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RESEARCH ARTICLE

Phylogenetic relationships within *Lactuca* L. (Asteraceae), including African species, based on chloroplast DNA sequence comparisons

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Abstract Lettuce (*Lactuca sativa* L.) belongs to the genus *Lactuca* L. and is an important vegetable worldwide. Over the past decades, there have been many controversies about the phylogeny of *Lactuca* species due to their complex and diverse morphological characters and insufficient molecular sampling. In this study we provide the most extensive molecular phylogenetic reconstruction of *Lactuca*, including African wild species, using two chloroplast genes (*ndhF* and *trnL-F*). The sampling covers nearly 40 % of the total endemic African *Lactuca* species and 34 % of the total *Lactuca* species. DNA sequences from all the subfamilies of Asteraceae in Genebank and those generated from *Lactuca* herbarium samples were used to establish the affiliation of *Lactuca* within Asteraceae. Based on the subfamily tree, we selected 33 *ndhF* sequences from 30 species and 79 *trnL-F* sequences from 48 species to infer relationships within the genus *Lactuca* using randomized axelerated maximum likelihood and Bayesian inference analyses. Biogeographical, chromosomal and morphological character states were reconstructed over the Bayesian tree topology. We conclude that *Lactuca* contains two distinct phylogenetic clades—the crop clade and the *Pterocypsela* clade. Other North American, Asian and widespread species either form smaller clades or mix with the *Melanoseris* species. The newly sampled African endemic species probably should be treated as a new genus.

Keywords African *Lactuca* · *Lactuca* phylogeny · Lettuce · *ndhF* · Phylogenetic relationships · *trnL-F*

Introduction

Domesticated lettuce (*Lactuca sativa* L.) is a member of the genus *Lactuca* L., which is grouped in the subtribe Lactucinae, tribe Cichorieae (Lactuceae), subfamily Cichorioideae of the family Asteraceae (Compositae; Judd et al. 2007; Kadereit et al. 2007). As one of the most important vegetables, lettuce is commercially produced worldwide, especially in Asia, North and Central America, and Europe (Lebeda et al. 2007). There are a large number of lettuce cultivars within *L. sativa*. These cultivars can be divided in seven distinct cultivar groups: Butterhead Group, Crisphead Group, Cos Group, Cutting Group, Stalk Group, Latin Group and Oilseed Group (de Vries 1997). Many studies have focused on domesticated

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lettuce (Hartman et al. 2012; Kerbiriou et al. 2013; Uwimana et al. 2012; Zhang et al. 2009a, b). However, there are still uncertainties about the phylogenetic relationships within *Lactuca*, mainly due to the complex and variable morphological characters of the species in the genus. Some of the controversies stem from the different circumscriptions proposed for the genus, which vary from extremely broad to very narrow concepts. Bentham (1873) included *Lactuca* species not only from the present subtribe Lactucinae, but also from the present subtribes Crepidinae and Hyoseridinae; this broad concept was maintained by Hoffmann (1890–1894). Stebbins (1937a, b, 1939), Feráková and Májovský (1977) and Lebeda et al. (2004, 2007) used a moderately wide concept of *Lactuca* that comprised a total of approximately 100 species. Tuisl (1968), Shih (1988a, b), and Kadereit et al. (2007) established a narrow circumscription. In this concept, Shih and Kilian (2011) consider there to be between 50 and 70 *Lactuca* species. However, all these authors mentioned before only dealt mostly with regional *Lactuca* species and the genus has never been revised in its entirety.

Lebeda et al. (2004) provided an overview of the biogeographical distribution of wild *Lactuca* species based on the available literature data and showed that Asia (containing 51 species) and Africa (containing 43 species) are the two centres of diversity for *Lactuca* species. Lebeda et al. (2004, 2009) elaborated a classification of *Lactuca* from taxonomic and biogeographical criteria and divided the genus into seven sections (*Lactuca* (subsection *Lactuca* and *Cyanicae* DC.), *Phaenixopus* (Cass.) Bentham, *Mulgedium* (Cass.) C.B. Clarke, *Lactucopsis* (Schultz Bip. ex Vis. et Pančić) Rouy, *Tuberosae* Boiss., *Micranthae* Boiss., *Sororiae* Franchet) and two geographical groups (African and North American). Recently, Wang et al. (2013) constructed a DNA-based phylogenetic tree of the *Lactuca* alliance with a focus on the Chinese centre of diversity. This study fills the gap in our understanding of Asian diversity centre of *Lactuca* species and related genera, especially for the Chinese species. However, a study of the African diversity centre of *Lactuca* species is still lacking.

Despite the lack of studies focused on the entire *Lactuca* genus, there have been a number of studies focused on cultivated lettuce and closely-related wild species. These studies concentrated on aspects of interest for lettuce breeding to improve growth related to abiotic and biotic stresses using genetic resources from wild lettuce species (Hartman et al. 2012, 2014; Jeuken et al. 2008; van Treuren et al. 2011). Zohary (1991) established a concept of the 'lettuce gene pool' and Koopman et al. (1998, 2001) modified Zohary's lettuce gene pool concept and provided the first molecular phylogenetic relationships among *Lactuca* species based on nrDNA ITS-1 and AFLPs. Koopman et al. (1998) described *L. sativa*, *L. serriola* L., *L. dregeana* DC., *L. aculeata* Boiss. and *L. altaica* Fischer et C.A. Meyer as the primary gene pool, *L. virosa* L. and *L. saligna* L. as the secondary gene pool, and *L. quercina* L., *L. viminea*, *L. sibirica* Benth. ex Maxim. and *L. tatarica* (L.) C.A. Meyer as the tertiary gene pool. Apart from Koopman et al. (2001) and Wang et al. (2013), there is limited information about the molecular phylogenetic relationships within the genus *Lactuca*, especially for the African species since they were first described (Jeffrey 1966; Stebbins 1937b).

More than 4000 years ago, the Egyptians started to cultivate wild lettuce (*L. serriola*) in Africa and this species is thought to be the ancestor of modern lettuce cultivars (Harlan 1986). Lindqvist (1960) doubted that only *L. serriola* was involved in the domestication of the cultivated lettuce, but he did not specify what species might have played a role. Kesseli et al. (1991) suggested a polyphyletic origin of *L. sativa* using RFLP loci. Mikel (2007) reported that apart from *L. serriola*, the current crisphead cultivar 'Salinas' was also derived from *L. virosa* for its robust root system and decreased leaf drop. Wei et al. (2014), using a recombinant inbred line population derived from *L. sativa* 'Salinas' (crop) and *L. serriola* (wild), found that alleles from the cultivated lettuce contribute more to lateral root development than those from wild lettuce.

The aim of this present study is to provide a DNA based phylogenetic tree of *Lactuca*, and 34 % of known *Lactuca* species and 40 % of the total endemic African *Lactuca* species were included in the taxon sampling. We reconstruct ancestral states for geographic areas, chromosome number and selected morphological characters over the phylogenetic trees. Novel potential genetic resources for lettuce breeding are proposed as well.

Materials and methods

Taxon sampling

Twenty-seven *Lactuca* species, including thirteen African endemic species, and four species from *Lactuca*-allied genera were sampled (Table 1). For the species *L. viminea* two samples representing two subspecies were included. Following the treatment of Lebeda et al. (2004), this sampling represents 34 % of the total *Lactuca* species and 40 % of the total endemic African species. The 32 samples come from fresh leaf, sillica-dried leaf and herbarium specimens (Table 1). Four of the fresh-collected materials were from Centre for Genetic Resources, the Netherlands (CGN, http://www.wageningenur.nl/en/Expertise-Services/Statutory-research-tasks/Centre-for-Genetic-Resources-the-Netherlands-1.htm). Herbarium materials were provided by the National Herbarium of the Netherlands (WAG) and the Botanic Garden and Botanical Museum Berlin-Dahlem (B), herbarium codes following Thiers (2011). All necessary permissions for the described plants and specimen samplings were obtained from the respective curators, dr. ir. J.J. Wieringa (Naturalis Biodiversity Center, Leiden) and dr. Norbert Kilian (Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin, Berlin).

