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Biogeographic history and diversification patterns in the Irano-Turanian genus *Acanthophyllum* s.l. (Caryophyllaceae)

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ABSTRACT

The Irano-Turanian (IT) floristic region in Asia contains three biodiversity hotspots and has been a source of xerophytic taxa for neighboring regions. Despite its species richness and large territory, the evolutionary history and biogeography of this floristic region is poorly understood. *Acanthophyllum*, encompassing ca. 70 subshrubby mostly thorny-cushion species, is one of the important components of the steppe and mountain vegetation of this region. In this study, we investigate the tempo and mode of diversification as well as the biogeographic patterns of this genus. The ancestral area analysis suggests that *Acanthophyllum* originated east of the Zagros Mountains in the Miocene, from where it expanded westwards. A shift in the speciation rate in the late Pliocene (ca. 3.24 Ma) was detected, based on a nrDNA ITS tree, affecting the lineage of the largest section in the genus, *Acanthophyllum* sect. *Oligosperma*. Parallelisms with another IT species-rich genera in aspects such as largely coincident ranges and habitats, presence of a thorny-cushion life-form, similar areas reconstructed for their MRCA, and the inference of a shift in speciation in the region suggest common underlying abiotic and biotic factors or even drivers for diversification and speciation in the high elevation dry continental landscape of IT region.

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Introduction

Plant distributions resulted from a combination of ecological and historical factors, and the arrangement in kingdoms, provinces, and other subdivisions recognized common distribution patterns across plant groups (Takhtajan 1986). These classic floristic divisions have the potential to orient evolutionary studies by suggesting the possible role of past geological, climatic and evolutionary processes (Vilhena and Antonelli 2015). From a phylogenetic perspective, species richness varies dramatically among clades across the tree of life. Evolutionary biology, and in particular biogeography, attempt to understand the causes for these variations over time, across space and along lineages taking into account underlying processes and intervening factors (Gotelli et al. 2009). This is particularly challenging in biotas evolved across spatially complex areas and geologically and climatically dynamic scenarios (Djamali et al. 2012a; Manafzadeh et al. 2017).

The Irano-Turanian (IT) region covers one third of Eurasia across highly fragmented highlands that have in common a continental climate (Djamali et al. 2012a). In this region, one of the largest in the world, species richness and endemism are not evenly distributed, but concentrated mainly along two hotspots sensu (Mittermeier et al. 2005)-the Irano-Anatolian area and the mountains of Central Asia-and the southern and

eastern parts of a third one, the Caucasus (Takhtajan 1986; Manafzadeh et al. 2017). Remarkable examples of species-rich groups with high incidence of endemism in the IT region are *Acantholimon* Boiss. (Plumbaginaceae) with ca. 200 species, *Acanthophyllum* C.A.Mey. (Caryophyllaceae) with ca. 70, and *Dionysia* Fenzl (Primulaceae) with ca. 30 species, often exhibiting cushion-like habits (Takhtajan 1986; Djamali et al. 2012b). The IT region has also been considered a source of xerophytic taxa for neighbouring areas and numerous domesticated plants originated here (Diamond 2002; Zohary et al. 2012; Manafzadeh et al. 2014).

Abiotic factors, in particular geology and paleoclimate, are determinant for current plant distribution (Zohary 1973). The roughly synchronous uplift of the Alborz and Zagros Mountains during the middle Miocene likely had a strong impact on the history of the IT floristic region. The Zagros are the largest mountain belt of the region and are part of the Alpine-Himalayan orogenic system. The onset of crustal thickening, caused by the first collision of the Arabian Plate with Eurasia, started about 25 Ma, but uplift, deformation and exhumation across the Zagros and throughout the Arabia-Eurasia collision zone took place later, between 15 and 5 Ma (e.g., Mouthereau 2011). The Alborz Mountains in northern Iran are an intra-continental, east-west oriented orogenic belt formed in response to crustal shortening and

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thickening induced by the ongoing Arabia–Eurasia convergence (Meulenkamp and Sissingh 2003). Palaeoclimate impact in the IT region was marked by the gradual trend of global cooling and aridification initiated during the Oligocene and continued in the Pliocene after the interruption by a warmer episode that peaked in the late-middle Miocene climatic optimum (van Dam 2006; Zachos et al. 2008). Regionally, this latter global warm episode was complemented with variations in the amount of aridity due to the uplift of the Iranian plateau (Ballato et al. 2010). The Pleistocene period was also important since climate-driven range shifts might have represented an opportunity for diversification rather than a threat for mountain cold-adapted xerophytic elements such as *Acanthophyllum* and *Acantholimon* (Bennett and Provan 2008; Djamali et al. 2012a; Manafzadeh et al. 2014; Moharrek et al. 2019).

