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Comparative pappus micromorphology of edelweiss (*Leontopodium*, Gnaphalieae, Asteraceae) with implications for taxonomy, ecology and evolution

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We analysed pappus characters in 31 of the *c.* 34 accepted *Leontopodium* spp. (edelweiss). Micromorphological pappus character states were useful for discriminating between individual species and intrageneric groups. The pappus differs in number, length, breadth, surface structure, colour and the tips of the bristles. Several features characterize single species, for example a unique fan-like tip that is only found in *L. franchetii*. *Leontopodium* section *Nobilia* is supported by unusual pappus characters. Experimental evidence shows that the pappus of *Leontopodium*, previously thought to be caducous, is well suited for wind dispersal of the fruit. One clear trend is that species growing in sparsely vegetated, high-altitude regions often have more numerous and longer pappus bristles, particularly on the female flowers. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **182**, 612–636

ADDITIONAL KEYWORDS: adaptation – Compositae – Cypselae – microscopy – wind dispersal.

INTRODUCTION

Leontopodium R.Br. ex Cass. (Gnaphalieae, Asteraceae), known as edelweiss, comprises *c.* 34 species (Blöch *et al.*, 2010; Safer *et al.*, 2011; Russell *et al.*, 2013; Stille *et al.*, 2014; W. B. Dickoré, unpubl. data). More species, however, were recognized in earlier surveys. In the only scientific monograph to date, by Handel-Mazzetti (1927), 41 species were treated, whereas *Flora of China* lists 58 species (Wu, Raven & Hong, 1994). Altogether, the number of species validly described in the genus has been *c.* 120, but most of these are synonyms. In particular, limited knowledge of patterns of growth and variation in natural populations has led to over-estimation of species diversity.

Leontopodium occupies many different habitats in the Asian mountains, being spread in a largely continuous distribution in central, temperate south-eastern and eastern Asia (Blöch *et al.*, 2010). About 15 species occur in the centre of diversity in the Sino-Himalayan region (Hengduan–Shan mountain region in south-western China; Meusel & Jaeger, 1992; Safer *et al.*, 2011). Two species grow much further west, as disjuncts in the European mountains (Meusel & Jaeger, 1992; Safer *et al.*, 2011): *Leontopodium alpinum* Cass. (Pyrenees, Alps, Carpathians, Balkan Peninsula) and *L. nivale* Ten. Huet ex Hand.-Mazz. (Apennines in Italy, Pirin Mountains in Bulgaria).

Within the *Leontopodium* pseudanthium, female (pistillate, male-sterile) and male (staminate,

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female-sterile) flowers occur in various compositions and arrangements. Most plants are monoecious with heterogamous inflorescences, but sometimes homogamous inflorescences and occasional dioecious plants occur. Beauverd initially believed that these sexual variations might be of taxonomic significance, but he later realized that this was not the case (Beauverd, 1909, 1910, 1911, 1912, 1914). Male and female flowers possess a pappus formed of bristles, which are connate in a ring at the base. Usually they are white and have barbs formed by single cells of the outer layer (Figs 1, 2).

The functions of the pappus of *Leontopodium* seem to be various. The pappus of Asteraceae is assumed to play a role in the wind-dispersal of the seeds (Azuma, 2006). In garden culture of *Leontopodium*, one commonly observes dry seeds being dispersed by wind from plants, hence revealing the efficiency of dispersal. In a small experiment that is part of this study (see Material and Methods), we tested this

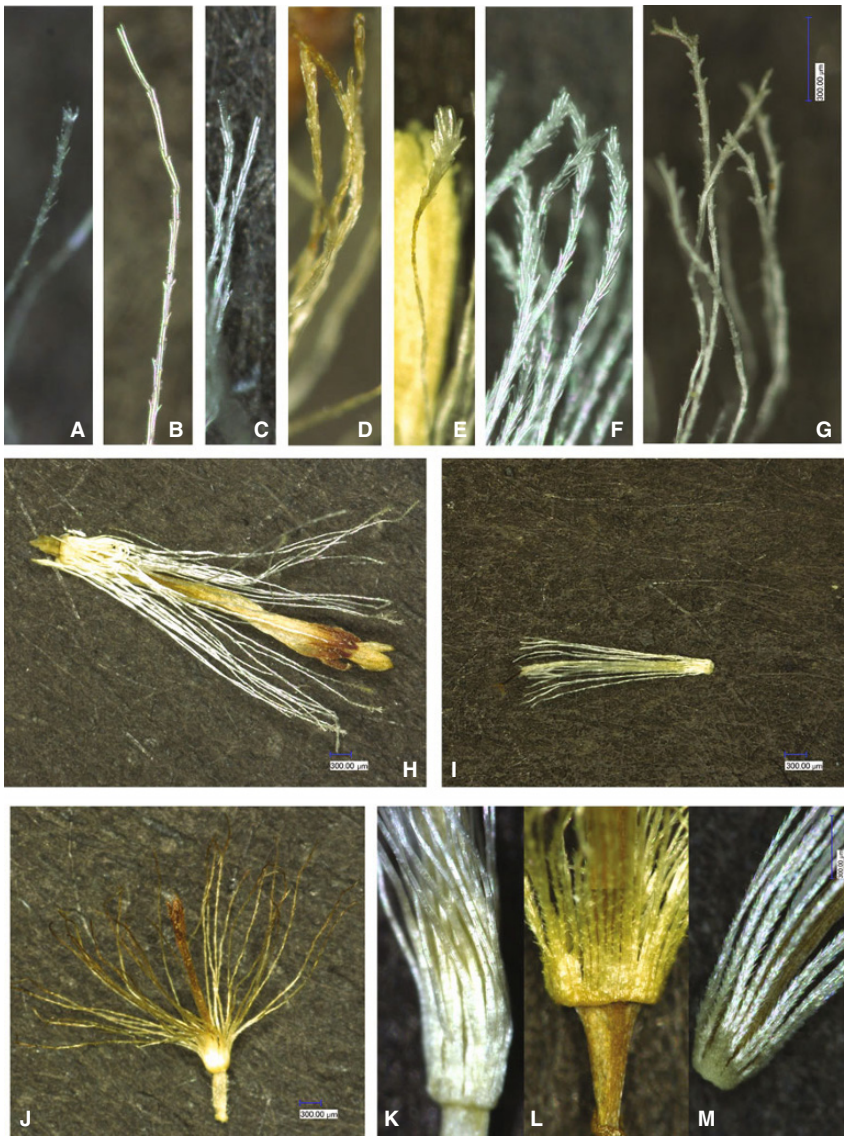

Figure 1. A, no structure at the end: *L. microphyllum* m. B, elongated 'end cells': *L. nanum* w. C, accumulation of elongated cells: *L. discolor* w. D, tip of round cells: *L. muscoides* m. E, fan tip: *L. franchetii* m. F, tree-like tip: *L. souliei* m. G, button-like barbs *L. roseum* m; two scale bars valid for A–G. H, bristle length: large *L. nanum* m. I, bristle length: small *L. artemisiifolium* w. J, coloured bristle *L. aurantiacum* w. K, number of barbs: nearly no barbs on the bristle *L. lingianum* w. L, number of barbs: some barbs on the bristle *L. jacotianum* m. M, number of barbs: many barbs on the bristle *L. discolor* w; scale bar valid for K–M. m, male; f, female.

