



# Molecular phylogenetic analysis of *Arenaria* (Caryophyllaceae: tribe Arenarieae) and its allies inferred from nuclear DNA internal transcribed spacer and plastid DNA *rps16* sequences

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The systematics and phylogeny of the genus *Arenaria* and allied genera are unresolved. The use of morphological data has resulted in contradictory taxonomic concepts in the past due to their homoplastic nature. We present a phylogenetic analysis based on internal transcribed spacer (ITS) and *rps16* sequence data of 140 (132 taxa) and 131 (120 taxa) accessions, respectively. Maximum parsimony and Bayesian analyses of each marker produced nearly congruent trees. Monophyly of *Arenaria s.s.* and *Eremogone* is confirmed here. Our results corroborate earlier results indicating that *Arenaria* subgenus *Odontostemma* is monophyletic, but outside the core group of *Arenaria*. *Arenaria* subgenus *Solitaria* is sister to *Odontostemma* and also not closely related to the latter; both of these subgenera are excluded from *Arenaria* and treated as distinct genera. The molecular data indicate that the ‘*Arenaria s.s.* clade’ consists of a few well-supported subgroups and that the current subgeneric classification of the genus does not reflect evolutionary history. *Arenaria* subgenus *Leiosperma* is clearly monophyletic, but we reduce it to sectional level. Our molecular data show that the monotypic *Arenaria* subgenera *Porphyrantha* and *Areniastrum* are nested in *A. subgenus Arenaria*, whereas subgenus *Eremogoneastrum* is included in *Eremogone*. None of the species-rich sections in subgenus *Arenaria* is monophyletic. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 178, 648–669.

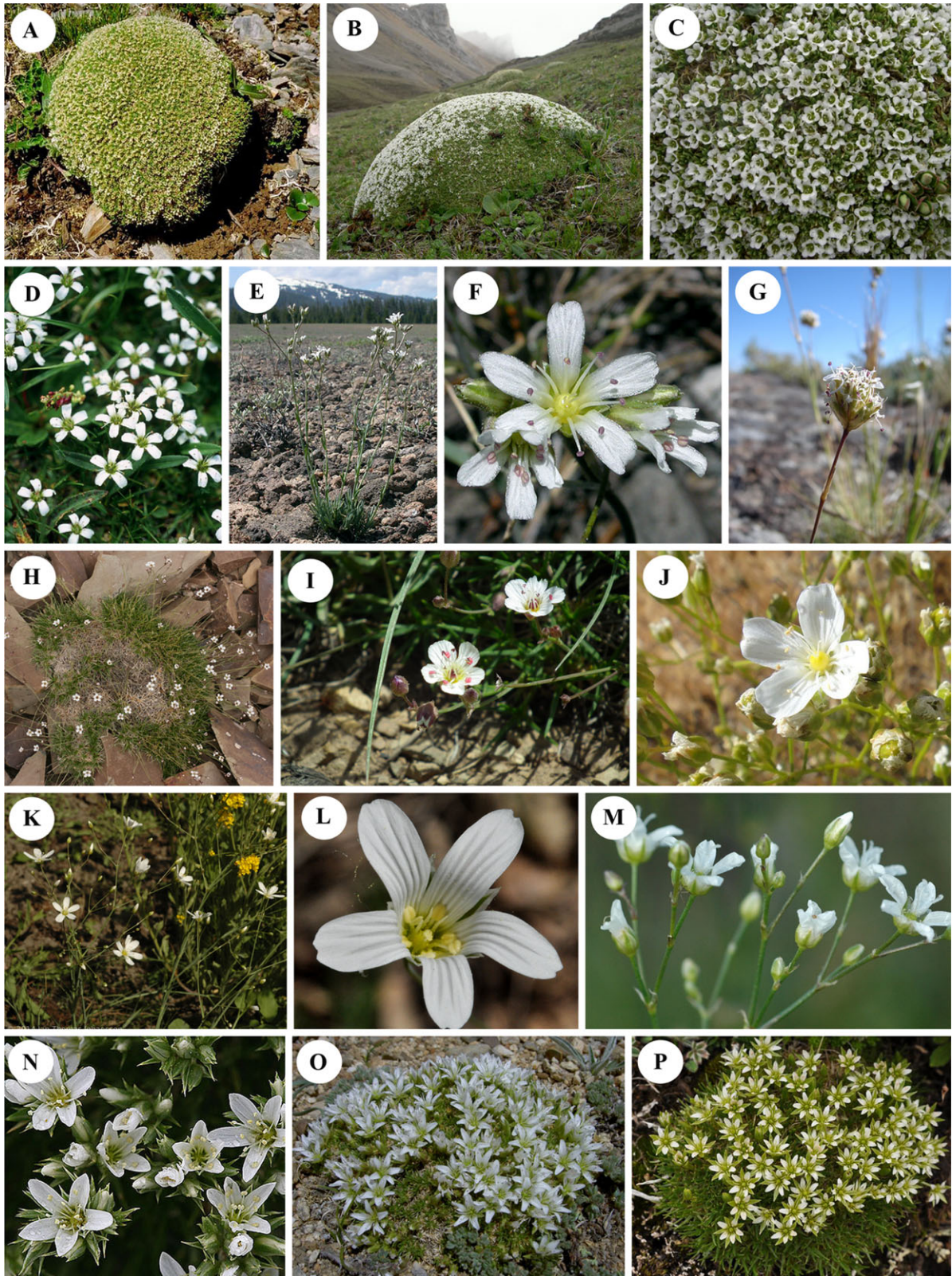
**ADDITIONAL KEYWORDS:** biogeography – Caryophyllales – determination key – *Eremogone* – *Moehringia* – *Odontostemma* – *Solitaria* – subgeneric classification – taxonomy – tribe Sileneae.

## INTRODUCTION

*Arenaria* L. comprises between 150 and more than 300 species, depending on the author and different generic concepts applied (McNeill, 1962; Bittrich, 1993; Charter & Halliday, 1993; Zhou, 1996; Zhengyi,

Zhou & Wagner, 2001; Rabeler & Hartman, 2005). They occur mainly in northern temperate to arctic regions (Zhengyi *et al.*, 2001). The highest number of species and subgenera inhabit areas in the Mediterranean and west-central Asia reaching eastward to the Qinghai–Tibetan Plateau in China (Zhou, 1996). *Arenaria* species show a variety of life forms (Figs 1, 2) consisting of small annuals, densely caespitose to pulvinate, spiny suffruticose perennials, succulent

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**Figure 1.** See caption on next page.

**Figure 1.** Habit and morphological variation in *Arenaria* and *Eremogone*. A, *Arenaria densissima* (subgenus *Dolophragma*), photo by David E. Boufford; B–C, *A. polytrichoides* (subgenus *Dolophragma*), photo by Harry Jans; D, *A. przewalskii* (subgenus *Dolophragma*), photo by David E. Boufford; E–F, *Eremogone pumicola* (subgenus *Eremogone*), photo by Kier Morse; G, *E. congesta* (subgenus *Eremogone*), photo by Sheri Hagwood; H, *E. insignis* (subgenus *Eremogone*), photo by Harald Pauli; I, *E. persica* (subgenus *Eremogone*), photo by M. Tehranian; J, *E. macradenia* (subgenus *Eremogone*), photo by Neal Kramer; K–L, *E. procera* (subgenus *Eremogone*), photo by Jan Thomas Johansson; M, *E. biebersteinii* (subgenus *Eremogone*), photo by Jan Thomas Johansson; N, *E. hookeri* (subgenus *Eremogoneastrum*), photo by Ernie Marx; O, *A. bryophylla* (subgenus *Eremogoneastrum*), photo by Harry Jans; P, *E. kansuensis* (subgenus *Eremogoneastrum*), photo by Harry Jans. Homepages: A, D: <http://hengduan.huh.harvard.edu/fieldnotes/specimens/>; B–C, O, P: <http://www.jansalpines.com/>; E–F, J: <http://calphotos.berkeley.edu/>; G: <http://www.wildflowersearch.com/>; H: <http://www.gloria.ac.at/>; K–M: <http://angio.bergianska.se/>; N: <http://www.easterncoloradowildflowers.com/>.

maritime herbs, xerophytes adapted to Mediterranean and steppe climates, and lax broadleaved mesophytes (McNeill, 1962). In the widely used taxonomic classification of Caryophyllaceae (Bittrich, 1993), *Arenaria* is included in subfamily Alsinoideae with 27 additional genera. According to most authors, *Arenaria* is a member of tribe Alsineae, consisting of 23 additional genera, and is closely related to *Moehringia* L. (McNeill, 1962; Bittrich, 1993; Fior & Karis, 2007), but the most recent classification system of Caryophyllaceae assigned it to tribe Arenarieae (Harbaugh *et al.*, 2010).

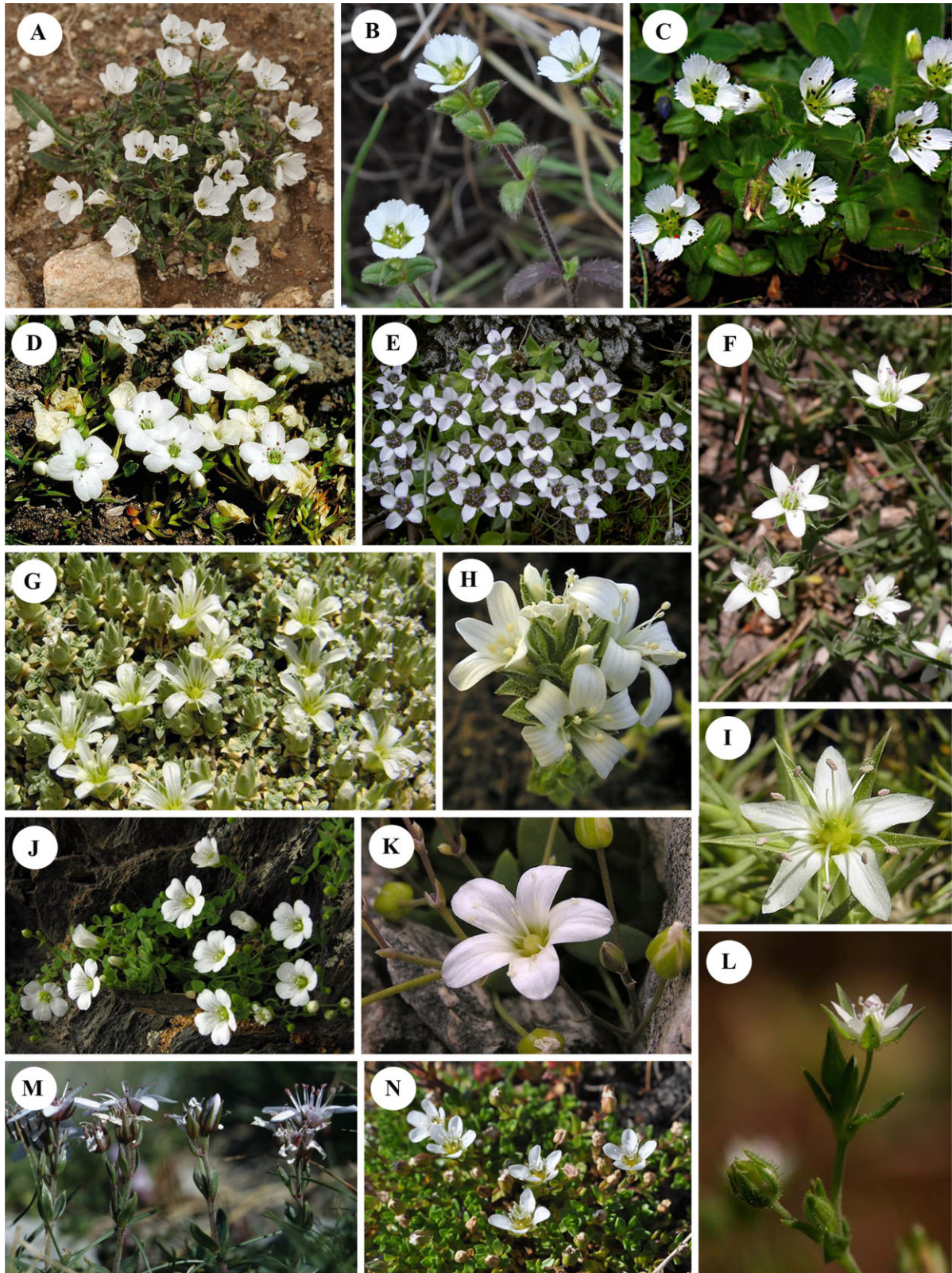
In the most comprehensive taxonomic treatment of Alsinoideae, McNeill (1962) divided Alsineae into three groups: the *Arenaria* group comprising *Arenaria*, *Brachystemma* D. Don, *Bufonia* L., *Gooringia* Williams, *Honckenya* Ehrh., *Lepyrodiclis* Fenzl, *Reicheella* Pax, *Minuartia* L., *Moehringia*, *Thurya* Boiss. & Balansa, *Thylacospermum* Fenzl and *Wilhelmsia* Rchb. (= *Merckia* Fisch.); the *Stellaria-Cerastium* group including *Cerastium* L., *Myosoton* Moench, *Holostium* L., *Moenchia* Ehrh., *Pseudostellaria* Pax and *Stellaria* L.; and the *Sagina* group consisting of *Sagina* L. and *Colobanthus* Bartl. Previous molecular phylogenetic data (Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011) have revealed that Alsineae (*sensu* Pax & Hoffmann, 1934; McNeill, 1962; Bittrich, 1993) and their subgroups are clearly polyphyletic except for the *Sagina* group, which is now best treated as a small unit called tribe Sagineae.