DNA extraction and purification

DNA was extracted from 10 to 30 mg of plant material using the cetyltrimethyl-ammonium-bromide (CTAB) method (Doyle and Doyle 1987), modified for herbarium specimens as in Särkinen et al. (2012) and Staats et al. (2011). The DNA extraction was then purified by Wizard DNA clean-up system (Promega Corp.) with a vacuum manifold (Promega Corp.) The quality of the DNA extractions was visualized on 1 % agarose gel and measured by Qubit 2.0 Fluorometer (Invitrogen). Polymerase chain reaction and Sanger sequencing were also performed for some of the herbarium samples to check for potential degradation of DNA. PCR amplifications were performed in 10 µl reactions using MyTaq^{TM} DNA polymerase (Bioline, London, UK). Thermal cycling for PCR included 2 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 50 °C, 1 min at 70 °C, and ended by 5 min at 72 °C. The forward and reverse primer sequences of *trnL-F* were 5′-GCAATCCTGAGCCAAATCC-3′ and 5′-GCTCGATGCATCATCCCGCTAAA-3′, respectively. Two pairs of primers (*ndhF* 5′ forward-1074 reverse and 913 forward-*ndhF* 3′ reverse) were used for the amplification of *ndhF* due to the large size of the gene (Karis et al. 2001). PCR products were then purified and sequenced as described in Schneider et al. (2014).

Next generation sequencing and de novo assembly

The dataset of plastid gene sequences presented in this work was generated as part of the SYNTHESYS Joint Research Activities 4 (JRA4: Plants/fungi herbarium DNA: http://www.synthesys.info/joint-research-activities/synthesys-2-jras/jra4-plantsfungi-optimised-dna-extraction-techniques/). The *Lactuca* samples were sequenced by National High-Throughput DNA Sequencing Centre of University of Copenhagen, using the next generation sequencing Illumina HiSeq 2000 platform (http://seqcenter.ku.dk/facilities/). The protocols for DNA library preparation and PCR amplification was described in Bakker et al. (2015). Contig assembly and read clean-up were performed using standard method similar to the 'MitoBIM' approach outlined in Hahn et al. (2013) for mitochondrial genomes. This method is called the *Iterative Organelle Genome Assembly* pipeline (IOGA), aiming to assemble paired-end reads into a series of candidate assemblies and selecting the best one based on likelihood estimation (Bakker et al. 2015). The IOGA pipeline can be briefly described in the following steps: (1) Trimmomatic was used to trim low quality, adapter and other Illumina-specific sequences from individual reads (Bolger et al. 2014); (2) chloroplast genome-derived reads were filtered out of the entire read pool in Bowtie 2, by aligning the latter to a range of reference Angiosperm chloroplast genome sequences (Langmead and Salzberg 2012); (3) de novo assemblies from the trimmed, filtered and corrected chloroplast reads, were performed in SOAPdenovo2, using k-mer values ranging from 37 to 97 (Luo et al. 2012); (4) 'best assemblies' were selected using the N50 criterion and then used as a new reference to find target-specific reads not selected in the first iteration; (5) step 4 was repeated until no more chloroplast genome-derived reads were found, followed by assembly of the final set of assemblies with SPAdes3.0 (Bankevich et al. 2012), under a range of different k-mer settings; (6) finally, Assembly Likelihood Estimation (Clark et al. 2013) was

Table 1 Taxon sampling information (including herbarium specimen, silica-dried and fresh materials)

No.	Taxon name	Collection number	Deposited in[a]	Collected year	Sample type[b]	Country of origin	Note[c]
1	*Lactuca aculeata* Boiss.	Koopman, W.J.M.; CGN15692	WAG	1995	F	Turkey	
2	*L. altaica* Fischer et C.A. Meyer (*L. serriola*)	Koopman, W.J.M.; CGN15711	WAG	1995	F	Georgia	
3	*L. attenuata* Stebbins	Lewalle, J.; 5982	WAG	1971	H	Burundi	*
4	*L. calophylla* C. Jeffrey	Pawek, J.; 12254	WAG	1977	H	Malawi	*
5	*L. formosana* Maximowicz	Zhu, S.X.; 2011-1576	HEAC	2011	S	China	3
6	*L. glandulifera* Hook.f.	Breteler, F.J.; 111	WAG	1962	H	Cameroon	*
7	*L. imbricata* Hiern	Witte, G.F. de; 7284	WAG	1949	H	Congo	2*
8	*L. indica* L.	Zhu, S.X.; 2010-1191	HEAC	2010	S	China	3
9	*L. inermis* Forssk.	Jongkind, C.C.H.; 2635	WAG	1996	H	Ghana	
10	*L. lasiorhiza* (O. Hoffm.) C. Jeffrey	Phillips, E.; 4048	WAG	1978	H	Malawi	*
11	*L. orientalis* Boiss.	Bayer, Ch.; B 100191996	B	1989	H	Jordan	2
12	*L. paradoxa* Sch.Bip. ex A. Rich.	Friis, I. et al.; 491	WAG	1970	H	Ethiopia	*
13	*L. perennis* L.	Wieringa, J.J.; 5779	WAG	2006	S	France	
14	*L. praevia* C.D. Adams	Simons, E.L.A.N.; 855	WAG	2012	H	Guinea	1*
15	*L. raddeana* Maximowicz	Zhu, S.X.; 09-208	HEAC	2009	S	China	3
16	*L. saligna* L.	Koopman, W.J.M.; CGN15705	WAG	1991	F	Georgia	
17	*L. schulzeana* Büttner	Pauwels, L.; 5453	WAG	1976	H	Cameroon	2*
18	*L. schweinfurthii* Oliv. et Hiern	Wilde, W.J.J.O. de; 2528	WAG	1964	H	Cameroon	*
19	*L. serriola* L. (1)	Jeuken, MJW; MJ19	L	2013	F	Turkey	3
20	*L. setosa* Stebbins ex C. Jeffrey	Blittersdorff, R. von; B100426945	B	2011	H	Tanzania	*
21	*L. tatarica* (L.) C.A. Meyer	Koopman, W.J.M.; 397	WAG	1996	H	Netherlands	
22	*L. tenerrima* Pourr.	Wilde, J.J.F.E. de; 3038	WAG	1961	H	Morocco	
23	*L. tinctociliata* I.M. Johnst (*Launaea cornuta* (Hochst. ex Oliv. et Hiern) C. Jeffrey)	Masens, B.; 180	WAG	1990	H	Congo	*
24	*L. ugandensis* C. Jeffrey (*Lactuca* sp.)	Wilde, W.J.J.O. de; 2457	WAG	1964	H	Cameroon	*
25	*L. viminea* subsp. *chondrilliflora* (Boreau) Malag.	Lewalle, J.; 10014	WAG	1981	H	Morocco	
26	*L. viminea* subsp. *ramosissima* (All.) Malag.	Wieringa, J.J.; 5974	WAG	2007	H	France	1
27	*L. virosa* L.	CGN09364	L	2013	F	Iran	**
28	*L. zambeziaca* C. Jeffrey	Niangadouma, R.; 391	WAG	2004	H	Gabon	*
29	*Cicerbita alpina* Wallr.	Breteler, F.J.; 7538	WAG	1977	H	France	
30	*Notoseris triflora* (Hemsl.) C. Shih	Zhu, S.X.; 2012-1818	HEAC	2012	S	China	3
31	*Paraprenanthes diversifolia* (Vaniot) N. Kilian	Zhu, S.X.; 2012-1817	HEAC	2012	S	China	3
32	*Prenanthes purpurea* (Vaniot) N. Kilian	Wieringa, J.J.; 5375	WAG	2004	H	France	

[a] Refer to Index Herbariorum (Thiers B 2011)

[b] *H* herbarium, *F* fresh, *S* silica-dried

[c] * African endemic species (Lebeda et al. 2004); ** seeds of the same accession can be required for free; 1 means the plastid gene sequences were obtained by Sanger sequencing; 2 indicates NGS and Sanger sequencing for this sample both failed; 3 voucher specimen are being submitted to herbarium

performed to select the best assembly (LnL score) among candidate assemblies as the final assembly. Chloroplast genes (*trnL-F* and *ndhF*) were annotated and extracted in DOGMA (Wyman et al. 2004). The IOGA script can be obtained from Github at https://github.com/holmrenser/IOGA.