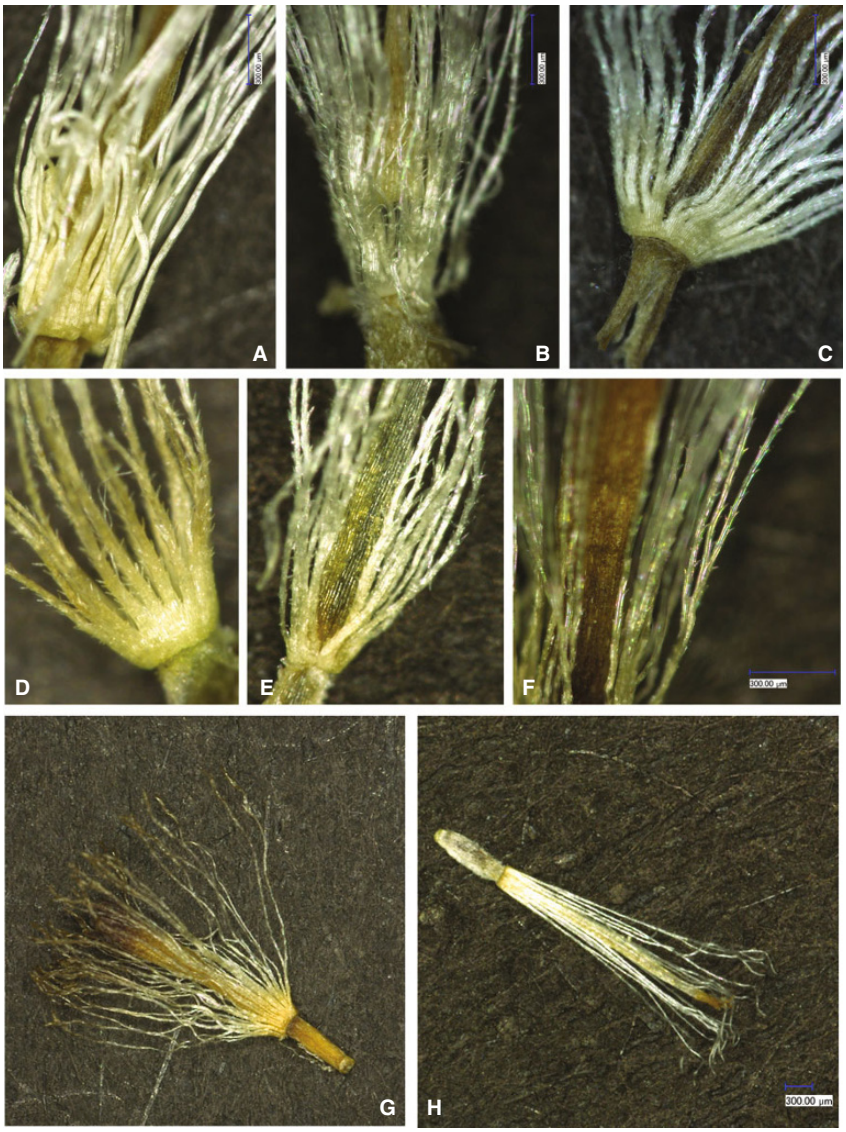

Figure 2. A, miniature barbs *L. nanum* m. B, long thin barbs *L. monocephalum* w. C, massive barbs *L. discolor* m. D, thickness: big *L. wilsonii* w. E, thickness: medium *L. franchetii* w. F, thickness: thin *L. ocroleucum* m; scale bar valid for D–F. G, number of pappus bristles, for example 42 *L. giraldii* m and 15 *L. sinense* w; scale bar valid for G and H. m, male; f, female.

assumption more precisely. Handel-Mazzetti (1927), however, pointed out that the pappus is also caducous in herbarium material, in which case it would be useless for dispersal, but this apparently occurs only after complete drying of the fruits. The pappus also plays an important role in aiding release of fruits from the maturing head, by spreading outward with hygroscopic movements and lifting the cypselae upward (Van der Pijl, 1982) (Fig. 3). Furthermore, the pappus of *Leontopodium* potentially also has a defensive function, offering apical protection against predation on the fruits or ovaries (Stuessy & Garver, 1996). The pappus bristles might also help retain moisture around the cypsela by cohesive attraction to avoid premature desiccation, as Stuessy & Spooner (1988) suggested for one of the roles of receptacular bracts in some members of the family (e.g. Heliantheae *s.l.*).

Although edelweiss is a well-known plant and important part of the cultural heritage of the international alpine region, little research on the genus has been conducted. In the second part of the 20th century, some studies investigated the biology, ecology and chorology of *L. alpinum* (Sokolowska-Kulczycka, 1959; Maugini, 1962; Tira, Galeffi & Dimodica, 1970; Erhardt, 1993; Hook, 1994). At the

Figure 3. The leontopodium collection of the botanical garden Gießen: A, *L. himalayanum*. B, *L. discolor*. C, *L. pusillum*. D, *L. calocephalum*. E, *L. haplophylloides*. F, *L. dedekensii*. G, *L. leontopodinum*. H, *L. nivale*. I, mature seeds of *L.* sp. leave the heads by hydroscopic movement of the pappus. J, anemochorie experiment with *L.* sp. in a sports hall.

beginning of the 21st century, several unknown secondary metabolites were found in several species (Stuppner *et al.*, 2002; Dobner *et al.*, 2003a, b, 2004; Schwaiger *et al.*, 2004, 2005; Speroni *et al.*, 2006; Hornick *et al.*, 2008; Reisinger *et al.*, 2009). Because some of these compounds now show biological activity (Safer *et al.*, 2011), interest in other species and the whole genus has increased. To understand better the phylogenetics and taxonomy of the genus, Blöch *et al.* (2010) and Safer *et al.* (2011) conducted molecular phylogenetic studies and Russell *et al.* (2013) and Stille *et al.* (2014) investigated karyotaxonomy. A modern comprehensive systematic treatment would be desirable, however, as little is known about Asian *Leontopodium* spp. A more predictive classification would also provide a better basis for further pharmaceutical research (Safer *et al.*, 2011).

Previous studies have specifically dealt with the pappus of *Leontopodium*. The structure of the pappus bristles has been examined as part of descriptions of many new taxa, for example by Handel-Mazzetti (1927), who used complex Latin adjectives, such as clavato-incrassatae, paulum incrassatae, modice incrassatae, vix incrassatae, paulum clavato-inflatae, apice cellulis inflatis, sensim incrassatae, subclavato-incrassatae and anguste clavato-incrassatae (Handel-Mazzetti, 1927). Handel-Mazzetti also compared pappus bristles with the aim of finding diagnostic

species characters (his prepared material is preserved in the Natural History Museum, Vienna). He concluded that special structures seem to be characteristic of several species: 'Für einige Arten erweist sich, soweit Material vorliegt, eine bestimmte Form als konstant' [In some species, to the extent that material exists, it proves that a certain form is constant] (Handel-Mazzetti, 1927). However, his observations were limited and he did not deal with all known species at that time.

Systematic comparative micromorphological studies on the pappus of *Leontopodium* are therefore the focus for the present investigation. Thirty-one of the 34 presently recognized species have been analysed micromorphologically. The results show that micromorphological data from the pappus are helpful for detecting new taxonomic characters, forming helpful categories, drawing systematic conclusions and inferring relationships among species, particularly in the context of recent phytochemical, molecular and karyological studies.

MATERIAL AND METHODS

Plant material

All investigated pappus specimens were taken from herbarium material from GI, GOET, W and WU. Thirty-one of the 34 *Leontopodium* spp. were investigated; only *L. fangingense* Ling, *L. kurilense* Takeda and *L.* sp. nov. (W. B. Dickoré unpubl. data), for which herbarium material was not available, were lacking. All examinations were carried out on fully developed dry fruits. Preliminary analyses of selected species had shown that pappus bristle structure did not differ in the investigated characters according to the position of the flower within the head. Male and female flowers (of most species) from three well-determined specimens from different geographical regions were used. The few exceptions were due to poor availability of material in some species. For specific character states (e.g. unique tip structure of *L. franchetii* Beauverd), more population samples were analysed. Taxa studied and detailed voucher information are listed in Table 1.

Micromorphological observations

Pappus structures were investigated using a three-dimensional (3D) light microscope (Keyence VHX-20000; Deutschland GmbH) and 3D digital pictures were made. At least three pictures were taken from each chosen pappus: one of the whole pappus (analysis of bristle quantity, length and colour); one of the tip (analysis of the structure of the tip); and one of the lower part of the bristle (analysis of the breadth and surface of the bristle) (Figs 1, 2).

