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ORIGINAL ARTICLE

Phylogenetic species delimitation unravels a new species in the genus *Sclerorhachis* (Rech.f.) Rech.f. (Compositae, Anthemideae)

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Abstract *Sclerorhachis* is a small genus and belongs to subtribe Handeliinae of tribe Anthemideae (Compositae). While according to the *Flora Iranica* only two species of the genus are indicated for Iran (i.e. *S. platyrachis* and *S. leptoclada*), the genus constitutes a taxonomically very interesting group here due to the presence of several isolated populations deviating from others morphologically. In the present study, we have used phylogenetic analyses as well as sequence-based species delimitation methods for clarifying species boundaries in *Sclerorhachis*. We used sequence information from the nrDNA regions ITS (ITS1–5.8S–ITS2) and ETS along with the plastid intergenic spacer region *rpl*32–*trn*L$^{(UAG)}$ in an array of sequence-based species delimitation methods: (1) the Bayesian implementation of generalised mixed Yule-coalescent (bGMYC) model and (2) a Bayesian implementation of the Poisson tree processes (bPTP) method. We compared the results of these methods with species delimitations derived from the statistical parsimony networks constructed with TCS and the application of a monophyletic species concept. When the results of phylogeny-based methods, species delimitation approaches, and morphological evidences are jointly considered, our study supports the classification of *S. leptoclada* as an independent species and reveals a new species of *Sclerorhachis* in the Binalud Mountains (described as *S. binaludensis*). It also indicates a new record of the species *S. caulescens* (formerly only known from Afghanistan) for Iran. Additionally, a morphologically deviating and phylogenetically independent population group of *S. platyrachis* was found along the NE boundary of Iran, which is considered being conspecific with the Turkmenistan species *S. kjurendaghi*. As a consequence, the present study indicates that *Sclerorhachis* is represented in the territory of Iran by five independent, evolutionary significant units (i.e. species).

Keywords Molecular phylogeny · Morphological characters · *Sclerorhachis* · Species delimitation

Introduction

Species are fundamental units in different fields of biology including taxonomy, evolutionary biology, biodiversity assessments, and ecology. As a consequence, a great number of different species concepts (Mayden 1997; De Pinna 1999; Wheeler and Meier 2000; De Queiroz 2007) and methods for species detection and delimitation (Sites and Marshall 2003) have been suggested. However, applying a single method for the delimitation of evolutionary significant units (species) might provide difficulties. Using the traditional taxonomy may lead to an underestimation of entities in the case of cryptic species, which have similar morphological attributes, but exhibit divergent phylogenetic signals (Abdelaziz

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et al. 2011; Dong et al. 2012). Likewise, in lineages with an extensive hybridization (introgression) or incomplete lineage sorting (ILS) history, using the phylogenetic/monophyletic species concept (MSC) (Rosen 1979; Cracraft 1983; Donoghue 1985) for clarifying species boundaries might be hampered by ample incongruence among gene trees (Jakob and Blattner 2006; Maddison and Knowles 2006; Degnan and Rosenberg 2009; Meng and Kubatko 2009).

Therefore, taking into account the complexity of relationship among species, employing more diagnostic approaches of species delimitation methods along with current taxonomy and phylogenetic analyses seems to be necessary. Presently, the coalescence-based species delimitation methods are increasingly used for species recognition. The common basis of these methods is finding the tipping point from species-level to population-level branching patterns in phylogenetic reconstructions. The generalised mixed Yule–coalescent (GMYC) method suggested by Pons et al. (2006) assumes both Yule (Nee et al. 1994; Barraclough and Nee 2001) and coalescent (Hudson 1990) processes to simulate divergence patterns of lineages along a time-calibrated tree to infer species boundaries. As an alternative to the GMYC method, the Poisson tree processes (PTP) method uses the branch lengths of a simple phylogenetic tree to model cladogenesis among and within species (Zhang et al. 2013). In order to take into account the uncertainty in the underlying tree topologies and branch lengths, Bayesian implementations of GMYC [i.e. bGMYC; (Reid and Carstens 2012)] and PTP [i.e. bPTP; (Zhang et al. 2013)] have been proposed. These models have been applied in species delimitation issues in several studies on plants (Leliaert et al. 2009; Hernández-León et al. 2013; Hu et al. 2015; Lang et al. 2015; Su et al. 2015) and animals (Domingos et al. 2014; Khan et al. 2014; Bagley et al. 2015; Dumas et al. 2015; Lecocq et al. 2015; Werneck et al. 2015; Eberle et al. 2016; Kuchta et al. 2016; Larson et al. 2016).

Sclerorhachis (Rech.f.) Rech.f. is a genus of the subtribe Handeliinae Bremer and Humphries from the tribe Anthemideae Cass. (Compositae) with a perennial life form and strongly dissected (3–4-pinnatisect) rosette and lower cauline leaves. Its discoid capitula are arranged in lax corymbs, exhibit paleate receptacles, and produce achenes with 4–7 inconspicuous ribs, an ecoronate to coronate apex, and a pericarp with myxogenic cells, but devoid of resin sacs or ducts (Oberprieler et al. 2007). The type species of *Sclerorhachis* [*S. caulescens* (Aitch. & Hemsl.) Rech.f.] has been firstly described as a species of *Anthemis* L. (Aitchison and Hemsley 1888) based on its paleate receptacle. Subsequently, Rechinger (1944) raised the assemblage to section rank within *Anthemis*, but then acknowledged it as an independent genus with an uncertain position within the tribe (Rechinger 1969). According to the taxonomical treatment of the genus in *Flora Iranica* (Rechinger 1986), *Sclerorhachis* comprises four species with two species being indicated for Iran [i.e. *S. platyrachis* (Boiss.) Podlech ex Rech.f. and *S. leptoclada* Rech.f.] and two other species for Afghanistan [i.e. *S. caulescens* and *S. polysphaera* Rech.f]. Subsequent studies, however, added two further species of *Tanacetopsis* (Tzvelev) Kovalevsk. from Turkmenistan to the genus, namely *T. paropamisica* (Krasch.) Kovalevsk. [as a synonym of *S. platyrachis* in Rechinger (1986)] and *T. kjurendaghi* Kurbanov [transferred to *Sclerorhachis* as *S. kjurendaghi* (Kurbanov) Kovalevsk. by Kovalevskaja (1987)].

While only two species of *Sclerorhachis* have been hitherto reported for Iran, a closer examination of the collected specimens from different geographical regions of the distribution range of the genus revealed considerable morphological variation leading to difficulties in species assignment of collections. As a consequence, the present study aims at a comprehensive study of *Sclerorhachis* and attempts to propose a phylogenetic framework and the inference of species boundaries in the genus. It is using sequence information from both the nuclear ribosomal DNA (nrDNA ITS and ETS) and the chloroplast DNA (cpDNA *rpl*32–*trn*L$^{(UAG)}$ intergenic spacer region) for the inference of the phylogeny of the genus and for a coalescent-based delimitation of evolutionary significant units (species).

Materials and methods

Taxon sampling

A total of 24 accessions as representatives for each of the previously recognised species and from populations covering the complete geographical range of *Sclerorhachis* were included in the present molecular study (Fig. 1, Table 1). Additionally, representatives of 12 genera from the subtribe Handeliinae [sensu Oberprieler et al. 2009; i.e. *Handelia* Heimerl, *Pseudohandelia* Tzvelev, *Lepidolopsis* Poljakov, *Microcephala* Pobed., *Allardia* Decne., *Richteria* Kar. & Kir., *Tanacetopsis* (Tzvelev) Kovalevsk., and *Trichanthemis* Regel & Schmalh.] or of genera allegedly related to this subtribe (i.e. *Cancrinia* Kar. & Kir., *Lepidolopha* C.Winkl., *Polychrysum* (Tzvelev) Kovalevsk., and *Ugamia* Pavlov) were also included to test for the monophyletic status of *Sclerorhachis*. For serving as further outgroups and calibration points in the phylogenetic analyses, six species from five genera of subtribe Artemisiinae Less. [i.e. *Artemisi*a L., *Ajania* Poljakov, *Dendranthema* (DC.) Des Moul., *Elachanthemum* Y.Ling & Y.R.Ling and *Kaschgaria* Poljakov] were also added to the dataset, following phylogenetic reconstructions of Oberprieler et al. (2009), which found this subtribe (together with the S African subtribe Pentziinae Oberpr. & Himmelreich) being the sister clade to Handeliinae. Finally,

