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ORIGINAL ARTICLE

Phylogeny and systematics of the Lactucinae (Asteraceae) focusing on their SW Asian centre of diversity

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Abstract

This study provides the first comprehensive molecular phylogenetic reconstruction of the lettuce alliance (Cichorieae subtribe Lactucinae of the sunflower family) in its SW Asia centre of diversity and assumed area of origin. The sampling contains multiple samples of all SW Asian Lactucinae except four unavailable rare taxa. One nuclear ribosomal and five plastid DNA markers were used for the reconstruction with maximum parsimony, maximum likelihood and Bayesian inference. A total of 716 individual sequences belonging to 56 taxa were newly generated. The nrDNA and plastid DNA gene trees show several hard topological incongruences at various levels of the trees, which make it very likely that the evolution of the subtribe was shaped by events of ancient and more recent reticulation, chloroplast capture and incomplete lineage sorting. The taxonomic conclusions from the phylogenetic analysis are drawn, and a revised inventory of the subtribe in SW Asia including new combinations and synonymies are provided.

Keywords Compositae · Cichorieae · cpDNA · *Lactuca* alliance · nrDNA

Introduction

The Asteraceae (sunflower family) is considered the largest flowering plant family with an estimated 25,000–35,000 species present in all continents except Antarctica (Funk et al. 2009). The family comprises some economically important species such as sunflower (*Helianthus annuus* L.), tarragon (*Artemisia dracunculus* L.), lettuce (*Lactuca sativa* L.), endive (*Cichorium endivia* L.) and chicory (*Cichorium intybus* L.). The last three species are members of the tribe Cichorieae Lam. & DC. formerly known as Lactuceae Cass. (Kilian et al. 2009a). The tribe contains more than 90 genera in 11 subtribes (Kilian et al. 2009b), has a mainly holarctic distribution and is characterized by milky latex and ligulate flowers (Kilian et al. 2009a). Whereas the tribe is recognized easily based on morphological features, delimitation of its phylogenetic lineages is often difficult because of the poverty of diagnostic morphological characters. The subtribe Lactucinae Dumort. poses particular problems in this respect (Kilian et al. 2017a). Formerly, its members were included in a large subtribe Crepidinae Dumort. (Lessing 1832; Hoffmann 1890–1894) and grouped in a single phylogenetic line (*Prenanthes-Lactuca* line) by Stebbins (1953). The Crepidinae were only separated by Bremer (1993, 1994), who divided them into four separate subtribes as Lactucinae, Crepidinae s.s., Hieraciinae Dumort. and Sonchinae K.Bremer based on morphological phylogenetics.

The first confirmation of Bremer's (1993, 1994) classification for subtribes of the Cichorieae came from Whitton et al. (1995) with phylogenetic analysis using chloroplast DNA restriction site variation, followed by analyses based on the nuclear ribosomal DNA (Internal Transcribed Spacer, nrDNA ITS) (Kilian et al. 2009a; Tremetsberger et al. 2012;

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Fernández-Mazuecos et al. 2016) which established the phylogenetic backbone of the tribe Cichorieae. The first comprehensive molecular phylogenetic study of the Lactucinae was performed by Wang et al. (2013) focusing on its Sino-Himalayan centre of diversity based on nrDNA ITS and five plastid DNA markers. Wei et al. (2015) conducted a molecular phylogenetic study on African Lactucinae using two chloroplast genes (*ndh*F and *trn*L-F). Kilian et al. (2017a) for the first time provided a global perspective of the Lactucinae phylogeny having continued the investigation with the same set of markers used by Wang et al. (2013), while Jones et al. (2018) investigated the migration of the subtribe onto the American continent.

The Lactucinae are widespread in Europe, Africa, Asia and North America (Kilian et al. 2009a, b). Their circumscription and infrageneric taxonomy have been uncertain and are still unsettled today. Consequently, the statements on the numbers of genera and species vary considerably in the literature, with 17 genera (ca. 270 species) according to Bremer (1994), 12 genera (ca. 179 species) according to Lack (2007), three genera (ca. 230 species) according to Kilian et al. (2009a) and nine lineages (ca. 200 species) according to Kilian et al. (2017a). The subtribe has two centres of diversity, one in the South-West Asian-East Mediterranean region, the other in China and the adjacent Himalayan region (Kilian et al. 2017a). The molecular phylogenetic studies of the Lactucinae in their Sino-Himalayan centre (Wang et al. 2013) and worldwide (Kilian et al. 2017a) have considerably improved our knowledge of the subtribe. Both studies have shown that convergent evolution of morphological features is a main cause for the long-standing disputes on the generic classification of the subtribe. They have, moreover, shown a surprising amount of hard topological incongruences (Wendel and Doyle 1998) between nuclear ribosomal and plastid DNA phylogenies, which very likely indicate a high significance of reticulation events in the evolution of the subtribe. A comparable study in its South-West Asian centre of diversity is a desideratum, the more as this region has been inferred as the area of origin of the subtribe in general (Kilian et al. 2017a) and of the wild lettuce relatives in particular (Kuang et al. 2008).

The investigation of the diversity of the Lactucinae in the East Mediterranean region and SW Asia dates back to the early years of modern botany in the eighteenth century, but the systematics of the group was shaped in the nineteenth century with the establishment of the genera *Cephalorrhynchus* Boiss. in 1844 and *Steptorhamphus* Bunge in 1852, and in particular with the first comprehensive and influential treatment by Boissier (1875), who classified its members in the four genera *Mulgedium* Cass., *Lactuca*, *Prenanthes* L. and *Cephalorrhynchus*. The two larger genera *Mulgedium* and *Lactuca* were subdivided in sections reflecting the closer relationships of the species as understood by Boissier. His generic classification received some important modification through a revised monographic treatment of *Cicerbita* Wallr., an older genus revived by Beauverd (1910) to accommodate all species with an outer pappus row of minute hairs. Later, the treatment of the "*Prenanthes-Lactuca* line", proposed by Stebbins (1953), by Kirpicznikov (1964) in the Flora URSS contributed the first comprehensive inventory and classification of the members of the subtribe in the eastern part of SW Asia and Middle Asia and was followed by revised comprehensive treatments for the Iranian Highlands (Tuisl 1968, 1977) and Turkey (Jeffrey 1975). A thorough treatment of the subtribe for the area between the Iranian Highlands and the Sino-Himalayan region followed much later only (Bano and Qaiser 2009, 2010, 2011).

The current paper addresses the phylogeny and systematics of the Lactucinae in their South-West Asian centre of diversity using the same set of DNA markers as Wang et al. (2013) and Kilian et al. (2017a). The aims of this study are (1) to test the hypothesis for the phylogenetic backbone of the Lactucinae by Kilian et al. (2017a) on the basis of a dense and comprehensive sampling in their inferred area of origin; (2) to gain deeper insights into the evolution and diversification of the lineages with a centre of diversity in SW Asia, including potential reticulation events; and (3) to revise the systematics of the subtribe in SW Asia with a special focus on the flora of Turkey.

Materials and methods

Plant material and sampling

An initial checklist for the members of the Lactucinae distributed in SW Asia (as defined below) was compiled from Kilian et al. (2009b), including 62 taxa that formed the basis for our sampling. The core of the samples was brought together by extensive field studies and collecting activities of the Turkish authors during the years 2013–2018. They are preserved at the herbarium of Karadeniz Technical University Department of Biology (KTUB), with some duplicates in the herbarium of the Botanic Garden and Botanical Museum Berlin (B). These collections were supplemented by material collected from voucher specimens preserved in herbaria of KTUB and B, the Botanische Staatssammlung München (M), the Natural History Museum Vienna (W), the Royal Botanic Garden Edinburgh (E), the Institute of Botany Ilia State University (TBI) and the University of Tabriz (HCAT). Only four rare SW Asian Lactucinae members (*Lactuca anatolica* Behçet & Yapar, *Lactuca azerbaijanica* Rech.f., *Cicerbita polyclada* (Boiss.) Beauverd and *Scariola amaurophyton* Podlech & Rech.f.) could not be sampled.

