

Molecular phylogeny of *Acanthophyllum* (Caryophyllaceae: Caryophylleae), with emphasis on infrageneric classification

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Abstract Despite being one of the larger genera of Caryophyllaceae with about 60 cushion-forming subshrubby species, *Acanthophyllum* is represented poorly in previous molecular phylogenetic studies. The genus is an important component of the subalpine steppe flora in Central to Southwest Asia. Although the placement of *Acanthophyllum* in the tribe Caryophylleae and a close relationship to *Allochrysa* has already been suggested, the monophyly of the genus and its infrageneric taxa, as well as its relation to other closely related genera, have not been addressed. We have assembled datasets of nuclear ribosomal internal transcribed spacer (ITS) sequences and intron sequences of the chloroplast gene *rps16* for 47 *Acanthophyllum* species and 63 species of 11 additional genera from Caryophylleae. Phylogenetic analyses were performed using maximum parsimony, maximum likelihood and Bayesian methods. Our analysis suggests that *Allochrysa*, *Diaphanoptera*, *Ochotonophila* and *Scleranthopsis* are nested within *Acanthophyllum* but that the traditionally recognized sections of *Acanthophyllum* are monophyletic after reassignment of a few species. Emarginate petals may be a synapomorphy for one of the two basal clades of *Acanthophyllum*. Moreover, non-monophyly of the genera *Gypsophila* and *Diaphanoptera* is suggested by the present study. The age of the crown clade of *Acanthophyllum* s.l. is estimated to be 11.1 Ma by *BEAST species tree analysis.

Keywords *Acanthophyllum*; Caryophyllaceae; Irano-Turanian; ITS; molecular phylogeny; *rps16*; species tree

■ INTRODUCTION

Acanthophyllum C.A.Mey., with ca. 60 species, is a mainly Irano-Turanian genus (Bittrich, 1993; Ghaffari, 2004) that inhabits areas between Syria and western China (Ghaffari, 2002). The diversity center of the genus is the Khorassan-Kopet dagh floristic province in NE Iran and neighboring areas in Afghanistan and Turkmenistan (Ghaffari, 1989, 2004; Mahmoudi-Shamsabad & al., 2012).

Acanthophyllum species are small, shrubby, cushion-forming perennials with spiny leaves. They grow in exposed habitats on sandy or stony hills and rocky slopes (Fig. 1A–B). They are important components of the steppe (Zohary, 1973) and mountain vegetation in Central and Southwest Asia.

Several biologically active triterpene saponins have been reported from different species of *Acanthophyllum* (Gaidi & al., 2000, 2004). The genus could be medicinally interesting due to the highly cytotoxic properties of many saponins. It has been shown that cytotoxic compounds have potential antitumor activity (Sparg & al., 2004). Saponins of some *Acanthophyllum* species have been suggested as substitutes for synthetic surfactants in shampoo (Aghel & al., 2007).

Although *Acanthophyllum* is one of the larger genera in the Caryophyllaceae, it has been subjected to few systematic

and phylogenetic studies and no inclusive monograph of the genus is available. The number of species was estimated to 56 in Bittrich (1993), based on *Flora Iranica* (Schiman-Czeika, 1988), but 14 species should be added based on *Flora of the U.S.S.R.* (Schischkin, 1936), *Flora of Uzbekistan* (Vvedensky, 1953) and *Flora of Tajikistan* (Ovchinnikov, 1968). With a few recently described species (Aytaç, 2001; Mahmoudi-Shamsabad & al., 2012; Pirani & al., 2013), the species number exceeds 70. On the other hand, several synonymies have been suggested by regional studies in Iran and Pakistan (Ghazanfar & Nasir, 1986; Basiri-Esfahani & al., 2011). Clearly, the taxonomy of *Acanthophyllum* is in need of revision.

Boissier (1867) recognized five groups within *Acanthophyllum*, delimited mainly on the basis of inflorescence and floral features. The basic framework of his classification was followed in the next three major taxonomic treatments of the genus by Golenkin (1893), Schischkin (1936) and Schiman-Czeika (1988). Golenkin (1893) recognized 19 species, including the genus *Allochrysa* Bunge as a sixth section. Schischkin (1936) classified the genus into two subgenera (*Euacanthophyllum* and *Allochrysa*), recognizing two sections in subg. *Allochrysa* (Bunge) Schischk. Schiman-Czeika (1988) transferred sect. *Pseudacanthophyllum* (Boiss.) Rech.f. from *Gypsophila* L. to *Acanthophyllum* and a new section, *Scapiflora*

Schiman-Czeika, was introduced. *Acanthophyllum* sect. *Scapiflora* includes four species, among which two were transferred from *Gypsophila* and *Saponaria* L. A comparison of the four major infrageneric classifications of *Acanthophyllum* (with type for each section) is given in Table 1.

Hitherto used characters in infrageneric classifications of *Acanthophyllum* are mostly related to inflorescence, shape and texture of bracts, and number of ovules. The general inflorescence type in *Acanthophyllum* is dichasial cymes arranged in terminal heads and/or axillary verticillasters. The exception is

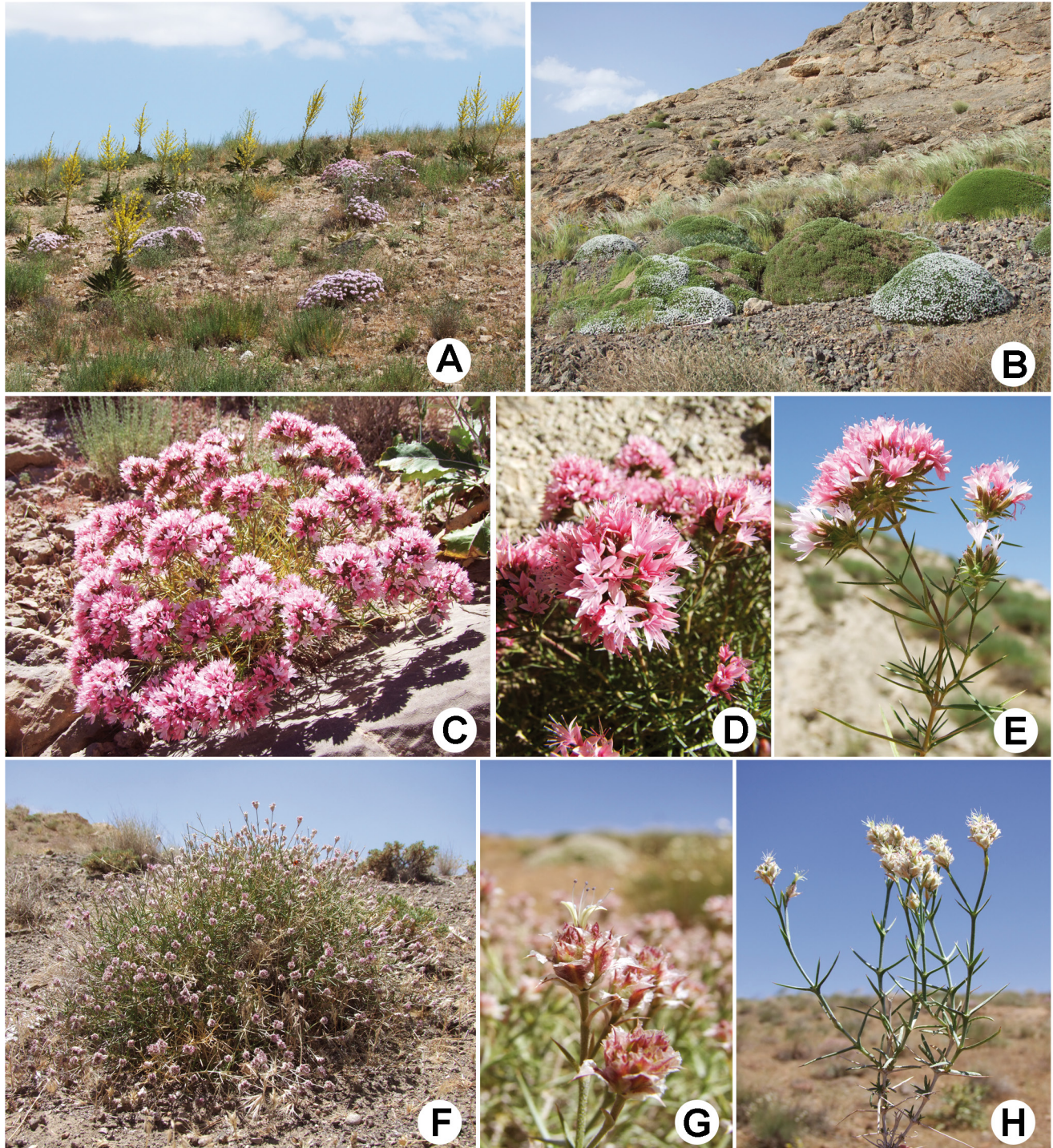
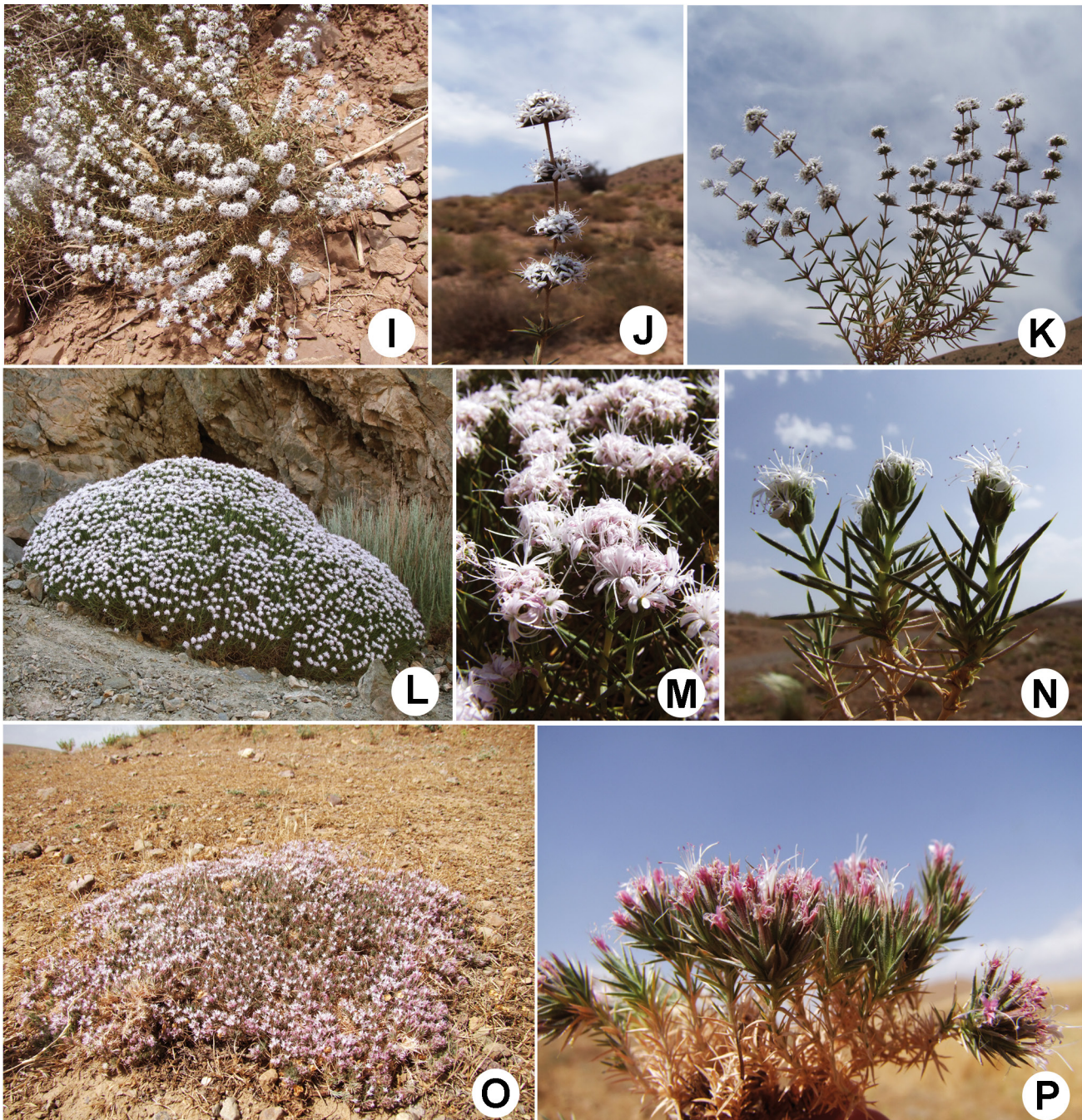