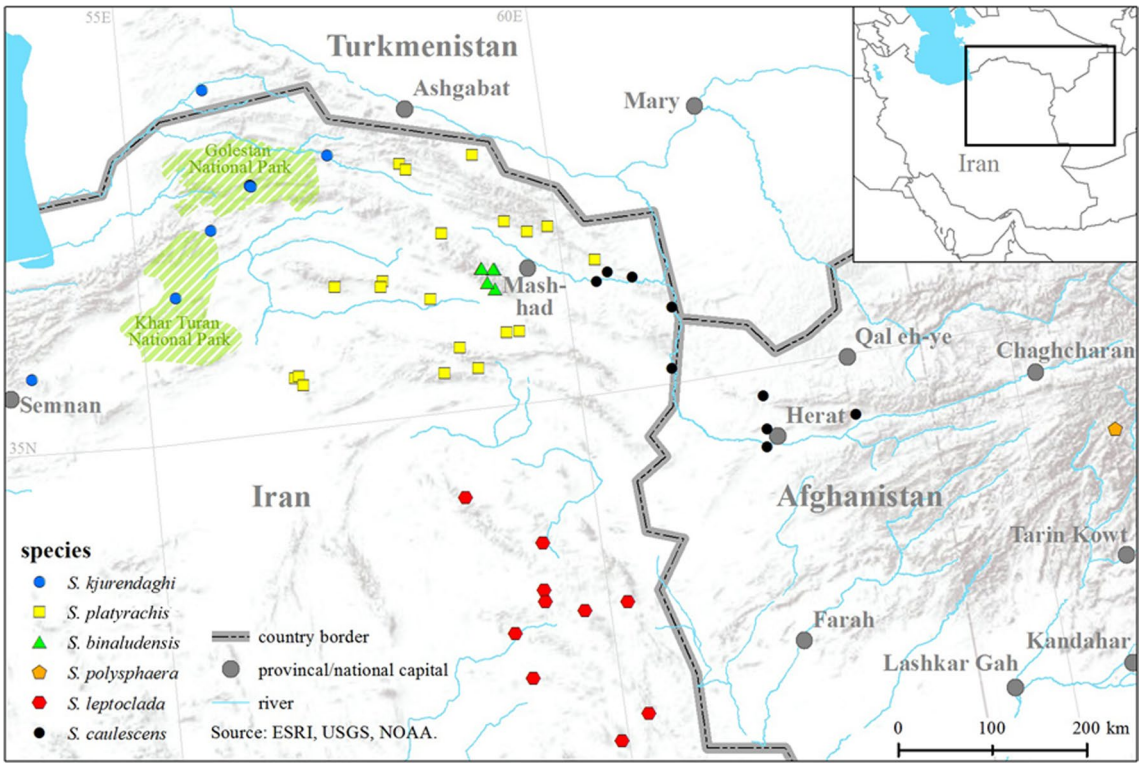

Fig. 1 Geographical distribution of *Sclerorhachis* specimens and their presently recognised affiliation to the molecularly and morphologically circumscribed species shown in different colours

a representative of the genus *Tanacetum* L. from subtribe Anthemidinae (Cass.) Dumort. was also included in the reconstructions. According to phylogenies published by Sonboli and Oberprieler (2010) and Sonboli et al. (2012), *Tanacetum* is a suitable outgroup for the present analyses because subtribe Anthemidinae is sister to the group of subtribes Handeliinae + Artemisiinae. Voucher information and accession numbers of all sequences are listed in Table 1.

DNA extraction, amplification, and alignment

Total genomic DNA was extracted using the Plant Genomic DNA Kit (Tiangen Biotech) from herbarium material following the protocols of the manufacturer. For some additional representatives, DNA extracts were gained through a traditional CTAB DNA extraction protocol (Doyle 1987; Doyle and Dickson 1987). Three spacer regions of the nuclear ribosomal repeat (nrDNA ITS1, ITS2, and ETS) were PCR amplified along with the plastid DNA *rpl*32–*trn*L$^{(UAG)}$ intergenic spacer region using primers ITS5mF and ITS4R (Sang et al. 1995; White et al. 1990), L-ETSF and 18s-2LR (Lee et al. 2002; Linder et al. 2000), and *rpl*32F and *trn*L$^{(UAG)}$R (Shaw et al. 2007), respectively, and Sanger sequenced by a commercial company (Macrogen Inc., www.macrogen.com). Additional sequences were retrieved from GenBank (NCBI, National Centre for Biotechnology Information, Bethesda, U.S.A.).

Sequences were subjected to an automated alignment procedure using Clustal W (Thompson et al. 1994) under BioEdit v7.0.5.3 (Hall 1999), and the resulting multiple alignment was corrected manually. Gaps were coded as informative sites in FastGap v1.2 (Borchsenius 2009) according to the simple gap-coding method of Simmons and Ochoterena (2000). The sequence alignments used in the analyses are found in Online Resources 1–2 (ESM 1–2). Statistics on variation and information content of datasets was calculated with MEGA v7.0.21 (Kumar et al. 2016).

Phylogenetic analyses

To infer phylogenetic relationships among *Sclerorhachis* accessions, sequences and indel data were treated as independent partitions in each dataset and were subjected to maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses. MP analyses were conducted using PAUP* v4.0b10 (Swofford 2002). The heuristic search with 1000 random addition of sequence replicates was employed for each dataset used, with the tree bisection–reconnection (TBR) branch swapping in action. Branch support was assessed by 1000 bootstrap replicates

Table 1 Accessions analysed in the present study, with clade affiliation, voucher information, and GenBank accession number

Taxa	Source/voucher data	Accession numbers		
		nrDNA ITS(ITS1/ITS2)	nrDNA ETS	cpDNA *rpl*32–*trn*L$^{(UAG)}$
***Sclerorhachis* (Rech.f.) Rech.f.**				
S. binaludensis Sonboli (clade D)	Iran, Razavi Khorasan, Neyshabur, Gerineh Mts., *Faghihnia* and *Zandgooei 27768* (FUMH)	LC313924	LC313962	LC313998
	Iran, Razavi Khorasan, Neyshabur, Kharv Mts., *Joharchi 44867* (FUMH)	LC313925	LC313963	–
	Iran, Razavi Khorasan, Mashhad, Feresgeh, *Faghihnia* and *Zandgooei 17888* (FUMH)	LC313926	LC313964	–
S. caulescens (Aitch. & Hemsl.) Rech.f. (clade A1)	Iran, Razavi Khorasan, Torbat-e-Jam, *Joharchi 34078* (FUMH)	LC313927	LC313965	LC313999
	Iran, Razavi Khorasan, Sarakhs, Mazdavand, *Joharchi* and *Zandgooei 16738* (FUMH)	LC313928	LC313966	LC314000
	Iran, Razavi Khorasan, Sarakhs, *Joharchi 44805* (FUMH)	LC313929	LC313967	LC314001
	Afghanistan, Herat, Koh-i-Zyarat, *Podlech* and *Jarmal 29232* (M)	LC313930	LC313968	LC314002
	Afghanistan, Herat, Chashma-i-Obeh, *Podlech* and *Jarmal 29700* (MSB)	LC313931	LC313969	LC314003
S. kjurendaghi (Kurbanov) Kovalevsk. (clade E)	Iran, North Khorasan, Gifan, *Memariani* and *Zangooie 38958* (FUMH)	LC313932	LC313970	LC314004
	Iran, North Khorasan, Ghorkhod, *Joharchi* and *Memariani 45030* (FUMH)	LC313933	LC313971	LC314005
	Iran, Semnan, Gardaneh-ye Ahuvan, *Gholipour 2209* (MPH)	LC313934	LC313972	LC314006
S. leptoclada Rech.f. (clade E)	Iran, South Khorasan, Gonabad-Ferdows road, *Ayatollahi* and *Zandgooei 15186* (FUMH)	LC313935	LC313973	LC314007
	Iran, South Khorasan, Birjand, *Joharchi 45005* (FUMH)	LC313936	LC313974	LC314008
	Iran, South Khorasan, Birjand, *Faghihnia* and *Zandgooei 30340* (FUMH)	LC313937	–	LC314009
S. platyrachis (Boiss.) Podl. (clade C2)	Iran, Razavi Khorasan, Faruj, *Faghihnia* and *Zandgooei 29325* (FUMH)	LC313938	LC313975	LC314010
	Iran, Razavi Khorasan, Quchan road, Ardak to Talqur, *Sonboli, Kanani* and *Gholipour 1101* (MPH)	LC313939	LC313976	LC314011
	Iran, Razavi Khorasan, Kalat Nader, *Joharchi* and *Zangooei 16821* (FUMH)	LC313940	LC313977	LC314012
	Iran, Razavi Khorasan, Kardeh, *Joharchi* and *Zangooei 12943* (FUMH)	LC313941	LC313978	LC314013
	Iran, Razavi Khorasan, Dargaz, *Amiri 45689* (FUMH)	LC313942	LC313979	LC314014

Table 1 (continued)