Sequence alignment and phylogenetic analyses

From GenBank we obtained 218 *ndhF* gene sequences from 211 species and 301 *trnL-F* gene sequences from 250 species by Blasting *L. sativa*, *L. inermis* Forssk., *L. paradoxa* Sch.Bip. ex A. Rich. and *L. canadensis* A. Gray (Table S1 and Table S2) against the NCBI nucleotide database. This sampling comprises a wide range of taxa from all the subfamilies in Asteraceae, according to the Angiosperm Phylogeny Website (http://www.mobot.org/MOBOT/research/APweb/). Together from with the *Lactuca* sequences generated in this study, we achieved 34 % taxonomic sampling for *Lactuca*. *Barnadesia caryophylla* was selected as outgroup based on the phylogenetic tree of Asteraceae in APG (http://www.mobot.org/MOBOT/research/APweb/trees/asteraceae.gif). All the DNA sequences were first automatically aligned with MAFFT (version 7, http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2002) and then manually adjusted in Mesquite 2.75 (Maddison and Maddison 2015), following the criteria used by Borsch et al. (2003), Bremer et al. (2002), Kim and Jansen (1995) and Taberlet et al. (2007). The alignments for *trn*L-F and *ndhF* genes were separately optimised by first performing Neighbour Joining in PAUP* version 4.0b10 (Swofford 2003). The following parameters were used: Outgroup: *Barnadesia caryophylla*, Dset Distance = GTR, Rates = Gamma. The vertical order of accessions in the two alignments was then adjusted according to the NJ tree in order to maintain a phylogenetic continuum and to see if local rearrangements in the alignment of nucleotides were needed. Presumably homologous indel events (gaps) were coded as additional presence/absence characters. Regions left doubts about the homology of indels or could not be aligned were treated as in Bremer et al. (2002).

Phylogenetic trees at the subfamily level were then reconstructed for *ndhF* and *trnL-F* regions separately using Randomized Axelerated Maximum Likelihood (RAxML)-HPC2 run on XSEDE (Stamatakis 2014) from the Cyber-infrastructure for Phylogenetic Research (CIPRES) Science Gateway (V. 3.3, available at http://www.phylo.org/; Miller et al. 2010; Figure S1 & S2). Simultaneously, MrBayes 3.2.2 on XSEDE from CIPRES Science Gateway was also used to perform phylogenetic analyses (Ronquist et al. 2012), using the same alignment (Figure S3 & S4).

In order to estimate phylogenetic relationships at the generic level, we then subsampled our subfamily level alignments based on the generated trees (Fig. S1–S4) and trees from Wang et al. (2013). 79 *trnL-F* and 33 *ndhF* accessions were selected to represent *Lactuca* and related genera. *Leontodon saxatilis* is the nearest sister group to *Lactuca* and related genera and therefore was chosen as the outgroup (Fig. S1 - S4). The subsampled sequences were re-aligned using MAFFT version 7. Indels were manually coded for *trnL-F* and *ndhF* genes following the Simple Indel Coding (SIC) method (Simmons and Ochoterena 2000) in Mesquite 2.75. The selected sequences were then concatenated using SequenceMatrix-Windows 1.7.8 (Vaidya et al. 2011).

The joined alignment, containing the two plastid DNA sequences, as well as the two separate gene alignments were used for further phylogenetic analyses. For the joined alignment, the dataset was analysed in three different ways for Bayesian Inference (BI): no partition, two partitions (*trnL-F*/*ndhF*) and three partitions (*trnL-F*/codon position1 + 2 of *ndhF*/codon position 3 of *ndhF*). The parameters for BI were as follows: outgroup *Leontodon saxatilis*; lset nst = mixed, rates = gamma; unlink statefreq = (all), revmat = (all), shape = (all), pinvar = (all); prset applyto = (all), ratepr = variable; mcmcp ngen = 50,000,000, relburnin = yes, burninfrac = 0.25, printfreq = 1000, samplefreq = 50,000 nchains = 4 temp = 0.05; Report tree = brlens. Other parameters were default settings. For the single gene alignments, the dataset of *ndhF* gene was treated in two ways for BI: no partition and two partitions (codon position1 + 2/codon position 3) and the alignment of *trnL-F* gene was not partitioned as it is not a coding sequence.

The Markov Chain output parameter files generated by MrBayes 3.2.2 were then used in Tracer v1.6 (available at http://tree.bio.ed.ac.uk/software/tracer/) to select the best partition for constructing phylogenetic trees by selecting the marginal density centred around the highest log likelihood (LnL). The chosen partition was then subjected to RAxML analysis using

default settings. TreeGraph 2 was used to add Bootstrap (BS) and Posterior Probability (PP) values on one tree (Stover and Muller 2010).

Biogeographical, chromosomal and morphological data analyses

Biogeographical distributions were inferred from The Cichorieae Portal (Hand et al. 2009+) and Lebeda et al. (2004). We used RASP (Reconstruct Ancestral State in Phylogenies) to reconstruct ancestral biogeographical areas whereby distribution areas were delineated as A(Asia), B(Europe), C(Africa) and D(North America) (Yu et al. 2015). We did not delineate more detailed distributions due to the restriction of the number of biogeographical areas in RASP. We used 1000 trees inferred from BI analyses and the condensed Bayesian tree in RASP. The Bayesian Binary MCMC (BBM; Experimental) method and the Fixed (JC) + Gamma model were used to reconstruct the biogeographical areas. Other settings were default.

Chromosome numbers were scored according to Koopman et al. (1993), Matoba et al. (2007) and the Index to Plant Chromosome Numbers (IPCN; Missouri Botanical Garden 2014). Selected morphological characters, such as floret number, achene winged or not and rib number were scored from The Cichorieae Portal (Hand et al. 2009+). We selected these characters because they are considered as important identification keys. Subsequently, we reconstructed the ancestral states for chromosomal and morphological characters over the same trees used for estimating the ancestral state of the biogeographical data in RASP. All the settings were the same.

Results

The *ndhF* and *trnL-F* sequences of 27 species were successfully sequenced by NGS, whereas the sequences of *L. praevia* C.D. Adams and *L. viminea* J. Presl & C. Presl subsp. *ramosissima* (All.) Malag. were failed for NGS and obtained using Sanger sequencing. In addition, the sequencing of *L. imbricata* Hiern, *L. orientalis* Boiss. and *L. schulzeana* Büttner was neither successful by NGS or Sanger. The *trnL-F* region had 863 (including indels)/853 characters in the alignment. Of the total 863/853 characters, 65(7.5 %)/58(6.8 %) were parsimony informative sites (Table 2). The alignment of *ndhF* gene contained 2251 (including indels)/2250 characters and 71(3.2 %)/70(3.1 %) of them were informative sites (Table 2). The total number of characters in the concatenated alignment was the sum of *trnL-F* and *ndhF* and 136(4.4 %)/128(4.1 %) of them were informative sites. The phylogenetic trees of 247 *ndhF* and 331 *trnL-F* gene sequences from different subfamilies using RAxML and BI analyses are shown in Fig. S1–S4. The no partition model for the concatenated dataset performed better than the partition models, as its marginal density was centred around a higher log likelihood (LnL), and therefore was chosen for further analyses. One 'best ML tree' for the concatenated sequences was inferred automatically from the RAxML analysis, which is generally congruent in topology with the BI 50 % majority rule consensus tree. We present the RAxML phylogram topology combined with BS and PP values (Fig. 1). The phylogenetic trees for single gene alignments are shown in Figs. S5 and S6. We also reconstructed ancestral states for biogeographical, chromosomal and morphological characters over the condensed Bayesian trees of the concatenated sequences (Figs. S7–S11).

The phylogenetic analyses showed that *L. tinctociliata* I.M. Johnst is outside the *Lactuca* clade and the sister group to all *Lactuca* and *Melanoseris* species, *Notoseris triflora* (Hemsl.) C. Shih, *Paraprenanthes diversifolia* (Vaniot) N. Kilian, *Cicerbita alpina* Wallr. and *Prenanthes purpurea* (Vaniot) N. Kilian (Fig. 1, name indicated with a star). A *Lactuca* clade (BS = 78, PP = 0.98) divides into three clades, Clade A, B and C. We will describe the clades in the following sections.