Whenever possible, at least three individuals from different populations were sampled for each taxon to account for

intraspecific DNA sequence variation. The sample numbers were increased to ten for some extensively variable taxa such as *Lactuca viminea* (L.) J. Presl & C. Presl and *L. serriola* L. Moreover, the sampling included (A) representative taxa of all major clades of the Lactucinae according to Kilian et al. (2017a), to represent the entire phylogenetic backbone of the subtribe. (B) Since several species present in the area are currently treated as members of *Prenanthes* and may not be members of the subtribe, the outgroup sampling was designed to include representatives of the related subtribes according to Kilian et al. (2009a), Tremetsberger et al. (2012) and Kilian et al. (2017a) to infer the systematic position of such taxa. The list of samples is provided in Online Resource 1.

Delimitation of the study area

The geographical boundaries of the SW Asian area of sampling mainly followed Zohary (1973), Rechinger (1990) and Akhani (2007), including Turkey, Transcaucasia (Armenia, Azerbaijan, Georgia and Abkhazia-east of Russia), Cyprus, Palestine/Israel, Lebanon, Syria, Jordan, the Arabian Peninsula, Iraq, Iran, Afghanistan and SW Pakistan. Although Caucasia is evaluated as a separate hotspot region of its own (Schatz et al. 2009), Transcaucasia is included into the study area because its Lactucinae inventory is very similar and closely related to that of SW Asia. For the pragmatic reason to cover the entire territory of Turkey, the Thrace region (Turkish European part) was also included in the study area (Fig. 1).

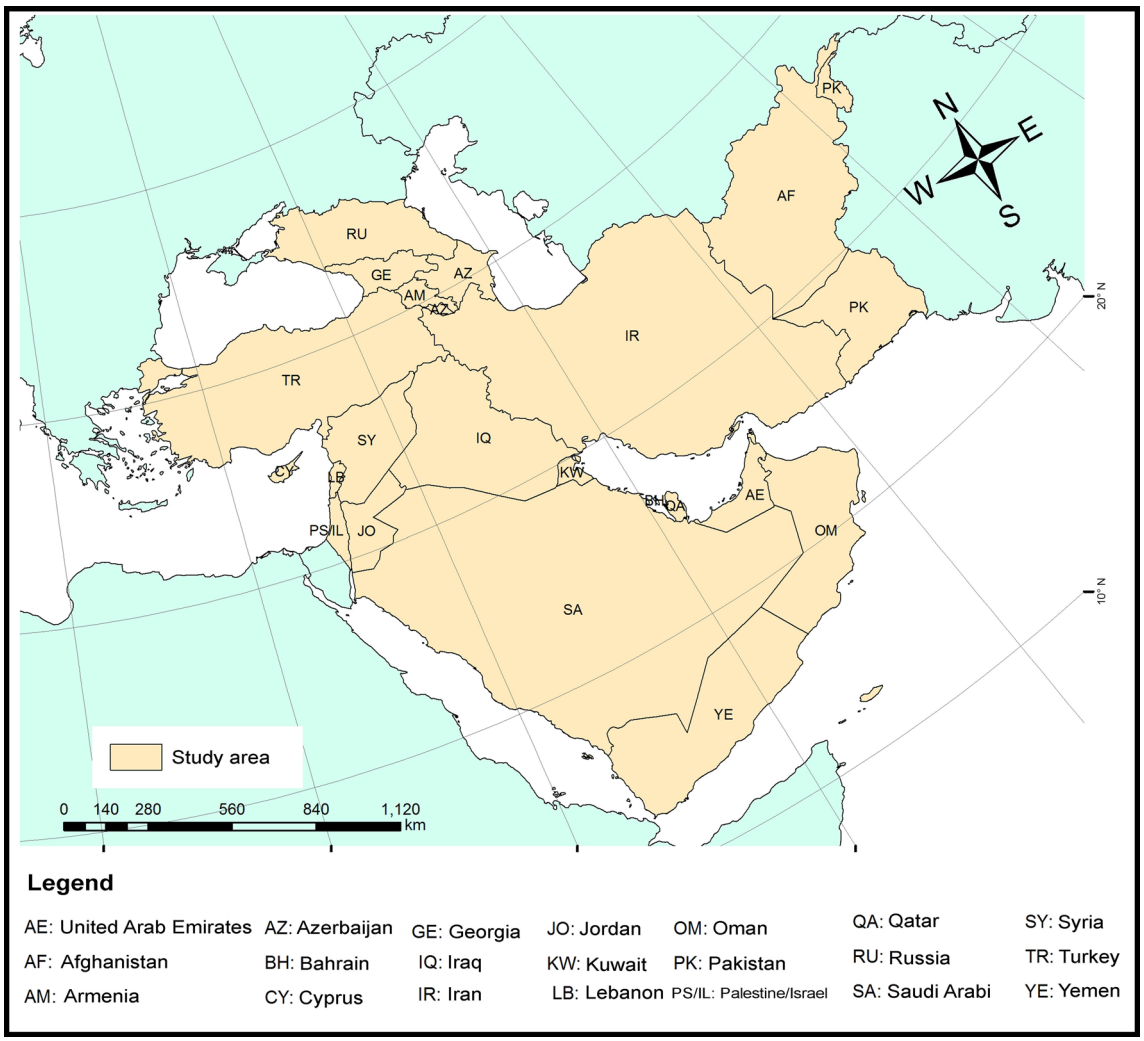

Fig. 1 Circumscription of the study area mapped with ArcGIS 10.2.2 (ESRI 2014)

DNA isolation, amplification and sequencing

Total genomic DNAs were extracted from herbarium material or c. 20 mg of silica-dried leaf sample following the modified CTAB extraction procedure of Doyle and Doyle (1987) or Plant Kit Rev. 03 (Macherey–Nagel GmbH & Co. KG, Germany) following the manufacturer's protocols. Amplification of the studied markers followed the protocols described by Wang et al. (2013).

Sequences of one nuclear (nrDNA ITS) and five plastid genome regions (*pet*D region, *psb*A-*trn*H spacer, 5′*trn*L*(UAA)*-*trn*F spacer, *rpl*32-*trn*L*(UAG)* and *trn*Q*(UUG)*-5′*rps*16 spacer) were used as phylogenetic markers. The entire nrITS region (ITS1, 5.8S rDNA, ITS2) was amplified using either the primer pairs ITS4/ITS5 (White et al. 1990) or ITSA/ITSB (Blattner 1999). The chloroplast markers were amplified using the following primers: (1) the *pet*D region (*pet*B-*pet*D spacer plus *pet*D intron) was co-amplified with the universal primers PIpetB1411F/PIpetD738R (Löhne and Borsch 2005); (2) the *psb*A-*trn*H spacer with the universal primers psbAF/trnHR (Sang et al. 1997); (3) the 5′*trn*L$^{(UAA)}$-*trn*F spacer with the universal primers trnC/trnF (Taberlet et al. 1991); (4) the *rpl*32-*trn*L$^{(UAG)}$ spacer with the primers rpl32-F/trnL$^{(UAG)}$ (Shaw et al. 2007) and (5) the 5′*rps*16-*trn*Q$^{(UUG)}$ spacer with the primers rps16 × 1/trnQ$^{(UUG)}$ (Shaw et al. 2007). PCR products were sequenced through Macrogen Inc. (Seoul, Korea) by use of the same primers for amplification. The list of samples and sequences included, with INSDC (International Nucleotide Sequence Database Collaboration) accession numbers, is given in Online Resource 1.