Taxa	Source/voucher data	Accession numbers		
		nrDNA ITS(ITS1/ITS2)	nrDNA ETS	cpDNA *rpl*32–*trn*L$^{(UAG)}$
S. platyrachis (Boiss.) Podl. (clade C1)	Iran, Razavi Khorasan, Torbat-e-Heydariyeh, *Hassanpour* and *Shahi-Shavvon 2363* (MPH)	LC313943	LC313980	LC314015
	Iran, Razavi Khorasan,Sabzevar, Soltan Abad, *Hassanpour* and *Shahi-Shavvon 2364* (MPH)	LC313944	LC313981	LC314016
	Iran, Razavi Khorasan, Kashmar, Bezgh Mts., *Assadi* and *Mozaffarian 35777* (TARI)	LC313945	LC313982	LC314017
	Iran, Razavi Khorasan, Sabzevar-Neyshabur road, *Rajamand* and *Bazargan 32097* (TARI)	LC313946	LC313983	LC314018
S. polysphaera Rech.f. (Isotype!) (clade A2)	Afghanistan, Deh Kundi, in W Shahrestan, *Rechinger 36811* (M)	LC313947	LC313984	LC314019
Subtribe Handeliinae				
Handelia trichophylla (Schrenk) Heimerl	Iran, Razavi Khorasan, Dargaz, *Amiri 1689* (MPH)	LC313948	LC313985	LC314020
Pseudohandelia umbellifera (Boiss.) Tzvelev	Iran, Razavi Khorasan, Mashhad, *Joharchi 37712-1745* (MPH)	LC313949	LC313986	LC314021
Microcephala lamellata (Bunge) Pobed.	Iran, Razavi Khorasan, Torbat-e-Jam, *Joharchi* and *Zangooei 39415* (FUMH)	LC313950	LC313987	LC314022
Richteria pyrethroides (Karelin & Kir.) B.Fedtsch. & H.Kraschen.	Tajikistan, Gorno-Badakhshan, Belyandkiik Mts, *Dickoré 18001* (MSB)	LC313951	LC313988	LC314023
Lepidolopsis turkestanica (Regel & Schmalh.) Polj.	Afghanistan, Maidan, Darsudyar, *Podlech 32259* (MSB)	LC313952	LC313989	LC314024
Allardia tomentosa Decne	Pakistan, Baru Gah, Panji Pass, *Miehe 6943* (MSB)	LC313953	LC313990	LC314025
Trichanthemis karataviensis Regel & Schmalh.	Kazakhstan, Terekty-Saj, *Goloskokov 4297* (S)	LC313954	LC313991	LC314026
Tanacetopsis mucronata (Regel & Schmalh.) Kovalevsk.	Uzbekistan, Tschulbair mountain supra pag."Sina", *Vvedensky s.n.,* 1984-0006023 (W)	LC313955	LC313992	LC314027
Subtribe Anthemidinae				
Tanacetum paleaceum Podl.	Afghanistan, Gardez, Altimur, *Rechinger 31859* (S)	LC313956	LC313993	LC314028
Subtribe Artemisiinae				
Artemisia vulgaris L.	Germany, Regensburg, *Konowalik s.n.* (WRSL)	LC313957	LC313994	LC314029
Artemisia tridentata Nutt. subsp. vaseyana (Rydb.) Beetle	GenBank	AF045411/AF079963	DQ028884	–
Kaschgaria komarovii (Krasch. & Rubtzov) Poljakov	GenBank	DQ028925/DQ028912	DQ028902	–
Ajania fastigiata (C.Winkler) Poljakov	GenBank	AF504169/AF504142	DQ028868	–
Dendranthema zawadskii (Herbich) Tzvelev	GenBank	DQ028924/DQ028911	DQ028901	–
Elachanthemum intricatum (Franch.) Y.Ling & Y.R.Ling	GenBank	AF504186/AF504159	DQ028869	–
Unassigned to the subtribe				
Cancrinia chrysocephala Karelin & Kir.	China, Xinjiang, Houxia—Ulastay, *Dickoré 81* (MSB)	LC313958	LC313995	LC314030

Table 1 (continued)

Taxa	Source/voucher data	Accession numbers		
		nrDNA ITS(ITS1/ITS2)	nrDNA ETS	cpDNA *rpl*32–*trn*L$^{(UAG)}$
Lepidolopha mogoltavica (H.Kraschen.) H.Kraschen.	Uzbekistan, Mogol-tau, Katar-bulak, *Popov* and *Vvedensky no 195* (S)	LC313959	LC313996	LC314031
Polychrysum tadshikorum (Kudr.) Kovalevsk.	Afghanistan, Kataghan, Pul-i Khumri, *Rechinger 33659* (S)	LC313960	LC313997	LC314032
Ugamia angrenica (Krasch.) Pavlov	Kyrgyzstan, Pskemski Ridge, *Lazkov s.n.,* 2007-0014400 (W)	LC313961	–	LC314033

(Felsenstein 1985) with the same settings as for the heuristic searches.

For the model-based inference methods (ML, BI), the best-fitted substitution models for the ITS, ETS, and the concatenated ITS + ETS dataset, along with the plastid intergenic spacer region was determined in jModelTest v2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012) under the Akaike information criterion (AIC). ML analyses were conducted using raxmlGUI v1.5 (Silvestro and Michalak 2012), the graphical interface of RAxML v7.2.6 (Stamatakis 2006), with 1000 replicates of bootstrap heuristic searches for nodal support. Bayesian inference (BI) was conducted with MrBayes v3.2.6_64 (Ronquist and Huelsenbeck 2003) on the CIPRES science gateway (Miller et al. 2010), using two parallel runs of 10-million generations with four Markov chains and a sampling frequency of 1000 generations. Tracer v1.6 (Rambaut et al. 2014) was used to check for the convergence of runs with accepting only the effective sample sizes > 200 for all parameters. After removing 10% of the sampled trees as burn-in, a fifty per cent majority rule consensus tree was constructed. Tree visualisation was done using TreeView v1.6.6 (Page 2001).

MEGA v7.0.21 (Kumar et al. 2016) was employed to access the genetic divergence (uncorrected p-distances) within and between resolved clades in phylogenetic trees with 500 bootstrap replicates. Finally, to assess potential conflicts between the two genomes (nuclear and plastid) in addition to visual inspection, a partition homogeneity test (i.e. the ILD test; Farris et al. 1994) as implemented in PAUP* was used with invariant characters excluded (Cunningham 1997) using TBR branch swapping with 1000 replicates. The maximum number of trees held in memory (MAXTREES) option was set to 1000 to allow the test to proceed to completion.

Tests for hybridisation

To detect possible hybridisation signals in the *Sclerorhachis* dataset, the method of Joly et al. (2009) implemented in the JML software programme v1.3.0 (Joly 2012) was used. This method calculates the probability for the minimum distance among sequences of two species being smaller than expected under a null hypothesis of a strictly bifurcating species tree (with no hybridisation). The software uses the posterior distribution of species trees with branch lengths and population sizes resulting from MCMC simulations in *BEAST (Heled and Drummond 2010) implemented in the BEAST v1.8.3 software package (Drummond et al. 2012) to estimate *p* values of minimum sequence pair distances. In the case of rejection of the null hypothesis, the assumption of hybridisation is accepted.

To sample from the posterior distribution of the species tree based on nrDNA ITS, nrDNA ETS, and cpDNA *rpl*32–*trn*L$^{(UAG)}$ in *BEAST, the following setting was applied: monophyletic clades found in the BI analysis of the nrDNA sequences and corresponding to putative morphospecies were used as priors for the taxon set. The three markers were loaded as unlinked partitions, each one with its specific substitution model. The uncorrelated lognormal relaxed-clock model was chosen to estimate divergence times. A Yule speciation process was assumed with a constant speciation rate and a constant population size through time. Two independent MCMC runs with 100-million generations, a sampling frequency of every 10,000th generation, and a 10% burn-in period were performed to prepare posterior distribution for the subsequent JML analysis. Convergence was assessed using Tracer v1.6 (Rambaut et al. 2014).

In order to infer possible hybrid signals, 5000 trees from the posterior distribution of *BEAST were used as input for the JML test. To generate the JML control file, the substitution models were estimated for each marker separately in the absence of outgroups. The locus rate was specified using the estimated parameters by *BEAST (found in the log file). The heredity scalars were set to 2.0 for the nrDNA and to 0.5 for the cpDNA markers. A significance level of 0.05 was considered for the analysis. Other parameters were left with their default values.

Species delimitation methods

Species boundaries in *Sclerorhachis* were determined using the coalescence-based species delimitation methods implemented in bGMYC (Reid and Carstens 2012) and bPTP (Zhang et al. 2013). For bGMYC, 100 random ultrametric

trees of the nrDNA ITS + ETS dataset obtained from the posterior distribution of the BEAST analysis outputs were sampled as an input to integrate over the uncertainty of tree topology. BEAST v1.8.3 (Drummond et al. 2012) was used to obtain the chronograms. The analysis was performed at the CIPRES science platform, assuming a Bayesian relaxed-clock model. Molecular rates were allowed to vary among lineages around an average value, by enforcing an uncorrelated lognormal clock of evolutionary rates. A Yule process prior was used in the analysis. Fossil pollen assigned to *Artemisia* from the Late Oligocene in the Qinghai Province (Zhu et al. 1985) was used as the calibration point. Since the age of this fossil cannot be ascribed to any of the species within the *Artemisia* group, it can only be used to suggest a minimum age for *Artemisia* and its closely related genera with an *Artemisia* pollen type (i.e. *Ajania*, *Dendranthema, Elachanthemum, Kaschgaria*). This calibration point was set with a lognormal prior to reflect the uncertainty in the fossil calibration as recommended by Ho and Phillips (2009), with the uppermost limit of the time interval as a minimum hard bound (offset = 22 Ma, mean = 1.04, standard deviation = 0.53) that includes the entire geological interval (23–28 Ma). The MCMC chain was run in two separate analyses for 50-million posterior iterations sampling every 5000 posterior iterations. LogCombiner v1.8 (Drummond et al. 2012) was used to combine the log and tree files. The initial 5-million iterations of each analysis were discarded as burn-in before combining. Tracer v1.6 (Rambaut et al. 2014) was used to assess effective sample sizes (ESS) with values greater than 200 considered indicating optimal convergence and tree likelihood stationarity. A maximum clade credibility (MCC) tree was constructed in TreeAnnotator v1.8.0 (Drummond et al. 2012) depicting the maximum sum of posterior clade probabilities. The MCC tree was visualised in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Subsequently, a bGMYC analysis was conducted with the R package "bGMYC" (Reid and Carstens 2012), with 50,000 MCMC generations, a thinning of 100 generations, and 40% burn-in. Starting parameters were set according to the default settings. Lower thresholds were adjusted to 43, corresponding to the number of tips. Applying the bPTP algorithm, also 100 random post-burn-in trees from the posterior distribution of Bayesian analysis were used to shed light on species boundaries. The bPTP analysis was executed with the python script "bPTP.py", running for with 500,000 generations, a thinning of 100 and a burn-in of 10%.