Fig. 1 (in two parts). A–B, Natural habitats of *Acanthophyllum*; C–E, *A. speciosum* (sect. *Oligosperma*); F–G, *A. gracile* (sect. *Macrostegia*); H, *A. bracteatum* (sect. *Macrostegia*); I–K, *A. crassifolium* (sect. *Acanthophyllum*); L–M, *A. glandulosum* (sect. *Pleiosperma*); N, *A. spinosum* (sect. *Pleiosperma*); O–P, *A. caespitosum* (sect. *Oligosperma*). — Photos by Hamid Moazzeni & Atefeh Pirani.

sect. *Macrodonta* Boiss. that includes species with 1-flowered (rarely up to 3-flowered) terminal inflorescences. Among inflorescence characters applied in sectional classification are (1) 1-flowered versus multi-flowered inflorescences, (2) terminal versus elongated inflorescences and (3) richly branched versus poorly branched inflorescences (richly branched inflorescences are composed of several partial dichasial units distributed along the stem or confined to several apical nodes of the stem, whereas poorly branched inflorescences are composed of one, or rarely up to three condensed dichasial unit(s) confined to only one terminal node of the stem; Fig. 2A–B). Inflorescence

variation in *A.* sect. *Acanthophyllum*, sect. *Macrostegia* Boiss., sect. *Oligosperma* Schischk. and sect. *Pleiosperma* Boiss. is shown in Fig. 1C–P.

As with many other genera in the family (Kurtto, 2001; Oxelman & al., 2001; Fior & al., 2006), the limits of *Acanthophyllum* in relation to closely related genera are controversial. There have been several transfers of species among *Acanthophyllum*, *Gypsophila*, *Saponaria* and *Dianthus* L. by different authors. Moreover, the relationships to the small genera, *Scleranthopsis* Rech.f., *Allochrusa*, *Ochotonophila* Gilli, *Diaphanoptera* Rech.f., and *Kuhitangia* Ovczinn. need to be elucidated.



Acanthophyllum has been included with few samples in a small number of molecular studies of Caryophyllaceae. In a phylogenetic analysis based on the chloroplast DNA *matK* region, *Allochrysa versicolor* Boiss. is sister to *Acanthophyllum sordidum* Bunge ex Boiss. (Fior & al., 2006), but as only one *Acanthophyllum* species was included, it is not possible to judge whether *Allochrysa* should be retained or not. Greenberg & Donoghue (2011) showed that *All. versicolor*, *A. sordidum* and *A. paniculatum* Regel & Herder make a monophyletic clade, reinforcing the idea that the two genera are closely related. *Allochrysa* was sister to *A. sordidum*, thus rendering *Acanthophyllum* paraphyletic. The latter work is the most comprehensive molecular study of Caryophyllaceae to date.

In the few divergence time studies that included *Acanthophyllum*, the approximate age of the *Acanthophyllum-Allochrysa*

clade was estimated to be 2.88 Ma (Frajman & al., 2009) or between 3 and 9 Ma (Valente & al., 2010). Since only one species each of *Allochrysa* and *Acanthophyllum* were sampled in these studies, there is a need for a focused study using a wider sampling.

The main goals of this study are: (1) to test the monophyly of *Acanthophyllum* and its infrageneric taxa using DNA sequence data; (2) to evaluate the infrageneric classification of *Acanthophyllum* in light of molecular data; (3) to check the evolution of morphological characters on the background of the molecular phylogeny; (4) to estimate the divergence time of the crown node of *Acanthophyllum*; (5) to investigate the phylogenetic position of *Acanthophyllum* within tribe Caryophylleae.

Table 1. Major infrageneric classifications of *Acanthophyllum* including types for sectional taxa.

Boissier (1867)	Golenkin (1893)	Schischkin (1936)		Schiman-Czeika (1988)	Type
		Subg. <i>Euacanthophyllum</i> (Boiss.) Schischk. ^a	Subg. <i>Allochrysa</i> (Bunge) Schischk.		
Sect. <i>Macrodonga</i>	Sect. <i>Macrodonga</i>	—	—	Sect. <i>Macrodonga</i> Boiss.	<i>A. grandiflorum</i> Stocks
Sect. <i>Macrostegia</i>	Sect. <i>Macrostegia</i> Boiss.	Sect. <i>Macrostegia</i> Boiss.	—	Sect. <i>Macrostegia</i> Boiss.	<i>A. bracteatum</i> Boiss.
Sect. <i>Turbinaria</i>	Sect. <i>Turbinaria</i> Boiss.	Sect. <i>Turbinaria</i> Boiss.	—	Sect. <i>Acanthophyllum</i>	<i>A. mucronatum</i> C.A.Mey.
Sect. <i>Pleiosperma</i>	Sect. <i>Pleiosperma</i> Boiss.	Sect. <i>Pleiosperma</i> Boiss.	—	Sect. <i>Pleiosperma</i> Boiss.	<i>A. spinosum</i> (Desf.) C.A.Mey.
Sect. <i>Euacanthophylla</i>	Sect. <i>Euacanthophylla</i>	Sect. <i>Oligosperma</i> Schischk.	—	Sect. <i>Oligosperma</i> Schischk.	<i>A. squarrosom</i> Boiss.
—	—	—	—	Sect. <i>Pseudacanthophyllum</i> (Boiss.) Rech.f. Syn.: <i>Gypsophila</i> sect. <i>Pseudacanthophyllum</i> Boiss.	<i>A. laxiflorum</i> Boiss.
—	—	—	—	Sect. <i>Scapiflora</i> Schiman-Czeika	<i>A. scapiflorum</i> (Akhtar) Schiman-Czeika
—	—	—	Sect. <i>Versicoloria</i> Schischk.	—	<i>A. versicolor</i> Fisch. & C.A.Mey.
—	Sect. <i>Paniculata</i> Golenk.	—	Sect. <i>Paniculata</i> Golenk.	—	<i>A. paniculatum</i> Regel & Herder

^a *Acanthophyllum* when described by Meyer (1831) included only one species which should automatically serve as the type of the genus. Boissier (1867) made the first infrageneric classification of the genus including five sections (indicated by “§”). He was not aware of typification rules and assigned the type to the genus in sect. *Turbinaria*. Most of earlier taxonomists ignored to put the type of the genus in section *Acanthophyllum* (or sect. *Euacanthophylla*). Schiman-Czeika (1988) recognized the problem and defined section *Acanthophyllum* with *A. mucronatum* as the type and including formerly described sect. *Turbinaria*.

■ MATERIALS AND METHODS

Sampling strategy and plant material. — All genera from Caryophylleae represented in former phylogenetic studies plus four small genera allied to *Acanthophyllum* were included. The genus *Silene* L. was chosen as outgroup (Sileneae is well established as sister group to Caryophylleae; Harbaugh & al., 2010; Greenberg & Donoghue, 2011).

New sequences were obtained from specimens deposited at FUMH, GAZI, GB, M, MSB, TARI, TMRC and TUH; a total of 108 sequences representing 47 species of *Acanthophyllum*, 4 of *Diaphanoptera*, 2 of *Allochrysa*, 1 of *Ochotonophila*, 1 of *Scleranthopsis* and 1 of *Gypsophila*. All sections of *Acanthophyllum* recognized by Schiman-Czeika (1988) and Schischkin (1936), covering the whole morphological variation within the genus, have been included. Amplification of the *rps16* region for *A. lilacinum* Schischk. and *D. afghanica* Podlech failed. There was no material of *A. paniculatum* available, therefore only ITS sequence for this species could be obtained from GenBank. Other ITS and *rps16* sequences representing 21 *Gypsophila*, 3 *Saponaria*, 23 *Dianthus*, 4 *Petrorhagia* (Ser.) Link, 1 *Velezia* L., 1 *Psammophilene* W.C.Wu & C.Y.Wu, 1 *Vaccaria* Medik., 1 *Bolbosaponaria* Bondarenko and 17 *Silene* species were obtained from GenBank. In several cases only the ITS or the *rps16* sequence was available for a species, so the ITS and *rps16* datasets do not strictly match.

The final dataset for ITS contained 117 sequences representing 117 taxa and for *rps16* 72 sequences representing 72 taxa. Voucher information is listed in Appendix 1.

DNA extraction, PCR, and sequencing. — Genomic DNA was extracted from herbarium specimens using the E.Z.N.A. SP Plant DNA Mini Kit (Omega Bio-Tek, Norcross,

Georgia, U.S.A.) according to the manufacturer's protocol, or using a modified Carlson/Yoon method (Oxelman & Lidén, 1995; Rautenberg & al., 2010). The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using primer pairs P17/26S-82R (Popp & Oxelman, 2001; Kool & al., 2012). The complete intron of the plastid *rps16* gene was amplified using primer pairs *rpsF/rpsR2R* (Oxelman & al., 1997; Petri & Oxelman, 2011; Kool & al., 2012) or *rpsF/rpsR3R*. Multi-screen PCR (Millipore, Billerica, Massachusetts, U.S.A.) was used to purify amplification products, according to the manufacturer's instructions. The ITS region was sequenced using primer pairs P16/ITS4 (Eggens & al., 2007; Popp & Oxelman, 2007), whereas the *rps16* region was sequenced using primer pairs *rpsF2a/rpsR3R* (Popp & al., 2005). Sequencing was performed by Macrogen (Amsterdam, Netherlands).

