EDINBURGH JOURNAL OF BOTANY 67 (2): 235–264 (2010)

doi:10.1017/S0960428610000065

MOLECULAR PHYLOGENY OF THE EDELWEISS (*LEONTOPODIUM*, ASTERACEAE – GNAPHALIEAE)

C. Blöch[1], W. B. Dickoré[2], R. Samuel[1] & T. F. Stuessy[1]

Leontopodium is a genus of approximately 30 species with a conspicuous Asian–European disjunct distribution. In this study samples from the Himalayan/Tibetan centre of diversity of the genus, as well as from Europe, were analysed to infer a phylogeny of the genus using sequences of nuclear ribosomal (ITS and ETS) and plastid (*mat*K and *trn*L/F) DNA. The Southeast Tibetan monotypic *Sinoleontopodium* [*Leontopodium lingianum* (Y.L.Chen) Dickoré, *comb. nov.*] falls into *Leontopodium*. Monophyly of *Leontopodium*, including *Sinoleontopodium*, is supported. Due to low rates of sequence divergence, intrageneric relationships in general are weakly supported, a pattern frequently observed in plant groups centred in the Tibetan Plateau. Three phylogenetic groups can be identified, however, and these are also supported by morphology. The low levels of nucleotide divergence suggest a young age for the group, which has been influenced by the turbulent geological history of the Tibetan Plateau. *Leontopodium* is a characteristic Sino-Himalayan element that appears to have found its way into the mountains of Europe in geologically recent times. The two European taxa, *Leontopodium alpinum* and *L. nivale*, form a genetically distinct group, which, considering the wide geographic disjunction, shows surprisingly little divergence from its Asian relatives.

Keywords. Asteraceae, ETS, Gnaphalieae, ITS, *Leontopodium*, *mat*K, molecular phylogeny, Sino-Himalayan flora, *Sinoleontopodium*, *trn*L/F.

Introduction

The alpine edelweiss (*Leontopodium alpinum* Cass. or *L. nivale* subsp. *alpinum* (Cass.) Greuter*) holds an important position in the cultural heritage of the European Alps. Starting from a tradition in folk medicine, phytochemical research has revealed the presence of various new or uncommon secondary compounds, some of which are of high biological activity (Grey *et al.*, 1999; Stuppner *et al.*, 2002; Dobner *et al.*, 2003a, 2003b, 2004; Schwaiger *et al.*, 2004). The present study has evolved under the growing need for an applicable taxonomy and systematics of the genus for further pharmaceutical research.

[1] Department of Systematic and Evolutionary Botany, Faculty Centre of Biodiversity, University of Vienna, Rennweg 14, 1030 Wien, Austria. E-mail for correspondence: cordula.bloech@univie.ac.at
[2] Department of Vascular Plants, Botanische Staatssammlung, Menzinger Str. 67, 80638 München, Germany.

* Although the alternative ranks are given here, this taxon is treated at species level in the rest of this paper.

Leontopodium (Pers.) R.Br. is a genus containing about 120 published binomials. Based on the earlier work of Franchet (1892) and Beauverd (1909, 1910, 1911, 1912, 1914), Handel-Mazzetti (1927) completed the only monograph to date, in which he accepted 41 species. Several new taxa have subsequently been described (e.g. Voroshilov, 1979; Akiyama, 1999). We found the species concepts of Handel-Mazzetti (1927) generally sound. However, several taxa he accepted, and almost all of those described since, are probably better reduced to synonymy. Many were known only from their types and, on account of more recent collections, are now known to fall within the often wide morphological variation of other species. Ongoing studies suggest there are fewer species, perhaps as few as 30 (W. B. Dickoré, unpubl.).

Most taxonomic treatments of *Leontopodium* in modern Asian Floras, such as the *Flora of the USSR* (Shishkin, 1999), *Flora Iranica* (Rechinger, 1980), *Flora of India* (Hajra *et al.*, 1995), *Flora of Pakistan* (Qaiser & Abid, 2003), *Flora of Japan* (Iwatsuki *et al.*, 1995), *Flora of Bhutan* (Grierson & Long, 2001) and for Central Asia (Grubov, 2003), do not seem fully satisfactory, because of their rather incompatible regional viewpoints.

Except for two taxa in the mountains of Central and Southern Europe (*Leontopodium alpinum* and *L. nivale*; author abbreviations for species are given in Table 1), the genus occupies an extensive and largely continuous distribution in the mountains of Central, temperate Southeastern, and Eastern Asia. This wide generic disjunction is unusual (Meusel & Jäger, 1992). Whereas *Leontopodium alpinum* is well known due to ample work on various aspects of its biology, ecology, chorology, and secondary chemistry (e.g. Uexküll-Gyllenband, 1901; Sokolowska-Kulczycka, 1959; Maugini, 1962; Tira *et al.*, 1970; Erhardt, 1993; Hook, 1994; Stuppner *et al.*, 2002), very little is known about the Asian members of the genus.

This study employs DNA sequences to understand affinities among species of *Leontopodium* and among some possible close generic relatives. More than two-thirds of the species of *Leontopodium* are sampled, hence providing a good taxonomic and geographical representation, plus taxa from related genera of Gnaphalieae and more distantly related taxa from other tribes of Asteraceae. Two nuclear ribosomal DNA markers (internal transcribed spacer (ITS) and external transcribed spacer (ETS)) and three plastid markers (*mat*K, *trn*L intron, and *trn*L/F intergenic spacer) were investigated. ITS1 and ITS2 have frequently been used for phylogenetic studies in Asteraceae especially at the generic level (Clevinger & Panero, 2000; Samuel *et al.*, 2003, 2006; Kimball & Crawford, 2004; Plovanich & Panero, 2004; Tremetsberger *et al.*, 2005), and for phylogenetic studies in Gnaphalieae (*Antennaria* Gaertn., Bayer *et al.*, 1996; *Anaphalioides* (Benth.) Kirp., Glenny, 1997; Australasian Gnaphalieae, Breitwieser *et al.*, 1999; Mediterranean *Helichrysum* Mill., Galbany-Casals *et al.*, 2004a). The ETS region of the 35S rDNA cistron belongs to the same functional unit as ITS (Baldwin & Markos, 1998), but so far ETS has been used less frequently (e.g. Baldwin & Markos, 1998; Clevinger & Panero, 2000; Plovanich & Panero, 2004). The *mat*K gene, which is one of the fastest

TABLE 1. Species names, localities, collectors and voucher numbers, and GenBank accession numbers of the analysed taxa. All vouchers are deposited in WU unless stated otherwise. Collectors are abbreviated as follows: AT = A. Tribsch; BD = B. Dickoré; EH = E. Hörandl; FE = F. Essl; GM = G. Miehe; GS = G. Schneeweiss; I = Iwatsuki; JR = J. F. Rock; K = Kleesadl; KR = K. Reiter; LK = L. Klimes; MD = M. Dobner; MW = M. Wiedermann; N = Narantuja; PS = P. Schönswetter; SE = S. Ertl; SM = S. Miehe; S = Smith; UW = U. Wündisch; WT = W. Till; Y = Yamazaki. Voucher information for sequences drawn from GenBank can be found in the indicated references

Species	Accession	Locality	Collector, collection number and herbarium acronym	ITS	ETS	*mat*K	*trn*L intron	*trn*L/F	Reference
Leontopodium alpinum Cass.	1	Italy, Piemonte	GS & PS 8914	FJ39910	–	–	–	–	This study
	2	Spain, Aragon	GS & PS 8959	FJ639911	–	–	–	–	This study
	3	Spain, Aragon	GS & PS 8881	FJ639912	–	–	–	–	This study
	4	Italy, Lombardia, Sondrio	MW 9282	FJ639913	FJ639981	–	–	–	This study
	5	France, Alpes de Haute Provence	MW 9029	FJ639914	–	–	–	–	This study
	6	Italy, Alto Aldige	PS & AT 9016	FJ639915	–	–	–	–	This study
	7	France, Savoie	SE 9377	FJ639916	–	–	–	–	This study
	8	Italy, Lombardia	GS & PS 8940	FJ639917	–	–	–	–	This study
	9	Italy, Cuneo	MW 9197	FJ639918	–	–	–	–	This study
	10	Austria, Kärnten	PS 9059	FJ639919	–	–	–	–	This study
	11	Italy, Trento	GS & PS 8809	FJ639920	–	FJ640024	–	–	This study
	12	–		–	–	–	–	AF141821	Bayer *et al.*, 2002
	13	–		–	–	–	AF141733	–	Bayer *et al.*, 2002

TABLE 1. (*Cont'd*)

Species	Accession	Locality	Collector, collection number and herbarium acronym	ITS	ETS	*mat*K	*trn*L intron	*trn*L/F	Reference
L. andersonii C.B.Clarke	1	China, Yunnan	MD et al. MD01-2394	FJ639921	–	–	–	–	This study
	2	China, Yunnan	BD 14068	FJ639922	FJ640006	–	–	–	This study
	3	China, Yunnan	BD 14570 (B, WU)	–	FJ640007	–	–	–	This study
	4	China, Yunnan	I et al. s.n. (BM)	–	FJ640008	FJ640038	–	–	This study
L. artemisiifolium (H.Lév.) Beauverd	1	China, Yunnan	MD et al. D01-2396	FJ639923	–	–	–	–	This study
	2	China, Yunnan	BD 14574	FJ639924	FJ640009	–	–	–	This study
	3	China, Yunnan	MD et al. D01-2384	FJ639925	FJ639982	–	FJ640041	FJ639971	This study
L. caespitosum Diels	1	China, Yunnan	BD 14040	FJ639926	FJ640010	–	–	–	This study
	2	China, Yunnan	MD et al. MD01-2354	FJ639927	FJ640011	FJ640025	–	–	This study
L. calocephalum (Franch.) Beauverd	1	China, Yunnan	MD et al. MD01-2395	FJ639929	FJ640012	–	–	–	This study
	2	China, Sichuan	UW 94-488-5	FJ639928	FJ639994	FJ640026	FJ640042	FJ639972	This study
L. dedekensii (Bureau & Franch.) Beauverd	1	China, Yunnan	MD et al. MD01-2358	FJ639930	FJ640001	–	FJ640043	FJ639973	This study
	2	China, Yunnan	BD 14159 (B, WU)	FJ639932	FJ640002	–	–	–	This study
	3	China, Tibet/Xizang	BD 8664 (B)	FJ639931	–	–	–	–	This study

