Pak. J. Bot., 54(4): 1461-1474, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-4(30)

ACHENE MICROMORPHOLOGICAL CHARACTERISTICS AND TAXONOMIC SIGNIFICANCE IN *LEONTOPODIUM* R. BROWN EX CASS. TAXA (ASTERACEAE: GNAPHALIEAE) IN CHINA

YI RAN MA[#], ZHEN WEI[#], YANG LU, ZHENG LI, QING FEI ZHAO AND SHI XIN ZHU[*]

School of Life Sciences, *Zhengzhou University*, *Henan*, *China*
**Corresponding author's email: sxzhu@zzu.edu.cn*

Abstract

Leontopodium R. Brown ex Cass.belongs to the tribe Gnaphalieae in the family Asteraceae; it comprises approximately 60 species worldwide, of which 40 are distributed in China. The morphological characteristics of the achene are relatively stable and can be used as taxonomic criteria for species classification within Asteraceae. In this study, scanning electron microscopy (SEM) was used to observe the achene micromorphological characteristics of 28 species and 1 variety of Chinese *Leontopodium*. The results showed that the achenes of *Leontopodium* species were elliptical or narrow elliptical, approximately 0.7–1.6-mm long × 0.1–0.6-mm wide. The surface ornamentation was reticulate or rippled with clavate twin hairs or smooth. The carpopodium base was constricted or unconstricted. Based on these characteristics, we provided a new key for Chinese *Leontopodium* taxa. The characteristics of the achene trichome, surface ornamentation, and carpopodium show important taxonomic value at different taxonomic levels, leading us to arrive at the following conclusions. 1. The shape of the achene trichome is valuable for the taxonomic delimitation of taxa between *Leontopodium* and related genera. 2. The achene surface ornamentation can be categorized into two types and is a taxonomic tool for classification at the section level within *Leontopodium*. 3. The characteristics of the carpopodium and trichome density can serve as important features under the section level. Thus, achene micromorphological characteristics provide significant morphological evidence that could play an important role in resolving various taxonomic problems within *Leontopodium* and among related genera in Asteraceae.

Key words: Achene micromorphology, Surface ornamentation, *Leontopodium*, Scanning electron microscopy, Gnaphalieae.

Introduction

Leontopodium R. Brown ex Cass. belongs to the tribe Gnaphalieae in the family Asteraceae and comprises approximately 60 species globally. It is the second-largest genus of Gnaphalieae in the northern hemisphere and is mainly distributed in Asia, Europe, and South America (Beauverd, 1909; Ward *et al*., 2009; Blöch *et al*., 2010), and there are 37 species of *Leontopodium* in China, of which 17 are endemic. *Leontopodium* species are widely distributed in the alpine meadows and mountain slopes of southwestern China (Chen & Bayer, 2011). The Himalayas, especially the Qinghai–Tibet Plateau, which contains approximately 15–18 species, is the diversity center of this genus (Safer *et al*., 2011; Lee *et al*., 2016a).

The tribe Gnaphalieae (comprising 178 genera and approximately 2100 species) was divided into two subtribes, Subtrib. Relhaniinae and Subtrib. Gnaphaliinae (Smissen *et al*., 2020). The tribe was distributed in temperate habitats around the world (Smissen *et al*., 2020), mainly in South America, South Africa, and Australia (Anderberg, 1994; Bayer *et al*., 2007). Galbany *et al*., (2010) indicated that the *Filago* group (Gnaphalieae) belonged to the FLAG clade (acronym of the first letters of the large genera *Filago*, *Leontopodium*, *Antennaria*, and *Gamochaeta*). Nie *et al*., (2016) performed a detailed study of Gnaphalieae species and divided them into seven clades. His results also supported the FLAG clade and showed the high support.

The genus *Leontopodium* was officially published by Cassini in 1819 and *L. alpinum* was the type species of the genus (Brown & Cassini, 1819; Flann *et al.*, 2010). In 1928, based on the morphology of the pappus and achene trichome, Handel-Mazzetti recorded 41 species of *Leontopodium* that could be classified into two subgenera (*Paragnaphalium* and *Euleontopodium*). The latter was divided into two sections, *Nobilia* and *Alpina* (Handel-Mazzetti, 1928). Lin (1965, 1979) generally followed the Handel-Mazzetti system of classifying the Chinese *Leontopodium* into two subgenera, *Paragnaphalium* and *Leontopodium*. The latter was divided into sections, *Nobilia* and *Leontopodium*. Sect. *Nobilia* includes four subsections: *Glandulosa*, *Haastata*, *Japonica*, and *Subulata*; Sect. *Leontopodium* was divided into subsections *Alpinoidea*, *Chromotricha*, *Haastioidea*, and *Pseudantennaria*. The two subsections consist of 12 series (Lin, 1965, 1979).

Numerous studies have shown that the morphological characteristics of Asteraceae achenes are stable and not easily changed by environmental conditions (Barthlott, 1984, 1990; Ghimire *et al*., 2016). The achene micromorphological characteristics were significantly different among various genera, which provided direct evidence of the species delimitation and systematic relationship within Asteraceae (Pak & Kawano, 1990a, 1990b; Zhang & Chen, 2008; Lu, 2013; Guan, 2014). The achene shape and size, surface ornamentation, and surface hairs have been proven to have taxonomic significance at the genus and species levels (Chen & Jing, 2007; Federico *et al*., 2017; Inceer *et al*., 2018). The achene in Gnaphalieae is oblong, oblanceolate or ellipsoid; yellowish-brown or dark brown; glabrous, papillate, with clavate or globose hairs. The pappus is uniseriate, white or yellow, and deciduous. The carpopodium is narrow or broad-round without interruption (Abid & Qaiser, 2008a; Ru, 2018). These previous studies observed the achenes of *L. dedekensii*, *L. souliei*, *L. calocephalum*, and *L. ochroleucum* using scanning electron microscopy (SEM), and the results showed that the achenes were elliptical, the carpopodium was round, and the surface ornamentation was rippled (Chen & Jing, 2007; Jing, 2007). These