All specimens of all species and of each sex were included to identify character states that were judged to be typical. In preliminary morphological investigations, we identified categories of character states that appeared to encompass the observed patterns of variation. Seven characters of the pappus were investigated (bristle length, colour, surface structure, type of barbs on the surface, tip structure and number of bristles). For each character, different character states were defined and investigated for each sex and each species. Some categories, such as miniature barbs, long thin barbs or massive barbs on the pappus bristles may well be functional, whereas others may not contribute directly to fitness (e.g. the angle between the bristle and a single barb). Patterns of morphological distinct character correlations were calculated and visualized using a principal coordinate analysis (PCoA) using GenAlEx version 6.5 (Peakall & Smouse, 2012).

Anemochory experiment

To test the efficacy of the *Leontopodium* pappus for wind dispersal, a small experiment was conducted. Several *Leontopodium* plants (*Leontopodium* sp. taken from the botanical garden of the Justus Liebig University Giessen) growing in flower pots were observed for months until the seeds became mature and the weather conditions were suitable for dispersal. We observed that the seeds left the fruiting heads by hygroscopic movement of the pappus. A hair-dryer was also used to blow wind on the heads from a safe distance and observations were made on fruit dispersal.

RESULTS

After observing the variety of pappus structure in *Leontopodium*, a system of different categories for the description and differentiation of typical character states was developed for each species. Other recent studies on the pappus in other groups of Asteraceae have suggested this as a useful way to deal with morphological variation and for acquisition of authoritative data (Semple & Hood, 2003). The different characters and states are presented in Table 2, organized by species and sexual conditions of the heads.

Characters and character states

Bristle tip

In many cases, the bristle tip of the pappus provides a diagnostic character for each species or subgroup

Table 1. Taxa and voucher information

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium alpinum Cass.	f/m	*M.Wiedermann 9282*	WU	38271	Italy	Piemonte, Sondrio, Livigno-Alpen	2630	2003-08-12
Leontopodium alpinum Cass.	f/m	*S.Safer & V.Vladimirov SS-SP-2*	WU	4989	Bulgaria	Plovdiv, Stara Planina	1640	2008-08-20
Leontopodium alpinum Cass.	f/m	*C.Gilli & A.Berger*	WU	67124	Austria	Kärnten, Bezirk Spittal an der Drau, Innerfragant		2010-07-16
Leontopodium andersonii C.B.Clarke	f	*S.Safer, S.Schwaiger & Y.Guo SSG31*	WU	44006	China (Yunnan)	Da Shi Village Heqing; Sang Gui District	2033	2008-09-08
Leontopodium andersonii C.B.Clarke	f	*S.Safer, S.Schwaiger & Y.Guo SSG27*	WU	43958	China (Yunnan)	Luquan; JiaoziShan	3145	2008-09-21
Leontopodium andersonii C.B.Clarke	m	*H.Handel-Mazzetti 289*	W	1924 4654	China (Yunnan)	Yunnanfu [Kunming] N	2200	1914-02-27
Leontopodium andersonii C.B.Clarke	m	*H.Handel-Mazzetti 5128*	W	1924 4851	China (Sichuan)	Lungdschu-shan Mt. [Liangshan], Huili	2100–3350	1914-09-16
Leontopodium andersonii C.B.Clarke	m	*H.Handel-Mazzetti 10048*	W	1924 2454	China (Yunnan)	Yangtse–Mekong divide, Weihsi – Djientschwan, Daidsedien – Schadien	2500–2900	1916-09-20
Leontopodium artemisiifolium (H.Lév.) Beauverd	f	*S.Safer, S.Schwaiger & Y.Guo SSG28*	WU	43959	China (Yunnan)	Luquan; JiaoziShan	3135	2008-09-21
Leontopodium artemisiifolium (H.Lév.) Beauverd	f	*S.Safer, S.Schwaiger & Y.Guo SSG13*	WU	43997	China (Yunnan)	Zhongdian; from Bita Lake to Haba village	2873	2008-09-11

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium artemisiifolium (H.Lév.) Beauverd	m	*W.B.Dickoré 14574*	WU	38295	China (Yunnan)	Lijiang Range E	3000	1996-07-31
Leontopodium artemisiifolium (H.Lév.) Beauverd	m	*E.E.Maire s.n.*	W	1913 5234	China (Yunnan)	Tong-tchouan [Dongchuan]	2600	1910-09-
Leontopodium artemisiifolium (H.Lév.) Beauverd	m	*H.Smith 12629*	W	1940 9650	China (Sichuan)	Taining (Ngata) – Maoniu (Ndröme), Tjedji Vy.	3200	1934-09-30
Leontopodium aurantiacum Hand.-Mazz.	f	*G.Forrest 27263*	W	1921 20051	Myanmar	N. E. Upper Burma	3600–4000	1925-09
Leontopodium aurantiacum Hand.-Mazz.	m	*G.Forrest 26832*	W	1921 20051	Myanmar	N. E. Upper Burma	3600–4000	1925-09
Leontopodium aurantiacum Hand.-Mazz.	m	*G.Forrest 24960*	W	1925 20052	Myanmar	NE Upper Burma, on the western flank of the Chimi-li N' Maikha-Salwin divide	3600	1924-09
Leontopodium calocephalum (Franch.) Beauverd	f/m	*H.Handel-Mazzetti 5492*	WU	69172	China (Sichuan)	inter oppidum Yenyüen et castellum Kwapi	–	1914-10-04
Leontopodium calocephalum (Franch.) Beauverd	f	*H.Handel-Mazzetti 8073*	WU	69173	China (Yunnan)	Ad confines Tibeticas sub jugo Dokerla	–	1915-09-17
Leontopodium calocephalum (Franch.) Beauverd	f	*U.Wündisch 94-488-5*	WU	38296	China (Sichuan)	Barkam	3350–3400	1994-10-03

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium calocephalum (Franch.) Beauverd	f/m	*Bot. Garten Gießen (J80)*	GI	007801	China (Sichuan)	Gansu, Min Shan	4100	2009-06-12
Leontopodium calocephalum (Franch.) Beauverd	f	*Bot. Garten Gießen (J10)*	GI	007795	China (Sichuan)	Min Shan	3500	2009-07-07
Leontopodium calocephalum (Franch.) Beauverd	f/m	*Bot. Garten Gießen (J56)*	GI	007795	China (Sichuan)	Min Shan, alpine meadows	3800	2009-07-07
Leontopodium calocephalum (Franch.) Beauverd	m	*H.Smith 12017*	W	1940 9644	China (Sichuan)	Sikang; between Taining and Taofu	3800	1934-10-09
Leontopodium calocephalum (Franch.) Beauverd	m	*Rück 18315*	W	1931 3503	China (Sichuan)	Mt Mitzuga	4460	1939-09
Leontopodium pusillum (Beauverd) Hand.-Mazz.	f/m	*G.&S.Miehe 7480*	GOET		China (Xinjiang)	Kunlun Shan, Tiznap Valley, N Kudi	3300	1992-07-09
Leontopodium pusillum (Beauverd) Hand.-Mazz.	m	*G.&S.Miehe 7614*	GOET		China (Xinjiang)	Kunlun Shan, Upper Tiznap Valley, SE Kudi Kunlun, Kudi	4200–4300	1992-07-10
Leontopodium pusillum (Beauverd) Hand.-Mazz.	f	*G.&S.Miehe 7476*	GOET		China (Xinjiang)	Kunlun Shan, Tiznap Valley, N Kudi	3080–3200	1992-07-09
Leontopodium pusillum (Beauverd) Hand.-Mazz.	m	*G.&S.Miehe 8220*	GOET		China (Xinjiang)	Aksai Chin	5150	1992-07-05