TABLE 1. (*Cont'd*)

L. discolor Beauverd		–		AF002168	–	–	–	–	Glenny & Wagstaff, 1997
L. franchetii Beauverd	1	China, Yunnan	MD et al. MD01-2411	FJ639933	–	–	–	–	This study
	2	China, Yunnan	BD 14499	FJ639934	FJ639983	–	–	–	This study
	3	China, Yunnan	BD 14495	FJ639935	FJ640003	–	–	–	This study
L. haastioides Hand.-Mazz.	1	China, Tibet/Xizang	BD 9538 (B)	FJ639936	–	–	–	–	This study
	2	China, Tibet/Xizang	BD 9892 (B, WU)	FJ639937	FJ640013	–	–	–	This study
L. himalayanum DC.	1	China, Tibet/Xizang	BD 5228 (B, WU)	FJ639938	FJ640004	–	FJ640044	FJ639974	This study
	2	China, Tibet/Xizang	BD 9945 (B)	FJ639939	–	–	–	–	This study
L. jacotianum Beauverd	1	Nepal, Langtang	KR 571	FJ639941	–	–	–	–	This study
	2	Nepal, Langtang	KR 25	–	–	–	–	–	This study
	3	China, Sichuan	MD et al. MD01-2445	FJ639940	FJ639995	–	FJ640046	FJ639976	This study
	4	Nepal, Langtang	KR518	FJ639942	–	–	–	–	This study
	5	China, Tibet/Xizang	BD 9167 (B)	–	FJ64001	–	–	–	This study
L. japonicum Miq.		Japan, Shinano	T 1228 (W)	–	FJ639984	–	–	–	This study
L. leontopodinum (DC.) Hand.-Mazz.		India, Jammu & Kashmir	Southampton University 51 (BM)	–	FJ639987	–	–	–	This study
L. leontopodioides (Willd.) Beauverd	1	China, Qinghai	GM & SM 9315/12 (B)	FJ639943	–	–	–	–	This study
	2	China, Gansu	JR 12239 (BM)	–	FJ639986	–	–	–	This study
	3	Mongolia, Hövgöl Aimak	N S-070800 (BM)	FJ639944	FJ640014	FJ640027	FJ640045	FJ639975	This study

TABLE 1. (*Cont'd*)

Species	Accession	Locality	Collector, collection number and herbarium acronym	ITS	ETS	*mat*K	*trn*L intron	*trn*L/F	Reference
L. microphyllum Hayata	1	Taiwan, Hualien	EH et al. 9549	FJ639946	FJ640015	FJ640040	FJ640047	FJ639977	This study
	2	Taiwan, Hualien	EH et al. 9555	FJ639947	FJ640016	FJ640039	–	–	This study
	3	Taiwan, Nanhuta shan	Y et al. 303 (BM)	–	FJ639989	–	–	–	This study
L. nanum Hand.-Mazz.	1	China, Xizang, Nagarze, Pomo Co.	BD 9539 (B)	FJ639951	FJ640005	–	–	–	This study
	2	China, Qinghai	GM et al. 98-34117 (B)	FJ639948	–	FJ640029	FJ640048	FJ639978	This study
L. nivale (Ten.) Huet ex Hand.-Mazz.	1	Italy, Abruzzo	GS & PS 8926	FJ639950	FJ640017	FJ640030	–	–	This study
	2	Italy, Abruzzo	EH et al. 6244	FJ639949	–	–	–	–	This study
L. ochroleucum Beauverd	1	India, Jammu & Kashmir	LK 03-21-30	FJ639954	FJ639997	FJ640032	–	–	This study
	2	Russia, Altay	AT 9523	FJ639952	FJ639996	FJ640031	–	–	This study
	3	Russia, Altay	AT 9594	FJ639953	FJ639990	FJ640033	–	–	This study
L. pusillum (Beauverd) Hand.-Mazz.	1	China, Tibet/Xizang	GM et al. 97-073-06 (B)	FJ639955	–	–	–	–	This study
	2	China, Qinghai	BD 4425 (B, WU)	FJ639956	–	–	–	–	This study
	3	China, Qinghai	GM et al. 98-35212 (B)	–	FJ640018	FJ640034	–	–	This study

TABLE 1. (*Cont'd*)

L. sinense Hemsl.	1	China, Yunnan	MD et al. MD01-2397	FJ639958	FJ639999	–	–	–	This study
	2	China, Yunnan	BD 14534 (B)	FJ639957	FJ640000	FJ640035	–	–	This study
	3	China, Sichuan	S 12219 (BM)	–	FJ639991	–	–	–	This study
L. souliei Beauverd		China, Yunnan	MD et al. MD01-2404	FJ639959	FJ639998	FJ640036	FJ640049	FJ639979	This study
L. stracheyi (Hook.f.) C.B.Clarke ex Hemsl.	1	China, Tibet/Xizang	GM & SM 98-09509 (B)	FJ639960	FJ640020	FJ640037	–	–	This study
	2	China, Tibet/Xizang	BD 3404 (B)	–	FJ640021	–	–	–	This study
L. cf. *stracheyi* (Hook.f.) C.B.Clarke ex Hemsl.	3	China, Tibet/Xizang	BD 10529 (B)	FJ639961	FJ640022	FJ640028	–	–	This study
Sinoleontopodium lingianum Y.L.Chen	1	China, Tibet/Xizang	BD 10836	–	FJ639988	–	–	–	This study
	2	China, Tibet/Xizang	BD 11363 (B, WU)	FJ639945	–	–	–	–	This study
OUTGROUPS									
Achillea millefolium L.	1	–		–	–	–	EU385030	EU385030	Panero & Funk, 2008
	2	–		–	–	EU385315	–	–	Panero & Funk, 2008
Anaphalioides subrigida (Colenso) Anderb.		–		U95279	–	–	–	–	Glenny & Wagstaff, 1997

TABLE 1. (*Cont'd*)

Species	Accession	Locality	Collector, collection number and herbarium acronym	ITS	ETS	*mat*K	*trn*L intron	*trn*L/F	Reference
Anaphalis javanica Sch.Bip. in Zoll.		–		U95292	–	–	–	–	Glenny & Wagstaff, 1997
Anaphalis margaritacea Benth. & Hook.f.		–		AF046937	–	–	–	–	Noyes & Rieseberg, 1999
Anaphalis nubigena DC.		India, Jammu & Kashmir, Ladakh	LK 04-01-9	FJ639962	FJ640023	–	–	–	This study
Antennaria anaphaloides Rydb.		–		L40857	–	–	–	–	Bayer *et al.*, 1996
Antennaria carpatica (Wahlenb.) Bluff & Fingerh.		Austria, Salzburg	EH Hö3-30.8.88	FJ639965	–	–	–	–	This study
Antennaria dioica (L.) Gaertn.	1	Austria, Niederösterreich	WT s.n.	FJ639964	FJ639985	–	–	–	This study
	2	Russia, Altay	AT & FE 004588-8978	–	FJ639992	–	FJ640050	FJ639980	This study
Antennaria luzuloides Torr. & Gray		–		–	–	AF456774	–	–	Bayer *et al.*, 2000
Castroviejoa frigida (Labill.) Galbany, L.Sáez & Benedi		–		AY445228	–	–	–	–	Galbany-Casals *et al.*, 2004a

TABLE 1. (*Cont'd*)

Castroviejoa montelinasana (Em.Schmid) Galbany, L.Sáez & Benedi		–	AY445229	–	–	–	–	Galbany-Casals *et al.*, 2004a
Ewartia argentifolia N.A.Wakef.		–	AF115910	–	–	–	–	Breitwieser *et al.*, 1999
Ewartia catipes (Hook.f.) Beauverd	1	–	–	–	AF151460	AF141698	AF141786	Bayer *et al.*, 2002
	2	–	–	FJ404694	–	–	–	R. D. Smissen, unpubl.
	3	–	U95290	–	–	–	–	Glenny & Wagstaff, 1997
Ewartia meredithiae (F.Muell.) Beauverd		–	AF115907	–	–	–	–	Breitwieser *et al.*, 1999
Ewartia planchonii (Hook.f.) Beauverd		–	AF115909	–	–	–	–	Breitwieser *et al.*, 1999
Ewartiothamnus sinclairii (Hook.f.) Anderb.		–	U95283	–	–	–	–	Glenny & Wagstaff, 1997
Filago pyramidata L.		–	AY445231	–	–	–	–	Galbany-Casals *et al.*, 2004a
Gamochaeta pensylvanica (Willd.) Cabrera		–	–	–	–	EU385070	EU385070	Panero & Funk, 2008

TABLE 1. (*Cont'd*)

Species	Accession	Locality	Collector, collection number and herbarium acronym	ITS	ETS	*mat*K	*trn*L intron	*trn*L/F	Reference
Gamochaeta spicata (Lam.) Cabrera		–		AF115917	–	–	–	–	Breitwieser *et al.*, 1999
Gnaphalium hoppeanum (L.) Hilliard & B.L.Burtt		Italy, Lombardia	PS & AT 5382	FJ639967	–	–	–	–	This study
Gnaphalium norvegicum Gunnerus		Austria, Oberösterreich	K s.n.	FJ639968	–	–	–	–	This study
Gnaphalium supinum L.		–		AY445230	–	–	–	–	Galbany-Casals *et al.*, 2004a
Gnaphalium sylvaticum L.		Austria, Niederösterreich	WT s.n.	FJ639969	FJ639993	–	–	–	This study
Helichrysum leucopsideum DC.		–		–	AF319712	–	–	–	Bayer *et al.*, 2002
Leucogenes grandiceps (Hook.f.) Beauverd	1	New Zealand, Canterbury	PS et al. 004137-8982a	FJ639970	–	–	–	–	This study
	2	–		–	–	–	AY606885	AY606896	Smissen *et al.*, 2004

TABLE 1. (*Cont'd*)

Leucogenes leontopodium (Hook.f.) Beauverd		–	AF543357	–	–	–	–		Smissen *et al.*, 2003
Leysera gnaphalodes (L.) L.	1	–	–	–	–	–	AF100473		Bayer *et al.*, 2000
	2	–	–	–	AF456793	–	–		Bayer & Cross, 2003
Pseudognaphalium luteoalbum (L.) Hilliard & B.L.Burtt		–	AY445227	–	–	–	–		Galbany-Casals *et al.*, 2004a
Oedera squarrosa (L.) Anderb. & K.Bremer	1	–	–	–	–	–	AF100475		Bayer *et al.*, 2000
	2		–	–	AF456795	–	–		Bayer & Cross, 2003
Relhania calycina L.Hér.		–	–	–	EU385392	EU385107	EU385107		Panero & Funk, 2008
Solidago rugosa Mill.		–	–	–	EU749421	–	–		Fazekas *et al.*, 2008
Symphyotrichum novae-angliae (L.) G.L.Nesom		–	–	–	–	–	U82019		Bayer & Starr, 1998

evolving genes of the chloroplast genome (Wolfe, 1991), is frequently employed for inferring plant phylogeny at the intergeneric level and above. Within Asteraceae *mat*K, *trn*L intron, and *trn*L/F spacer have been used to infer higher level phylogenies, for example the *trn*L intron and *trn*L/F spacer at the tribal level (Bayer & Starr, 1998), *mat*K for South African Gnaphalieae (Bayer *et al.*, 2000), and *mat*K, *trn*L intron, and *trn*L/F spacer for Australian Gnaphalieae (Bayer *et al.*, 2002). We employed these plastid markers to reveal intergeneric relationships involving *Leontopodium* and relatives.