Sequence alignment and coding of length mutational events

The boundaries of the nrITS region (ITS1, 5.8S rDNA, ITS2) and the *pet*D region (*pet*D intron, 5′*pet*B-*pet*D spacer) were defined according to Goertzen et al. (2003) and Löhne and Borsch (2005), respectively. The boundaries of the other markers were taken from, and their designation corresponds to, the annotated complete chloroplast genome sequence of *Lactuca sativa* (INSDC: DQ383816) by Timme et al. (2007), following Wang et al. (2013) and Kilian et al. (2017a).

The nrITS sequences were aligned using Muscle v.3.8.31 (Edgar 2004) and edited in PhyDE v.0.9971 (Müller et al. 2010). The plastid sequences were first automatically aligned using Muscle, then edited and adjusted manually to a motif-based alignment in PhyDE following the criteria outlined by Kelchner (2000), Borsch et al. (2003) and Löhne and Borsch (2005). Hypervariable sequence portions with homology uncertainties were excluded from the final alignment, and inversions were re-inverted prior to phylogenetic reconstruction.

Two separate datasets were built for the nrDNA ITS region and the five concatenated noncoding chloroplast DNA (cpDNA) regions. Indels were coded as binary characters according to the simple indel coding (SIC) method (Simmons and Ochoterena 2000) implemented in the program SeqState v.1.40 (Müller 2005a). Five reinverted inversions in the plastid matrix were coded manually as a single binary character (0 = absent, 1 = present).

Phylogenetic reconstruction

The nuclear and plastid datasets were analysed separately because of the hard topological incongruences between their gene trees found by Wang et al. (2013) and Kilian et al. (2017a). Phylogenetic relationships were reconstructed using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). MP analyses were carried out using the Parsimony Ratchet (Nixon 1999) implemented in PRAP (Müller 2004), which was run with 200 ratchet iterations with 25% of the positions randomly upweighted (weight = 2) during each replicate and ten random addition cycles. The generated command files also including the nexus data matrix were run in PAUP* version 4.0b10 (Swofford 2003) using heuristic search with the following parameters: all characters have equal weight, gaps are treated as "missing", simple addition of sequences, TBR branching swapping, maxtrees setting to 100 and auto-increased by 100, one nonbinary starting tree arbitrarily dichotomized before branch swapping, only one tree saved. A majority rule consensus tree was calculated from the most parsimonious trees received. Jackknife (JK) support values for the nodes found by the MP analysis were calculated in PAUP* version 4.0b10 with the recommended settings (Müller 2005b) of 10,000 jackknife replicates with the TBR branch swapping algorithm, 36.788% of characters deleted and one tree held during each replicate.

For the model-based phylogenetic approaches, the matrices were partitioned according to the functional elements: the nrDNA matrix was divided into the three partitions ITS1, 5.8S and ITS2, and the plastid DNA matrix into five partitions corresponding to the five markers; to either matrix, a binary partition including the coded indels and inversion was added. The ML analyses were performed with RAxML-HPC2 (Stamatakis 2006) on the Cipres Gateway (Miller et al. 2010). The analyses were done after removal of identical sequences by RAxML. Rapid bootstrapping (with the maximum set of 1000 replicates) integrated with a thorough ML search for the optimal tree was carried out using the resource-efficient CAT approximation (Stamatakis 2006) of the general time-reversible (GTR) model of nucleotide substitution under the gamma model of rate heterogeneity as the predefined substitution model in RAxML for all DNA partitions and BINCAT for the binary partitions.

Prior to the BI analyses, the nucleotide substitution model that best fit for the dataset was determined separately for each of the three partitions of the nrITS dataset and each of the five cpDNA partitions with MrModeltest 2.3 (Nylander 2004). The optimal model revealed under the Akaike Information Criterion (AIC) for ITS1 and ITS 2 was GTR + I + G, for 5.8S SYM + G, and for the cpDNA partitions GTR + G. A binary (restriction site) model was implemented for the coded indels. BI analyses were performed in MrBayes 3.2 (Ronquist et al. 2012) with four simultaneous runs of Metropolis-coupled Markov Chains Monte Carlo (MCMCMC), each with four parallel Markov chains. Each chain was run for 10 million generations, saving one tree every 1000th generation. To ensure convergence of the run, a conservative burn-in of 0.2 (i.e. discarding the first 20% of the trees) was applied, during which the average standard deviation of the split frequencies between the runs dropped below 0.01 and after which the effective sampling size (ESS) for all parameters was well above 200 in either run. The post-burn-in trees were used to generate a majority consensus tree, of which the nodes with less than 0.5 posterior probability supports were collapsed.

TreeGraph v.2 (Stöver and Müller 2010) was used to assess the tree topologies and to visualize the trees with node supports.

Results

Molecular datasets

Our analyses included 55 of the 59 taxa we recognize for SW Asia (Online Resource 4), most of them with multiple samples. The two molecular matrices comprise a total of 1300 individual sequences, of which 716 were newly generated in this study (Online Resource 1). The newly generated sequences belong to nrITS (125), *pet*D intron (121), *psb*A-*trn*H spacer (122), *5′trn*L$^{(UAA)}$-*trn*F spacer (119), *rpl*32-*trn*L$^{(UAG)}$ (119) and *trn*Q$^{(UUG)}$-*5′rps*16 spacer (110). The nrITS matrix included a total of 222 samples, 211 in-group members and 11 outgroup members. The length of the nrITS region varied between 619 and 644 bp. The coded indels and inversions added 79 binary characters to the nrITS matrix, resulting in a total of 755 characters. The plastid DNA matrix included 225 samples, 113 in-group members and 12 outgroup members, and the length of the combined plastid sequences varied between 3862 and 4015 bp. The coded indels and inversions added 449 binary characters to the plastid DNA matrix, resulting in a total of 5567 characters.

Phylogenetic analyses

The aligned nrITS matrix has 755 characters, of which 391 were parsimony informative. The MP analysis resulted in 356 most parsimonious trees (L = 1963, CI = 0.4121, RI = 0.8698, RC = 0.3585, HI = 0.6309). The aligned concatenated plastid DNA matrix has 5296 characters, of which 901 were parsimony informative. The MP analysis resulted in 87 most parsimonious trees (L = 2897, CI = 0.6890, RI = 0.9076, RC = 0.6254, HI = 0.3110). The MP, ML and BI analyses revealed trees with almost identical topology for either matrix. Therefore, only BI trees (Figs. 2 and 3) with posterior probabilities (PP), and the MP jackknife support values (JK) and ML bootstrap values (BS) added, are presented for each datasets. The trees were rooted with *Scorzonera hispanica* L. The final aligned datasets belonging to the nrITS and the five concatenated cpDNA regions are available in Online Resource 2 and Online Resource 3, respectively.

nrDNA ITS phylogeny

The BI tree (Fig. 2) revealed a weakly supported (JK = 55, BS = 63, PP = 1) trichotomous clade 1, which includes the *Lactucinae* (clade 2) except *Prenanthes purpurea* L., which is resolved in a separate clade, and *Leontodon tuberosus* L., representing the subtribe Hypochaeridinae. Clade 1 in turn is part of a polytomy, else with a Hyoseridinae clade, a Crepidinae clade and *Prenanthes abietina* (Boiss. & Balansa) Kirp., forming a clade of its own. Clade 2, with the Lactucinae except *P. purpurea*, revealed strong support (JK = 95, BS = 91, PP = 1) but itself is unresolved. It consists of three E and Central Asian clades outside the scope of this study and is therefore represented by each a single member only and of three clades, which include all SW Asian Lactucinae members apart from the monotypic Cypriot endemic *Astartoseris* N.Kilian, Hand, Hadjik., Christodoulou & Bou Dagh.-Kharr., which forms a clade of its own. Clade C with strong support (JK = 72, BS = 83, PP = 1) comprises the *Cicerbita* lineage members as defined by Kilian et al. (2017a) plus *Cephalorrhynchus rechingerianus* Tuisl currently listed under the *Lactuca* lineage (Kilian et al. 2009b). Clade M with strong support (JK = 91, BS = 95, PP = 1) comprises the *Melanoseris* lineage members as defined by Kilian et al. (2017a) plus *Lactuca haimanniana* E.A.Durand & Barratte, currently placed in the *Lactuca* lineage (Kilian et al. 2009b). Clade L with good support values (JK = 73, BS = 81, PP = 1) comprises the *Lactuca* lineage members as defined by Kilian et al. (2017a). Nested in this clade are *Lactuca adenophora* Boiss. & Kotschy, *L. boissieri* Rouy, *L. fenzlii* N.Kilian & Greuter, *L. mulgedioides* (Vis. & Pančić) Boiss. & Kotschy, *L. quercina* subsp. *wilhemsiana* (DC.) Feráková, *L. scarioloides* Boiss., *L. leucoclada* Rech.f. & Tuisl and *Lactuca*

