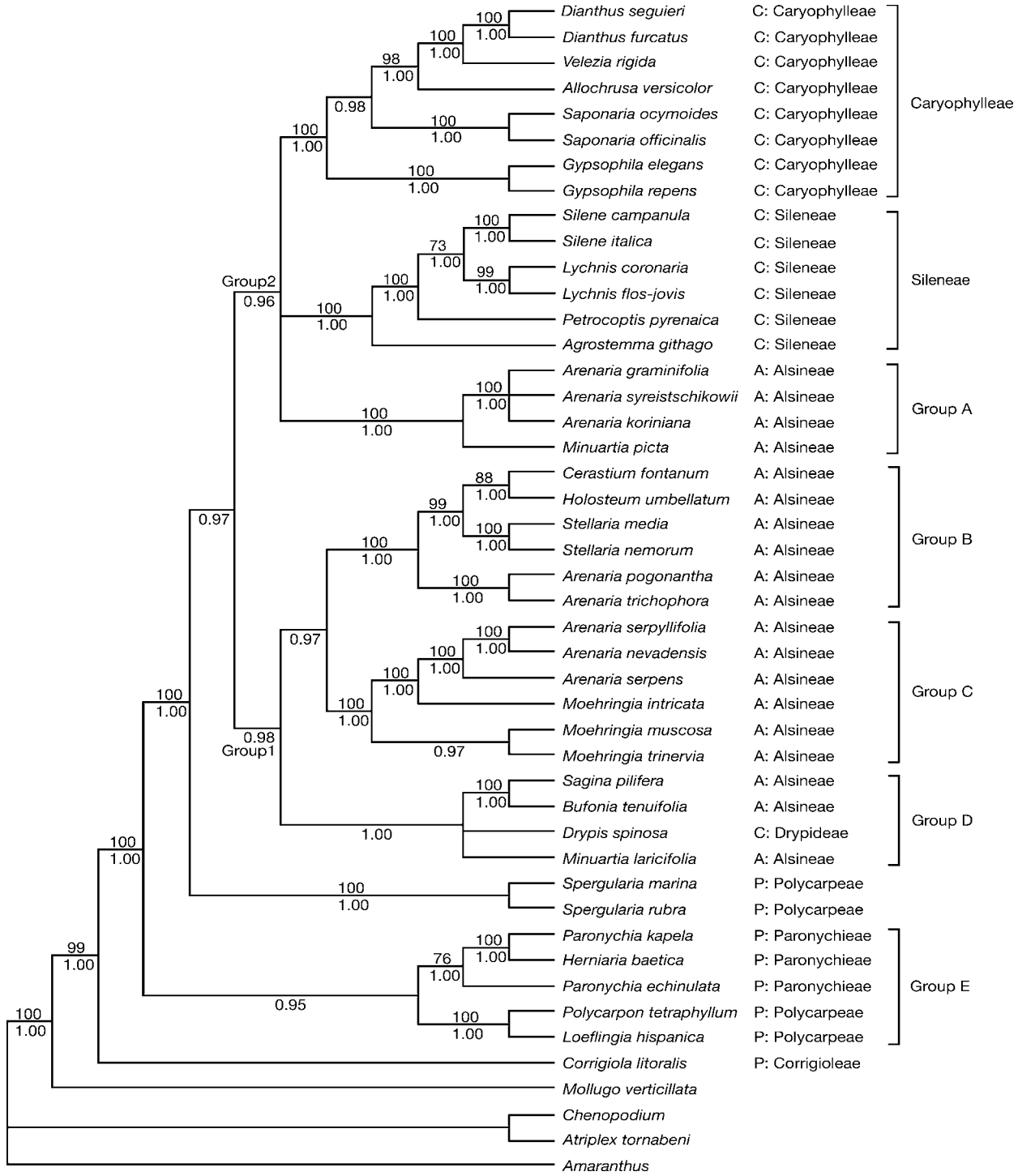

Fig. 1. Parsimony strict-consensus tree based on simultaneous analysis of ITS and *matK* data. Bootstrap and posterior probability values are marked above and below branches, respectively. Branches are retained for BP $\geq$ 70% and PP $\geq$ 0.95. Groups 1, 2, A, B, C, D, E, Caryophylleae, and Sileneae are discussed in the text. Classification of genera (following Bittrich, 1993) is indicated by an abbreviation for subfamily (P = Paronychioideae, A = Alsinoideae, C = Caryophylloideae) to the right of genus names followed by the tribe name.

includes *Spergularia* associated with *Spergula* (100% BP, 1.00 PP) and *Minuartia* subg. *Rhodalsine* (1.00 PP). Other representatives of subfamily Paronychioideae constitute a polytomy at the base of the tree, whereas the two genera of tribe Corrigioleae (i.e., *Corrigiola* and *Telephium*; 0.98 PP) are sister to the rest of the family (91% BP, 1.00 PP). As in the combined tree, tribe Corrigioleae do not group with *Mollugo*.

ITS data set—The large Alsinoideae + Caryophylloideae clade is supported only by the bootstrap analysis (79% BP.,