The evolution of biotas in the IT region remains poorly explored (Manafzadeh et al. 2017) and studying its highly diverse taxa can help to better understand the evolution of biodiversity in this vast and complex area as well as to identify possible internal and external factors triggering strong diversification (Bouchenak-Khelladi et al. 2015). In recent years, a few studies scrutinized the evolution of species-rich IT key elements (e.g., López-Vinyallonga et al. 2009; Djamali et al. 2012b; Pirani et al. 2014; Manafzadeh et al. 2014; Amirahmadi et al. 2016; Moharrek et al. 2017; 2019), but they are insufficient to build a comprehensive picture of the biogeography of the IT region because scenarios are diverse. For instance, the initial diversification of the xerophytic genus *Haplophyllum* A.Juss. (Rutaceae) in the Central Asian part of the IT region was estimated to have happened in the early Oligocene, followed by its secondary, Mediterranean, diversification in the early Pliocene (Manafzadeh et al. 2014). By contrast, diversification of *Cousinia* Cass. was estimated to start in the Miocene, ca. 8.7 Ma (López-Vinyallonga et al. 2009). In this genus, allopatric speciation seems to have driven diversification along a complex topography over the Miocene–Pliocene and was also facilitated during Pleistocene glacial periods alternating with isolation during interglacials (Djamali et al. 2012b). *Acantholimon* probably diversified under the same dynamic allopatric scenario although more recently, ca. 4.1 Ma (Moharrek et al. 2019). The colonization of water-stressed, substrate-poor mountainous habitats, which were abundant in the IT region during the Pleistocene, seems to have been spurred by the evolution of morphological innovations such as the thorny-cushion habit, which was associated to accelerated diversification rates in this genus. Such a habit could have also facilitated the colonization of IT highlands and diversification of other groups (Boucher et al. 2016), including *Acanthophyllum* (Ghaffari 2004; Mahmoudi Shamsabad et al. 2018).

Acanthophyllum, an IT element encompassing ca. 70 shrubby species many of them being thorny cushions, inhabits areas between Syria and western China (Schiman-Czeika 1988; Ghaffari 2004; Mahmoudi Shamsabad et al. 2018) but is mostly concentrated in northeastern Iran and adjacent regions in Afghanistan and Turkmenistan. Fifty-six species of *Acanthophyllum* s.s. occur in the territory covered by *Flora*

Iranica (Schiman-Czeika 1988), i.e., Iran, Afghanistan and adjacent territories of Turkmenistan, Azerbaijan, Pakistan and Iraq. *Acanthophyllum* species grow on exposed habitats on sandy or stony hills and rocky slopes. The available karyological information reports three ploidy levels (Ghaffari 2004). Most species in *Acanthophyllum* sections *Oligosperma* and *Macrostegia* are diploid ($2n = 2x = 30$), but *A. caespitosum* exhibits a different number ($2n = 2x = 28$) together with a distinct inflorescence morphology. The species in sect. *Acanthophyllum* are tetraploid ($2n = 4x = 60$) and distributed in central and western parts of the IT region. The hexaploid level ($2n = 6x = 90$) is found in sect. *Pleiosperma* consisting of species distributed in eastern and northern parts of the IT region (Ghaffari 2004).

Molecular phylogenetic studies revealed that three small genera *Allochrusa* Bunge, *Ochotonophila* Gilli and *Scleranthopsis* Rech.f. as well as three out of four species analysed of another one, *Diaphanoptera* Rech.f., and *Gypsophila cerastoides* D.Don are all nested within *Acanthophyllum*, and that its diversification started ca. 11.1 Ma (Pirani et al. 2014). We here follow such an enlarged circumscription of the genus and refer to it as *Acanthophyllum* s.l.