Possible species boundaries were also inferred by using the statistical parsimony network reconstruction software TCS (Templeton et al. 1992; Clement et al. 2000) applied to the nrDNA dataset and treating indels as fifth character state. We applied the 95 and 98% connection limits to define haplotype groups that were separated from each other sufficiently to merit consideration as an independent evolutionary entity (species).

Reliability of species delimitations inferred through the different applied methods was assessed by measuring the genealogical sorting index *gsi* (Cummings et al. 2008). The degree of exclusive ancestry of predefined lineages on 100 random trees from the Bayesian inference of nrDNA ITS + ETS (gsi_T) was quantified with the R package "genealogical sorting index" (Bazinet et al. 2008). gsi_T values vary from 0 to 1, which indicates the absence of exclusive ancestry and monophyly, respectively. The *p* values of the obtained *gsi* values for each clade were inferred with 1000 repeats of randomizing the association of members to the different groups.

Morphological examination

The corona of achenes was examined by scanning electron microscopy (SEM). SEM was performed on representative individuals of each population and photographed using a Philips XL30 scanning electron microscope at an accelerating voltage of 20 kV after covering the mounted achenes on stubs with 30 nm of gold.

Results

Phylogenetic analyses

Statistics of sequences, lengths, numbers, and consistency and retention indices of the most parsimonious trees (MPT), as well as the best-fit model for each of the datasets, are summarised in Table 2. The observation of congruent topologies obtained from maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) for nrDNA regions, ITS and ETS (not shown here), in addition with the non-significant result of the partition homogeneity test ($p = 0.1$) led us to combine these markers. On the other hand, the visual inspection and also the result of the partition homogeneity test ($p = 0.01$) showed that the trees obtained from the nrDNA and the plastid datasets were not congruent, and thus these datasets were not combined for downstream analyses.

Since MP, ML, and BI analyses of the combined nrDNA ITS + ETS dataset gave very similar results, only the Bayesian tree along with bootstrap (BS) values from the ML analysis and Bayesian posterior probabilities (PP) is shown (Fig. 2). These phylogenetic reconstructions identified *Sclerorhachis* as a well-supported, monophyletic genus (BS = 100%, PP = 1.0) and recovered five main clades (A–E) with high to moderate support. While clade A (BS = 88%, PP = 1.0) comprised specimens from Iran and Afghanistan morphologically ascribable to *S. caulescens* (clade A1; BS = 94%, PP = 0.91) and *S. polysphaera* (clade A2), clade B corresponds to *S. leptoclada* (BS = 100%, PP = 1.0), and clade C with moderate statistical support

Table 2 Statistics and homoplasy measures of most parsimonious tree (MPT) and model selection for each marker/combination

	ITS	ETS	*rpl*32–*trn*L$^{(UAG)}$	ITS_ETS
Number of analysed sequences	43	41	36	43
Sequences length	651	474	1055	1125
Number of variable positions	185	163	99	348
Number of PI characters	102	82	44	184
Number of PI indels	13	6	27	19
Overall divergence genetic	0.045	0.038	0.013	0.045
Number of MPTs	11,124	91,543	116	204,721
Length of MPTs	211	171	67	391
C.I. of MPT	0.607	0.614	0.664	0.596
R.I. of MPT	0.841	0.831	0.844	0.824
Model of evolution for complete dataset	SYM + G	GTR + G	GTR + I + G	GTR + G
Model of evolution for *Sclerorhachis* dataset	K80 + I	HKY + I	F81	–

(BS = 80%, PP = 0.68) comprised specimens morphologically classified as *S. platyrachis*. Lineage D (BS = 97%, PP = 1.0) comprised specimens from the Binalud Mountain Range (Iran, Razavi Khorasan Province) and lineage E (BS = 100%, PP = 1.0) from the Kopet Dagh and southern Alborz Mountains (Iran, Northern Khorasan and Semnan Provinces). The estimated genetic diversity (uncorrected p-distances) within monophyletic clades identified by phylogenetic analyses ranged from complete identity (within clades A2, B, C1, C2, and D) to 0.50% (within clade A1), while distances among clades ranged between 0.17% (between clades C1 and aggregation C2) and 2.05% (between clades A1 and E; Table 3).

The phylogeny based on the cpDNA *rpl*32–*trn*L$^{(UAG)}$ intergenic spacer region showed less resolution than the nrDNA ITS + ETS trees, and all of the monophyletic groups described above found no support in the cpDNA analyses (Fig. 3). However, the plastid data corroborated the monophyly of the genus *Sclerorhachis* (BS = 60%, PP = 0.99) and provided some interesting hints for maternal relationships among the nrDNA clades through sharing of similar chloroplast haplotypes.

The BEAST analyses of the concatenated nrDNA ITS + ETS dataset generated a well-resolved time-calibrated phylogeny of *Sclerorhachis* (Fig. 4, Table 4). This BEAST chronogram was consistent with the result from the Bayesian phylogenetic reconstruction. The analysis suggested that divergence of *Sclerorhachis* from the related genera occurred about 34.8 Ma (95% HPD: 19.4–53.6 Ma), while the onset of differentiation within the genus was dated to the early Oligocene and late Miocene at around 18.3 Ma (95% HPD: 8.7–31.2 Ma).

Hybridisation test

The results obtained from the JML simulations on the whole dataset (ITS, ETS and *rpl*32–*trn*L$^{(UAG)}$) revealed four pairs of clades with observed minimum genetic distance values lower than expected at the 5% level ($p < 0.05$; Table 5). While the nrDNA ITS data revealed no hint for hybridisation among monophyletic lineages, nrDNA ETS pinpointed to some sequences of clades B, C, and D with a hybrid signal. Since the same is true for some members of clades C, D, and E in the cpDNA *rpl*32–*trn*L$^{(UAG)}$ dataset, hybridisation as an explanation for the phylogenetic incongruence between nuclear and plastid markers appears very plausible.

Species delimitation methods

The results of the two coalescent-based species delimitation approaches (bGMYC, bPTP), along with delimitations resulting from the parsimony network reconstruction of haplotypes (TCS) and the application of a monophyletic species concept (MSC) in the nrDNA ITS + ETS phylogenetic analysis are summarised in Fig. 4. The bGMYC method, considering the probability threshold > 0.5 to confirm segregated entities as a discrete species, identified ten entities. This method revealed the specimen aggregations C1 and C2 as independent species and split clades A1 and E into three and two independent evolutionary lineages, respectively (entities A1-1, A1-2, A1-3, E1, and E2). The bPTP approach united clade C1 and clade C2 into a single species (C) and corroborated the other five monophyletic clades (A1, A2, B, D, and E) as independent evolutionary units.

Using TCS to find independent networks at a 95% probability of accuracy, only clade D was identified as an independent haplotype lineage (species) while all other haplotypes were grouped together. However, at a level of 98% probability of accuracy, haplotype groups identified corresponded mostly to the results of the bPTP analysis (see above), except for clade A1, which was divided into two independent haplotype groups (A1-1 and A1-2).

Subjecting the different evolutionary units suggested by the species delimitations from the bGMYC, bPTP, and TCS