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium pusillum (Beauverd) Hand.-Mazz.	f	*G.&S.Miehe 8231*	GOET		China (Xinjiang)	Aksai Chin	5100	1992-07-05
Leontopodium pusillum (Beauverd) Hand.-Mazz.	f	*G.&S.Miehe 7875a*	GOET		China (Xinjiang)	Karakash Valley, SE Dahongliutan	4800–4850	1992-07-05
Leontopodium dedekensii (Bureau & Franch.) Beauverd	f/m	*G.Miehe 07-012-02*	WU	43965	China (Qinghai)	35.55°N/99.85°E	3280	2007-08-31
Leontopodium dedekensii (Bureau & Franch.) Beauverd	m	*G.Miehe 07-012-01*	WU	43964	China (Qinghai)	35.55°N/99.85°E	3280	2007-08-31
Leontopodium dedekensii (Bureau & Franch.) Beauverd	f/m	*S.Safer, S.Schwaiger & Y.Guo 17*	WU	43994	China (Yunnan)	Benzilan, Dongzhulin Monastery	2780	2008-09-13
Leontopodium discolor Beauverd	f/m	*Faurie 8489*	WU	8982 13-83/1	Japan	Rebunshiri	–	1899-08-01
Leontopodium discolor Beauverd	f/m	*Faurie 3405*	WU	8982 13-83/2	Japan	Rebunshiri	–	1892-07-31
Leontopodium franchetii Beauverd	f	*S.Safer, S.Schwaiger & Y.Guo SSG09*	WU	44016	China (Yunnan)	Zhongdian	3287	2008-09-10
Leontopodium franchetii Beauverd	f/m	*S.Safer, S.Schwaiger & Y.Guo SSG15*	WU	44008	China (Yunnan)	Zhongdian	3746	2008-09-12
Leontopodium franchetii Beauverd	f	*S.Safer, S.Schwaiger & Y.Guo SSG15*	WU	44008	China (Yunnan)	Zhongdian	3746	2008-09-12

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium franchetii Beauverd	m	*H.Hand.-Mazz. 4616*	W	1923 3629	China (Yunnan)	Inter vicos Alo et Halao-Dschungdien	3500	1914-08-09
Leontopodium franchetii Beauverd	m	*H.Smith 11762*	W	1940 9633	China/	Sikang, Taofu, distr.: Taining	3600	1934-09-04
Leontopodium giraldii Diels	m	*G.Giraldis.n.*	W	1926 1875	China (Shaanxi)	T'ai pa shan		1893-08
Leontopodium haastioides Hand.-Mazz.	f	*W.B.Dickoré 9892*	WU	38291	China (Xizang)	Xizang, S Tibet. Nagarze – Pomo Co. S of Dalung	4700	1994-07-23
Leontopodium haastioides Hand.-Mazz.	m	*F.E.Younghusband s.n.*	W	-	China (Xizang)	[Tibet austr.,] Gyamtsoma (near source of Teesta)	4876	1903-07-19
Leontopodium haplophylloides Hand.-Mazz.	f/m	*Bot. Garten Gießen (J11)*	GI	007826	China (Sichuan)	Shaluli Shan	4200	2009-08-15
Leontopodium haplophylloides Hand.-Mazz.	m	*Bot. Garten Gießen (J157)*	GI	010306	China (Sichuan)	Between Zaige and Songpan	3600	2010-10-12
Leontopodium haplophylloides Hand.-Mazz.	f/m	*HS 12131*	W	1940 9625	China (Sichuan)	Sikang, between Taining and Taofu	3900	1934-09-13
Leontopodium haplophylloides Hand.-Mazz.	f/m	*HS 4046*	W	1924 2068	China (Sichuan)	Huang-chen-huan	3300	1922-08-19
Leontopodium himalayanum DC.	f/m	*BD W.B.Dickoré 5228*	WU	38287	China (Xizang)	SE Tibet, Namchabarwa, Nam La Tso	4400	1989-09-14
Leontopodium himalayanum DC.	f	*S.Safer, S.Schwaiger & Y.Guo SSG18*	WU	43993	China (Yunnan)	Deqen; from Zhongdian to Deqen, pass facing the MeiLi Snow Mountain group	4316	2008-09-13

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium himalayanum DC.	f	*S.Safer, S.Schwaiger & Y.Guo SSG12*	WU	44000	China (Yunnan)	Zhongdian; from Bita Lake to Haba village	3582	2008-09-11
Leontopodium jacotianum Beauverd	f/m	*H.Handel-Mazzetti 9672*	WU	69171	China (Yunnan)	In montium inter fluvios Landsang-djiang (Mekong) et Lu-djiang (Salween)	3450	1916-08-04
Leontopodium jacotianum Beauverd	m	*K.Reiter 571*	WU	38297	Nepal	Langtang Valley, Kyangjin	3780	1995-08-24
Leontopodium japonicum Miq.	f/m	*G.-Y.Rao 01*	WU	43992	China (Hubei)	Shennongjia Mt	2600	2008-08-08
Leontopodium japonicum Miq.	f/m	*Faurie 183*	WU	8982 13-83/5	Japan	Asamayama	–	1897-07-20
Leontopodium japonicum Miq.	f/m	*U.J.Faurie 183*	WU	8982 13-83/8	Japan	Asamayama	–	1897-07-20
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*U.Wündisch 462*	GOET		China (Xinjiang)	SW Xinjiang, Upper Ojtagh Valley	2805	1998-07-18
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f	*U.Wündisch 371*	GOET		China (Xinjiang)	SW Xinjiang, Upper Ojtagh Valley	2885	1998-07-16
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*U.Wündisch 258*	GOET		China (Xinjiang)	SW Xinjiang, Upper Ojtagh Valley	2615	1998-07-16
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*E.Eberhardt 98-533*	GOET		Pakistan	Karakorum, Hunza, Khoorzoie	3400	1998-07-16

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f	*E.Eberhardt 98-422*	GOET		Pakistan	Karakorum, Hunza, Kirgaswashk	3200	1998-07-15
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*U.Schickhoff*	GOET		Pakistan	NW Himalaya, Diamir Nullah	3150	1994-07-16
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*E.Eberhardt 1131*	GOET		Pakistan	Karakorum, Batura, Wudmur	3850	1994-07-19
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*E.Eberhardt 1182*	GOET		Pakistan	Karakorum, Batura, Wudmur	3700	1994-07-19
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*E.Eberhardt 3348*	GOET		Pakistan	Karakorum, Batura, Guchism	3955	1994-08-13
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f	*E.Eberhardt 3349*	GOET		Pakistan	Karakorum, Batura, Guchism	3955	1994-08-13
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*E.Eberhardt 1176*	GOET		Pakistan	Karakorum, Batura, Wudmur	3700	1994-07-19
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*E.Eberhardt 1276*	GOET		Pakistan	Karakorum, Batura, Wudmur	3800	1994-07-20
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f/m	*U.Wündisch 29*	GOET		China (Xinjiang)	SW Xinjiang, Upper Ojtagh Valley	3050	1998-06-22

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium leontopodinum (DC.) Hand.-Mazz.	f	*Tribsch & Essel 9802*	WU	38279	Russian Federation	Altay. Ridge between the valley of river Sarzhematy (Sarżematy) and the valley of river Kalanegyr	2500–3348	2003-08-05
Leontopodium leontopodinum (DC.) Hand.-Mazz.	m	*J.F. Duthie 11223*	WU	69174	India (Jammu and Kashmir)	Kashmir, Gulmarg	2450–2750	1892-05-25
Leontopodium leontopodinum (DC.) Hand.-Mazz.	m	*S.A.Harriss 16255*	WU	69175	Pakistan (Khyber Pakhtunkhwa)	Guger	2750	1895-05-18
Leontopodium leontopodioides (Willd.) Beauverd	f	*G.Miehe & al. 07-006-02*	WU	43969	China	No loc. 35.56°N/ 99.85°E	3180	2007-08-31
Leontopodium leontopodioides (Willd.) Beauverd	m	*G.Miehe & al. 07-006-03*	WU	43968	China	No loc. 35.56°N/ 99.85°E	3180	2007-08-31
Leontopodium leontopodioides (Willd.) Beauverd	f/m	*G.Miehe & al. 07-006-05*	WU	43967	China	No loc. 35.56°N/ 99.85°E	3180	2007-08-31
Leontopodium lingianum (Y.L.Chen) Dickoré	f	*W.B.Dickoré 11363*	WU	38301	China (Xizang)	SE Tibet. Gyala Peri N, Ridge W of Gyala Peri-N Glacier	4550	1994-08-19
Leontopodium lingianum (Y.L.Chen) Dickoré	m	*W.B.Dickoré 10836*	WU	38302	China (Xizang)	SE Tibet. Tsangpo tributary, Nangxian - Mainling. Lilung Chu Eastern branch (High Camp)	4850	1994-08-11