We assembled datasets with published sequences of the internal transcribed spacers of the nuclear ribosomal DNA (ITS) and the intron of the plastid gene *rps16* for 75% of the *Acanthophyllum* s.l. species, to investigate the tempo and mode of diversification and reconstruct the spatiotemporal evolution of this genus. Specifically, we aimed to (1) estimate the age of divergence of major lineages within *Acanthophyllum* s.l. using relaxed molecular clocks and two different calibration strategies; (2) infer changes in diversification rates in the evolutionary history of *Acanthophyllum* s.l. over time and across clades; (3) reconstruct the biogeographic pathways by which this genus reached its current range.

Materials and methods

Taxon sampling, nucleotide matrix data assembly and model selection

A two-marker dataset (ITS and *rps16*) for 52 *Acanthophyllum* s.l. taxa was obtained from previously published sequences deposited in GenBank, most of them from Pirani et al. (2014) but also three from Mahmoudi Shamsabad et al. (2019): 46 species of *Acanthophyllum* s.s., four of *Diaphanoptera*, two of *Allochrusa*, one of *Ochotonophila*, one of *Scleranthopsis* and one of *Gypsophila* (*G. cerastoides*). Samples from these five genera have not been here included in *Acanthophyllum* despite the phylogenetic results in Pirani et al. (2014) since a comprehensive taxonomic review of *Acanthophyllum* s.l. would be required before the taxonomic changes are made. In addition, *Diaphanoptera afghanica* Podlech did not fall within *Acanthophyllum* clade in such study. Fifteen other Caryophyllaceae were included as outgroups: *Cerastium arvense* L. and *Holosteum umbellatum* L. from tribe Alsineae, *Gypsophila repens* L., *G. elegans* M.Bieb., *Saponaria officinalis* L. and *S. ocymoides* L. from tribe Caryophylleae, *Drypis spinosa* L. from tribe Sagineae, *Minuartia loricifolia* (L.) Schinz & Thell. from tribe Scleranthaeae, *Lychnis coronaria* (L.) Desr.,

Silene italica (L.) Pers., *S. campanula* Pers. and *S. rothmaleri* P. Silva from tribe Sileneae, *Minuartia geniculata* (Poir.) Thell., *Spergula arvensis* L. and *Spergularia marina* (L.) Besser from tribe Sperguleae. Tribe adscription follows Harbaugh et al. (2010).

We created three datasets: (1) ITS comprising 73 terminals; (2) *rps16* with 62 terminals; and (3) a combined dataset comprising 52 terminals. The sampling encompasses the major distribution areas of *Acanthophyllum* in the IT region, including its main diversity center in Iran and Afghanistan, as well as Anatolia and Pakistan. All samples, along with locality data, voucher information, and GenBank accession numbers are listed in [Supplementary Appendix S1](#).

The nucleotide sequences were aligned using Muscle (Edgar 2004) followed by manual adjustment implemented in MEGA version 7.0 (Kumar et al. 2016) and then refined by visual inspection. The best substitution model identified by jModeltest 2.1.7 (Darriba et al. 2012) was GTR + G model for both markers.

Divergence time estimation

Divergence times were inferred with BEAST v1.8.0 (Drummond et al. 2012), ran at the CIPRES Science Gateway (<http://www.phylo.org/>; Miller et al. 2010) assuming a Bayesian relaxed molecular clock. The dated Bayesian analyses followed two different approaches: First, a node dating approach was applied to the individual markers and to the combined data set. The trees were calibrated using the age of a described fossil inflorescence from the Middle–Late Eocene (Jordan and Macphail 2003). Following a detailed phylogenetic study (Jordan and Macphail 2003), this fossil was placed within or as sister to subfamilies Alsinoideae and Caryophylloideae (Frajman et al. 2009). Given the uncertainty regarding the positioning of the fossil with respect to these subfamilies, we chose the most conservative approach (the one that produces the oldest ages) and applied an age constraint at the crown node of Alsinoideae and Caryophylloideae. We calibrated the root node of our trees using a lognormal distribution prior with offset = 33.9 Ma, and SD = 1 Ma, covering the entire Middle–Late Eocene geological interval (33.9–47.8 Ma). A general time reversible model with rate variation across sites, modeled using a gamma distribution (GTR + G), was applied to each data matrix.