The aims of this molecular study of *Leontopodium*, therefore, were to (1) elucidate phylogenetic relationships among species within the genus (including monophyly and infrageneric classification), (2) clarify relationships with other genera, including the recently described monotypic *Sinoleontopodium* Y.L.Chen (Chen, 1985; Chen & Yang, 2009), and (3) address the origin and evolutionary history of the alpine edelweiss (*L. alpinum*), a prominent taxon of the European mountain flora.

Materials and Methods

Sampling

We have included in our study 22 species of *Leontopodium* in 65 accessions (Table 1) from a broad geographic range and with a good representation from the Himalayan/Tibetan centre of diversity. Less material was available from North and East Asia. Since many taxa occur only in remote areas, most DNA samples were obtained from herbarium specimens. Silica gel dried material from natural populations was available only from the European taxa (*Leontopodium alpinum*, *L. nivale*) and from the Taiwanese *L. microphyllum*. Vouchers are deposited in B and WU. Additional sequences, mostly of outgroup taxa, were drawn from the NCBI GenBank (Table 1).

Several outgroup taxa were included to investigate the position of *Leontopodium* within Gnaphalieae: *Anaphalioides subrigida*, *Anaphalis javanica*, *A. margaritacea*, *A. nubigena*, *Antennaria anaphaloides*, *A. carpatica*, *A. dioica*, *A. luzuloides*, *Castroviejoa frigida*, *C. montelinasana*, *Ewartia argentifolia*, *E. catipes*, *E. meredithiae*, *E. planchonii*, *Ewartiothamnus sinclairii*, *Filago pyramidata*, *Gamochaeta pensylvanica*, *G. spicata*, *Gnaphalium hoppeanum*, *G. norvegicum*, *G. supinum*, *G. sylvaticum*, *Helichrysum leucopsideum*, *Leucogenes grandiceps*, *L. leontopodium* and *Pseudognaphalium luteoalbum*. In addition, several accessions from the '*Relhania* clade' (Gnaphalieae) and from other Asteraceae tribes were added to root the plastid phylogenies, namely *Leysera gnaphalodes*, *Oedera squarrosa* and *Relhania calycina* (*Relhania* clade, Gnaphalieae), *Achillea millefolium* (Anthemideae), *Solidago rugosa* and *Symphyotrichum novae-angliae* (Astereae).

DNA extraction and amplification

Total genomic DNA was extracted from one leaf of herbarium material with the modified CTAB protocol of Doyle & Dickson (1987). The complete ITS (ITS1, 5.8S

rRNA gene, ITS2), the 3′ part of ETS, a partial sequence of the plastid gene *mat*K and the *trn*L/F region (*trn*L 5′-exon, intron, 3′-exon, intergenic spacer and *trn*F gene) were amplified with the universal primers ITS4, ITS5, ITS2, ITS3 (White *et al.*, 1990), 17SE and 26SE (Sun *et al.*, 1994) for ITS; ETS-Hel-1 and 18SR (Baldwin & Markos, 1998) for ETS; *trn*L-*c* and *trn*L-*c*-1F/*trn*L-F (Taberlet *et al.*, 1991) for *trn*L (UAA) intron and intergenic spacer between *trn*L (UAA), as well as 3′-exon and *trn*F (GAA) and 800F/1710R (Samuel *et al.*, 2005) for a partial *mat*K. Polymerase chain reaction was carried out in a volume of 25 µl, containing 10–50 ng of genomic DNA, 10 pmol of each primer, 1× Reddy Mix TM PCR Master Mix (including 2.5 mM $MgCl_2$; Abgene, Vienna) and 0.04% dimethyl sulfoxide (DMSO; for ITS and ETS) or 0.016% bovine serum albumin (BSA; for *trn*L/F and *mat*K). The PCR profile consisted of an initial pre-melt at 94°C and 35 cycles of 1 min denaturing at 94°C, 30 s annealing at 48°C and 1 min extension at 72°C followed by a final extension of 10 min at 72°C. Amplified fragments were checked on 1% agarose gel.

Sequencing

The amplification product was gel purified using Invitek Invisorb® Spin DNA Extraction Kit (Invitek Biotechnology & Biodesign Ltd, Berlin). The purified fragments were directly sequenced using dye terminator chemistry following the manufacturer's protocol and run on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA). The cycle sequencing reactions were performed with the same primers as used for PCR amplification. The programs Sequence Navigator version 1.0.1 and AutoAssembler version 1.4.0 (Applied Biosystems, Foster City, CA) were used to edit and assemble the complementary sequences. Alignments were obtained using the program Clustal X 1.5b (Thompson *et al.*, 1997) and improved by manual refinement using the program BioEdit 7.09 (Hall, 1999). Pairwise genetic distances were calculated in the program BioEdit (Hall, 1999) using the feature Identity Matrix. Sequences are deposited in GenBank (www.ncbi.nlm.nih.gov).

Phylogenetic analyses

Fitch parsimony analyses (Fitch, 1971) were performed using PAUP* 4.0b10 (Swofford, 2002). Four datasets were analysed: (1) ITS, (2) ETS, (3) partial sequence of *mat*K, and (4) the *trn*L intron sequence and the *trn*L/F intergenic spacer. Heuristic search was conducted with equal weights, 1000 replicates of random sequence addition, tree bisection reconnection (TBR) branch swapping, and MulTrees on permitting 10 trees to be held in each step. Indels were treated as missing data, except for the ETS dataset where an additional parsimony analysis was conducted adding characters from gap coding using the MCIC method (Müller, 2006) as implemented in SequState (Müller, 2005). Trees were rooted in the plastid datasets using outgroups from the Gnaphalieae '*Relhania* clade' (*Leysera* L., *Oedera* L., *Relhania* L'Hér.; Ward *et al.*, 2009) and from more distantly related tribes, namely *Achillea* L.

(Anthemideae) and *Solidago* L. (in the *mat*K dataset) or *Symphyotrichum* Nees (in the *trn*L/F dataset; both Astereae). The root for the nuclear markers was chosen using results of the plastid *trn*L/F dataset, in which *Ewartia* Beauverd was not sister to *Leontopodium*. To test the monophyly of *Leontopodium*, the following putatively close relatives from Gnaphalieae (according to Merxmüller *et al.*, 1977; Anderberg, 1991; Bayer *et al.*, 1996; Glenny & Wagstaff, 1997; Breitwieser *et al.*, 1999) were included: *Anaphalis* DC. (ITS, ETS), *Anaphalioides* (ITS), *Antennaria* (ITS, ETS, *mat*K, *trn*L/F), *Castroviejoa* Galbany, L.Sáez & Benedí (a recently recognised segregate of *Helichrysum*; ITS), *Ewartia* (ITS, ETS, *mat*K, *trn*L/F), *Ewartiothamnus* Anderb. (ITS), *Filago* L. (ITS), *Gamochaeta* Wedd. (ITS, *trn*L/F), *Gnaphalium* L. (ITS, ETS), *Helichrysum* (ETS), *Leucogenes* Beauverd (ITS, *trn*L/F), and *Pseudognaphalium* Kirp. (ITS). Nodal support was assessed via bootstrap values (Felsenstein, 1985), which were calculated using PAUP* 4.0b10 with 10,000 bootstrap replicates each with 20 random sequence addition replicates holding a maximum of 10 trees per replicate, SPR branch swapping, and MulTrees on.

To find the best-fit substitution models for the Bayesian analysis, all four datasets were tested using MrModeltest 2.2 (Nylander, 2005). The following partitionings were used for the analysis: (1) the ITS region was split into two partitions (the genic and the spacer regions of the ribosomal cistron) with GTR+I and GTR+G substitution models; (2) the *mat*K/*trn*K region was also split into two partitions (the genic partial *mat*K and the partial intron of the *trn*K gene) with GTR+G and F81 substitution models; (3) the *trn*L/F region was split into three partitions (the *trn*L intron, the *trn*L gene and the *trn*L/F spacer) with F81+G, the Jukes-Cantor, and GTR+G substitution models. For the ETS dataset Bayesian analysis was performed using the HKY+G with Mr Bayes (Ronquist & Huelsenbeck, 2003), running four simultaneous Markov chains for 10,000,000 generations using four chains. Trees were sampled after every 1000 generations with a total of 40,000 trees. The first 10% of trees was discarded to ensure that sufficient allowance was made for the chains to become stationary, thereby reducing the number of trees to 36,000.

