

## A NEW LINEAGE-BASED TRIBAL CLASSIFICATION OF THE FAMILY CARYOPHYLLACEAE

Danica T. Harbaugh,<sup>1\*</sup> Molly Nepokroeff,<sup>†</sup> Richard K. Rabeler,<sup>‡</sup> John McNeill,<sup>§</sup> Elizabeth A. Zimmer,<sup>\*</sup> and Warren L. Wagner<sup>\*</sup>

<sup>\*</sup>Department of Botany, National Museum of Natural History, Smithsonian Institution, MRC166, P.O. Box 37012, Washington, D.C. 20013-7012, U.S.A.; <sup>†</sup>Department of Biology, University of South Dakota, 177 Churchill-Haines, Vermillion, South Dakota 57069, U.S.A.; <sup>‡</sup>University of Michigan Herbarium, 3600 Varsity Drive, Ann Arbor, Michigan 48108-2228, U.S.A.; and <sup>§</sup>Royal Botanic Garden, Edinburgh EH3 5LR, Scotland, United Kingdom

Understanding the relationships within the Caryophyllaceae has been difficult, in part because of arbitrarily and poorly defined genera and difficulty in determining phylogenetically useful morphological characters. This study represents the most complete phylogenetic analysis of the family to date, with particular focus on the genera and relationships within the large subfamily Alsinoideae, using molecular characters to examine the monophyly of taxa and the validity of the current taxonomy as well as to resolve the obscure origins of divergent taxa such as the endemic Hawaiian *Schiedea*. Maximum parsimony and maximum likelihood analyses of three chloroplast gene regions (*matK*, *trnL-F*, and *rps16*) from 81 newly sampled and 65 GenBank specimens reveal that several tribes and genera, especially within the Alsinoideae, are not monophyletic. Large genera such as *Arenaria* and *Minuartia* are polyphyletic, as are several smaller genera. The phylogenies reveal that the closest relatives to *Schiedea* are a pair of widespread, largely Arctic taxa, *Honckenya peploides* and *Wilhelmsia physodes*. More importantly, the three traditional subfamilies (Alsinoideae, Caryophylloideae, and Paronychioideae) are not reflective of natural groups; we propose abandoning this classification in favor of a new system that recognizes major lineages of the molecular phylogeny at the tribal level. A new tribe, Eremogoneae Rabeler & W.L. Wagner, is described here.

**Keywords:** Alsinoideae, Caryophyllaceae, Eremogoneae, molecular phylogeny, *Schiedea*.

**Online enhancement:** appendix table.

### Introduction

The family Caryophyllaceae Juss., the pink or carnation family, is cosmopolitan and includes a number of common ornamental plants, such as carnations (*Dianthus* L.) and baby's breath (*Gypsophila* L.). The family is primarily Holarctic in distribution, with diversity centered in the Mediterranean and Irano-Turanian regions (Bittrich 1993), and includes ~3000 species distributed among 88 genera (Rabeler and Hartman 2005). The number of genera declines to 82 if one accepts a broad concept of *Silene* (see Greuter 1995; Morton 2005a) or could increase to more than 120 if one accepts all the segregates of several of the large genera that have been proposed (see Oxelman et al. 2001; Tzvelev 2001). The most common classification (Pax and Hoffman 1934; Bittrich 1993) of Caryophyllaceae includes three subfamilies based on characters of the stipules, petals, sepals, and fruits: Alsinoideae Burnett [Minuartioideae DC.], Caryophylloideae Arn., and Paronychioideae A. St. Hil. ex Fenzl [Illecebroideae Arn.].<sup>2</sup> Rabeler

and Hartman (2005) recognized a fourth subfamily, Polycarpoideae Burnett [Polycarpaoideae Beilschm.], including there the species of the Paronychioideae with capsular fruits. This scheme is also consistent with treating the Paronychioideae sensu stricto as a separate family, the Illecebraceae R. Br. (Hutchinson 1973). Takhtajan (1997) recognized the “traditional” three subfamilies as well as Scleranthoideae Arn., into which *Scleranthus* L. and *Pentastemonodiscus* Rech.f. were segregated from the Alsinoideae. He reverted to three subfamilies in his most recent work (Takhtajan 2009), returning these genera to a tribe within the Alsinoideae.

Understanding the relationships within the Caryophyllaceae has been difficult, in part because many of the genera are not well defined morphologically and are difficult to distinguish (Bittrich 1993). The definition of monophyletic groups within the family has been problematic because of difficulty in determining phylogenetically useful characters and the possibility of widespread convergence of characters used in formal classifications, including embryogeny, leaf morphology, chromosome

<sup>1</sup> Author for correspondence; current address: Department of Integrative Biology, University and Jepson Herbaria, 1001 Valley Life Sciences Building, University of California, Berkeley, California 94720-2465, U.S.A.; e-mail: danicah@berkeley.edu.

<sup>2</sup> Review of the recently released work by Takhtajan (2009) revealed that, unfortunately, three of the subfamily names used throughout

*Manuscript received August 2009; revised manuscript received October 2009.*

this paper are not the oldest available names. The correct names, following Reveal (2007), are as follows: Alsinoideae Burnett (1835) is predated by Minuartioideae DC. in Beilschm. (1833), Paronychioideae A. St. Hil. ex Fenzl (1839) is predated by Illecebroideae Arn. (1832), and Polycarpoideae Burnett (1835) is predated by Polycarpaoideae Beilschm. (1833).

numbers, number of floral parts, and the presence and nature of nectaries.

Possible morphological homoplasy and difficulty in determining morphological synapomorphies make the use of molecular phylogenetic data critical in understanding the relationships within the Caryophyllaceae (Smitsen et al. 2002; Fior et al. 2006). Until recently, most of the molecular studies that have included members of the Caryophyllaceae have been focused on the relationships of the families within the order Caryophyllales rather than on relationships within the family (Rettig et al. 1992; Downie and Palmer 1994; Downie et al. 1997; Cuénoud et al. 2002); these studies have revealed that the family is monophyletic (Rettig et al. 1992; Downie and Palmer 1994; Downie et al. 1997; Fior et al. 2006), supporting the continued inclusion of members of the Paronychioideae. Many of the more focused studies have been on the tribe Sileneae DC., especially the genus *Silene* L. (Desfeux and Lejeune 1996; Oxelman and Lidén 1995; Oxelman et al. 1997, 2001; Egens et al. 2007; Erixon and Oxelman 2008). In addition, a number of studies on genera within the Caryophyllaceae have been completed (*Schiedea* Cham. & Schldl.: Soltis et al. 1996; *Scleranthus*: Smitsen et al. 2003; *Schiedea*: Wagner et al. 2005; *Arenaria* L. and *Moebringia* L.: Fior and Karis 2007; *Polycarpon* L.: Kool et al. 2007; *Silene*: Popp and Oxelman 2001) and are beginning to provide an understanding of the complexities of the family. Finally, a recent study by Brockington et al. (2009) has advanced our understanding of the complexities of the family. Finally, a recent study by Brockington et al. (2009) has advanced our understanding of the order Caryophyllales and the placement of the Caryophyllaceae.

Relationships within the Caryophyllaceae and monophyly of the subfamilies were first investigated with molecular data by Smitsen et al. (2002), who produced a phylogeny of 15 genera based on the chloroplast *ndhF* gene. A more comprehensive study of 38 genera in the Caryophyllaceae was performed by Fior et al. (2006), using a combination of chloroplast (*matK*) and nuclear ribosomal DNA (nrDNA; ITS) data. Both of these studies revealed that none of the three traditional subfamilies (Alsinoideae, Caryophylloideae, and Paronychioideae) is monophyletic. Despite limited sampling, Smitsen et al. (2002) concluded that all three subfamilies are polyphyletic. While the molecular phylogenies in Fior et al. (2006) are not well resolved, they do suggest that Alsinoideae (minus tribe Pyncnophylleae Mattf.) and Caryophylloideae together form a monophyletic group, with Paronychioideae forming a basal grade. Both studies demonstrate that the Alsinoideae tribe Scleranthae Link ex DC. is clearly separated from tribe Alsineae Lam. & DC., which itself is polyphyletic (Smitsen et al. 2002; Fior et al. 2006).

At a very basic level, members of subfamily Alsinoideae are distinguished from the Caryophylloideae by their free sepals and from the Paronychioideae by their exstipulate leaves (McNeill 1962). Members of subfamily Alsinoideae are thought to be most closely related to subfamily Caryophylloideae, on the basis of “caryophyllad type” embryogeny, the development of diverticles of the embryo sac, and the shared character of sheathing leaf bases (Bittrich 1993). However, the monophyly of the Alsinoideae has been questioned because it may be either monophyletic, based on nectary gland characteristics, or paraphyletic, based on chromosome numbers. Fernandes and Leitão (1971) assumed that the Silenoideae (Caryophylloideae) are nested within the Alsinoideae and that

they represent an increase in chromosome number from the base number of  $n = 9$  for the family. Characters that have been used to circumscribe subfamily Alsinoideae include exstipulate leaves; perigynous or hypogynous flowers; often conspicuous petals; free sepals; mostly open or semiclosed petal venation; epipetalous stamens, often with a nectar gland at the base and mainly capsulate; and dehiscent fruit. As Bittrich (1993) suggested, the circumscription of the Alsinoideae could be less clear if genera (e.g. *Polycarpon* and *Spergula* L.) with capsular fruits, which are most often regarded as members of the subfamily Paronychioideae, are included in the Alsinoideae; Leonhardt (1951) did so in his seven-subfamily classification of the family. This lack of clarity could also occur when authors recognize a narrow sense of the Paronychioideae as the family Illecebraceae (Hutchinson 1973). These genera differ from the “core” Alsinoideae in characters of embryology, leaf venation, and presence of stipules, suggesting that capsular fruits may have arisen more than once in the Caryophyllaceae. Furthermore, the genus *Geocarpon* Mack. (Alsinoideae) has tiny petals that could be interpreted as staminodes, a diagnostic feature of some of the Paronychioideae (Bittrich 1993).