Table 2 Characteristics of individual gene alignment and concatenated plastid matrix

Data set	No. of char.[a]/ No. of char.[b]	No. of parsimony inform. sites[a]/no. of inform. sites[b]
trnL-F	863/853	65(7.5 %)/58(6.8 %)
ndhF	2251/2250	71(3.2 %)/70(3.1 %)
trnL-F + *ndhF*	3114/3103	136(4.4 %)/128(4.1 %)

char. character, *inform.* informative

[a] With indel

[b] Without indel

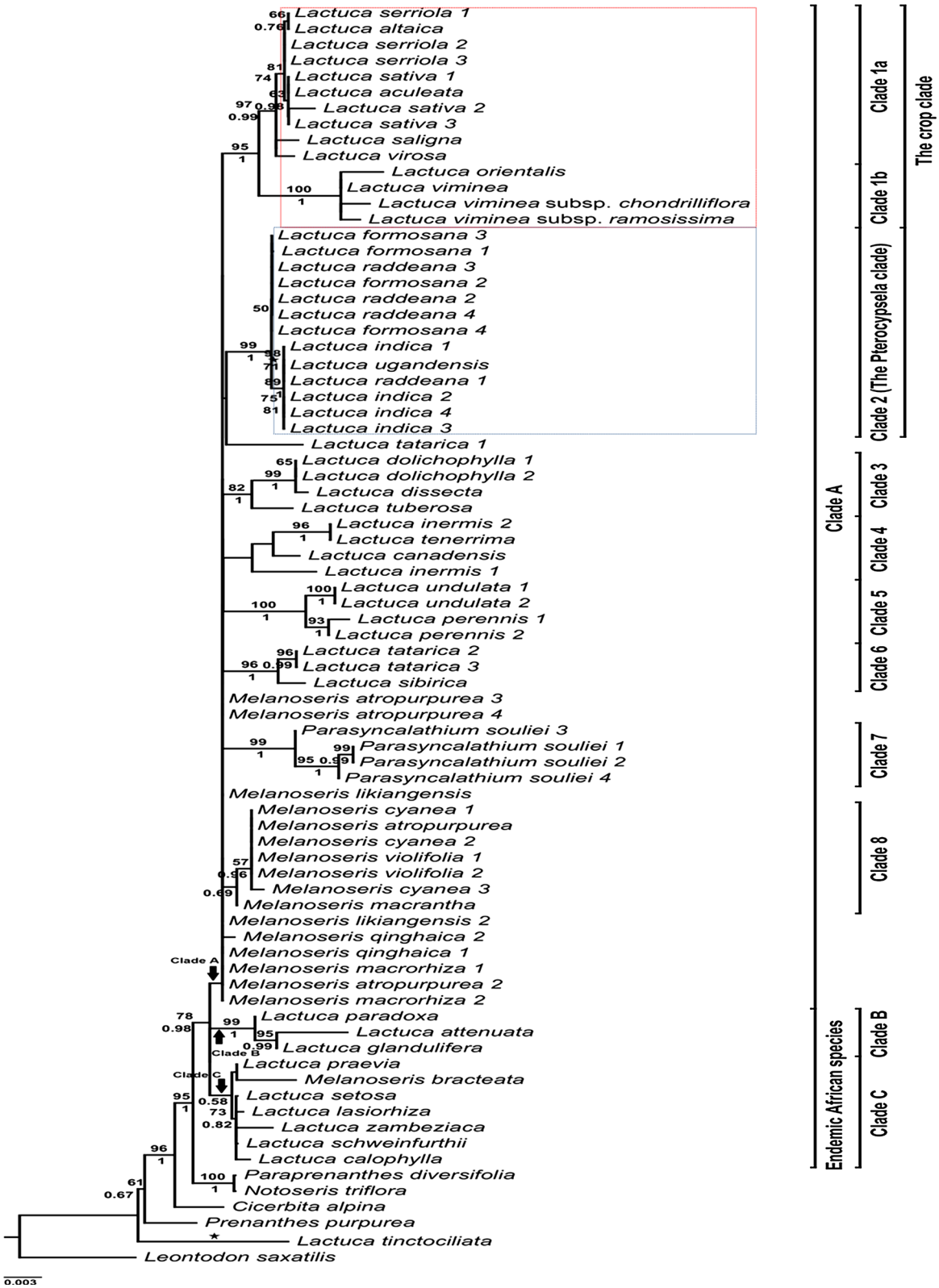
Clade 1 (BS = 95, PP = 1) includes the lettuce crop and closely related wild lettuce species. It contains two subclades. Clade 1a (BS = 97, PP = 0.99) consists of the domesticated lettuce *L. sativa* and its closest relatives *L. serriola*, *L. altaica*, *L. aculeata*, *L. saligna* and *L. virosa*. One *L. serriola* accession is the sister group to *L. altaica* (BS = 66, PP = 0.76). *L. aculeata* and *L. sativa* are grouped together (BS = 63, PP = 0.98). *L. saligna* and *L. virosa* are the sister groups of *L. serriola*, *L. altaica*, *L. aculeata* and *L. sativa*. Clade 1b (BS = 100, PP = 1) comprises *L. orientalis*, *L. viminea* J. Presl et C. Presl, *L. viminea* J. Presl et C. Presl subsp. *chondrilliflora* (Boreau) Malag. and *L. viminea* subsp. *ramosissima*. Clade 1 (PP = 1) comprises widely spread *Lactuca* species from Asia, Europe and Africa (Figure S7). The species in Clade 1 have a chromosome number of eighteen (2n = 18) except *L. orientalis* (2n = 18 or 36; Figure S8). Most species in Clade 1a have a floret number between 6 and 15 (20) or even more than 20 florets (Figure S9). Other species in Clade 1b have less than 6 florets (Figure S9). The achenes of most species in Clade 1 are not winged except *L. virosa* (Figure S10). Most species in Clade 1 have a rib number between 3 and 9 (Figure S11).

Clade 2 (BS = 99, PP = 1) comprises of ex-*Pterocypsela* C. Shih species, including *L. indica* L., *L. raddeana* Maximowicz, *L. formosana* Maximowicz and *L. ugandensis* C. Jeffrey (not ex-*Pterocypsela* species). Four *L. indica* accessions, one *L. raddeana* accession and *L. ugandensis* are in one subclade (BS = 89, PP = 1) whereas the other three *L. raddeana* accessions and four *L. formosana* accessions are in one clade (BS = 50). In addition, one *L. tatarica* accession is the sister group to Clade 2, though the BS support is very low (BS < 50). This clade contains Asian species and one African species *L. ugandensis* clade (PP = 1; Figure S7). *Lactuca* species in Clade 2 have eighteen chromosomes (2n = 18) but this information for *L. ugandensis* is missing (Figure S8). They usually have a floret number between 6 and 15 (sometimes more than 20; Figure S9). Most species in Clade 2 (excluding *L. ugandensis*) have winged achenes (Figure S10) and a rib number between 1 and 7 (Figure S11).

Clade 3 (BS = 82, PP = 1) consists of *L. dolichophylla* Kitamura, *L. dissecta* D. Don and *L. tuberosa* Jacq. Clade 4 (lacking support) is composed of *L. tenerrima* Pourr., *L. inermis* and *L. canadensis*. *L. inermis* 1 from Ghana is the sister group of *L. tenerrima*, *L. canadensis* and *L. inermis* 2 from Togo. Clade 5 (BS = 100, PP = 1) includes *L. undulata* Ledebour and *L. perennis* L. Clade 6 (BS = 96, PP = 1) contains two *L. tatarica* accessions and *L. sibirica*. Clade 3 and 4 (PP = 1) include species from Asia and widespread species (Figure S7). Most species in Clade 5 and 6 are from Asia, North America or widespread species (Figure S7). The *Lactuca* species in Clade 3 have sixteen chromosomes (2n = 16; Figure S8). *Lactuca* species in Clade 5 and 6 have a chromosome number of eighteen (2n = 18). *L. tenerrima* and *L. inermis* in Clade 4 have sixteen chromosomes (2n = 16) while *L. canadensis* has thirty-four chromosomes (2n = 34; Figure S8). Most species in Clade 3–6 have a floret number usually between 6 and 15 (sometimes more than 20; Figure S9) and non-winged achenes (excluding *L. canadensis* and *L. tuberosa* (Figure S10). Most species in Clade 3 and 4 have a rib number between 3 (1) and 7. Species in Clade 5 and 6 have 1–3 ribs (Figure S11).