RESULTS

Nuclear markers

The data matrix of the combined ITS region (ITS1, 5.8S, ITS2) included 22 ingroup taxa in 53 accessions (including one accession of *Sinoleontopodium*) and 23 outgroup accessions from 11 currently accepted genera (see Table 2 for analysis statistics and Table 3 for sequence statistics). Pairwise genetic distance in the complete ITS region (ITS1, 5.8S, ITS2) ranged from 0.2% to 6.8% among the *Leontopodium* species. Pairwise sequence divergences between ingroup and outgroup were between 6.2% and 17%. Maximum parsimony and Bayesian analysis did show congruent results, with the latter providing considerably more resolved nodes. Therefore, phylogenetic

TABLE 2. Sequence statistics for ITS, ETS, *mat*K and *trn*L/F

Marker	Length	Var. char. (%)	PICs (%)	CI	RI	No. of trees	Tree length
ITS	649	30.8	21.4	0.68	0.88	710	381
ETS	400/415[a]	35.8/38.3[a]	13/13.5[a]	0.9/0.91[a]	0.86/0.87[a]	9540/9200[a]	181/202[a]
Partial *mat*K	829–832	1.8/8.1[b]	0.84/3.6[b]	1/0.9[b]	1/0.9[b]	11/31[b]	15/79[b]
*trn*L intron, *trn*L/F spacer	792	2.6/13.4[b]	0.5/3.5[b]	1/0.94[b]	1/0.91[b]	1/8[b]	22/125[b]

[a]Including coded gap characters.
[b]Including outgroups from tribes Anthemideae, Astereae and Gnaphalieae (*Relhania* clade).
Abbreviations: var. char. = number of variable characters; PICs = number of parsimonious informative characters; CI = Consistency Index; RI = Retention Index.

results of the ITS region are presented showing the Bayesian majority rule consensus tree (Fig. 1).

Leontopodium was supported as monophyletic, with the inclusion of *Sinoleontopodium lingianum*, the only species in *Sinoleontopodium*, with 100% bootstrap support (BS) and 1.00 posterior probability (PP) (Fig. 1). Some weakly (PP $\geq$ 0.70) supported clades could be identified (clades A–C, Fig. 1), although the ingroup was not well resolved. Sister to the ingroup (BS 60, PP 0.91) were *Antennaria*, *Castroviejoa*, *Gnaphalium*, *Filago* and *Gamochaeta* (OG I). Sister (BS 87, PP 0.98) to *Leontopodium* and the taxa of OG I were *Anaphalis* and *Pseudognaphalium luteoalbum*, which together formed OG II (BS 100/PP 1.00). A third group of outgroup taxa comprised the Australian and New Zealand Gnaphalieae: *Anaphalioides*, *Ewartia*, *Ewartiothamnus* and *Leucogenes* (BS 87, PP 0.98; OG III).

The dataset of the ETS consisted of 39 ingroup accessions of 22 species and six outgroup accessions of five genera, namely *Anaphalis*, *Antennaria*, *Gnaphalium*, *Ewartia* and *Helichrysum* (Fig. 2). Generally, sequence divergence within the ETS dataset (Tables 2, 3) was extremely low except for *Helichrysum* and *Ewartia*. *Antennaria dioica* and *Gnaphalium sylvaticum* were nested within the ingroup. One clade (BS 57, PP 0.91) shared almost identical sequences and included *Antennaria*

TABLE 3. ITS and ETS sequence characteristics showing length of the different ITS and ETS regions and GC content for ingroup and outgroup

Region	Length (aligned)	Length ingroup	Length outgroup	GC mean (%)	GC ingroup (%)	GC outgroup (%)
ITS1	257	252	251–254	43.7	42.7–45.5	41.5–45.5
5.8S	254	254	254	52.2	52.5	52.16
ITS2	228	214–217	214–223	50.9	50.23–51.63	45.2–53.6
ETS	408	260–390	377–389	43.2	42–43.8	42.7–43.9

FIG. 1. Phylogenetic relationships of species of *Leontopodium* and related genera of tribe Gnaphalieae inferred from Bayesian and maximum parsimony analysis of the nuclear ITS region. Branches collapsing in the strict consensus tree of 710 equally most parsimonious trees are indicated by arrowheads. Bootstrap values/posterior probabilities are indicated at the nodes. Clades discussed in the text are marked with A–C and OG I–OG III.

dioica, *Gnaphalium sylvaticum*, as well as most *Leontopodium* species (*L. alpinum*, *L. artemisiifolium*, *L. franchetii*, *L. japonicum*, *L. leontopodinum*, *L. leontopodioides*, *L. microphyllum*, *L. nivale*, *L. ochroleucum*, *L. pusillum*, *L. sinense*) and *Sinoleontopodium lingianum*. Two groups that were supported in the ITS analysis could be identified in the ETS analysis as well: Group B′ (BS 74, PP 1.00) showed almost

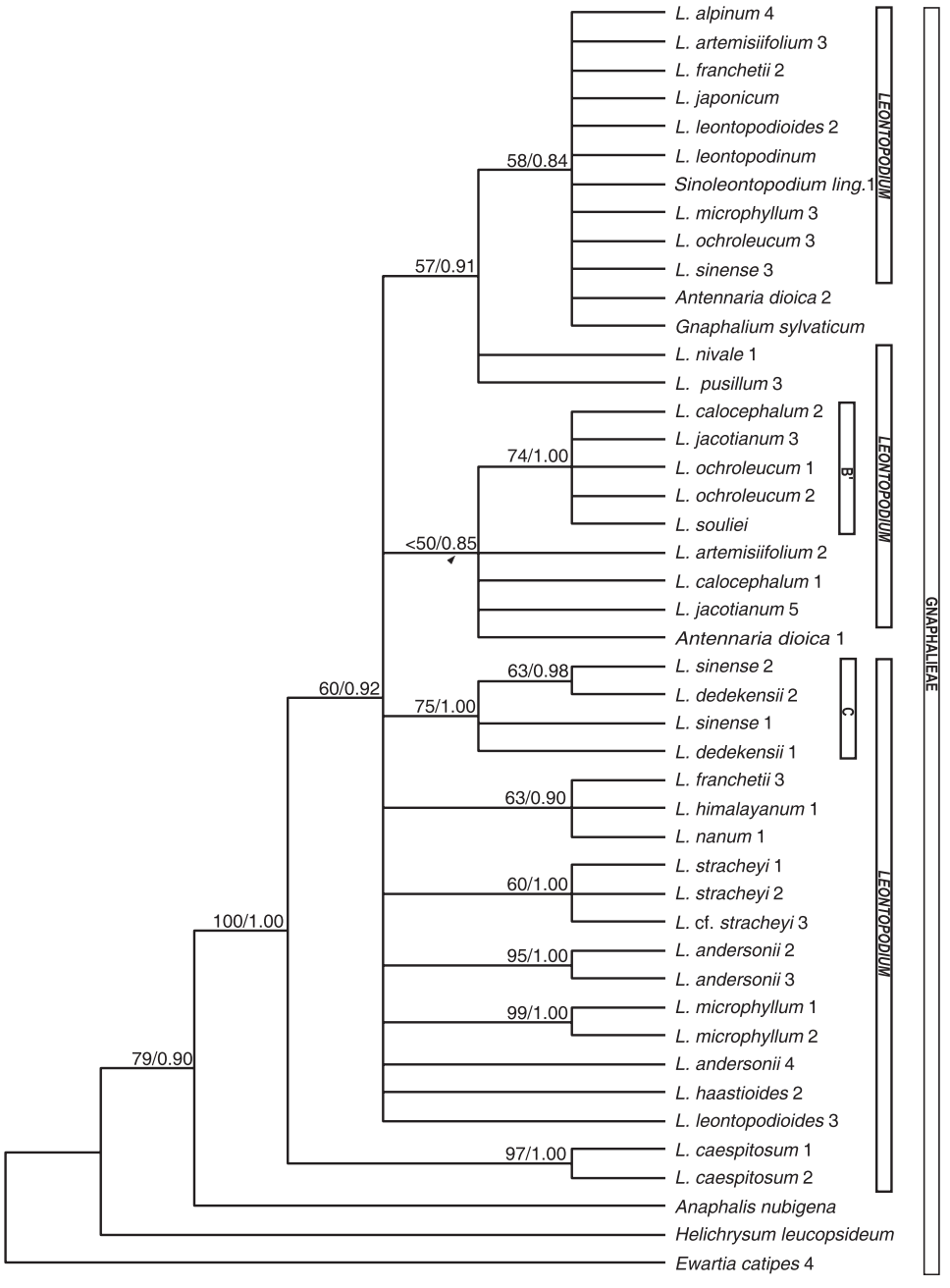

FIG. 2. Phylogenetic relationships of species of *Leontopodium* and related genera of tribe Gnaphalieae inferred from Bayesian and maximum parsimony analysis of the nuclear 3′ ETS region. Branches collapsing in the strict consensus tree of 9200 equally most parsimonious trees are indicated by arrowheads. Bootstrap values/posterior probabilities are indicated at the nodes. Clades discussed in the text are marked with B′ and C.

identical sequences and included *Leontopodium calocephalum*, *L. ochroleucum*, *L. souliei* and *L. jacotianum* (the last came out in a different position in the ITS analysis). Group C contained *Leontopodium sinense* and *L. dedekensii* (BS 75, PP 1.00) which share almost identical sequences. *Leontopodium caespitosum* appeared as sister to the ingroup comprised of *Leontopodium*, *Antennaria* and *Gnaphalium* (which were supported with BS 60, PP 0.92). The ingroup was supported with BS 100, PP 1.00, when rooted with *Ewartia*, having *Helichrysum leucopsideum*, *Ewartia catipes* and *Anaphalis nubigena* as clearly defined outgroups.

Plastid markers

Both plastid markers used in this study (*mat*K, *trn*L intron–*trn*L/F spacer) had very low sequence divergences, with ingroup sequences being identical or almost identical. The *mat*K dataset comprised 17 accessions of the ingroup and two outgroup accessions from the Gnaphalieae, namely *Antennaria luzuloides* and *Ewartia catipes* (for analysis details refer to Table 2). Furthermore, five outgroup accessions from other tribes of Asteraceae were included.

The two outgroup genera from Gnaphalieae were clearly separated by three synapomorphic substitutions as compared to *Leontopodium*. Considering the low variation within the whole dataset, these substitutions provide further support for monophyly of the ingroup (BS 77, PP 0.98; Fig. 3).

Within *Leontopodium*, one group consisting of *L.* cf. *stracheyi* (3), *L. ochroleucum* (1 and 2), *L. andersonii* (4), and *L. leontopodioides* (3) was supported (BS 64, PP 0.98). However, the relevance of this group might be in doubt as they share only a single nucleotide substitution not present in other *Leontopodium* accessions. This well-supported group, which is not supported by either morphology or chorology, may be an artefact of the low sequence divergence. *Leontopodium microphyllum* appears sister to the remainder of *Leontopodium* (BS 52, PP 0.88).