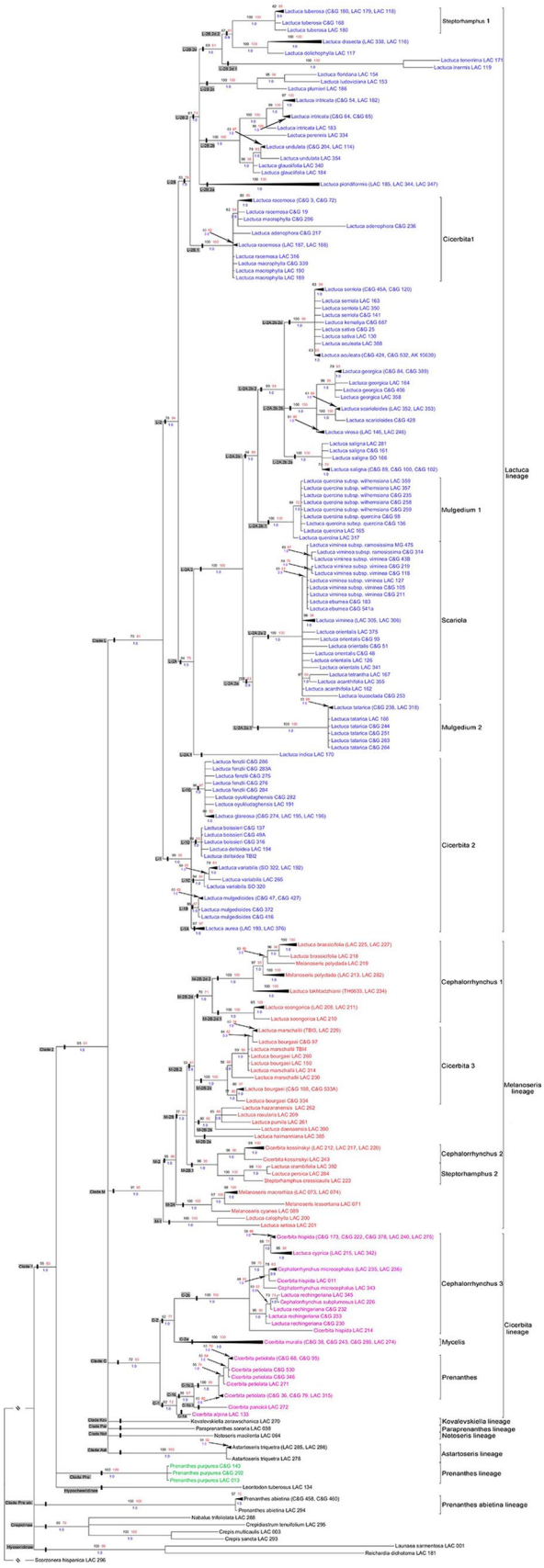

Fig. 2 Majority consensus phylogram of the Lactucinae from the BI ▶ analysis based on the nrITS dataset (support values: first-line MP jackknife, and ML bootstrap, second-line BI posterior probability). Branch colours designate the phylogenetic lineages (blue *Lactuca* lineage, red *Melanoseris* lineage, purple *Cicerbita* lineage, green *Prenanthes purpurea* lineage). Kilian et al. (2009b) is adopted for the taxa names, and the most general conventional genera names are shown

viminea subsp. *ramosissima* (All.) Arcang., taxa not so far included in any molecular phylogenetic analysis but already placed in the *Lactuca* linage based on their morphological features (Kilian et al. 2009b).

cpDNA phylogeny

The BI tree (Fig. 3) revealed a strongly supported (JK = 99, BS = 98, PP = 1) clade 1, which comprises a *Prenanthes purpurea* clade and a large clade with almost all other Lactucinae. Clade 1 in turn is part of a polytomy including three more clades with the representatives of the subtribes Hyoseridinae, Hypochaeridinae and Crepidinae. *Prenanthes abietina* and *Astartoseris triquetra* (Labill.) N.Kilian, Hand, Hadjik., Christodoulou & Bou Dagh.-Kharr. are resolved as members of the Crepidinae clade. Clade 2 with the core Lactucinae members in the sense of Wang et al. (2013) received full statistical support. In contrast to the nrITS tree, the core Lactucinae clade is resolved and shows the *Cicerbita* clade (clade C) and the *Notoseris–Paraprenanthes* clade as consecutive sisters to the remainder of the subtribe in clade 4. Clade C has full support and fully corresponds to Clade C in the nrITS tree (Fig. 2). The last clade (Clade 4) is a trichotomy of the Central Asian *Kovalevskiella* clade (Clade Kov), the *Lactuca rosularis* clade (M-2B.2b) of the Iranian Highlands and a large clade comprising a complex assemblage of the *Lactuca* and *Melanoseris* lineages, which were resolved as separate clades in the nrITS tree.

Incongruences between nuclear and plastid DNA phylogeny: The hard topological incongruences between nuclear and plastid DNA phylogeny found by Wang et al. (2013) and Kilian et al. (2017a) are confirmed in the present study as far as they are within its scope. A striking single further incongruence revealed concerns the little known *Lactuca haimanniana*, which is included in a molecular study for the first time. This Cyrenaican endemic is found in the *Melanoseris* clade in the nrITS tree (clade 6; JK = 58, BS = 84, PP = 0.9), but resolved in the plastid DNA tree in the *Lactuca* lineage and surprisingly as sister to *Lactuca plumieri* (L.) Gren. & Godr., a chiefly SW European species.