Sequence assembly, alignment and analysis. — Sequence editing, contig assembly and alignments (see TreeBase, <http://purl.org/phylo/treebase/phyloids/study/TB2:SI5296>) were performed using Geneious v.5.5.8. For multiple alignments, we used the MUSCLE plug-in with default settings. The alignments were checked and adjusted manually. Indels were coded using SeqState v.1.4.1 (Müller, 2005), under the simple indel-coding option (Simmons & Ochotorena, 2000). The best substitution model for each alignment was selected using jModelTest v.0.1.1 (Posada, 2008), under the Bayesian information criterion (BIC). The GTR+G model was determined as the best-fit model for both nuclear and chloroplast markers. The binary data model (Lewis, 2001), as implemented in MrBayes, was used for indel characters. Bayesian inference (BI) of the individual gene analyses was performed using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001), with default prior settings, for ten million MCMC generations in four parallel runs, each

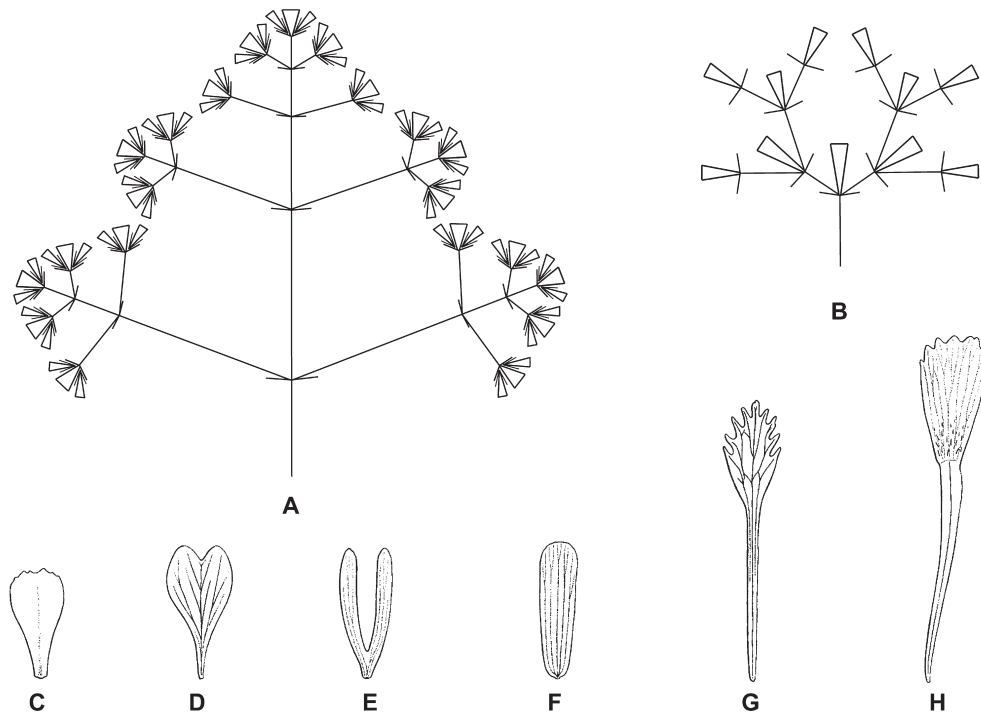


Fig. 2. **A**, richly branched inflorescence; **B**, poorly branched inflorescence; **C**, dentate petal; **D**, emarginate petal; **E**, bifid petal; **F**, entire petal; **G**, fimbriate petal; **H**, sinuate petal. — A–B after Schiman-Czeika, 1988; C–H after Láinz & Garmendia (1990).

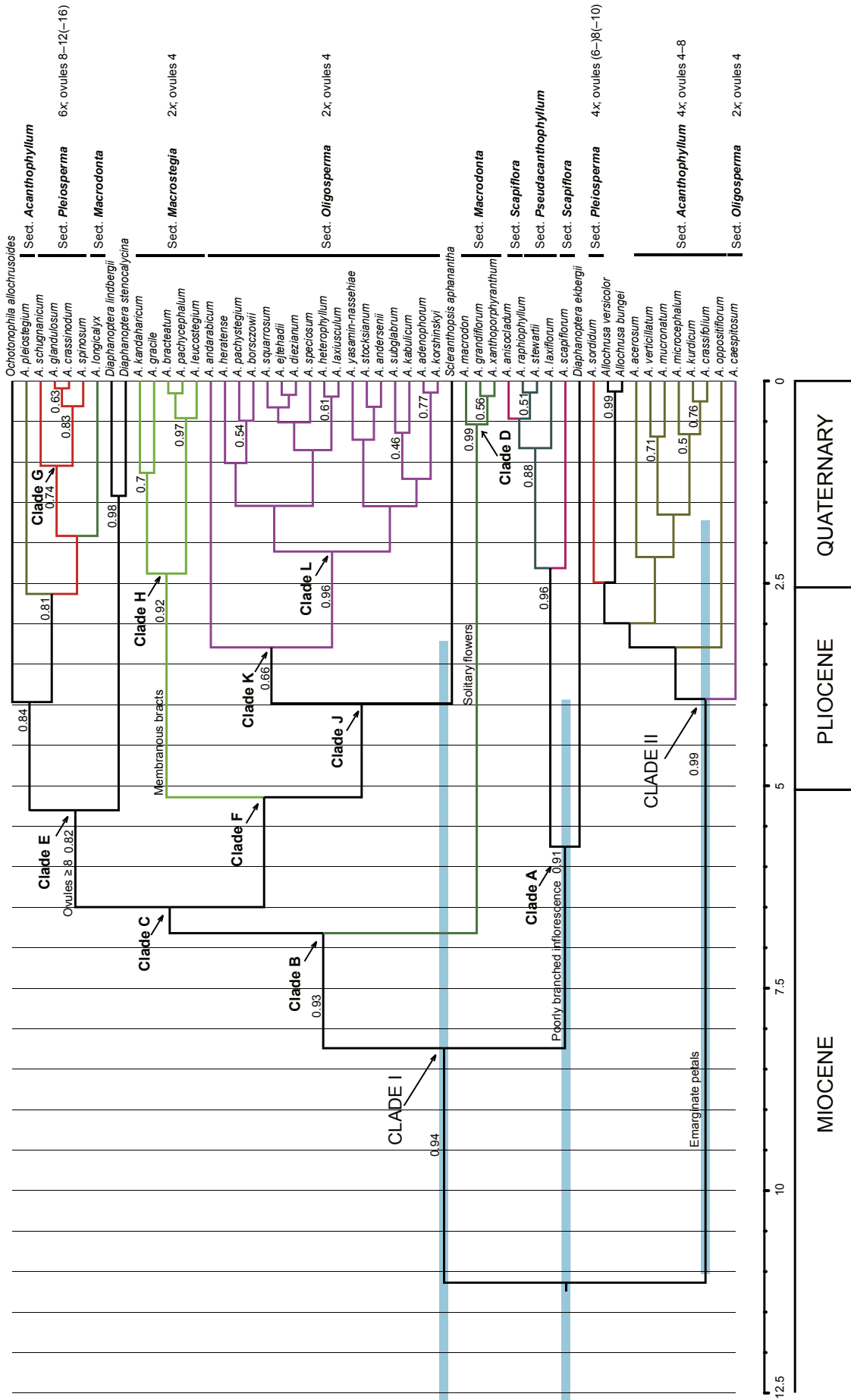


Fig. 3. *BEAST species tree of *Acanthophyllum* with node bars representing 95% HPD intervals for the root, Clade I and Clade II nodes. Bayesian posterior probabilities are indicated above branches. Values below 50% are not shown.

with four parallel chains for *rps16*, and four runs each with eight chains under increased temperature of $t = 0.1$ for the ITS dataset. Tracer v.1.5 (Rambaut & Drummond, 2009) was used to check convergence of runs and diagnose MCMC chains. Trees were summarized in TreeAnnotator v.1.6.1 (Drummond & Rambaut, 2007) with the 25% first generations discarded as burn-in. Maximum likelihood (ML) analyses for individual nuclear and chloroplast datasets were conducted using RAxML-HPC2 v.7.3.2 on the Cipres Science Gateway (Miller & al., 2010) under the GTRCAT model with 1000 bootstrap replicates. Bootstrap proportions on the ML tree were summarized using Sumtrees from the Python Dendropy library (Sukumaran & Holder, 2010). Maximum parsimony (MP) analyses were performed on both datasets after excluding outgroup taxa, using PAUP* v.4.0b10 (Swofford, 2003), which was also used to calculate consistency and retention indices, and tree length. Parameters for the parsimony heuristic searches included 100 random addition replicates, tree bisection-reconnection branch swapping and multrees off. Trees were visualized using FigTree v.1.3.1 (Rambaut, 2009).

Species tree inference and dating. — The *BEAST method as implemented in BEAST v.1.7.3 (Drummond & al., 2006; Drummond & Rambaut, 2007) was used to estimate the species tree for 52 species (covering those species common to both plastid and nuclear datasets except for *Gypsophila cerastioides* D. Don). The data matrix was composed of two unlinked partitions: (1) ITS sequences, (2) *rps16* sequences, using the GTR+G as substitution model for both partitions, and an uncorrelated relaxed lognormal clock. One MCMC chain was run for 20 million generations, with tree sampling every 1000 steps. Since there is no known fossil record for the study group, we used published ITS and chloroplast substitution rates. The prior probability of the clock rate was set to a truncated normal distribution with a mean of 2.15×10^{-3} , ranging from 0.38×10^{-3} to 7.83×10^{-3} substitutions per site per Ma for ITS and a truncated normal distribution with a mean of 0.67×10^{-3} , ranging from 0.5×10^{-4} to 2.6×10^{-3} substitutions per site per Ma for *rps16*. ITS substitution rates were set according to previous estimates for perennial woody plants (Kay & al., 2006). The *rps16* rates could have been independently estimated, but in order to speed up convergence (particularly by excluding highly suboptimal starting rates) we instead provided rate priors where the lower and upper rates of ITS were divided by three (substitution rates in the chloroplast genome are typically slower by similar magnitudes compared to rates in the nuclear genome in plants; Wolfe & al., 1987; Gaut & al., 1996; Yue & al., 2010). The *rps16* prior standard deviation was set to 0.7×10^{-3} that fits in the truncated normal distribution. The value of standard deviation is larger than the mean and unlikely to bias its posterior estimate. Then the estimated rates were checked and modified to the values that allowed the *rps16* posteriors to be able to fluctuate within these priors. The ploidy level for *rps16* was set to haploid.