The combined dataset of the *trn*L intron and the *trn*L/F spacer region included 10 ingroup accessions and three additional accessions from the crown group of the Gnaphalieae (Ward *et al.*, 2009), namely *Antennaria carpatica*, *Ewartia catipes* and *Leucogenes grandiceps*. The trees were rooted using three accessions from the '*Relhania* clade' of Gnaphalieae (Ward *et al.*, 2009) and one accession each from Astereae and Anthemideae (analysis details in Table 2). All accessions of the ingroup had identical sequences for the intron, exon and spacer regions, except for *Leontopodium microphyllum*, which showed two autapomorphic substitutions and one substitution shared with the other included genera of Gnaphalieae. The ingroup was supported by BS 67 and PP 0.99. Sister to *Leontopodium* was *Antennaria dioica* (BS 68, PP 0.97; Fig. 4). *Ewartia*, *Gamochaeta* and *Leucogenes* were together sister to *Antennaria* and *Leontopodium* (BS 97, PP 1.00). The outgroup taxa of Gnaphalieae shared one synapomorphic substitution.

Despite the lack of resolution, the plastid sequences support the monophyly of *Leontopodium*. Furthermore, the sequences from *Leontopodium microphyllum* were

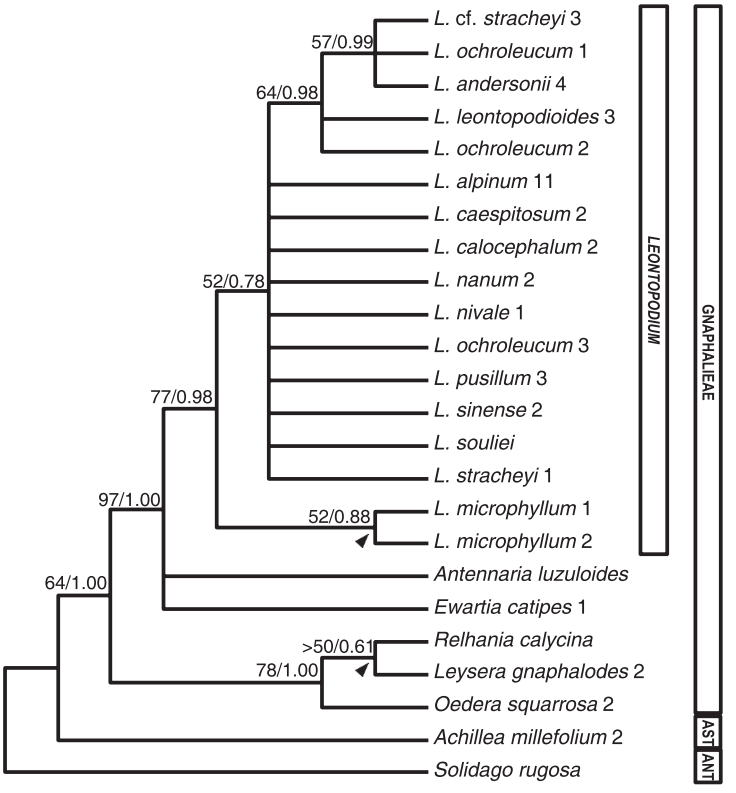

FIG. 3. Phylogenetic relationships of species of *Leontopodium* and related genera of tribe Gnaphalieae, as well as of additional accessions from the tribes Astereae and Anthemideae inferred from Bayesian and maximum parsimony analysis of the partial plastid *mat*K region. Branches collapsing in the strict consensus tree of 31 equally most parsimonious trees are indicated by arrowheads. Bootstrap values/posterior probabilities are indicated at the nodes. AST = Astereae, ANT = Anthemideae.

distinct from the other sequences of the ingroup in both regions. Further infrageneric grouping could not be inferred using these markers. However, it is noteworthy that the ingroup sequences were clearly different from the outgroup sequences of *Antennaria*, *Ewartia*, *Gamochaeta* and *Leucogenes* and that according to the *trn*L/F dataset, *Antennaria* was the closest relative of *Leontopodium* of the four included genera of the crown radiation of Gnaphalieae (according to Ward *et al.*, 2009).

DISCUSSION

Intergeneric relationships

The genus *Leontopodium* is monophyletic if the monospecific *Sinoleontopodium* is included. Three of the four employed markers support its monophyly: ITS (BS 100,

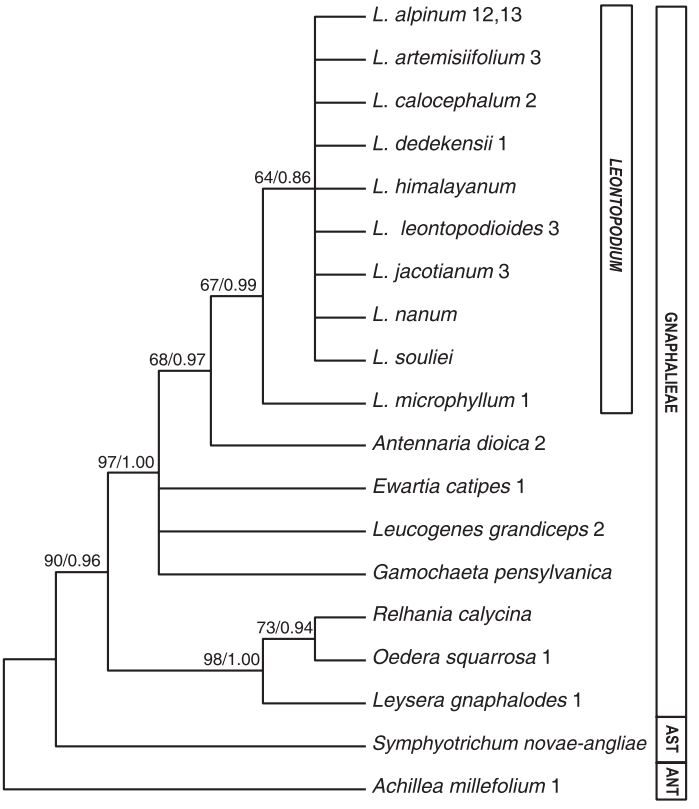

FIG. 4. Phylogenetic relationships of species of *Leontopodium* and related genera of tribe Gnaphalieae, as well as of additional accessions from the tribes Astereae and Anthemideae inferred from Bayesian and maximum parsimony analysis of the plastid *trn*L gene and *trn*L/F spacer region. Branches collapsing in the strict consensus tree of 8 equally most parsimonious trees are indicated by arrowheads. Bootstrap values/posterior probabilities are indicated at the nodes. AST = Astereae, ANT = Anthemideae.

PP 1.00), *mat*K (BS 77, PP 0.98) and *trn*L/F (BS 67, PP 0.99). The ETS dataset included part of *Antennaria* and *Gnaphalium* in the ingroup (BS 100, PP 1.00), probably due to the lack of sequence divergence and homoplasy.

The position of *Sinoleontopodium* within *Leontopodium* is morphologically and chorologically supported and, therefore, the following new combination is made:

Leontopodium lingianum (Y.L.Chen) Dickoré, **comb. nov.** – *Sinoleontopodium lingianum* Y.L.Chen, Novon 19: 24 (2009) [*Sinoleontopodium lingianum* Y.L.Chen, Acta Phytotaxon. Sin. 23: 458, fig. 1 (1985), nom. inval. (two types cited)].

Leontopodium lingianum is a local endemic of the high mountains around the Tsangpo Gorge of Southeast Tibet (Mainling, Mt Namcha Barwa, Mt Gyala Peri), where it occurs at 4400–4850 m in a perhumid alpine belt. *Leontopodium lingianum*

displays distinct morphological adaptations: a dense cushion of columnar shoots; leaves many, imbricate, small, ovate-spathulate, long and densely lanate; calathidia sessile, solitary, without a star. However, all individual characters and its distribution are contiguous to other species of *Leontopodium*. The peculiar habit of *Leontopodium lingianum* is closely matched by *L. aurantiacum*, a species from the Burma/Yunnan border, which was not available for the present study. Complete dioecy, one of the main distinguishing characters of *Sinoleontopodium*, occurs facultatively in *Leontopodium haastioides*, another acaulescent, cushion-forming species from the Central Himalayas, and in several other species of *Leontopodium*. Hirsute corollas, although not to the extent of *Leontopodium lingianum*, are also found in other species of *Leontopodium*. The exact position of *Leontopodium lingianum* within the genus is still unclear; ITS1 data place it within a large polytomy.

Relationships of *Leontopodium* within Gnaphalieae are currently largely unresolved. Based on cladistic analysis of morphological data, Anderberg (1991) concluded that the 'New Zealand edelweiss' *Leucogenes* is sister to *Leontopodium*. This hypothesis was, however, rejected by the molecular phylogenetic analyses of Glenny & Wagstaff (1997) and Breitwieser *et al.* (1999). Based on morphology Merxmüller *et al.* (1977) placed *Leontopodium* within an '*Anaphalis*-group' consisting of *Antennaria*, *Anaphalis* and *Leontopodium*. However, it grouped with *Antennaria* in the molecular study of Glenny & Wagstaff (1997) as sister to *Pseudognaphalium* and *Anaphalis*. The latter findings are partly concordant with Bayer *et al.* (1996) who identified *Antennaria* as sister to *Leontopodium* and both as sister to the Australian–New Zealand genus *Ewartia*. The studies of Ward *et al.* (2009) on the phylogeny of Gnaphalieae, with a focus on African and Australasian taxa, show that relationships within Gnaphalieae are not well resolved, that taxa from the so-called *Relhania* clade are basal to the rest of the Gnaphalieae, and that *Leontopodium* is sister to *Antennaria*. Our combined plastid and nuclear data indicate that *Leontopodium* is closely related to a group comprised of representatives from the bicentric (south and north hemisphere) genus *Gnaphalium*, the northern hemisphere genus *Antennaria*, the newly described genus *Castroviejoa* (Galbany-Casals *et al.*, 2004b), and *Filago*. Accordingly, none of the solely southern hemisphere (mainly Australian–New Zealand) taxa, which form a distinct clade in our study and include *Ewartia* which was sister to *Leontopodium* and *Antennaria* in the study of Bayer *et al.* (1996), is sister to *Leontopodium*. Despite strong morphological similarities with *Leontopodium*, the large genus *Anaphalis*, primarily of temperate East Asian–North American distribution but also with a considerable representation in tropical Indo-Malaysia, seems to be even more distantly related to *Leontopodium* and instead is rather close to *Pseudognaphalium*.