The taxonomic history of *Arenaria* is complex and has, during the course of time, been intertwined with several other genera (Table 1). One of the major questions in subfamily Alsinoideae relates to the generic boundaries around *Arenaria* (Pax & Hoffmann, 1934; Maguire, 1951; McNeill, 1962) and whether it should be broadly or more narrowly circumscribed. If one accepts a narrow concept of *Arenaria*, the 'Arenaria complex' includes three large genera, *Arenaria*, *Minuartia* and *Moehringia*, and several smaller ones including *Brachystemma*, *Cherleria* L., *Gouffea* Robill. & Castagne ex DC., *Greniera* J. Gay, *Honckenya*, *Lepy-*

*rodiclis*, *Moehringella* H. Neumayer, *Queria* L., *Rhodalsine* J. Gay, *Triplateia* Bartl. (= *Hymenella* Ses. & Moc. ex DC.) and *Wilhelmsia* (McNeill, 1962). Some authors even split *Minuartia* into additional genera (Löve & Löve, 1975), a notion recently confirmed by a molecular survey of *Minuartia* (Dillenberger & Kadereit, 2014). A broad concept of *Arenaria* comprises all of the above-mentioned genera as synonyms (Fernald, 1919; Maguire, 1951) of *Arenaria*.

The infrageneric classification of *Arenaria* s.s. has also been problematic and included such groups as *Eremogone*, *Dolophragma* and *Odontostemma*, one or more of which have been treated either as genera (Fenzl, 1840, 1842; Ikonnikov, 1973; Rabeler & Hartman, 2005) or as subgenera by others (McNeill, 1962; Bittrich, 1993) (Table 1). Additionally, some species were described more than once under different names, reflecting the widespread occurrence of the genus worldwide.

In one of the oldest and most comprehensive treatments on *Arenaria*, Fenzl (1840, 1842) placed 11 genera referable to the 'Arenaria complex' into three tribes (Table 1) mainly based on the type of capsule dehiscence and the number of its valves proportional to the styles. Bentham & Hooker (1862) preserved only three genera, *Arenaria*, *Brachystemma* and *Queria*, and transferred the other genera into unranked subgeneric taxa under *Arenaria*. Boissier (1867) mainly followed Fenzl's (1840) system and arranged 39 *Arenaria* species known to him in the area of the *Flora Orientalis* into three unranked groups (indicated by '§'), *Eremogoneae*, *Sclerophyllae* and *Euthaliae*. In the first comprehensive revision of *Arenaria*, Williams (1898) classified 168 *Arenaria* species into seven subgenera, again following Fenzl's system. Maguire (1951), in his synopsis of the North American members of *Arenaria*, treated *Honckenya*, *Minuartia*, *Moehringia* and *Wilhelmsia* as sections of *Arenaria*. McNeill (1962) split *Arenaria* into ten subgenera, which in turn were further divided into 24 sections and several series. The morphological features characterizing subgenera recognized in



**Figure 2.** See caption on next page.

**Figure 2.** Habit and morphological variation in *Arenaria* and *Eremogone*. A, *A. roseiflora* (subgenus *Odontostemma*), photo by Harry Jans; B, *A. barbata* var *hirsutissima* (subgenus *Odontostemma*), photo by Jan Thomas Johansson; C, *A. pogonantha* (subgenus *Odontostemma*), photo by David E. Boufford; D, *A. forrestii* (subgenus *Solitaria*), photo by David E. Boufford; E, *A. glanduligera* (subgenus *Solitaria*), photo by Harry Jans; F, *A. lanuginosa* (subgenus *Leiosperma*), photo by Brad Boyle; G, *A. tetraquetra* (subgenus *Arenaria*), photo by Andrés Ivorra; H, *A. arcuatociliata* (subgenus *Arenaria*), photo by Andrés Ivorra; I, *A. pungens* (subgenus *Eremogone*), photo by Andrés Ivorra; J, *A. fontqueri* (section *Pseudomoehringia*), photo by Andrés Ivorra; K, *A. suffruticosa* (section *Pseudomoehringia*), photo by Andrés Ivorra; L, *A. serpyllifolia* (subgenus *Arenaria*), photo by Patrick J. Alexander; M, *A. purpurascens* (subgenus *Arenaria*), photo by Harald Pauli; N, *A. norvegica* (subgenus *Arenaria*), photo by Jan Thomas Johansson. Homepages: A, E: <http://www.jansalpines.com>; B: <http://angio.bergianska.se>; C, D: <http://hengduan.huh.harvard.edu/fieldnotes/specimens>; F: <http://tcf.bh.cornell.edu>; G–K: <http://www.almerinatura.com>; L: <http://plants.usda.gov/java>; M: <http://www.gloria.ac.at>; N: <http://angio.bergianska.se>.

*Arenaria* by McNeill (1962) are summarized in Table 2. Since then, most authors have followed McNeill and treated this complex as a set of several different genera. Several authors have amended McNeill's concept by segregating the genus *Eremogone* Fenzl (Ikonnikov, 1973; Löve & Löve, 1975; Rabeler & Hartman, 2005).

Despite considerable efforts to clarify the delimitation of *Arenaria* and closely related genera, problems in determining useful morphological characters have hindered producing definitive treatments; comparable to many other genera in the family (Kurtto, 2001; Oxelman *et al.*, 2001), the limits of *Arenaria* in relation to its closely related genera are somewhat uncertain. There have been several recent transfers of species between *Arenaria* and *Eremogone*, *Minuartia* and *Moehringia* (e.g. McNeill, 1980; Fior & Karis, 2007; Dillenberger & Kadereit, 2014) as these features have been reinterpreted.

Although Caryophyllaceae as a whole are well defined by apomorphic characters, infrafamilial classification is still in a state of flux. Bittrich (1993) suggested that the relationships between subfamilies and tribes are highly blurred, owing to the apparent convergent evolution of morphological characters. Thus, the high level of homoplasy in morphological characters and problems in determining morphological synapomorphies indicate the importance of molecular data in understanding the relationships in the family (Smitsen *et al.*, 2002; Fior *et al.*, 2006; Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011). In subfamily Caryophylloideae (shown to be non-monophyletic; see Harbaugh *et al.*, 2010), molecular phylogenetic studies of tribe Sileneae (Oxelman & Liden, 1995; Oxelman, Lidén & Berglund, 1997) have revealed the inadequacy of current classifications and the problematic process in distinguishing well-supported monophyletic lineages.

In recent years, the use of DNA sequence data has proved useful in inferring phylogenetic relationships in Caryophyllaceae (Nepokroeff *et al.*, 2001; Smitsen *et al.*, 2002; Fior *et al.*, 2006; Harbaugh *et al.*, 2010;

Greenberg & Donoghue, 2011), including relationships in and among the few genera of Alsinoideae (Minuartieae) (Smitsen, Garnock-Jones & Chambers, 2003; Scheen *et al.*, 2004; Fior & Karis, 2007; Dillenberger & Kadereit, 2014). Molecular phylogenetic studies have confirmed the close relationship between *Moehringia* and *Arenaria* and provided some insights into the phylogeny of both genera (Fior *et al.*, 2006; Fior & Karis, 2007; Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011). In the systems of Harbaugh *et al.* (2010) and Greenberg & Donoghue (2011), a new tribal classification in Caryophyllaceae was proposed, including at least 11 tribes representing well-supported monophyletic clades. These studies showed that *Arenaria* is polyphyletic and three of its subgenera, *Odontostemma* (Benth. ex G. Don) Williams, *Eremogone* (Fenzl) Fenzl and *Eremogoneastrum* F.N. Williams, should be excluded and treated as two genera. They placed *Arenaria* s.s. [*Arenaria* subgenus *Arenaria*, *A.* subgenus *Leiosperma* McNeill and *A.* subgenus *Porphyrantha* (Fenzl) McNeill] and *Moehringia* into tribe Arenarieae Kitt. and described tribe Eremogoneae, including genera *Eremogone* (*Arenaria* subgenera *Eremogone* and *Eremogoneastrum*), *Thylacospermum* and *Minuartia* subgenus *Spergella* (Fenzl) McNeill. In a parallel molecular analysis of Caryophyllaceae as a whole, Greenberg & Donoghue (2011) described the clade 'Plurcaryophyllaceae' including the traditional Alsinoideae and Caryophylloideae (tribes Alsineae, Arenarieae, Caryophylleae, Eremogoneae, Sagineae, Scleranthae and Sileneae) plus Sperguleae (*sensu* Harbaugh *et al.*, 2010) characterized by the synapomorphies of presence of petals, ten stamens and capsular fruit.

The only investigation focusing specifically on phylogenetic relationships within *Arenaria* to date is that of Valcárcel, Vargas & Feliner (2006) and covers only section *Plinthine* (Reichenb.) McNeill. That study confirmed that a combination of morphological, molecular and ecological data provides useful information for resolving taxonomic controversies within this group.