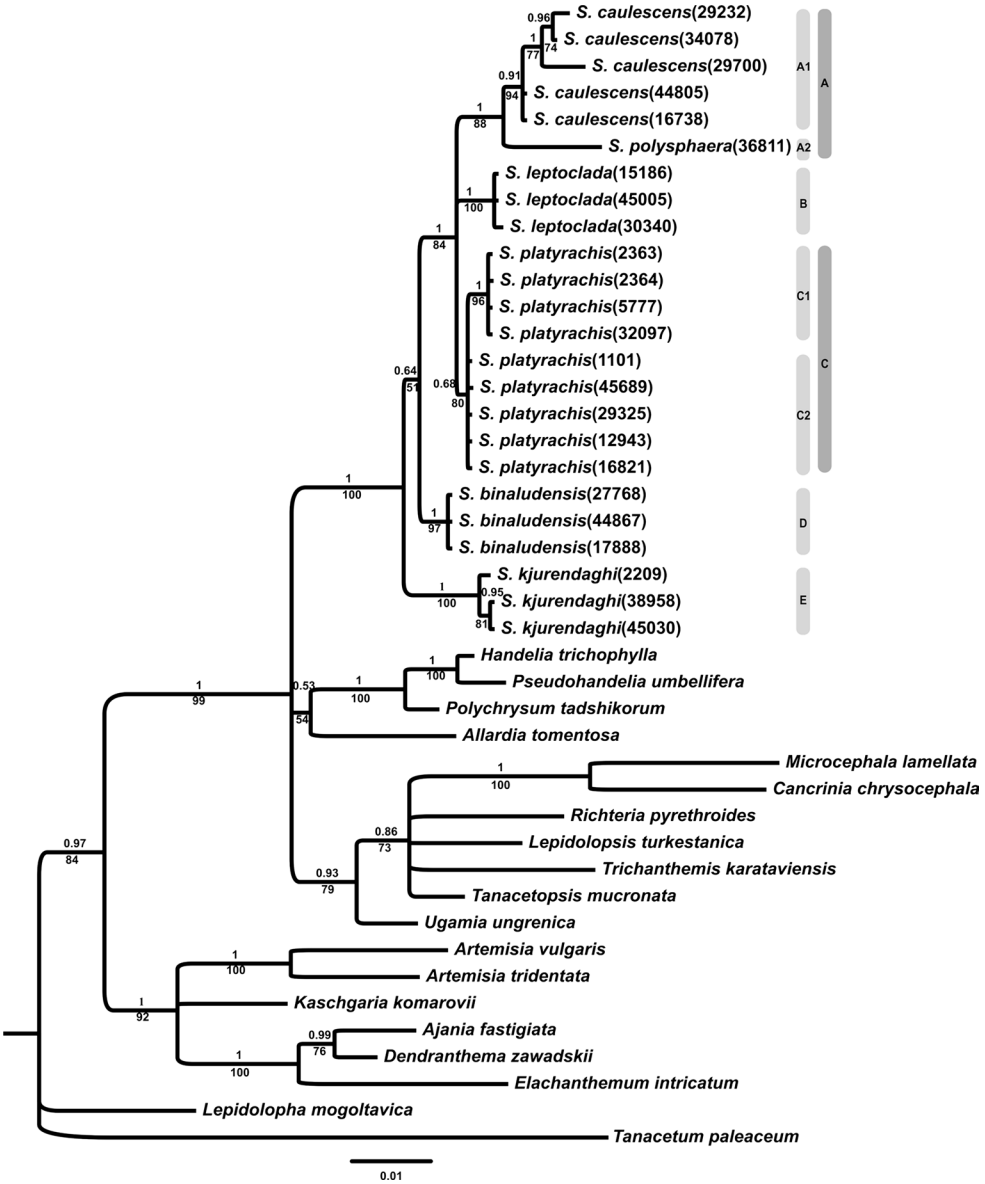

Fig. 2 50% majority rule consensus tree resulting from the Bayesian phylogenetic analysis of the combined nrDNA ITS + ETS dataset. Numbers above and below the branches are posterior probability (PP) from the BI and bootstrap support (BS) values from a ML analysis, respectively. Values below 0.5 (PP) or 50% (BS) are not shown

Table 3 Genetic distances between and within the seven clades reconstructed through phylogenetic analysis, based on nrDNA ITS + ETS dataset

	A1	A2	B	C1	C2	D	E
A1	0.005						
A2	0.0115	0.0000					
B	0.0115	0.0135	0.0000				
C1	0.0081	0.0102	0.0068	0.0000			
C2	0.0064	0.0085	0.0051	0.0017	0.0000		
D	0.0132	0.0118	0.0152	0.0118	0.0102	0.0000	
E	0.0205	0.0192	0.0192	0.0158	0.0141	0.0107	0.0010

analyses to calculations of the genealogical sorting index gsi_T, most identified entities received high support from these tests, except entities A1-2, A1-3, and C2 (Table 6).

Discussion

Despite differences in the power of resolution among infrageneric assemblages, the two datasets (nrDNA ITS + ETS and cpDNA *rpl*32–*trn*L$^{(UAG)}$) supported the monophyly of

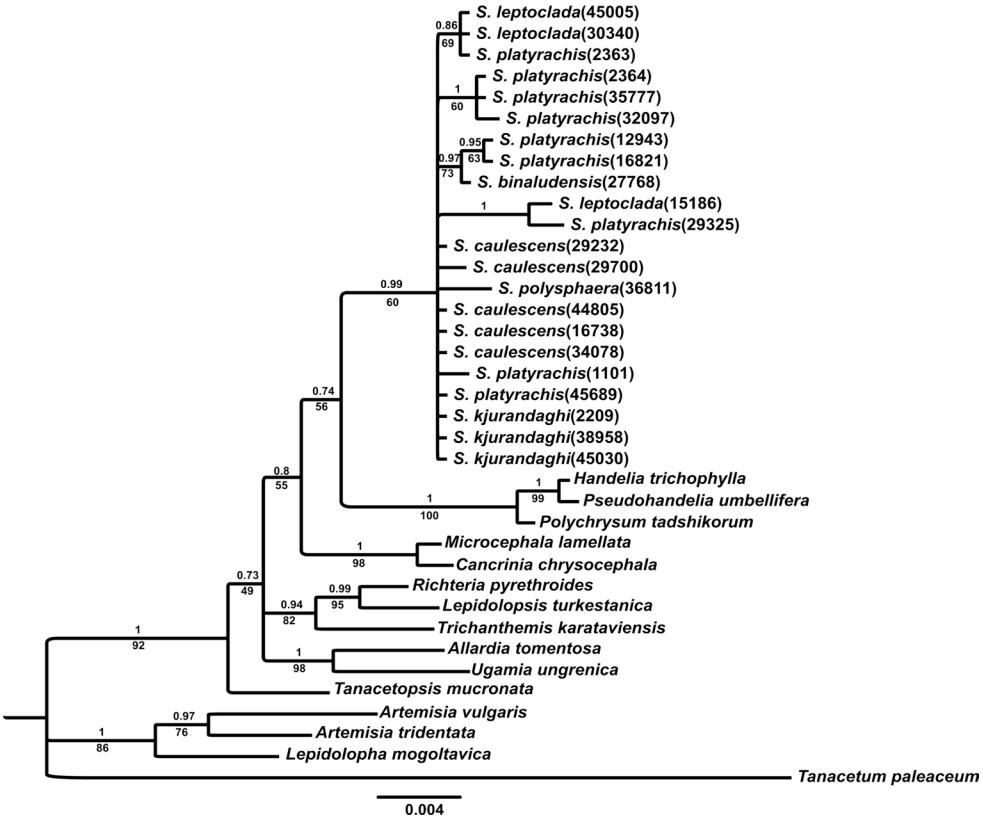

Fig. 3 50% majority rule consensus tree resulting from the Bayesian phylogenetic analysis of the cpDNA *rpl*32–*trn*L$_{UAG}$ dataset. Numbers above and below the branches are posterior probability (PP) from the BI and bootstrap support (BS) values from a ML analysis, respectively. Values below 0.5 (PP) or 50% (BS) are not shown

the genus. While both datasets failed to pinpoint to a well-supported sister-group relationship with another genus of the tribe, the nesting of *Sclerorhachis* among members of the subtribe Handeliinae received high statistical support. However, in contrast to the morphologically defined, narrowly circumscribed subtribe Handeliinae sensu Bremer and Humphries (1993), comprising only *Handelia*, *Lepidolopsis*., *Pseudohandelia*, *Polychrysum*, and *Sclerorhachis,* the present phylogenetic reconstructions rather support the broader subtribal circumscription of Oberprieler et al. (2007, 2009). The latter is characterised by adding *Allardia*, *Cancrinia*, *Richteria*, *Trichanthemis,* and *Ugamia* (subtribe Cancriniinae Bremer & Humphries), along with *Microcephala* (subtribe Matricariinae Bremer & Humphries) and *Tanacetopsis* (provisional subtribe "Tanacetinae" Bremer & Humphries) to a strongly supported monophyletic group, which is found being sister to subtribe Artemisiinae (represented by *Ajania, Artemisia, Dendranthema, Elachanthemum,* and *Kaschgaria* in the present analysis). The lack of a well-supported subtribe Handeliinae s. str. (i.e. sensu Bremer and Humphries 1993) in our present analyses casts doubts on the apomorphic status of the thick and villous-tomentose stems with a soft pith considered decisive for the alleged evolutionary closeness of the five genera of the subtribe in its narrow sense. On the other hand, as stated by Oberprieler et al. (2007) the subtribe in its broader sense lacks obvious synapomorphies from morphology or anatomy due to its considerable phenotypic diversity.

In contrast to the infrageneric taxonomy proposed in *Flora Iranica* by Rechinger (1986) for the genus *Sclerorhachis* with the four morphologically distinct species *S. caulescens, S. leptoclada, S. platyrachis,* and *S. polysphaera,* the phylogenetic analysis based on nrDNA ITS + ETS (Fig. 2) revealed two further well-supported monophyletic entities (clades D and E) in sister-group relationship to the clade of the four mentioned species. While the phylogenetic analysis based on the cpDNA intergenic spacer region *rpl*32–*trn*L$^{(UAG)}$ did not reveal the same entities—presumably due to the lack of sufficient variation in this marker for a better resolution—a statistical parsimony network analysis with the software programme TCS corroborated the distinctness of nrDNA clades in nearly all cases. With the exception of haplotypes found in accessions of *S. caulescens,* which were separated into two haplotype groups according to the 98% connection limit criterion (clades A1-1 and A1-2 in Fig. 4), all other monophyletic entities of the nrDNA phylogram were separated into statistically independent haplotype groups (from clade A2 to clade E).