Species delimitation and infrageneric classification

The ITS dataset, and to a lesser degree the other markers, reveals information about infrageneric relationships within *Leontopodium*. Three groups may be recognised but

these do not correspond well to the hierarchical scheme of Handel-Mazzetti (1927). While species delimitations within *Leontopodium* were partly supported by our study (not all taxa were monophyletic), broader infrageneric relationships were not fully resolved, indicating close species relationships and suggesting recent speciation. Hybridisation seems probable in *Leontopodium*, due to nested distributions of species and often contiguous variation, especially in the Himalayas and the Hengduan Shan. We could not confirm any of the hybrids proposed by Handel-Mazzetti (1927). Furthermore, species delimitation in *Leontopodium* seems to be complicated by the possible occurrence of apomixis. This was mentioned, for example, in the *Flora of Pakistan* (Qaiser & Abid, 2003). The only species where apomixis has been studied and found is in *Leontopodium alpinum* (Sokolowska-Kulczycka, 1959; Maugini, 1962). In a review of apomixis in Asteraceae, Noyes (2007) mentions that apomixis within *Leontopodium* has been poorly studied so far. On the basis of available data, therefore, no further conclusion on apomixis can be drawn.

We have identified the following molecular clades of *Leontopodium*, each with morphological integrity:

Group A contains the European taxa (*Leontopodium alpinum*, *L. nivale*). These taxa belong to the type section *Leontopodium* [*Alpina* Hand.-Mazz.]. They are genetically distinct from all Asian species in the ITS analysis, although perhaps not as distinctly separated as might be expected from the geographic distance. In ETS, *mat*K and combined *trn*L intron–*trn*L/F spacer datasets, sequences of the European *Leontopodium alpinum* were the same as for many Asian *Leontopodium* species. In contrast, the similarly geographically isolated *Leontopodium microphyllum* from Taiwan had clearly distinct sequences in all three datasets. The question is still unresolved as to whether the European taxa comprise two distinct species (Handel-Mazzetti, 1927), subspecies (Tutin, 1973; Greuter, 2003), or a series of varieties or unclassifiable forms. We recommend recognising *Leontopodium alpinum* and *L. nivale* at species rank until more sophisticated population-level analyses may clarify their status (S. Safer *et al.*, unpubl.). Similar problems also occur in Asian *Leontopodium*.

Group B, consisting of *Leontopodium calocephalum*, *L. leontopodioides*, *L. ochroleucum* and *L. souliei* (all in *Leontopodium* sect. *Leontopodium* in Handel-Mazzetti, 1927), is moderately supported by ITS and ETS, in the latter with the inclusion of *L. jacotianum*. Morphologically, this group (except *Leontopodium jacotianum*) is quite coherent and close to the European representatives. Except for *Leontopodium jacotianum*, group B species occur mostly in areas with cold, humid or mesic conditions in Central and Northern Asia and are largely absent from the warm and humid to perhumid areas of the Sino-Himalayan region and the dry-cold core of Central Asia. Morphological, ecological and chorological properties would thus make group B species (except for *Leontopodium jacotianum*) candidates for the closest Asian relatives of the European taxa.

Group C, supported by both the ITS and ETS datasets, consists of *Leontopodium sinense* and *L. dedekensii*. Both species belong to *Leontopodium* sect. *Nobilia* (Handel-Mazzetti, 1927) and are very similar in habit and range, being partly sympatric, from subtropical Southwest China to subalpine altitudes of the Eastern Tibetan Plateau. Although *Leontopodium sinense* and *L. dedekensii* are morphologically and ecologically sufficiently distinct, they share identical sequences in all the markers used in this study. Although Handel-Mazzetti's *Leontopodium* sect. *Nobilia* is morphologically well defined, the remaining species of the section are in unsupported positions in both the ETS and ITS phylogeny.

Origin and evolution

In the absence of any fossil record, a hypothesis for the origin of *Leontopodium* needs to take into account our molecular findings plus inferences from present-day ecology, distribution and diversification. In the molecular data two morphologically similar species, *Leontopodium caespitosum* (from the Hengduan Shan) and *L. microphyllum* (Taiwan), were basal, suggesting they are part of an ancestral stock within the genus. The East Himalaya-Hengduan Shan region on the south and east rim of the Tibetan Plateau, home of *Leontopodium caespitosum*, comprises a major hotspot of biodiversity outside the tropics and is renowned for the occurrence of numerous 'relict' taxa (Liu *et al.*, 2006). The island flora of Taiwan, where *Leontopodium microphyllum* occurs, is dominated by paleotropical elements, while its mountains show strong biogeographic parallels to the Himalaya-Hengduan Shan region (Wu & Wu, 1998). The southern edge of the temperate Sino-Japanese (Grisebach, 1872; Good, 1974) or East Asiatic floristic region (Diels, 1901; Takhtajan, 1969) towards the paleotropics of Indo-Malesia includes the highest present-day species diversity in *Leontopodium* and may also have been its centre of origin.

Many species of *Leontopodium* show distinct ecological adaptations to high altitudes and dry-cold conditions, but this may not have been the climate of its origin. In its Southwest Chinese centre of diversity, the genus spans a wide range of elevations and climates. The ecology of *Leontopodium* shows strong links to what is commonly called an 'Arcto-Tertiary laurel forest flora' (Takhtajan, 1969), and the highest concentration of species occurs at or near the subtropical/meridional laurel forest region of Southwest China. The unique floristic richness of this region can be explained by an unbroken connection between tropical and temperate, generally rather favourable, climates, with wide altitudinal gradients and relative stability throughout a long geological timespan (Axelrod *et al.*, 1998; Kubitzki & Krutzsch, 1998). The modern stock of *Leontopodium* may have evolved from plants adapted to an equable, subtropical climate, which prevails in part of this area.

Speciation of *Leontopodium* has been influenced by the turbulent geological history in its centre of distribution, the Tibetan Plateau. Following the collision of the Indian Plate with Eurasia, the Tibetan Plateau began to uplift between 70 Ma

(Unsworth *et al.*, 2005) and 50 Ma ago (Searle, 1991; Royden *et al.*, 2008). The uplift of the Himalayas and the Tibetan Plateau led to large-scale climate changes in Asia, basically by establishing the monsoon system, but also causing a general transition towards cooler, more pronounced seasonal climates over large areas (Jen, 1982; Axelrod *et al.*, 1998; Kubitzki & Krutzsch, 1998). During the Pleistocene several glaciations affected the region (Zhou *et al.*, 2006). While the ('Sino-Japanese') flora of the Hengduan Shan is considered to be derived from the ancient Laurasian flora, epibiontic and relict in nature, the Himalayan ('Sino-Himalayan') flora may be characterised as being mixed with neoendemic elements derived from several (Laurasian, Tethyan and Gondwanic) geoelements (Wu & Wu, 1998). Species diversity in *Leontopodium* is higher on the Hengduan Shan eastern edge of the Tibetan Plateau, and individual species' distribution patterns would also imply a subsequent colonisation of the Himalayas, approximately separated by the Mekong–Salween divide (Ward, 1921). However, a considerable distribution gap between the regions, such as in the genus *Roscoea* Sm. (Ngamriabsakul *et al.*, 2000), is absent in *Leontopodium*, possibly because this genus spread much more into the cold-arid hinterland. Distribution and diversification of *Leontopodium* aligns to climatic and geomorphological patterns around the Tibetan Plateau, and repeated, partial re-colonisations of large high altitude areas (i.e. during the Pleistocene glaciation cycles) would seem most likely.

Recent molecular phylogenetic studies in various Asian alpine plant groups have also assumed that extreme abiotic factors related to the geological and climatic history of this region have substantially affected biota and speciation processes (Liu *et al.*, 2002, 2006; Wang & Liu, 2004; Y. J. Wang *et al.*, 2004, 2007; A. L. Wang *et al.*, 2005). In all these studies levels of nucleotide substitution and support for internal branches in the phylogenetic analyses are low, and morphologically diverse species often group together. These patterns could also be detected in this study. They have perhaps arisen due to rapid radiation and hybridisation triggered by multiple, severe climatic changes, and habitat fragmentation and rejoining. Ecological constraints might also have been responsible for the occurrence of similar morphological characters in unrelated lineages, resulting in the grouping of taxa with few morphological similarities.

The origin of the European taxa of *Leontopodium* cannot be inferred with high confidence on the basis of available data. Nevertheless, a migration from Southeast Asia via Middle Asia seems most probable. While there are no extant intermediate occurrences between Central Asia and Eastern Europe, long-distance dispersal spanning this gap does not seem likely as the pappus of achenes in *Leontopodium* cypselas is not well suited for wind dispersal. The geological history of Eurasia and the alpine orogenic belt, however, might suggest migration during cooler climatic cycles of the Pleistocene. A continuous distribution from Central Asia to Eastern Europe might have been possible during the last interglacial (120,000 years ago) along with widespread herb-grass and *Artemisia*-grass steppe formations (Grichuk, 1992).

Acknowledgements

This work was funded by the Austrian Academy of Science (KIÖS grant) to R.S. We are grateful to a number of people who provided us with plant material: E. Hörandl, G. Schneeweiss, P. Schönswetter, A. Tribsch (WU), G. Miehe, S. Miehe (Faculty of Geography, University of Marburg), L. Klimeš (PRA) and M. Dobner (Institute for Pharmacy, University of Innsbruck). The authors wish to thank E. Grasserbauer, G. Kadlec and V. Klejna (WU) for technical support. We are grateful to S. Zorinyants (IMBA, University of Vienna) for translation of Russian publications and to W. Till (Institute of Botany and the Botanical Garden, University of Vienna) for his advice on nomenclatural issues. We also thank H. Weiss-Schneeweiss (Institute of Botany and the Botanical Garden, University of Vienna), G. Wagenitz (Albrecht-von-Haller-Institute, University of Göttingen), A. Susanna (Institute of Botany, Barcelona), M. Galbany-Casals (University of Barcelona), Y. Kadota (Department of Botany, National Museum of Science and Nature, Tokyo), M. Kriechbaum and G. Karrer (both University of Natural Resources and Applied Life Sciences, Vienna), and an anonymous reviewer, for their advice and discussion.