Relationships within subfamily Alsinoideae, the largest of the Caryophyllaceae subfamilies, are poorly known. Bittrich (1993) placed the 28 genera of the Alsinoideae into five tribes, the Alsineae (23 genera), the Geocarpeae E.J. Palmer & Steyerl. (consisting of a single species, *Geocarpon minimum* Mack.), the Habrosieae Endl. (consisting of a single species, *Habrosia spinuliflora* Fenzl), the Scleranthae (two genera), and the Pyncnophylleae Mattf. (consisting of the single genus *Pyncnophyllum* E.J. Remy; see also table 1). Characters of floral variation (loculicidal vs. both loculicidal and septicidal dehiscence, carpel number, presence or absence of petals, and sepal characteristics) have been used extensively in generic delimitation in the Alsinoideae. However, such characters are known to be highly evolutionarily labile or plastic and may not represent homologous characters for use in defining phylogenetic groups (Endress 1996; Hufford 1996). Because of possible homoplasy, the subfamilial classification, in particular relationships within the Alsinoideae, has remained problematic and should be examined by use of molecular characters. For example, McNeill (1973) described an example from Turkey that illustrates the difficulty, due to the convergence of morphological characters, of segregating two genera in different subfamilies, *Stellaria* L. (Alsinoideae) and *Gypsophila* L. (Caryophylloideae).

One of the major questions within subfamily Alsinoideae involves the delimitation of the widespread genus *Arenaria* (Fernald 1919; Pax and Hoffman 1934; Maguire 1951; McNeill 1980) and whether it should be broadly or more narrowly circumscribed. Another problem is that certain genera, such as the North American *Geocarpon* and the Hawaiian *Schiedea* Cham. & Schldl., have obscure relationships with the remainder of the family. The single species of *Geocarpon* (*G. minimum*) was originally placed outside of the Caryophyllaceae in the Aizoaceae but also possesses some characters of the subfamilies Alsinoideae and Paronychioideae, and it was placed in its own tribe by Palmer and Steyerl. (1950). *Schiedea*, which represents the fifth-largest radiation of angiosperms in the Hawaiian flora (Wagner et al. 1995, 2005; Soltis et al. 1996), is one of the most striking examples of adaptive radiation in the islands. Previous studies based on morphological

**Table 1**  
**Classification of the Caryophyllaceae (from Bittrich 1993) and Taxa Sampled in Smissen et al. (2002), Fior et al. (2006), and This Study**

Subfamily, tribe	Total genera	Genera (species) sampled in Smissen et al. 2002	Genera (species) sampled in Fior et al. 2006	Genera (species) sampled in this study
Alsinoideae:				
Alsineae Lam. & DC.	28	4 (4)	9 (32)	18 (74)
Geocarpeae E.J.Palmer & Steyerm.	1	0 (0)	0 (0)	1 (1)
Habrosieae Endl.	1	0 (0)	0 (0)	0 (0)
Pycnophylleae Mattf.	1	0 (0)	1 (1)	1 (2)
Scleranthae Link ex DC.	2	1 (1)	1 (3)	1 (3)
Caryophylloideae:				
Caryophylleae Lam. & DC.	17	1 (1)	7 (11)	8 (15)
Drypideae Fenzl	1	0 (0)	1 (1)	1 (1)
Sileneae DC.	6	1 (1)	4 (9)	4 (13)
Paronychioideae:				
Corrigioleae Dumort.	2	0 (0)	2 (2)	2 (3)
Paronychieae Dumort.	15	4 (4)	7 (13)	4 (6)
Polycarpaceae DC.	16	4 (4)	6 (7)	6 (8)
Total	90	15 (15)	38 (79)	46 (126)

and molecular data suggest that *Schiedea* is monophyletic and the result of a single ancestral colonization to the archipelago (Wagner et al. 1995, 2005; Weller et al. 1995; Soltis et al. 1996); however, their ancestor and source area remain unresolved. The use of traditional classifications of the Alsinoideae and Caryophyllaceae to identify the closest relatives of the Hawaiian lineage is problematic, and a molecular phylogenetic analysis should contribute immensely to both subfamilial circumscription and placement of the Hawaiian Alsinoideae within the family.

Morphologically, the Hawaiian Alsinoideae appear to be most closely aligned to the large “*Arenaria* complex,” a group that traditionally includes the large genera *Arenaria* and *Minuartia* and up to nine smaller genera (McNeill 1962; Wagner et al. 1999). The specialized morphology of the nectaries of the Hawaiian genera suggest a possible relationship with *Minuartia* sect. *Greniera* Mattf., which consists of two serpentine endemic species in the western United States, one of which (*Minuartia douglasii* Mattf.) has a similar unusual nectary extension (Harris and Wagner 1995). Current studies of the ontogeny of nectary characteristics in Hawaiian Alsinoideae and *Minuartia* sect. *Greniera* (Harris and Wagner 1995; Wagner and Harris 2000) suggest that development of nectary tissue follows the same pathway in both lineages. Molecular phylogenetic analysis based on nrDNA sequences suggests, however, that *Minuartia* sect. *Greniera* is not as closely related to the Hawaiian genera as previously believed (Wagner et al. 2005); this is consistent with our results as well. Carlquist (personal communication to S. Weller) has suggested a New World origin for the group, while Ballard and Sytsma (2000) pointed to the Arctic region as the possible origination of *Schiedea*, after the surprising discovery, based on molecular data, of a subarctic origin for *Viola* L. These results necessitate identification of the sister group to the Hawaiian lineage and, as a consequence, reevaluation of relationships within the Alsinoideae on a worldwide basis by means of independently derived, molecularly based phylogenies. Thus, a more comprehensive phyloge-

netic perspective will allow us to address the possibility of convergent evolution in a complex, well-characterized morphological trait until recently considered a synapomorphy for the Hawaiian Alsinoideae and *Minuartia*.

We examine the phylogenetic utility of morphological characters used in traditional classifications of Caryophyllaceae, focusing on subfamily Alsinoideae and the origin of the Hawaiian endemic *Schiedea* and using the historical context provided by a phylogeny derived from molecular characters, including three chloroplast gene regions (*matK*, *trnL-F*, and *rps16*). The primary goals of this study are (1) to examine the monophyly and relationships of the three traditional subfamilies of Caryophyllaceae, (2) to examine the relationships within subfamily Alsinoideae (Caryophyllaceae) on a worldwide basis, and finally, (3) to clarify the position of the Hawaiian Alsinoideae (*Schiedea*) and their ancestral source area.

## Material and Methods

### Taxonomic Sampling

This study represents the most comprehensive sampling of Caryophyllaceae taxa in a molecular phylogenetic study to date and includes a total of 126 species from 46 genera in the Caryophyllaceae worldwide (including 81 newly sequenced specimens), with particular emphasis on subfamily Alsinoideae and tribe Alsineae, from which 18 of the 28 genera were sampled (table 1). Sampling was initially designed to cover all putative outgroups to the Hawaiian *Schiedea* species in order to determine the source area for colonization. This included a focus on the “*Arenaria* complex” (McNeill 1962); we also sampled a number of the smaller genera in the Alsinoideae (e.g., *Honckenya* Ehrh. and *Wilhelmsia* Rchb., but not all were available, including *Brachystemma* D. Don) as well as six of the 10 subgenera of *Arenaria* and each of the four subgenera of *Minuartia*. Nine outgroup sequences from three families in the Caryophyllales (Achatocarpaceae, Amaranthaceae,

and Molluginaceae) were used to root the phylogenies. See table A1 in the online edition of the *International Journal of Plant Sciences* for a list of all specimens used in this study, including voucher and source information as well as GenBank accession numbers.

#### DNA Extraction, Amplification, and Sequencing

For the specimens newly sampled in this study, DNA was isolated from fresh, silica-dried, or herbarium leaf material with the Qiagen DNeasy DNA Plant Mini Kit (Qiagen, Valencia, California). For most specimens, the *matK*, *trnL-F*, and *rps16* chloroplast gene regions were analyzed; three independent regions were chosen to test for incongruence that may have resulted from chloroplast recombination, which was detected in the Sileneae by Erixon and Oxelman (2008), or from hybridization or lineage sorting, as hypothesized by Rautenberg et al. (2008). To amplify *matK*, the entire *matK* coding exon was amplified, together with a portion of the 5' and 3' exons for *trnK* (and associated intronic regions), with the primers *trnK1F* and *trnK2R* (see table 2 for primer source information) under the following polymerase chain reaction (PCR) parameters: 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2.5 min. The cycling ended with 72°C for 7 min; the preparation then was held at 4°C. In some cases, because of the large fragment size generated from the above primers, two smaller overlapping fragments were amplified with 710F and Als11R for the 5' end and with 980F and *trnK2R* for the 3' end. Alsinoideae-specific (Als11F, Als11R) and Caryophyllaceae-wide (Car11F, Car11R) primers were designed for sequencing the 3' end of the coding region. In the middle of the coding region, the primers 980F and 980R were designed for sequencing Caryophyllaceae-wide samples. In addition, the primers 710F and *trnK2R* were used to sequence some samples. Amplification and sequencing of *rps16* and *trnL-F* were more straightforward; *rps16* was amplified with primers *rpsF* and *rpsR*, while *trnL-F* was amplified with *trnL-F* primers C and F and sequenced with primers C and F as well as E and D (Taberlet

et al. 1991). Standard protocols were used for the amplifications and sequencing.

The total number of specimens analyzed for each of the three chloroplast regions is included in table 3. For the *matK* analysis, of the 135 total sequences, 70 were generated in this study and 65 were taken from previous studies and accessed through GenBank; most of these were analyzed in Fior et al. (2006). The combined data set included only those specimens that were newly sequenced in this study (81) and not those taken from GenBank, in order to have a more complete matrix; most of the newly sequenced specimens had all three genes sequenced, while seven were missing an *rps16* sequence and 13 were missing a *trnL-F* sequence (table 3).