characteristics distinguished *Leontopodium* from its related genera. Stille *et al*., (2016) performed cluster analysis using a principal coordinate analysis (PCoA) based on six characteristics of the achene pappus (bristle number, bristle length, color, surface structure, tip structure, and type of surface barb) from 31 *Leontopodium* species. Monti *et al*., (2013) revised the genera *Gnaphalium* and *Pseudognaphalium* based on the morphological characteristics of the achene surface trichomes and pappus. They found that the *Gnaphalium* pappus was dimorphic and the achene surface had papillate hairs, whereas the *Pseudognaphalium* pappus was monomorphic and the achene surface was glabrous or with papillary hairs. Based on these characteristics, they transferred four *Gnaphalium* species (*G. aldunateoides*, *G. andicola*, *G. glanduliferum*, and *G. perpusillum*) to *Pseudognaphalium*. Xu *et al*., (2017, 2021) observed the surface micromorphological characteristics of achenes from 29 *Anaphalis* taxa using SEM. The surface ornamentation of the achenes was categorized into two types. Type I was reticulate with rod-like bulges; Type II had ligule bulges.

The number of *Leontopodium* species in China accounts for two-thirds of the total *Leontopodium* species; therefore, to further examine and characterize the phylogenetic relationships of *Leontopodium*, it is important to study Chinese *Leontopodium*. However, current *Leontopodium* studies have mainly focused on karyotype (Meng *et al*., 2012; Russell *et al*., 2013); molecular phylogeny (Blöch *et al*., 2010; Safer *et al*., 2011), and chemical composition (Dobner *et al*., 2004; Ma *et al*., 2018; Xu *et al*., 2019). Studies on *Leontopodium* achene morphology have primarily focused on nine species (Chen & Jing, 2007; Jing, 2007; Abid & Qaiser, 2008b; Lee *et al*., 2016b; Ru, 2018). Therefore, this study aimed to conduct an in-depth micromorphological study of the achenes of 29 *Leontopodium* taxa from China to explore the taxonomic value of achene micromorphological characteristics. The results provide evidence for the taxonomic and systematic research of *Leontopodium* at the species level and the relationship between *Leontopodium* and its related genera.

Materials and Methods

Plant material: Mature achene specimens representing 28 species and 1 variety of *Leontopodium* were mainly collected in the field (voucher specimens were deposited at Herbarium Zhengzhou University (ZZU)) or obtained from Herbarium, Institute of Botany, Chinese Academy of Sciences (PE) (Table 1).

Table 1. Taxa of *Leontopodium* from China examined for achene structures, together with locality and voucher specimens details.

Taxon	Locality	Voucher specimens (Herbarium)
L. andersonii C.B. Clarke	Yunnan	S.X. Zhu *et al*., DS15068 (ZZU)
L. brachyactis Gand.	Unknown	Anonymous 5612 (PE)
L. caespitosum Diels	Yunnan	H.C. Wang 1056 (PE)
L. calocephalum Beauverd	Yunnan	J.F. Rock 5865 (PE)
L. campestre Hand. -Mazz.	Unknown	Anonymous 3534 (PE)
L. conglobatum Hand. -Mazz.	Neimenggu	J. Zhou 135 (PE)
L. dedekensii Beauverd	Sichuan	S.X. Zhu *et al*., DS15016 (ZZU)
L. fangingense Y. Ling	Guizhou	Wulingshan group 1322 (PE)
L. forrestianum Hand. -Mazz.	Xizang	FLPH Tibet Expedition 12-1758 (PE)
L. franchetii Beauverd	Yunnan	S.X. Zhu *et al*., 1907307 (ZZU)
L. giraldii Diels	Shanxi	Y.S. Chen 8128 (PE)
L. haplophylloides Hand. -Mazz.	Gansu	J.F. Rock 13390 (PE)
L. himalayanum DC.	Yunnan	Hengduanshan Expedition 3587 (PE)
L. jacotianum Beauverd	Sichuan	X.Y. Zhu *et al*., 10203 (ZZU)
L. japonicum Miq.	Hubei	S.X. Zhu *et al*., DS14526 (ZZU)
L. japonicum var. *microcephalum* Hand. -Mazz.	Shanxi	T.P. Wang W. 3959 (PE)
L. junpeianum Kitam.	Sichuan	S.X. Zhu *et al*., 1908198 (ZZU)
L. leontopodioides Beauverd	Xinjiang	R.C. Qin 5016 (PE)
L. microphyllum Hayata	Taiwan	T.Y.A. Yang 002498 (TNM)
L. monocephalum Edgew.	Xizang	Anonymous s.n. (PE)
L. nanum Hand. -Mazz.	Xinjiang	Y. Lu 201801 (ZZU)
L. ochroleucum Beauverd	Xinjiang	R.C. Qin 5016 (PE)
L. omeiense Y. Ling	Sichuan	X.L. Sun 486 (PE)
L. pusillum Hand. -Mazz.	Xizang	D.H. Liu *et al*., BNU2019XZ061 (ZZU)
L. sinense Hemsl.	Sichuan	S.X. Zhu *et al*., 1908004 (ZZU)
L. smithianum Hand. -Mazz.	Unknown	P. Licent S.J. 9948 (PE)
L. souliei Beauverd	Sichuan	S.X. Zhu *et al*., 1908168 (ZZU)
L. stracheyi C.B. Clarke ex Hemsl.	Sichuan	S.X. Zhu *et al*., DS15006 (ZZU)
L. wilsonii Beauverd	Sichuan	W.B. Ju *et al*., AZH00802 (CDBI)

Micromorphology: Achene micromorphological examinations (Table 2) were performed using an automated digital three-dimensional microscope (Zeiss Smartzoom 5; Zeiss, Germany) and SEM (SU3500; Hitachi High-Tech America, Inc., Japan). Five–six achene specimens were collected from each taxon, from which three–five mature and complete achenes were selected and soaked in distilled water for 3 h. The achenes were dehydrated in an ethanol series (35%, 50%, 70%, 85%, 95%, and 100%). After dehydration, they were attached to the sample table using conductive glue and sprayed with gold (approximately 20-nm thick). SEM imaging was performed at 5 kV.