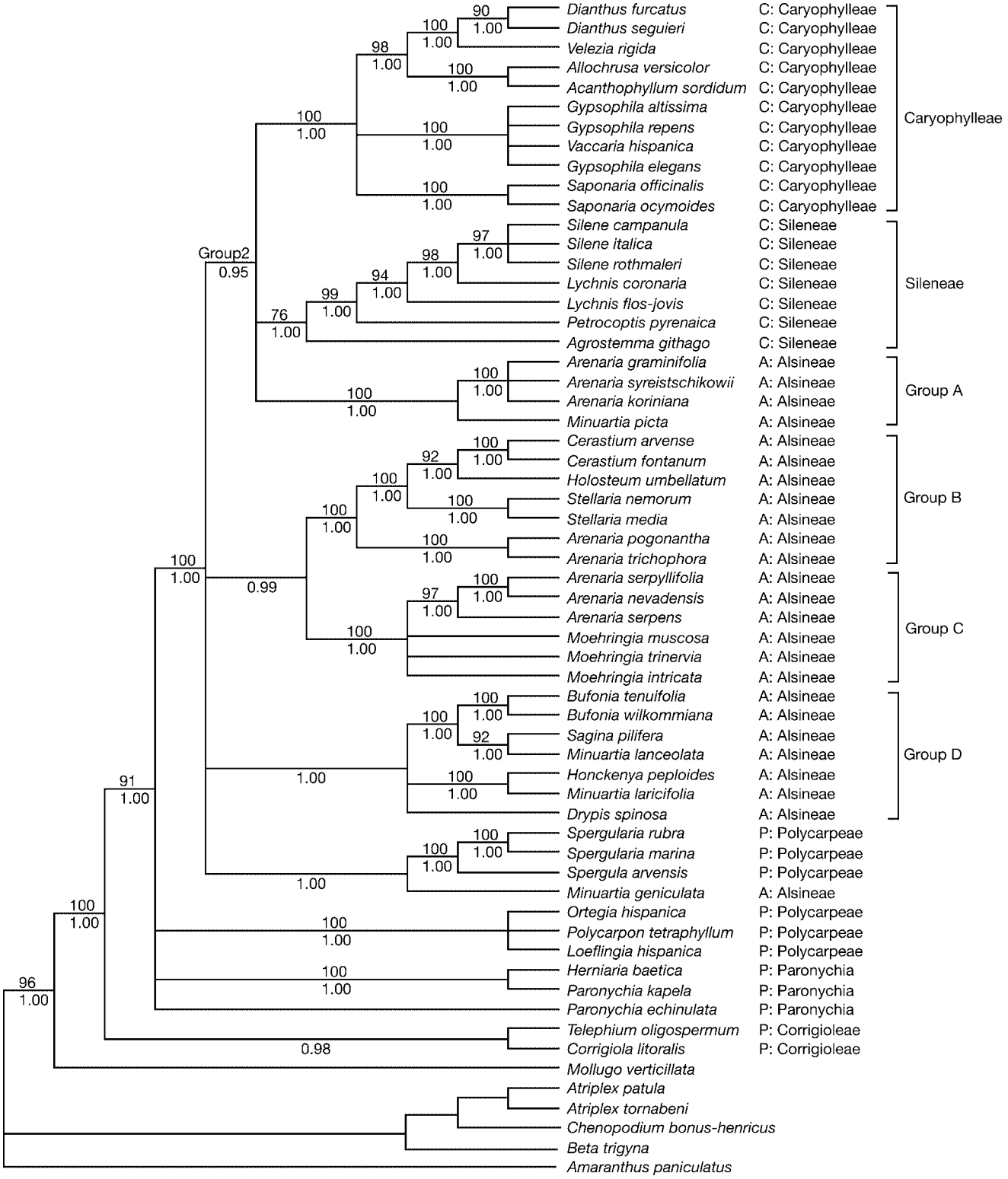

Fig. 2. Parsimony strict-consensus tree based on *matK* data. Bootstrap and posterior probability values are marked above and below branches, respectively. Branches are retained for BP $\geq$ 70% and PP $\geq$ 0.95. Groups 2, A, B, C, D, Caryophylleae, and Sileneae are discussed in the text. Classification of genera (following Bittrich, 1993) is indicated by an abbreviation for subfamily (P = Paronychioideae, A = Alsinoideae, C = Caryophylloideae) to the right of genus names followed by the tribe name.

Fig. 3). Within this, Bayesian analysis supports group 1 (0.97PP), but the internal resolution of this clade remains scarce. Groups B (100% BP, 1.00 PP) and C (99% BP, 1.00 PP) are still recognized, together with a number of minor clades among which figures *Scleranthus*, absent from the other data sets. Group 2 is not recognized as a monophylum by the nuclear genome, but tribe Sileneae (84% BP, 0.99 PP), tribe Caryophylleae (97% BP, 1.00 PP), and group A (100% BP, 1.00 PP) are recovered. Representatives of subfamily Paronychioideae are left in a broad polytomy at the base of the tree. Because of the larger number of paronychioid taxa present in the nuclear data set, new relationships within the subfamily are seen. A low bootstrap value is received by a clade including some members of tribe Paronychieae (73% BP) where the nature of large genera such as *Herniaria* and *Paronychia* is called into question by their polyphyly. The other representatives of tribe Paronychieae (*Pteranthus*, *Dicheranthus*, *Sphaerochoma*) form a group with *Polycarpon* and *Loeflingia* belonging to tribe Polycarpeae (75% BP). *Spergularia* is collapsed at the basal node of the tree, along with *Corrigiola* (Corrigioleae) and the unexpected grouping of *Drymaria* (Polycarpeae) with the alsinoid *Pycnophyllum* (79% BP, 0.98 PP). *Mollugo* is also unresolved.

DISCUSSION

In this study, the use of ITS sequence data at a deeper taxonomic level than its "near-universal usage" (Álvarez and Wendel, 2003) at the species- and genus-level has revealed a great potential to resolve phylogenies within the Caryophyllaceae. Like previous authors (Hershkovitz and Zimmer, 1999; Soltis et al., 2001) who have attempted to reconstruct intrafamiliar phylogenetic relationships from this region, we maintain that ITS sequences contain cladistically valuable information. Though extensive work is certainly required to explore the reliability of the phylogenetic signal and its stability in dependence of different alignment decisions, we concur with Hershkovitz and Zimmer (1999), Soltis et al. (2001), and Simmons and Freudenstein (2001) that well-supported groups are not affected by altering the alignment or discarding ambiguously aligned sites. Highly variable molecules like the ITS region are likely to accumulate a great amount of mutations that can potentially record the sequential divergence of the lineages in an extensive taxon sampling. However, this will also increase the level of homoplasious changes, thus the data contain more "noise" compared to sequences from more conserved protein-coding regions. The search strategies employed in this study have revealed a strong phylogenetic signal retained by the ITS sequences despite the noise that inevitably accompanies rapidly evolving sequences. Furthermore, inclusion/deletion of ambiguously aligned sites and "hard-to-align" terminals have little or no effect on well-supported parts of our ITS topology. The high consistency of the ITS tree topology to that of the more conserved plastid region *matK* is further evidence that some shared phylogenetic information has been captured, and the general agreement of the two phylogenies induced us to combine all available data. As a result, we obtained a more resolved tree with an increase in support values on the basal nodes of the tree. We regard this topology as a more comprehensive phylogenetic hypothesis where the information from the nuclear and chloroplast sequence data jointly provide a strong phylogenetic signal.

The results of the analyses give support for the recognition of a number of clades in the Caryophyllaceae. The separate data sets produced less resolved trees (Figs. 2 and 3) than the one obtained from the combined analysis, and, if not specified otherwise, the discussion will focus on this topology (Fig. 1). The two methods used in the study to assess phylogeny (i.e., parsimony analysis and Bayesian inference) are consistent in supporting many of the nodes of the tree, except those leading to the Paronychioideae (except *Corrigiola* and *Spergularia*), Groups 1 and 2 as well as the node immediately below, where bootstrap values are missing.

The phylogeny emerging from this study reflects the inadequacy of the current classification of the Caryophyllaceae, on all levels. As suggested by previous investigations (Nepokroeff et al., 2001; Smissen et al., 2002), the morphological characters used with taxonomic implications within the family seem to be subject to a high degree of homoplasy, but this is hardly surprising in such a species-rich group. This is due to the apparent convergent evolution or reversal of key morphological characters in isolated species, small groups of species, or larger parts of the family. Optimization of these characters on the molecular topology therefore reflects an overall pattern of evolution where repeated reversals and parallelisms occur at various taxonomic levels (Fig. 4). The role of DNA-based trees is crucial for a proper assessment of homoplasious morphological characters in the Caryophyllaceae.

None of the three subfamilies as circumscribed here appear monophyletic, but it should be pointed out that subfamily Caryophylloideae is not contradicted either (provided *Drypis* is excluded), leaving for future investigations to scrutinize the relationships between this subfamily and group A including *Arenaria* subg. *Eremogone* and *Minuartia* subg. *Spergella*.

Species representing subfamily Paronychioideae form a basal grade, with *Corrigiola* sister to the rest of the family in our combined analysis (99% BP, 1.00 PP). *Corrigiola* and its close ally *Telephium* are resolved in a clade by BI in the *matK* data set (0.98 PP), suggesting monophyly of the digeneric tribe Corrigioleae as outer sister of the Caryophyllaceae. This topology is consistent with previous molecular investigations based on plastid *trnL* and *matK* (Nepokroeff et al., 2002) and a combination of nuclear 18S and plastid *rbcL*, *atpB*, and *matK* (Cuénod et al., 2002) DNA sequences, which contradicted the hypothesis of closer affinities between Corrigioleae and Molluginaceae as occasionally proposed in the literature (Gilbert, 1987). The placement of Corrigioleae in the Caryophyllaceae is also favored by the common type of sieve-element plastids (Behnke, 1976, 1993), which thus assumes relevant taxonomic importance as a synapomorphic character for the family.