Clade 7 contains four *Parasyncalathium souliei* (Franch.) J.W. Zhang, Boufford et H. Sun accessions with a good support value (BS = 99, PP = 1; Fig. 1). Clade 8 lacks support (BS < 50, PP = 0.69) but may become stronger after adding more taxonomic sampling. It includes *Melanoseris cyanea* Edgew, *M. violifolia* (Decne.) N. Kilian, *M. atropurpurea* (Franch.) N. Kilian et Ze H. Wang and *M. macrantha* (C.B. Clarke) N. Kilian et J.W. Zhang. Other *Melanoseris* species, *M. atropurpurea*, *M. qinghaica* (S.W. Liu et T.N. Ho) N. Kilian et Ze H. Wang, *M. macrorhiza* (Royle) N. Kilian, *M. likiangensis* (Franch.) N. Kilian et Ze H. Wang are in a huge polytomy. *Melanoseris* and *Parasyncalathium* species are from Asia or widespread species (Figure S7). They have sixteen chromosomes (2n = 16; Figure S8). *Melanoseris* species have a floret number between 6 and 15 (sometimes more than 20) while *Parasyncalathium souliei* has a floret number less than 6 (Figure S9). *Melanoseris* and *Parasyncalathium* species do not have winged achenes (Figure S10). The rib number of most *Melanoseris* species is unknown (Figure S11). *Parasyncalathium souliei* in Clade 8 has 1–3 ribs.

Clade B (BS = 99, PP = 1) contains three scandent African species, *L. glandulifera* Hook.f., *L. attenuata* Stebbins and their sister group *L. paradoxa* (Figure S7). Clade C (PP = 0.58) includes the African


Lactuca serriola 1
Lactuca altaica
Lactuca serriola 2
Lactuca serriola 3
Lactuca sativa 1
Lactuca aculeata
Lactuca sativa 2
Lactuca sativa 3
Lactuca saligna
Lactuca virosa
Lactuca orientalis
Lactuca viminea
Lactuca viminea subsp. chondrilliflora
Lactuca viminea subsp. ramosissima
Lactuca formosana 3
Lactuca formosana 1
Lactuca raddeana 3
Lactuca formosana 2
Lactuca raddeana 2
Lactuca raddeana 4
Lactuca formosana 4
Lactuca indica 1
Lactuca ugandensis
Lactuca raddeana 1
Lactuca indica 2
Lactuca indica 4
Lactuca indica 3
Lactuca tatarica 1
Lactuca dolichophylla 1
Lactuca dolichophylla 2
Lactuca dissecta
Lactuca tuberosa
Lactuca inermis 2
Lactuca tenerrima
Lactuca canadensis
Lactuca inermis 1
Lactuca undulata 1
Lactuca undulata 2
Lactuca perennis 1
Lactuca perennis 2
Lactuca tatarica 2
Lactuca tatarica 3
Lactuca sibirica
Melanoseris atropurpurea 3
Melanoseris atropurpurea 4
Parasyncalathium souliei 3
Parasyncalathium souliei 1
Parasyncalathium souliei 2
Parasyncalathium souliei 4
Melanoseris likiangensis
Melanoseris cyanea 1
Melanoseris atropurpurea
Melanoseris cyanea 2
Melanoseris violifolia 1
Melanoseris violifolia 2
Melanoseris cyanea 3
Melanoseris macrantha
Melanoseris likiangensis 2
Melanoseris qinghaica 2
Melanoseris qinghaica 1
Melanoseris macrorhiza 1
Melanoseris atropurpurea 2
Melanoseris macrorhiza 2
Lactuca paradoxa
Lactuca attenuata
Lactuca glandulifera
Lactuca praevia
Melanoseris bracteata
Lactuca setosa
Lactuca lasiorhiza
Lactuca zambeziaca
Lactuca schweinfurthii
Lactuca calophylla
Paraprenanthes diversifolia
Notoseris triflora
Cicerbita alpina
Prenanthes purpurea
Lactuca tinctociliata
Leontodon saxatilis
Clade A
Clade B
Clade C
0.003
Clade 1a
Clade 1b
Clade 2 (The Pterocypsela clade)
The crop clade
Clade 3
Clade 4
Clade 5
Clade 6
Clade 7
Clade 8
Endemic African species

◀**Fig. 1** RAxML phylogram ('best ML tree') of the concatenated sequences of *ndhF* gene and *trnL-F* gene used in this study; Bootstrap (BS > 50) support values are given above the branches and Posterior Probability (PP > 0.5) support values are below; the names of Chinese taxa are referred to Wang et al. (2013); star *L. tinctociliata* was mis-identified and it could be *Launaea cornuta*; *L. ugandensis* should be *Lactuca* sp.

species *L. lasiorhiza* (O. Hoffm.) C. Jeffrey, *L. schweinfurthii* Oliv. et Hiern, *L. calophylla* C. Jeffrey, *L. zambeziaca* C. Jeffrey, *L. setosa* Stebbins ex C. Jeffrey, *L. praevia* and *Melanoseris bracteata* (Hook.f. et Thomson ex C.B. Clarke) N. Kilian. Chromosome number is only available for *L. attenuata* (2n = 32) and *L. glandulifera* (2n = 16; Figure S8). Species in Clade B and C have a floret number less than 6 (Figure S9) and they do not have winged achenes (Figure S10). Most species in Clade B have a rib number between 3 and 7. Species in Clade C have 1–3 ribs (Figure S11).

Discussion

Lettuce is an economically important crop and consequently most studies have mainly focused on *L. sativa* and closely related wild species (Koopman et al. 1993, 1998, 2001). Conversely, the entire *Lactuca* genus is poorly studied, especially for the two regions with the highest diversity, Asia (51 species) and Africa (43 species; Lebeda et al. 2004). Recently, a publication focused on the Chinese centre of diversity, including 15 Asian *Lactuca* species (Wang et al. 2013). However, the African *Lactuca* center of diversity remains unstudied. We here present the first study focused on the phylogenetic relationships within *Lactuca* and related genera with extensive sampling of the African diversity centre, based on plastid genes. This is the first molecular phylogeny for 40 % of the endemic African *Lactuca* species, especially for the scandent species since they were described and revised by Stebbins (1937b).

The mapping of biogeographical, chromosomal and morphological character states lend additional supports to the topologies of the RAxML trees. For biogeographical data, Clade B and Clade C only contain *Lactuca* species endemic to African continent, although other clades do not show distinctive pattern. The chromosome numbers (excluding the accessions with unknown chromosome number in Clade 8) supported the topology of the RAxML tree. *Lactuca* species in Clade 1, 2, 5 and 6 have a chromosome number of eighteen (2n = 18) except *L. orientalis* (2n = 18 or 36). Species in Clade 3, and *Melanoseris* species have sixteen chromosomes (2n = 16). *L. tenerrima* and *L. inermis* in Clade 4 have sixteen chromosomes (2n = 16) while *L. canadensis* has thirty-four chromosomes (2n = 34). In Clade B, *L. glandulifera* has sixteen chromosomes (2n = 16) while *L. attenuata* has thirty-two (2n = 32). The floret number also validated the topology of the RAxML tree. Most species in Clade 1a, 2–6 and C have a floret number usually between 6 and 15 (sometimes more than 20). Other species in Clade 1b, 7, B and C have a floret number less than 6. For the state of achene, most species in the *Lactuca* clade do not have winged achenes. Only *L. virosa*, *L. canadensis*, *L. tuberosa* and species in Clade 2 (excluding *L. ugandensis*) have winged achenes. For rib number, most species in Clade 1, 4 and B have a rib number between 3 and 9. Species in Clade C, 5, 6 and Clade 8 have 1–3 ribs. Species in Clade 2 and 3 have a rib number between 1 and 7. The rib number of most *Melanoseris* species is unknown.