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium microphyllum Hayata	f/m	*E.Hörandl, Chien-I-Huang Huang, Chien-Hua Liu 9549*	WU	4795 13-83/13	Taiwan	Nantou County, Central Mountains, Mt Hohuan, along central cross-island highway, from Wuling to Hohuan Lodge	3230	2004-09-29
Leontopodium monocephalum Edgew.	f	*Staubinger N16/3*	W	2005 01062	Nepal	Chukhung, Solo Khumbu	4800	1997-10-18
Leontopodium monocephalum Edgew.	m	*J.F.Duthie 816*	W	1924 12098	India (Uttarakhand)	NW Himalaya, Tehri Garhwal	4500–4900	1883-08-09
Leontopodium monocephalum Edgew.	f	*Smith & G.H.Cave*	W	1924 12098	India (Sikkim)	Sikkim	4700	1909-08-06
Leontopodium muscoides Hand.-Mazz.	f/m	*G.Forrest 20724*	W	1924 1117	China (Yunnan)	24°30′N, 98°56′E	4400 m	1921-09-
Leontopodium muscoides Hand.-Mazz.	f	*F.K.Ward 5375*	W	1924 1127	China (Yunnan)	Danyon	5180 (17 000 ft)	1922-09
Leontopodium muscoides Hand.-Mazz.	f/m	*G.Forrest 12989*	W	1924 1120	China (Yunnan)	Mekang – Yangtze	3960 (13 000 ft)	1914-08-
Leontopodium nanum (C.B.Clarke ex Hook.f. & Thomson) Hand.-Mazz.	f	*Dawnogorski 121*	W	1925 3808	China (Xinjiang)	Kaschgar	–	1909-06-22

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium nanum (C.B.Clarke ex Hook.f. & Thomson) Hand.-Mazz.	m	*N.M.Przewalski*	W	1925 10255	China (Qinghai)	Tibet, fl. Yangze	3960 (13 000 ft)	1884-06-13 to 25
Leontopodium nanum (C.B.Clarke ex Hook.f. & Thomson) Hand.-Mazz.	f/m	*N.M.Przewalski 154*	W	1925 10255	China (Qinghai)	Tibet, fl. Yangze	–	1884-06-12 to 24
Leontopodium nanum (C.B.Clarke ex Hook.f. & Thomson) Hand.-Mazz.	f/m	*W.Koelz 2179*	W	1935 1322	India (Jammu & Kashmir)	Puga, Rupshu	4570 (15 000 ft)	1931-07-04
Leontopodium nivale (Ten.) Huet ex Hand.-Mazz.	m	*M. de Sardagna, Plantae Italicae s.n.*	WU	69169	Italy	Abbruzzo		1887
Leontopodium nivale (Ten.) Huet ex Hand.-Mazz.	f/m	*H.Groves s.n.*	WU	69170	Italy	Majella	2750	1880-08
Leontopodium nivale (Ten.) Huet ex Hand.-Mazz.	f	*S.Safer, V.Vladimirov SS-P-1*	WU	43988	Bulgaria	Bansko, Pirin	2385	2008-08-13
Leontopodium ochroleucum Beauverd	f	*G.&S.Miehe 2746*	GOET		Pakistan	Karakorum	4270	1990-08-26
Leontopodium ochroleucum Beauverd	f	*G.&S.Miehe 2780*	GOET		Pakistan	Karakorum	4260	1990-08-26
Leontopodium ochroleucum Beauverd	f/m	*G.&S.Miehe 2866*	GOET		Pakistan	Karakorum	4260–4370	1990-08-27

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium ochroleucum Beauverd	m	*G.&S.Miehe 8442*	GOET		China (Xinjang)	Kunlun-Aksai Chin Tiznap	4200	1990-07-09
Leontopodium ochroleucum Beauverd	m	*G.&S.Miehe 8373*	GOET		China (Xinjiang)	Kunlun-Aksai Chin, Kunlun	4880	1990-07-10
Leontopodium ochroleucum Beauverd	f	*G.&S.Miehe 951206*	GOET		Pakistan	Deosai Lake	4050	1995-09-23
Leontopodium ochroleucum Beauverd	f	*W.B.Dickoré 17837*	WU	43979	Tajikistan	Gorno-Badakshan, East Pamir, Takhtakorum, N Gudara Mts, Uruchbulak-Ailutek P.S.	4200	2002-09-06
Leontopodium roseum Hand.-Mazz.	f/m	*H.Smith 12190*	W	1940 9601	China (Sichuan)	Sikang, Taofu	2900	1934-09-17
Leontopodium sinense Hemsl.	f	*S.Safer, S.Schwaiger & Y.Guo SSG04*	WU	43976	China (Yunnan)	Dali; Cang Shan Mt	2216–2482	2008-09-07
Leontopodium sinense Hemsl.	f/m	*H.Hand.-Mazz. 9780*	WU	69167	China (Yunnan)	Ad fluvium Lu-djiang (Salween)	2050	1916-08-09
Leontopodium sinense Hemsl.	f	*S.Safer, S.Schwaiger & Y. Guo 04*	WU	43976	China (Yunnan)	Dali; Cang Shan Mt	2216–2482	2008-09-07
Leontopodium souliei Beauverd	f	*W.B.Dickoré 13167*	GOET		Pakistan	NW Himalaya, Hazara, Mt Makra	3800	1995-09-14
Leontopodium souliei Beauverd	f	*W.B.Dickoré 13144*	GOET		Pakistan	NW Himalaya, Hazara, Mt Makra	3610	1995-09-14
Leontopodium souliei Beauverd	f	*W.B.Dickoré 13129*	GOET		Pakistan	NW Himalaya, Hazara, Mt Makra	3570	1995-09-14

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium souliei Beauverd	m	*H.Hand.-Mazz. 7515*	WU	69165	China (Sichuan)	Muli – Yungning, Hwayi	3200	1915-08-07
Leontopodium souliei Beauverd	m	*S.Safer, S.Schwaiger & Y.Guo SSG30*	WU	44014	China (Yunnan)	Zhongdian	3347	2008-09-12
***L.* sp. nov.**	f/m	*W.B.Dickoré 10529*	WU	38293	China (Xizang)	SE Tibet. Tsangpo Vy., Nangxian – Mainling. Vy. 10 km W of Gyemdong (Camp 13)	3350	1994-08-04
***L.* sp. nov.**	f	*H.Hand.-Mazz. 8038*	W	1924 2445	China (Yunnan)	Ad confines Tibeticas sub jugo Dokerla	3600	1915-09-19
Leontopodium stracheyi (Hook.f.) C.B.Clarke ex Hemsl.	f	*S.Safer, S.Schwaiger & Y.Guo MSMt 1*	WU	44005	China (Yunnan)	Deqen; National Park Mei Li Snow Mt	3055	2008-09-14
Leontopodium stracheyi (Hook.f.) C.B.Clarke ex Hemsl.	f	*H.Hand.-Mazz. 80832*	WU	69164	China (Yunnan)	Ad confines Tibeticas sub jugo Dokerla	3750	1915-09-16
Leontopodium stracheyi (Hook.f.) .B.Clarke ex Hemsl.	m	*H.Smith 11925*	W	1940 9556	China (Sichuan)	Sikang, Taofu, distr.: Taining	3600	1934-09-08
Leontopodium stracheyi (Hook.f.) C.B.Clarke ex Hemsl.	m	*H.Smith 13765*	W	1940 9554	China (Sichuan)	Sikang, Taofu, distr.: Taining cult. e sem. in hort. Upsal.	2700	1934-08-12
Leontopodium villosum Hand.-Mazz.	f/m	*K.L.Chu, 3357*	W	1950 15874	China (Sichuan)	Pao-hsing-hsien	4000	1936-08-12

Table 1. *Continued*

Taxon	Sex	Collector no.	Herbarium	Herbarium no.	Country (state)	Locality	Altitude (m)	Date
Leontopodium wilsonii Beauverd	f/m	*S.Safer, S.Schwaiger & Y.Guo 26*	WU	43960	China (Yunnan)	Luquan; on top of Jiaozi Shan	4162	2008-09-21
Leontopodium wilsonii Beauverd	f/m	*W.B.Dickoré 14040*	WU	38300	China (Yunnan)	Yunnan NW, Dali Xian. Cangshan W of Dali	3440	1996-07-29

in the genus. A group of cells forms distinctive structures at the end of the tip that can be divided into the following subcategories.