As to the remainder of the Paronychioideae, it is difficult to propose a sound reclassification, but one striking result is undoubtedly the full support yielded by both analytical methods to the inclusion of *Spergularia* (currently belonging to tribe Polycarpeae) in the large clade formed by Alsinoideae + Caryophylloideae (100% BP, 1.00 PP). This result is consistent with previous investigations inferred from plastid *ndhF* DNA sequences analyzed both with distance and parsimony methods (Smissen et al., 2002); but a novel resolution within the clade is here given by the BI that places *Spergularia* as sister to the remainder of the group (0.97 PP). *Spergularia* shows some alsinoid characters such as capsular fruit and free styles that have always made its position dubious (Bittrich, 1993; Smissen et al., 2002). In fact, connate styles are

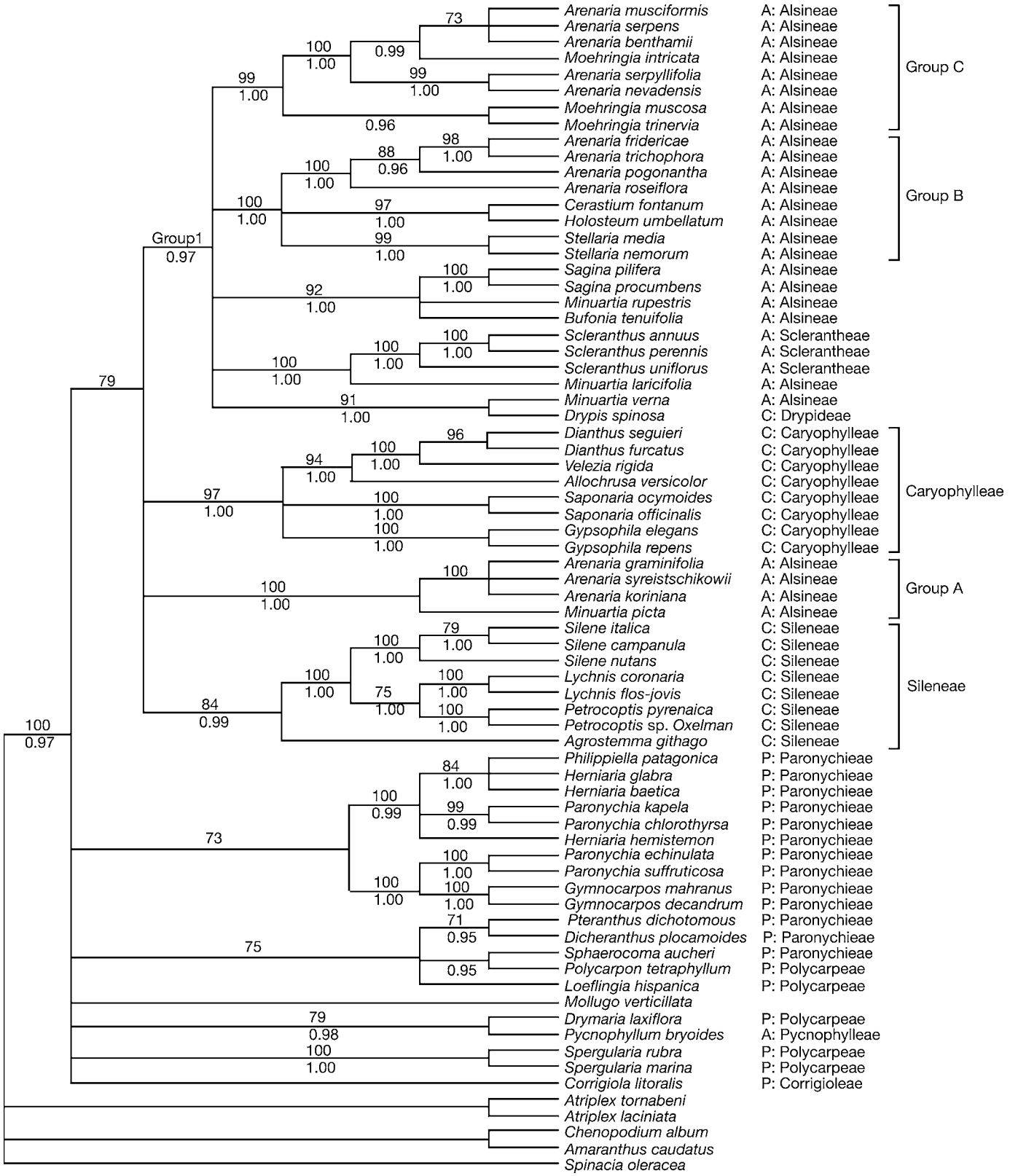

Fig. 3. Parsimony strict-consensus tree based on ITS data. Bootstrap and posterior probability values are marked above and below branches, respectively. Branches are retained for BP $\geq$ 70% and PP $\geq$ 0.95. Groups 1, A, B, C, Caryophylleae, and Sileneae are discussed in the text. Classification of genera (following Bittrich, 1993) is indicated by an abbreviation for subfamily (P = Paronychioideae, A = Alsinoideae, C = Caryophylloideae) to the right of genus names followed by the tribe name.

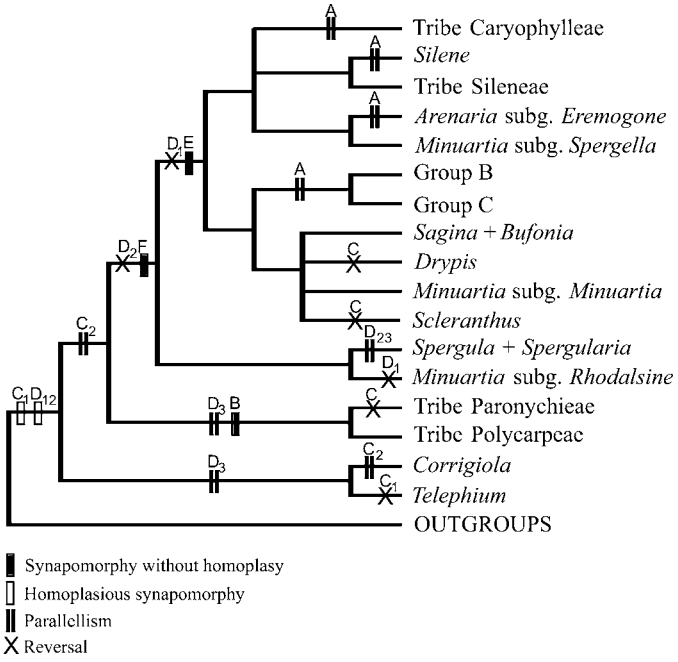

Fig. 4. Optimizations of the morphological characters discussed in the text. Characters are optimized on the topology from the combined tree but with the addition of critical taxa missing in the combined analyses (viz. *Scleranthus*, *Telephium*, *Spergula*, and *Minuartia* subg. *Rhodalsine*). These were placed according to their position in either separate analyses. *Mollugo* is excluded. A = bifid capsular valves; B = connate styles; C = dehiscent fruit, where C_1 and C_2 represent ACCTRAN and DELTRAN optimizations, respectively; D = stipulate leaves, where D_1 and D_2 represent ACCTRAN and D_3 DELTRAN optimizations; E = Caryophyllad-type of embryogeny; F = stamen number twice the number of sepals.