The following achene morphological parameters were examined: shape, size (length and width), surface ornamentation, trichome (shape, density, and length), and carpopodium (shape, diameter, and height). Three pictures of each achene were taken: one general view (70×), one surface view (800×), and one of the carpopodium (500×). For each taxon, the sizes of three–five achenes were measured. The trichome density and length and carpopodium diameter and height of each achene were measured in different field views.

The terminology proposed by Barthlott (1990) and Liu (2004) was used to describe the achene morphological characteristics and surface micromorphological characteristics.

Results

Achene morphological characteristics: The achene shape, size (length and width), and surface ornamentation; trichome shape, density, and length; carpopodium size (diameter and height); and shape of the carpopodium base were observed and measured (Fig. 1, Table 2). The results showed that the *Leontopodium* achenes were approximately 0.7–1.6-mm long and approximately 0.1–0.6-mm wide, elliptical or narrow elliptical, mostly with sparse or dense clavate twin hairs, but smooth in some species. The diameter of the carpopodium was approximately 0.07–0.18 mm, and the height was approximately 0.02–0.14 mm. The carpopodium was round, and the base was constricted or unconstricted. The micromorphological descriptions of the achenes of each taxon are given in Table 2. Achene length was shortest in *L. giraldii* (0.61 mm), whereas it was longest in *L. smithianum* (1.68 mm). Achene width was narrowest in *L. andersonii* and widest in *L. himalayanum*. *L. himalayanum* had the longest trichome. Both the diameter and height of the *L. omeiense* carpopodium were greater than those in the other species (Fig. 1).

Achene micromorphological characteristics: The surface ornamentation of the *Leontopodium* achene was categorized into two types, Type I and Type II. Type I is reticulate, with edges forming several regular or irregular grids, whereas Type II is rippled, with undulate thickened edges (Fig. 2). Type I includes 14 taxa: *L. andersonii*, *L. calocephalum*, *L. forrestianum*, *L. sinense*, *L. microphyllum*, etc. Type II includes 15 taxa: *L. campestre*, *L. junpeianum*, *L. leontopodioides*, *L. nanum*, *L. smithianum*, etc. (Table 2).

Type I can be divided into three subtypes based on trichome density: Type I-1 (dense type, ≥4/100 × 100 μm^2), Type I-2 (sparse type, 1-3/100 × 100 μm^2), and Type I-3 (smooth type, no trichomes). Type I-1 includes *L. andersonii*, *L. dedekensii*, *L. franchetii*, *L. omeiense*, *L. sinense*, and *L. stracheyi*; Type I-2 includes *L. caespitosum*, *L. calocephalum*, *L. conglobatum*, *L. fangingense*, *L. forrestianum*, and *L. himalayanum*; and Type I-3 includes *L. microphyllum* and *L. wilsonii*. Based on the same features, Type II can also be divided into three subtypes: Type II-1 (dense type, ≥4/100 × 100 μm^2), Type II-2 (sparse type, 1-3/100 × 100 μm^2) and Type II-3 (smooth type). Type II-1 includes *L. japonicum*, *L. japonicum* var. *microcephalum*, *L. nanum*, and *L. smithianum*; Type II-2 includes *L. campestre*, *L. haplophylloides*, *L. jacotianum*, *L. junpeianum*, *L. leontopodioides*, and *L. ochroleucum*; and Type II-3 includes *L. brachyactis*, *L. giraldii*, *L. monocephalum*, *L. pusillum*, and *L. souliei*.

Different achene surface ornamentations and carpopodium sizes showed some correlation with trichome density. In Type I-1, the carpopodium diameter was approximately 0.08–0.11 mm and the height was approximately 0.02–0.04 mm (except in *L. omeiense* and *L. sinense*). In Types I-2 and I-3, the carpopodium diameter was approximately 0.12–0.18 mm, and the height was approximately 0.05–0.09 mm (except in *L. conglobatum* and *L. himalayanum*). The carpopodium diameter in Type II-1 was approximately 0.12–0.13 mm, while it was smaller (approximately 0.08–0.11 mm) in Types II-2 and II-3. However, the carpopodium diameters of *L. giraldii*, *L. monocephalum*, *L. ochroleucum*, and *L. pusillum* were approximately 0.13–0.22 mm. The mean carpopodium height in Type II was approximately 0.02–0.07 mm, with the carpopodium of *L. giraldii* and *L. jacotianum* showing the greatest height (approximately 0.11 mm) (Table 2).

Key to species of *Leontopodium* taxa in China: Based on the achene micromorphological characteristics, we have compiled a new key for 29 *Leontopodium* taxa in China.