No structure at the end (Fig. 1A): the structure of the tip cannot be distinguished from the rest of the bristle; it ends bluntly.

Elongated end cells (Fig. 1B): the bristle ends in one or a maximum of two thin, elongated end cells.

Accumulation of elongated cells (Fig. 1C): the bristle ends in an accumulation of elongated cells that form small barbs; the tip is not clavate.

Tip of round cells (Fig. 1D): the cells of a clearly distinguishable tip are not elongated but round instead; they do not stick out of the bristle and form no barbs.

Fan tip (Fig. 1E): the tip is extremely clavated, and the cells are elongated and spread out, appearing like a fan, because it is broader and shorter than the tree-like tips.

Tree-like tip (see Fig. 1F): many cells of the more or less clavate bristle stick out of the bristle and form a clearly defined tree-like tip.

Button-like barbs (Fig. 1G): some cells form button-like barbs in the tip region.

Bristle length (Fig. 1H, I): Three character states were established: < 3 mm; 3–4 mm; and > 4 mm.

Colour of the pappus bristles (Fig. 1J): The sexes and species were divided into two groups: those with normal white bristles; and those with coloured bristles, usually slightly brown or golden especially at the tip.

Number of barbs on the bristle (Fig. 1K–M): The three categories in ascending order are: no (or nearly no) barbs; some barbs; or many barbs. To make sure that this category does not interfere with the tip, the surface of the pappus bristle in the lowermost third was examined.

Kind of barbs on the bristle (Fig. 2A–C): There are three kinds of barbs: miniature; long and thin; and massive.

Thickness of the pappus bristle (Fig. 2D–F): The thickness was determined by counting the numbers of cells visible in top view in the outer layer of the pappus bristle. If containing up to three rows of cells, the bristle was designated as thin, with four or five cell rows as medium, and with five or more cell rows as large.

Number of pappus bristles (Fig. 2G, H): The number of pappus bristles was counted only on an undamaged pappus. For this character intraspecific

Table 2. Characters and character states

species	sex	Subgroups section *Nobilia* (n), Himalayan Group (him), plants forming soft and dense cushions (cu) distribution Europe (eur)	1 Bristle lenght: large	2 Bristle lenght: medium	3 Bristle lenght: small	4 Thickness: thin	5 Thickness: medium	6 Thickness: big	7 Elongated "end cells"	8 Accumulation of elongated cells	9 Tip of round cells	10 No structure at the end	11 Treelike tip	12 Button-like barbs	13 Fan Tip	14 nearly no barbs on the bristle	15 some barbs on the bristle	16 many barbs on the bristle	17 long thin barbs	18 miniature barbs	19 massive barbs	20 coloured	21 number of bristels (PCoA : number ≤ 24 vs. number ≥ 25)	1:finest conditions for wind dispersal, 2:wind dispersal might work in a diminished way, 3:wind dispersal not possible.	1: alp. heathland (low, dense, wet, acidic), 2: alp. steppe (open, dry, disperse, medium high, usually alkaline) ,3: alp. turf (closed, moderately moist, usually low), 4: mont. steppe, 5: subalp. steppe, 6: mont. heathland, moist, acidic, 7: mont.-subalp. steppe forest, 8: subtrop. heathland/low scub, seasonally dry, 9: subtrop. grassland/meadow, sesonally moist, 10: subalp. steppe /steppe forest, 11: temp. scrub/open forest.
character number (PCoA)			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
L. discolor	f			x				x	x								x				x		16 20 25	1	3
L. jacotianum	f			x			x		x								x		x				31	2	7
L. microphyllum	f				x		x					x					x		x					1	1
L. monocephalum	f			x			x			x								x	x				39 13	1	2
L. roseum	f			x			x							x			x		x				15 22	2	7
L. villosum	f		x				x		x									x			x	x	32	1	6
L. wilsonii	f			x				x	x									x			x		25 20 20 18	1	6
L. discolor	m			x				x					x					x			x		26 23 26	1	3
L. giraldii	m			x			x				x						x				x	x	42	1	6
L. jacotianum	m			x			x						x				x		x				39 17 23 13	2	7
L. microphyllum	m				x			x				x					x		x					1	1
L. monocephalum	m			x				x					x			x				x			17 15	1	2
L. roseum	m			x			x							x		x				x			21	2	7
L. villosum	m		x				x		x								x				x	x	32	1	6
L. wilsonii	m			x				x	x									x			x		22 21	1	6
L. aurantiacum	f	cu		x			x		x								x			x		x	33	1	1
L. haastioides	f	cu		x			x			x						x				x			31	1	2
L. lingianum	f	cu	x					x		x						x				x			24	1	1
L. muscoides	f	cu	x				x		x							x					x		21 19 24	1	1
L. aurantiacum	m	cu		x			x			x						x				x		x	23 23	1	1
L. haastioides	m	cu		x			x			x						x				x			62	1	2
L. lingianum	m	cu		x				x					x			x				x			25 30	1	1
L. muscoides	m	cu	x					x			x						x			x		x	26 28	1	1
L. alpinum	f	eur	x					x	x									x			x		21 22 18 15 16	1	3
L. nivale	f	eur	x					x	x								x		x				29 25 17	1	2
L. alpinum	m	eur	x					x					x					x			x			25 27 27 14 15	1	3
L. nivale	m	eur	x					x					x				x		x				24 28	1	2
L. calocephalum	f	him	x					x	x									x	x				30 36 25 26 29	1	3
L. himalayanum	f	him	x				x		x								x		x				26 30 18	1	3
L. leontopodioides	f	him	x						x							x				x			26	1	4
L. leontopodinum	f	him	x				x		x									x			x		16 21 20 20 19 21	1	5
L. nanum	f	him	x				x		x							x				x			36 28 17	1	3
L. ochroleucum	f	him		x					x								x		x				25 29 24 29 23 25 28 36 28	1	2
L. pusillum	f	him	x						x							x				x			24 33 25 24 23	1	2
L. souliei	f	him		x					x								x		x				23 23 20	1	3
L. calocephalum	m	him	x				x			x							x				x		26 26 28 23 20	1	3
L. himalayanum	m	him	x				x						x				x		x				31	1	3

Table 2. *Continued*

L. leontopodioides	m	him	x				x					x				x				x		24 23 22	1	4
L. leontopodinum	m	him	x				x					x					x			x		21 18 20 28 22 18 23 24	1	5
L. nanum	m	him	x				x					x			x				x			25 28 27	1	3
L. ochroleucum	m	him		x			x					x				x			x			26 26 15 20 20 23	1	2
L. pusillum	m	him	x				x					x			x				x			26 23 17 26	1	2
L. souliei	m	him		x			x					x				x				x		35 34 30	1	3
L. andersonii	f	n		x				x	x							x		x				22 11	3	8
L. artemisiifolium	f	n			x		x		x								x	x				16 16	2	9
L. dedekensii	f	n		x			x		x								x			x		22 20	2	7
L. franchetii	f	n			x		x		x							x		x				13 14 18 18 18 13	2	10
L. haplophylloides	f	n			x			x	x								x			x		14 14 16 13 22 15	2	7
L. japonicum	f	n			x			x	x								x			x		21 19 19	2	9
L. sinense	f	n		x			x		x							x		x				13 17 15	3	8
L. spec. nov.	f	n		x			x		x								x			x		23 20	3	11
L. stracheyi	f	n		x				x	x								x	x				20 20 19	2	7
L. andersonii	m	n		x				x				x				x				x		18 19	3	8
L. artemisiifolium	m	n			x			x				x					x			x		10 24 24	2	9
L. dedekensii	m	n		x				x				x					x			x		16 19	2	7
L. franchetii	m	n			x		x							x			x			x		18/15 12	2	10
L. haplophylloides	m	n		x				x				x					x			x		18 20 24 17 20 19	2	7
L. japonicum	m	n		x				x				x					x			x		21 16 19	2	9
L. sinense	m	n		x				x				x					x	x				14	3	8
L. spec. nov.	m	n		x				x				x					x			x		22	3	11
L. stracheyi	m	n		x				x				x					x			x		15	2	7

Different colors indicate that the infrageneric subgroups.

and intra-individual variation was detected. Only consistent counts, however, have been listed in Table 2.