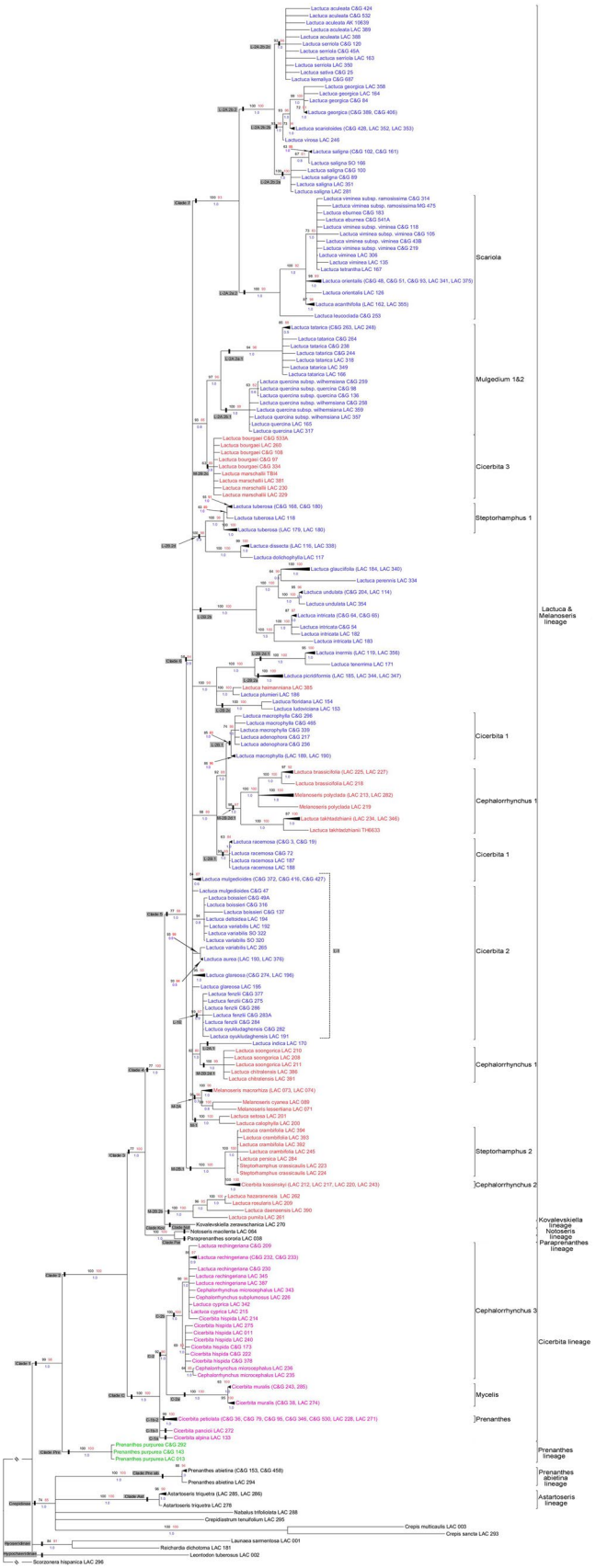

Fig. 3 Majority consensus phylogram of the Lactucinae from the BI analysis based on the plastid DNA dataset (support values: first-line MP jackknife, and ML bootstrap, second-line BI posterior probability). Designations and branch colours of the lineages as in Fig. 2

Discussion

Our phylogenetic reconstruction treated the Lactucinae in their SW Asia centre of diversity and area of origin in a comprehensive way, including 95% of all taxa, mostly represented by multiple samples. In this way, it provides an essential facet for the picture of this subtribe in addition to the previous studies. In the following, we will discuss the main findings of the nuclear and plastid phylogenies (Figs. 2 and 3) and the partly different phylogenetic histories for the subtribe they revealed.

Prenanthes clades

Prenanthes purpurea, which provides the type of the generic name *Prenanthes*, and *P. abietina* are resolved in distant clades and are corroborated as clearly being no congeners (Kilian et al. 2017b). *Prenanthes purpurea,* distributed from W Europe to the Caucasus, was shown by Kilian et al. (2017a) to form the earliest diverging, isolated lineage of the Lactucinae, which is found in ITS phylogenies often near members of the Hypochaeridinae due to long-branch attraction. *P. abietina*, a yellow-flowered endemic of the Caucasus ecoregion originally described from Rize province (NE Turkey), has gone through a taxonomic odyssey, after it was first described as *Mulgedium abietinum* Boiss. & Balansa by Boissier (1875). It was treated as *Lactuca abietina* (Boiss. & Balansa) Bornm. (Bornmüller 1904–1905), as *Crepis abietina* (Boiss. & Balansa) Beauverd (1910), as *Cicerbita abietina* (Boiss. & Balansa) Grossh. (Grossheim 1934) and finally as *Prenanthes abietina* by Kirpicznikov (1964). Three accessions of *P. abietina* form a separate clade in the basal polytomy among the representatives of the subtribes Hyoseridinae, Crepidinae and Lactucinae + Hypochaeridinae in the nrITS tree, whereas *P. abietina* is nested in the Crepidinae clade in the plastid DNA tree with good support (JK = 74, BS = 85, PP = 1). This is largely in accordance with the finding by Kilian et al. (2017a) based on a single sample of *P. abietina*, except that we find it even more remote from the Lactucinae in the nrITS tree. It seems justified to assume that *P. abietina* (near or at the base of the Crepidinae) along with *Astartoseris* (Kilian et al. 2017b) and *P. purpurea* (at the base of the Lactucinae) and *Avellara* Blanca & C.Díaz and *Urospermum* Scop. (Fernández-Mazuecos et al. 2016, at the base of the Hypochaeridinae) are early diverging orphan lineages of the Chondrillinae–Crepidinae–Hypocharidinae–Hyoseridinae–Lacrtucinae main clade of the Cichorieae (Tremetsberger et al. 2012).

Cicerbita lineage (Clade C)

This lineage, already resolved by Wang et al. (2013) and later confirmed by Kilian et al. (2017a) as the first diverging lineage of the core-Lactucinae, has its centre of diversity in SW Asia. Plastid and nuclear phylogeny revealed this lineage as a well-supported clade, the circumscription of which stands in striking contrast to the morphology-based traditional delimitations of the genus *Cicerbita*. This holds equally true for both its wide concept by Beauverd (1910) and its narrow concept by Tuisl (1968). The *Cicerbita* clade comprises three well-supported major terminal clades in the nrITS tree, one of it being collapsed in the plastid DNA tree: (1) the *Cephalorrhynchus* clade in the narrow sense of the type of the generic name *C. glandulosus* (= *C. hispidus*), the monospecific *Mycelis* clade, and the *Cicerbita* clade in the narrow sense of the type of the generic name *C. alpina*. The morphological variation shown in this early diverging clade already displays in a nutshell much of the variety of the subtribe in its entirety: many- to few-flowered (*M. muralis*) capitula, blue- to yellow-flowered corollas (*M. muralis*), terete to compress (*M. muralis*) and truncate to beaked (*M. muralis, Cephalorrhynchus* spp.) achenes, pappus white to pale brown (*C. petiolata*), with or without (*M. muralis*) an outer series of minute hairs. On the other hand, the clade includes only perennials with 2n = 18 chromosomes. The Caucasian endemic *C. petiolata* of the *Cicerbita* s.s. clade is included with multiple samples. It shows no variation in the plastid sequences but exhibits some phylogenetic structure in the nrITS tree (Fig. 2), which does not correspond to any morphological differences. The *Cephalorrhynchus* clade only includes a portion of the species placed in this genus by Tuisl (1968) and Rechinger (1977), who diagnosed it by the presence of an outer row of minute hairs combined with fusiform terete to elliptical compressed and beaked achenes. The phylogenetic structure of the *Cephalorrhynchus* clade is poorly resolved in both trees, which may be due to its young age (3.7 mya; Kilian et al. 2017a). Grouping of the samples is partly incongruent between the trees; therefore the taxonomy of the members of this clade needs attention. The finding of a close relationship of *Cephalorrhynchus subplumosus* Kovalevsk. and *C. rechingerianus* Tuisl in both trees agrees with the observation by Tuisl (1968) that they share what he, with some exaggeration, termed a "subplumose" pappus, actually having bristles with fimbriae of 1–1.5 × the length of the bristle diameter instead of usually less or rarely more than 0.5 × the bristle diameter.

Melanoseris lineage (Clade M)

This lineage was first identified by Wang et al. (2013) and further explored by Kilian et al. (2017a) based on additional dense sampling from Africa to Asia. Whereas the lineage forms a strongly support clade (JK = 91, BS = 95, PP = 1) in the nrITS tree (Fig. 2), it forms in the plastid DNA tree a series of consecutive sister groups together and partly merged with the *Lactuca* clade, while at the same time several deeper nodes remain unresolved (Fig. 3). The principal topology of the *Melanoseris* clades in both trees agrees with the findings by Wang et al. (2013), Wei et al. (2015) and Kilian et al. (2017a). The *Melanoseris* clade in the nrITS tree shows a distinct geographic structure (Kilian et al. 2017a), which is also evident in our limited sampling: a tropical African clade (M-1) is sister to a chiefly Sino-Himalayan (M-2A) and a chiefly SW Asian clade (M-2B). This last clade is composed of a polytomy of four clades and the *Steptorhamphus* clade (M2-B.1), which is sister to the former clade (M-1). These five clades are discussed in the following.