Monophyly of the subtribe Lactucinae

Our RAxML tree for concatenated sequences shows that *C. alpina*, *Faberia*, *P. purpurea* and *L. tinctociliata* should be excluded to maintain the monophyly of the subtribe Lactucinae (Figs. S1–S4). *L. tinctociliata* is placed outside Lactucinae and nested in Hyoseridinae (Figs. S1–S4). It is clustered with *Launaea sarmentosa* (Willd.) Kuntze with a very high support (BS = 100, PP = 1) in the *trnL-F* tree and is sister group of *Sonchus oleraceus* L. in the *ndhF* trees (BS < 50, PP = 0.64; Figs. S1–S4). This species was first published and described by I.M. Johnst in 1925 (Jeffrey 1966; Anonymous 1925). No detailed description or molecular data have been made available since then. According to I.M. Johnst, *L. tinctociliata* is very well characterized by its narrow firm purple leaf-margins which commonly bear purplish-tinged teeth and fleshy cilia, the capitula with about 12 yellow flowers, a very compressed achene, marginal, oblong-ovate or oblanceolate 5–6 mm long, thin beak >1 mm long, about 12 ribs, bristle white pappus, 5–6 mm long (Anonymous 1925). From the image of the *L. tinctociliata* specimen used in this study, we can see

(image available at http://medialib.naturalis.nl/file/id/WAG.1288514/format/large?width=800px&height=800px) that it has broader leaves than the type specimen (image available at http://plants.jstor.org/stable/10.5555/al.ap.specimen.gh00009514) and does not have purple leaf-margins. Although we could only compare the specimen images, the '*L. tinctociliata*' used in our study is clearly not *L. tinctociliata*. Based on our molecular data and the woody habit (typical of the species), the specimen is most likely *Launaea cornuta* (Hochst. ex Oliv. et Hiern) C. Jeffrey.

Wang et al. (2013) indicated that when *Faberia* and *P. purpurea* lineages are excluded, the subtribe Lactucinae is monophyletic. Moreover, they suggested that *C. alpina* should be disregarded while the other *Cicerbita* species are placed inside the Lactucinae. A narrow circumscription of *Prenanthes* L. was proposed making it a probably monospecific genus (Kilian and Gemeinholzer 2007; Kilian et al. 2009). Wang et al. (2013) transferred species from *Prenanthes* to *Notoseris* Shih and confirmed this narrow concept of *Prenanthes*. The BI tree of *ndhF*, including species from different subfamilies (Figure S3), shows that the genus *Tolpis* Adanson from the subtribe Cichoriinae is the sister group of the clade comprising *P. purpurea*, *C. alpina*, *N. triflora*, *Paraprenanthes diversifloria* and the genus *Lactuca* (PP = 0.54), but support for this pattern is lacking. The RAxML *ndhF* tree indicates *P. purpurea* is the sister group of *Tolpis* species (Figure S1). In our *trnL-F* trees, *P. purpurea* is the sister group of *Ixeridium gracile* (DC.) C. Shih, a species from the subtribe Crepidinae (BS = 61, PP = 0.93; Figs. S2, S4). Although all BS and PP values involved are low, these results would confirm the narrow concept of *Prenanthes* and indicate that *P. purpurea* probably belongs to the subtribe Cichoriinae or Crepidinae and is far away from the subtribe Lactucinae.

Our RAxML tree reveals that *Notoseris* and *Paraprenanthes* C. C Chang ex C. Shih are the sister groups to *Lactuca* in the subtribe Lactucinae (Fig. 1). When the genus *Notoseris* was first described, it comprised 12 species, with shared morphological characters such as capitula with 3–5 florets, beakless achene apices and 6–9 ribs on each side of achene (Shih 1987). Shih (1997) then reduced the number of species to 11. Wang et al. (2013) recently removed several species from *Notoseris* and transferred two scandent species from *Prenanthes* to *Notoseris*, based on ITS and plastid DNA sequences. *Paraprenanthes* was first proposed by C. C. Chang and formally established by Shih (1988a), who added new species and transferred some species from *Lactuca*, *Crepis* L. and *Mycelis* Cass. based on morphological characters, e.g. capitula with 6–23 cyanic florets, achenes with 5 main ribs and two rather similar secondary ribs in-between, and a single pappus (1988a). Shih and Kilian (2011) maintained the circumscription of *Paraprenanthes* but used a wider species concept and separated three species from the genus. Recently, Wang et al. (2013) revised the genus by reducing the species recognized by Shih and Kilian (2011) to six and adding four new species. Although the phylogenetic relationships among *Paraprenanthes* and *Notoseris*species remains unresolved based on *trnL-F* DNA sequence comparisons (Figs. S2, S4), our results indicate that *Notoseris* and *Paraprenanthes* are closely related to *Lactuca*.

Circumscription of *Lactuca* and its subgeneric classification

The phylogenetic tree for the concatenated sequences indicates that the *Lactuca* species, autochthonous to the African continent, are far away from the other *Lactuca* species. Meanwhile, the other *Lactuca* species (not endemic to Africa), *Melanoseris* and *Paracyncalathium* are nested within Clade A (lacking support) as part of the large polytomy (Fig. 1).

The African Lactuca species (Clade B and C, 2n = 16, 32 or ?) The African species include *L. paradoxa*, *L. attenuata*, *L. glandulifera*, *L. lasiorhiza*, *L. schweinfurthii*, *L. calophylla*, *L. zambeziaca*, *L. setosa* and *L. praevia*. Of all of these species we present, as far as we know, the first molecular phylogeny since they were summarized and described by Jeffrey (1966). Jeffrey (1966) elaborated a total of 33 African *Lactuca* species but Lebeda et al. (2004) reported that this group contains at least 43 species and 75 % of the group (31 in total) can be considered as endemic. In our sampling, only autochthonous African *Lactuca* species are included in these two clades with one exception—*M. bracteata*. The support between *L. praevia* and *M. bracteata* is very low), hence it is difficult to tell if *M. bracteata* does or does not belong to Clade C. Other species occuring in Africa but not endemic to the African continent, such as *L. inermis*, *L. tenerrima*, *L. saligna* and *L. virosa*, are distributed in other clades. This may indicate an independent

evolution of the African endemic species. Based on their scandent or herbal habits, these endemic species can be divided into two groups: the scandent group and the herbal group. According to Stebbins (1937b), there were seven scandent *Lactuca* species in Africa: *L. stipulata* Stebbins, *L. elgonensis* Stebbins, *L. paradoxa*, *L. attenuata*, *L. semibarbata* Stebbins, *L. wildemaniana* Stebbins, and *L. glandulifera*. Jeffrey (1966) combined the last two species as *L. glandulifera* and added *L. attenuatissima* Robyns to the scandent group. Our scandent samples include *L. paradoxa*, *L. attenuata* and *L. glandulifera*. These scandent species are not related to the two scandent species from *Notoseris*, which indicates two independent evolutions of the scandent habit in Lactucinae (Figs. S2, S4). These African species share some characters, such as capitula with less than 6 yellow florets (an exception from *L. lasiorhiza* with 10–14 florets) and 1–3 ribs on each side of achene. Chromosome number is only available for *L. attenuata* ($2n = 32$) and *L. glandulifera* ($2n = 16$; Missouri Botanical Garden 2014). Wang et al. (2013) used the same dataset of *Melanoseris* species as in our study and showed that the genus *Melanoseris* is closely related to the genus *Lactuca*. In our results, *Melanoseris* and *Parasyncalathium* species are in Clade A and the African *Lactuca* species in Clade B and C are even further away from other *Lactuca* species in Clade A than *Melanoseris* and *Parasyncalathium* species. Our molecular, biogeographical, chromosomal and morphological data all show that the endemic African *Lactuca* species have a unique position and evolved independently. We suggest that the African species in Clade B and Clade C could be removed from *Lactuca* and treated as a new genus. However, further taxonomic, cytological and molecular studies are still needed to do an official taxonomic revision.