**Table 1.** Classification history of the *Arenaria* complex and overview of previous infrageneric classifications of *Arenaria*

Linnaeus (1753)	Fenzl (1840, 1842)	Bentham & Hooker (1862)	Williams (1895)	McNeill (1962)	Rabaler & Hartman (2005)	Harbaugh <i>et al.</i> (2010)
Tribe (upper row, bold) and genera (lower row, non-bold)						
	<b>Stellarineae</b>	<b>Alsineae</b>	<b>Stellarineae</b>	<b>Alsineae*</b>	<b>Alsinoideae</b>	<b>Arenarieae</b>
<i>Cherleria</i>	<i>Odontostemma</i>	<i>Querria</i>			<i>Eremogone</i>	
<i>Minuartia</i>	<i>Brachystemma</i>	<i>Brachystemma</i>		<i>Honckenya</i>	<i>Honckenya</i>	
<i>Moehringia</i>	<i>Moehringia</i>		<i>Moehringia</i>	<i>Lepyrodiclis</i>	<i>Lepyrodiclis</i>	
<i>Arenaria</i>	<i>Arenaria</i>	<i>Arenaria</i>	<i>Arenaria</i>	<i>Wilhelmsia</i>	<i>Wilhelmsia</i>	
<b>Merckieae</b>				<i>Brachystemma</i>		
<i>Dolophragma</i>				<i>Minuartia</i>	<i>Minuartia</i>	
<i>Merckia</i>				<i>Moehringia</i>	<i>Moehringia</i>	<i>Moehringia</i>
<b>Sabulineae</b>				<i>Arenaria</i>	<i>Arenaria</i>	<i>Arenaria</i> s.s.
<i>Lepyrodiclis</i>						<b>Eremogoneae</b>
<i>Alsine</i>						<i>Thylacospermum</i>
<i>Buffonia</i>						<i>Eremogone</i>
<i>Honckenya</i>						<b>Alsineae</b>
<i>Querria</i>						<i>Lepyrodiclis</i>
Subgenera of <i>Arenaria</i>						<i>Pseudostellaria</i>
<i>Euthalia</i>	<i>Moehringia</i>		<i>Euaenaria</i>	<i>Arenaria</i>	<i>Arenaria</i>	<i>Arenaria</i>
<i>Goffeia</i>	<i>Rhodalsine</i>		<i>Pentadenaria</i>	<i>Leiosperma</i>	<i>Leiosperma</i>	<i>Leiosperma</i>
<i>Dicranilla</i>	<i>Alsine</i>		<i>Macrogyn</i>	<i>Porphyrantha</i>	<i>Porphyrantha</i>	<i>Porphyrantha</i>
<i>Porphyrantha</i>	<i>Minuartia</i>		<i>Dicranilla</i>	<i>Dolophragma</i>	<i>Dolophragma</i>	
<i>Eremogone</i>	<i>Cherleria</i>		<i>Arenariastrum</i>	<i>Solitaria</i>	<i>Solitaria</i>	
	<i>Hymenella</i>		<i>Odontostemma</i>	<i>Dicranilla</i>	<i>Dicranilla</i>	
	<i>Goffeia</i>		<i>Eremogoneastrum</i>	<i>Arenariastrum</i>	<i>Arenariastrum</i>	
	<i>Lepyrodiclis</i>			<i>Odontostemma</i>	<i>Odontostemma</i>	
	<i>Ammodenia</i>			<i>Eremogoneastrum</i>		
	<i>Merckia</i>			<i>Eremogone</i>		

\*Only those genera are listed that are related to *Arenaria* complex.

**Table 2.** Diagnostic morphological characteristics of the ten subgenera of genus *Arenaria sensu McNeill (1962)*

	<i>Arenaria</i>	<i>Arenariastrum</i>	<i>Dicranilla</i>	<i>Dolophragma</i>	<i>Eremogone</i>	<i>Eremogoneastrum</i>	<i>Leiosperma</i>	<i>Odontostemma</i>	<i>Porphyrantha</i>	<i>Solitaria</i>
Type	<i>A. serpyllifolia</i>	<i>A. massiliensis</i>	<i>A. dicranoides</i>	<i>A. globiflora</i>	<i>A. graminifolia</i>	<i>A. festucoides</i>	<i>A. musciformis</i>	<i>A. blinkworthii</i>	<i>A. purpurascens</i>	<i>A. cilicollata</i>
No. of species	ca. 106*	1	ca. 12	ca. 7*	ca. 70*	ca. 20*	ca. 32	ca. 65*	1	ca. 6*
Habit	annual or perennial herb, sometimes densely caespitose	annual or biennial herb, slender	perennial, densely pulvinate	perennial, densely pulvinate (sometimes caespitose)	perennial, pulvinate	perennial, densely caespitose to pulvinate	perennial herb	annual, biennial or perennial herb, diffuse	perennial, caespitose	perennial, caespitose (sometimes densely)
Leaves	lanceolate to ovate	linear to subulate	subulate to ovate, imbricate at base	linear to subulate or linear-lanceolate	linear to subulate, graminoid	subulate to linear; never setiform or graminoid	linear-lanceolate	linear to ovate, rarely subulate	ovate to lanceolate	ovate, often overlapping
Inflorescence type/position	terminal or axillary cyme, rarely reduced to 1–3 flowers	cyme	solitary	terminal, 1–3-flowered	terminal cyme or panicle of cymes, rarely head or solitary	solitary or terminal cluster	axillary cyme	various	terminal cyme, 1- to few-flowered	solitary
Sepal	lanceolate to broadly ovate	lanceolate	ovate-lanceolate to lanceolate	ovate to ovate-lanceolate or elliptic	linear-lanceolate to ovate	lanceolate to ovate-lanceolate	lanceolate to ovate	excurred	lanceolate	elliptic or lanceolate to orbicular
Sepal apex	obtuse or acute to acuminate	acute to acuminate	inflexed at the apex	obtusely acute, acuminate, or spinose	obtusely acute, acuminate, or spinose	long acuminate-acute	acute to acuminate	truncate	acute	acute
Petal	entire or slightly emarginate	entire	entire	entire or slightly emarginate	entire or slightly emarginate	entire	entire	retuse, emarginate or fimbriate	entire	entire
Petal size	often shorter than sepals	partly longer than sepals	shorter than sepals	usually longer than sepals	various	shorter than or as long as sepals, rarely longer than them	longer than sepals	usually longer than sepals	½ times as long as sepals	twice as long as sepals
Style	3	2	2 (3)	3	3	3	3	2 (3)	3	3
Special features	–	indecisive capsule	seeds usually smooth and shiny	leaf margin slightly swollen	sepal markedly hardened at the base; cotyledons accumbent; $x = 11$	leaf margin scarious	seeds smooth and shiny	sepal saccate at the base; winged seed	elongated capsule (twice as long as sepals); smooth seed	sepal hardened at the apex and margin
Distribution	N & E Africa, C Asia, and North America & Europe	SE France	S & C America	Sino-Himalaya	E Asia & W North America & Europe	Sino-Himalaya & W North America	S & C America, extending into N. America	Sino-Himalaya	Spain, Cantabrian Mountains	Sino-Himalaya

\* According to Zhengyi, Zhou &amp; Wagner 2001.

To investigate the phylogenetic relationships of *Arenaria* and its allied genera and to evaluate the phylogenetic importance of morphological characters used for subgeneric classification of *Arenaria*, nuclear (internal transcribed spacer region, ITS) and plastid (*rps16*) DNA sequences are used in the present study. We selected species representing all patterns of morphological divergence and covering the geographical range in an attempt to generate reliable phylogenetic trees. The aims of this study are briefly: (1) a detailed molecular phylogenetic investigation based on a broad taxon sampling on *Arenaria* and the genera around it; (2) re-circumscription of *Arenaria* and allied genera based on the results obtained; and (3) evaluation of the subgeneric classification of *Arenaria* and other related genera.

## MATERIAL AND METHODS

### TAXON SAMPLING

Sequence data in the present study were generated from specimens held at Botanische Staatssammlung München (M), München Systematische Botanik (MSB) and Central Herbarium of the University of Tehran (TUH). The sampling strategy was to include all generic names allied or attributed once to *Arenaria*, all recognized subgenera of *Arenaria* (*sensu* McNeill, 1962) except subgenus *Dicranella* (Fenzl) Williams for which no material was available, and a few species representing *Moehringia* and *Lepyrodiclis* known to be related or among the species of *Arenaria* in previous investigations (e.g. Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011). We generated 201 DNA sequences, of which 118 represent 56 taxa of *Arenaria* (57 ITS and 61 *rps16* sequences), 80 represent 38 taxa of *Eremogone* (40 ITS and 40 *rps16* sequences) and three sequences represent two species of *Lepyrodiclis* (one ITS and two *rps16* sequences). To cover the taxonomic diversity known in *Arenaria* and its allies, several additional sequences were downloaded from GenBank. Furthermore, the GenBank sequences of tribes Polycarpaeae and Paronychieae were used as outgroups according to former molecular phylogenetic studies of Caryophyllaceae (e.g. Greenberg & Donoghue, 2011). All taxa and summarized sources, voucher information and GenBank accession numbers of the sequences of all specimens used in this investigation are given in Appendix S1.

### DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The phylogenetic study was conducted based on sequence data of the ITS (comprising ITS1, 5.8S rDNA, ITS2) of nuclear ribosomal (nr) DNA and the plastid *rps16* intron. DNA extractions were performed using dried leaf material. Whole genomic DNA was extracted

using a NucleoSpin Plant DNA Extraction kit (Macherey-Nagel) according to the manufacturer's protocol. The ITS region was amplified using primer pairs ITS1/ITS4 (Popp & Oxelman, 2001; Kool, Perrigo & Thulin, 2012). For amplification of the complete intron of the plastid *rps16* gene we used primer pairs *rpsF/rpsR2R* (Oxelman *et al.*, 1997; Petri & Oxelman, 2011; Kool *et al.*, 2012) or *rpsF/rpsR3R*. PCR reactions were performed according to Salmaki *et al.* (2012).

### SEQUENCE EDITING AND PHYLOGENETIC RECONSTRUCTION

All sequences were first aligned using default parameters in Mafft v.7 (Katoh & Standley, 2013) and then alignment errors were identified and manually corrected in Mesquite v.1.12 (Maddison & Maddison, 2006). This method was especially effective for improving the ITS alignment. The beginning and end of the alignments where the majority of taxa did not provide complete data were excluded. When a minority of taxa lack some positions we treated them as missing (see Appendix S2, S3). The polymorphic sites in the ITS dataset were minor and were treated as missing when present. Ambiguously aligned regions were minor in both data matrices and have a negligible effect on the tree topology in the form of diminishing the resolution in few terminal branches and consequently they were kept in the data matrix. Bayesian phylogenetic inference (BI) and maximum parsimony (MP) approaches were used for phylogenetic reconstruction. An alignment for each ITS and *rps16* sequence was analysed without indels coded.

Bayesian analyses of the individual matrices were conducted using the Markov chain Monte-Carlo (MCMC) algorithm of MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001). The best nucleotide substitution model was selected using the Akaike information criterion (AIC) in jModelTest v.0.1.1 (Posada, 2008). The general time reversible model of nucleotide substitution with gamma-shaped rate variation and a proportion of invariable sites (GTR+I+G) was the estimated best-fit model for ITS and a simpler model, GTR+G, was chosen for *rps16*. Trees were sampled every 1000th generation with the default of three 'heated' and one 'cold' chain for 12 million MCMC generations for ITS and 3 million generations for *rps16* as the convergence of runs had been reached earlier and the standard deviation of split frequencies fell significantly below 0.01. Burnin was set to 3000 and 750, respectively. Bayesian search results were summarized by the 50% majority rule consensus tree, and posterior probability (PP) values ('clade credibility') are indicated at the branches.