prevalent in the Paronychioideae, with the only exceptions in *Spergularia* and *Spergula* (sister to *Spergularia* in the *matK* tree) and *Sanctambrosia* (Pedersen, 1984; Bittrich, 1993), as well as in some isolated cases in *Paronychia*. Styles are connate in Amaranthaceae, but free in Corrigioleae and in the basalmost lineages of the core Caryophyllales. In the rest of the Caryophyllaceae, styles are free in Caryophylloideae and Alsinoideae. Hence, provided that the doubtful group E (0.95 PP) is confirmed to be monophyletic, the optimization of the style on the topology of the combined tree suggests the free styles as plesiomorphic, whereas the connate styles may be interpreted as apomorphic, diagnosing a major part of the Paronychioideae (Fig. 4). Furthermore, the "anomalous" genus *Pycnophyllum* with connate styles is currently placed in the Alsinoideae, but in the monogeneric tribe Pycnophylleae. Bittrich (1993) justifies this tribal assignment mainly because styles otherwise are free in the Alsinoideae. In our study, however, where *Pycnophyllum* is present only in the ITS tree, it is placed in a clade with *Drymaria* (79% BP, 0.98 PP), which is currently included in the Polycarpeae, with connate styles prevailing. Further resolution of the Paronychioideae is needed to pinpoint the stylar evolution in the Caryophyllaceae, but again, we suggest that connate styles could be regarded as a possible synapomorphy for a conspicuous number of taxa.

Indehiscent fruits are very common in tribe Paronychieae (here as part of group E), as well as a number of genera usually included in the Alsinoideae and Caryophylloideae (e.g., *Scleranthus*, *Habrosia*, *Drypis*). If credit is given to the poorly supported group E, Paronychieae and Polycarpeae may still form a monophyletic group, though both tribes are paraphyletic. Optimizing the character on this topology, it seems appropriate to accept Bittrich's hypothesis (1993) of the capsule as plesiomorphic for the Caryophyllaceae (based on Rohweder, 1970), followed by parallel events of evolution towards indehiscence and reduced ovule number in different lineages (Fig. 4). However, because of the small number of representatives in the combined dataset, the monophyly of group E may represent an artefact caused by insufficient sampling.

The delimitation of large genera such as *Paronychia* and *Herniaria*, as currently described, seems artificial according to our results. Strong indication is given by nuclear ITS sequences of the complex relationships between the two genera, whose representatives are distributed in different branches within a monophyletic group, but this is supported only by PA (73% BP). The two genera are indeed difficult to discern and the current taxonomy is based mainly on minor differences in bract morphology. Another indication of the inadequate delimitation within the "*Herniaria-Paronychia* complex" is the position of the Patagonian endemic *Philippiella* among the representatives of this complex, and with which it shares little of the morphology and certainly not the distribution.

Another unexpected result, albeit based on a partial sequence of the nuclear data alone, is the close relationship between *Drymaria* and *Pycnophyllum*, as was also shown by Smissen et al. (2003). Both genera are morphologically aberrant and Bittrich (1993) calls into question the reliability of their position in the family. As discussed, *Pycnophyllum* has connate styles instead of the alsinoid free condition, as well as paronychioid-like indehiscent fruit and inconspicuous petals (sometimes referred to as staminodes) and lacks the nectary glands at the abaxial base of the episepalous stamens, characters that are suspiciously atypical within the Alsinoideae. The current position of *Pycnophyllum* in the Alsinoideae relies mainly on its estipulate leaves, but Smissen et al. (2002) already proposed its exclusion from the group on the basis of the particular morphology of the genus. On the other hand, *Drymaria* shows some clear alsinoid characters such as the dehiscent fruit and deeply bifid petals with complex venation, which made Bittrich (1993) include the genus in the Paronychioideae but with hesitation. Moreover, according to Bittrich (1993) the stipule ontogeny of *Drymaria* differs from that of other Paronychioideae, raising doubts on the homology of the structures. The same observation concerns the auriculate stipules of the outer sister of the family, *Corrigiola*. At best, stipules should be regarded as plesiomorphic, and their mere presence is certainly insufficient for elucidating the phylogeny of the Paronychioideae, a subfamily that should be recircumscribed and possibly dismantled.

According to our results, it is difficult to interpret the presence/absence of stipules in *Spergula* and *Spergularia*, because their evolution in the family can involve different equally parsimonious scenarios (Fig. 4), and the Solanad-type of embryogeny in these two genera should simply be regarded as symplesiomorphic with the rest of the Paronychioideae. On the other hand, one putative synapomorphy for the *Spergularia*-Alsinoideae-Caryophylloideae clade includes the stamen number being twice the number of sepals (usually diplostemonous, but obdiplostemonous in *Spergula* due to ontogenetic displacement; Bittrich, 1993), whereas the clade Alsinoideae-Caryophylloideae in turn might be diagnosed by the Caryophyllad-type of embryogeny (e.g., Bittrich, 1993; Fig.

4). Yet other likely synapomorphies for this clade are the connate leaves and basic chromosome numbers (Bittrich, 1993). However, all these characters are subject to homoplasy, which again, is not surprising when considering the large number of species involved.

Among alsinoid members resolved in group 1 (0.98 PP), BI recognizes two major clades. The first (group D; 1.00 PP) includes *Sagina*, *Minuartia laricifolia* (L.) Schinz & Thell., *Bufonia*, *Drypis*, but supposedly also *Honckenya* (only in the *matK* data set), and *Scleranthus* (only in the ITS data set). According to Bittrich's classification (1993), the Mediterranean *Drypis* currently belongs to the Caryophylloideae, though placed in a monogeneric tribe Drypideae on account of its unclear relationship and several aberrant characters (e.g., zygomorphic flowers, semifused styles and indehiscent fruit). Although the placement of *Drypis* outside the Caryophylloideae is only supported by the BI, this is in accordance with previous results (Oxelman and Lidén, 1995), and the possibility of parallel gains of certain caryophylloid features, such as the calyx tube, clawed petals, and corolla scales should not be ruled out.

The second large clade within group 1 includes the sister relationship between what can possibly be identified with the historical aggregations referred to as the *Stellaria-Cerastium* group (including *Holosteum*, group B; 100% BP, 1.00 PP) and part of the *Arenaria* complex (group C; 100% BP, 1.00 PP; McNeill, 1962). *Minuartia* is doubtlessly a genus among the hardest to diagnose within the entire family (McNeill, 1962), and some authors have suggested it should be split into several genera (Gay, 1845; Williams, 1898; Löve and Löve, 1974). *Minuartia* was monographed by Mattfeld (1921, 1922), who proposed a complex classification still in use today (Bittrich, 1993). No wider conclusions can be drawn on the fate of *Minuartia* due to the insufficient number of representatives included in this study. Its supposed polyphyletic nature is only indicated by the different position acquired by all of the included terminals, which represent three of four acknowledged subgenera (Bittrich, 1993; Figs. 1, 2). This heterogeneity is fully consistent with the results by Nepokroeff et al. (2002), and McNeill's (1962) note that *Minuartia* subg. *Rhodalsine* is more similar to *Spergularia* than any group of *Minuartia* is supported by our results (*matK*, 1.00 PP). A number of morphological characters, such as whorled leaves, pink subperigynous flowers, accumbent cotyledons, and tricolpate pollen, coincide with this grouping (McNeill, 1962). It is more difficult to find grounds to justify the grouping of *Minuartia* subg. *Spergella* with representatives of *Arenaria* subg. *Eremogone* in the fully supported group A (100% BP, 1.00 PP), where the only synapomorphic character could be found in the accumbent cotyledons, a feature that has therefore evolved twice within the family.