References

Akiyama, S. (1999). *Leontopodium montisganeshii*, a new species of Asteraceae from Ganesh Himal, central Nepal. *Bull. Natl. Sci. Mus. Tokyo, B* 25: 1–4.

Anderberg, A. A. (1991). Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). *Opera Bot.* 104: 1–195.

Axelrod, D. I., Al-Shehbaz, I. & Raven, P. H. (1998). History of the modern flora of China. In: Zhang, A. L. & Wu, S. G. (eds) *Floristic Characteristics and Diversity of East Asian Plants*, pp. 43–55. Beijing and Berlin: China Higher Education Press and Springer-Verlag.

Baldwin, B. G. & Markos, S. (1998). Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molec. Phylogenet. Evol.* 10: 449–463.

Bayer, R. J. & Cross, E. W. (2003). A reassessment of tribal affinities of *Craystylis* and *Haegelia* (Asteraceae) based on three chloroplast DNA sequences. *Plant Syst. Evol.* 236: 207–220.

Bayer, R. J. & Starr, J. R. (1998). Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trn*L intron and *trn*L/*trn*F intergenic spacer. *Ann. Missouri Bot. Gard.* 85: 242–256.

Bayer, R. J., Soltis, D. E. & Soltis, P. S. (1996). Phylogenetic inferences in *Antennaria* (Asteraceae: Gnaphalieae: Cassiniinae) based on sequences from nuclear ribosomal DNA internal transcribed spacers (ITS). *Amer. J. Bot.* 83: 516–527.

Bayer, R. J., Puttock, C. F. & Kelchner, S. A. (2000). Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. *Amer. J. Bot.* 87: 259–272.

Bayer, R. J., Greber, D. G. & Bagnall, N. H. (2002). Phylogeny of Australian Gnaphalieae (Asteraceae) based on chloroplast and nuclear sequences, the *trn*L intron, *trn*L/*trn*F intergenic spacer, *mat*K, and ETS. *Syst. Bot.* 27: 801–814.

Beauverd, G. (1909). Nouvelles espèces euroasiatiques du genre *Leontopodium*. *Bull. Soc. Bot. Genève* 1: 185–196.

BEAUVERD, G. (1910). Contribution à l'étude des Composées. Suite IV. *Bull. Soc. Bot. Genève* 2: 244–252.

BEAUVERD, G. (1911). Contribution à l'étude des Composées. Suite V. *Bull. Soc. Bot. Genève* 3: 353–359.

BEAUVERD, G. (1912). Contribution à l'étude des Composées. Suite VI. Nouveaux *Leontopodium* et *Raoulia*. *Bull. Soc. Bot. Genève* 5: 12–55.

BEAUVERD, G. (1914). Contribution à l'étude des Composées. Suite IX. *Bull. Soc. Bot. Genève* 6: 142–148.

BREITWIESER, I., GLENNY, D. S., THORNE, A. & WAGSTAFF, S. J. (1999). Phylogenetic relationships in Australasian Gnaphalieae (Compositae) inferred from ITS sequences. *N. Zeal. J. Bot.* 37: 399–412.

CHEN, Y.-L. (1985). *Sinoleontopodium*, a new genus of Compositae from China. *Acta Phytotaxon. Sin.* 23: 457–459.

CHEN, Y.-S. & YANG, Q.-E. (2009). Validation of the name *Sinoleontopodium lingianum* (Asteraceae, Gnaphalieae). *Novon* 19: 23–24.

CLEVINGER, J. A. & PANERO, J. L. (2000). Phylogenetic analysis of *Silphium* and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. *Amer. J. Bot.* 87: 565–572.

DIELS, L. (1901). Die Flora von Central-China. Nach der vorhandenen Literatur und neu mitgeteiltem Original-Materiale. *Bot. Jahrb. Syst.* 29: 169–659.

DOBNER, M. J., ELLMERER, E. P., SCHWAIGER, S., BATSUGKH, O., NARANTUYA, S., STUTZ, M. & STUPPNER, H. (2003a). New lignan, benzofuran, and sesquiterpene derivatives from the roots of *Leontopodium alpinum* and *L. leontopodioides*. *Helv. Chim. Acta* 86: 733–738.

DOBNER, M. J., SCHWAIGER, S., JENEWEIN, I. H. & STUPPNER, H. (2003b). Antibacterial activity of *Leontopodium alpinum* (Edelweiss). *J. Ethnopharmacol.* 89: 301–303.

DOBNER, M. J., SOSA, S., SCHWAIGER, S., ALTINIER, G., LOGGIA, R. D., KANEIDER, N. C. & STUPPNER, H. (2004). Anti-inflammatory activity of *Leontopodium alpinum* and its constituents. *Planta Med.* 70: 502–508.

DOYLE, J. J. & DICKSON, E. (1987). Preservation of plant samples for DNA restriction: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Taxon* 36: 715–722.

ERHARDT, A. (1993). Pollination of the Edelweiss, *Leontopodium alpinum*. *Bot. J. Linn. Soc.* 111: 229–240.

FAZEKAS, A. J., BURGESS, K. S., KESANAKURTI, P. R., GRAHAM, S. W., NEWMASTER, S. G., HUSBAND, B. C. *et al.* (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE* 3: E2802.

FELSENSTEIN, J. (1985). Confidence-limits on phylogenies – an approach using the bootstrap. *Evolution* 39: 783–791.

FITCH, W. M. (1971). Toward defining course of evolution – minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.

FRANCHET, A. R. (1892). Observations sur le groupe des *Leontopodium*. *Bull. Soc. Bot. France* 39: 126–136.

GALBANY-CASALS, M., GARCIA-JACAS, N., SUSANNA, A., SAEZ, L. & BENEDI, C. (2004a). Phylogenetic relationships in the Mediterranean *Helichrysum* (Asteraceae, Gnaphalieae) based on nuclear rDNA ITS sequence data. *Aust. Syst. Bot.* 17: 241–253.

GALBANY-CASALS, M., SAEZ, L. & BENEDI, C. (2004b). Taxonomy of *Castroviejoa*, a new genus of Gnaphalieae (Asteraceae), endemic to the Mediterranean islands Corsica and Sardinia. *Aust. Syst. Bot.* 17: 581–591.

GLENNY, D. (1997). A revision of the genus *Anaphalioides* (Asteraceae: Gnaphalieae). *N. Zeal. J. Bot.* 35: 451–477.

GLENNY, D. S. & WAGSTAFF, S. J. (1997). Evolution and biogeography of New Zealand *Anaphalis* DC. (Asteraceae: Gnaphalieae) inferred from rDNA sequences. *N. Zeal. J. Bot.* 35: 441–449.

GOOD, R. (1974). *The Geography of the Flowering Plants*. London: Longman.

GREUTER, W. (2003). The Euro+Med treatment of Gnaphalieae and Inuleae (Compositae) – generic concepts and required new names. *Willdenowia* 33: 239–244.

GREY, A. I., HOOK, I. L., JAMES, P. & SHERIDAN, H. (1999). Sesquiterpenes from *Leontopodium alpinum*. *Phytochemistry* 50: 1057–1060.

GRICHUK, V. P. (1992). *Main Types of Vegetation (Ecosystems) during the Maximum Cooling of the Last Glaciation*. Budapest: INQUA/Hungarian Academy of Sciences.

GRIERSON, A. J. C. & LONG, D. G. (2001). *Flora of Bhutan (including a record of plants from Sikkim & Darjeeling)*, Vol. 2(3), pp. 1508–1515. Edinburgh: Royal Botanic Garden Edinburgh and Royal Government of Bhutan.

GRISEBACH, A. (1872). *Die Vegetation der Erde nach ihrer klimatischen Anordnung: Ein Abriss der vergleichenden Geographie der Pflanzen*. Leipzig: Verlag von Wilhelm Engelmann.

GRUBOV, V. (2003). Conspectus generis *Leontopodium* (Pers.) R. Br. (Compositae) Asiae centralis [in Russian]. *Sist. Vyssh. Rast.* 35: 188–197.

HAJRA, P. K., RAO, R. R., SINGH, D. K. & UNIYAL, B. P. (1995). *Flora of India*, Vol. 13, pp. 103–111. Calcutta: Botanical Survey of India.

HALL, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41: 95.

HANDEL-MAZZETTI, H. (1927). Systematische Monographie der Gattung *Leontopodium*. *Beih. Bot. Centralbl.* 44: 1–178.

HOOK, I. (1994). Secondary metabolites in hairy root cultures of *Leontopodium alpinum* Cass. (Edelweiss). *Pl. Cell Tiss. Org.* 38: 321–326.

IWATSUKI, K., YAMAZAKI, T., BOUFFORD, D. E. & OHBA, H. (1995). *Flora of Japan*, Vol. 3b, pp. 106–108. Tokio: Kondasha.

JEN, H. (1982). The uplift of the Qinghai-Xizang (Tibet) Plateau in relation to the vegetational changes in the past. *Acta Phytotaxon. Sin.* 20: 385–391.

KIMBALL, R. T. & CRAWFORD, D. J. (2004). Phylogeny of Coreopsideae (Asteraceae) using ITS sequences suggests lability in reproductive characters. *Molec. Phylogenet. Evol.* 33: 127–139.

KUBITZKI, K. & KRUTZSCH, W. (1998). Origins of east and southeast Asian plant diversity. In: ZHANG, A. L. & WU, S. G. (eds) *Floristic Characteristics and Diversity of East Asian Plants*, pp. 56–70. Beijing and Berlin: China Higher Education Press and Springer-Verlag.

LIU, J. Q., GAO, T. G., CHEN, Z. D. & LU, A. M. (2002). Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic *Nannoglottis* (Asteraceae). *Molec. Phylogenet. Evol.* 23: 307–325.

LIU, J. Q., WANG, Y. J., WANG, A. L., HIDEAKI, O. & ABBOTT, R. J. (2006). Radiation and diversification within the *Ligularia–Cremanthodium–Parasenecio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau. *Molec. Phylogenet. Evol.* 38: 31–49.