MP of the individual genes was performed using PAUP\* v.4.0b10 (Swofford, 2003). Searches were



**Table 3.** Sequence characteristics and statistics of maximum parsimony analysis for ITS and *rps16*

	ITS	<i>rps16</i>
No. of accessions	140	131
BIC model choice	GTR+I+G	GTR+G
Sequence length (bp)	626	810
Aligned length (bp)	651	1174
Invariable sites (bp)	239	559
Variable sites (bp)	412	615
Variable sites (%)	63.29	52.38
Potentially parsimony-informative characters (bp)	340	422
Potentially parsimony-informative characters (%)	52.23	35.94
Consistency index, excluding uninformative characters	0.3167	0.5494
Retention index	0.7994	0.9024
Tree length	2362	1508

performed separately on each data set, as the taxon composition of plastid DNA and nrDNA matrices was not the same and as the two genomes are different in evolutionary history. MP bootstrapping was done using the following settings: hsearch addseq = random, nchuck = 10, chuckscore = 1, nreps = 10, bootstrap nreps = 1000. The bootstrap results are summarized in a 50% majority-rule consensus cladogram. TreeGraph 2.0.54–364 beta (Stöver & Müller, 2010) was used for tree presentation. The alignments used in this study are available in Appendices S2 and S3.

## RESULTS

### SEQUENCE CHARACTERISTICS AND ALIGNMENT

The data sets included a total of 271 sequences; 201 produced by this study and 70 obtained from GenBank (Appendix S1). For the following taxa, we were not able to produce any ITS sequences: *Arenaria conimbricensis* Brot. subsp. *viridis* Font Quer, *A. grandiflora* L., *A. merckioides* Maxim., *A. serpyllifolia* L., *A. speluncarum* McNeill, *Eremogone zargariana* (Parsa) Holub and *Lepyrodiclis holosteoides* Fenzl ex Fisch. & C.A.Mey. For *E. saxatilis* (L.) Ikonn., the amplification of *rps16* was not successful. Ultimately, the final dataset of ITS included 140 accessions, and the final *rps16* matrix contained 131 sequences. Detailed information about alignment characteristics and statistics of MP analyses is given in Table 3.

### PHYLOGENETIC RELATIONSHIPS

Phylogenetic trees generated using parsimony were congruent with those produced by Bayesian analysis.

Therefore, only the BI cladograms are presented and discussed in detail here (Figs 3–5). Some information from MP analyses, including tree length, number of potentially informative characters, and consistency and retention indices, are summarized in Table 3. The trees gained from both ITS and *rps16* markers reveal that *Arenaria s.l.* (as defined by McNeill, 1962) is divided into three major clades: (1) the ‘*Odonostemma* clade’ (Figs 3, 4, with PP = 0.926, BS = 66% and PP = 1.00, BS = 91%, respectively) including *A.* subgenus *Odonostemma* and *A.* subgenus *Solitaria* McNeill plus representatives of the genera *Lepyrodiclis* and *Pseudostellaria*; (2) the so-called ‘*Arenaria s.s.* clade’ (Figs 3, 4, with PP = 1.00, BS = 86% and PP = 0.995, BS = 88%, respectively) embracing the vast majority of the species attributed to *A.* subgenus *Arenaria*, *A.* subgenus *Arenariastrum* Williams, *A.* subgenus *Porphyrantha*, *A.* subgenus *Leiosperma*, *A.* section *Pseudomoehringia* McNeill and the monotypic *A.* section *Pungentes* McNeill; and (3) the ‘*Eremogone* clade’ (Figs 3, 4, with PP = 1.00, BS = 100% and PP = 1.00, BS = 100%, respectively) representing the genus *Eremogone* and the former *A.* subgenus *Eremogoneastrum*. More details of the branching pattern are discussed below (see Discussion).

The monophyletic crown groups recognized in ITS (Figs 3, 5) and *rps16* (Figs 4, 5) topologies were highly congruent, but the *rps16* tree (Fig. 4) is more resolved. Most cases of incongruence involved the position of a few terminal branches in the two topologies; some of these differences are outlined below.

#### ‘*Eremogone* clade’

In the ITS cladogram it is sister to the members of ‘*Arenaria s.s.*’ and ‘*odontostemma*’ clade (with low support: BS = 0.52%; Fig. 3), whereas in the *rps16* tree it is sister to *A. densissima* ex Edgew. & Hook.f., which is in turn sister to the members of Caryophylleae (Fig. 4).

#### *Arenaria densissima*

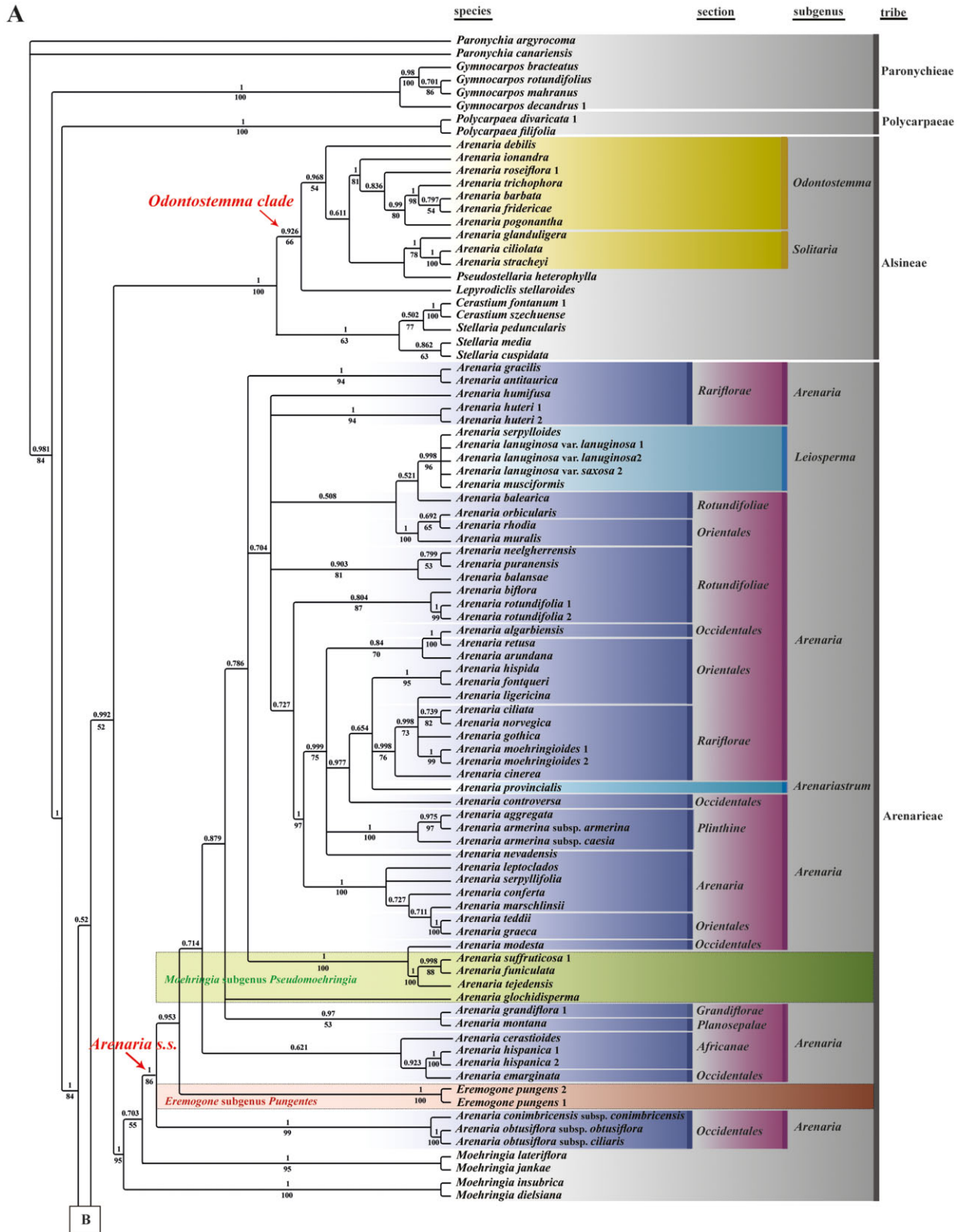
In the ITS topology this species is sister to the members of Sileneae and Caryophylleae (with weak support: PP = 0.676; Fig. 3), whereas in the *rps16* tree it is sister to the ‘*Eremogone* clade’ (Fig. 4, PP = 0.914, BS = 67%).

#### *The placement of Arenaria antitaurica* McNeill and *A. gracilis* Waldst. & Kit.

These taxa form a clade in the ITS tree (Fig. 3, PP = 1.00, BS = 94%), whereas they are nested in two separate clades in *rps16* tree (Fig. 4).

#### *Moehringia lateriflora* Fenzl

In the *rps16* phylogenetic tree this species is nested within the ‘*Arenaria s.s.*’ clade (with moderate



**Figure 3.** Majority-rule consensus tree inferred from Bayesian analysis of ITS data. Posterior probability values are indicated above branches, MP bootstrap values below. Values below 50% are not shown.

B

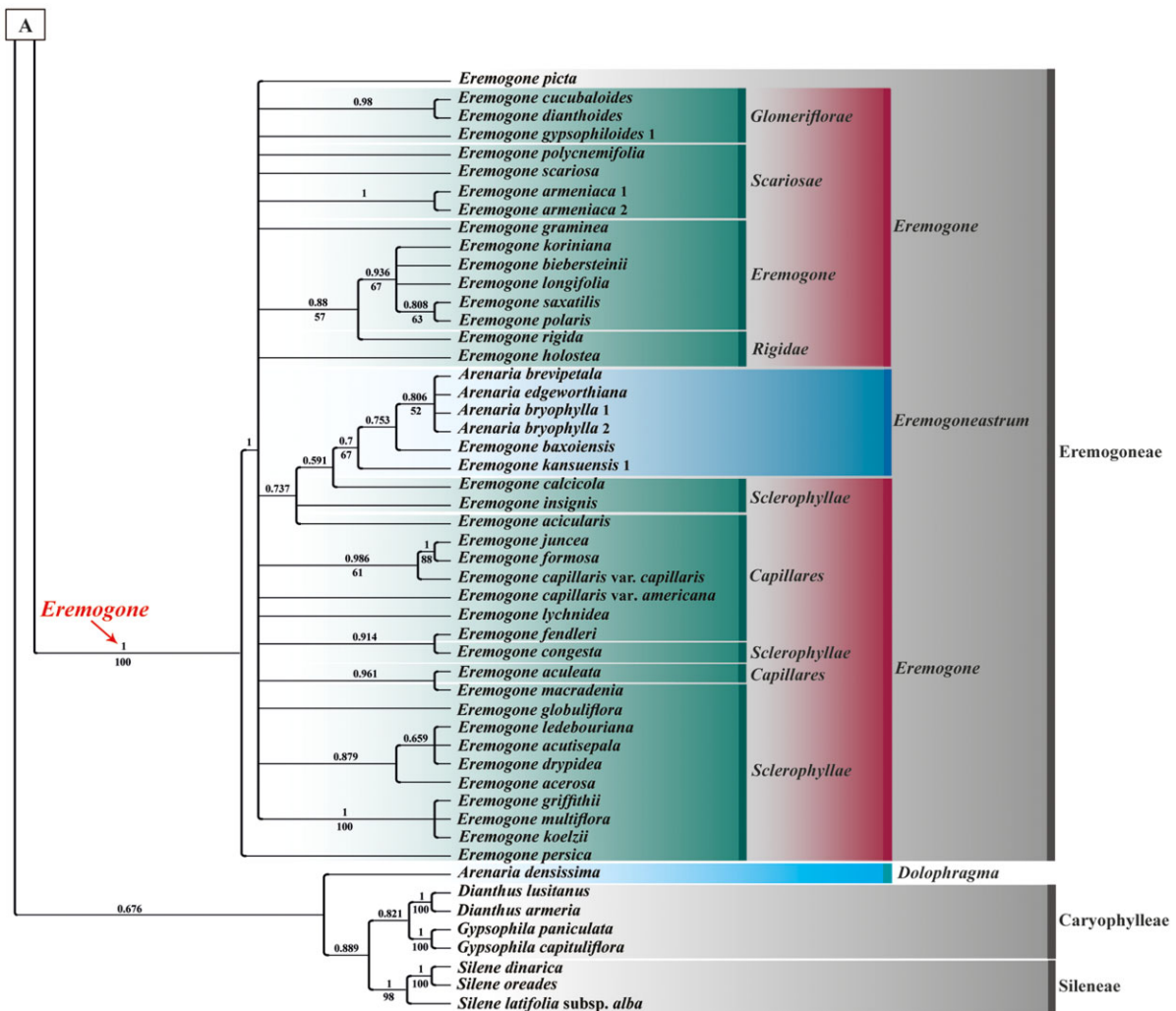


Figure 3. Continued.