The two Bayesian coalescent-based, single-locus species delimitation algorithms bGMYC and bPTP applied to the trees based on nrDNA sequence variation (Fig. 4) further corroborated the evolutionary independence of many of the mentioned clades, with an obviously stronger splitting by

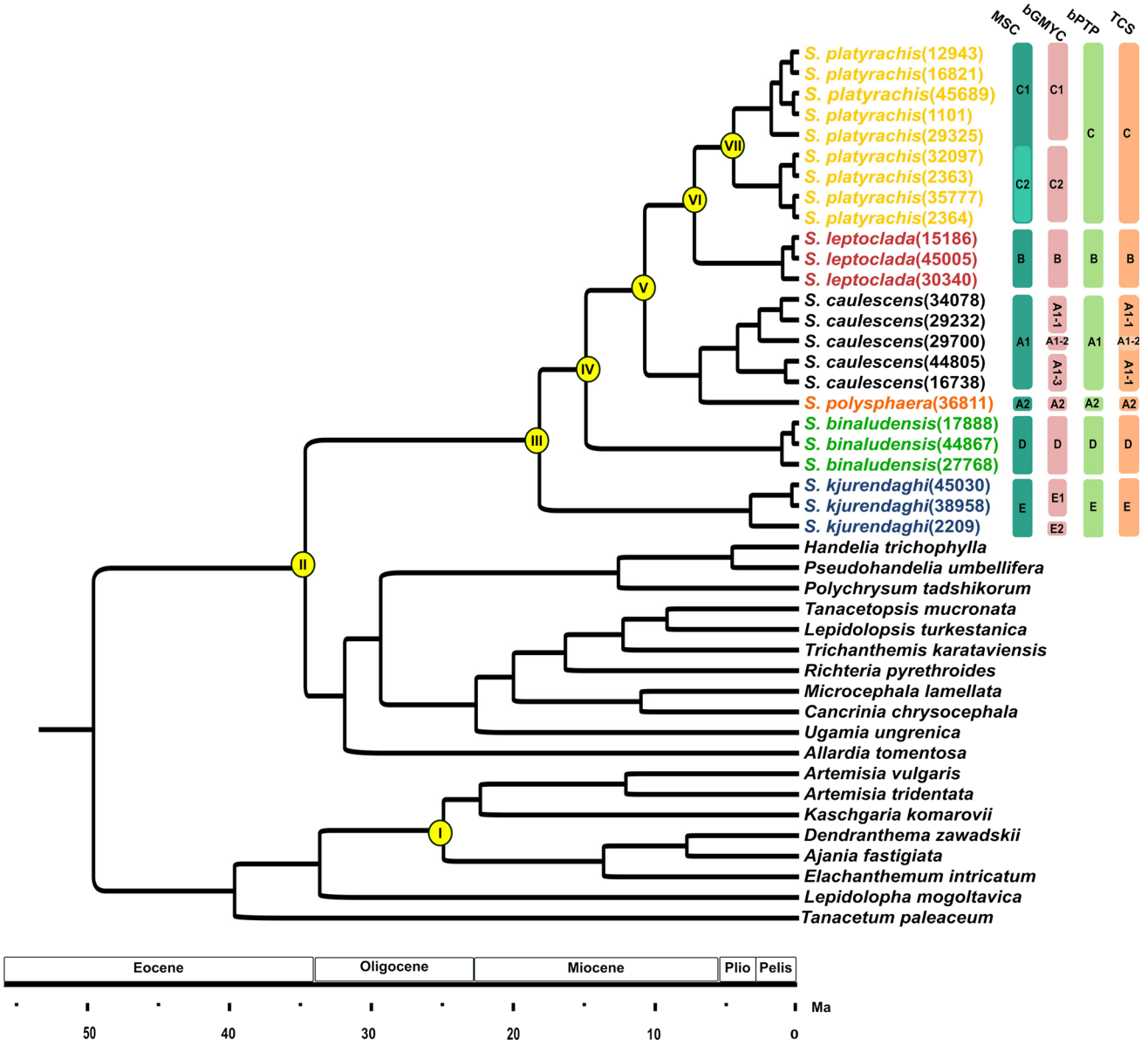

Fig. 4 BEAST chronogram depicting relationships among *Sclerorhachis* accessions based on a Bayesian analysis of nrDNA ITS + ETS using a Yule model and a relaxed-clock model. Coloured columns to the right represent the results of different species delimitation methods applied. The first column to the right of the tree indicates putative phylogenetic species delimited based on a monophyletic species concept (MSC). The second column indicates clusters recovered by bGMYC, while the third (light green) indicates species identified by bPTP. The last column shows TCS haplotype groups based on the nrDNA ITS + ETS dataset. The digits in tips of tree represent herbarium accession codes of each population. Colour-coded tips match colour-coded accessions in Fig. 1. The branch length represents time in millions of years

the former of the two methods. This is in accordance with studies of Zhang et al. (2013) who reported on the outperformance of PTP over GMYC in evenly and unevenly sampled simulated datasets because the former method resulted in more conservative estimates of species numbers than the latter. Other studies (e.g. Lang et al. 2015), however, found both methods prone of exaggerated splitting compared to the morphologically or ecologically circumscribed species concepts. The fact that our present *Sclerorhachis* dataset is far from an even sampling of taxa (with one species, *S. polysphaera,* being represented by only a single accession from the type collection) and the better correspondence between the bPTP results with haplotype groups found by TCS and morphological evidence (see below) argues also for a more conservative approach in species circumscription in the genus.

The incongruence between the two model-based phylogenetic reconstructions (gene trees) based on nrDNA

Table 4 Node numbers refer to Fig. 4. Posterior mean ages and 95% HPD intervals of divergence times are in millions of years before the present

Nodes of interest	Description	Mean nodal age (Ma)	95% HPD (Ma)
I	Calibration point	25.05	22.68–28.37
II	Split of *Sclerorhachis* from Handeliinae	34.79	19.36–53.58
III	onset diversification within the *Sclerorhachis*	18.29	8.68–31.16
IV	The second diversification within the *Sclerorhachis*	15.02	6.78–25.19
V	The third diversification within the *Sclerorhachis*	10.87	5.1–18.5
VI	The fourth diversification within the *Sclerorhachis*	7.38	2.72–13.47
VII	The fifth diversification within the *Sclerorhachis*	4.61	1.27–8.99

Table 5 JML results from ETS and *rpl*32–*trn*L$^{(UAG)}$ datasets

	seq1	seq2	Distance	Probability
ETS				
B versus D	*S. leptoclada*(15186)	*S. binaludensis*(27768)	0.004255	0.0046
B versus D	*S. leptoclada*(15186)	*S. binaludensis*(44867)	0.006383	0.0174
B versus D	*S. leptoclada*(45005)	*S. binaludensis*(27768)	0.004255	0.0046
B versus D	*S. leptoclada*(45005)	*S. binaludensis*(44867)	0.006383	0.0174
C2 versus D	*S. platyrachis*(45689)	*S. binaludensis*(27768)	0.004255	0.004
C2 versus D	*S. platyrachis*(45689)	*S. binaludensis*(44867)	0.006383	0.0176
C2 versus D	*S. platyrachis*(12943)	*S. binaludensis*(27768)	0.006383	0.0176
C2 versus D	*S. platyrachis*(16821)	*S. binaludensis*(27768)	0.006383	0.0176
C1 versus D	*S. platyrachis*(2364)	*S. binaludensis*(27768)	0.006383	0.0174
*rpl*32–*trn*L$^{(UAG)}$				
C2 versus D	*S. platyrachis*(12943)	*S. binaludensis*(27768)	0	0.0314
C2 versus E	*S. platyrachis*(1101)	*S. kjurendaghi*(2209)	0	0.0294
C2 versus E	*S. platyrachis*(1101)	*S. kjurendaghi*(38958)	0	0.0294
C2 versus E	*S. platyrachis*(45689)	*S. kjurendaghi*(45030)	0	0.0294

All the pairwise sequence distances which have a p value smaller than significance level of 0.05 (with hybridization signals) are depicted

Table 6 Genealogical sorting index (gsi_T) and p values of identified species by bGMYC, bPTP, and TCS analyses

	C1	C2	C	A1	A1-1	A1-2	A1-3	A1-4	A2	B	D	E	E1	E2
bGMYC	1**	0.76**			1*	1**	0.53		1*	1**	1**		1**	1**
bPTP			0.97**	0.99**					1**	1**	1**	1**		
TCS			0.97**			0.71**		1*	1*	1**	1**	1**		

* $p < 0.01$; ** $p < 0.001$

ITS + ETS (Fig. 2) and cpDNA (Fig. 3) can be the consequence of incomplete lineage sorting (ILS) or hybridisation. In order to discriminate between these two processes, a number of approaches have been suggested in the literature, reaching from statistical tests based on gene tree topologies (Huson and Kloepper 2005) to coalescent simulation-based algorithms (e.g. Maureira-Butler et al. 2008; Blanco-Pastor et al. 2012; Konowalik et al. 2015). In our present contribution, we used the JML method proposed by Joly et al. (2009) to detect accession pairs from different predefined species (clades) that are more similar to each other than expectable under ILS alone and therefore may indicate hybridisation events. The results of this method (Table 5) suggested hybridisation between members of clades C2 and D (nrDNA, cpDNA), clades B and D (nrDNA), clades C1 and D (nrDNA), and clades C2 and E (cpDNA), respectively. Since these alleged gene-flow events make sense in both temporal–spatial and morphological perspective (see below), hybridisation among *Sclerorhachis* species may be considered a further explanation for infraspecific differentiation

and over-splitting by bGMYC as a species delimitation algorithm, arguing also for a rather conservative interpretation of the species circumscription results gained.