Tracer was used to check the convergence and mixing of MCMC chains and the effective sample sizes for all parameters to be above 200. Trees were summarized in TreeAnnotator with the 10% first generations discarded as burn-in, and visualized using FigTree.

RESULTS

Species tree inference and dating. — Two major clades are retrieved within *Acanthophyllum* in a wide sense (see below) by the *BEAST species tree analysis (Fig. 3). These clades are referred to as Clade I and Clade II hereafter. Two clades are recognized within Clade I (PP 0.94): (1) clade A (PP 0.91), including *A. sect. Pseudacanthophyllum* and *sect. Scapiflora*, with *Diaphanoptera ekbergii* Hedge & Wendelbo as sister to these sections; (2) clade B (PP 0.93), including *A. sect. Macrodonia*, *sect. Macrosteigia*, *sect. Pleiosperma* and *sect. Oligosperma* as well as *Ochotonophila allochrusoides* Gilli, *Scleranthopsis aphanantha* (Rech.f.) Rech.f., *Diaphanoptera stenocalycina* Rech.f. & Schiman-Czeika and *D. lindbergii* Hedge & Wendelbo. A single species of *A. sect. Acanthophyllum* (*A. pleiostegium* Schiman-Czeika) also nests within clade B. Clade II (PP 0.99) largely corresponds to *A. sect. Acanthophyllum*. *Allochrusa bungei* Boiss. and *All. versicolor* form a subclade within Clade II with strong support (PP 0.99). *Acanthophyllum sordidum* of *A. sect. Pleiosperma* and *A. caespitosum* Boiss. of *A. sect. Oligosperma* also resolve within Clade II.

This analysis estimates the age of the *Acanthophyllum* clade (crown node) to be 11.1 Ma (3.8–32.4 Ma, 95% HPD interval). Clades I and II are estimated to be 8.2 Ma (3.2–22.2 Ma, 95% HPD interval) and 3.9 Ma (1.7–11.1 Ma, 95% HPD interval), respectively.

Phylogenetic analyses. — BI, ML and MP analyses of individual nuclear and plastid markers yielded mostly congruent trees within each marker, with no strongly supported differences. Therefore, the results of BI are presented and discussed in detail, whereas those of ML and MP analyses including tree length, invariable characters, number of informative characters, number of indels, consistency and retention indices, and likelihood bootstraps are summarized in Table 2 and Figs. 4–5.

Table 2. Models chosen by ModelTest, sequence and parsimony statistics.

	ITS	<i>rps16</i>
Number of sequences (number of ingroup taxa)	56	53
BIC model choice	GTR+G	GTR+G
Sequence length [bp]	674	839
Number of coded indels	63	40
Invariable characters [bp]	446	742
Variable sites [bp]	228	97
Variable sites [%]	33.8	11.6
Parsimony-informative characters [bp]	140	43
Parsimony-informative characters [%]	20.7	5.1
Consistency index, excl. uninformative characters	0.57	0.78
Retention index	0.8	0.94

Two major well-supported clades are recognized within *Acanthophyllum* in both the ITS (Fig. 4) and the *rps16* (Fig. 5) trees. These clades are congruent with the Clades I and II obtained by *BEAST species tree analysis. The genera *Ochotonophila*, *Scleranthisopsis*, *Diaphanoptera* (pro majora parte) and one species of *Gypsophila* are nested within *Acanthophyllum*. However, *Allochrusa* is sister to *Acanthophyllum* in the ITS

phylogeny (PP 0.86; Fig. 4), whereas it nests within *Acanthophyllum* Clade II in the *rps16* phylogeny (PP 1.0; Fig. 5). Clade I is composed of *A.* sect. *Oligosperma*, sect. *Pleiosperma*, sect. *Scapiflora*, sect. *Pseudacanthophyllum*, sect. *Macrostegia* and sect. *Macrodonata*. Clade I also contains representatives of the genera *Diaphanoptera*, *Ochotonophila*, *Scleranthisopsis* and *Gypsophila*. Moreover, monophyly of *A.* sect. *Oligosperma*,

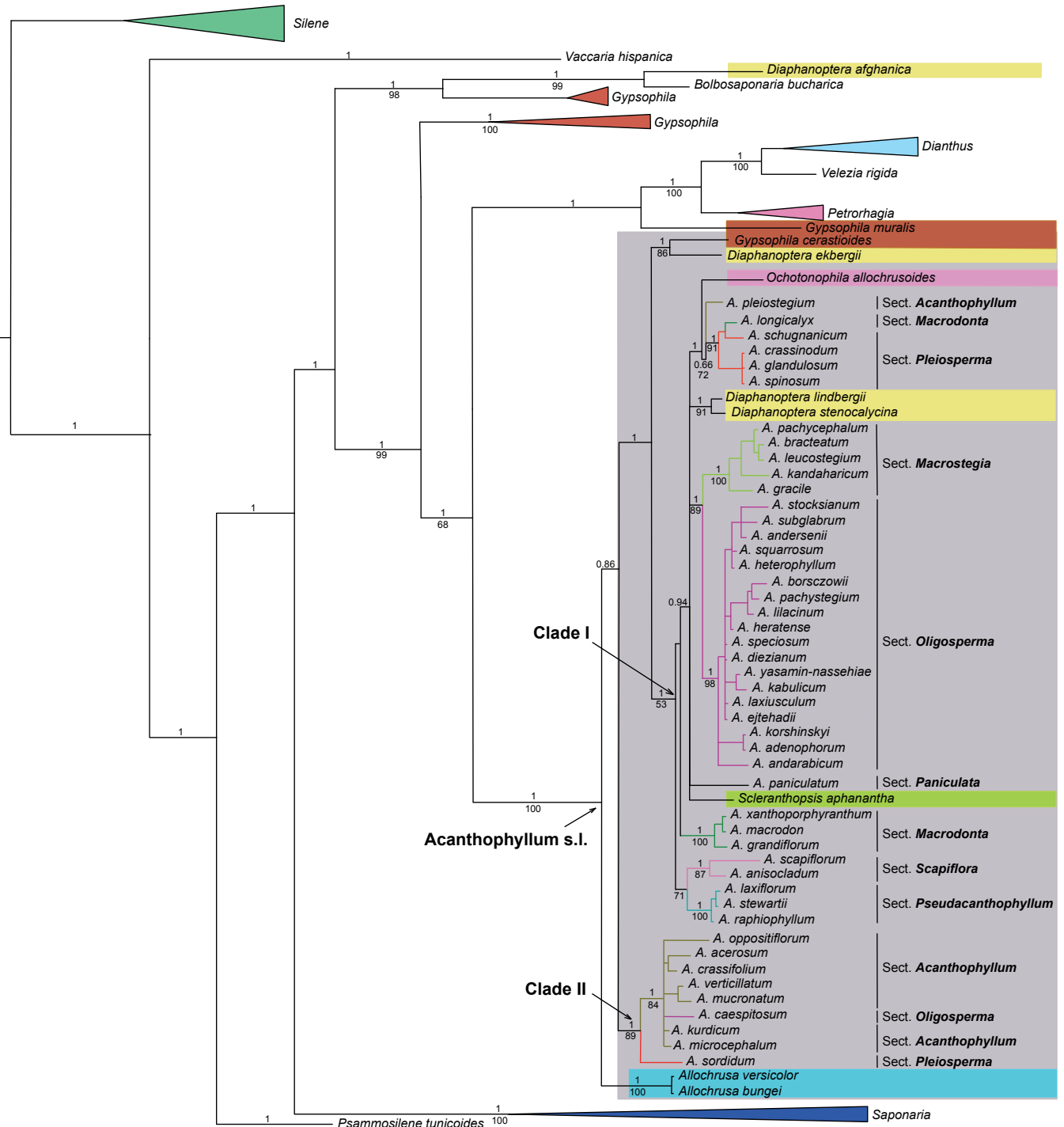


Fig. 4. Majority-rule consensus tree inferred from Bayesian analysis of ITS data. Posterior probability values are indicated above branches, ML bootstrap values below. Values below 50% are not shown.

sect. *Macrodon* and sect. *Macrostegia* is not obtained in the *rps16* tree. Clade II includes all examined species of *A.* sect. *Acanthophyllum* (except for *A. pleiostegium*) as well as *A. sordidum* (sect. *Pleiosperma*) and *A. caespitosum* (sect. *Oligosperma*). The *Allochrusa* species are included in this clade in the *rps16* tree. The resolution within Clade II is low in both datasets.

■ DISCUSSION

Phylogenetic position of *Acanthophyllum* within tribe Caryophylleae. — *Acanthophyllum* has gained little attention in previous phylogenetic analyses of tribe Caryophylleae. The most comprehensive molecular study included only two species (Fior & al., 2006; Harbaugh & al., 2010; Valente & al.,