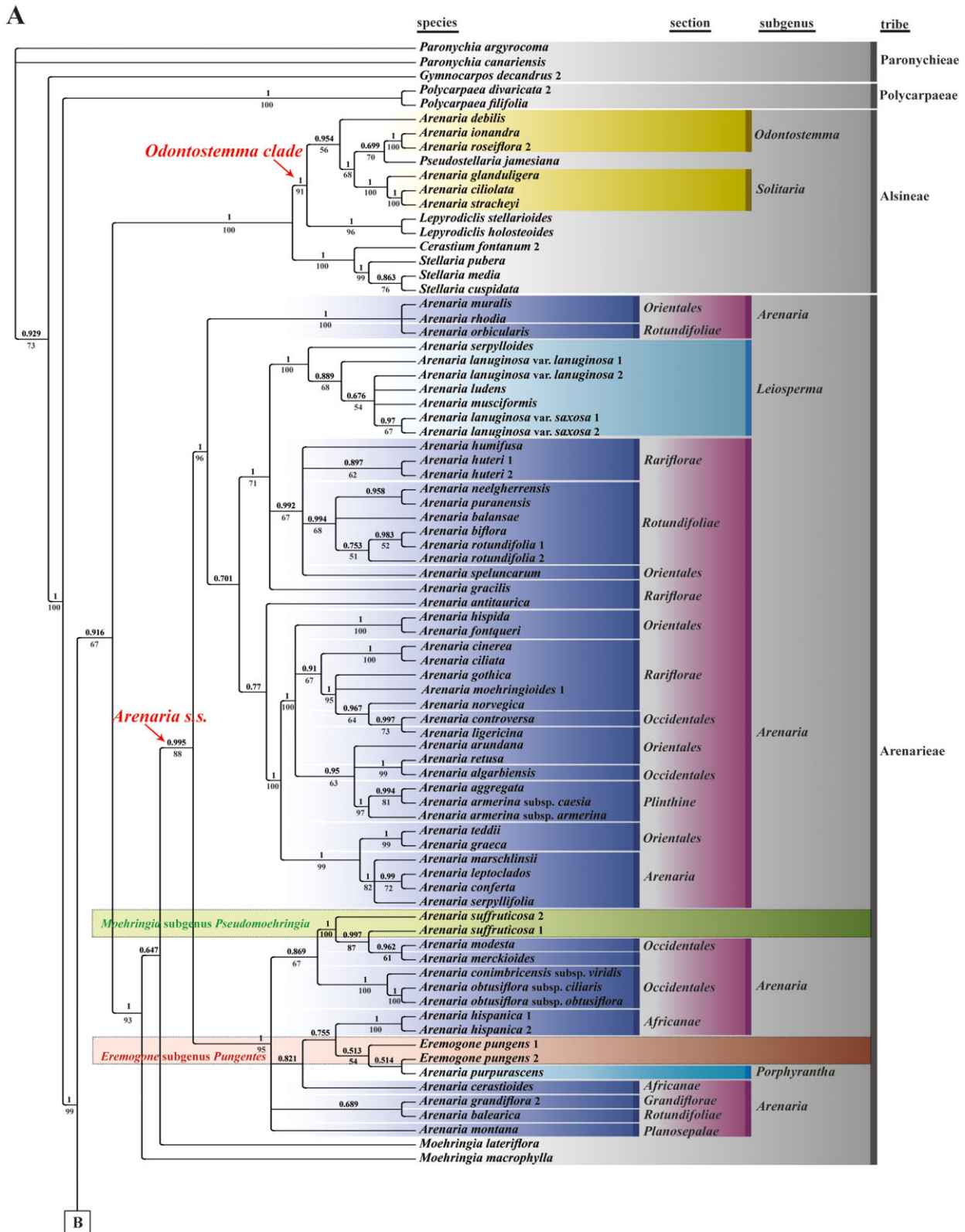
support: PP = 0.647; Fig. 4), whereas in the ITS topology it is sister to the same clade (Fig. 3).

## DISCUSSION

Our data, with a rather different taxon sampling and using one plastid DNA marker (*rps16*) and nrDNA ITS sequences, corroborate the findings of former molecular phylogenetic studies on Caryophyllaceae and reject the application of tribe Alsineae in their older circumscription (Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011). Our analyses recover the three tribes recognized by Harbaugh *et al.* (2010): Eremogoneae, Alsineae and Arenarieae. The species attributed to *Arenaria* in earlier treatments are scattered through these three clades. The composition of each of these main clades is explained in detail below.

## TRIBE EREMOGONEAE

The earlier molecular phylogenetic studies using several plastid DNA markers and the nrDNA ITS sequences provided new insights into the delimitation of *Eremogone* (Nepokroeff *et al.*, 2001; Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011) and resulted in the creation of tribe Eremogoneae. In all our phylogenetic analyses, the former *Arenaria* subgenera *Eremogone* and *Eremogoneastrum* are united in a strongly supported single clade representing tribe Eremogoneae (Figs 3, 4, with PP = 1.00, BS = 100% and PP = 1.00, BS = 100%, respectively), which is characterized by narrow, grass-like leaves (Fig. 1E–M). Whereas the ITS phylogenetic trees support Sileneae as sister to this clade (Fig. 4), the plastid trees do not exactly address the placement of Eremogoneae.



**Figure 4.** Majority-rule consensus tree inferred from Bayesian analysis of *rps16* data. Posterior probability values are indicated above branches, MP bootstrap values below. Values below 50% are not shown.

B

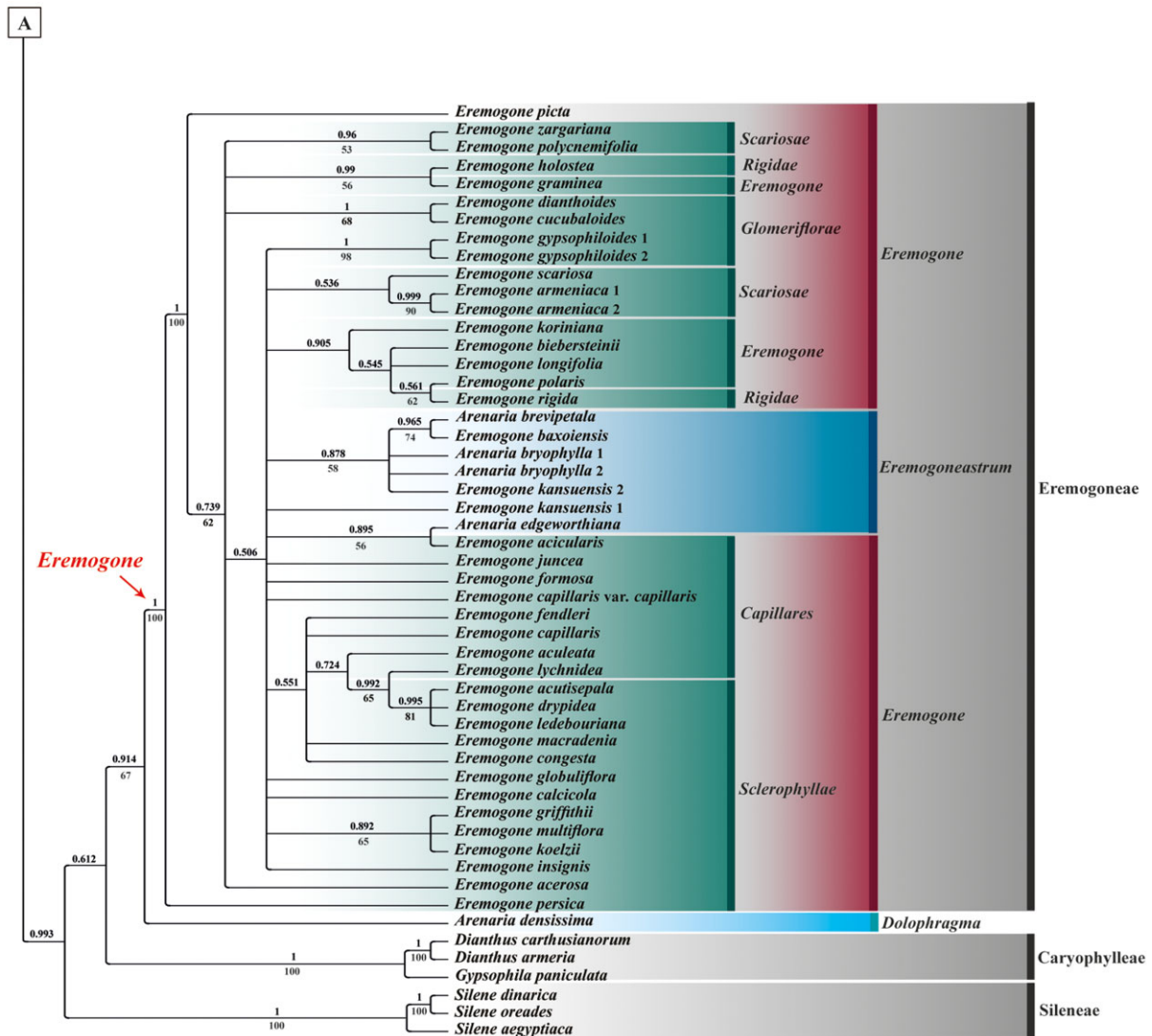
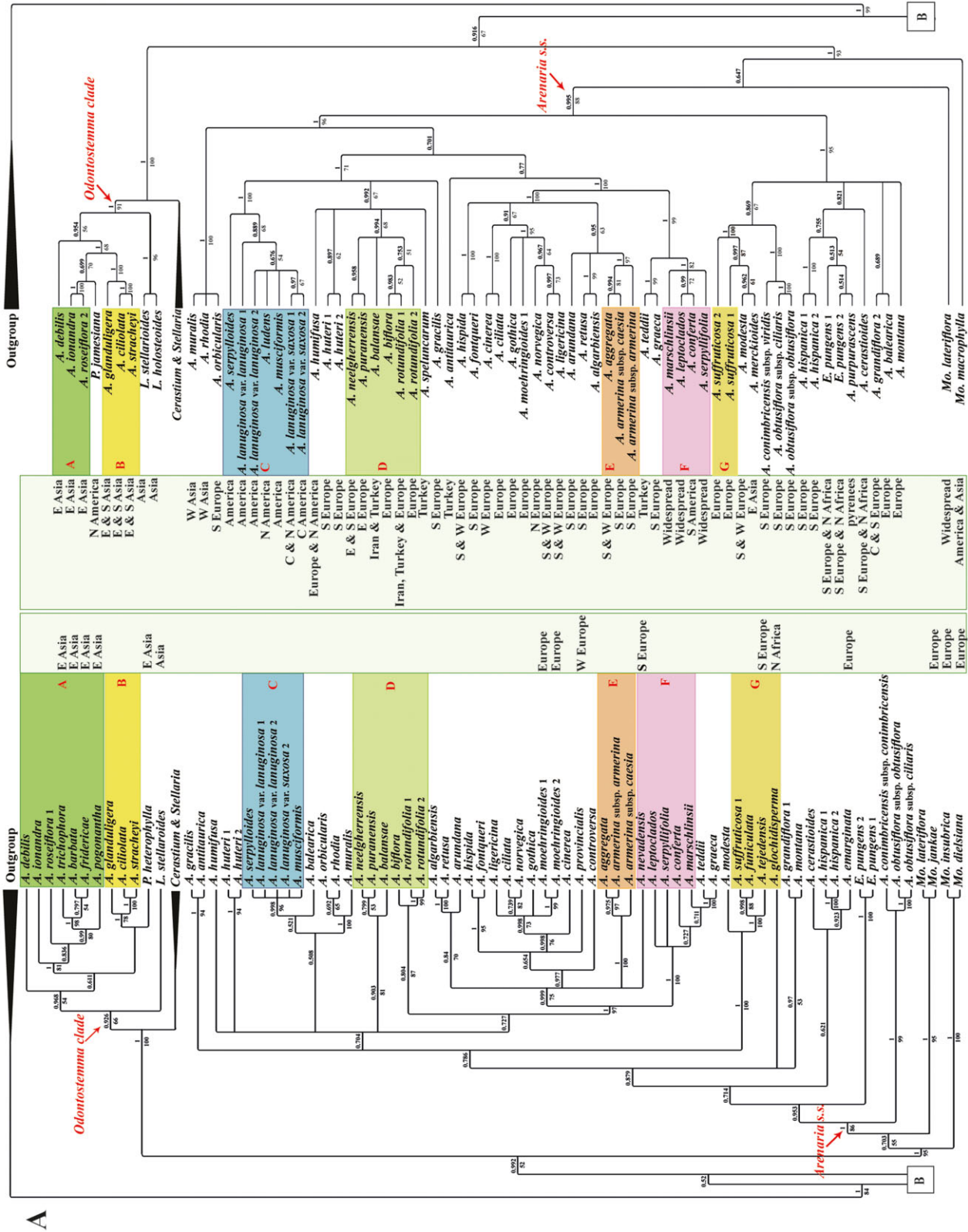