The PCoA depicted a distinct cluster for *Leontopodium* section *Nobilia* (Handel-Mazzetti, 1927) if male and female fruit characteristics were evaluated seperately. Characters 1, 4, 5, 7, 8, 10, 11, 12, 14, 18 and 21 (Table 2) for the male pappi were correlated and depict a clear cluster for section *Nobilia*. The first three coordinates accounted for 36.27, 21.25 and 19.13% of the morphological variation, respectively (Fig. 4).

The correlation of 13 female fruit characters (characters 1, 4, 7, 8, 9, 10, 11, 12, 13, 14, 18, 20 and 21; Table 2) also resulted in a distinct cluster of taxa belonging to section *Nobilia*. The first three principal coordinates accounted for 41.36, 24.59 and 18.23% of the morphological variation, respectively (Fig. 5). No other section could be detected by character correlation using the PCoA.

DISCUSSION

Diagnostic characters for delimitation of species

Morphological data from the pappus have been shown to be taxonomically useful for assessing relationships among species within genera of Asteraceae, e.g in *Solidago* L. (Semple & Hood, 2003). In the present study on *Leontopodium*, pappus characters have also been revealed to be significant for inferring relationships. Several features are seen to be diagnostic for particular species and these observations provide new distinguishing characters.

The pappus tips of male flowers of *L. franchetii* possess a tip that is extremely clavate, with elongated and spread-out cells as in a fan (Fig. 1E). This structure has been found in all investigated specimens of this species from different geographical localities and it can be regarded as a new distinctive character.

Leontopodium microphyllum Hayata (male and female flowers) shows a unique kind of bristle tip, the structure of which can be seen as a newly discovered distinctive feature. The structure of the tips cannot be distinguished from the rest of the bristle: the tip is the same and it ends abruptly and bluntly (Fig. 1A).

The type of barbs found on the pappus bristle tips of *L. roseum* Hand.-Mazz. (male and female flowers) is characteristic for the species. These unique barbs (Fig. 1G) are button-like, and they provide a newly discovered diagnostic character.

Leontopodium wilsonii Beauverd and *L. villosum* Hand.-Mazz. are the only two species in which bristles in male flowers do not form unique tips, but instead are similar to the bristles of female flowers. Because the bristles of *L. wilsonii* are white and those of *L. villosum* are coloured, however, it is easy to distinguish the pappus between them.

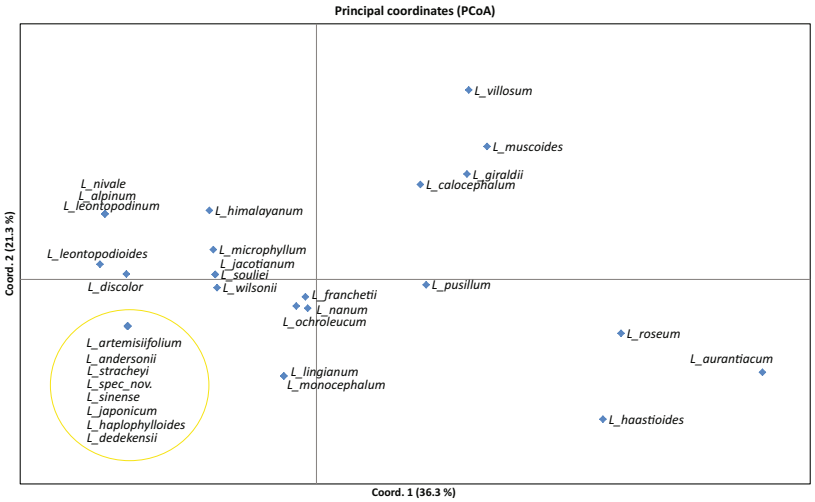

Figure 4. Principal coordinate analysis depicting morphological distances between the 31 analysed *Leontopodium* species by using 11 pappi characters (see Table 2) from the male pappi. The correlation depicts sectional delimitation (section *Nobilia*, marked in yellow) with the exception of *L. franchetii*.

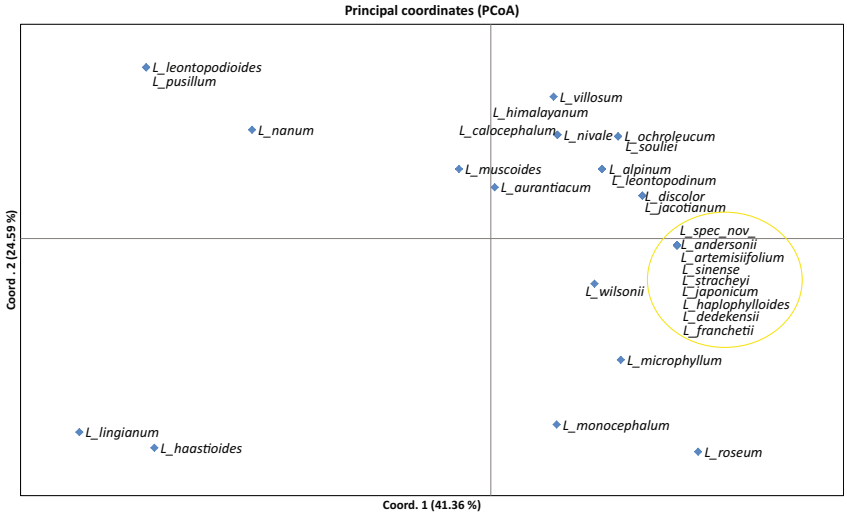

Figure 5. Principal coordinate analysis depicting morphological distances between the 31 analysed *Leontopodium* species by using 13 pappi characters (see Table 2) from the female pappi. The correlation depicts sectional delimitation (section *Nobilia*, marked in yellow).

Recognition of infrageneric subgroups

On different systematic levels, morphological data from the pappus in Asteraceae have helped in other taxa to reveal evolutionary relationships among and within genera. Mukherjee & Sarkarj (2001) showed the utility of pappus features among genera broadly in tribe Astereae and Semple (2006) likewise showed their efficacy in subtribe Chrysopsidinae of the same tribe. Semple & Hood (2005) also showed the positive contributions of pappus structure for inferring affinities in *Symphyotrichum* Nees and related genera of Astereae. This study in *Leontopodium* also provides evidence for the utility of pappus features for recognition of evolutionary subgroups in the genus.

Section *Nobilia* has been a previously accepted subgroup (Handel-Mazzetti, 1927), and it is confirmed by the results of this study. Female flowers in section *Nobilia* are characterized by pappus bristles that are never bald and never show a special

structure on their tip. The highest number of bristles can reach 23, but usually there are < 20. Some of the bristles are medium-long and thick, but frequently they are small and thick. The pappus bristles of male flowers in section *Nobilia* are completely uniform, except for three minimal deviations: they are normally thick, medium-long to short, have many large barbs on their surface and form tree-like tips. *Leontopodium franchetii*, however, is an exception because it forms a unique fan-like tip (Fig. 1E). The barbs of *L. sinense* Hemsl. are longer and thinner and those of *L. franchetii* fewer than those of the other members of section *Nobilia*. The maximum count of pappus bristles for male flowers in section *Nobilia* was 24, but < 20 is usually found. This uniform appearance of male and female pappus bristles separates section *Nobilia* from the rest of the genus.

Four *Leontopodium* spp. form soft, dense, ± hemisphaerical cushions [*L. lingianum* (Y.L.Chen) Dickoré, *L. aurantiacum* Hand.-Mazz., *L. haastioides* Hand.-Mazz., *L. muscoides* Hand.-Mazz.]. They are often seen as a subgroup, but proper evidence for their affiliation is still missing. The results of this study suggest that they are closely related to each other because they share particular characters. They have a large number of pappus bristles (always > 21) and almost no barbs on their more or less thick bristles. It is also remarkable that, with the exception of *L. lingianum* (Y.L.Chen) Dickoré, the male flowers do not form tree-like tips as the great majority of male flowers in the genus do.