#### Sequence Alignments and Phylogenetic Analyses

Sequences were aligned manually by eye in PAUP\* 4.0b10 (Swofford 2002). Regions for which homology assessments were ambiguous between a few taxa were replaced by question marks, whereas ambiguous regions across the majority of taxa were removed from the matrices before analyses. Insertions and deletions (indels) of more than 2 bp were scored as characters with the simple gap-coding method of Simmons and Ochoterena (2000) and were included in the maximum parsimony (MP) analyses (see table 3 for the numbers of indels scored). All MP analyses were performed in PAUP\* 4.0b10 (Swofford 2002). In all MP analyses, characters were treated as unordered and equally weighted. For separate *matK*, *rps16*, and *trnL-F* data sets, a heuristic search with 10,000 random-addition replicates, tree bisection reconnection (TBR) branch swapping, Multrees on, and no more than 100 trees held at each step was performed, with all characters unordered and unweighted; these parameters were identical to those used in Fior et al. (2006). A combined analysis of all three chloroplast regions (not including GenBank *matK* sequences) was also performed with a heuristic search using 10,000 random-addition replicates, TBR branch swapping, and Multrees on. The total aligned lengths of the four matrices, including the number of indels, are listed in table 3. MP bootstrap analyses were run on all three separate and one

Table 2

Primers Used for Amplification and Sequencing in This Study

Name	Sequence (5' to 3')	Source
<i>trnK1F</i>	CTC AAC GGT AGA GTA CTC	Manos and Steele 1997
<i>trnK2R</i>	AAC TAG TCG GAT GGA GTA G	Steele and Vilgalys 1994
Als11F	ATC TTT CGC ATT ATT ATA G	This study
Als11R	GCA CGT ATA GCA CTT TTG T	This study
Car11F	GTG CTA GAA CTT TGG CTC G	This study
Car11R	CGA GCC AAA GTT CTA GCA C	This study
980F	TGG TCT CAA CCA AGA AGA AT	This study
980R	ATT TCT TCT TGG TTG AGA CCA	This study
710F	GTA TCG CAC TAT GTW TCA TTT GA	Johnson and Soltis 1995
<i>rpsF</i>	GTG GTA GAA AGC AAC GTG CGA CTT	Popp and Oxelman 2001
<i>rpsR</i>	TCG GGA TCG AAC ATC AAT TGC AAC	Popp and Oxelman 2001
<i>trnL-F</i> , primer C	CGA AAT CGG TAG ACG CTA CG	Taberlet et al. 1991
<i>trnL-F</i> , primer D	GGG GAT AGA GGG ACT TGA AC	Taberlet et al. 1991
<i>trnL-F</i> , primer E	GGT TCA AGT CCC TCT ATC CC	Taberlet et al. 1991
<i>trnL-F</i> , primer F	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. 1991

Table 3

## Statistics from the Maximum Parsimony Analyses

Spacer	Sequences	bp	Indels	PI characters	Trees	L (steps)	CI	RI
<i>matK</i>	135	1764	7	861 (48.8%)	236,819	4067	.4699	.7573
<i>rps16</i>	74	768	2	338 (43.9%)	88,989	1512	.5357	.7059
<i>trnL-F</i>	68	888	2	380 (42.7%)	13,155	1881	.5183	.6448
Combined	81	3420	11	1454 (42.5%)	576	6474	.5263	.7008

Note. Table shows the total aligned length (bp), the number of parsimony-informative (PI) characters, the total number of trees, the shortest tree length (L), the consistency index (CI), and the retention index (RI) for all separate and combined data sets.

combined data set; for the separate data sets, the same parameters as those used in Fior et al. (2006) were used, which involved a heuristic search with 10,000 bootstrap replicates, with 10 random-addition replicates and TBR branch swapping, saving 10 trees at each replicate; for the combined data set, a heuristic search was conducted with 1000 bootstrap replicates, TBR branch swapping, Multrees on, and 10 random-addition replicates, holding 10 trees at each step. Maximum likelihood (ML) analyses also were performed for all three chloroplast regions separately and combined with the Web-based program RAxML (Stamatakis et al. 2005) using the GTR model with 1000 bootstrap replicates (Stamatakis et al. 2008); they were performed at least twice for each data set to ensure the stability of the topology.

## Results

## Phylogenetic Analyses

The statistics from the MP analyses, including the total number of sequences in the matrices and their aligned length, the number of indels, the number of parsimony-informative characters, tree length, and the consistency and retention indices for the separate chloroplast and combined data sets, are listed in table 3. The ML analyses resulted in trees with the following likelihood ( $-\ln L$ ) scores: *matK* =  $-24,507.8522$ ; *rps16* =  $-8985.9028$ ; *trnL-F* =  $-10,683.8514$ ; combined =  $-39,025.4385$ . Results from the separate and combined ML analyses are consistent with, but somewhat better resolved and better supported than, the strict consensus trees from the MP analyses; results from the two ML analyses resulted in consistent topologies and indicate that the ML analyses did not get caught in local optima. The separate *rps16* and *trnL-F* ML and MP analyses are not shown but are consistent with the *matK* and combined analyses in the main Alsinoideae groupings (figs. 1, 2, clades A–E); however, the deeper nodes are unresolved.

Finally, observations of the relative positions of the outgroup families (Achatocarpaceae, Amaranthaceae, and Molluginaceae) are incongruent between the *matK* and combined analyses (figs. 1, 2), possibly because of limited sampling. Both, however, place Molluginaceae in a proximal position to the Caryophyllaceae, which is not consistent with the results of Cuénoud et al. (2002), who sampled widely within the order.

## Taxonomic Groupings

The phylogenetic analyses presented here (figs. 1, 2) demonstrate that subfamilies Alsinoideae and Paronychioideae as

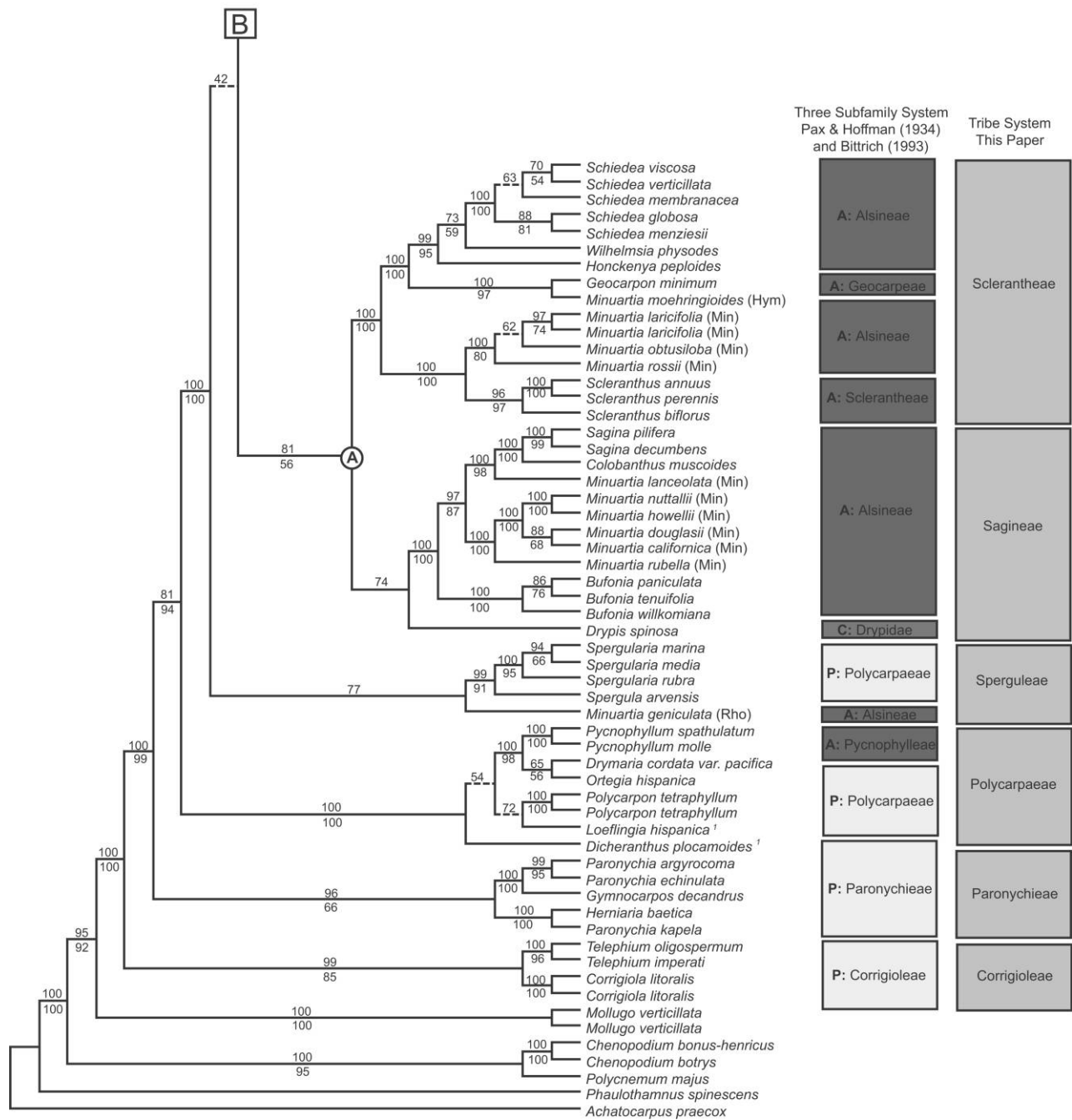
usually delimited are not monophyletic; subfam. Paronychioideae is a basal paraphyletic grade, while tribe Pycnophylleae, usually included in subfam. Alsinoideae, is nested within it. In both the *matK* and combined analyses, members of tribe Corrigioleae Dumort. (*Corrigiola* L. and *Telephium* L.) are monophyletic and sister to the rest of the family. The tribe Paronychieae Dumort. is polyphyletic, with one main clade including *Paronychia* Miller, *Gymnocarpos* Forssk., and *Herniaria* L., while the genus *Dicheranthus* Webb is nested within a clade of the polyphyletic tribe Polycarpaeae DC. Tribe Polycarpaeae consists of a clade including *Polycarpon* L. and *Loeflingia* L. and a clade including *Spergula* and *Spergularia* L.; the latter clade is more closely related than the former to the Alsinoideae + Caryophylloideae clade. The South American Alsinoideae genus *Pycnophyllum* is nested within the first Polycarpaeae clade, sister to *Drymaria*.

In the *matK* analysis (fig. 1), *Paronychia* Mill. is itself paraphyletic, with *Gymnocarpos* and *Herniaria* nested within it. Chaudhri (1968) recognized three subgenera in his revision of the *Paronychia*: the chiefly New World *Paronychia*, the southeastern United States endemic *Siphonychia* (Torrey & A. Gray) Chaudhri, and the Old World *Anoplonychia* (Fenzl) Chaudhri. The “divergent” species in our analysis, *Paronychia kapela* (Hacq.) A. Kern, is a member of the Old World assemblage.

In both the *matK* and combined analyses (figs. 1, 2), the Alsinoideae are also demonstrated to be paraphyletic, because the monophyletic Caryophylloideae are nested within, but the two together are well supported as a monophyletic group. Rapid diversification of the family, inferred from extremely short branch lengths, obscures relationships among the major clades of the Alsinoideae (A–C) and Caryophylloideae (D and E); however, a number of important results are elucidated. The Caryophylloideae consist of two main lineages, which include (1) *Dianthus*, *Saponaria* L., *Gypsophila*, and their relatives (clade D) and (2) *Silene* L. and its relatives (clade E); these correspond to tribes Caryophylleae Lam. & DC. and Sileneae DC., respectively. In the *matK* analysis (fig. 1), *Dianthus* is paraphyletic, with *Velezia* L. nested within it, and *Lychnis* and *Silene* are both polyphyletic, with *Lychnis* nested within *Silene*; although there is reduced sampling in the combined analysis (fig. 2), results are consistent, suggesting that *Lychnis* is paraphyletic, with *Silene* within it. If *Viscaria* Bernh. is treated as a separate genus (Lidén et al. 2001), i.e., *Lychnis viscaria* L. in the combined analysis (fig. 2), then *Lychnis* would be a sister group to *Silene*, with *Viscaria* as a sister to *Lychnis* + *Silene*.