Figure 4. Continued.

First used in recent treatments by Ikonnikov (1973) and especially in North American floras by Weber, Johnston & Wittmann (1981), Dorn (2001) and more recently by Rabeler & Hartman (2005), *Eremogone* is a taxonomic segregate from the polyphyletic *Arenaria* s.l. *Eremogone* is a morphologically distinctive group characterized by several synapomorphies (e.g. accumbent cotyledons and chromosome number  $x = 11$ ; Table 2). Several investigations have attempted to provide an infrageneric classification of the genus (Maguire, 1947, 1951; McNeill, 1962; Hickman, 1971), but due to its heterogeneous morphology none seems to be appropriate (Rabeler & Hartman, 2005). McNeill (1962) recognized eight sections in his *Arenaria* subgenus *Eremogone*. Thirty-seven representa-

tives of all sections of *Eremogone* (except the monotypic section *Monogone* Maxim.) were included in our ITS and *rps16* analysis (see Figs 3–5). Both ITS data (with higher resolution) and *rps16* show that hardly any of the sections recognized by McNeill (1962) could be considered as monophyletic with certainty. In the case of section *Pungentes*, this taxon should be removed from *Eremogone* and placed in *Arenaria* (see further discussion under tribe Alsineae). A few clades matching certain sections (such as section *Eremogone*) could be recovered by both markers (when ignoring *E. graminea* C.A.Mey. ex Fisch. & Mey.) (Fig. 5, compare box H). *Arenaria* section *Capillares* McNeill with about nine species was described by McNeill (1962) to encompass those



**Figure 5.** Bayesian consensus trees from the ITS dataset (left side) compared with the rps16 dataset (right side). For better comparison of positions of species in the trees certain species groups are highlighted with coloured boxes. Abbreviations: *A.*, *Arenaria*; *E.*, *Eremogone*; *L.*, *Lepyrodiclis*; *Mo.*, *Moehringia*; *P.*, *Pseudostellaria*.

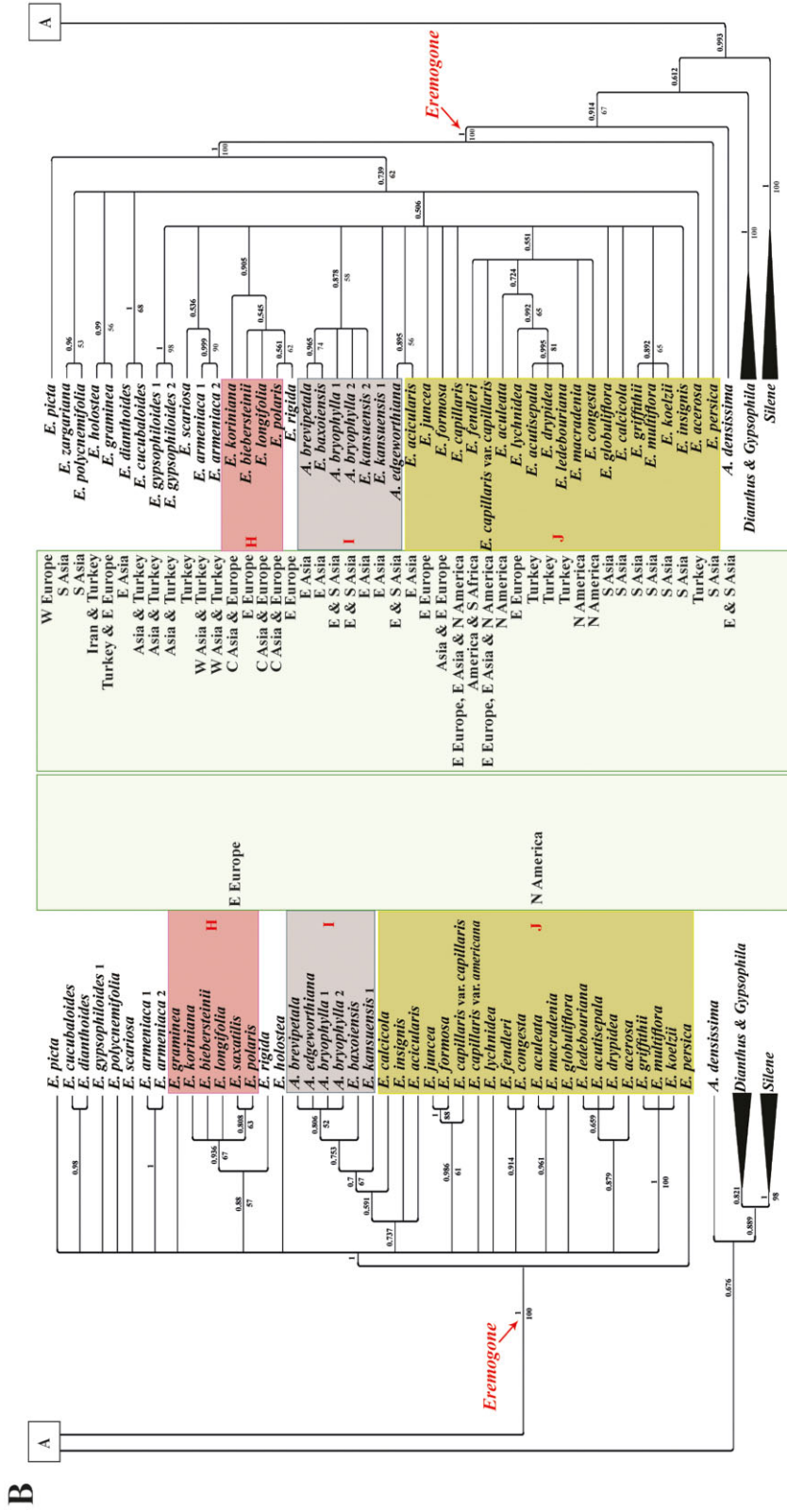


Figure 5. Continued.

dense caespitose species having long linear or setaceous leaves and acute to acuminate sepals. According to McNeill (1962), members of this section appear to be closely related to species of *A.* section *Sclerophyllae* (Boiss.) McNeill, so close that it is sometimes difficult to distinguish the sections (Fig. 5, box J). However, due to low support values and incomplete sampling, it is premature to propose a new infrageneric classification for *Eremogone*. Therefore, we highlight here only one example indicating the need for additional molecular phylogenetic study in *Eremogone*.

A densely caespitose to densely pulvinate habit also characterizes members of subgenus *Eremogoneastrum* (Fig. 1N–P). However, these taxa have scarious leaf margins and acuminate sepals, separating them from *Dolophragma* (Fig. 1A–C). In the topology acquired from the ITS sequence analysis, the species of subgenus *Eremogoneastrum* form a moderately supported monophyletic group, but this group is not completely recovered by the *rps16* trees (Fig. 5, box I). Our data indicate that members of subgenus *Eremogoneastrum* should clearly be included in *Eremogone*. Therefore, we transfer the species of subgenus *Eremogoneastrum* in our analysis to *Eremogone*.

Greenberg & Donoghue (2011) reported that *A. lancangensis* L.H.Zhou and *A. roborowskii* Maxim., included in *Arenaria* subgenus *Eremogoneastrum* by Zhengyi, Zhou & Wagner (2001), were found in a clade containing more than 20 *Cerastium* species. We did not include these species in our analysis, but believe that their result should be verified. We found the same results as Dillenberger & Kadereit (2014) regarding placement of *Eremogone picta* (Sibth. & Sm.) Dillenb. & Kadereit, confirming the inclusion of *Minuartia* subgenus *Spergella* in *Eremogone*.

#### PLACEMENT OF *ARENARIA* SUBGENUS *DOLOPHRAGMA*

The single species of *A.* subgenus *Dolophragma* included in the present study (*Arenaria densissima*) shows a sister relationship to *Eremogoneae* in the *rps16* tree and falls in the same clade as tribes Sileneae and Caryophylleae in the ITS tree (but with distinctly lower support; Figs 3–5). Another species (*A. przewalskii* Maxim.; Fig. 1D) assigned to this subgenus by McNeill (1962) was nested in the ‘*Odontostemma* clade’ (Greenberg & Donoghue, 2011), making it likely that this subgenus is polyphyletic. At least in the case of *A. densissima*, it probably should not be maintained and should instead be *Dolophragma juniperinum* (D. Don) Fenzl. As material of the type species of *A.* subgenus *Dolophragma* [*Dolophragma globiflorum* Fenzl  $\equiv$  *A. globiflora* (Fenzl) Wall. ex Edgew. & Hook.f.] was not available, we cannot completely confirm this usage. However, due to the close morphological resemblance of *A. densis-*

*sima*, *A. polytrichoides* Edgew. and *A. globiflora* characterized by dense pulvinate habit, swollen coriaceous leaf margin and short obtuse sepals (Fig. 1A–C), it seems likely that the genus *Dolophragma* should be reinstated and recircumscribed to exclude other species with dense cushion-forming but caespitose habit having linear leaves (Fig. 1D), such as *A. przewalskii*, which are probably related to the members of the ‘*Odontostemma* clade’. The dense cushion habit has most probably arisen independently in this group of species.