Minuartia has always been an unquestioned part of the large *Arenaria* complex, along with 14 other smaller genera united by consistent floral similarities (McNeill, 1962). Mattfeld (1922) stressed the supposed constancy of the capsule dehiscence to be the only reliable character from which to infer relationships within this complex, and McNeill (1962) further suggested that the two types of capsule openings could represent separate lines of evolutionary development, viz. one leading to taxa with as many valves as carpels, and another leading to taxa with twice as many valves as carpels. An optimization of the valve number on our topology reveals that a valve number equal with the carpel number is plesiomorphic, but that a doubling occurs as four parallel gains, viz. for the Caryophylleae, for *Silene*, for *Arenaria* subg. *Eremogone*, and for clades B + C (Fig. 4). We point out, however, that this might be an oversimplified view on capsule morphology, especially when considering the notification by McNeill (1962) that underlines that some variation occurs in some taxa (e.g., *Arenaria* subg. *Eremogone*) in relation with the texture of the capsule at the time of dehiscence.

Another genus that has been notoriously difficult to circumscribe adequately is *Arenaria* itself (McNeill, 1962), and its morphological complexity alone indicates its supposed nonmonophyly. This is indeed verified by our results, where the *Arenaria* terminals are positioned in three different clades (Fig. 1) corresponding to subg. *Eremogone* (sister to Caryophylloideae, group 2; 0.96 PP), subg. *Odontostemma* (sister to *Stellaria-Cerastium*, Group B; 100% BP, 1.00 PP), and the subgenera *Arenaria* and *Leiosperma* (both nested within *Moehringia*, Group C; 100% BP, 1.00 PP), respectively. Although being anomalous within *Arenaria*, characters such as accumbent cotyledons, grass-like leaves, glomerate inflorescence, and basally lignified sepals (McNeill, 1962) are not of any guidance for inferring a close relationship between *Arenaria* subg. *Eremogone* and the Caryophylloideae. In this respect, Bittrich's hypothesis (1993) of the evolution of the Caryophylloideae from an "advanced" group of Alsinoideae appears very attractive, as the *Arenaria* subg. *Eremogone-Minuartia* subg. *Spergella* clade might be interpreted as the "diverging" alsinoid taxa that are more closely related to the Caryophylloideae than to the Alsinoideae, provided the grouping is corroborated in future studies. Bittrich (1993) bases this hypothesis mainly on the evolution of chromosome number, where an ascending series starting from the base number $x = 6$ in the Alsinoideae would lead to the Caryophylloideae ancestral number, which is assumed to be $x = 12$. Within *Arenaria* subg. *Eremogone*, virtually all species have $x = 11$ (Favarger, 1962; Hartman and Rabeler, 2004), but chromosome numbers within the Alsinoideae are the most variable in the Caryophyllaceae (Bittrich, 1993), and many more taxa are needed to depict any evolutionary lineage in this respect.

Similarly, it is difficult to pinpoint putative synapomorphies for *Arenaria* subg. *Odontostemma* and the *Stellaria-Cerastium* complex, if not the toothed or cleft petal apex in the former, when interpreted as homologous with the more properly bifid petals of the latter. Recognition of *Odontostemma* at generic level is supported by our results, and the truncate calyx, winged seeds, laterally folded cotyledons, and two carpels, that were originally put forward as grounds for this assignment (McNeill, 1962), constitute autapomorphies and parallelisms, which still diagnose only these *Arenaria* species in question.

Overall similarity probably rationalized the suggested close relationship between *Arenaria* subgenera *Arenaria-Leiosperma* and *Moehringia*, but McNeill (1962) discussed the intricate morphology of the seeds and especially the strophiole of *Moehringia*, suggesting this structure to be reduced in some stepwise manner in *M. intricata* R. de Roemer ex Willk., with further reduction in *Arenaria* subg. *Leiosperma* and *Arenaria* subg. *Arenaria* (*A. nevadensis* Boiss. & Reuter, *A. serpyllifolia* L.). Although the relationship between *Moehringia* and *Arenaria* should be examined cladistically with an enlarged sampling, as well as through detailed studies on seed morphology, the strophiole might be interpreted as a synapomorphy for this clade, but with subsequent modifications or more drastically, simply in terms of a reversal. As the type of

Arenaria, *A. serpyllifolia*, is nested within *Moehringia*, the taxonomy of this clade must also be amended.

Conclusions—The evidence presented here indicates that rDNA spacers may retain a stable phylogenetic signal and therefore may be used at higher taxonomic levels. This is in disagreement with the suggestion put forward by Álvarez and Wendel (2003), namely that the ITS should be abandoned as a source for phylogenetic information. We believe that it is wrong to state a priori that a particular region is obsolete for obtaining a useful phylogenetic signal.

Even though the monophyly of the subfamily Caryophylloideae is strongly indicated by a firm set of morphological synapomorphies, it is notable that this clade has not received unanimous support from molecular data, neither previously (Nepokroeff et al., 2001, 2002; Smissen et al., 2002) nor in this study, regardless of mode of analysis. However, the monophyly of the Caryophylloideae is not contradicted by this study. Our results show clearly that none of the three subfamilies within the Caryophyllaceae constitute clades as currently circumscribed, and major re-arrangements and amendments are required in order to arrive at a phylogenetic subfamilial and tribal classification. Paronychioideae form a grade at the base of the tree, with Corrigioleae as sister to the rest of the family. A close relationship between *Mollugo* and Corrigioleae is discredited. This study suggests that *Spergularia* and *Spergula* should be moved from the Paronychioideae to a new systematic position with the Alsinoideae and Caryophylloideae. Extensive work is certainly required for elucidating the phylogenetic relationships of clades currently assigned to the large, polyphyletic genera *Arenaria* and *Minuartia* as well as *Paronychia/Herniaria*, and our study indicates at least partly how to proceed with this task. Many morphological features may still diagnose a number of nodes, but none of the features are likely to be without homoplasy. Our study clearly shows molecular phylogenies to be of extreme value in understanding the evolutionary history of complex families such as the Caryophyllaceae.

LITERATURE CITED

ÁLVAREZ, I., AND J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

BEHNKE, H.-D. 1976. Die Siebelement-Plastiden der Caryophyllaceae, eine weitere speizifische Form der P-Typ-Plastiden bei Centrispermen. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 95: 327–333.

BEHNKE, H.-D. 1993. Further studies of the sieve-element plastids of the Caryophyllales including *Barbeuia*, *Corrigiola*, *Lyallia*, *Microtea*, *Sarcobatus*, and *Telephium*. *Plant Systematics and Evolution* 186: 231–243.

BITTRICH, V. 1993. Caryophyllaceae. *In* K. Kubitzki, J. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, Magnoliid, Hamamelid, and Caryophyllid families, 206–236. Springer Verlag, Berlin, Germany.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, USA.

CUÉNOD, P., V. SAVOLAINEN, L. W. CHATROU, M. POWELL, R. J. GRAYER, AND M. W. CHASE. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *American Journal of Botany* 89: 132–144.

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND K.-Y. CHO. 1997. Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF 2280 homolog sequences. *American Journal of Botany* 84: 253–273.

ECKARDT, T. H. 1964. Caryophyllaceae. *In* H. Melchior [ed.], Syllabus der Pflanzenfamilien, 12th ed., 93–96. Gebrüder Borntraeger, Berlin, Germany.