In both the *matK* and combined analyses, the Alsinoideae are polyphyletic, composed of major clades A–C (figs. 1, 2), as well as having the Caryophylloideae tribes Caryophylleae

A



**Fig. 1** Maximum likelihood (ML) phylogeny from analysis of *matK* sequences from GenBank and those generated in this study (table A1). Numbers above the branches represent ML bootstrap (BS) values, while those below are for maximum parsimony (MP) BS values (a missing value represents an MP BS value of <50). Branches with dashed lines collapse in the MP strict consensus tree. Circled letters indicate major clades discussed in the text; squared letter refers to the other part of the figure (A or B). The taxonomy shown here along the right-hand side of the phylogeny indicates the three-subfamily system (Alsinoideae, Caryophylloideae, and Paronychioideae) of Pax and Hoffmann (1934) and the associated tribes from Bittrich (1993); alongside this is the tribal alignment proposed in this article. Subgenera of *Arenaria* and *Minuartia* follow McNeill (1962) and are denoted by three-letter codes. *Arenaria*: *Are* = *Arenaria*, *Ere* = *Eremogone*, *Ers* = *Eremogoneastrum*, *Lei* = *Leiosperma*, *Odo* = *Odontostemma*, *Por* = *Porphyrantha*. *Minuartia*: *Hym* = *Hymenella*, *Min* = *Minuartia*, *Rho* = *Rhodalsine*, *Spr* = *Spergella*. Superscripts next to taxon names indicate instances of differing topology in the MP strict consensus tree. 1: *Loeflingia hispanica* and *Dicheranthus plocamoides* are sister to each other (BS = 54) and are basal in the clade. 2: *Silene campanula* and *Silene italica* are sister to each other (BS = 60) and sister to *Silene acaulis* + *Silene rothmaleri*.

B

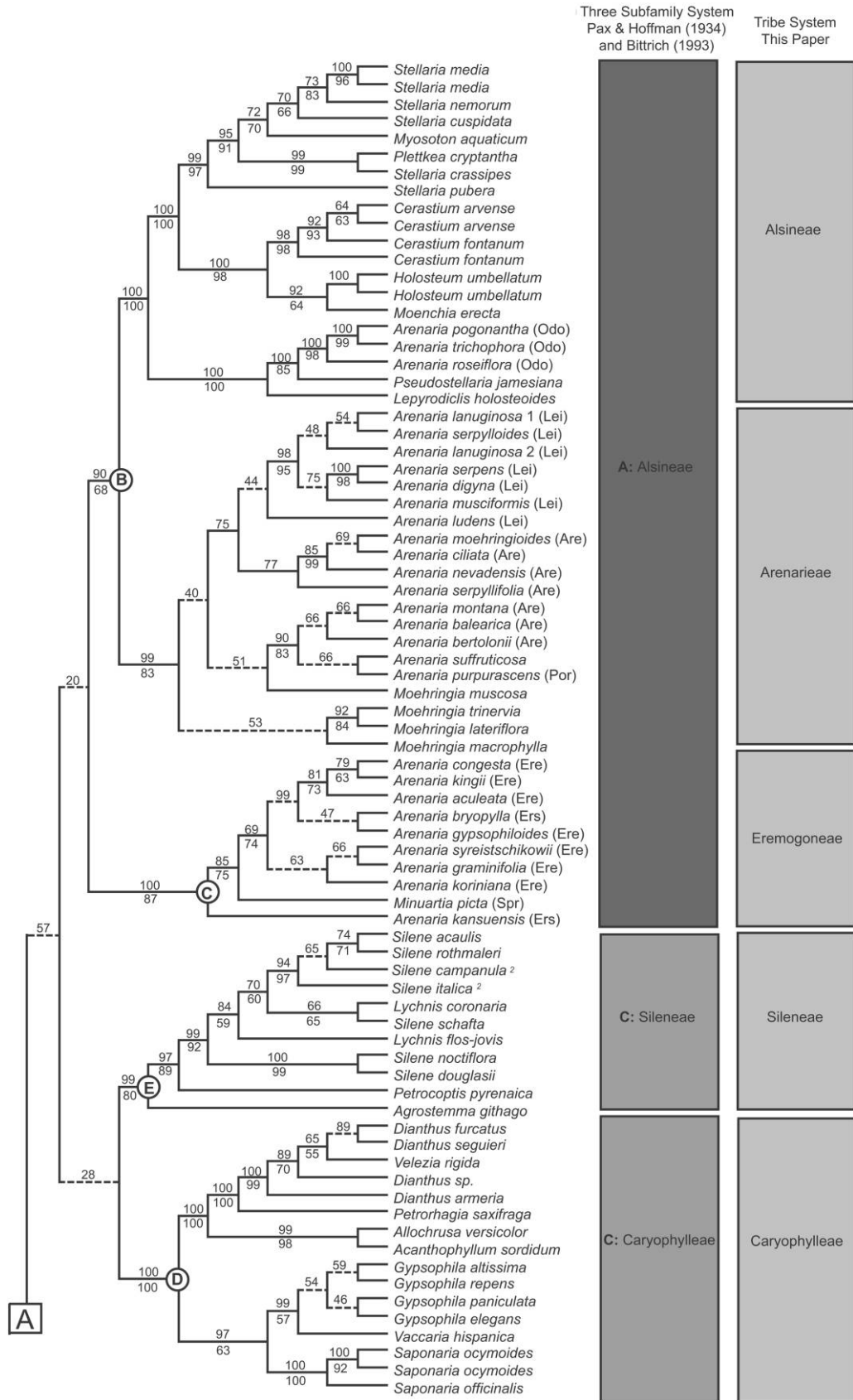
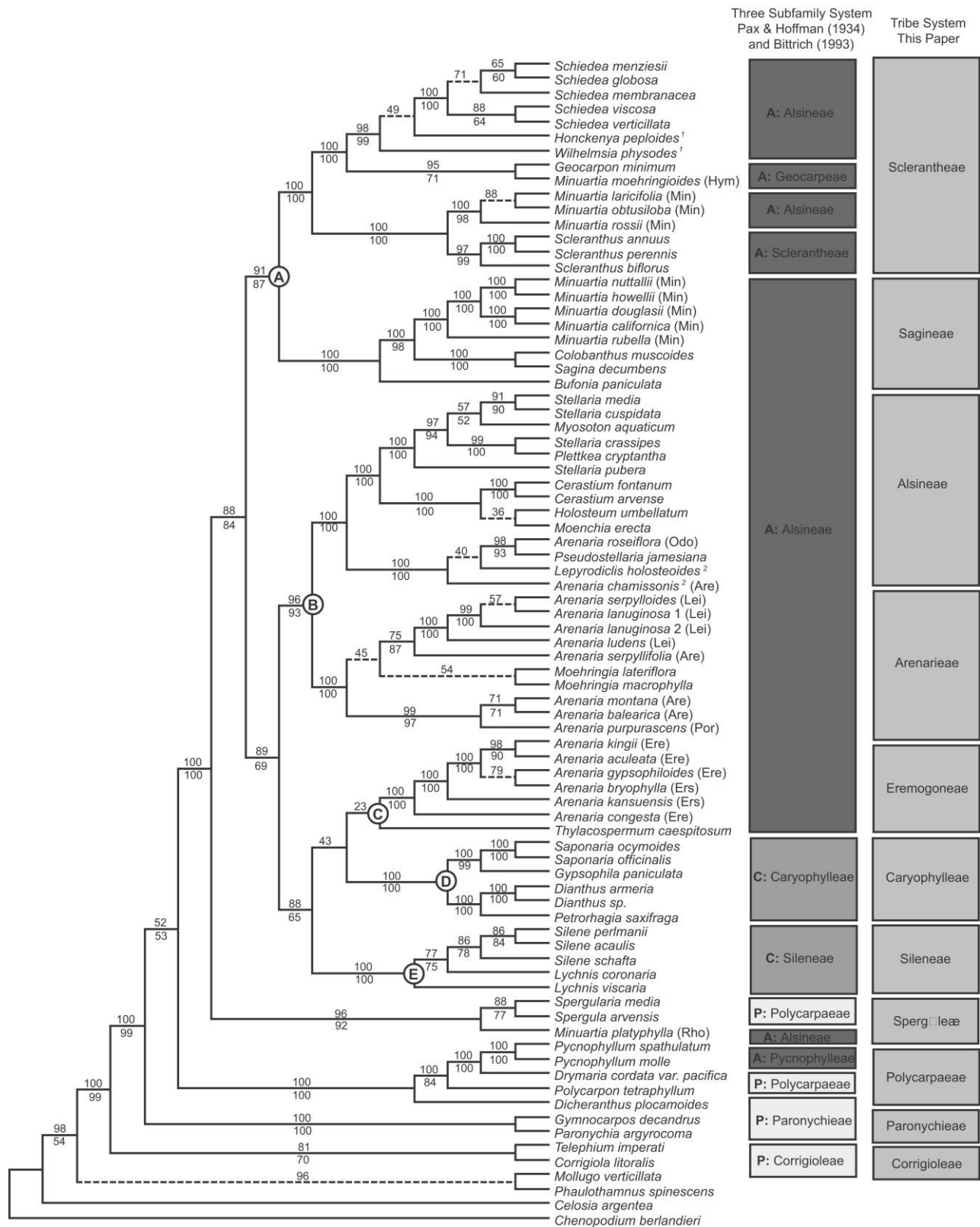


Fig. 1 (Continued)



**Fig. 2** Maximum likelihood (ML) phylogeny from analysis of combined *matK*, *trnL-F*, and *rps16* sequences. Numbers above the branches represent ML bootstrap (BS) values, while those below are for maximum parsimony (MP) BS values (a missing value represents an MP BS value of <50). Branches with dashed lines collapse in the MP strict consensus tree. Circled letters indicate major clades discussed in the text. The taxonomy



(clade D), Sileneae (clade E), and Drypidae Fenzl nested within them. In addition, *Minuartia geniculata* (Poir.) Thellung, *Minuartia platyphylla* (Christ) McNeill (both members of *Minuartia* subg. *Rhodalsine* (J. Gay) Graebner), and tribe Pycnophylleae nest within the Paronychioideae. All of our phylogenetic analyses demonstrate that the members of the Alsinoideae within clades A–C are consistent and indicate that the subfamily Alsinoideae consists of at least two main lineages: (1) several morphologically divergent taxa, including most of the *Minuartia* taxa sampled, with capsules dehiscent by entire valves (clade A), and (2) most of the sampled *Arenaria*, plus *Cerastium*, *Stellaria* L., and several smaller related genera, all but one (*Lepyrodiclis*) dehiscent by split capsule valves (clades B and C). These results suggest that tribes in the Alsinoideae as currently defined (Bittrich 1993; table 1) are not supported; in the *matK* analysis (fig. 1), tribe Alsineae is clearly polyphyletic, with Alsinoideae tribes Geocarpeae and Sclerantheae, as well as Caryophylloideae tribe Drypidae (*Drypis spinosa* L.), nested within it, while one species, *M. geniculata*, that has sometimes been placed in the separate genus *Rhodalsine* J. Gay is nested within the Paronychioideae.