#### TRIBE ALSINEAE

Among the genera once attributed to this tribe, *Odontostemma* was treated as a subgenus of *Arenaria* by McNeill (1962). However, two recent molecular phylogenetic studies both suggested that this genus has to be resurrected (Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011). In the present study and based on both analyses, *Odontostemma*, *Lepyrodiclis* and *Pseudostellaria* and members of *Arenaria* subgenus *Solitaria* are assigned to the ‘*Odontostemma* clade’ (Figs 3–5). These genera are clearly apart from *Arenaria s.s.* in both trees obtained. The genera forming this clade are morphologically well defined (Fig. 2A–E). Most genera in this clade show an East Asian (Sino-Himalayan) distribution and are characterized by petals mostly conspicuous overtopping the sepals and entire or emarginate at the apex (only rarely absent in some *Pseudostellaria*), leaves relatively large, linear to ovate and capsules dehiscent by twice as many valves as styles (except for *Lepyrodiclis*). Due to the  $\pm$  sharp boundaries between these genera, uniting them into one genus would make the group heterogeneous. Furthermore, such a treatment may cause the disappearance of several generic names that are currently in common use. Whereas the *rps16* data (Fig. 4) show *Pseudostellaria* to be nested in *Odontostemma*, both consensus trees (Fig. 5) show a segregation of the genera similar to the results obtained by Harbaugh *et al.* (2010). Greenberg & Donoghue’s (2011) figure 3 suggested that *Pseudostellaria* may be paraphyletic. Our sampling of *Pseudostellaria* was not sufficient to confirm or refute this possibility, providing another reason for retaining current usage until additional studies can be made. A more plausible taxonomic treatment with the fewest nomenclatural changes would be the resurrection of *Odontostemma* as a genus (type species: *O. glandulosa* Benth. ex G. Don) and treating *A.* subgenus *Solitaria* as a new genus (genus *Solitaria*, type species: *A. ciliolata* Edgew.; see below). A new taxonomic system involving the members of the ‘*Odontostemma* clade’ along with a key to the accepted genera in this clade is presented at the end of the Discussion.



## TRIBE ARENARIEAE

The members of the tribe have mostly conspicuous entire petals, capsules dehiscing by twice as many valves as styles and often  $\pm$  broad leaves ovate to lanceolate in shape (Fig. 2F–L). In all trees we obtained, a core group of *Arenaria*, the so-called *Arenaria s.s.*, is recovered with *Moehringia s.s.* as sister (Figs 3, 4; PP = 1.00, BS = 95% and PP = 1.00, BS = 93%, respectively). This finding is in agreement with previous studies (Fior & Karis, 2007; Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011). However, contrary to those studies, *Moehringia* is paraphyletic in our analyses (see results, Figs 3–5). This fact could be caused by the lack of enough informative signals and/or the low sampling. The greater number of markers used in the previous studies and the greater number of taxa sampled (Harbaugh *et al.*, 2010; Greenberg & Donoghue, 2011) probably resulted in different topologies obtained in those studies versus our results.

There are few clades in *Arenaria s.s.* with adequate support from both markers that would allow recognition as natural taxonomic groups. Most of the extant subgenera and sections are not monophyletic. Given our results, we consider the rank of subgenus in *Arenaria* is not justified, but there is enough support for several sections, as smaller natural groups, within *Arenaria*. The main supported monophyletic clades inside *Arenaria* which are in accordance with already recognized subgeneric taxa in the genus are as follows.

1. *Arenaria* subgenus *Leiosperma* (Fig. 5, box C). Both morphological and molecular data strongly support this clade as a distinct group. Furthermore, with about 32 species, it is distributed in the New World with centre of diversity in the Andes. The members of this subgenus are homogeneous in vegetative and floral traits and are also characterized by smooth and shiny seeds (Sadeghian, Zarre & Heubl, 2014). Although several features support the monophyly of this group, questions regarding its proper taxonomic rank could not be addressed until now. Based on the trees obtained from ITS and *rps16* sequence data, it seems that accepting *Leiosperma* as a subgenus would make other subgenera and sections in *Arenaria* paraphyletic. Based on our results, treating *Leiosperma* as a section (section *Leiosperma* F.N. Williams) is more appropriate.
2. *Arenaria* section *Rotundifoliae* McNeill (Fig. 5, box D). The members of this section are conveniently recognizable by rounded leaves and creeping habit. Our analyses imply that section *Rotundifoliae* may possibly be monophyletic, if *A. orbicularis* Vis and *A. balearica* L. are excluded. The latter species differ from other species of the section by having solitary flowers with obtuse sepals on long pedicels. *Arenaria orbicularis* was placed by McNeill (1962) in section *Rotundifoliae* but has subsequently been transferred to section *Orientalis* McNeill by Jalas & Suominen (1988). Our results also show a possible relationship between this species and some members of section *Orientalis*.
3. *Arenaria* section *Plinthine* (Fig. 5, box E). In our study, the monophyly of section *Plinthine* is supported in the ITS and plastid DNA phylogenetic trees, confirming the results of previous molecular phylogenetic studies (Valcárcel *et al.*, 2006; Greenberg & Donoghue, 2011). There are also several morphological synapomorphies supporting the monophyly of section *Plinthine* (Valcárcel *et al.*, 2006). This natural group is geographically restricted to the western Mediterranean and the morphology of these species is distinctive compared with the rest of *Arenaria* (Valcárcel *et al.*, 2006; Fig. 2G, H). Our results suggest that the members of section *Plinthine* are related to a group of other Mediterranean species such as *A. retusa* Boiss. and *A. arundana* Gallego.
4. *Arenaria* section *Arenaria* (Fig. 5, box F). This is a distinct and natural group of annual plants. In the ITS and *rps16* trees, two short-lived perennial species, *A. teddii* Turrill and *A. graeca* (Boiss.) Halácsy (previously considered as members of *A. section Orientalis*), are nested in the same clade with the species of section *Arenaria*. As it appears that the annual habit seems to be derived several times in *Arenaria*, the circumscription of this section could be extended to also encompass some perennial species. However, due to the low number of taxa sampled in the present study (five of 15 species recognized), it is premature to suggest any formal treatment of this section and its members.
5. Section *Pseudomoehringia* (Fig. 5, box G). The members of this section were originally placed in *Moehringia* due to the papillo-strophiolate seeds. In accordance with Fior & Karis (2007), our ITS and *rps16* phylogenetic trees indicate that these Iberian taxa should be transferred to *Arenaria*. From a morphological point of view, the seeds of the members of this section resemble *Moehringia*, but the seed surface is rather tuberculate instead of being smooth (McNeill, 1962; Fior & Karis, 2007). Our results indicate high support for this section. Furthermore, the species assigned to this section are homogeneous morphologically. Therefore, this group should be considered as a well-supported monophyletic group within *Arenaria* and, by the new combination made below, we treat it as a formal section under *Arenaria*. The plants

are mostly annual with sepals obscurely veined and show a western Mediterranean distribution pattern (centred in Spain and North Africa)

Most other subgenera (*sensu* McNeill, 1962) are not monophyletic according to the results of our molecular phylogenetic analyses. These groups are explained below.

1. *Arenaria* subgenus *Arenaria*. In McNeill's (1962) classification system on Alsinoideae, the species-rich *Arenaria* subgenus *Arenaria*, with at least 100 species, was divided into 11 sections. These sections share several morphological characters, such as  $\pm$  entire petals, three carpellate capsules, six capsule teeth and incumbent cotyledons. Our results from ITS and *rps16* sequence analyses indicate that this large subgenus is paraphyletic with several other subgenera (such as *Arenaria* subgenera *Leiosperma* and *Arenariastrum*) nested in it. Therefore, the above-mentioned shared characters are probably symplesiomorphies. As mentioned above under *Arenaria* subgenus *Leiosperma*, the application of the rank of subgenus in *Arenaria* is not helpful; sections would provide a better interpretation of the natural groups present in the genus. The single species of *Arenaria* subgenus *Arenariastrum* (*A. provincialis* Chater & G.Halliday) is closely related to some members of *Arenaria* section *Orientalis*, *Arenaria* section *Occidentales* McNeill and *Arenaria* section *Rariflorae* (*Arenaria* subgenus *Arenaria sensu* McNeill, 1962) in our analyses and its separation from these sections, or at least part of them, is not supported. The affinity of this species with members of *Arenaria* was also previously determined by detailed morphological and biogeographical analyses as well as molecular data (Youssef *et al.*, 2011).
2. The monotypic *Arenaria* subgenus *Porphyrantha*, restricted to the Pyrenees and the Cantabrian Mountains, appears to share a similar fate. Our molecular data clearly place the type species of this subgenus (*A. purpurascens* Ramond ex DC.) in *Arenaria s.s.*, although the species seems to be unique in *Arenaria* by its odd morphological characters (e.g. elongated capsule and smooth seeds having a tuft of white hairs at the hilum).
3. Although with low support, the *rps16* sequence analysis places *Eremogone pungens* (Clemente ex Lag.) Fenzl (the type of McNeill's monotypic *Arenaria* section *Pungentes*) close to *A. purpurascens* and members of *Arenaria* section *Africanae* McNeill. Therefore, *Eremogone pungens* is not related to other species of *Eremogone* and should instead be treated as a species of *Arenaria*, i.e. *A. pungens* Clemente ex Lag. This is consistent with Ikonnikov (1973), who excluded *E. pungens* from his review of *Eremogone*.

4. *Arenaria* sections *Orientalis*, *Occidentales* and *Rariflorae* F.N. Williams. The species once assigned to sections *Orientalis* and *Occidentales* are scattered among various groups of *Arenaria s.s.* As outlined by McNeill (1962), the 11 species recognized in *Arenaria* section *Occidentales* are mainly distributed in the western Mediterranean. Presence of linear to subulate leaves is the most important morphological characteristic separating the species of this section from the members of the allied *Arenaria* section *Orientalis*, which encompasses about 20 species with somewhat ovate leaves and an eastern Mediterranean distribution. The close relationship between these sections has been expressed by several authors (e.g. López Gonzáles, 1990). The phylogenetic trees depicted here show that the species of both sections are mixed with each other and the members of several other sections such as *Arenaria* section *Rariflorae*. In general, our results suggest that the subgeneric classification of *Arenaria s.s.* needs to be revised substantially and that the available system, which is mainly according to McNeill (1962), does not present a natural classification. For this purpose, it is necessary to conduct a detailed morphological study along with an inclusive molecular analysis using several markers and denser sampling.

#### TAXONOMIC CONCLUSION

Although our study does not provide a complete phylogenetic basis for a revised taxonomy for *Arenaria*, it indicates that some infrageneric taxa may be natural, whereas others are paraphyletic or polyphyletic. In addition, our results highlight several taxonomic ambiguities that may be corrected by transferring some taxa to other genera. The present study sheds light on the borders between *Arenaria* and allied genera. In general, the results of our molecular phylogenetic investigation demonstrate the need for a substantial revision of *Arenaria* to include the majority of its species. Our phylogenetic analyses of ITS and *rps16* were unable to resolve fully relationships among species of *Arenaria* and *Eremogone*. The application of additional plastid and nuclear markers could help to resolve some of the polytomies detected here. The following taxonomic treatments could be proposed based on our results.

- Section *Pseudomoehringia* should be transferred from *Moehringia* to *Arenaria*:

*Arenaria* section *Pseudomoehringia* (McNeill) Rabeler & Zarre, **comb. nov.** = *Moehringia* section *Pseudomoehringia* McNeill, Notes Roy. Bot. Gard. Edinburgh 24: 131. 1962. Type: *Arenaria suffruticosa*

Fior & P.O.Karis = *Moehringia intricata* Willk., non *A. intricata* (Ser.) Rivas Mart. & M.J.Costa.