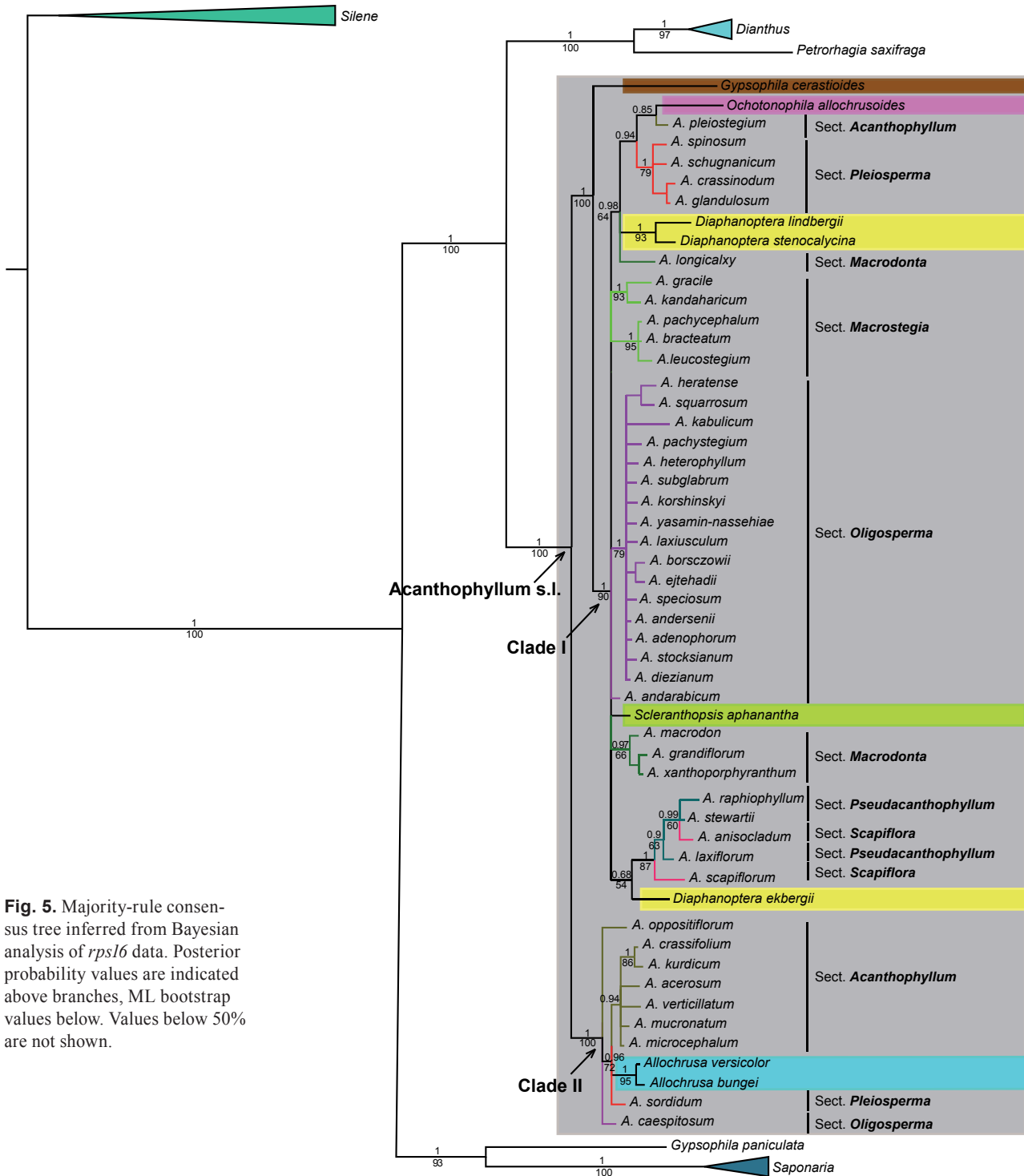


Fig. 5. Majority-rule consensus tree inferred from Bayesian analysis of *rps16* data. Posterior probability values are indicated above branches, ML bootstrap values below. Values below 50% are not shown.

2010; Greenberg & Donoghue, 2011) and the small genera allied to *Acanthophyllum*, i.e., *Scleranthopsis*, *Ochotonophila* and *Diaphanoptera*, were ignored. In our trees these small genera are embedded in *Acanthophyllum* s.l. (Figs. 4–5). Cushion-forming subshrubby to somewhat woody habit is a character that distinguishes the *Acanthophyllum* s.l. clade (*Acanthophyllum*, *Scleranthopsis*, *Allochrusa*, *Ochotonophila*, *Diaphanoptera* p.p.) from the rest of tribe Caryophylleae. We conclude that the morphological characters used for delimiting *Acanthophyllum* s.str., *Allochrusa*, *Scleranthopsis*, *Ochotonophila* and *Diaphanoptera* are either not congruent with the phylogenetic patterns or need to be reassessed. Therefore, we suggest a new classification for this group of taxa based on more reliable characters and the evidence from molecular phylogenetics (see below under Circumscription of *Acanthophyllum*).

The analyses of nuclear and plastid loci show that the *Acanthophyllum* s.l. clade (including *Allochrusa*, *Ochotonophila*, *Diaphanoptera* p.p. and *Scleranthopsis*), (PP 1.0; Figs. 4–5) is sister to a clade holding *Dianthus* and *Petrorragia* (and *Velezia* only in ITS tree) (PP 1.0; Figs. 4–5). This is in agreement with previous studies (Fior & al., 2006; Harbaugh & al., 2010; Valente & al., 2010; Greenberg & Donoghue, 2011). *Gypsophila cerasioides* nests within *Acanthophyllum* s.l.

The placement of *G. muralis* L. as sister to the *Dianthus-Petrorragia* clade in the present ITS tree is consistent with the results of Greenberg & Donoghue (2011).

Circumscription of *Acanthophyllum*. — This study reveals that *Acanthophyllum* as currently circumscribed (Schiman-Czeika, 1988; Bittrich, 1993; Takhtajan, 2009) is not monophyletic. The circumscription of *Acanthophyllum* is discussed in relation to its closely related genera residing within *Acanthophyllum* s.l. clade.

***Scleranthopsis*.** — The monospecific *Scleranthopsis* is distributed in SW, E and central Afghanistan. The position of this genus has been uncertain and was discussed by Rechinger (1957, 1967). It was first described as *Acanthophyllum aphananthum* Rech.f. and classified in the monotypic sect. *Aphanantha* Rech.f. (Rechinger, 1957). It was later raised to generic level, *Scleranthopsis*, based on the following characters: non-clawed petals, short petals enclosed within the calyx, and unequal calyx teeth (Rechinger, 1967).

Our study supports returning *Scleranthopsis* to *Acanthophyllum* and re-establishing the sect. *Aphanantha*.

***Allochrusa*.** — *Allochrusa* has approximately seven species distributed in Armenia, Turkey, NW Iran, NE Afghanistan, Tajikistan and Turkmenistan. It was first proposed as distinct from *Acanthophyllum* (Boissier, 1867) based on non-spiny leaves and enclosed stamens but was included in *Acanthophyllum* by Golenkin (1893) and Schischkin (1936). Golenkin made *Allochrusa* a section, whereas Schischkin (1936) treated it as a subgenus. He divided subg. *Allochrusa* into two sections, sect. *Versicoloria* Schischk. and sect. *Paniculata* Golenk. (Table 1). The species of the latter section have a short calyx and petals, as well as loose inflorescences. Barkoudah (1962) followed Schischkin. However, *Allochrusa* has later been accepted as a separate genus (Yukhananov, 1974; Schiman-Czeika, 1987; Bittrich, 1993; Takhtajan, 2009).

Allochrusa versicolor and *All. bungei* form a strongly supported clade within *Acanthophyllum* s.l. (Figs. 3–5). The Bayesian analysis of the plastid marker as well as the results of the *BEAST species tree analyses are congruent in resolving this clade within Clade II. However, Bayesian analysis of ITS suggests that the *Allochrusa* clade is sister group to *Acanthophyllum* (PP 1.0, but note PP only 0.86 for monophyly of the latter). We cannot resolve the reason for this incongruence at the moment. Emarginate petals is a possible synapomorphy for Clade II, including *Allochrusa bungei* and *All. versicolor* (= *Allochrusa* s.str.). Our ITS dataset also included *Acanthophyllum paniculatum*, which was classified in subg. *Allochrusa* sect. *Paniculata* by Schischkin (1936) and in the genus *Allochrusa* by Schiman-Czeika (1988). *Acanthophyllum paniculatum* which has entire petals, is placed in Clade I in the ITS tree, together with other species with entire petals.

Interestingly, in Clade II, *All. versicolor* and *bungei* that are distributed in NW Iran and Armenia come together with *Acanthophyllum* species with a mainly western distribution pattern, i.e., Syria, Turkey, Iraq and W Iran, whereas the more eastern *A. paniculatum* (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan) is placed in Clade I, which includes taxa with an eastern distribution. Our study supports the inclusion of *Allochrusa* in the *Acanthophyllum* s.l. clade.

***Ochotonophila*.** — *Ochotonophila* comprises two species inhabiting central and E Afghanistan (Gilli, 1956; Hedge & Wendelbo, 1963; Schiman-Czeika, 1988). They are low-growing multi-stemmed perennials with a woody base. In the original description by Gilli (1956) the genus (with the single species *O. allochrusoides*) was considered intermediate between *Acanthophyllum* and *Gypsophila*. Hedge & Wendelbo (1963) described *O. eglandulosa* Hedge & Wendelbo, again emphasizing similarities to *Acanthophyllum* and *Gypsophila*. Later work by Schiman-Czeika (1988) suggested that *Ochotonophila* is more closely related to *Acanthophyllum*, *Scleranthopsis* and *Allochrusa*. The tubular calyx and the presence of bracteoles are features shared by *Ochotonophila*, *Allochrusa*, *Scleranthopsis* and *Acanthophyllum* but not *Gypsophila*. *Ochotonophila* differs from *Acanthophyllum* by its non-spiny leaves, enclosed stamens and, non-appressed bracteoles. *Ochotonophila allochrusoides* is placed close to *A. sect. Pleiosperma*, *A. pleiostegium* (*A. sect. Acanthophyllum*) and *A. longicalyx* Hedge & Wendelbo (*A. sect. Macrodonata*) in our analyses (Figs. 3–5). An important feature shared by *Ochotonophila* and *A. sect. Pleiosperma* is the high number of ovules (10–21). We suggest reducing *Ochotonophila* to a section under *Acanthophyllum* s.l.

***Diaphanoptera*.** — *Diaphanoptera* has six species and is distributed in NE Iran, Turkmenistan and Afghanistan (Schiman-Czeika, 1988). They are perennial multi-stemmed plants with a woody base. The genus was described by Rechinger (1940) with the single species *D. khorassanica* Rech.f., distinguished from *Acanthophyllum* by diaphanous wings on the calyx (Rechinger, 1940; Hedge & Wendelbo, 1963). However, the other five currently accepted species of the genus lack diaphanous wings. Lack of bracteoles and a non-tubular calyx also separate *Diaphanoptera* from *Acanthophyllum* (Schiman-Czeika, 1988). *Diaphanoptera afghanica*

(included only in the ITS dataset) is sister to *Bolbosaponaria*, a genus closely related to *Gypsophila* s.str., whereas *Diaphanoptera stenocalycina*, *D. lindbergii* and *D. ekbergii*, nest inside *Acanthophyllum* Clade I in all trees (Figs. 3–5). *Diaphanoptera lindbergii* and *D. stenocalycina* have 10–12 ovules in each ovary, in accordance with its placement in clade E (PP 0.82; Fig. 3) that contains taxa with more than eight ovules. However, the support for clade E is not strong and is due only to *rps16* (PP 0.98; Fig. 5). The placement of *D. ekbergii* as sister to *A.* sects. *Pseudacanthophyllum* and *Scapiflora* is likewise poorly supported in the species tree (PP 0.91; Fig. 3) and the *rps16* tree (PP 0.68; Fig. 5) and not at all in the ITS tree (Fig. 4), where it is resolved as sister to *Gypsophila cerastioides*. We were not successful in generating high-quality sequences for *D. khorassanica*, the type of the genus. Therefore, the delimitation of *Diaphanoptera* from *Acanthophyllum*, *Gypsophila* and *Ochotonophila* cannot be ascertained, but it is clear that *Diaphanoptera* as currently circumscribed contains unrelated elements.