The *Steptorhamphus* clade (M2-B.1) includes *S. crambifolius* Bunge (*Lactuca crambifolia* (Bunge) Boiss.), which provides the type of the name *Steptorhamphus*, *S. persicus* (Boiss.) O.Fedtsch. & B.Fedtsch. (*Lactuca persica* Boiss.) from Iran, *S. crassicaulis* (Trautv.) Kirp. from Russia, and *Cicerbita kossinskyi* Krasch. (≡ *Cephalorrhynchus kossinskyi* (Krasch.) Kirp.). In the plastid DNA tree, the *Steptorhamphus* clade is resolved as sister to the entire *Lactuca-Melanoseris* clade except for the *Lactuca rosularis* clade (M-2B.2b), see below. *Steptorhamphus* is diagnosed by large, winged and beaked achenes, and a pappus with an outer row of minute hairs, *C. kossinskyi*, however, has slender and fusiform achenes. On the other hand, *Steptorhamphus tuberosus* (Jacq.) Grossh., which fully shares the diagnostic features of *Steptorhamphus*, is placed in the *Lactuca* subclade L-2B of the nrITS tree with *Lactuca dissecta* D.Don and *L. dolichophylla* Kitam., which have unwinged narrowly ellipsoid to obcolumnar achenes. Evidently, *Steptorhamphus* and *Cephalorrhynchus* are highly artificial taxa, the diagnostic features of which are homoplastic.

Lactuca haimanniana forms a subclade of its own in the polytomy (M-2B.2) that is sister to the *Steptorhamphus* clade in the nrITS tree. For the first time this species, which is restricted to the coastal mountains of Cyrenaica in Libya, is included in a molecular phylogenetic study. It is of special interest, being the only Lactucinae species restricted to a N African area. Durand and Barratte (1910) plausibly, according to morphology, assumed a relationship with *L. mulgedioides* (≡ *Cicerbita mulgedioides* (Vis. & Pančić) Beauverd), which is, however, resolved in a distant position as a member of the *Lactuca aurea* clade (L-1). The incongruent placement of *L. haimanniana* in the plastid DNA tree as sister to the chiefly SW European *L. plumieri*, and in a polytomy of the *Lactuca* s.s. clade with the North American clade, the W Mediterranean *L. tenerrima*, the African *L. inermis* and the SW Asian *L. picridiformis,* indicates a rather complex evolutionary history of this species, which is worth further

investigation. The enigmatic disjunct relic occurrence of *L. plumieri* in the S Bulgarian Rila Mts (Wegmüller 1994) may be of some significance in this respect.

A second clade in the *Melanoseris* polytomy M-2B.2 is the *Lactuca rosularis* clade (M-2B.2b). It comprises inconspicuous rosette herbs confined and adapted to rocky crevices and screes of the arid Iranian–Afghan highlands (Kilian et al. 2012; Doostmohammadi and Kilian 2017). The long-neglected Iranian *Lactuca daenaensis* N.Kilian & Djavadi (Kilian et al. 2012) is another species here included for the first time in a molecular phylogenetic study, and both trees confirm it as a member of the *L. rosularis* group. Remarkably, this clade was resolved by Kilian et al. (2017a) in the plastid DNA tree as sister to the entire *Lactuca-Melanoseris* clade, which in principal agrees with our finding here, except that the basal node of our Clade 4 is not resolved, likely as an effect of our more selective sampling.

The *Lactuca bourgaei* clade (M-2B.2c), which is a well-supported sister to the *L. quercina-L. tatarica* clade in the plastid DNA tree (see also Kilian et al. 2017a), only includes *L. bourgaei* (C&G 533, C&G 108, C&G 97, C&G 334 and LAC 260) and *Lactuca marschallii* Stebbins (≡ *Cicerbita prenanthoides* (M.Bieb.) Beauverd) (TBI4, LAC 381, LAC 230, LAC 229). These were recognized as two W Caucasian species by Kirpicznikov (1964), Gagnidze (1967) and (Jeffrey 1975) but considered as conspecific later by Sennikov (1997). On the other hand, *L. marschallii* is a doubtful record for Turkey (Jeffrey 1975). The subtle distinction of *L. bourgaei* from *L. marschallii* by its divided lyrate leaves, a stronger developed synflorescence, the presence of a cover of thin arachnoid hairs on the pappus disk and a pappus with a hardly visible outer row of minute hairs (Kirpicznikov 1964) was already questioned by Jeffrey (1975), who also noted that despite the statement of Kirpicznikov (1964), all Turkish material of *L. bourgaei* (including isotypes) has a glabrous (not arachnoid hairy) pappus disk, which according to our observations also holds true for the Caucasian material. Our molecular findings also do not support the recognition of two separate species. Hence, corroborating Sennikov (1997), both should be treated as conspecific.

The well-supported clade M-2B.2d in the nrITS tree comprises a group of species formerly placed in *Cephalorrhynchus* and characterized by non-compressed short-beaked and comparatively small achenes (Tuisl 1968; Kirpicznikov 1964). The members of *Cephalorrhynchus* are thus dispersed over three clades: the *Cicerbita* clade (with *C. glandulosus* (= *C. hispidus*) which provides the type of the name *Cephalorrhynchus,* see above), the *Steptorhamphus* clade (see above) and this clade. The members of this clade are distributed in the Iranian–Afghan highlands, the adjacent Caucasus region and further into central Asia. *Lactuca soongorica* Regel (≡ *Cephalorrhynchus soongoricus* (Regel) Kovalevsk.), which is sister to the remainder in this clade and distributed from Afghanistan to Kirgizstan, is resolved in the plastid DNA tree together with *Lactuca chitralensis* (Tuisl) Ghafoor, Qaiser & Roohi Bano (≡ *Cephalorrhynchus chitralensis* Tuisl) (included for the first time but only in the plastid DNA tree) as sister to the E Asian *L. indica*. The diversification of the small-fruited *Cephalorrhynchus* clade was estimated to have taken place around 5–6 mya; the branching of the *L. indica* lineage from the core *Lactuca* clade is estimated at c. 8 mya, but the diversification of the *L. indica* clade in its current members only at c. 2 mya (Kilian et al. 2017a). We therefore assume a reticulation event during the inferred eastward migration of the *L. indica* ancestor with a *L. soongorica* ancestor. *L. soongorica* is also sister to the other species of the *L. indica* clade (Kilian et al. 2017a). The rest of this *Cephalorrhynchus* clade forms, in contrast, the sister group to the *L. macrophylla* clade in the plastid DNA tree (Fig. 3).

The SW Asian *Melanoseris* clade provides a main source of incongruence between the nrITS and plastid DNA trees. It is the only major *Melanoseris* clade with members having sister group relationships with *Lactuca* members in the plastid DNA tree (see also Kilian et al. 2017a). Since both the *Melanoseris* and the *Lactuca* lineage originated in the E Mediterranean-SW Asian region (see also Kilian et al. 2017a), this provides strong evidence for various reticulation events during their early states of diversification and likely accounts to some extent for the levelling of morphological discontinuities between both lineages.

Lactuca lineage (Clade L)

Whereas monophyly of this linage is well supported (JK = 73, BS = 81, PP = 1) in the nrITS tree, the relationships of the *Kovalevskiella*, *Melanoseris* and *Lactuca* lineages to each other remained partly unresolved in the plastid DNA tree. As discussed above, moreover several *Melanoseris* clades are nested in the plastid DNA tree within the *Lactuca* clade. The backbone of the *Lactuca* lineage in both trees agrees with those reported by Wang et al. (2013), Wei et al. (2015) and Kilian et al. (2017a). Therefore, we discuss here only some new findings.