Accepting the bPTP results as the most conservative species delimitation scheme, a clear-cut geographical representation of the genus with six allopatrically distributed species emerges (Fig. 1). The three representatives of the early-diverging clade E all come from the westernmost part of the distribution range of *Sclerorhachis,* namely the Kopet Dagh Mts and the southern Alborz Range, along the Iranian–Turkmenistan border between Semnan and Ashgabat. Despite its geographical proximity to representatives of clade C2 (and some hybridisation between the two entities), there is an obvious phylogenetic gap between these two clades, which were united under *S. platyrachis* in the revision of the genus by Mozaffarian (2008). Since the type locality of *S. platyrachis* ("Persia, Khorasan, prope Miandescht, Sabzewar et inter Nischapur et Mesched") falls into the range of clade C1 and D, clade E—if accepted on species level—should be named *S. kjurendaghi* The type locality of the latter ("Kjuren Dagh Range") is localised in Turkmenistan (Kurbanov 1984; Kovalevskaja 1987), but it is close to the Iranian border and in geographical proximity and morphological similarity with clade E specimens.

The three members of clade D, diverging next in the phylogenetic reconstructions based on nrDNA, were all collected in the very limited area of the Binalud Mountains Range in the west of Mashhad (NE Iran) and are surrounded in the south and the north by members of clade C (Fig. 1), for which the name *S. platyrachis* is usually used. Due to its phylogenetic independence, its geographical isolation, and its morphological distinctness from *S. platyrachis,* we here acknowledge and describe the Binalud Mts populations as an independent species, *S. binaludensis* Sonboli (see below). Khorassan-Kopet Dagh (KK) floristic province which encompasses Binalud Mts (located in northeastern Iran and southern Turkmenistan) is one of the important centres of plant endemism in Irano–Turanian region. Recently, an updated and annotated checklist of 356 endemic vascular plant taxa has been published, of which 105 taxa belong to the Asteraceae (Memariani et al. 2016). The high rate of diversification and endemism in this area could be explained by long-time geographical isolation and subsequent reduced gene flow provided by interglacial refuges of Neogene periods (Djamali et al. 2012).

The further evolutionary history of *Sclerorhachis* is characterised by a split into an eastern lineage with the two species *S. caulescens* (clade A1) and *S. polysphaera* (clade A2), and a western lineage with *S. platyrachis* (clade C) in the north and *S. leptoclada* (clade B) in the south; all four acknowledged on species level in the *Flora Iranica* treatment of Rechinger (1986). Within clade C (*S. platyrachis*), a further subdivision into a southern (C1; Sabzewar-Kashmar and Torbat-e Heydarieh Mts) and a northern (C2; Hezar Masjed Mts) population group is suggested by the bGMYC species delimitation algorithm (Fig. 4). However, despite the fact that there are some similarities between the plants from the northern group C2 and the description of *Tanacetopsis paropamisica*. (based on *Pyrethrum paropamisicum* Krasch.) from the Paropamis Mts. in adjacent Turkmenistan (e.g. rarely hairy receptacle and coronate achenes; Fig. 5) [see Tzvelev (1961), sub *Cancrinia paropamisica* (Krasch.) Tzvel.] and the latter taxon is alleged being a synonym of *S. platyrachis* by Rechinger (1986), we refrain here from taxonomical and nomenclatural consequences because of some uncertainties with the type locality and our ignorance of the type material.

While hybridisation signals found between *S. kjurendaghi* (clade E) and the northern populations of *S. platyrachis* (clade C2) could be easily explained by distributional shifts along the NE Iranian mountain ranges and chloroplast capture by some populations of the latter species, the additionally detected hybrid signal between *S. binaludensis* (clade D) with *S. platyrachis* (clade C2) on the one hand and with *S. leptoclada* (clade B) on the other hand is biogeographically counterintuitive.

The presented results gathered from phylogenetic and species delimitation analyses together with the mentioned morphological data argue for the description of *Sclerorhachis* populations from the Binalud Mts. as a species new to science, which is clearly separated from *S. platyrachis.*

Taxonomic treatment

Sclerorhachis binaludensis Sonboli, **sp. nov.**—HOLOTYPE: Iran, Razavi Khorasan, Neyshabur, Garineh Mts., 1900 m a. s. l., 35°43′N, 53°02′E, 25 May 2007, *Sonboli 1106* (MPH!; Isotype: W!) (Figs. 5, 6).

Etymology: The epithet "binaludensis" has been selected because the new species inhabits the Binalud Mountain Range.

Description: Perennial plants of 40–60 cm height, with erect, tenuous, slender and sparsely leafy stems. Basal leaves numerous and up to 11 cm long, 2–3 cm wide, tripinnatisect, on rather long petiole slightly thickened at base, alternately arranged, cauline leaves more or less reduced about 3.6 cm long and 1.5 cm wide, bi-pinnatisect. Capitula about 4–7 per stalk, on rather long (up to 12 cm) peduncles, involucre 6–11 mm in diameter and 3–4 mm high. Receptacle convex, with sessile glands and rarely hairy (paleate). Achenes 1.5–2.2 mm long and 0.3–0.5 mm wide, with five longitudinal ribs, corona around 0.1–0.3 mm long and formed by blunt teeth.

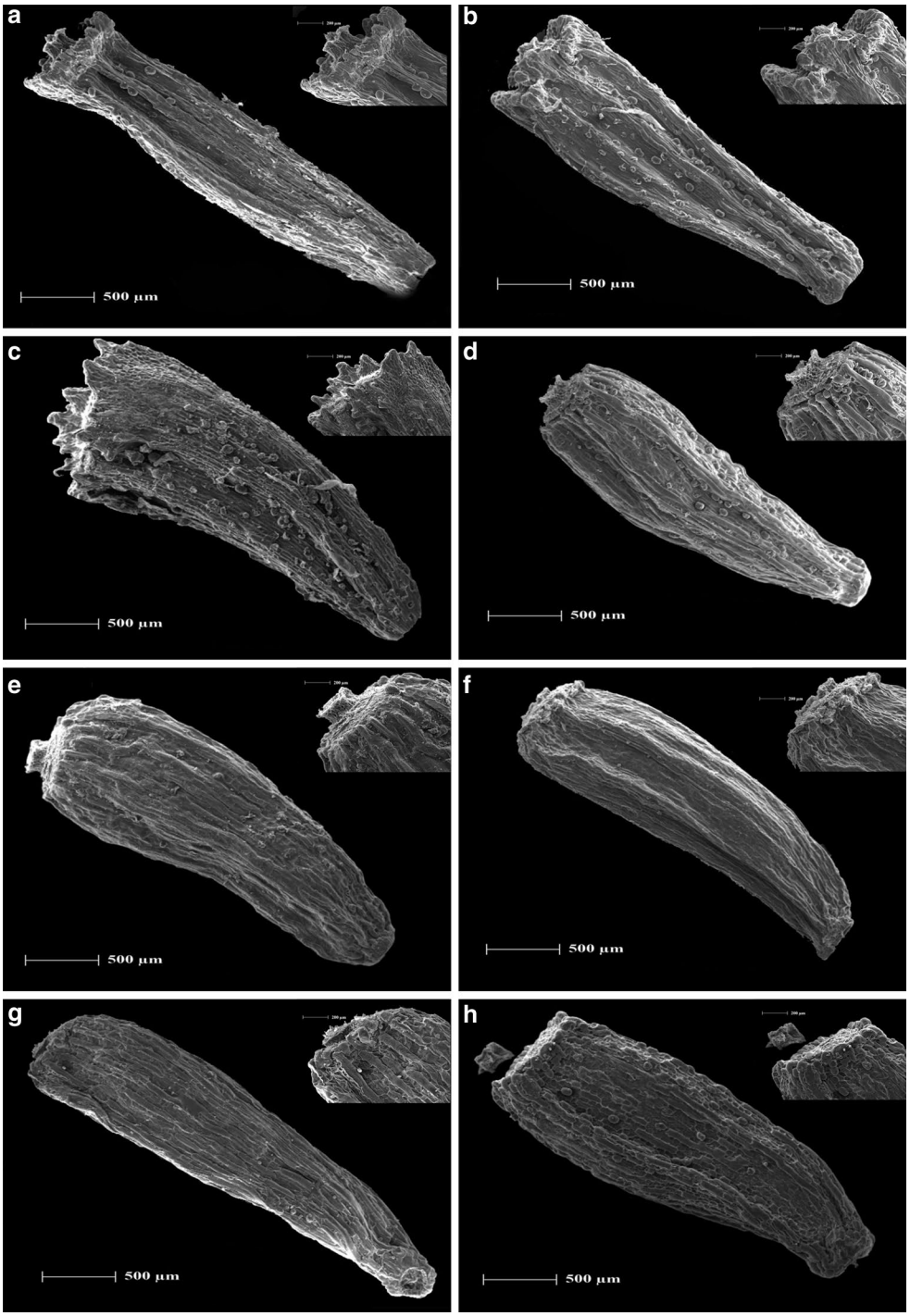

Fig. 5 SEM micrographs of the achene corona status in *Sclerorhachis* species. **a** clade D [*S. binaludensis*(44867)]; **b**, **c** aggregation C2 [*S. platyrachis*(29325), *S. platyrachis*(45689)]; **d** clade E [*S. kjurendaghi*(45030)]; **e** clade C1 [*S. platyrachis*(2364)]; **f** clade B [*S. leptoclada*(30340)]; **g** clade A1 [*S. caulescens*(34078)]; **h** clade A2 [*S. polysphaera*(36811)]

Diagnosis: *Sclerorhachis binaludensis* is closely related to *S. platyrachis,* but differs from it by thinner stems and a laxly tomentose indumentum, 10.6 mm diameter and sparsely wooly collar (not 28 mm and strongly wooly), very thin cauline leaf raches (not thick), capitula broad, 4–12 mm in diameter (not 12–20 mm), and achenes 1.2–2.2 mm long (not c. 4 mm) with an apical corona longer than 0.1 mm (not < 0.1 mm).