The second approach consisted in a multispecies coalescent model implemented in *BEAST (Drummond and Rambaut 2007; Drummond et al. 2012) to estimate the species tree for 52 species of *Acanthophyllum* s.l. To convert genetic branch lengths into absolute times, we used published ITS and plastid substitution rates following the procedures in Pirani et al. (2014). The data matrix was composed of the ITS and the *rps16* unlinked partitions and a GTR + G substitution model was applied for both. The prior probability of the clock rate was set to a truncated normal distribution with a mean of 2.15×10^{-9} , ranging from 0.38×10^{-9} to 7.83×10^{-9} mutations per site per year for ITS estimated for perennial woody plants (Kay et al. 2006), 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 million years for *rps16*. For the *rps16*, we provided rate priors by dividing the lower and upper rates of ITS by three (following the typical magnitudes for plastid substitution rates compared to nuclear rates in plants; Wolfe et al. 1987; Gaut et al. 1996; Yue et al. 2010). This was done to speed up convergence (particularly by excluding highly suboptimal starting rates). The *rps16* prior standard deviation was set to 0.7×10^{-3} , which fits in the truncated normal distribution, is larger than the mean and unlikely to bias its posterior estimate. 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. For comparison this approach was also applied to the individual datasets.

In the tree dating analyses, molecular rates were allowed to vary among lineages around an average value, by enforcing an uncorrelated relaxed lognormal clock of evolutionary rates. A birth–death branch process prior was used. The MCMC chain was run in two separate analyses for 50 million generations sampling every 1000th generation. LogCombiner v1.7.5 (Drummond et al. 2012) was used to combine the log and tree files after discarding the initial 5 million iterations of each analysis as burn-in. Tracer v1.6 (Rambaut and Drummond 2013) was used to assess that Effective Sample Sizes (ESS) were above 200 for optimal convergence and tree likelihood stationarity. Tracer automatically removed the first 10% of the log statistics from the combined sample, so we correspondingly discarded the first 10% of trees. A maximum clade credibility (MCC) tree was constructed with TreeAnnotator v1.7.5 (Drummond et al. 2012), depicting the maximum sum of posterior clade probabilities (nexus file in [Supplementary Appendix S2](#)), and visualized in FigTree v1.4.4 (Rambaut 2018).

Ancestral area reconstruction analyses

The biogeographic history of *Acanthophyllum* was explored using dispersal-extinction-cladogenesis (DEC) method (Ree and Smith 2008) implemented in RASP (Reconstruct Ancestral State in Phylogenies) v. 3.2 (Yu et al. 2015). This method has been widely used to study the biogeographic patterns in closely related taxa with restricted geographic ranges. Also, DEC method allows estimating the probability of ancestral areas at each node of a phylogenetic tree and assumes extinction or dispersal by contraction or expansion of the ancestral geographic range, respectively (Ree and Smith 2008). Several IT species (including *Silene goniocaula* Boiss., *Cousinia lasiolepis* Boiss., *Primula capitellata* Boiss., *Pedicularis cabulica* Benth., *Gagea capillifolia* Vved. ex Czerniak.) currently display disjunct west-east distribution patterns in the alpine zones of Asian mountain chains: between the Zagros, in the west, and either the mountains of Afghanistan or the west Himalayas and central Asia, in the east (Noroozi et al. 2008; Manafzadeh et al. 2017). However, this is not the case in *Acanthophyllum* since most species are concentrated in northeastern Iran and adjacent regions in Afghanistan and Turkmenistan, and species richness severely

decreases both from eastern Afghanistan to China and from western Iran to Turkey to and Syria (Supplementary Figure S1) (Ghaffari 2004; Mahmoudi Shamsabad et al. 2012). Therefore, in defining the boundaries and number of our operational areas, we considered the current distribution patterns of *Acanthophyllum* s.l. species, i.e., geographic areas defined by the congruent distribution of two or more species. We noted a pattern of species occurring east but not west of the chain and vice versa as well as a sharp decline in number of species west of Iran and east of Afghanistan. This is consistent with the different climatic conditions east and west of the Zagros Mountains since this chain blocks the moist air masses from entering in the interior parts of Iran (Alijani 2008). Accordingly, the range of *Acanthophyllum* was divided into two areas, the regions west (A) and east (B) of the Zagros Mountains (Figure 1). To avoid biases related to incomplete taxon sampling in the outgroup, we pruned the BEAST MCC trees by removing outgroups and leaving only 52 terminals of *Acanthophyllum* s.l.