***Gypsophila*.** — *Gypsophila* has about 150 species and is distributed in temperate Asia and Europe, Egypt, Australia, North America and China (Amini & al., 2011). The delimitation of *Acanthophyllum* from *Gypsophila* has long been controversial. General habit, calyx shape, petal shape in terms of developing a claw, leaf texture, duration of bracts and bracteoles, calyx shape and mode of capsule dehiscence (Barkoudah, 1962; Gilli, 1964; Schiman-Czeika, 1987) are the most commonly used characters for discriminating the two genera. Emphasis on different characters has led to several transfers of species. *Acanthophyllum* sect. *Pseudacanthophyllum* is one of the most controversial groups transferred from *Gypsophila* to *Acanthophyllum* (Oliver, 1859; Bentham & Hooker, 1862; Boissier, 1867; Williams, 1889; Barkoudah, 1962; Schiman-Czeika, 1988). The examined species of this section nest within *Acanthophyllum* in our analyses (Figs. 3–5).

In both analyses of ITS and *rps16* (Figs. 4–5) *G. cerastioides* falls within *Acanthophyllum* s.l. *Gypsophila muralis* resolves as sister to the *Dianthus-Petrorrhagia* clade in the ITS tree (consistent with Greenberg & Donoghue, 2011), and the remaining species of *Gypsophila* are found in two additional clades. This shows that *Gypsophila* is non-monophyletic. A broad molecular study (Zarre & al., in prep.) shows that apart from *G. cerastioides* no other species of *Gypsophila* appear to belong to the *Acanthophyllum* clade. Therefore, the poor sampling of *Gypsophila* is not likely to affect our taxonomic conclusions.

Infrageneric classification of *Acanthophyllum* s.str. — The sections in individual ITS and *rps16* trees do not fully match. *Acanthophyllum* sect. *Pseudacanthophyllum* and sect. *Scapiflora* are intermingled in the plastid tree, whereas they are sister sections in the nuclear tree. *Acanthophyllum* sect. *Macrostegia* appears monophyletic only in the ITS tree. *Acanthophyllum* sect. *Pleiosperma* is monophyletic only in *rps16* tree, except for one species. The large section *Oligosperma* forms a clade in both nuclear and plastid trees, except for one and two species, respectively. *Acanthophyllum* sect. *Macrodonta* is monophyletic in both gene trees if *A. longicalyx* is excluded. Most

species of *A.* sect. *Acanthophyllum* form a clade in the ITS tree; only one species is excluded from this clade while one species of *A.* sect. *Oligosperma* is nested within it. In *rps16*, *A.* sect. *Acanthophyllum* is also monophyletic except for two species.

The differences between the *rps16* and ITS trees can be explained by different numbers of informative characters in plastid and nuclear datasets. It can also be attributed to stochastic effects, hybridizations, or incomplete lineage sorting. The implementation of the multispecies coalescent model in *BEAST takes the latter into account (Heled & Drummond, 2010), and by using the information from all datasets simultaneously, the stochastic effects can be expected to decrease. Therefore, we will from now on discuss the phylogenetic status of the taxa in relation to the *BEAST species tree (Fig. 3).

Emarginate petals appear to be a synapomorphy for Clade II, whereas Clade I holds taxa with mainly entire petals (some other petal types, e.g., dentate-sinuate in *A. pleiostegium* and deeply bifid in *O. allochrusoides* are rarely seen in Clade I). The common petal shapes in outgroups are entire, bifid, emarginate, fimbriate and sinuate. Some common petal shapes in Caryophylloideae are shown in Fig. 2C–H.

Clade I. — *Acanthophyllum* sect. *Scapiflora*, sect. *Pseudacanthophyllum*, sect. *Macrodonta*, sect. *Pleiosperma*, sect. *Macrostegia* and sect. *Oligosperma* together form Clade I (PP 0.94; Fig. 3). *Acanthophyllum* sects. *Scapiflora* and *Macrodonta* are not resolved as monophyletic. A single species of *A.* sect. *Acanthophyllum*, *A. pleiostegium*, also nests within Clade I. *Acanthophyllum* sect. *Scapiflora*, sect. *Pseudacanthophyllum*, and sect. *Macrodonta*, sect. *Pleiosperma*, sect. *Macrostegia* and sect. *Oligosperma* correspond to the subclades A, D, G, H and K, respectively. Clade A and clade B, the latter including clades D, E and H, have posterior probabilities from 0.82 to 0.99, and are in the following discussed in relation to putative synapomorphies. The other clades have low support and are not discussed.

Clade A. — Clade A (PP 0.91; Fig. 3) includes *A.* sect. *Pseudacanthophyllum* and sect. *Scapiflora* and *D. ekbergii*. A putative synapomorphy for *Acanthophyllum* sections in this clade is poorly branched inflorescence (Fig. 2B) of one (rarely up to three in *A. laxiflorum* Boiss.) condensed unit(s) confined to only one terminal node of the stem and composed of multi-flowered partial cymes (with more than seven flowers). These sections are almost confined to Afghanistan.

Acanthophyllum anisocladum Schiman-Czeika and *A. scapiflorum* (Akhtar) Schiman-Czeika of sect. *Scapiflora* and *A. laxiflorum*, *A. raphiophyllum* (Rech.f.) Barkoudah and *A. stewartii* (Thoms. ex Edgew. & Hook.f.) of *A.* sect. *Pseudacanthophyllum* constitute clade A. The two sections do not form separate clades in the *BEAST tree (Fig. 3), despite morphological differences that make them easily recognizable. Species of *A.* sect. *Scapiflora* have elongated, leafless peduncles whereas species of *A.* sect. *Pseudacanthophyllum* have a condensed habit with the inflorescence set close to the leaves. The two sections are, however, resolved as monophyletic in the ITS tree (Fig. 4). It will require sampling of other nuclear loci to resolve whether the incongruence should be attributed to stochasticity or needs a biological explanation.

Clade B. – Clade B (PP 0.93; Fig. 3) includes *A.* sect. *Macrodonia* (clade D), sect. *Pleiosperma* (clade G), sect. *Macrostegia* (clade H) and sect. *Oligosperma* (clade K), a single species of *A.* sect. *Acanthophyllum*, as well as *Diaphanoptera*, *Ochotonophila* and *Scleranthopsis*. No morphological synapomorphy for this clade could be found.

Clade D. – Within clade B, subclade D is strongly supported (PP 0.99; Fig. 3) and holds all examined species of *A.* sect. *Macrodonia* except *A. longicalyx*. Clade D is characterized by solitary flowers and four ovules. *Acanthophyllum longicalyx* has one to three terminal flowers and eight ovules and resolves within clade E. This species also possesses the longest calyx and the shortest calyx teeth among the taxa assigned to sect. *Macrodonia* (Hedge & Wendelbo 1963; Schiman-Czeika 1988).

Clade E. – Clade E (PP 0.82; Fig. 3) holds *Ochotonophila allochrusoides*, *A.* sect. *Pleiosperma* (except *A. sordidum*), *A. pleiostegium* (sect. *Acanthophyllum*), *A. longicalyx* (sect. *Macrodonia*) and *Diaphanoptera lindbergii* and *D. stenocalycina*. Apart from *A. pleiostegium* with 4–6 ovules in each ovary, the rest of the taxa in this clade produce eight or more ovules (up to 21 ovules in *O. allochrusoides*).

Clade H. – Clade H (PP 0.92; Fig. 3) corresponds to *A.* sect. *Macrostegia*. Broadly ovate to lanceolate membranous bracts and bracteoles constitute a synapomorphy for this clade. It is widespread in Iran, Iraq, Turkmenistan, Tajikistan, Kyrgyzstan, Uzbekistan, Kazakhstan, Afghanistan and Pakistan.

***Acanthophyllum* sect. *Oligosperma*.** — Clade K (PP 0.66; Fig. 3) has poor support but is more or less identical to the large and taxonomically complicated *A.* sect. *Oligosperma* (except for *A. caespitosum* which is resolved as member of Clade II in all analyses). However, without *A. andarabicum* Podl. ex Schiman-Czeika, the rest of the clade (clade L) has strong support in all trees. Only the ITS phylogeny (Fig. 4) has strong support (PP 1.0) for clade K.

Schischkin (1936) divided *A.* sect. *Oligosperma* into five series. Series *Elatiora* Schischk. (including *A. elatius* Bunge and *A. borsczowii* Litv.) was later elevated to sectional rank (Zakirov & Musaeva, 1981). However, Schiman-Czeika (1988) included *A. elatius* and *A. borsczowii* within sect. *Oligosperma*. We can confirm the latter opinion, as *A. borsczowii* falls within the core group of *A.* sect. *Oligosperma* in the species (PP 0.96; Fig. 3), ITS (PP 1.0; Fig. 4) and *rps16* (PP 1.0; Fig. 5) trees.

Clade II. — Clade II (PP 0.99; Fig. 3) is characterized by emarginate petals. A large part of this clade corresponds to *A.* sect. *Acanthophyllum* which is recognised by branched elongated inflorescences of white flowers (bright pink flowers are rarely seen in some populations of *A. acerosum* Sosn.). However, the section is not resolved as monophyletic, with *A. oppositiflorum* Aytac falling outside this group including *A. acerosum*, *A. verticillatum* Hand.-Mazz., *A. mucronatum* C.A.Mey., *A. microcephalum* Boiss., *A. kurdicum* Boiss. & Hausskn. ex Boiss. and *A. crassifolium* Boiss. *Acanthophyllum oppositiflorum* possesses elongated inflorescences with only two opposite flowers at each node, whereas the other species of *A.* sect. *Acanthophyllum* have inflorescences with multi-flowered verticillasters.

Acanthophyllum sect. *Acanthophyllum* shows a mainly western distribution and inhabits Syria, Iraq, Turkey, Armenia, Azerbaijan, Iran and Turkmenistan. The Afghan species *A. pleiostegium* was assigned to sect. *Acanthophyllum* by Schiman-Czeika (1988), but belongs to clade E (see above).

Acanthophyllum caespitosum (sect. *Oligosperma*) and *A. sordidum* (sect. *Pleiosperma*) also nest in Clade II. They are the only species of their sections that have emarginate petals. *Acanthophyllum caespitosum* is an odd member of *A.* sect. *Oligosperma* also in its procumbent densely branched habit, extremely reduced stems and imbricate leaves. The other taxa of *A.* sect. *Oligosperma* have tall stems and non-imbricate leaves. Further, *A. caespitosum* is the only known species of *Acanthophyllum* with a haploid base chromosome number of $x = 14$ instead of $x = 15$ (Ghaffari, 2004). Ghaffari discussed these morphological and base chromosome number differences and suggested that *A. caespitosum* should form a section of its own. Exclusion of *A. caespitosum* from *A.* sect. *Oligosperma* is corroborated by our analyses and it is placed together with the species of *A.* sect. *Acanthophyllum* (Figs. 3–5). However, *A. caespitosum* differs from *A.* sect. *Acanthophyllum* in 4-ovulate ovaries, reduced stems and reduced inflorescences. Furthermore, all examined representatives of sect. *Acanthophyllum* are tetraploids (Ghaffari, 2004), whereas *A. caespitosum* is diploid. Taking these complexities into account, it is not possible to decisively judge the taxonomic placement of *A. caespitosum* in *A.* sect. *Acanthophyllum*.