FAVARGER, C. 1962. Contribution a l'étude cytologique des genres *Minuartia* et *Arenaria*. *Bulletin de la Société neuchâteloise des Sciences Naturelles* 85: 53–81.

FERNANDES, A., AND M. T. LEITAO. 1971. Contribution à la connaissance cytotaxonomique des Spermatophyta du Portugal. Caryophyllaceae. *Boletim da Sociedade Broteriana* 45: 167–176.

FIOR, S., P. O. KARIS, AND A. A. ANDERBERG. 2003. Phylogeny, taxonomy, and systematic position of *Clethra* (Clethraceae, Ericales) with notes on biogeography: evidence from plastid and nuclear DNA sequences. *International Journal of Plant Sciences* 164: 997–1006.

GAY, J. 1845. Holostei Caryophyllearum Alsinearum generis, monographia. *Annales de la Société Royale des Sciences Medicales et Naturelles de Bruxelles, séries 3,* 4: 23–44.

GILBERT, M. G. 1987. The taxonomic position of the genera *Telephium* and *Corrigiola*. *Taxon* 36: 47–49.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41: 95–98.

HARTMAN, R. L., AND R. K. RABELER. 2004. New combinations in North American *Eremogone* (Caryophyllaceae). *Sida* 21: 237–247.

HERSHKOVITZ, M. A., AND E. A. ZIMMER. 1997. On the evolutionary origin of the cacti. *Taxon* 46: 217–232.

HERSHKOVITZ, M. A., AND E. A. ZIMMER. 1999. Ribosomal DNA evidence and disjunctions of western American Portulacaceae. *Molecular Phylogenetics and Evolution* 15: 419–439.

HERSHKOVITZ, M. A., W. J. HAHN, AND E. A. ZIMMER. 1999. Ribosomal DNA and angiosperm evolution. *In* P. M. Hollingsworth, R. M. Bateman, and R. Gornall [eds.], Molecular systematics and plant evolution, 268–326. Cambridge University Press, Cambridge, UK.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

HUTCHINSON, J. 1974. The families of flowering plants. Oxford University Press, Oxford, UK.

JUDD, W. S., C. S. CAMPBELL, E. A. KELLOGG, P. F. STEVENS, AND M. J. DONOGHUE. 2002. Plant systematics: a phylogenetic approach, 2nd ed. Sinauer, Sunderland, Massachusetts, USA.

JUDD, W. S., AND R. G. OLMSTEAD. 2004. A survey of tricolpate (eudicot) phylogenetic relationship. *American Journal of Botany* 91: 1627–1644.

KIM, K.-J., AND R. K. JANSEN. 1995. *ndhF* sequence evolution and the major clades in the sunflower family. *Proceedings of the National Academy of Sciences, USA* 92: 10379–10383.

KRON, K. A., W. S. JUDD, P. F. STEVENS, D. M. CRAYN, A. A. ANDERBERG, P. A. GADEK, C. J. QUINN, AND J. L. LUTEYN. 2002. Phylogenetic classification of Ericaceae: molecular and morphological evidence. *Botanical Review* 68: 335–423.

KURTTO, A. 2001. Caryophyllaceae. *In* B. Jonsell [ed.], Flora nordica, vol. 2, Chenopodiaceae to Fumariaceae, 83–84. Bergius Foundation, Royal Academy of Sciences, Stockholm, Sweden.

LANAVE, C., G. PREPARATA, C. SACCONE, AND G. SERIO. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* 20: 86–93.

LÖVE, A., AND D. LÖVE. 1974. Nomenclatural adjustments in the Yugoslavian flora. II. Pteridophytes and dicotyledons. *Preslia* 46: 123–138.

MATTFELD, J. 1921. Enumeratio specierum generis *Minuartia* (L.) emend. Hiern. *Botanische Jahrbucher 57, Beiblätter* 126: 27–33.

MATTFELD, J. 1922. Geographisch-genetische Untersuchungen über die Gattung *Minuartia* (L.) Hiern. *Feddes Repertorium Specierum Novarum Regni Vegetabilis*. Beiheft 15.

MCNEILL, J. 1962. Taxonomic studies on the Alsinoideae. I. Generic and infra-generic groups. *Notes from the Royal Botanic Garden Edinburgh* 24: 79–155.

MEIMBERG, H., P. DITTRICH, G. BRINGMANN, J. SCHLAUER, AND G. HEUBL.

2000. Molecular phylogeny of Caryophyllidae s.l. based on *matK* sequences with special emphasis on carnivorous taxa. *Plant Biology* 2: 121–252.

NEPOKROEFF, M., W. L. WAGNER, E. A. ZIMMER, S. G. WELLER, A. K. SAKAI, AND R. K. RABELER. 2001. Origin of the Hawaiian subfam. Alsinoideae and preliminary relationships in Caryophyllaceae inferred from *matK* and *trnL C-F* sequence data. Botany 2001: The annual meeting of the Botanical Society of America, in Albuquerque, New Mexico, website http://www.botany2001.org/section12/abstracts/196.shtml (Abstract).

NEPOKROEFF, M., W. L. WAGNER, R. K. RABELER, E. A. ZIMMER, S. G. WELLER, AND A. K. SAKAI. 2002. Relationships within Caryophyllaceae inferred from molecular sequence data. Botany 2002: The annual meeting of the Botanical Society of America, in Madison, Wisconsin, website http://www.botany2002.org/sympos12/abstracts/6.shtml (Abstract).

NYLANDER, J. A. A. 2002. MrModeltest, version 1.0b. Computer program distributed by the author, available at website http://www.ebc.uu.se/systzoo/staff/nylander.html. Department of Systematic Zoology, Uppsala University, Uppsala, Sweden.

OXELMAN, B., AND M. LIDEN. 1995. Generic boundaries in the tribe Sileneae (Caryophyllaceae) as inferred from nuclear ribosomal DNA sequences. *Taxon* 44: 525–542.

OXELMAN, B., M. LIDEN, AND D. BERGLUND. 1997. Chloroplast *rps*16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.

OXELMAN, B., M. LIDEN, R. K. RABELER, AND M. POPP. 2001. A revised generic classification of the tribe Sileneae (Caryophyllaceae). *Nordic Journal of Botany* 20: 743–748.

OXELMAN, B., B. AHLGREN, AND M. THULIN. 2002. Circumscription and phylogenetic relationships of *Gymnocarpos* (Caryophyllaceae-Paronychioideae). *Edinburgh Journal of Botany* 59: 221–237.

PAX, F., AND K. HOFFMANN. 1934. Caryophyllaceae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilen, 2nd ed., 275–364. Engelmann, Leipzig, Germany.

PEDERSEN, T. M. 1984. Caryophyllaceae. *In* M. N. Correa [ed.], Flora patagonica, vol. 4A. INTA, Buenos Aires, Argentina.

PETRUSSON, L., AND M. THULIN. 1996. Taxonomy and biogeography of *Gymnocarpos* (Caryophyllaceae, Paronychioideae). *Edinburgh Journal of Botany* 53: 1–26.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

RABELER, R. K., AND V. BITTRICH. 1993. Suprageneric nomenclature in the Caryophyllaceae. *Taxon* 42: 857–863.

RODRÍGUEZ, F., J. L. OLIVER, A. MARÍN, AND J. R. MEDINA. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* 142: 485–501.