Diversification analyses

To understand the diversification dynamics of *Acanthophyllum* s.l., we used the Bayesian Analysis of Macroevolutionary Mixtures program (BAMM) (Rabosky 2014), which estimates diversification rate through time and identifies shifts in diversification rate. This method employs MCMC and Bayes Factor comparisons as well as Compound Poisson Process (CPP) models to explore alternative diversification dynamic regimes, allowing for both rate-heterogeneity across lineages and variation in speciation rates over time. As for ancestral area reconstruction analyses, we removed all outgroup taxa. To account for incomplete taxon sampling, we specified a global sampling probability of 0.75. Priors for BAMM were generated using `setBAMMpriors` function in BAMMtools package based on the MCC tree (Rabosky et al. 2014). It has been suggested that the likelihood function of BAMM might be incorrect and that the program suffers from strong prior sensitivity and unreliable rate estimates (Moore et al. 2016). However, Rabosky et al. (2017) recently reported that they were unable to reproduce the prior sensitivity and that the shortcomings indicated by Moore et al. (2016) were likely due to a misapplication of the program and poorly designed test data. Sensitivity to the choice of the CPP prior, which informs the expected number of rate shifts, was assessed by performing multiple BAMM analyses with varying CPP priors (1, 5, and 10). We ran the analyses for 5 million generations, sampling every 5000th generation. Since priors had little effect on the results, we ran the final analysis with the priors estimated by BAMMtools and four independent chains of 10 million generations each, sampled every 10,000th generation. The convergence of MCMC was assessed using the coda package in R (Plummer et al. 2006) by computing the effective sample sizes (ESS) of log likelihoods. An ESS value above 200 was considered to reach convergence and the first 10% sampling data was then discarded as burn-in. The posterior distribution was used to summarize and visualize diversification rates over the branches of the phylogeny and through

evolutionary time. Results were analyzed and plotted using the BAMMtools package (Rabosky et al. 2014).

Results

Divergence time and ancestral area reconstruction

The three datasets yielded overall similar topologies for the main clades, but there were incongruences affecting some genera and species within those clades. For instance, the clade formed by *Allochrysa bungei* Boiss. and *A. versicolor* Boiss. is sister to the remainder of Clade II in the ITS and combined trees (Figure 2A, Supplementary Figure S2A,B,D) but nested in it in the *rps16* trees (Figure 2B, Supplementary Figure S2C). Two other dissimilarities affecting positions of the close genera in Clade I are *Ochotonophila allochrysoidea* Gilli and *Scleranthopsis aphanantha* (Rech.f.) Rech.f. Phylogenetic relationships within the *Acanthophyllum* s.l. clade recovered in the present study largely agree with those inferred by Pirani et al. (2014), including the two main clades I and II (Figures 1 and 2), and thus here we do not focus on topological aspects. Divergence times and HPD intervals for *Acanthophyllum* s.l. clade obtained with the two calibration approaches for the three datasets (Figure 2 and Supplementary Figure S2) are summarized in Table 1. Estimated average divergence times for the crown node of *Acanthophyllum* s.l. ranged from 9.44 to 14.29 Ma, whereas those for the crown nodes of clade I and clade II ranged from 6.47 to 12.9 Ma and from 3.18 to 9.1 Ma, respectively. HPD intervals also showed considerable variation across datasets and Bayesian approach: from 4.75 to 21.7 myr for the crown node of *Acanthophyllum* s.l., 4.39 to 18.9 myr for the crown nodes of clade I, and 3.4 to 16.6 myr for the crown nodes of clade II. Unlike the average divergence times, for the amplitude of the HPD intervals there is a noticeable pattern such that the combined datasets produced the larger intervals than the individual datasets (Table 1).

In the ancestral area reconstruction, the eastern Iran–Afghanistan region (area B) was inferred to be the most likely ancestral area of *Acanthophyllum* s.l., as well as of the clade I (Figure 1). The ancestral area of the MRCA of the clade II was widespread in western Iran and eastern Iran–Afghanistan (areas AB), implying a dispersal event to the west. Furthermore, there is a strong geographic concordance for these two clades since a majority of the species occurring east and west of the Zagros Mountains fell in separate clades (Figure 1). Specifically, of the 52 species sampled of *Acanthophyllum* s.l., 42 consistently occur in area A and were positioned in clade II or occur in area B and belong to clade I. Of the remaining species, nine occur in both areas and were positioned either in clade I or in clade II (6 and 3, respectively). The only discordant pattern is represented by *A. sordidum* Bunge ex Boiss., which occurs in area B but belongs to clade II.