Within the Alsinoideae, there are a number of important taxonomic issues that were resolved in our phylogenetic analyses, especially related to the large genera *Arenaria* and *Minuartia*. In both the *matK* and combined analyses (figs. 1, 2), *Arenaria* is polyphyletic. Clade C consists of *Arenaria* subg. *Eremogone* (Fenzl) Fenzl and *Eremogoneastrum* F. Williams, along with *Minuartia* subg. *Spergella* (Fenzl) McNeill. Within clade B, *Arenaria* subg. *Odonostemma* (G. Don) F. Williams is included in a clade with *Pseudostellaria* Pax and *Lepyrodiclis*, while members of *Arenaria* subg. *Arenaria*, *Leiosperma* McNeill, and *Porphyrantha* (Fenzl) McNeill, as well as members of the genus *Moebria*, form a clade; *Arenaria* subg. *Arenaria* is itself polyphyletic, consisting of two lineages in the combined analysis (fig. 2) and three in the *matK* analysis (fig. 1). In the *matK* analysis (fig. 1), *Moebria* is also demonstrated to be polyphyletic.

The genus *Minuartia* is also polyphyletic in both analyses. *Minuartia* subg. *Minuartia* is polyphyletic and consists of two clades within clade A; one includes three Holarctic-Eurasian species (*Minuartia laricifolia* [L.] Schinz. & Thell., *Minuartia obtusiloba* [Rydb.] House, and *Minuartia rossii* R. Br. ex Richardson [Graebner]) and is sister to *Scleranthus*. The second clade includes *Sagina* L. and *Colobanthus* Bartl., nested within a group of six mostly North American species (*Minuartia lanceolata* [All.] Mattf., *Minuartia nuttallii* [Pax] Briq., *Minuartia howellii* [S. Wats.] Mattf., *Minuartia douglasii* [Fenzl ex Torrey & A. Gray] Mattf., *Minuartia californica* [A. Gray] Mattf., and *Minuartia rubella* [Wahlenb.] Hiern). The other three subgenera of *Minuartia* are widely dispersed in

our analyses. *Minuartia* subg. *Hymenella* (Moc. & Sesse ex Ser.) McNeill (*Minuartia moebrioides* [DC.] Mattf.) is also in clade A, sister to *Geocarpon minimum* (tribe Geocarpeae). *Minuartia* subg. *Spergella* is in clade C, showing affinity to *Arenaria* subg. *Eremogone* and subg. *Eremogoneastrum*. *Minuartia* subg. *Rhodalsine* (J. Gay) Graebner is very distantly related, appearing in a clade of Paronychioideae that includes part of the tribe Polycarpeae (*Spergularia* and *Spergula*).

Another genus within the Alsinoideae that was shown to be paraphyletic is *Stellaria*. In the *matK* analysis (fig. 1), *Stellaria* has *Myosoton aquaticum* (L.) Moench (sometimes treated as *Stellaria aquatica* [L.] Scop.) and *Plettkea cryptantha* Mattf. nested within it.

The results from these phylogenetic analyses have shown that the genera most closely related to the endemic Hawaiian genus *Schiedea* are *Honckenya* and *Wilhelmsia*. In both the *matK* and combined analyses, the genus *Schiedea* is monophyletic, with bootstrap support of 100, and related to the unspecific genera *Honckenya* and *Wilhelmsia*. In the *matK* analysis (fig. 1), it is sister to *Wilhelmsia*, followed by *Honckenya*, in both the MP and ML analyses. In the combined analysis, the ML tree places *Honckenya* as more closely related to *Schiedea*, while in the MP tree, *Honckenya* and *Wilhelmsia* are sister to each other and to *Schiedea*. In all analyses, *Schiedea*, *Honckenya*, and *Wilhelmsia* are in clade A, with members of Alsinoideae tribes Geocarpeae, Sclerantheae, and Alsineae.

## Discussion

Results from the phylogenetic analysis of three chloroplast gene sequences (*matK*, *trnL-F*, and *rps16*) reveal several important taxonomic and biogeographic discoveries, including that (1) the subfamilies within Caryophyllaceae as currently delimited are not natural groups and should be abandoned, (2) the species-rich alsinoid genera *Arenaria* and *Minuartia* and several other genera are not natural groups and require both reorganization and further study to better reflect phylogenetic relationships, (3) several smaller genera are nested within larger genera, such as *Myosoton* Moench and *Plettkea* Mattf. (in *Stellaria*) and *Velezia* (in *Dianthus*), and (4) the closest relatives to the endemic Hawaiian genus *Schiedea* are a pair of circumboreal taxa, *Honckenya peploides* (L.) Ehrh. and *Wilhelmsia physodes* (Fisch. ex Ser.) McNeill.

The traditional three-subfamily subdivision of the Caryophyllaceae is not reflected in our results, suggesting that the morphological characters used to delimit them are unreliable because of extensive convergent evolution. This is consistent with results from prior phylogenetic studies, including Smis-

shown here along the right-hand side of the phylogeny indicates the three-subfamily system (Alsinoideae, Caryophylloideae, and Paronychioideae) of Pax and Hoffmann (1934) and the associated tribes from Bittrich (1993); alongside this is the tribal alignment proposed in this article. Subgenera of *Arenaria* and *Minuartia* follow McNeill (1962) and are denoted by three-letter codes. *Arenaria*: *Are* = *Arenaria*, *Ere* = *Eremogone*, *Ers* = *Eremogoneastrum*, *Lei* = *Leiosperma*, *Odo* = *Odonostemma*, *Por* = *Porphyrantha*. *Minuartia*: *Hym* = *Hymenella*, *Min* = *Minuartia*, *Rho* = *Rhodalsine*. Superscripts next to taxon names indicate instances of differing topology in the MP strict consensus tree. 1: *Honckenya peploides* and *Wilhelmsia physodes* are sister to each other (BS = 59) and sister to the *Schiedea* clade. 2: *Lepyrodiclis holosteoides* and *Arenaria chamissonis* are sister to each other (BS = 59) and sister to *Pseudostellaria jamesiana* and *Arenaria roseiflora*.

sen et al. (2002) and Fior et al. (2006). One possible scenario would be to treat the three tribes of the Paronychioideae as subfamilies and have the species-rich part of the family, ~2500 of the ~3000 species (R. K. Rabeler, unpublished data), constitute one large subfamily (Alsinoideae + Caryophylloideae); this arrangement seems rather unconventional at best. We believe that a better course of action would be to abandon subfamilies within the Caryophyllaceae and instead recognize at least 11 tribes based on the well-supported lineages from our phylogenetic analyses. Outside of those, we propose to accommodate the relationships we have found within the Alsinoideae. Most of these tribes are in current use in the sense we use here (Bittrich 1993) and have a recognized morphological basis as well. There is some uncertainty in this classification, which we summarize below.

Within subfamily Paronychioideae, results from this study agree with those of Fior et al. (2006), in which this subfamily is a basal grade of taxa, with tribe Corrigioleae monophyletic and sister to the remainder of the subfamily, a monophyletic tribe Paronychieae, and a polyphyletic tribe Polycarpeae. Smissen et al. (2002) found that subfamily Paronychioideae did not form a basal grade but was polyphyletic, with *Spergularia* more closely related to alsinoid taxa than to members of the tribe Polycarpeae. Recognizing tribe Sperguleae Dumort., as Pax and Hoffman (1934) and Eckhardt (1964) did, is appropriate because *Spergula* and *Spergularia* clustered outside of the Polycarpeae, closer to but not inside of the Alsinoideae-Caryophylloideae clade. This intermediate position seems consistent because these taxa share some alsinoid characters (Bittrich 1993; Smissen et al. 2002).

*Paronychia*'s being paraphyletic is consistent with the ITS results of Oxelman et al. (2002), where the "divergent" species in our analysis, *Paronychia kapela* (Hacq.) A. Kern, also clustered with *Herniaria* L. While this suggests that the Old World subg. *Anoplonychia* (Fenzl) Chaudhri, a group of more than 40 species (Chaudhri 1968), may deserve generic recognition, we agree with Oxelman et al. (2002) that further study is warranted, since no study has yet been focused on sampling the diversity within either *Paronychia* or *Herniaria*, genera of ~110 and 45 species (Bittrich 1993), respectively.

In our new tribal classification, the subfamily Paronychioideae is abandoned and replaced by four tribes: Corrigioleae (the clade *Corrigiola* + *Telephium*), Paronychieae (the clade *Gymnocarpus* + *Herniaria* + *Paronychia*), Polycarpeae (the clade containing *Dicheranthus*, *Loeflingia*, *Ortegaia* L., *Polycarpon*, and *Drymaria*, as well as *Pycnophyllum*, formerly in the Alsinoideae), and Sperguleae (the clade including *Spergularia*, *Spergula*, and *Rhodalsine* [*Minuartia* subg. *Rhodalsine*]; figs. 1, 2; table A1). The connate styles of *Pycnophyllum*, an anomaly in the Alsinoideae (Bittrich 1993), are also found in other members of the Polycarpeae. One other consequence of this classification is that the presence of stipules can no longer be used as a defining character for the Polycarpeae and the Sperguleae; only the Corrigioleae and the Paronychieae will be consistently stipulate.