ROHWEDER, O. 1970. Centrospermen-Studien. 4. Morphologie un Anatomie del Blünten, Früchte und Samen bei Alsinoideen und Paronychioideen s. lat. (Caryophyllaceae). *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 90: 201–271.

SCHMITZ-LINNEWEBER, C., R. M. MAIER, J. P. ALCARAZ, A. COTTET, R. G. HERMANN, AND R. MARCHE. 2001. The plastid chromosome of spinach (*Spinacia oleracea*): complete nucleotide sequence and gene organization. *Plant Molecular Biology* 45: 307–315.

SCHÖNENBERGER, J., AND H. CONTI. 2003. Molecular phylogeny and floral evolution of Penaeaceae, Oliniaceae, Rhyncocalycaceae, and Alzateaceae (Myrtales). *American Journal of Botany* 90: 293–309.

SIMMONS, M. P., AND J. V. FREUDENSTEIN. 2003. The effects of increasing genetic distance on alignment of, and tree construction from, rDNA internal transcribed spacer. *Molecular Phylogenetics and Evolution* 26: 444–451.

SMISSEN, R. D. 1999. Systematics of *Scleranthus*. Ph.D. dissertation, Victoria University of Wellington, New Zealand.

SMISSEN, R. D., J. C. CLEMENT, P. J. GARNOCK-JONES, AND G. K. CHAMBERS. 2002. Subfamilial relationships within Caryophyllaceae as inferred from 5′ *ndhF* sequences. *American Journal of Botany* 89: 1336–1341.

SMISSEN, R. D, P. J. GARNOCK-JONES, AND G. K. CHAMBERS. 2003. Phylogenetic analysis of ITS sequences suggests a Pliocene origin for the bipolar distribution of *Scleranthus* (Caryophyllaceae). *Australian Systematic Botany* 16: 301–315.

SOLTIS, D. E., P. S. SOLTIS, M. W. CHASE, M. E. MORT, D. C. ALBACH, M. ZANIS, V. SAVOLAINEN, W. H. HAHN, S. B. HOOT, M. F. FAY, M. AXTELL, S. M. SWENSEN, L. M. PRINCE, W. J. KRESS, K. C. NIXON, AND J. S. FARRIS. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society* 133: 381–461.

SOLTIS, D. E., R. K. KUZOFF, M. E. MORT, M. ZANIS, M. FISHBEIN, L. HUFFORD, J. KOONTZ, AND M. K. ARROYO. 2001. Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplast and nuclear DNA regions. *Annals of the Missouri Botanic Garden* 88: 669–693.

STADEN, R., K. BEAL, AND J. K. BONFIELD. 1998. The Staden package. *In* S. Misener and S. A. Krawets [eds.], Bioinformatic methods and protocols: computer methods in molecular biology, 115–130. Humana, Totowa, New Jersey, USA.

SWOFFORD, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

TAVARÉ, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876–4882.

WILLIAMS, F. N. 1898. A revision of the genus *Arenaria* Linn. *Journal of the Linnean Society* 38: 395–407.

APPENDIX. Taxa used in this study, citation and voucher information, and GenBank accession numbers for ITS and *matK*. A dash indicates the region was not sampled. Voucher specimens are located in the following herbaria: FI = Museo di Storia Naturale dell'Università, Firenze, Italy; GE = Università di Genova, Genova, Italy; JACA = Instituto Pirenaico de Ecología, Jaca, Spain; S = Swedish Museum of Natural History, Stockholm, Sweden. Classification follows Bittrich (1993). Subgeneric classification of *Arenaria* and *Minuartia* follows McNeill (1962): *Arenaria—ARE = Arenaria*, *ERE = Eremogone*, *LEI = Leiosperma*, *ODO = Odontostemma*; *Minuartia—MIN = Minuartia*, *RHO = Rhodalsine*, *SPE = Spergella*.

FAMILY
Subfamily
Tribe
Taxon; Citation and/or *Voucher*, Collection locality (Herbarium); GenBank accession nos.: ITS, *matK*.

CARYOPHYLLACEAE
Alsinoideae
Alsineae

Arenaria benthamii Fenzl ex Torr. & Gray (*ARE*); Smissen et al. (2003); AY286524, —. ***A. nevadensis*** Boiss. & Reuter (*ARE*); *Quer & Cuartrec s.n.*, Spain (S); AY936280, AY936303. ***A. serpyllifolia*** L. (*ARE*); *Fior s.n.*, Switzerland (GE); AY936279, AY936302. ***A. graminifolia*** Schrad. (*ERE*); *Skvortsov & Schanzer s.n.*, Russia (FI); AY936263, AY936316. ***A. koriniana*** Fisch (*ERE*); *Bochkin, Klinkova & Sogalaev s.n.*, Russia (FI); AY936265, AY936318. ***A. syreistschikovii*** P.A. Smirn (*ERE*); *Tichomirov & Anoheeva s.n.*, Russia (FI); AY936264, AY936317. ***A.***

musciformis Triana & Planch. (*LEI*); *Killip & Smith 20654*, Columbia (S); AY936334, —. ***A. serpens*** Kunth (*LEI*); *Bocher, Hjerting & Rahu 1183*, Argentina (S); AY936275, AY936304. ***A. fridericae*** Hand.-Mazz. (*ODO*); *Handel & Mazzetti 1211,* China (S); AY936332, —. ***A. pogonantha*** W.W. Sm. (*ODO*); *Forrest 27217*, Tibet (S); AY936333, AY936300.***A. roseiflora*** Sprague (*ODO*); *Forrest 20036*, Tibet (S); AY936244, —. ***A. trichophora*** Franchet (*ODO*); *Maire 6687*, China (S); AY936243, AY936301. ***Bufonia tenuifolia*** L.; *Montserrat 4964/69*, Spain (FI); AY936238, AY936289. ***B. willkommiana*** Boiss.; *Zubizarreta 14916*, Spain (FI); —, AY936290. ***Cerastium arvense*** L.; *Minuto & Fior s.n.*, Italy (GE); —, AY936295. ***C. fontanum*** Baumg.; *Alanko 58340*, Finland (FI); AY936241, AY936296. ***Holosteum umbellatum*** L. **subsp.** ***umbellatum***; *Hoste 8*, Belgium (FI); AY936242, AY936297. ***Honckenya peploides*** (L.) Ehrh.; Sosa et al. (unpub.); —, AY042602. ***Minuartia lanceolata*** (All.) Mattf. (*MIN*); Pascale s.n., Italy (FI); —, AY936292. ***M. laricifolia*** (L.) Schinz & Thell. (*MIN*); Martini s.n., Italy (FI); AY936282, AY936294. ***M. rupestris*** (Scop.) Schinz & Thell. **subsp.** ***rupestris*** (*MIN*); *Minuto & Fior s.n.*, Italy (GE); AY936237, —. ***M. verna*** (L.) Hiern; *Minuto s.n.* (*MIN*), Italy (GE); AY936239, —. ***M. geniculata*** (Poiret) Thell. (*RHO*); H.M.P. N.Rec.It. 151/88b, Spain (FI); —, AY936307. ***M. picta*** (Sibth. & Sm.) Bornm. (*SPE*); *H.M.P. N.Rec.It. 1587/91*, Cyprus (FI) AY936266, AY936319. ***Moehringia intricata*** R. de Roemer ex Willk.; *Reverchon 1106*, Spain (S); AY936276, AY936305. ***M. muscosa*** L.; *Minuto & Casazza s.n.*, Italy (GE); AY936277, AY936306. ***M. trinervia*** (L.) Clairv.; Cuénod et al. (2002); —, AY042615. ***M. trinervia*** (L.) Clairv.; *Viciani s.n.*, Italy (FI); AY936278, —. ***Sagina pilifera*** (DC.) Fenzl; *Foggi s.n.*, France (FI); AY936235, AY936291. ***S. procumbens*** L.; *Auquier 5056*, Belgium (FI); AY936236, —. ***Stellaria media*** (L.) Vill.; *Minuto s.n.*, Italy (GE); AY936245, AY936299. ***S. nemorum*** L.; *Luccioli & Padovani s.n.*, Italy (FI); AY936246, AY936298.