Phenology: Flowering time from May to June.

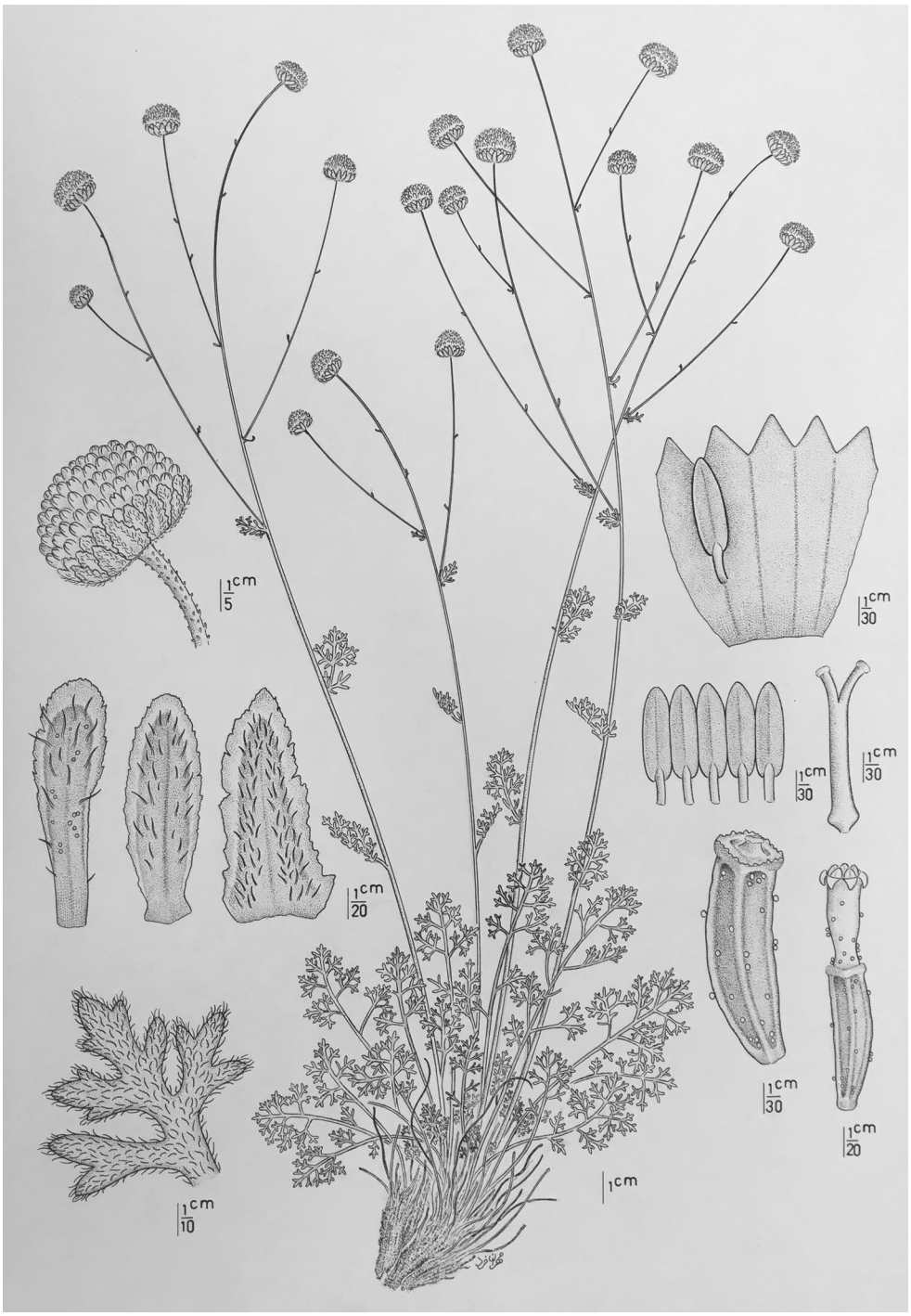

Fig. 6 *Sclerorhachis binaludensis* Sonboli from *Sonboli 1106* (MPH)

Distribution and habitat: *Sclerorhachis binaludensis* grows on rocky hills and mountain regions in the NE of Iran (Binalud Mountains) at an elevation of 1600–2400 m a. s. l.

Additional specimens examined: IRAN. Razavi Khorasan, Neyshabur, Baghshan, Garineh Mts., 1900 m a. s. l., 36°07′0.75″N, 59°10′59.3″E, 3 Jul 1996, *Faghihnia* and *Zangooei 27768* (FUMH); Razavi Khorasan, Neyshabur, Kharv Mts., 1690 m a. s. l., 36°12′17.7″N, 59°06′01.9″E, 13 Jun 2012, *Joharchi 44867* (FUMH); Razavi Khorasan, Neyshabur, Ghadamgah, Dizbad village, 1700–2200 m a. s. l., 4 Aug 1983, *Mozaffarian 45579* (TARI); Razavi Khorasan, Neyshabur, Shah-taghi, Dizbad village, 1800 m a. s. l., 4 Aug 1984, *Mozaffarian 48929* (TARI); Razavi Khorasan, Chenaran, Golmakan, Cheshme-Sabz, 2330 m a. s. l., 36°20′44.43″N, 59°03′23.80″E, 26 Jun 1985, *Ayatollahi* and *Zangooei 13257* (FUMH); Razavi Khorasan, Chenaran, Golmakan, Cheshme-Sabz, 36°20′44.43″N, 59°03′23.80″E,

3 Aug 1992, *Ayatollahi* and *Zangooei 22598* (FUMH); Razavi Khorasan, Mashhad, Zoshk Mts., 36°16′56.50″N, 59°06′35.91″E, 3 Aug 2007, *Joharchi 39217* (FUMH); Razavi Khorasan, Mashhad, N slope of Binalud Mts., above Zoshk village, Abdullah River, 2100–3000 m a. s. l., 3 Apr 1984, *Mozaffarian 48852* (TARI); Razavi Khorasan, Mashhad, Binalud Mts., Feresgeh, 2000 m a. s. l., 36°17′30.84″N, 59°14′0.07″E, 3 Jul 1989, *Faghihnia* and *Zangooei 17888* (FUMH).

Lectotypification of *Sclerorhachis platyrachis*

Sclerorhachis platyrachis (Boiss.) Podlech ex Rech.f., Fl. Iranica 158: 47 (1986) ≡ *Pyrethrum platyrachis* Boiss., *Fl. Orient* 3: 356 (1875). Described from: "Hab. In Persiae prov. Khorassan in regione montana et alpine prope Miandescht, Sebsewar et inter Nischapur et Mesched (Bge!). Fl. Jun." —LECTOTYPE (**designated here**): Herbar. Bungeanum. Iter persicum. 99. [Iran] "pr. Sebsewar, Chorassan, inter Schahrud et Nischapur. Juni 1858", *[Bunge]* (G00764721!).

Notes: The syntype specimens of *Sclerorhachis platyrachis* (i.e. *Pyrethrum platyrachis*) mentioned in the protologue (Boissier 1875) and housed in the G-Boissier herbarium in Geneva consist of three different elements: (α) a specimen labelled with a hand-written collection number "98." on the otherwise printed label reading "Herbar. Bungeanum. Iter persicum./Regio montana et alpine inter Nischapur et Mechhed./Jun., Jul. 1858" (G00764196); (β) a specimen labelled with a hand-written collection number "99." and an equally hand-written location "pr. Sebsewar" on the otherwise printed label reading "Herbar. Bungeanum. Iter persicum./Chorassan, inter Schahrud et Nischapur. Juni 1858" (GG00764721; with three other sheets recently barcode-labelled "a", "b", and "c"); and (γ) a specimen labelled with a hand-written collection number "100." and an equally hand-written location "pr. Miandescht" on the otherwise printed label reading "Herbar. Bungeanum. Iter persicum./Chorassan, inter Schahrud et Nischapur. Juni 1858".

We here propose element (β) as lectotype of the name *Pyrethrum platyrachis* Boiss. because (a) due to its consistence of four plant fragments allotted to four herbarium sheets this specimen is the most complete one of the three candidate elements, and (b) compared with the other two elements, element (β) has larger capitula (c. 12–15 mm in diameter) than the other two elements (α: c. 8 mm; β: c. 10 mm) and therefore fits best the mentioning of rather large capitula in Boissier's (1875) diagnosis ("[…] capitulis tandem piso duplo majoribus."). Additionally, element (β) has been collected around Sebsewar, which is in close proximity to localities of specimens representing clade C1 in the present analysis, while element (α) has been collected in the Binalud Mts between Nischapur and Maschhad and corresponds to the new species *S. binaludensis* (clade D), and element (γ) has been collected around Mian Dashd ("Miandescht"), which is close to the distribution range of *S. kjurendaghi* (clade E).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online resource 1. Alignment of concatenated nrDNA ITS + ETS sequences in nexus format. Gaps coded as absent = "0", present = "1", and unknown = "N".
Online resource 2. Alignment of cpDNA *rpl*32–*trn*L$^{(UAG)}$ sequences in nexus format. Gaps coded as absent = "0", present = "1", and unknown = "N".

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