Some species of *Arenaria* subgenus *Eremogoneastrum* are transferred to the genus *Eremogone*:

1. *Eremogone brevipedata* (Y.W.Tsui & L.H.Zhou) Sadeghian & Zarre, **comb. nov.** = *Arenaria brevipedata* Y.W.Tsui & L.H.Zhou, Acta Phytotaxon. Sin. 18:360. 1980 – Type: China, Sichuan, Kangding, Yingguanzhai, 3650 m, Dept. of Agriculture, Sichuan Agriculture College (58)100 (holotype, KUN).
2. *Eremogone bryophylla* (Fernald) Sadeghian & Zarre, **comb. nov.** = *Arenaria bryophylla* Fernald, Rhodora 21:5. 1919, nom. nov. = *Arenaria musciformis* Wall. ex Edgew. & Hook.f. Fl. Brit. India 1(2): 237. 1874, not Triana & Planch., 1862 – Syntypes: Alpine Eastern and Western Tibet, 16–18000 ft, Webb & Jacquemont s.n.; Balch pass, R. Strachey. & J.E. Winterbottom, s.n.; Karakoram and Parang passes, T. Thomson s.n.; Tibetan Sikkim, 15–16000 ft, J.D. Hooker s.n. (K?).
3. *Eremogone edgeworthiana* (Majumdar) Sadeghian & Zarre, **comb. nov.** = *Arenaria edgeworthiana* Majumdar, J. Indian Bot. Soc. 44:141. 1965 = *A. monticola* Edgew. – Type: India, Himalya, Sikkim Lama Krupa, J.D. Hooker, Hooker & Thomson, Herb. Ind. Orient. 4 (holotype: K, K000723993 [photo!]).

The genus *Odontostemma* Benth. ex G.Don should be reinstated to include the members of *Arenaria* subgenus *Odontostemma*:

*Odontostemma* Benth, ex G.Don, Gen. Syst. 1: 449. 1831 = *Arenaria* subgenus *Odontostemma* (Benth. ex G.Don) F.N.Williams, Bull. Herb. Boissier 3:603. 1895 – Type: *O. glandulosum* Benth. ex G.Don = *Gooringia* F.N.Williams, Bull. Herb. Boissier 5: 530, 1897 – Type: *G. littledalei* (Hemsley) F.N.Williams (= *Arenaria littledalei* Hemsley).

As a major conclusion of our study, the genus is described in detail below:

Herbs annual, biennial, or perennial, densely branched. Leaves linear to ovate, rarely subulate and never setaceous. Inflorescences various, but often with vegetative branches arising within them. Sepals curved, often saccate, veins inconspicuous, margin broadly membranous, apex truncate. Petals usually longer than sepals (but sometimes smaller) cleistogamous flowers sometimes present, apex emarginate or fimbriate. Styles usually two, rarely three. Seeds often inflated, winged, rough on surface. About 65 species, with a Sino-Himalayan distribution.

Accepted species:

1. *Odontostemma glandulosum* Benth. ex G.Don, Gen. Syst. 1: 449. 1831 = *Arenaria blinkworthii*

McNeill, Notes Roy. Bot. Gard. Edinburgh 24: 128. 1962 = *Arenaria glandulosa* (Benth. ex G.Don) F.N.Williams, Bull. Herb. Boissier 5: 603. 1895, not Jacquin (1798) = *Arenaria benthamii* Edgew., Fl. Brit. India. 1: 242. 1874, not Fenzl ex Torrey & A. Gray (1840) – Type: India: Kamoun, R. Blinkworth s.n., Wallich Cat. 645 (holotype: K, K000742186 [photo!]; isotype: E, E00317569 [photo!]).

= *Arenaria debilis* Hook.f., Fl. Brit. India. 1: 242. 1874 – Type: India, Sikkim, Kankok, 15000 ft, 27 Aug 1849, J.D. Hooker, Hooker & Thomson, Herb. Ind. Orient. s.n. (lectotype, designated here; K, K000742183, right-hand plant on sheet [photo!]; isolectotypes; K, K000742185, excl. plants immediately above label [photo!]; GH, GH00353888 [photo!]).

2. *Odontostemma barbata* (Franch.) Sadeghian & Zarre, **comb. nov.** = *Arenaria barbata* Franch., Bull. Soc. Bot. France. 33: 430. 1886 – Type: China, Yunnan, in lapidosis calcareis, ad pedem montis Yang-in-chan, supra Lankong, 2500 m, 14 Sep 1885. Delavay 1901 (P?).
3. *Odontostemma fridericae* (Hand.-Mazz.) Sadeghian & Zarre, **comb. nov.** = *Arenaria fridericae* Hand.-Mazz., Anz. Akad. Wiss. Wien. 57: 142. 1920. – Type: China, Yunnan bor.occid.: in lateris occid. montis Piepun ad austro-orient. pagi Dschungdien ('Chungtien') regione alpina, in glarea mobile, 4300–4650 m, 11 Aug 1914, H.R.E. von Handel-Mazzetti 4684 (holotype; (WU, WU0043547 [photo!]).
4. *Odontostemma ionandra* (Diels) Sadeghian & Zarre, **comb. nov.** = *Arenaria ionandra* Diels, Notes Roy. Bot. Gard. Edinburgh. 5: 182. 1912. – Type: China, Lichiang Range, 11–12000 ft, lat. 27°20'N, Sep 1906. G. Forrest 2902A (holotype; E, E00313709 [photo!]).
5. *Odontostemma pogonantha* (W.W.Smith) Sadeghian & Zarre, **comb. nov.** = *Arenaria pogonantha* W.W.Smith, Notes Roy. Bot. Gard. Edinburgh. 11: 198. 1920. – Type: China, Yunnan, western flank of Shweli Salween Divide, 10000 ft, lat 25°20' N, Aug. 1912, G. Forrest 8935 (holotype; E, E00117574 [photo!]).
6. *Odontostemma roseiflora* (Sprague) Sadeghian & Zarre, **comb. nov.** = *Arenaria roseiflora* Sprague, Bull. Misc. Inform. Kew: 33. 1916 = *Moehringella roseiflora* (Sprague) H.Neumayer, Verh. Zool.-Bot. Ges. Wien, 73: 14. 1923 – Type: China: Forrest 13225, described from cultivated plant at K (holotype; K, K000723896 [photo!]). = *Arenaria atuntziensis* var. *stenopetala* Y.W.Cui ex L.H.Zhou, Acta Biol. Plateau Sin. 6: 27. 1987. Type: China: Yunnan: Dequin, 2700 m, C.W. Wang 70086 (holotype, KUN).

KEY TO THE GENERA OF THE 'CLADE *ODONTOSTEMMA*'

- 1a. Flowers of two types: chasmogamous at stem apex, with five large, entire or rarely bifid petals and an ovary of three carpels, usually sterile; cleistogamous at stem base, with petals small or absent; leaves relatively large, fertile; plants with fleshy root tubers; plants with habit of *Stellaria* (Alps; C. & E. Asia; North America)..... *Pseudostellaria*
- 1b. Flowers of one type: cleistogamous flowers absent; plants usually without fleshy root tubers; plants not creeping, or when creeping not similar to *Stellaria*..... 2
- 2a. Capsule teeth as many as styles: styles two, opening by two valves (SW Asia to C. Asia)..... *Lepyrodiclis*
- 2b. Capsule teeth twice as many as styles: styles two or three, opening by four or six valves or teeth, rarely eight-, or ten-valved or -toothed..... 3
- 3a. Styles usually two, sometimes three (four or five); capsule four-valved or -toothed, rarely six-, eight-, or ten-valved or -toothed; sepals truncate at apex and  $\pm$  saccate at base, excurved; inflorescence various; diffuse annual, biennial or perennial herbs (Sino-Himalaya)..... *Odontostemma*
- 3b. Styles three; capsules six-valved or -toothed; sepal apex acute, acuminate, or obtuse at apex, non-saccate at base; flower solitary; plants densely caespitose but never pulvinate (Sino-Himalaya)..... *Solitaria*

**7. *Odontostemma trichophora*** (Franch.) Sadeghian & Zarre, **comb. nov.**  $\equiv$  *Arenaria trichophora* Franch., Bull. Soc. Bot. France, 33: 431. 1886  $\equiv$  *Arenaria yunnanensis* var. *trichophora* (Franch.) F.N.Williams – Type: China, Yunnan, in monte Hee-chan-men, supra Lankong, 11 Jul 1883, Delavay 110 (P?).

*Arenaria* subgenus *Solitaria* should be elevated to generic rank:

***Solitaria* (McNeill) Sadeghian & Zarre, comb. nov.**  $\equiv$  *Arenaria* subgenus *Solitaria* McNeill, Notes Roy. Bot. Gard. Edinburgh. 24: 128, 1962.  $\equiv$  *Arenaria* section *Sikkimenses* F.N.Williams, Bull. Herb. Boissier 3, 600, 1895. – Type: *S. ciliolata* (Edgew.) Sadeghian & Zarre

As another major conclusion of our study, the genus is described in detail below:

Herbs perennial, caespitose, sometimes densely branching, but never pulvinate. Stems clustered, short. Leaves relatively large (> 5 mm long), leaf blade remote or sometimes approximate (covering each other), but never imbricate in four rows, lanceolate to ovate-orbicular, base narrowed, margin usually slightly hard. Flowers solitary, rarely paired, terminal, large and showy. Sepals elliptic or lanceolate to narrowly orbicular, margin membranous, usually cartilaginous, apex acute, about twice as long as sepals. Petals white, pink or violet, obovate to obovate-elliptic. Styles three. About six species, with a Sino-Himalayan distribution.

Accepted species:

**1. *Solitaria ciliolata*** (Edgew.) Sadeghian & Zarre, **comb. nov.**  $\equiv$  *Arenaria ciliolata* Edgew., Fl. Brit. India. 1: 240. 1874. – Type: India, Sikkim Lama Kengna, 15000 ft, 24 Jul 1849, J.D.Hooker, Hooker & Thomson, Herb. Ind. Orient. 11 (K, lectotype, designated here, K000742194 [photo!]; isolectotype; GH, GH00353887 [photo!]).

**2. *Solitaria glanduligera*** (Edgew.) Sadeghian & Zarre, **comb. nov.**  $\equiv$  *Arenaria glanduligera* Edgew., Fl. Brit. India. 1: 240. 1874. – Syntypes: Kashmir, H. Falconer s.n. (K, K000742189 [photo!]; India, Kumaon, Barji Kang pass, 14 500 ft, R. Strachey & J.E. Winterbottom (K?); India, Sikkim, 12–16000 ft, J.D.Hooker, Hooker & Thomson, Herb. Ind. Orient. 12 (E, E00317564 [photo!]; GH, GH00353890 [photo!]; K?).

**3. *Solitaria stracheyi*** (Edgew.) Sadeghian & Zarre, **comb. nov.**  $\equiv$  *Arenaria stracheyi* Edgew., Fl. Brit. India. 1: 240. 1874. – Type: China, Tibet near Rakas Tal., R. Strachey & J.E. Winterbottom 3 (lectotype, designated here, K, K000723873 [photo!]).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Voucher information and GenBank accession numbers for the sequences analysed in this study. For the accessions obtained from GenBank, the collection data were not available.

**Appendix S2.** Aligned data matrix of ITS sequences of *Arenaria* and allied genera used in the phylogenetic study in nexus format.

**Appendix S3.** Aligned data matrix of *rps16* sequences of *Arenaria* and allied genera used in the phylogenetic study in nexus format.