Key to Chinese *Leontopodium* species based on achene characteristics

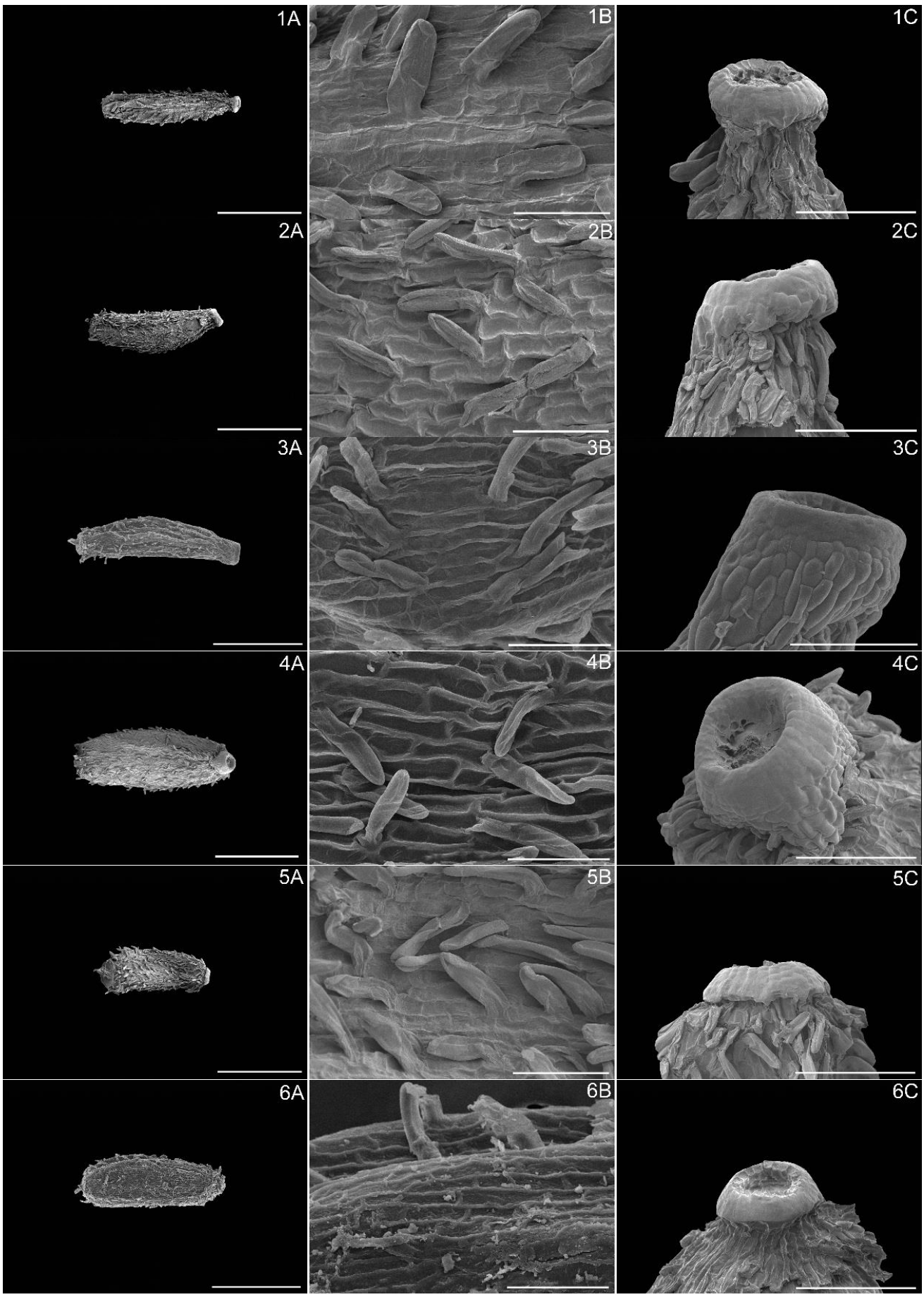
1 + Surface ornamentation of achenes reticulate (Type I) ..…..…..…… 2
- Surface ornamentation of achenes rippled (Type II) ……...…… 15
2 + Achenes surface with trichomes ……...…..…… 3
- Achenes surface smooth (Type I-3) ……..……... 14
3 + Achenes surface dense trichomes, ≥4/100 × 100 μm^2 (Type I-1) ……..…… 4
- Achenes surface sparse trichomes, 1–3/100 × 100 μm^2 (Type I-2) …….....…… 9