This study provided a better-resolved phylogeny of the Caryophylloideae and Alsinoideae than the previous study by Fior et al. (2006), as a result of both increased taxonomic sampling and additional molecular characters. While the "backbones" of the separate and combined analyses were not

resolved in this study, they likewise were neither well supported nor resolved in the phylogenies of Fior et al. (2006). This may be due to short branch lengths after rapid radiation. Most of the Alsinoideae and a nested Caryophylloideae form a monophyletic group; this is consistent with the view of Bittrich (1993), who hypothesized this on the basis of caryophyllad type embryogeny in these subfamilies, while Paronychioideae have the solanad type.

Subfamily Caryophylloideae was shown to be monophyletic (except for tribe Drypidae) and nested within Alsinoideae in this study, consistent with the findings of Fior et al. (2006) but not those of Smissen et al. (2002); in the latter study, tribe Sileneae was resolved as sister to *Scleranthus* (subfamily Alsinoideae tribe Scleranthae). Treating the Caryophylloideae as tribes Caryophylleae and Sileneae (clades D and E, respectively; figs. 1, 2) is consistent with both traditional and molecular studies and current usage (Bittrich 1993). The increased sampling of *Dianthus* in our *matK* analysis showed that the genus *Dianthus* is paraphyletic, with *Velesia rigida* nested within it (fig. 1); just like *Paronychia*, *Dianthus* is a very large genus (~300 species; Bittrich 1993) that has not been broadly sampled. Our *matK* analysis identified a paraphyletic *Silene* with *Lychnis* nested within it, which is consistent with results of other studies, including Oxelman and Lidén (1995; ITS and 5.8S), Oxelman et al. (1997; *rps16*), Erixon and Oxelman (2008), and Rautenberg et al. (2008; *SIX1* and *YI*). Our combined analysis showed that *Silene* and *Lychnis* were sister to each other, consistent with findings of Popp and Oxelman (2004; RNA polymerase gene family, ITS, and *rps16*), Oxelman et al. (2001; ITS and *rps16*), and Fior et al. (2006); this may be due to the more limited sampling in those analyses. However, independent data indicate that the incongruence between these molecular phylogenies may be due to a reticulate history (Delichère et al. 1999; Frajman et al. 2007). Oxelman (personal communication 2009) indicates that part of the problem may also rest with a misidentified sequence of *Lychnis coronaria* in GenBank, which may be the *matK* sequence used in our study. We could not test whether this sequence is identical to one from *Silene gallica* L. because none are currently available. Further work with new sequences and better sampling should help resolve the issue. The results of the combined analysis (fig. 2) do concur with those of Frajman et al. (2009; *rps16* and ITS) in showing that the genus *Viscaria*, here shown as *Lychnis viscaria*, should be recognized because it is a sister taxon to *Silene* + *Lychnis*.

Within subfamily Alsinoideae, as frequently defined, there are a number of important taxonomic conclusions that arise from this study. The increased resolution of this study confirms the polyphyly of tribe Alsineae (sensu Bittrich 1993), which was not well resolved in Fior et al. (2006); this conclusion is consistent with the findings of Smissen et al. (2002; *ndhF*). We also demonstrated that the tribes Geocarpeae and Scleranthae are nested with taxa in Bittrich's Alsineae, suggesting that the tribal alignment in the Alsinoideae must be reconsidered and most likely more finely subdivided; we have proposed such a revision (figs. 1, 2; table A1). Our results are consistent with those of Smissen et al. (2003; ITS) in showing tribe Pycnophylleae more closely related to the formerly circumscribed tribe Polycarpeae, into which we now include *Pycnophyllum* (figs. 1, 2; table A1).

Before molecular investigations of the Alsinoideae, there were various attempts at grouping the genera within tribe Alsineae. Pax and Hoffmann (1934) recognized two subtribes on the basis of whether the capsules opened by entire or split valves. This concept matches, with only one exception (the genus *Lepyrodiclis*), the members of clade A (entire valves) and clades B + C (split valves) that have traditionally been included in that tribe. McNeill (1962) recognized three “aggregations,” not necessarily representing natural relationships, within the Alsineae, along with a divergent *Schiedea* (which he hypothesized was aligned with the Paronychioideae): (1) the *Stellaria-Cerastium* group (including *Myosoton*, *Holosteum* L., *Moenchia* Ehrh., and maybe *Pseudostellaria*), (2) the *Sagina* group (including *Colobanthus*), and (3) the *Arenaria* group (including *Minuartia*, *Moehringia*, *Wilhelmsia*, *Lepyrodiclis*, *Brachystemma*, and *Honckenya* as well as *Thylacospermum* Fenzl, *Thurya* Boiss. & Balansa, *Gooringia* Williams, *Reicheella* Pax, and *Bufonia* L.).

Our study confirms that McNeill’s first group, with the addition of *Arenaria* subg. *Odonostemma* and *Lepyrodiclis* (in clade B; figs. 1, 2), would constitute tribe Alsineae sensu stricto (type: *Stellaria media* [L.] Vill.). Most of these genera have petals deeply cleft (rarely jagged or nearly entire) or rarely absent and, except for *Lepyrodiclis*, capsules dehiscent by twice as many valves as styles. In both the *matK* and combined analyses, the Andean South American *Plettkea* is nested inside *Stellaria*, sister to the Arctic North American *Stellaria crasipes* Hultén (figs. 1, 2). The nesting of *Plettkea* and *Myosoton* (one species native to Eurasia) within *Stellaria* illustrates a third example of a genus of Caryophyllaceae of more than 100 species that should be the focus of broad sampling and is one of several examples of taxa with indehiscent fruits clustering with genera with capsular fruits. The appearance of *Arenaria chamissonis* Maguire in this clade in the combined analysis reinforces Morton’s (2005b) comments on the uncertain generic placement of this taxon; recent treatments have considered it to be a species of either *Arenaria* or *Stellaria*, but it was originally described as *Cherleria dicranoides* Cham. & Schldl., a genus described by Linnaeus to accommodate a single species now included in *Minuartia*.

McNeill’s (1962) second group, *Sagina* and *Colobanthus*, is a monophyletic group in this study; however, it is clustered with a portion of *Minuartia* subg. *Minuartia* in a clade that also includes *Bufonia* and *Drypis* (clade A, fig. 1), to form what we propose as tribe Sagineae. Each of these genera, except *Drypis*, have petals entire or rarely absent and capsules dehiscent by as many valves as styles. *Sagina*, *Colobanthus*, and *Bufonia* have mostly four or five styles, while the *Minuartia* species here have three. The inclusion of *Drypis*, a spiny perennial from the eastern Mediterranean with hooded sepals, bifid petals, and an indehiscent fruit, is an unexpected result. It is often placed in its own tribe (Drypideae Fenzl) within the Caryophylloideae because of a number of anomalous features (see Bittrich 1993). While it clearly clusters away from the rest of the Caryophylloideae, we suggest that further study is warranted to determine whether it should be retained in the Sagineae or tribe Drypideae should be recognized.

The *Arenaria* “group” does not form a cohesive group, let alone a monophyletic one, as *Arenaria* and *Minuartia* are both polyphyletic and genera such as *Wilhelmsia*, *Lepyrodi-*

*clis*, and *Honckenya* are not closely aligned (figs. 1, 2). *Schiedea* may indeed have a number of morphological features that are “divergent” from many Alsinoideae; based on his anatomical study of flowers and fruits, Rohweder (1970) proposed, but did not publish, the tribe “Alsinidendreae” to segregate it within the Alsinoideae. Our study does show that, contrary to McNeill’s (1962) suspicion, *Schiedea* is clearly not related to the Paronychioideae. The morphologically diverse taxa *Schiedea*, *Wilhelmsia*, *Honckenya*, *Scleranthus*, and *Geocarpon*, as well as members of *Minuartia* subg. *Hymenella* and several species of *Minuartia* subg. *Minuartia*, form a highly supported monophyletic clade that is here treated as the tribe Scleranthae. The inclusion of *Geocarpon* and *Scleranthus*, genera that were formerly in separate tribes of the Alsinoideae, complicates the morphological diagnosis of this clade, in part because of the highly reduced morphology of these taxa. Other than the fact that all of the genera except *Scleranthus* have as many capsule valves as styles, it is difficult to locate morphological features that would unite the assemblage. Most are apetalous, with *Honckenya*, *Wilhelmsia*, and *Minuartia* having entire petals. In both the combined and separate analyses, results are consistent with those of Smissen et al. (2003; ITS) in demonstrating that in *Scleranthus*, Northern Hemisphere species (*Scleranthus perennis*, *Scleranthus annuus*) are sister to Southern Hemisphere ones (*Scleranthus biflorus*), while we did not find that *Sagina* and *Colobanthus* were as closely related to *Scleranthus* as they are to other taxa. Further study, especially involving *Minuartia* sensu lato, is required in this clade; it may reveal morphological and/or molecular data that support further splitting of this tribe.

The delimitation of groups within *Arenaria* has often been disputed, with concepts ranging from a broad, all-inclusive *Arenaria* (Fernald 1919; Maguire 1951), recognizing *Minuartia*, *Arenaria*, *Moehringia*, and up to nine other genera (McNeill 1962), to splitting *Minuartia* into many segregates, e.g., six additional genera to cover the Arctic taxa (Löve and Löve 1976). We demonstrate here not only that these genera should not be united into an all-inclusive “*Arenaria* group” but also that *Arenaria* and *Minuartia* are both polyphyletic.

Three of the subgenera of *Arenaria* that we sampled should be recognized as genera: *Arenaria*, *Odontostemma* Benth. (*Arenaria* subg. *Odontostemma*), and, especially via its placement in a separate clade, *Eremogone* Fenzl (*Arenaria* subg. *Eremogone* and *Eremogoneastrum*). *Eremogone* has been adopted for the recent *Flora of North America* treatment (Hartman and Rabeler 2004; Hartman et al. 2005). We propose that *Eremogone* (clade C, figs. 1, 2) and *Thylacospermum* (clade C, fig. 2) should constitute a new tribe Eremogoneae (Rabeler & W.L. Wagner; *Thylacospermum* is tentatively included in this tribe because it is absent from the *matK* analysis and weakly supported in the combined analysis (fig. 2)).

We place *Arenaria* sensu stricto and *Moehringia* in tribe Arenarieae Kitt. (figs. 1, 2; table A1), the plants having petals entire or rarely absent and capsules dehiscent by twice as many valves as styles. While members of the Eremogoneae have narrow, grasslike leaves, most species of *Arenaria* have broader, often ovate to lanceolate leaves. Although *Moehringia* is easily characterized by a unique appendaged seed, we also demonstrate that there is little support for recognizing it

as a genus separate from *Arenaria*; this is consistent with the results of Fior and Karis (2007; ITS and *matK*).