Diversification rates

In the BAMM analyses, we confirmed convergence of the MCMC chains after discarding the burn-in and that effective

Figure 1. Ancestral Area Reconstruction of *Acanthophyllum* s.l. using (A) ITS and (B) *rps16* datasets. Current genus distribution has been divided into two areas for analysis indicated by color-codes: regions west (A) and east (B) of the Zagros Mountains. Current species distribution in the two areas is indicated by color-coded circles on the tree tips, and pie charts at nodes represent alternative range probabilities.

Figure 2. Phylogenetic relationships within *Acanthophyllum* s.l. based on (A) ITS and (B) *rps16*, inferred with BEAST. Chronograms represent the maximum clade credibility trees estimated using a fossil calibrated approach, with mean divergence times in million years ago shown for important nodes. Blue bars represent 95% highest posterior densities for node ages. Species in *Acanthophyllum* sect. *Oligosperma* (excluding *A. caespitosum*; see text) are marked in red.

sample sizes were >900 for both the number of shifts and log likelihoods. The results supported a diversity-dependent speciation process across *Acanthophyllum* s.l. with a net diversification rate $r=0.30$ species/Ma (95% quantile = 0.01–0.42), speciation rate $\lambda=0.51$ species/Ma (95% quantile = 0.28–0.62), and extinction rate $\mu=0.21$ species/Ma (95% quantile = 0.01–0.44) for the ITS dataset. The *rps16* dataset resulted in a net diversification rate $r=0.29$ species/Ma (95% quantile = 0.02–0.43), speciation rate $\lambda=0.50$ species/Ma (95% quantile = 0.21–0.65), and extinction rate $\mu=0.21$ species/Ma (95% quantile = 0.01–0.44).

For the ITS dataset, the 95% credible set of rate shift configurations sampled with BAMM included four distinct ones; the configuration with the highest probability (0.95) exhibited one shift in sect. *Oligosperma* (except for *A. caespitosum* Boiss.) within clade II (Figures 1 and 2). Rate-through-time plots showed that there are relatively accelerated speciation and diversification rates and a flat extinction rate using the ITS dataset (Figure 3).

The *rps16* dataset did not show any significant shift (Figure 3) and the rate-through-time plots of speciation, extinction and diversification remained almost flat across the evolutionary history of *Acanthophyllum* s.l. for this marker (Figure 3).

Discussion

Divergence times and biogeography

Divergence time estimates within *Acanthophyllum* s.l. vary across datasets—based on independent or concatenated markers—and Bayesian approaches, i.e., root node fossil calibrated vs. relaxed clock model using published substitution rates implemented in *BEAST, also when compared to Pirani et al. (2014) (Table 1; Figure 2 and Supplementary Figure S2). Under the first approach, there is a sharp contrast between the dates based on the ITS and combined data sets, which are close to each other and higher than those based on the plastid region. Under the second approach, estimates based on the ITS and combined data sets are also close to each other. However, unlike the first approach, the estimated date based on the *rps16* dataset is older than those based on the ITS and combined datasets both for the crown nodes of *Acanthophyllum* s.l. and clade I (Table 1). The divergence time for the split of the lineages leading to *Acanthophyllum sordidum* and *Allochrusa versicolor* estimated in Frajman et al. (2009) using the same fossil for node calibration of a *matK* dataset of Caryophyllaceae, 2.8 Ma, is consistent with those found here in the fossil-calibrated *rps16* dataset (Figure 2B), but not with the ITS or concatenated datasets, where it is much older (Figure 2A and Supplementary Figure S2D). The pattern found regarding the amplitude of the HPD intervals (Table 1), which is consistently wider in the trees resulting from a combined data set, also holds when compared to those intervals reported in Pirani et al. (2014). The HPD intervals in the latter study, based on a combined dataset, are even wider than those found here and span the Oligocene, Miocene and Pliocene. The likely explanation for such a pattern is that topological differences (and thus phylogenetic

signal) between the ITS and plastid trees increase the uncertainty and thus the amplitude of the confidence intervals. Since the multispecies coalescence approaches, such as *BEAST, account for different coalescence times for the genes, but not for other causes such as hybridization, the different topological position of *Allochrusa bungei*, *A. versicolor*, *Ochotonophila allochrusoides* and *Scleranthopsis aphanantha* in the ITS and combined dataset (Figure 2A, Supplementary Figure S2A,B,D) vs. the *rps16* trees (Figure 2B, Supplementary Figure S2C) may be due to hybridization. However, the short branches in the *rps16* topology recommend caution and further analyses based on a larger representation of the genome.