Acanthophyllum sordidum has been assigned to *A.* sect. *Pleiosperma* (Boissier, 1867; Schischkin, 1936; Golenkin, 1893; Schiman-Czeika, 1988; Basiri-Esfahani & al., 2011), but our analysis shows that it is remotely related to the rest of the section, which resides within Clade I (Figs. 3–5). The non-clawed linear emarginate petals of *A. sordidum* are not seen in any other taxa of *A.* sect. *Pleiosperma*. The ploidy level reported for *A. sordidum* is tetraploid (Ghaffari 2004), the same as for *A.* sect. *Acanthophyllum*, whereas the rest of the examined species of *A.* sect. *Pleiosperma* are hexaploid (Ghaffari, 2004). However, non-elongated inflorescences and non-clawed petals of *A. sordidum* separate it from *A.* sect. *Acanthophyllum*. We conclude that *A. sordidum* should be excluded from *A.* sect. *Pleiosperma*.

Some conclusions with regard to the circumscription and infrageneric classification of *Acanthophyllum* s.l. and the geographical distribution of its infrageneric taxa can be derived from our study: (1) The majority of the morphological characters used in infrageneric classifications of *Acanthophyllum* show low levels of homoplasy, and are thus useful in interpreting phylogenetic patterns within the genus. We conclude that the value of petal morphology has been underestimated. Emarginate petals, for instance, characterizes one of the two major clades. However, the state of “clawed petal” which is frequent among the studied group, is lost in several places in the trees to form the “non-clawed petals” in *A. aphananthum* (see above under *Scleranthopsis*), *O. allochrusoides* (see above under *Ochotonophila*), *A. pachycephalum* Schiman-Czeika, *A. pleiostegium*, *A. sordidum* and *A. oppositiflorum*; (2) Increase in ploidy level is accompanied by increase in the number

of ovules (see Fig. 3). *Acanthophyllum* sect. *Macrostegia* and sect. *Oligosperma*, which have diploid chromosome numbers, always produce four ovules per ovary, whereas tetraploid *A.* sect. *Acanthophyllum* produces 4–8 ovules, and hexaploid *A.* sect. *Pleiosperma* produces 8–16 ovules; (3) The number of *Acanthophyllum* species is estimated to amount to about 80–90. *Acanthophyllum* sect. *Acanthophyllum* constitutes the westernmost limit of the genus. The diversity center of this section is in NW Iran and neighboring areas in Turkey and Iraq. The examined species of this section reside within Clade II in our trees. The easternmost limit is set by *A. pungens* Boiss. (sect. *Oligosperma*), which inhabits Central Asia to W China. *Acanthophyllum* sect. *Oligosperma* is otherwise most diverse in NE Iran and adjacent areas of Afghanistan and Turkmenistan, corresponding to the diversity center of the genus as a whole. *Acanthophyllum* sect. *Oligosperma* falls into Clade I in our analyses. Generally, most *Acanthophyllum* species are in Clade I, found in NE Iran or further east. Afghanistan harbors the highest number of *Acanthophyllum* sections, including three endemic sections (*A.* sect. *Scapiflora*, sect. *Ochotonophila* and sect. *Aphanantha*; see below).

Divergence time of *Acanthophyllum*. — Valente & al. (2010) obtained an approximate age of 5 Ma (3–9 Ma, 95% HPD interval) for the *Acanthophyllum*-*Allochrusa* clade. BEAST analysis of *matK* data by Frajman & al. (2009) estimated the age to 2.88 Ma. These studies included only one species each of *Acanthophyllum* and *Allochrusa*: *A. sordidum* and *All. versicolor*. In our *BEAST species tree (Fig. 3) *Allochrusa* nests within *Acanthophyllum* and *A. sordidum* is resolved as sister to *All. versicolor* and *All. bungei*). Although weakly supported, the *A. sordidum*-*Allochrusa* clade has an approximate age of 2.48 Ma (0.1–7.7 Ma, 95% HPD interval) in our analysis. The species tree analysis suggests a somewhat younger age for the *A. sordidum*-*Allochrusa* clade, compared to the age estimated by Valente & al. (2010) using gene trees, although the credibility intervals of both estimates largely overlap. The age of 2.48 Ma estimated by the present species tree analysis for the *Allochrusa*-*Acanthophyllum* clade is very close to the age (2.88 Ma) gained by the BEAST analysis of *matK* (Frajman & al., 2009).

The *BEAST analysis estimated the age of the *Acanthophyllum* s.l. crown clade as 11.1 Ma (3.8–32.4 Ma, 95% HPD interval), a time span covering the Oligocene, Miocene and Pliocene (Fig. 3).

There are some fossils assigned to Caryophyllaceae (Jordan & Macphail, 2003; Huang & al., 2012), but we did not use them as the taxa were phylogenetically far from our focal group, which would either introduce long branches to our tree or require an extremely increased taxon sampling. Therefore, we used only clock rate calibration analysis.

■ TAXONOMIC IMPLICATIONS

Acanthophyllum C.A.Mey., Verz. Pfl. Casp. Meer.: 210. 1831
– Type: *A. mucronatum* C.A.Mey.
= *Ochotonophila* Gilli in Feddes Repert. Spec. Nov. Regni Veg. 59: 169. 1956, **syn. nov.** – Type: *O. allochrusoides* Gilli.

= *Allochrusa* Bunge in Boissier, Fl. Orient. 1: 559. 1867 ≡ *Acanthophyllum* subg. *Allochrusa* (Bunge) Schischk., Fl. U.S.S.R. 6: 800. 1936 – Type (designated by Schiman-Czeika, 1988): *All. versicolor* (Fisch. & C.A.Mey.) Boiss.

New names and combinations

Acanthophyllum* sect. *Ochotonophila (Gilli) Pirani, **comb. & stat. nov.** ≡ *Ochotonophila* Gilli in Feddes Repert. Spec. Nov. Regni Veg. 59: 169. 1956 – Type: *A. allochrusoides* (Gilli) Pirani

Perennial low-growing plants, multi-stemmed. Leaves non-spiny. Bracteoles distant from flowers. Ovary stipitate, ovules 10–18; capsule many-seeded.

Acanthophyllum allochrusoides (Gilli) Pirani, **comb. nov.** ≡ *Ochotonophila allochrusoides* Gilli in Feddes Repert. Spec. Nov. Regni Veg. 59: 169. 1956 – Holotype: AFGHANISTAN. In montibus calc. NE Bamian, 3000 m, 1949, Gilli 1269 (W [photo!]).

Acanthophyllum eglandulosum (Hedge & Wendelbo) Pirani, **comb. nov.** ≡ *Ochotonophila eglandulosa* Hedge & Wendelbo in Aarbok Univ. Bergen, Mat.-Naturvitensk. Ser. 18: 21. 1964 – Holotype: AFGHANISTAN. Kabul, in decl. orientalibus jugi Shibar, 2750 m, 1962, Hedge & Wendelbo W-4239 (E n.v.; isotypes: BG n.v., W [photo!]).

Section to be reinstated

Acanthophyllum sect. *Aphanantha* Rech.f. in Oesterr. Bot. Z. 104: 174. 1957 ≡ *Scleranthopsis* Rech.f. in Ann. Naturhist. Mus. Wien 70: 37. 1967 – Type: *Acanthophyllum aphananthum* Rech.f. in Oesterr. Bot. Z. 104: 174. 1957.

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Appendix 1. Voucher information: species name, geographical origin, collector(s), voucher (herbarium), GenBank accession numbers for ITS and *rps16*, respectively (* indicates sequences new for this study).

Acanthophyllum acerosum Sosn., Iran, W Azarbaijan, Zarre & al. 41900 (TUH), *KF924680, *KF924732; *Acanthophyllum adenophorum* Freyn, Iran, Khorassan, Pirani & Moazzeni 1947 (TMRC), *KF924679, *KF924731; *Acanthophyllum andarabicum* Podl. ex Schiman-Czeika, Afghanistan, Baghlan, Podlech 10985 (MSB), *KF924678, *KF924730; *Acanthophyllum andersenii* Rech.f. & Schiman-Czeika, Iran, Khorassan, Anders & Peterson 320 (GB), *KF924677, *KF924729; *Acanthophyllum anisocladum* Schiman-Czeika, Afghanistan, Lugar, Ekberg W9180 (GB), *KF924676, *KF924728; *Acanthophyllum borsczowii* Litv., Iran, Khorassan, Zarre & al. 41034 (TUH), *KF924675, *KF924727; *Acanthophyllum bracteatum* Boiss., Iran, Kohghiluyeh & Boyer-Ahmad, Pirani & Moazzeni 2104 (TMRC), *KF924674, *KF924726; *Acanthophyllum caespitosum* Boiss., Iran, Hamadan, Zarre & al. 41903 (TUH), *KF924673, *KF924725; *Acanthophyllum crassifolium* Boiss., Iran, Mazandaran, Pirani & Moazzeni 41702 (TUH), *KF924672, *KF924724; *Acanthophyllum crassinodum* Yukhan. & J.R. Edm., Iran, Kerman, Pirani & Moazzeni 2923 (TMRC), *KF924671, *KF924723; *Acanthophyllum diezianum* Hand.-Mazz., Iran, Khorassan, Zarre & al. 41040 (TUH), *KF924670, *KF924722; *Acanthophyllum ejtehadii* Mahmoudi & Vaezi, Iran, Khorassan, Pirani & Moazzeni 2181 (TMRC), *KF924669, *KF924721; *Acanthophyllum glandulosum* Bunge ex Boiss., Iran, Khorassan, Zarre & al. 41037 (TUH), *KF924668, *KF924720; *Acanthophyllum gracile* Bunge ex Boiss., Iran, Semnan, Pirani & Moazzeni 2989 (TMRC), *KF924667, *KF924719; *Acanthophyllum grandiflorum* Stocks, Afghanistan, Bamian, Podlech 1340 (MSB), *KF924666, *KF924718; *Acanthophyllum heratense* Schiman-Czeika, Iran, Isfahan, Pirani & Moazzeni 2152 (TMRC), *KF924665, *KF924717; *Acanthophyllum heterophyllum* Rech.f., Iran, Kerman, Pirani & Moazzeni 2921 (TMRC), *KF924664, *KF924716; *Acanthophyllum kabulicum* Schiman-Czeika, Afghanistan, Ghazni, Frey s.n. (GB), *KF924663, *KF924715; *Acanthophyllum kandaharicum* Gilli, Iran, Khorassan, Joharchi & Zangouei 36245 (FUMH), *KF924662, *KF924714; *Acanthophyllum korshinskyi* Schischk., Iran, Khorassan, Pirani & Moazzeni 2123 (TMRC), *KF924661, *KF924713; *Acanthophyllum kurdicum* Boiss. & Hausskn. ex Boiss., Iran, Ilam, Hamzehee & Lashkarbolooki 1756 (TARI), *KF924660, *KF924712; *Acanthophyllum laxiflorum* Boiss., Afghanistan, Lugar, Ekberg W9184 (GB), *KF924659, *KF924711; *Acanthophyllum*