We have extensively sampled the members of the *Lactuca aurea* clade L-1, and most species are evaluated for the first time with multiple sampling in the present study. The clade is of some significance as the strongly supported (JK = 99, BS = 95, PP = 1) sister to the remainder of the *Lactuca* clade in the nrITS tree (Fig. 2, see also Kilian et al. 2017a). This early diverging clade exclusively comprises species distributed in the SW Asian-E Mediterranean region of origin of the subtribe and comprises the Turkish endemics *Lactuca fenzlii*, *L. glareosa*, *L. oyukludagensis*, *L. boissieri* and *L. variabilis*, moreover *L. deltoidea* endemic to the Caucasus, *L. mulgedioides* endemic to Turkey, Syria and Lebanon and

the Balkan endemic *L. aurea*. In contrast to Kilian et al. (2017a) where this clade is also resolved in the plastid DNA tree, it is fragmented into seven separate clades in our final plastid DNA-based analysis with extensive sampling. Apparently, the accumulated genetic variation of the final number of samples in comparison with the sister clade significantly exceeds the range compatible for resolution as a single clade, either due to incomplete sorting of the variation at the time of the clade divergence or due to later reticulation.

Jeffrey (1975) noted that *L. variabilis*, *L. mulgedioides* and *L. fenzlii* are closely related taxa. This author also stated that both *L. variabilis* and *L. mulgedioides* have a pappus with an outer row of minute hairs, but this is clearly erroneous in the case of the first species (unpublished data) and also *L. fenzlii* does not have this outer row. In addition, *L. mulgedioides* has a longer beak than *L. fenzlii* and *L. variabilis*. *L. mulgedioides* is resolved as a separate subclade in both trees (with one sample, C&G 47, in a second separate subclade in the plastid DNA tree). Also *L. variabilis* forms a distinct clade in the nrITS tree with strong supported values (JK = 94, BS = 94, PP = 1), whereas in the plastid DNA tree one accession is nested with *L. aurea* and the others are nested with *L. boissieri* and *L. deltoidea*. *L. fenzlii* finally forms a strongly supported clade with *L. glareosa* and *L. oyukludaghensis* in the nrITS tree, but without *L. glareosa* in the plastid DNA tree. *L. oyukludaghensis* was described from rock and scree communities in Karaman by Parolly (1995) under *Prenanthes*. We sampled *L. oyukludaghensis* from the locus classicus and adjacent areas and examined them morphologically, including the type specimen stored at B, in comparison with material from *L. fenzlii*. We did not find any morphological difference except in plant height and habitat. Apparently, *L. fenzlii* has been used as the name for plants growing in forest communities and have a height from 30 to 100 cm (Jeffrey 1975), whereas the name *L. oyukludaghensis* was applied to the high montane plants of rock and scree communities with a height from 17 to 46 cm (Parolly 1995). Plants of both habitats actually form a morphological continuum in every respect. The phylogenetic results fully support the morphological findings: seven accessions of *L. fenzlii* and *L. oyukludaghensis* form a strongly supported polytomy in both trees. Therefore, we consider *Lactuca oyukludaghensis* as a synonym of *L. fenzlii*. Moreover, our results support *L. variabilis*, *L. mulgedioides* and *L. fenzlii* as distinct species.

Lactuca boissieri and *L. deltoidea,* which have some morphological affinities, form another polytomy in the ITS tree, and a polytomy together with *L. variabilis* in the plastid DNA tree. Further studies are needed to elucidate their taxonomy.

The core *Lactuca* clade (L-2A.2) of the nrITS tree comprises members of the former genera *Mulgedium* and *Scariola*, the economically important *L. sativa* and its primary, secondary and tertiary gene pool (Koopman et al. 1998). In contrast to the nrITS tree, the *Lactuca tatarica* (≡ *Mulgedium tataricum*) clade (L-2A.2a.1) and the *Lactuca quercina* (L-2A.2b.1) clade are clustered with *L. bourgaei* of the *Melanoseris* lineage. Similar topological incongruences were reported by Kilian et al. (2017a). *Mulgedium salicifolium* K. Koch (≡ *Lactuca kochiana* Beauverd) described from Oltu/Erzurum/Turkey (Koch 1850) and later treated as a synonym of *M. tataricum* by Jeffrey (1975) was recently listed as an accepted species of *Lactuca* in the Vascular Plant Checklist of Turkey by Ekim (2012). We examined the type specimen stored at B and also several samples collected from the locus classicus and adjacent areas by the present authors and corroborated it as conspecific with *L. tatarica*.

Both phylogenies revealed with full support the monophyly of the former genus *Scariola* (L-2A.2a.2) deeply nested in the *Lactuca* lineage. *Lactuca leucoclada,* only recently recorded from Turkey (Güzel et al. 2018) and for the first time included in a phylogenetic study, is resolved in the plastid DNA phylogeny as sister to the remainder of the *Scariola* group. This remainder forms a polytomy and comprises *L. acanthifolia*, *L. orientalis* and the *L. viminea* complex with *L viminea* (≡ *Scariola viminea*), its subspecies and *L. tetrantha* in a separate polytomous subclade. Further studies are needed to clarify their internal relationships.

Lactuca acanthifolia (Willd.) Boiss. is another member of the *Scariola* group and was questionably reported for the flora of Turkey based on specimens collected from Marmaris by P.H. Davis (D. 41122 photo! stored at E) and Carlström (1987). According to the type specimens at K and LD (photographs seen) and several specimens from the locus classicus (Rhodos) stored at B, *L. acanthifolia* is characterized by vivid-yellow flowers and pale-brown achenes, but the Turkish specimen (D. 41122) referred to *L. acanthifolia* is sterile, only contains basal leaves and was anonymously identified as *L. eburnea* Rech. f. and later revised as *Scariola acantifolia* (Willd.) Soják by Jeffrey. We collected between 2014 and 2017 several specimens from Marmaris and Datça, and most of them as well as most of the further Turkish specimens examined clearly represent *L. viminea,* whereas the remaining specimens without flowers and achenes were identified as *L. eburnea;* no material referable to *L. acanthifolia* was found. Although the specimens collected at the beginning of the vegetation period are healthy with dense decurrent cauline foliage, they did not bloom and therefore did not produce any achenes and stayed sterile during the year. Achenes were absent also from the type collection of *L. eburnea* as is stated in the protologue. *L. eburnea* is listed among the heterotypic synonym of *L. acanthifolia* (Kilian et al. 2009b), but the Turkish material of *L. eburnea* included in our phylogenetic analysis is nested in both trees in the polytomous subclade that includes all but two *L. viminea*, accessions, whereas our samples of *L. acanthifolia* from Rhodes (Greece) are nested

in a polytomy with *L. orientalis, L. tetrantha* and *L. leuco-clada*. We conclude that *L. eburnea* is not conspecific with *L. acanthifolia* and the latter species not present in Turkey.

All *L. quercina* accessions form a separate subclade (L-2A.2b.1) in both trees with strong to full support. Neither dataset provides any resolution at subspecies rank for *L. quercina*. Whereas *L. quercina* is sister to the *L. sativa* clade (L-2A.2b.2) in the nrITS tree, it is clustered with *L. tatarica* and *L. bourgaei* in the plastid DNA tree.

The *L. sativa* clade (L-2A.2b.2) is a trichotomy with strong to full support in both trees and comprises the primary and secondary gene pool of *L. sativa*. One of its subclades comprises only *L. saligna.* A second one includes *L. scarioloides,* which is distributed from Turkey to Afghanistan, and for the first time subject of a molecular phylogenetic study. It is nested in this clade together with *L. georgica* and *L. virosa* in both trees.