If we focus on the results of the ITS tree from the fossil-calibrated approach, which is based on more informative characters than the *rps16* but carries less uncertainty than the two regions concatenated, and has a specific calibration scheme for this group compared to the second approach that is based on published rates, we can propose the following scenario (Figure 2A). *Acanthophyllum* s.l. seem to have diverged from its sister group during the early Miocene and started to diversify sometime during the late Miocene. Such a timing is consistent with the idea that the uplift of the Alborz and Zagros Mountains, which occurred along that period, might have promoted high levels of alpine endemism in this area (Noroozi et al. 2008, 2019; Pirani et al. 2014; Moharrek et al. 2019).

The biogeographic analysis suggests that *Acanthophyllum* s.l. as well as clade I most likely originated in eastern Iran–Afghanistan (area B) (Figure 1). This possibility is consistent with the fact that the Khorassan–Kopet Dagh floristic province in NE Iran and neighboring Afghanistan and Turkmenistan is the major center of taxonomic diversity of the genus. The reconstructed ancestral area for clade II, supported by both ITS and *rps16* analyses, spanned across western Iran and eastern Iran–Afghanistan (areas A and B). We suggest that easterly adjacent Syria and westerly adjacent Western China were colonized by westward and eastward dispersal events, followed by peripatric speciation. This scenario, including the origin in eastern Iran–Afghanistan was also reported for other IT xerophyte taxa such as *Acantholimon* (Moharrek et al. 2019) and is consistent with a pattern of xerophytes migration from east to west (Manafzadeh et al. 2014).

Clade II is far less species-rich than the Clade I and includes the only clear exception to the concordance between phylogenetic placement and geographic distribution: *A. sordidum* (Ghaffari 2004; Pirani et al. 2014, 2019). Of the five species here sampled belonging to *Acanthophyllum* section *Pleiosperma*, only *A. sordidum* is placed in clade II whereas *A. crassinodum* Yukhan. & J.R.Edm., *A. glandulosum* Bunge ex Boiss., *A. schuganicum* Schischk. and *A. spinosum* C.A.Mey are placed in clade I. The first two of these four species have been reported as hexaploids whereas *A. sordidum* is tetraploid (Ghaffari 2004). Although this raises the possibility that *A. sordidum* might have been involved in an allopolyploid event—with diploids from section *Oligosperma* from clade I—to give rise to hexaploids in sect. *Pleiosperma*, the

Figure 3. Diversification rate shifts within *Acanthophyllum* s.l. through time. (A) Rate shift configuration with the highest posterior probability model showing one significant rate shift in speciation (λ)—color coded by λ —, rates-through-time analysis of speciation (λ), extinction (μ) and net diversification (r) in *Acanthophyllum* s.l. using nrDNA ITS. (B) Rate shift configuration with the highest posterior probability model showing no significant rate shift in *rps16* dataset. rates-through-time analysis of speciation (λ), extinction (μ) and net diversification (r) in *Acanthophyllum* s.l. using *rps16* dataset.

consistent nuclear and plastid affinities of those four species within clade I do not support such a merger. To support such hypothesis we would have to assume that signal of *A. sordidum* was lost in sect. *Pleiosperma* via concerted evolution in the ITS (Felinier and Rosselló 2012) and/or via plastid capture if *A. sordidum* contributed to such hypothetical allopolyploid event as ovule donor (Tsitrone et al. 2003). The alternative is that the traditional placement of *A. sordidum* in sect. *Pleiosperma* based on some morphological characters (Schiman-Czeika 1988) is simply wrong and their relationships are distant as phylogenetic evidence suggests. Our

biogeographic and diversification results are compatible with both hypotheses and thus a more thorough sampling including chromosome numbers is needed.