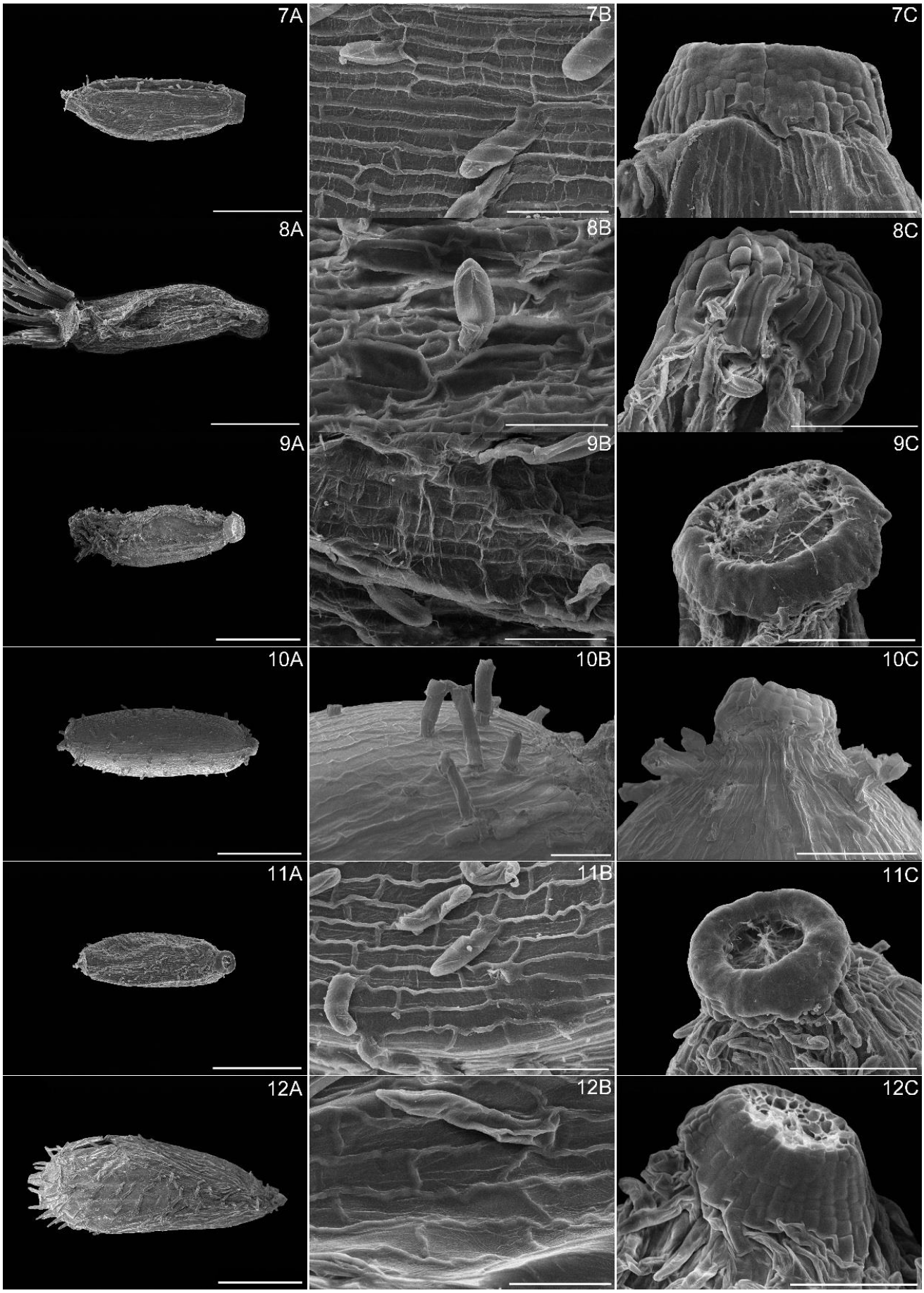
4 + Carpopodium base constricted 5
- Carpopodium base unconstricted 6
5 + Achene width 0.19–0.36 mm, elliptical, aspect ratio < 3 ..... *L. franchetii*
- Achene width 0.18–0.19 mm, narrow elliptical, aspect ratio > 3 ..... *L. andersonii*
6 + Carpopodium diameter < 0.12 mm, carpopodium height < 0.05 mm 7
- Carpopodium diameter > 0.12 mm, carpopodium height > 0.05 mm 8
7 + Achene elliptical, aspect ratio < 3 ..... *L. stracheyi*
- Achene narrow elliptical, aspect ratio > 3 ..... *L. dedekensii*
8 + Carpopodium height 0.13–0.14 mm ..... *L. omeiense*
- Carpopodium height 0.07–0.08 mm ..... *L. sinense*
9 + Carpopodium base constricted 10
- Carpopodium base unconstricted 13
10 + Carpopodium diameter < 0.12 mm 11
- Carpopodium diameter ≥ 0.12 mm 12
11 + Trichome length 0.05–0.06 mm ..... *L. conglobatum*
- Trichome length 0.08–0.10 mm ..... *L. himalayanum*
12 + Achene elliptical, aspect ratio < 3 ..... *L. calocephalum*
- Achene narrrow elliptical, aspect ratio > 3 ..... *L. caespitosum*
13 + Achene elliptical, aspect ratio < 3; carpopodium height < 0.07 mm ..... *L. forrestianum*
- Achene narrow elliptical, aspect ratio > 3; carpopodium height > 0.07 mm ..... *L. fangingense*
14 + Carpopodium base constricted ..... *L. microphyllum*
- Carpopodium base unconstricted ..... *L. wilsonii*
15 + Achenes surface with trichomes 16
- Achenes surface smooth (Type II-3) 25
16 + Achenes surface dense trichomes, ≥4/100 × 100 μm^2 (Type II-1) 17
- Achenes surface sparse trichomes, 1–3/100 × 100 μm^2 (Type II-2) 20
17 + Carpopodium base constricted 18
- Carpopodium base unconstricted ..... *L. japonicum* var. *microcephalum*
18 + Achene length 1.60–1.69 mm ..... *L. smithianum*
- Achene length < 1.40 mm 19
19 + Achene length 0.98–1.19 mm, carpopodium height ≥ 0.05 mm ..... *L. nanum*
- Achene length 0.85–1.28 mm, carpopodium height < 0.05 mm ..... *L. japonicum*
20 + Carpopodium base constricted 21
- Carpopodium base unconstricted 24
21 + Carpopodium diameter ≥ 0.12 mm 22
- Carpopodium diameter < 0.12 mm 23
22 + Achene elliptical, aspect ratio < 3 ..... *L. ochroleucum*
- Achene narrrow elliptical, aspect ratio > 3 ..... *L. leontopodioides*
23 + Carpopodium height ≥ 0.05 mm ..... *L. campestre*
- Carpopodium height < 0.05 mm ..... *L. junpeianum*
24 + Carpopodium height > 0.10–0.12 mm ..... *L. jacotianum*
- Carpopodium height 0.02–0.04 mm ..... *L. haplophylloides*
25 + Carpopodium base constricted 26
- Carpopodium base unconstricted ..... *L. giraldii*
26 + Carpopodium diameter ≤ 0.12 mm 27
- Carpopodium diameter > 0.12 mm 28
27 + Achene length 1.10–1.24 mm, elliptical, aspect ratio < 3 ..... *L. brachyactis*
- Achene length 1.35–1.49 mm, narrow elliptical, aspect ratio > 3 ..... *L. souliei*
28 + Achene elliptical, aspect ratio < 3 ..... *L. pusillum*
- Achene narrow elliptical, aspect ratio > 3 ..... *L. monocephalum*