Similarly, our results show that three of the subgenera of *Minuartia* should also be removed from *Minuartia*. *Minuartia platyphylla* and several other closely related species (not sampled here) from the Canary Islands and Mediterranean coasts, currently placed in *Minuartia* subg. *Rhodalsine*, would be better treated as members of the genus *Rhodalsine*. These taxa appear to be related to *Spergularia*, which, as McNeill (1962) noted, they closely resemble except for lacking stipules; we place both of these genera in the tribe Sperguleae. Because of the distinctive quadrangular stems and spreading sepals and capsule valves of the Mexican endemic *Minuartia moebringioides*, its retention in *Minuartia* as the only member of *Minuartia* subg. *Hymenella* was questioned by McNeill (1962). It is clear from our results that it should now be removed and regarded as the only member of a unispecific genus, *Triplateia* Bartl. (*Hymenella* Moç. & Sessé ex Ser. in DC. [1824] non E.M. Fries [1822]). Although our results suggest that *Minuartia* subg. *Spergella* belongs within *Eremogone*, no previous study of this distinctive taxon gives support for this; reestablishment of *Phlebanthia* Rchb. to accommodate the two or three species currently recognized in the subgenus *Spergella* may be a wiser interim step.

While results also suggest that *Minuartia* subg. *Minuartia* should be split (figs. 1, 2) into a Eurasian-Holarctic and a North American clade, this result must be tempered by the limited sampling in this study. Sampled taxa belong to seven of the 12 sections recognized by McNeill (1962). Since one of the “missing” five sections is *Minuartia*, a group of ~50 mostly Mediterranean species, we cannot postulate into which clade *Minuartia* sensu stricto would be placed. Another missing section is sect. *Uninerviae* (Fenzl) Mattf., a section of six to eight species most diverse in southeastern North America. Given its occurrence near the native range of *Geocarpon minimum* and the North American *Minuartia* taxa clustering with *Sagina*, it would be most interesting to see where *Minuartia* sect. *Uninerviae* would be placed. Our results show that molecular data do support some of the segregates proposed by Löve and Löve (1976), which were primarily based on differences in base chromosome number. Since the nine taxa sampled here would be placed in eight(!) different genera by Löve and Löve (1976), it is evident that *Minuartia* must be more completely sampled before any additional conclusions can be drawn.

Results from this study confirmed prior phylogenetic analyses that showed that the Hawaiian genus *Schiedea* is monophyletic and the result of a single colonization event to the islands (Soltis et al. 1996; Wagner et al. 2005). An important and surprising result from this study was the identification of the closest living relatives of Hawaiian *Schiedea*: the unispecific genera *Honckenya* and *Wilhelmsia*, primarily from the Arctic and subarctic regions of both Eurasia and North America. *Wilhelmsia* is similar to *Honckenya* in habit, but their resemblance was previously hypothesized to be due to convergence associated with their riparian or maritime habitats (McNeill 1962, 1980; Wagner 2005a, 2005b). Further study with the addition of samples of *Minuartia* sect. *Uninerviae* will help to resolve whether the Hawaiian *Schiedea* did originate from the Arctic via North America.

While we have proposed a classification with 11 tribes, we realize that there are several limitations that could expand it to include additional tribes. Several taxa, if/when sampled, could produce changes in the scheme. The placement of *Drypis* within the Sagineae is tentative; it may be better segregated as tribe Drypideae. While we were unable to obtain *Habrosia spinuliflora*, an eastern Mediterranean herb and the only member of tribe Habrosieae, Smitsen et al. (2003) included it in their ITS2 study. The sequence most closely related to *Habrosia* was *Drypis*; should tribe Habrosieae be maintained, or do these taxa together form a tribe? From a short ITS2 sequence, Smitsen et al. (2003) was able to place *Pentastemonodiscus monochlamydeus* Rech.f., a highly reduced plant from Afghanistan, within the family but not within a subfamily; placement near *Scleranthus*, as suggested by Bittrich (1993), was not confirmed. The current composition of the Scleranthae would be subject to change if a broadly based study of *Minuartia* were to be completed. As noted above, we did not sample a member of *Minuartia* sect. *Minuartia*; resolution of the placement of *Minuartia* sensu stricto might allow subdividing our Scleranthae into more morphologically consistent groups.

Our results provide the most in-depth understanding of the relationships within the Caryophyllaceae, especially within the large, often recognized subfamily Alsinoideae, and clarify the position of some morphologically divergent lineages as well as confirming that the classification within the family is in need of significant revision. Several steps were taken in this article to revise the classification on the basis of molecular data, including abandoning the traditional three-subfamily system in favor of one based on tribes. We propose segregating the Caryophyllaceae into at least 11 tribes based on highly supported monophyletic groups; further study with increased sampling and morphological data may warrant recognizing additional tribes within the family.

#### Description of Tribe Eremogoneae Rabeler & W.L. Wagner, tribus nov.

Plantae perennes, raro annuae. Folia filiformia ad subulata, saepe longissima linearia, graminiformia, in rosulis vegetativis et prope basim caulium floriferorum congesta; apices saepe apiculati. Inflorescentia cymis solitariis vel paucis terminalis constata; cymae interdum confertae, capituliformae, interdum ad florem unicum reductae. Flores infirme, raro valde (*Thylacospermum*), perigyni. Sepala margine scarioso, saepe late scarioso.

Plants perennial, rarely annual. Leaves filiform to subulate, often long-linear and grasslike, congested in the vegetative rosettes and at or near base of flowering stems, apex often apiculate. Inflorescence of one or more terminal cymes, sometimes compressed to headlike, sometimes flowers solitary. Flowers weakly perigynous, rarely strongly so (*Thylacospermum*). Sepals with scarios margins, often broad.

Type: *Eremogone* Fenzl (*Eremogone* Fenzl, Vers. Darst. Verbr. Alsin.: 13. 1833—Lectotype [see McNeill in Notes Roy. Bot. Gard. Edinburgh 24:120. 20 Sep 1962]; *Eremogone graminifolia* Fenzl, Vers. Darst. Verbr. Alsin.: 37. 1833).

### Acknowledgments

This study was funded in part by a grant from the Smithsonian Institution Scholarly Studies program to W. L. Wagner, M. Nepokroeff, and E. A. Zimmer and a fellowship from the Botany Department to D. T. Harbaugh, as well as by funds from the Office of Research, University of South Dakota, to M. Nepokroeff. In addition, M. Nepokroeff was supported by the National Tropical Botanical Garden McBryde Senior Research Fellowship program during analysis of these data and by funds from NSF grant DEB 0445410. We thank B. Jacobs, C. Krogman, and C. Griffith at the University of South Da-

kota, as well as M. Van Horn and C. McCracken at the Smithsonian, for laboratory assistance. We also thank the following individuals, who supplied leaf material for this study: L. Ahart, F. Ashby, C. Best, B. Castro, M. Chase, L. Consaul, S. Downie, L. Gillespie, S. Gonzales, J. R. Grant, M. Hershkovitz, L. Janeway, D. Lorence, K. McCue, R. Muriillo, D. Murray, C. Parker, S. Perlman, A. Sakai, A. Santos, P. Sklenar, R. Smissen, R. Soreng, M. E. Timana, S. Weller, R. Worthington, and S. Zamudio. Finally, we thank B. Baldwin and C. Hobbs for assisting D. T. Harbaugh with phylogenetic analyses and P. Julian and T. Wendt for assisting W. L. Wagner with field collections.

### Literature Cited

- Appelquist WL, WL Wagner, EA Zimmer, M Nepokroeff 2006 Molecular evidence resolving the systematic position of *Hectorella* (Portulacaceae). *Syst Bot* 31:310–319.
- Ballard HE, KJ Sytsma 2000 Evolution and biogeography of the woody Hawaiian violets (*Viola*, Violaceae): Arctic origins, herbaceous ancestry and bird dispersal. *Evolution* 54:1521–1532.
- Bittrich V 1993 Caryophyllaceae. Pages 206–230 in K Kubitzki, V Bittrich, J Rohwer, eds. *The families and genera of vascular plants*. Vol 2. Springer, Berlin.
- Brockington SF, R Alexandre, J Ramdial, MJ Moore, S Crawley, A Dhingra, K Hilu, DE Soltis, PS Soltis 2009 Phylogeny of the Caryophyllales sensu lato: revisiting hypotheses on pollination biology and perianth differentiation in the core Caryophyllales. *Int J Plant Sci* 170:627–643.
- Chaudhri M N 1968 A revision of the Paronychiinae. *Mededelingen van het Botanisch Museum en Herbarium Van Rijksuniversiteit te Utrecht*, no. 285. Gianotten, Tilburg. 440 pp.
- Cuénoud P, V Savolainen, LW Chatrou, M Powell, RJ Grayer, MW Chase 2002 Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcl*, *atpB*, and *matK* DNA sequences. *Am J Bot* 89:132–144.
- Delichère C, J Veuskens, M Hernould, N Barbacar, A Mouras, I Negrutiu, F Monéger 1999 *SIY1*, the first active gene cloned from a plant Y chromosome, encodes a WD-repeat protein. *EMBO J* 18:4169–4179.
- Desfeux C, B Lejeune 1996 Systematics of Euromediterranean *Silene* (Caryophyllaceae): evidence from a phylogenetic analysis using ITS sequences. *C R Acad Sci Ser III Sci Vie* 319:351–358.
- Downie D, S Katz-Downie, K Cho 1997 Relationships in the Caryophyllales as suggested by phylogenetic analysis of partial chloroplast DNA ORF2280 homolog sequences. *Am J Bot* 84:252–273.
- Downie SR, JD Palmer 1994 A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. *Syst Bot* 19:236–252.
- Eckhardt T 1964 Centrospermae. Pages 79–101 in H Melchior, ed. *A. Engler's Syllabus der Pflanzenfamilien*. Borntraeger, Berlin.
- Eggs F, M Popp, M Nepokroeff, WL Wagner, B Oxelman 2007 The origin and number of introductions of the Hawaiian endemic *Silene* species (Caryophyllaceae). *Am J Bot* 94:210–218.
- Endress PK 1996 Homoplasy in angiosperm flowers. Pages 303–325 in MJ Sanderson, L Hufford, eds. *Homoplasy: the recurrence of similarity in evolution*. Academic Press, San Diego, CA.
- Erixon P, B Oxelman 2008 Reticulate or tree-like chloroplast DNA evolution in *Sileneae* (Caryophyllaceae)? *Mol Phylogenet Evol* 48:313–325.
- Fernald ML 1919 The unity of the genus *Arenaria*. *Rhodora* 21:1–22.
- Fernandes A, MT Leitão 1971 Contribution à la connaissance cytotaxonomique des Spermatophyta du Portugal. III. Caryophyllaceae. *Bol Soc Broteriana*, ser 2, 45:143–176.
- Fior S, PO Karis 2007 Phylogeny, evolution and systematics of *Moehringia* (Caryophyllaceae) as inferred from molecular and morphological data: a case of homology reassessment. *Cladistics* 23:362–372.
- Fior S, PO Karis, G Casazza, L Minuto, F Sala 2006 Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast *matK* and nuclear rDNA ITS sequences. *Am J Bot* 93:399–411.
- Frajman B, F Eggen, B Oxelman 2007 Hybrid origins and homoploid reticulate evolution within *Heliosperma* (Sileneae, Caryophyllaceae): a multigene phylogenetic approach with relative dating. In B. Frajman, ed. *Taxonomy and reticulate phylogeny of Heliosperma and related genera* (Sileneae, Caryophyllaceae). PhD diss. Uppsala University.
- Frajman B, N Heidari, B Oxelman 2009 Phylogenetic relationships of *Atocion* and *Viscaria* (Sileneae, Caryophyllaceae) inferred from chloroplast, nuclear ribosomal, and low-copy gene DNA sequences. *Taxon* 58:811–824.
- Greuter W 1995 *Silene* (Caryophyllaceae) in Greece: a subgeneric and sectional classification. *Taxon* 44:543–581.
- Harris EM, WL Wagner 1995 Outgroup determination for the problematic genera *Schiedea* and *Alsinodeudron* (Alsinoideae: Caryophyllaceae) using floral ontogeny. *Am J Bot* 82 (suppl.):19.
- Hartman RL, RK Rabeler 2004 New combinations in North America *Eremogone* (Caryophyllaceae). *Sida* 21:237–241.
- Hartman RL, RK Rabeler, FH Utech 2005 *Eremogone*. Pages 56–70 in *Flora of North America Editorial Committee, eds. Flora of North America North of Mexico*. Vol 5. Oxford University Press, New York.
- Holmgren PK, NH Holmgren 1998 Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden, Bronx. <http://sweetgum.nybg.org/ih/>.
- Hufford L 1996 Ontogenetic evolution, clade diversification, and homoplasy. Pages 271–302 in MJ Sanderson, L Hufford, eds. *Homoplasy: the recurrence of similarity in evolution*. Academic Press, San Diego, CA.
- Hutchinson J 1973 The families of flowering plants arranged according to a new system based on their probable phylogeny. 3d ed. Clarendon, Oxford.
- Johnson LA, DE Soltis 1995 Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. *Ann Mo Bot Gard* 82:149–175.
- Kool A, A Bengtson, M Thulin 2007 Polyphyly of *Polycarpon* (Caryophyllaceae) inferred from DNA sequence data. *Taxon* 56:775–788.
- Leonhardt R 1951 Phylogenetisch-systematische Betrachtungen. II.