Diversification of Irano-Turanian mountain xerophytes

Scenarios with one (ITS) or zero (*rps16*) shifts in macroevolutionary rate regimes were inferred most frequently by our diversification analyses (Figure 3). The scenario with one shift suggests an increase in speciation rates in section

Oligosperma, comprising part of clade I, around 3.24 Ma in the Pliocene. This is the largest section in *Acanthophyllum* including more than 20 species, of which 17 have been sampled in this study. The incidence of such increase in speciation rate leading to a rapid formation of species with close and overlapping ranges is consistent with the species' identification problems in this section (Mahmoudi Shamsabad et al. 2019). The fact that no shift was identified by *rps16* and that the speciation rate inferred with this marker is relatively flat could suggest that a species under-sampling effect on this clade had biased the shift position in BMM analyses in the ITS tree. However, BMM takes into account possible species under-sampling effects (see material and methods section), and 75% of the species of this clade included in our analyses do not represent a significant under-sampling. We thus suggest that the absence of the shift in the plastid tree is due to different evolutionary rates in ITS and plastid regions, which is in accordance with the lower number of informative characters and the low sequence divergence values between the plastid sequences (Pirani et al. 2014).

Our results suggest that during the late Miocene, *Acanthophyllum* s.l., experienced a relatively high speciation rate and the evolution of the cushion-thorny habit may have spurred such increase in speciation rates. Interestingly, a strikingly similar morphological syndrome including life form and leaves is exhibited by another IT genus, *Acantholimon* (Plumbaginaceae), occurring in similar habitats (Moharrek et al. 2019). The interesting parallelism between the two genera goes beyond their morphological adaptations to the same habitats. They have similar distribution ranges although that of *Acantholimon* is larger (Rechinger and Schiman-Czeika 1974). The reconstructed ancestral area for both is Eastern Iran-Afghanistan (Moharrek et al. 2019). Both genera experienced shifts in diversification although in *Acantholimon* there were two shifts associated with the evolution of the cushion-thorny habit whereas in *Acanthophyllum* this habit is predominant also outside the lineage that experienced the shift (*A.* sect. *Oligosperma*). In addition, both genera required enlarging their taxonomic circumscription to accommodate their phylogenetic history, which is probably due to disparate evolution rates in different morphological characters-affected directly or not by the diversification shifts-which obscure the existing synapomorphies used to define large clades.

Such similarities in genera from two separate families suggest common environmental underlying factors playing an important role in their diversification. Based on the habitats where species of both genera mostly occur, it is likely that water-stressed, substrate-poor mountainous habitats-such as stony mountain slopes or summit areas with poorly developed soils, or even rocky outcrops-exerted strong selection pressures, in addition to grazing. We thus hypothesize that the acquisition of the cushion-thorny habit facilitated the colonization of vast territories of seemingly homogeneous continental high elevation environments and spurred diversification. These environments where *Acanthophyllum* and *Acantholimon* species occur are frequent and even dominant in the IT region, and host also other species-rich

genera, such as *Astragalus* L., *Cousinia*, *Haplophyllum*, *Onobrychis* Mill. (Podlech and Maassoumi 2003; Ranjbar and Karamian 2003; López-Vinyallonga et al. 2009; Djamali et al. 2012b; Manafzadeh et al. 2014; Pirani et al. 2014; Amirahmadi et al. 2016). This scenario is consistent with three additional facts: (1) nearly three quarters of the endemic species in Iran are restricted to mountain ranges and the rate of endemism increases along an elevational gradient (Noroozi et al. 2019); (2) in the niche model of *Acanthophyllum squarrosum* cold and dry environments are determinant for increasing habitat suitability (Mahmoudi Shamsabad et al. 2018); (3) both cold and dry environments are the drivers of repeated evolution of the cushion life form in general and Caryophyllaceae is the family having the highest number of cushion species (Boucher et al. 2016).

To further refine the historical scenarios for *Acanthophyllum* s.l. and other IT-rich groups, methods for projecting ancestral ecological niches onto palaeogeographical configurations need to be improved and implemented (Crisp and Cook 2012). Within *Acanthophyllum* s.l., the application of niche modelling tools to a wide sample of species will be useful to better explain the relative roles of geological versus climatic factors in speciation processes, and of niche conservatism versus niche evolution in shaping distributional patterns in the IT region. A more thorough representation of the genus in molecular- and karyological-based studies, including intraspecific sampling, would also be helpful particularly for taxa with conflicting positions in the inferred phylogenies. In addition, new molecular data, preferably covering a wide genomic representation, is necessary for exploring the incongruences between the nuclear and plastid trees.

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