Another subgroup comprises eight *Leontopodium* spp. that grow in the Himalayan range, the Tibetan Plateau and bordering regions. They differ with respect to the surface of the pappus bristles in number of barbs and kind of barbs, but they are conspicuously uniform in terms of length (nearly all long): the bristles of male flowers are medium or thick and form tree-like tips [with the exception of *L. calocephalum* (Franch.) Beauverd]; those of female flowers are medium or thin and end in elongated cells. The micromorphological uniformity of this group supports their coherence as a subgroup. The extraordinary tip of *L. calocephalum*, being not tree-like but instead with an accumulation of elongated cells, helps distinguish this species from the others of this group.

In Figure 6, pictures of pappi (one male and one female) show typical characters for each infrageneric subgroup.

Phylogenetic relationships

Safer *et al.* (2011) published the most elaborate molecular study on phylogenetic relationships in *Leontopodium*, but < 50% of the species of the genus were included. Nevertheless, a comparison of the results shows that the data based on amplified fragment length polymorphisms and the micromorphological data of this study lead to congruent results regarding affinities. A neighbour-joining bootstrap consensus tree (50% majority rule) shows that the European species and the Mongolian/central Chinese species (groups A and B) are monophyletic [bootstrap support (BS) 100%]. For geographical reasons the European species were not included in the Himalayan group, but because of identical morphological pappus characters, it is possible to place them closely together. *Leontopodium leontopodioides* (Willd.) Beauverd and *L. leontopodinum* (DC.) Hand.-Mazz. [≡ *L. campestre* (Ledebour) Hand.-Mazz.] form a separate group according to genetic data. These two species are also part of the same Himalayan group in this study (see above). The additional species of that morphological group were not investigated genetically. Section *Nobilia* is again delimited clearly by the molecular data.

Figure 6 shows the overlap of genetic analysis (Blöch *et al.*, 2010; Safer *et al.*, 2011) with morphological groups pointed out in this study.

Dispersal ecology

Our small, non-quantitative anemochory experiment showed that the pappus of *Leontopodium* can serve as a mechanism for wind dispersal. The pappus with attached fruits flew or slipped on the ground several metres from the blowing hair-dryer. The pappus always remained intact, suggesting that it seems functional for at least short-distance dispersal. More elaborate and quantified experiments might be done in the future.

Based on the results of this experimental and morphological study, conclusions concerning the dispersal biology of the species in the genus *Leontopodium* can be drawn. The distributional areas for all examined *Leontopodium* spp. were compiled and supplemented with relevant eco-factors. Areas were divided according to potential wind dispersal into three groups: (1) best conditions for wind dispersal; (2) wind dispersal might possibly be efficacious; and (3) wind dispersal not possible.

Leontopodium spp. that grow in regions where wind dispersal seems to have little prospect of success (Table 2), such as in forested regions of the subtropical zone, have short, thin and inconspicuous pappus bristles. If a plant is surrounded by plants that are as high as the plant or even higher, wind dispersal loses its efficiency (Sheldon & Burrows, 1973).

Species that are located in little or sparsely vegetated mountainous regions (Table 2) often possess a

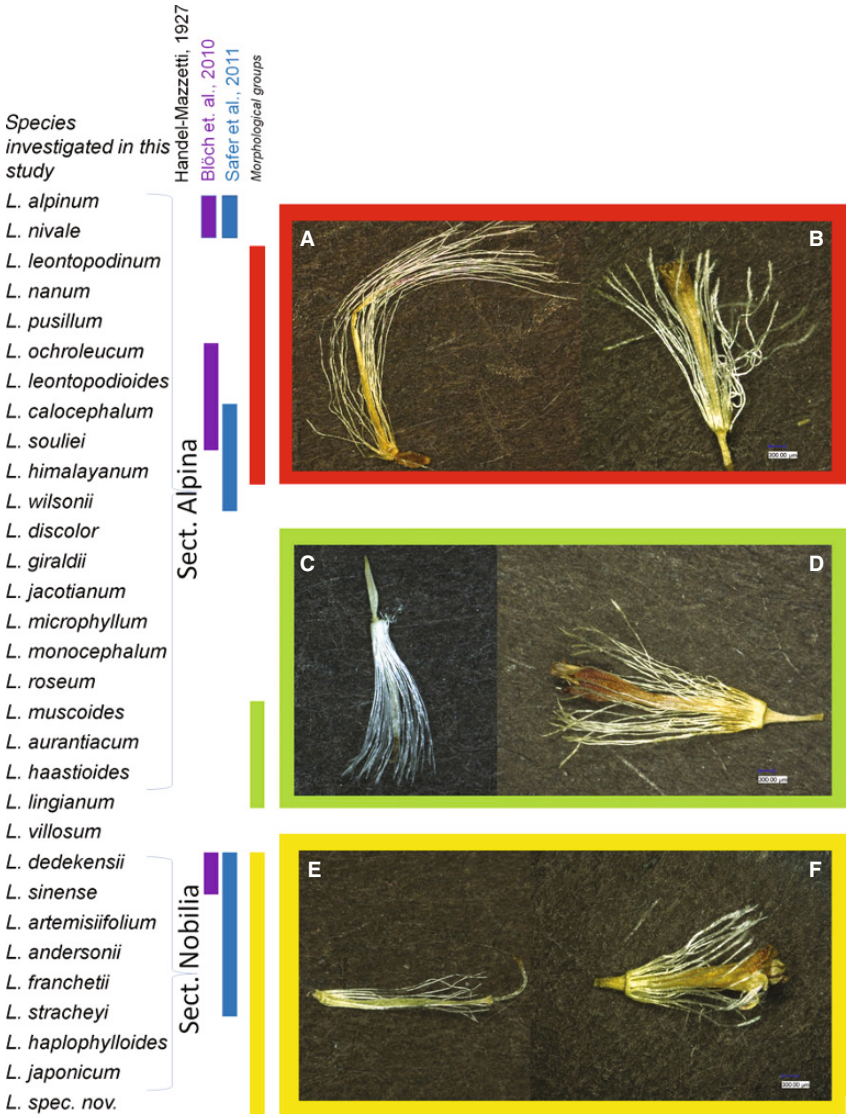

Figure 6. Species investigated in this study arranged according to the morphological groups established in 1927 (Handel-Mazzetti). The overlap with results of genetic analysis (Blöch *et al.*, 2010; Safer *et al.*, 2011) and with morphological groups pointed out in this study is marked. Pictures of two pappi (one male and one female) show typical characters for each of those groups. A, *L. pusillum* f. B, *L. ochroleucum* m. C, *L. haastioides* f. D, *L. aurantiacum* m. E, *L. andersonii* f. F, *L. haplophylloides* m. Scale bars are valid for A–F. m, male; f, female.

large number of long pappus bristles, especially on the female flowers, which are well suited for dispersal of the achenes. Minami & Azuma (2003) found in a theoretical–mathematical flight dynamics study that these features significantly increase the effectiveness of the pappus as a flight apparatus. Pappus with seeds are known to make parachuting flights which utilize the drag force acting on the pappus. With longer bristle hairs this force becomes stronger and the seeds are dispersed over a wider area. This might be an evolutionary advantage by helping colonization of larger geographical regions.

Defensive function

The structure of male pappus bristles and especially their tips obviously cannot be interpreted in the context of dispersal biology, because these florets produce no fertile achenes. Nearly all pappus bristles of male flowers show a tree-like tip. One might hypothesize that these tips have a defensive function to protect the infertile ovaries and/or adjacent fertile ovaries in commonly heterogamous inflorescences from predators and water (Stuessy & Spooner, 1988; Stuessy & Garver, 1996).

Pappus structure and polyploidy

In some genera of Asteraceae a correlation between pappus length and polyploidy has been documented and Semple (2006) showed such a relationship in subtribe Chrysopsidinae (tribe Astereae). Such a correlation, however, does not apply for *Leontopodium*. The pappus of section *Nobilia*, for example, is remarkably uniform even though different chromosome numbers and even ploidies exist in the group (Russell *et al.*, 2013; Stille *et al.*, 2014).

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