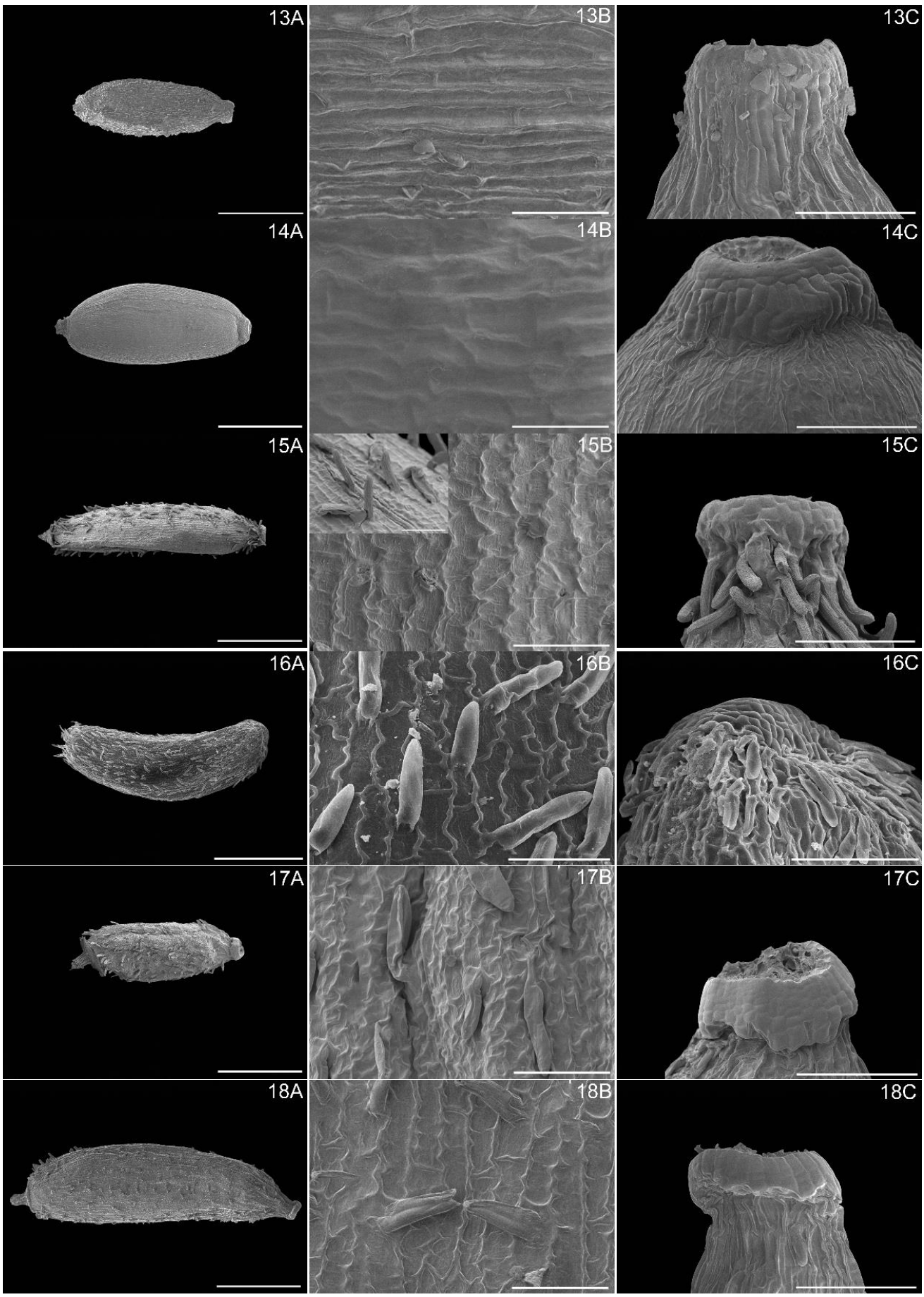
Table 2. Summary of micromorphological characteristics of achenes in Chinese *Leontopodium*.