The *L. sativa* subclade (L-2A.2b.2c) includes besides *L. sativa* the primary gene pool members *L. serriola* and *L. aculeata.* Considering that E Turkey and the adjacent areas have been corroborated as the centre of diversity and putative region of origin of the primary lettuce gene pool (Kuang et al. 2008), we have extensively sampled this clade. Morphologically, *L. aculeata* can be distinguished by the glandular hairs on pedicels and the lax paniculiform synflorescence from *L. serriola* and *L. sativa*, and also the last two species can be morphologically distinguished, but their accessions are not resolved in either tree. *L. kemaliya* Yıld. described from NE Turkey by Yıldırımlı (2010) and synonymized with *L. serriola* by Kandemir et al. (2015) because it cannot be separated morphologically, was sampled from the locus classicus (C&G 687 in the trees) and is also found nested in this clade.

Lactuca racemosa, L. macrophylla and L. *adenophora* (the last species for the first time included in a molecular phylogenetic study) show clear morphological affinity to each other and are nested together in clade L-2B.1 of the nrITS tree with full support. In the plastid DNA tree, however, the small-fruited *Cephalorrhyncus* clade of the *Melanoseris* lineage is sister to a *L. macrophylla-L. adenophora* clade, which in turn is sister to a *L. racemosa* clade. *L. racemosa* and *L. macrophylla* have a chiefly Caucasian distribution (Kirpicznikov 1964), and their distribution partly overlaps. *L. adenophora,* in contrast, is distributed in SE Turkey and Iraq without contact to either of the other species. Güzel et al. (2018) reported sterile individuals belonging to *L. macrophylla* in Şavşat/Artvin/NE Turkey, where also *L. racemosa* occurs. The different gene tree topologies of *L. racemosa, L. macrophylla* and *L. adenophora* hint to ancient reticulation, chloroplast capture or introgressive hybridization between these species.

Lactuca picridiformis Boiss. (≡ *Cephalorrhynchus picridiformis* (Boiss.) Tuisl) was resolved by Kilian et al. (2017a), based on a single sample from Afghanistan without close relatives. We included three samples, which were resolved in a separate clade labelled as L-2B.2a in the nrITS tree, and as sister to the *L. inermis* clade in the plastid DNA tree they appear, confirming the findings by Kilian et al. (2017a).

The *L. perennis* clade L-2B.2b is formed in both trees in accordance with characteristic achene morphology (a distinct rib on each face and filiform beak) and comprises *Lactuca perennis*, *L. glauciifolia* Boiss., *L. intricata* Boiss. and *L. undulata* Ledeb. which are chiefly distributed in the E Mediterranean and SW Asia, which is in agreement with Kilian et al. (2017a). The accessions of *L. intricata* from Greece (LAC 183) and four accessions from the Mediterranean region of Turkey show a well-supported corresponding geographic structure, but we failed to find morphological discontinuities between them.

Taxonomic conclusions

This study deepens our insights into the phylogeny of the Lactucinae in its area of origin and provides the basis for a taxonomic revision of the 59 taxa currently recognized in SW Asia. The phylogenetic backbone of the subtribe revealed by Kilian et al. (2017a) on the global scale is perfectly confirmed with the denser sampling of our study for the SW Asian area of origin of the Lactucinae. This also holds fully true for the massive gene tree incongruence at different levels of the phylogenetic trees found by Kilian et al. (2017a), which hinders a straightforward transformation of the phylogenetic reconstruction of the subtribe into a taxonomic treatment. With respect to the subtribe in SW Asia, this difficulty is largely restricted to the taxonomic treatment of the *Melanosoeris* lineage. Likely as a consequence of the assumed various reticulation events, possibly coupled with incomplete lineage sorting between the *Lactuca* and the *Melanoseris* lineages, their morphological distinction seems, for the time being, not sufficiently expressed to justify separation at generic rank. This problem is, however, to be addressed on a global scale. For the taxonomic revision of the subtribe in SW Asia, we have decided to take a conservative approach and treat the *Melanoseris* lineage members as an only informal group under *Lactuca*. Necessary new combinations are listed below, and a revised checklist with the accepted names and the most important synonyms for SW Asian Lactucinae is provided as Online Resource 4. Further information on the taxa and full synonymies are available through the Cichorieae Systematics Portal (Kilian et al. 2009b).

1. *Cicerbita rechingeriana* (Tuisl) Coskunç., M.Güzel, & N.Kilian, **comb. nov.** ≡ *Cephalorrhynchus rechingerianus* Tuisl, Ann. Naturhist. Mus. Wien 72: 614. 1968.—Holotype: "Iraq: Sulaimanya: Montes Avroman ad conf. Pers., in ditione pagi Tawilla", *K.H. Rechinger 10386a* (W barcode W19700008713 [web!]).

2. *Cicerbita subplumosa* (Kovalevsk.) M.Güzel, Coşkunç., & N.Kilian, **comb. nov.** ≡ *Cephalorrhynchus subplumosus* Kovalevsk., Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. SSR 15: 53. 1959.—Holotype: [Uzbekistan] "Dolina r. Chatkal. Bass. r. Ak-Bulak Nyzhe sliyaniya r. Ak-Bulak i Serkalisaya. Turay", 17 Jun 1957, *Butkov 755* (TASH [n.v.])

3. *Cicerbita cyprica* (Rech. f.) M.Güzel, Coşkunç., & N.Kilian, **comb. nov.** ≡ *Cephalorrhynchus cypricus* Rech. f., Ark. Bot., ser. 2, 1: 435. 1951 [– *Cicerbita cyprica* Beauverd in Bull. Soc. Bot. Genève 26: 156. 1936, nom. inval. (Art. 36.1)].—Syntypes: "Insula Cyprus: In silvis montis Troodos", 18 Jun 1880, *Sintenis* and *Rigo 798* (W [n.v.]); "in summis Troodi latere boreali, 6000′", 20 May 1862, T. Kotschy 785 (W [n.v.]).

4. *Cicerbita microcephala* (DC.) M.Güzel, Coşkunç., & N.Kilian, **comb. nov.** ≡ *Lactuca microcephala* DC., Prodr. 7: 134. 1838.—Holotype: [Iran] *Aucher-Eloy 3517* (G-DC barcode G00498775 [web!]).

5. *Lactuca amaurophyton* (Podlech & Rech. f.) N.Kilian, **comb. nov.** ≡ *Scariola amaurophyton* Podlech & Rech. f., Rechinger, Fl. Iran. 122: 207. 1977.—Holotype: "Afghanistan: C: Ghorat: In parte summa vallis Lal prope Dahane Bum ad viam Panjao—Lal", 2950 m, 31 Jul 1970, *Podlech 19085* (M barcode M0030837 [web!]; isotypes: MSB barcode MSB003297 [web!], W barcode W19840010219 [web!]).

6. *Lactuca kossinskyi* (Krasch.) Coskunç., M.Güzel, & N.Kilian, **comb. nov.** ≡ *Cicerbita kossinskyi* Krasch., Izv. Glavn. Bot. Sada SSSR 26(2): 115. 1927.—Lectotype (designated by Kirpicznikov 1964: 348): "Turcomania (reg. Transcaspia), Ashkhabad District, Kopet dagh, Firjuza", 3 May 1912, *V.I. Lipsky [Ekspeditsiya v Zakaspiikuyu Oblast' 1912] 1516* (LE [n.v.]).

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Compliance with ethical standards

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

Availability of data and material The newly generated sequences will be submitted to NCBI.

Information on Electronic Supplementary Material

Online Resource 1. Taxon sampling, voucher data and accession numbers of taxa used in this study.
Online Resource 2. Final alignment of the nrITS dataset.
Online Resource 3. Final alignment of the cpDNA dataset.
Online Resource 4. Revised checklist for SW Asian Lactucinae.

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