Pycnophylleae

Pycnophyllum bryoides (Phil.) Rohrb.; Smissen et al. (2003); —, AY286527.

Sclerantheae

Scleranthus annuus L.; Smissen et al. (2003); AY286513, AY286533. ***S. perennis*** L.; *Hern 1975/95*, Spain (FI); AY936281, —. ***S. uniflorus*** P.A. Will.; Smissen et al. (2003); AY286522, AY286542.

Caryophylloideae

Caryophylleae

Acanthophyllum sordidum Bunge ex Boiss.; *Kiseleva & Proskuriakova s.n.*, Russia (FI); —, AY936324. ***Allochrusa versicolor*** Boiss.; *Nydegger 435976*, Turkey (FI); AY936270, AY936323.

Dianthus furcatus Balbis; *Minuto & Fior s.n.*, Italy (GE); AY936268, AY936320. ***D. seguieri*** Vill.; *Minuto s.n.*, Italy (GE); AY936267, AY936321. ***Gypsophila altissima*** L.; Cuénod et al. (2002); —, AY042597. ***G. elegans*** Bieb.; *Hillyihugseanov s.n.*, Armenia (FI); AY936273, AY936327. ***G. repens*** L.; *Minuto & Casazza s.n.*, Italy (GE); AY936274, AY936326. ***Saponaria ocymoides*** L.; *Minuto & Fior s.n.*, Italy (GE); AY936271, AY042651. ***S. officinalis*** L.; *Minuto s.n.*, Italy (GE); AY936272, AY936325. ***Vaccaria hispanica*** (Miller) Rauschert; *H.M.P. N.Rec. It. 0267*, Spain (FI); —, AY936328. ***Velezia rigida*** L.; *H.M.P. N. Rec.It. 1302*, Cyprus (FI); AY936269, AY936322.

Drypideae

Drypis spinosa L.; *Moggi, Luccioli & Tosi s.n.*, Italy (FI); AY936240, AY936293.

Sileneae

Agrostemma githago L.; *Minuto s.n.*, Italy (GE); AY936257, AY936315. ***Lychnis coronaria*** (L.) Desr. in Lam.; Cuénod et al. (2002); —, AY042612. ***L. coronaria*** (L.) Desr. in Lam.; Oxelman and Liden (1995); X86891, —. ***L. flos-jovis*** (L.) Desr. in Lam.; *Minuto & Casazza s.n.*, Italy (GE); AY936261, AY936313. ***Petrocoptis pyrenaica*** (J.P. Bergeret) A. Braun ex Walpers; *Montserrat & Villar s.n.*, Spain (JACA); AY936262, AY936314.

P.* sp. *Oxelman Oxelman; Oxelman and Liden (1995); X86875, —. ***Silene campanula*** Pers.; *Minuto s.n.*, Italy (GE); AY936259, AY936311. ***S. italica*** (L.) Pers.; Minuto s.n., Italy (GE); AY936258, AY936312. ***S. nutans*** L.; *Minuto & Fior s.n.*, Italy (GE); AY936260, —. ***S. rothmaleri*** P. Silva; Cuénod et al. (2002); —, AY042656.

Paronychioideae

Polycarpeae

Drymaria laxiflora Benth.; Smissen et al. (2003); AY286528, —. ***Loeflingia hispanica*** L.; *H.M.P. N.Rec.It. 1121/88*, Spain (FI); AY936251, AY936288. ***Ortegia hispanica*** Loefl. ex L.; *Valdes & Bermejo s.n.*, Spain (FI); —, AY936286. ***Polycarpon tetraphyllum*** (L.) L.; *Minuto s.n.*, Italy (GE); AY936252, AY936287. ***Spergula arvensis*** L.; *Baldini s.n.*, Italy (FI); —, AY936310. ***S. marina*** (L.) Griseb.; *Minuto s.n.*, Italy (GE); —, AY936309. ***S. marina*** (L.) Griseb.; Smissen et al. (2003); AY286529, —. ***S. rubra*** J. & C. Presl; *Minuto & Fior s.n.*, Italy (GE); —, AY936308. ***S. rubra*** J. & C. Presl; Oxelman et al. (2002); AJ310981, —.

Paronychieae

Dicheranthus plocamoides Webb; Oxelman et al. (2002); AJ310976, —. ***Gymnocarpos decandrum*** Forssk.; Oxelman et al. (2002); AJ310988, —. ***G. mahranus*** Petrusson & Thulin; Oxelman et al. (2002); AJ310987, —. ***Herniaria baetica*** Boiss. & Reuter; *H.M.P. N. Rec.It. 2405/88*, Spain (FI); AY936248, AY936283. ***H. glabra*** L.; Oxelman et al. (2002); AJ310986, —. ***H. hemistemon*** J. Gay; Oxelman et al. (2002); AJ310984, —. ***Paronychia chlorothyrsa*** Murb.; Oxelman et al. (2002); AJ310982, —. ***P. echinulata*** Chater; *Selvi s.n.*, Italy (FI); AY936249, AY936285. ***P. kapela*** (Hacq.) A. Kern.; *Minuto & Fior s.n.*, France (GE); AY936247, AY936284. ***P. suffruticosa*** (L.) Lam.; Oxelman et al. (2002); AJ310981, —. ***Philippiella patagonica*** Speg.; Oxelman et al. (2002); AJ310989, —. ***Pteranthus dichotomus*** Forssk.; *Podlech 49042*, Morocco (FI); AY936250, —. ***Sphaerocoma aucheri*** Boiss.; Oxelman et al. (2002); AJ310979, —.

Corrigioleae

Corrigiola litoralis L. **subsp.** ***foliosa*** (Pérez-Lara ex. Willk.) Chaudhri; *Lewalle 10564*, Morocco (FI); AY936254, AY936331. ***Telephium oligospermum*** Steud. ex Boiss.; Cuénod et al. (2002); —, AY042664.

MOLLUGINACEAE

Mollugo verticillata L.; *Barchiesi 0202*, USA, South Carolina (FI); AY936253, AY936330.

AMARANTHACEAE

Amaranthus caudatus L.; Song et al. (unpub.); AF210907, —. ***A. paniculatus*** L.; Meimberg et al. (2000); —, AF204866. ***Atriplex laciniata*** L.; *Buggenhout s.n.*, UK (FI); AY936256, —. ***A. patula*** L.; Cuénod et al. (2002); —, AY042550. ***A. tornabeni*** Tineo; *Lambinon 98/733*, France (FI); AY936255, AY936329. ***Beta trigyna*** Waldst. & Kit.; Cuénod et al. (2002); —, AY042555. ***Chenopodium album*** L.; Hershkovitz and Zimmer (1997); L78088, —. ***C. bonus-henricus*** L.; Meimberg et al. (2000); —, AF204864. ***Spinacia oleracea*** L.; Li and Guy (unpub.); AF062088, —.