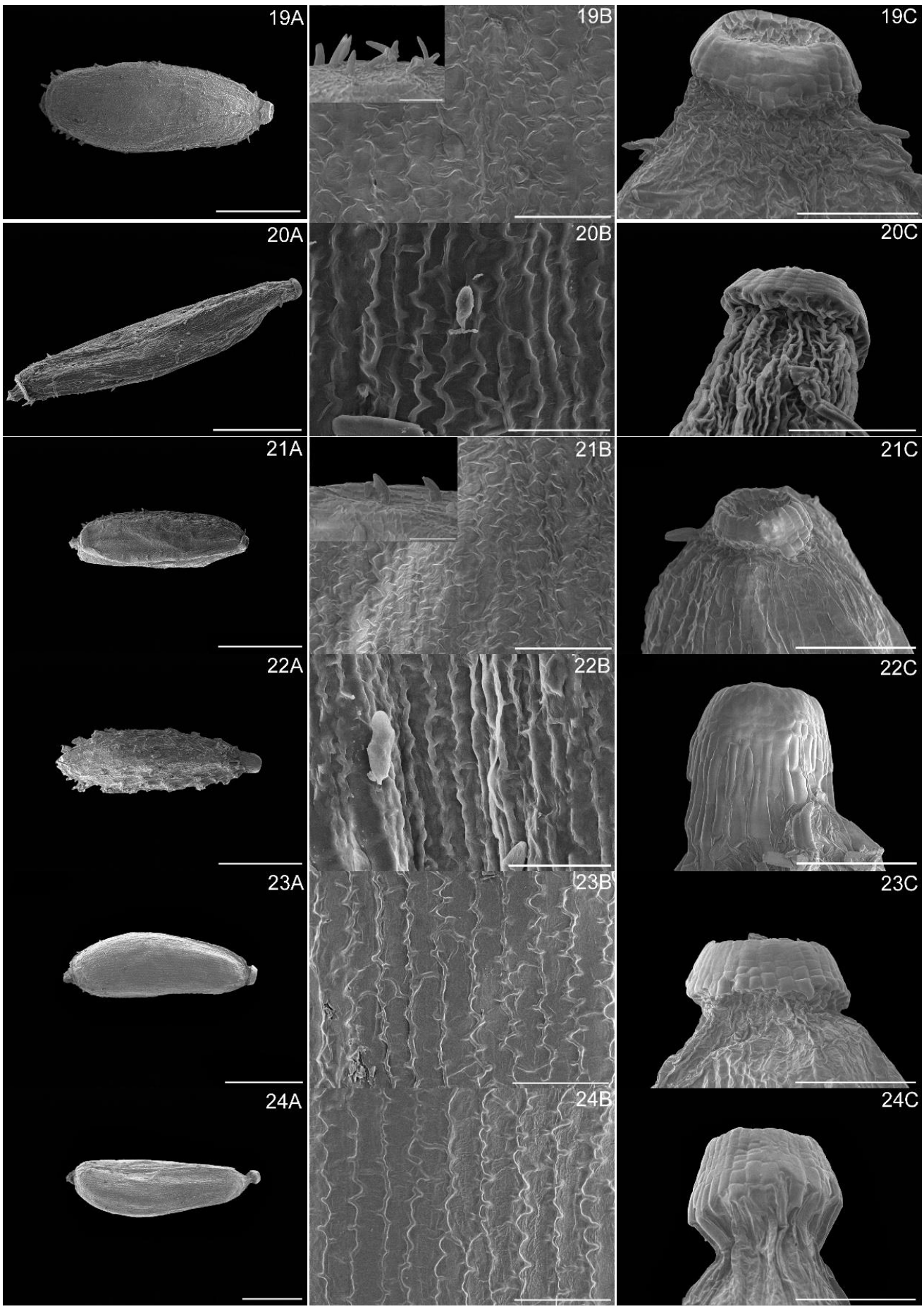
Taxon	Shape	Body size		Surface ornamentation	Trichome			Carpopodium		
		Length (mm) mean ± SD	Width (mm) mean ± SD		Shape	Density (per 100×100 μm^2) mean ± SD	Length (mm) mean ± SD	Shape	Height (mm)	Diameter (mm)
L. andersonii	NE	0.807 ± 0.005	0.183 ± 0.005	RE (Type I-1)	CT	4.667 ± 0.471	0.043 ± 0.005	BC	0.038	0.098
L. brachyactis	E	1.170 ± 0.072	0.442 ± 0.014	RI (Type II-3)	S	0	null	BC	0.026	0.085
L. caespitosum	NE	1.123 ± 0.076	0.340 ± 0.054	RE (Type I-2)	CT	3.333 ± 0.471	0.063 ± 0.005	BC	0.049	0.164
L. calocephalum	E	0.792 ± 0.046	0.318 ± 0.025	RE (Type I-2)	CT	3.333 ± 0.471	0.040 ± 0.005	BC	0.048	0.121
L. campestre	NE	1.323 ± 0.218	0.334 ± 0.096	RI (Type II-2)	CT	1.333 ± 0.471	/	BC	0.050	0.108
L. conglobatum	E	1.268 ± 0.148	0.428 ± 0.018	RE (Type I-2)	CT	2.667 ± 0.471	0.057 ± 0.005	BC	0.028	0.097
L. dedekensii	NE	0.839 ± 0.039	0.217 ± 0.013	RE (Type I-1)	CT	6.000 ± 0.816	0.040 ± 0.008	NBC	0.041	0.112
L. fangingense	NE	1.153 ± 0.003	0.376 ± 0.006	RE (Type I-2)	CT	1.333 ± 0.471	0.043 ± 0.005	NBC	0.091	0.168
L. forrestianum	E	0.953 ± 0.024	0.353 ± 0.019	RE (Type I-2)	CT	2.667 ± 0.471	0.040 ± 0.008	NBC	0.068	0.178
L. franchetii	E	0.627 ± 0.177	0.274 ± 0.089	RE (Type I-1)	CT	3.667 ± 0.471	0.043 ± 0.005	BC	0.020	0.082
L. giraldii	E	0.609 ± 0.212	0.346 ± 0.036	RI (Type II-3)	S	0	null	NBC	0.105	0.223
L. haplophylloides	NE	1.624 ± 0.102	0.373 ± 0.037	RI (Type II-2)	CT	1.333 ± 0.471	0.047 ± 0.005	NBC	0.022	0.105
L. himalayanum	E	1.358 ± 0.055	0.604 ± 0.053	RE (Type I-2)	CT	3.333 ± 0.471	0.090 ± 0.008	BC	0.052	0.071
L. jacotianum	NE	0.956 ± 0.187	0.266 ± 0.060	RI (Type II-2)	CT	2.667 ± 0.471	0.047 ± 0.005	NBC	0.110	0.099
L. japonicum	NE	1.062 ± 0.216	0.292 ± 0.007	RI (Type II-1)	CT	3.667 ± 0.471	0.047 ± 0.005	BC	0.035	0.128
L. japonicum var. *microcephalum*	E	1.200 ± 0.050	0.418 ± 0.005	RI (Type II-1)	CT	5.333 ± 0.471	0.047 ± 0.005	NBC	0.031	0.115
L. junpeianum	NE	1.017 ± 0.035	0.328 ± 0.016	RI (Type II-2)	CT	1.333 ± 0.471	0.033 ± 0.005	BC	0.021	0.078
L. leontopodioides	NE	1.152 ± 0.072	0.399 ± 0.044	RI (Type II-2)	CT	1.333 ± 0.471	0.020 ± 0.005	BC	0.041	0.124
L. microphyllum	E	1.123 ± 0.037	0.473 ± 0.007	RE (Type I-3)	S	0	null	BC	0.065	0.156
L. monocephalum	E	1.197 ± 0.063	0.419 ± 0.082	RI (Type II-3)	S	0	null	BC	0.043	0.131
L. nanum	NE	1.085 ± 0.108	0.283 ± 0.035	RI (Type II-1)	CT	4.667 ± 0.471	0.033 ± 0.005	BC	0.055	0.129
L. ochroleucum	E	1.307 ± 0.153	0.457 ± 0.060	RI (Type II-2)	CT	1.667 ± 0.471	0.037 ± 0.005	BC	0.052	0.136
L. omeiense	NE	0.871 ± 0.065	0.272 ± 0.025	RE (Type I-1)	CT	4.667 ± 0.471	0.047 ± 0.005	NBC	0.140	0.133
L. pusillum	NE	1.052 ± 0.008	0.318 ± 0.032	RI (Type II-3)	S	0	null	BC	0.068	0.140
L. sinense	E	1.003 ± 0.104	0.346 ± 0.039	RE (Type I-1)	CT	5.667 ± 0.471	0.033 ± 0.005	NBC	0.069	0.133
L. smithianum	NE	1.681 ± 0.085	0.444 ± 0.031	RI (Type II-1)	CT	3.667 ± 0.471	0.043 ± 0.005	BC	0.033	0.121
L. souliei	NE	1.417 ± 0.069	0.356 ± 0.040	RI (Type II-3)	S	0	null	BC	0.049	0.102
L. stracheyi	E	0.670 ± 0.037	0.242 ± 0.009	RE (Type I-1)	CT	7.667 ± 0.471	0.043 ± 0.005	NBC	0.031	0.101
L. wilsonii	E	0.934 ± 0.017	0.317 ± 0.004	RE (Type I-3)	S	0	null	NBC	0.072	0.137