The Melanoseris species (Clade 7 and 8, 2n = 16 or ?) Clade 7 contains *Parasyncalathium souliei* accessions with a very high support value (BS = 99, PP = 1; Fig. 1). This implication is in line with Stebbins (1940) and Zhang et al. (2009a, b, 2011). However, Wang et al. (2013) preferred to put this species in *Melanoseris* while Zhang et al. (2011) proposed that this species should be either put back in *Lactuca* or treated as a new genus. Clade 8 includes *M. cyanea*, *M. violifolia*, *M. atropurpurea* and *M. macrantha*. One *M. atropurpurea* accession is in this clade while other three *M. atropurpurea* accessions are in an unresolved polytomy together with *M. macrorhiza*, *M. likiangensis* and *M. qinghaica*. The name *Melanoseris* was first proposed by Decaisne in 1843 for two species from the Himalayas, which are now treated as *M. lessertiana*. Edgeworth (1846) then added more Himalayan species to *Melanoseris*. Shih (1991) established two new genera from Sino-Himalayan region, *Chaetoseris* C. Shih and *Stenoseris* C. Shih, by transferring species from *Lactuca* and *Cicerbita*. *Chaetoseris* was distinguished from *Lactuca* and *Cicerbita* because of its achene corpus with broad and thickened lateral ribs and a pappus with an outer ring of minute hairs (Shih 1991, 1997). *Stenoseris* was established with five species and circumscribed by 3–5 flowered capitula and an achene with an outer ring of minute hairs (Shih 1991). Shih and Kilian (2011) revised this lineage and reused the name *Melanoseris* for the lineage based on their molecular data. They transferred species that were formerly placed in *Chaetoseris*, *Cicerbita*, *Lactuca*, *Mulgedium* Cass., *Prenanthes* and the genus *Stenoseris* to *Melanoseris*. Furthermore, Wang et al. (2013), using nrITS1 and plastid genes, concluded that *Melanoseris* could be divided into three groups: *M. cyanea* group, *M. macrorhiza* group and *M. gracilifiora* group. Although our results do not separate the *Melanoseris* lineage from *Lactuca* species, they reveal a close relationship between *Lactuca* and *Melanoseris.* Compared with previous molecular and morphological investigations, we still think *Melanoseris* and *Lactuca* are two separate but closely related genera (Shih and Kilian 2011; Wang et al. 2013).

We will now discuss the clades (1–6) that can be highlighted within *Lactuca*:

Clade 1 (The Crop Clade) (2n = 18 or 36) This clade comprises Clade 1a and 1b. Clade 1a contains the cultivated lettuce and can be referred to as *Lactuca* section *Lactuca* subsect. *Lactuca* (Lebeda et al. 2009). This clade includes *L. serriola*, *L. altaica*, *L. aculeata*, *L. virosa* and *L. saligna*. All the species in Clade 1a are interfertile or partly interfertile with *L. sativa* (Hartman et al. 2012; Thompson et al. 1941). Koopman et al. (1998) considered *L. serriola* and *L. altaica* to be conspecific based on their identical ITS-1 sequences and the results of crossing experiments. Our phylogenetic tree confirms his conclusion and also show that *L. aculeata* is closer to *L. sativa* than *L. serriola*. *L. sativa*, *L. serriola*, *L. altaica* and *L. aculeata* comprise the primary lettuce gene pool (Koopman et al. 1998).

L. virosa and *L. saligna* are the sister groups to the species in the primary gene pool and form the secondary lettuce gene pool (Koopman et al. 1998). Crosses between *L. serriola* and *L. saligna*, and between *L. sativa* and *L. saligna* were shown to be partly fertile or self-fertile (Jeuken et al. 2001; Thompson et al. 1941; Zohary 1991). Chromosomal studies have demonstrated that *L. saligna* is potentially more closely related to *L. sativa—L. serriola* than *L. virosa* (Koopman et al. 1993; Matoba et al. 2007). Conversely, nrITS1 and AFLP fingerprints with moderate support indicated that *L. virosa* is closely-related to *L. sativa—L. serriola* (Koopman et al. 1998, 2001). Although the cross between *L. virosa* and *L. sativa* often failed, it was still possible to obtain the cross and the hybrid was found to be self-sertile (Thompson et al. 1941; Whitaker and Thompson 1941; Zohary 1991). All the species in Clade 1a are widespread and share some characters, like a floret number >6 (Figs. S7–S11).

Clade 1b includes *L. orientalis* and *L. viminea* and refers to section *Phaenixopus* (Lebeda et al. 2009). *L. orientalis* and *L. viminea* belonged to the genus *Scariola* but recently they were both treated as *Lactuca* species (Flann et al. 2010; Shih 1997; Shih and Kilian 2011; Wang et al. 2013). *L. orientalis* (2n = 18, 36) is a subshrub, which is very rare in *Lactuca*, all the other *Lactuca* species are herbs (Shih and Kilian 2011). It has whitish, rigid, intricately and divaricately branched stems, glaucous green leaves, solitary capitula with 4 or 5 pale yellow florets and a narrowly cylindrical involucre, and narrowly ellipsoid achenes with 5–7 ribs on either side (Shih and Kilian 2011). *L. viminea* subsp. *viminea*, *L. viminea* subsp. *chondrilliflora* and *L. viminea* subsp. *ramosissima* (2n = 18) share many morphological characters although they differ from each other in certain characteristics. For example, *L. viminea* subsp. *chondrilliflora* has a beak length as long as ¼–½ of the achene body while *L. viminea* subsp. *viminea* and *L. viminea* subsp. *ramosissima* have a beak length equal to the achene body. Furthermore, *L. viminea* subsp. *viminea* branches only in the upper part of the stem whereas *L. viminea* subsp. *ramosissima* branches mostly in the basal part (Feráková and Májovský 1977). According to Koopman et al. (1998), *L. viminea* from the section *Phaenixopus* belongs to the tertiary lettuce gene pool, which also contains *L. quercina* from section *Lactucopsis*, *L. sibirica* and *L. tatarica* from section *Mulgedium*. In our phylogentic inferences, *L. quercina* was not included and *L. sibirica* and *L. tatarica* form a seperate Clade 4. Wang et al. (2013) using their nrITS1 sequences indicated a tertiary gene pool similar to Koopman's but showed that *L. sibirica* and *L. tatarica* form a well-supported seperate clade using their plastid gene sequences. Hybridization experiments showed that *L. viminea* is partly fertile with *L. virosa* (Groenwold 1983) and *L. tatarica* could be somatically hybridized with *L. sativa* (Chupeau et al. 1994; Maisonneuve et al. 1995). As the chance of generating fertile seeds from hybrids of *L. tatarica* and *L. sativa* is very low in nature (Chupeau et al. 1994; Maisonneuve et al. 1995), we consider *L. orientalis* and the three *L. viminea* subspecies as the tertiary gene pool and keep *L. sibirica* and *L. tatarica* beyond the tertiary gene pool.

The lettuce gene pool can provide rich genetic resources for improving lettuce growth, e.g. with respect to resistance to abiotic and biotic stresses. For example, *L. serriola* from the primary gene pool has been proven to possess interesting alleles for acquiring water and fertilizer in soil, increasing germination and seed longevity (Argyris et al. 2005; Johnson et al. 2000; Schwember and Bradford 2010). *L. aculeata* from the primary gene pool, *L. saligna* and *L. virosa* from the secondary gene pool, *L. viminea* from the tertiary gene pool, and *L. tatarica*, *L. biennis*, *L. canadensis*, *L. homblei*, *L. indica* and *L. perennis* beyond the lettuce gene pool all showed high resistance to downy mildew (Jeuken et al. 2008; van Treuren et al. 2011). These species may provide rich genetic resources for the crop lettuce. *L. orientalis*, belonging to the tertiary gene pool, could be a potential resource to improve the growth, development and resistance to diseases of the lettuce crop as well.