Appendix 1. Continued.

laxiusculum Schiman-Czeika, Iran, Qom, Pirani & Moazzeni 1941 (TMRC), *KF924658, *KF924710; *Acanthophyllum leucostegium* Schiman-Czeika, Iran, Bandar Abbas, Ghahreman & Mozaffarian 5656 (TUH), *KF924657, *KF924709; *Acanthophyllum lilacinum* Schischk., Afghanistan, Badghis, Podlech & Jarmal 29855 (MSB), *KF924656, –; *Acanthophyllum longicalyx* Hedge & Wendelbo, Afghanistan, Jawzjan, Freitag 6580 (MSB), *KF924655, *KF924708; *Acanthophyllum macrodon* Edgew., Afghanistan, Kandahar, Hedge & al. W7641 (GB), *KF924654, *KF924707; *Acanthophyllum microcephalum* Boiss., Iran, Tehran, Rajamand & Bazargan 32055 (TARI), *KF924653, *KF924706; *Acanthophyllum mucronatum* C.A.Mey., Iran, W Azarbaijan, Assadi & Olfat 68668 (TARI), *KF924652, *KF924705; *Acanthophyllum oppositiflorum* Aytaç, Turkey, Sivas, Aytaç 7476 (GAZI), *KF924651, *KF924704; *Acanthophyllum paniculatum* Regel & Herder, JN589016, –; *Acanthophyllum pachycephalum* Schiman-Czeika, Iran, Tehran, Ganjalizadeh 6106 (TUH), *KF924650, *KF924703; *Acanthophyllum pachystegium* Rech.f., Afghanistan, Badakhshan, Hedge & Wendelbo W9282 (GB), *KF924649, *KF924702; *Acanthophyllum pleiostegium* Schiman-Czeika, Afghanistan, Kataghan, Grey-Wilson & Hewer 1320 (GB), *KF924648, *KF924701; *Acanthophyllum raphiophyllum* (Rech.f.) Barkoudah, Afghanistan, Kapisa, Podlech 12548 (MSB), *KF924647, *KF924700; *Acanthophyllum scapiflorum* (Akhtar) Schiman-Czeika, Afghanistan, Kabul, Podlech 31232 (MSB), *KF924646, *KF924699; *Acanthophyllum schugnanicum* Schischk., Afghanistan, Bamian, Wendelbo & Ekberg W9796 (GB), *KF924645, *KF924698; *Acanthophyllum sordidum* Bunge ex Boiss., Iran, Isfahan, Pirani & Moazzeni 2147 (TMRC), *KF924644, *KF924697; *Acanthophyllum speciosum* Rech.f. & Schiman-Czeika, Iran, Khorassan, Pirani & Moazzeni 2186 (TMRC), *KF924643, *KF924733; *Acanthophyllum spinosum* C.A.Mey., Iran, Isfahan, Pirani & Moazzeni 2150 (TMRC), *KF924642, *KF924696; *Acanthophyllum squarrosus* Boiss., Iran, Semnan, Pirani & Moazzeni 2974 (TMRC), *KF924641, *KF924695; *Acanthophyllum stewartii* (Thoms. ex Edgew. & Hook.f.) Barkoudah, Afghanistan, Khost, Anders 8994 (MSB), *KF924640, *KF924694; *Acanthophyllum stocksianum* Boiss., Afghanistan, Kandahar, Toncev s.n. (MSB), *KF924639, *KF924693; *Acanthophyllum subglabrum* Schischk., Afghanistan, Nangarhar, Hedge & al. W7483 (GB), *KF924638, *KF924692; *Acanthophyllum verticillatum* Hand.-Mazz., Iran, Markazi, Mozaffarian & Sardabi 42175 (TARI), *KF924637, *KF924691; *Acanthophyllum xanthoporphyranthum* Hedge & Wendelbo, Afghanistan, Herat, Hedge & al. W8003 (GB), *KF924636, *KF924690; *Acanthophyllum yasamin-nassehiae* Joharchi & Pirani, Iran, Khorassan, Memariani & Zangouei 41448 (FUMH), *KF924635, *KF924689; *Allochrysa bungei* Boiss., Iran, E Azarbaijan, Rechinger 43834 (M), *KF924634, *KF924688; *Allochrysa versicolor* Boiss., Turkey, Kars, Nydegger 43597b (MSB), *KF924633, *KF924687; *Bolbosaponaria bucharica* Bondarenko, JN589057, –; *Dianthus anatolicus* Boiss., MA-690057, GU440777, –; *Dianthus armeria* L., –; FJ404903; *Dianthus capitatus* J.St.-Hil., GU440792, –; *Dianthus carthusianorum* L., –; EF674194; *Dianthus charidemi* Pau, GU440795, –; *Dianthus ciliatus* Guss., GU440798, –; *Dianthus corymbosus* Sibth. & Sm., GU440801, –; *Dianthus costae* Willk., GU440802, –; *Dianthus crassipes* De Roem., GU440803, –; *Dianthus crinitus* Sm., GU440805, –; *Dianthus cyri* Fisch. & C.A.Mey., GU440808, –; *Dianthus diffusus* Sm., GU440811, –; *Dianthus erinaceus* Boiss., GU440814, –; *Dianthus gracilis* Sm., JN589061, –; *Dianthus hyssopifolius* L., GU440826, –; *Dianthus laricifolius* Boiss. & Reut., GU440831, –; *Dianthus microlepis* Boiss., GU440840, –; *Dianthus micropetalus* Ser., GU440841, –; *Dianthus orientalis* Adams, GU440847, –; *Dianthus pyrenaicus* Bernh. ex Steud., GU440854, –; *Dianthus serratifolius* Sm., GU440858, –; *Dianthus thunbergii* S.S.Hooper, GU440872, –; *Dianthus turkestanicus* Preobr., GU440876, –; *Dianthus versicolor* Fisch. ex Link, GU440878, –; *Diaphanoptera afghanica* Podlech, Afghanistan, Baghlan, Podlech 21075 (MSB), *KF924632, –; *Diaphanoptera ekbergii* Hedge & Wendelbo, Afghanistan, Takhar, Podlech 11848 (MSB) & 11760 (MSB), *KF924631, *KF924686; *Diaphanoptera lindbergii* Hedge & Wendelbo, Afghanistan, Fariab, Hedge & al. W8336 (GB), *KF924630, *KF924685; *Diaphanoptera stenocalycina* Rech.f. & Schiman-Czeika, Iran, Golestan, Attar & Mehdigholi 24422 (TUH), *KF924629, *KF924684; *Gypsophila arrostii* Guss., JN589043, –; *Gypsophila aucheri* Boiss., JN589077, –; *Gypsophila bicolor* Grossh., JN589151, –; *Gypsophila capituliflora* Rupr., JN589143, –; *Gypsophila cephalotes* (Schrenk) Raikova, JN589105, –; *Gypsophila cerastioides* D.Don., Pakistan, Hazar, Ewald & Zetterlund 6227 (GB), *KF924628, *KF924683; *Gypsophila curvifolia* Fenzl, JN589159, –; *Gypsophila desertorum* Fenzl, JN589021, –; *Gypsophila elegans* M.Bieb., JN589130, –; *Gypsophila fastigiata* L., JN589144, –; *Gypsophila heteropoda* Freyn, JN589110, –; *Gypsophila montserratii* Fern.Casas, JN589155, –; *Gypsophila muralis* L., JN589037, –; *Gypsophila paniculata* L., JN589150, FJ404908; *Gypsophila patrinii* Ser., JN589076, –; *Gypsophila pilulifera* Boiss. & Heldr, JN589132, –; *Gypsophila pinifolia* Boiss. & Hausskn. ex Boiss., JN589050, –; *Gypsophila scorzonrifolia* Ser., JN589100, –; *Gypsophila silenoides* Rupr., JN589049, –; *Gypsophila steveni* Besser, JN589022, –; *Gypsophila violacea* Fenzl, JN589068, –; *Ochotonophila allochrosoides* Gilli, Afghanistan, Bamian, Wendelbo & Ekberg W9801 (GB), *KF924627, *KF924682; *Petrorhagia prolifera* (L.) P.W.Ball & Heywood, GU440883, –; *Petrorhagia saxifrage* Link., –; FJ404930; *Petrorhagia thessala* (Boiss.) P.W. Ball & Heywood, GU440885, –; *Petrorhagia velutina* (Guss.) P.W. Ball & Heywood, AY857974, –; *Psammosilene tunicoides* W.C.Wu & C.Y.Wu, JN589122, –; *Saponaria ocymoides* L., AY936271, FJ404936; *Saponaria officinalis* L., AY594313, FJ404937; *Saponaria pumila* Hayek, AY594311, –; *Scleranthopsis aphanantha* (Rech.f.) Rech.f., Afghanistan, Kabul, Rechinger 31265 (M), *KF924626, *KF924681; *Silene ampullata* Boiss., EF060223, –; *Silene austroiranica* Rech.f., Aellen & Esfand., EF060204, EF061364; *Silene campanulata* Pers., –; DQ908812; *Silene cariensis* Boiss., EF060205, EF061365; *Silene conoidea* L., FN821101, –; *Silene corinthiaca* Boiss., EF060206, EF061366; *Silene dioica* (L.) Clairv., –; FN821276; *Silene echinosperma* Boiss. & Heldr., X86845.1, Z83196; *Silene fruticosa* L., X86865, Z83188.1; *Silene latifolia* Poir., –; Z83171; *Silene martyi* Emb. & Maire, EF060213, EF061373; *Silene mentagensis* Coss., EF060236, EF061396; *Silene nana* Kar. & Kir, EF060217, EF061377; *Silene nutans* L., EF061361; *Silene reticulata* Desf., EF060216, EF061376; *Silene vulgaris* (Moench) Garcke, –; EF674192; *Vaccaria hispanica* (Mill.) Rauschert, AY857969, –; *Velezia rigida* L., GU440888, –.