MAUGINI, E. (1962). Morfologia fiorale, embriologia ed embriogenesi in *Leontopodium alpinum* Cass. var. α typicum Fiori e Paoletti. *Giorn. Bot. Ital.* 96: 1–18.

MERXMÜLLER, H., LEINS, P. & ROESSLER, H. (1977). Inuleae – systematic review. In: HEYWOOD, V. H., HARBORNE, J. B. & TURNER, B. L. (eds) *The Biology and Chemistry of the Compositae*, Vol. 1, pp. 577–602. London: Academic Press.

MEUSEL, H. & JÄGER, E. J. (1992). *Vergleichende Chorologie der zentraleuropäischen Flora*. Jena, Stuttgart, New York: Fischer Verlag.

MÜLLER, K. (2005). SeqState – primer design and sequence statistics for phylogenetic DNA data sets. *Appl. Bioinformatics* 4: 65–69.

MÜLLER, K. (2006). Incorporating information from length-mutational events into phylogenetic analysis. *Molec. Phylogenet. Evol.* 38: 667–676.
NGAMRIABSAKUL, C., NEWMAN, M. F. & CRONK, Q. C. B. (2000). Phylogeny and disjunction in *Roscoea* (Zingiberaceae). *Edinburgh J. Bot.* 57: 39–61.
NOYES, R. D. (2007). Apomixis in Asteraceae: diamonds in the rough. *Funct. Plant Sc. Biotechn.* 1: 207–222.
NOYES, R. D. & RIESEBERG, L. H. (1999). ITS sequences support a single origin for the North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster* s.l. *Amer. J. Bot.* 86: 398–412.
NYLANDER, J. A. A. (2005). *MrModeltest v.2.2* [Program distributed by the author]. Uppsala University, Uppsala: Department of Systematic Zoology.
PANERO, J. & FUNK, V. (2008). The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. *Molec. Phylogenet. Evol.* 47: 757–782.
PLOVANICH, A. E. & PANERO, J. L. (2004). A phylogeny of the ITS and ETS for *Montanoa* (Asteraceae: Heliantheae). *Molec. Phylogenet. Evol.* 31: 815–821.
QAISER, M. & ABID, R. (2003). *Flora of Pakistan*, Vol. 210, pp. 147–161. Karachi and St Louis: Department of Botany, University of Karachi and Missouri Botanical Press.
RECHINGER, K. H. (1980). *Flora Iranica. Compositae IV – Inuleae*, Vol. 145, pp. 28–30. Graz: Akademischer Drucks- und Verlagsgesellschaft.
RONQUIST, F. & HUELSENBECK, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
ROYDEN, L. H., BURCHFIEL, B. C. & VAN DER HILST, R. D. (2008). The geological evolution of the Tibetan Plateau. *Science* 321: 1054–1058.
SAMUEL, R., STUESSY, T. F., TREMETSBERGER, K., BAEZA, C. M. & SILJAK-YAKOVLEV, S. (2003). Phylogenetic relationships among species of *Hypochaeris* (Asteraceae, Cichorieae) based on ITS, plastid *trn*L intron, *trn*L-F spacer, and *mat*K sequences. *Amer. J. Bot.* 90: 496–507.
SAMUEL, R., KATHRIARACHCHI, H., HOFFMANN, P., BARFUSS, M. H. J., WURDACK, K. J., DAVIS, C. C. & CHASE, M. W. (2005). Molecular phylogenetics of Phyllanthaceae: evidence from plastid *mat*K and nuclear *phy*C sequences. *Amer. J. Bot.* 92: 132–141.
SAMUEL, R., GUTERMANN, W., STUESSY, T. F., RUAS, C. F., LACK, H. W., TREMETSBERGER, K. *et al.* (2006). Molecular phylogenetics reveals *Leontodon* (Asteraceae, Lactuceae) to be diphyletic. *Amer. J. Bot.* 93: 1193–1205.
SCHWAIGER, S., ADAMS, M., SEGER, C., ELLMERER, E. P., BAUER, R. & STUPPNER, H. (2004). New constituents of *Leontopodium alpinum* and their in vitro leukotriene biosynthesis inhibitory activity. *Planta Med.* 70: 978–985.
SEARLE, M. P. (1991). *Geology and Tectonics of the Karakoram Mountains*. Chichester: John Wiley & Sons.
SHISHKIN, B. K. (1999). *Flora of the USSR*, Vol. 25, pp. 318–337. Washington: Bishen Singh Mahendra Pal Singh and Koeltz Scientific Books.
SMISSEN, R. D., BREITWIESER, I., WARD, J. M., MCLENACHAN, P. A. & LOCKHART, P. J. (2003). Use of ISSR profiles and ITS-sequences to study the biogeography of alpine cushion plants in the genus *Raoulia* (Asteraceae). *Plant Syst. Evol.* 239: 79–94.
SMISSEN, R. D., BREITWIESER, I. & WARD, J. M. (2004). Phylogenetic implications of transspecific chloroplast DNA sequence polymorphisms in New Zealand Gnaphalieae. *Plant Syst. Evol.* 249: 37–53.
SOKOLOWSKA-KULCZYCKA, A. (1959). Apomiksja u *Leontopodium alpinum* Cass. [Apomixis in *Leontopodium alpinum* Cass.]. *Acta Biol. Cracov., Ser. Bot.* 2: 51–63.
STUPPNER, H., ELLMERER, E. P., ONGANIA, K. H. & DOBNER, M. (2002). Bisabolane derivatives from *Leontopodium alpinum*. *Helv. Chim. Acta* 85: 2982–2989.

SUN, Y., SKINNER, D. Z., LIANG, G. H. & HULBERT, S. H. (1994). Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theor. Appl. Genet.* 89: 26–32.

SWOFFORD, D. L. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (*and other Methods), Version 4*. Sunderland, MA: Sinauer Associates.

TABERLET, P., GIELLY, L., PAUTOU, G. & BOUVET, J. (1991). Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Plant Mol. Biol.* 17: 1105–1109.

TAKHTAJAN, A. (1969). *Flowering Plants: Origin and Dispersal*. Edinburgh: Oliver and Boyd.

THOMPSON, J. D., GIBSON, T. J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 24: 4876–4882.

TIRA, S., GALEFFI, C. & DIMODICA, G. (1970). Flavonoids of Gnaphalieae – *Leontopodium alpinum* Cass. *Experientia* 26: 1192.

TREMETSBERGER, K., WEISS-SCHNEEWEISS, H., STUESSY, T., SAMUEL, R., KADLEC, G., ORTIZ, M. A. & TALAVERA, S. (2005). Nuclear ribosomal DNA and karyotypes indicate a NW African origin of South American *Hypochaeris* (Asteraceae, Cichorieae). *Molec. Phylogenet. Evol.* 35: 102–116.

TUTIN, T. G. (1973). Flora Europaea. Notulae systematicae. No. 14. *Bot. J. Linn. Soc.* 67: 283.

UEXKÜLL-GYLLENBAND, M. (1901). Phylogenie der Blütenformen und Geschlechterverteilung bei den Compositen. *Biblioth. Bot.* 52: 27–32.

UNSWORTH, M. J., JONES, A. G., WEI, W., MARQUIS, G., GOKARN, S. G. & SPRATT, J. E. (2005). Crustal rheology of the Himalaya and Southern Tibet inferred from magnetotelluric data. *Nature* 438: 78–81.

VOROSHILOV, V. N. (1979). Novyi vid edel'veisa [A new species of the genus *Leontopodium* L.]. *Byull. Mosk. Obshch. Ispyt. Prir. Biol.* 84: 102–104.

WANG, A. L., YANG, M. H. & LIU, J. Q. (2005). Molecular phylogeny, recent radiation and evolution of gross morphology of the rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA *trn*L-F sequences. *Ann. Bot.* 96: 489–498.

WANG, Y. J. & LIU, J. Q. (2004). Phylogenetic analyses of *Saussurea* sect. *Pseudoeriocoryne* (Asteraceae: Cardueae) based on chloroplast DNA *trn*L-F sequences. *Biochem. Syst. Ecol.* 32: 1009–1023.

WANG, Y. J., LI, X. J., HAO, G. & LIU, J. Q. (2004). Molecular phylogeny and biogeography of *Androsace* (Primulaceae) and the convergent evolution of cushion morphology. *Acta Phytotaxon. Sin.* 42: 481–499.

WANG, Y. J., LIU, J. Q. & MIEHE, G. (2007). Phylogenetic origins of the Himalayan endemic *Dolomiaea*, *Diplazoptilon* and *Xanthopappus* (Asteraceae: Cardueae) based on three DNA regions. *Ann. Bot.* 99: 311–322.

WARD, F. K. (1921). The Mekong–Salween divide as a geographical barrier. *Geogr. J.* 1: 385–404.

WARD, J., BAYER, R. J., BREITWIESER, I., SMISSEN, R., GALBANY-CASALS, M. & UNWIN, M. (2009). Gnaphalieae. In: FUNK, V. A., SUSANNA, A., STUESSY, T. E. & BAYER, R. J. (eds) *Systematics, Evolution and Biogeography of Compositae*, pp. 539–588. Vienna: International Association for Plant Taxonomy.

WHITE, T. J., BRUNS, T., LEE, S. & TAYLOR, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INIS, M. A., GELFAND, D. H., SNINSKY, J. J. & WHITE, T. J. (eds) *PCR Protocols: A Guide to Methods and Applications*, pp. 315–322. San Diego, CA: Academic Press.

WOLFE, K. H. (1991). *Protein-coding Genes in Chloroplast DNA: Compilation of Nucleotide Sequences, Data Base Entries and Rates of Molecular Evolution*. San Diego, CA: Academic Press.

WU, Z. Y. & WU, S. K. (1998). A proposal for a new floristic kingdom (realm) – the Asiatic kingdom, its delineation and characteristics. In: ZHANG, A. L. & WU, S. G. (eds) *Floristic Characteristics and Diversity of East Asian Plants*, pp. 3–42. Beijing and Berlin: China Higher Education Press and Springer-Verlag.

ZHOU, S., WANG, X., WANG, J. & XU, L. (2006). A preliminary study on timing of the oldest Pleistocene glaciation in Qinghai-Tibetan Plateau. *Quatern. Int.* 154/155: 44–51.

Received 8 September 2008; accepted for publication 22 February 2010