- Gedanken zur systematischen Stellung, bzw. Gliederung einiger Familien der Choripetalen. Oesterr Bot Z 98:1–43.
- Lidén M, M Popp, B Oxelman 2001 A revised generic classification of the tribe *Sileneae* (Caryophyllaceae). Nord J Bot 20:513–518.
- Löve A, D Löve 1976 Nomenclatural notes on Arctic plants. Bot Not 128:497–523.
- Maguire B 1951 Studies in the Caryophyllaceae. V. *Arenaria* in America North of Mexico: a conspectus. Am Midl Nat 46:493–511.
- Manos PS, KP Steele 1997 Phylogenetic analyses of higher Hamamelididae based on plastid sequence data. Am J Bot 84:1407–1419.
- McNeill J 1962 Taxonomic studies in the Alsinoideae. I. Generic and infra-generic groups. Notes R Bot Gard Edinb 24:79–155.
- 1973 *Gypsophila* and *Stellaria*: an unexpected problem in generic delimitation. Notes R Bot Gard Edinb 32:389–395.
- 1980 The delimitation of *Arenaria* (Caryophyllaceae) and related genera in North America with 11 new combinations in *Minuartia*. Rhodora 82:495–502.
- Morton JK 2005a *Silene*. Pages 168–216 in Flora of North America Editorial Committee, eds. Flora of North America North of Mexico. Vol 5. Oxford University Press, New York.
- 2005b *Stellaria*. Pages 96–114 in Flora of North America Editorial Committee, eds. Flora of North America North of Mexico. Vol 5. Oxford University Press, New York.
- Oxelman B, B Ahlgren, M Thulin 2002 Circumscription and phylogenetic relationships of *Gymnocarpos* (Caryophyllaceae-Paronychioideae). Edinb J Bot 59:221–237.
- Oxelman B, M Lidén 1995 Generic boundaries in the tribe *Sileneae* (Caryophyllaceae) as inferred from nuclear rDNA sequences. Taxon 44:525–542.
- Oxelman B, M Lidén, D Berglund 1997 Chloroplast *rps16* intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). Plant Syst Evol 206:393–410.
- Oxelman B, M Lidén, RK Rabeler, M Popp 2001 A revised generic classification of the tribe *Sileneae* (Caryophyllaceae). Nord J Bot 20: 743–748.
- Palmer EJ, JA Steyermark 1950 Notes on *Geocarpon minimum* Mackenzie. Bull Torrey Bot Club 77:268–273.
- Pax F, K Hoffmann 1934 Caryophyllaceae. Pages 275–364 in A Engler, H Harms, eds. Die natürlichen Pflanzenfamilien. 2d ed. Engelmann, Leipzig.
- Popp M, B Oxelman 2001 Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. Mol Phylogenet Evol 20:474–481.
- 2004 Evolution of a RNA polymerase gene family in *Silene* (Caryophyllaceae): incomplete concerted evolution and topological congruence among paralogues. Syst Biol 53:914–932.
- Rabeler RK, RL Hartman 2005 Caryophyllaceae. Pages 3–8 in Flora of North America Editorial Committee, eds. Flora of North America North of Mexico. Vol 5. Oxford University Press, New York.
- 2007 *Eremogone cliftonii* (Caryophyllaceae), a new species from California. Madroño 54:329–333.
- Rautenberg A, D Filatov, B Svennblad, N Heidari, B Oxelman 2008 Conflicting phylogenetic signals in the SIX1/Y1 gene in *Silene*. BMC Evol Biol 8:299.
- Rettig JH, HD Wilson, JR Manhart 1992 Phylogeny of the Caryophyllales: gene sequence data. Taxon 41:201–209.
- Reveal JL 2007 Index nominum supragenericorum plantarum vascularium. Alphabetical listing by family of validly published suprageneric names: families Cabombaceae through Cytinaceae. <http://www.plantsystematics.org/reveal/pbio/fam/famC.html>.
- Rohweder O 1970 Centrospermen-Studien. 4. Morphologie und Anatomie der Blüten, Früchte und Samen bei Alsinoideen und Paronychioideen s. lat. (Caryophyllaceae). Bot Jahrb Syst 90:201–271.
- Simmons MP, H Ochoterena 2000 Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381.
- Smitsen RD, JC Clement, PJ Garnock-Jones, GK Chambers 2002 Subfamilial relationships within Caryophyllaceae as inferred from 5' *ndhF* sequences. Am J Bot 89:1336–1341.
- Smitsen RD, PJ Garnock-Jones, GK Chambers 2003 Phylogenetic analysis of ITS sequences suggests a Pliocene origin for the bipolar distribution of *Scleranthus* (Caryophyllaceae). Aust Sys Bot 16:301–315.
- Soltis PS, DE Soltis, SG Weller, AK Sakai, WL Wagner 1996 Molecular phylogenetic analysis of the Hawaiian endemics *Schiedea* and *Alsinodendron* (Caryophyllaceae). Syst Bot 21:365–379.
- Stamatakis A, P Hoover, J Rougemont 2008 A fast bootstrapping algorithm for the RAXML web-servers. Syst Biol 57:758–771.
- Stamatakis A, M Ott, T Ludwig 2005 RAXML-OMP: an efficient program for phylogenetic inference on SMPs. Pages 288–302 in Proceedings of 8th International Conference on Parallel Computing Technologies (PaCT2005). Lecture Notes in Computer Science, no. 3506. Springer, Berlin.
- Steele KP, R Vigalys 1994 Phylogenetic analysis of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. Syst Bot 19: 126–142.
- Swofford DL 2002 PAUP\*: phylogenetic analysis using parsimony and other methods, v 4.0b10. 2d ed. Sinauer, Sunderland, MA.
- Taberlet P, L Gielly, G Pautou, J Bouvet 1991 Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109.
- Takhtajan A 1997 Diversity and classification of flowering plants. Columbia University Press, New York.
- 2009 Flowering plants. 2nd ed. Springer, Berlin.
- Tzvelev N 2001 De generibus tribus *Sileneae* DC. (Caryophyllaceae) in Europa orientali. Nov Syst Plant Vasc 33:90–113.
- Wagner WL 2005a *Honckenya*. Pages 137–140 in Flora of North America Editorial Committee, eds. Flora of North America North of Mexico. Vol 5. Oxford University Press, New York.
- 2005b *Wilhelmsia*. Pages 136–137 in Flora of North America Editorial Committee, eds. Flora of North America North of Mexico. Vol 5. Oxford University Press, New York.
- Wagner WL, EM Harris 2000 A unique Hawaiian *Schiedea* (Caryophyllaceae: Alsinoideae) with only five fertile stamens. Am J Bot 87: 153–160.
- Wagner WL, DR Herbst, SH Sohmer 1999 Caryophyllaceae. Pages 498–528 in Manual of the flowering plants of Hawai'i. Rev ed. Vol 1. University of Hawai'i Press and Bishop Museum Press, Honolulu.
- Wagner WL, SG Weller, AK Sakai 1995 Phylogeny and biogeography in *Schiedea* and *Alsinodendron* (Caryophyllaceae). Pages 221–258 in WL Wagner, VA Funk, eds. Hawaiian biogeography: evolution on a hot spot archipelago. Smithsonian Institution Press, Washington, DC.
- 2005 Monograph of *Schiedea* (Caryophyllaceae-Alsinoideae). Syst Bot Monogr 72:1–169.
- Weller SG, WL Wagner, AK Sakai 1995 A phylogenetic analysis of *Schiedea* and *Alsinodendron* (Caryophyllaceae: Alsinoideae): implications for the evolution of breeding systems. Syst Bot 20:315–337.