Clade 2 (The Pterocypsela Clade) (2n = 18 or ?) This clade comprises species mostly distributed in Asia: *L. indica* [2n = 18, although Lebeda et al. (2004) indicate it is also in Africa based on floras], *L. raddeana* (2n = 18) and *L. formosana* (2n = 18; Hand et al. 2009+; Jeffrey 1966). The only exception is *L. ugandensis* (2n = ?) from Africa. The first three species belonged to the genus *Pterocypsela*, which was established by Shih (1988b) with type species *Pterocypsela indica* (L.) Shih. They have some shared characters, such as involucral bracts in 4–5 rows, capitula with 9–25 florets, broadly winged achenes with 1 or 3(5) prominent ribs on either side of the

achene body and double pappus (Shih 1988b, 1997). Shih and Kilian (2011) transferred these three *Pterocypsela* species to *Lactuca*. Although *L. ugandensis* is grouped together with these ex-*Pterocypsela* species, it is depicted without winged achene (Jeffrey 1966; Jeffrey and Beentje 2000). This *L. ugandensis* specimen could be mis-identified. Therefore we treat it as *Lactuca* sp. Clade 2 confirms the nrITS-1 and plastid gene trees of Wang et al. (2013) and is also comparable to section *Tuberosae* (Lebeda et al. 2007, 2009). In addition, *L. indica* (Indian lettuce) has been cultivated for its edible leaves (Kadereit et al. 2007). Somatic hybridizations between *L. sativa* and *L. indica* have shown that a viable callus can be generated but it cannot produce a viable plant (Mizutani et al. 1989). Moreover, *L. indica* is resistant to downy mildew (van Treuren et al. 2011). Thus, *L. indica* could be a useful genetic resource for lettuce breeding.

Clade 3 (2n = 16) This clade is composed of *L. dolichophylla*, *L. dissecta* and *L. tuberosa* (BS = 82, PP = 1). The support value between *L. dolichophylla* and *L. dissecta* (BS = 99, PP = 1) is even higher. These three species all have a chromosome number of 16 (Shih and Kilian 2011; Vogt and Aparicio 1999). *L. dolichophylla* and *L. dissecta* have some shared characters such as capitula with 6–15(20) blue florets and 3–5 ribs on either side of the achene while *L. tuberosa* has tuberous roots and broadly winged achenes (Hand et al. 2009+; Shih and Kilian 2011). *L. dolichophylla* and *L. dissecta* are distributed in Asia, mainly in South Asia and East Asia, whereas *L. tuberosa* occurs in Asia and Europe (Geltman 2003; Hand et al. 2009+).

Clade 4 (2n = 34, 16) This clade includes *L. canadensis* (2n = 34) originating from North America, *L. tenerrima* (2n = 16) and *L. inermis* (2n = 16). *L. inermis* 1 (collected in Ghana) is the sister group to *L. canadensis*, *L. tenerrima* and *L. inermis* 2 (collected in Togo) while *L. tenerrima* and *L. inermis* 2 is close to each other (BS = 96, PP = 1; Fig. 1). This could be the result of mis-identification of any of the *L. inermis* accessions or not enough evidence to distinguish these species. The American *Lactuca* group includes 12 species, 7 of them are endemic with 34 chromosomes (2n = 34) and different relative DNA content (Babcock et al. 1937; Doležalová et al. 2002; Lebeda and Astley 1999). *L. tenerrima* and *L. inermis* (treated as *L. capensis* before) have been shown to cluster together due to their low DNA content while *L. canadensis* is far away from them as a result of high DNA content (Doležalová et al. 2003). The crosses between *L. canadensis* and *L. tatarica* (2n = 18), and between *L. canadensis* and *L. raddeana* (2n = 18) can generate self-sterile hybrid plants (Thompson et al. 1941). Other North American *Lactuca* species, *L. graminifolia* Michx. (2n = 34), *L. floridana* (L.) Gaertn. (2n = 34) and *L. spicata* Hichc. (2n = 34) could be crossed with *L. indica*, *L. laciniata* Roth (now treated as *L. indica*), *L. raddeana*, and *L. tatarica* and produce self-sterile or partly fertile hybrid plants (Thompson et al. 1941; Wang et al. 2013). In addition, *L. canadensis*, *L. raddeana* and *L. indica* share a distinctive character, broadly winged achene, from other *Lactuca* species although their beak length are clearly different. The North American *Lactuca* species are supposed to have an amphidiploid origin and arose by subsequent crossings, doubling of chromosomes and hybrid stabilization. Their chromosome complement can be represented by the formula AABB (A = 8, B = 9; Feráková and Májovský 1977). Our phylogenetic inferences and all these experimental hybridizations support the assumption that the North American *Lactuca* species could have a possible origin from the hybridization between *Lactuca* species with a haploid chromosome number of 8 (e.g. *L. tenerrima*) and 9 (e.g. *L. tatarica*, *L. raddeana* and *L. indica*).

Clade 5 (2n = 18) This clade comprises *L. undulata* from the section *Micranthae* and *L. perennis* from the section *Lactuca* subsect. *Cyanicae* (Lebeda et al. 2007, 2009). *L. undulata* shares characters with *L. perennis*, for example, 1–3 ribs per side of achene and beak as long as achene body (Feráková and Májovský 1977; Shih 1997). This close relationship between *L. undulata* and *L. perennis* is supported by Wang et al. (2013). According to Lebeda et al. (2007), species in the section *Micranthae* have a chromosome number of 16, which is not the case for *L. undulata*. Therefore, we suggest placing *L. undulata* into the section *Lactuca* subsect. *Cyanicae*.

Clade 6 (2n = 18) This clade contains *L. tatarica* and *L. sibirica* from Asia. These species are considered to belong to the section *Mulgedium* (Lebeda et al. 2007, 2009). Shih (1988b) revised the concept of genus *Mulgedium* (including *L. tatarica*) and considered *Lagedium* Soják (only including *L. sibirica*) as a monospecific genus, based on the absence of a true beaked achene and a weakly compressed achene body.

But Shih's concept of *Mulgedium* and *Lagedium* is not accepted by most taxonomists. Shih and Kilian (2011) revised these two genera and transferred these species into *Lactuca*. *L. sibirica* is fully fertile with *L. tatarica*, indicating a close relationship between these two species (Koopman et al. 2001). However, another European *L. tatarica* 1 is the sister group to Clade 2 (Fig. 1). This accession is the sister group to Clade 2 in the *ndhF* tree (Figure S5) and the sister group to the whole *Lactuca* clade in the *trnL-F* tree (Figure S6). *L. indica* in Clade 2 can be crossed with *L. tatarica*, although producing self-sterile seeds (van Treuren et al. 2011). The conflicting positions of *L. tatarica* accessions could be the consequence of hybridization. More samples and evidence are needed to solve the problem.

Conclusions

This work presents the first molecular phylogeny of *Lactuca* with representatives of African species and includes the most extensive sampling of *Lactuca* species analyzed to date. Based on the results of the phylogenetic trees, we draw the following conclusions:

1. The genus *Lactuca* contains two well-distinguished clades: the crop clade and the *Pterocypsela* clade. Other North American, Asian and widespread species either form small clades or are mixed with the *Melanoseris* species. However, we still think *Melanoseris* and *Lactuca* are two separate but closely related genera based on previous studies. The newly identified African endemic species could be treated as a new genus, though more evidence is still needed.
2. We confirm the primary and secondary lettuce gene pool and modify the tertiary gene pool concept: adding *L. orientalis* and three *L. viminea* subspecies to the tertiary gene pool while excluding *L. sibirica* and *L. tatarica*.
3. *L. indica*, *L. orientalis* and *L. viminea* could be useful genetic resources for lettuce breeding.
4. *L. undulata* should be transferred from section *Micranthae* to the section *Lactuca* subsect. *Cyanicae* based on our molecular data and its chromosome number.
5. There are at least two independent origins of the scandent habit in Lactucinae.

Although the sampling used in this study only covers 34 % of the total known *Lactuca* species, we provide the most extensive molecular sampling for *Lactuca* species to date. Until now, most species in *Lactuca* have never been revised or sequenced since they were published. In the future, we will sample more species and use whole chloroplast genome data to resolve the polytomy in *Lactuca*.

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