NE, narrrow elliptical; E, elliptical; RE, reticulate; RI, rippled; CT, clavate trichome; S, smooth; BC, base constricted; NBC, no base constricted; /, no data been measured yet


1A
1B
1C
2A
2B
2C
3A
3B
3C
4A
4B
4C
5A
5B
5C
6A
6B
6C


7A
7B
7C
8A
8B
8C
9A
9B
9C
10A
10B
10C
11A
11B
11C
12A
12B
12C


13A
13B
13C
14A
14B
14C
15A
15B
15C
16A
16B
16C
17A
17B
17C
18A
18B
18C


19A
19B
19C
20A
20B
20C
21A
21B
21C
22A
22B
22C
23A
23B
23C
24A
24B
24C

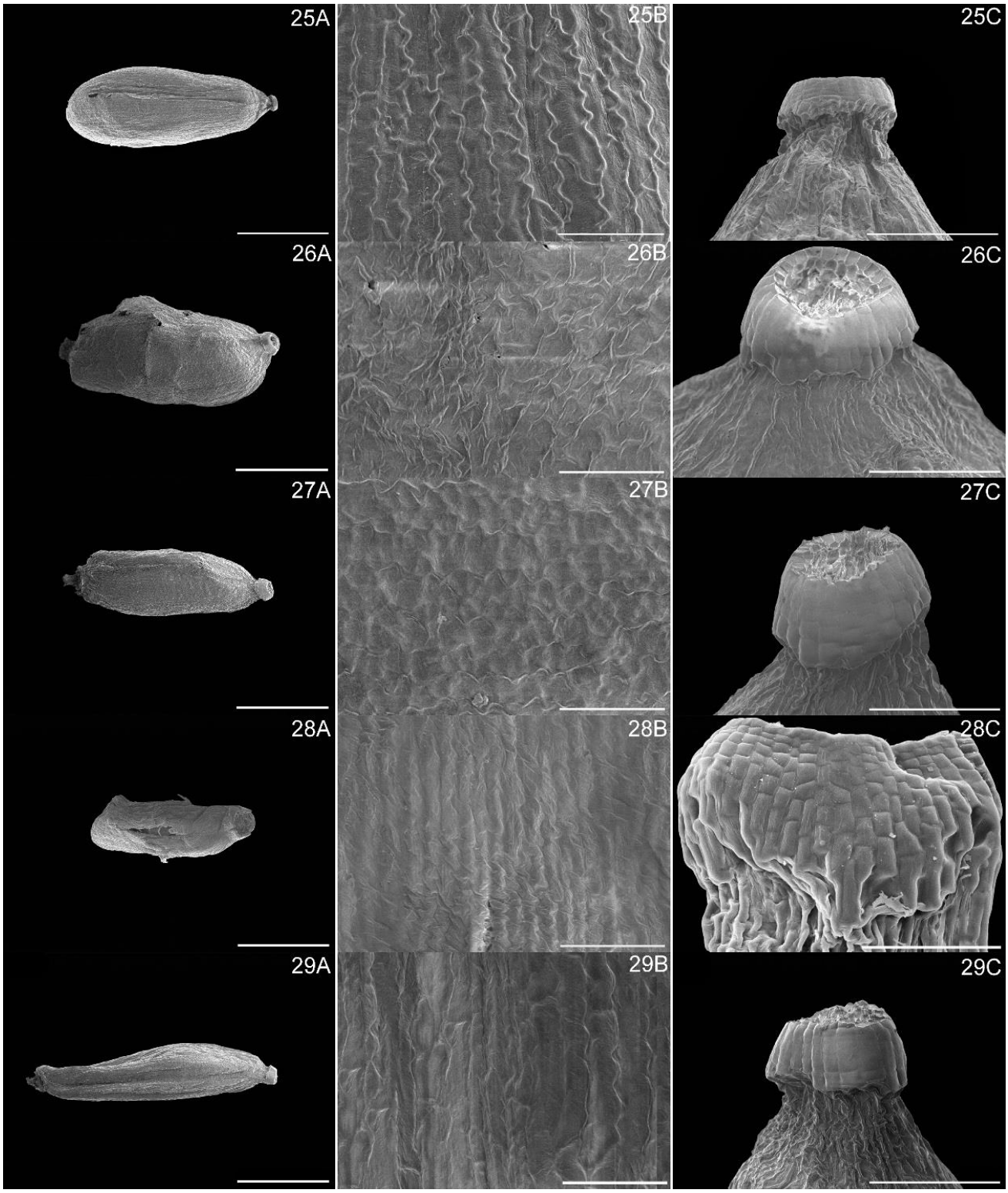


Fig. 1. Scanning electron micrographs of achenes of *Leontopodium* taxa studied from China. The pictures in the upper left corner of 15B, 19B and 21B represent the surface trichomes. A, general view of achene (scale bars = 500 μm); B, achene surface (scar bars = 50 μm); C, achene carpopodium (scar bars = 100 μm).

1, *L. andersonii*; 2, *L. dedekensii*; 3, *L. omeiense*; 4, *L. sinense*; 5, *L. stracheyi*; 6, *L. franchetii*; 7, *L. forrestianum*; 8, *L. fangingense*; 9, *L. caespitosum*; 10, *L. conglobatum*; 11, *L. calocephalum*; 12, *L. himalayanum*; 13, *L. wilsonii*; 14, *L. microphyllum*; 15, *L. japonicum*; 16, *L. japonicum* var. *microcephalum*; 17, *L. nanum*; 18, *L. smithianum*; 19, *L. ochroleucum*; 20, *L. haplophylloides*; 21, *L. junpeianum*; 22, *L. jacotianum*; 23, *L. leontopodioides*; 24, *L. campestre*; 25, *L. brachyactis*; 26, *L. monocephalum*; 27, *L. pusillum*; 28, *L. giraldii*; 29, *L. souliei*

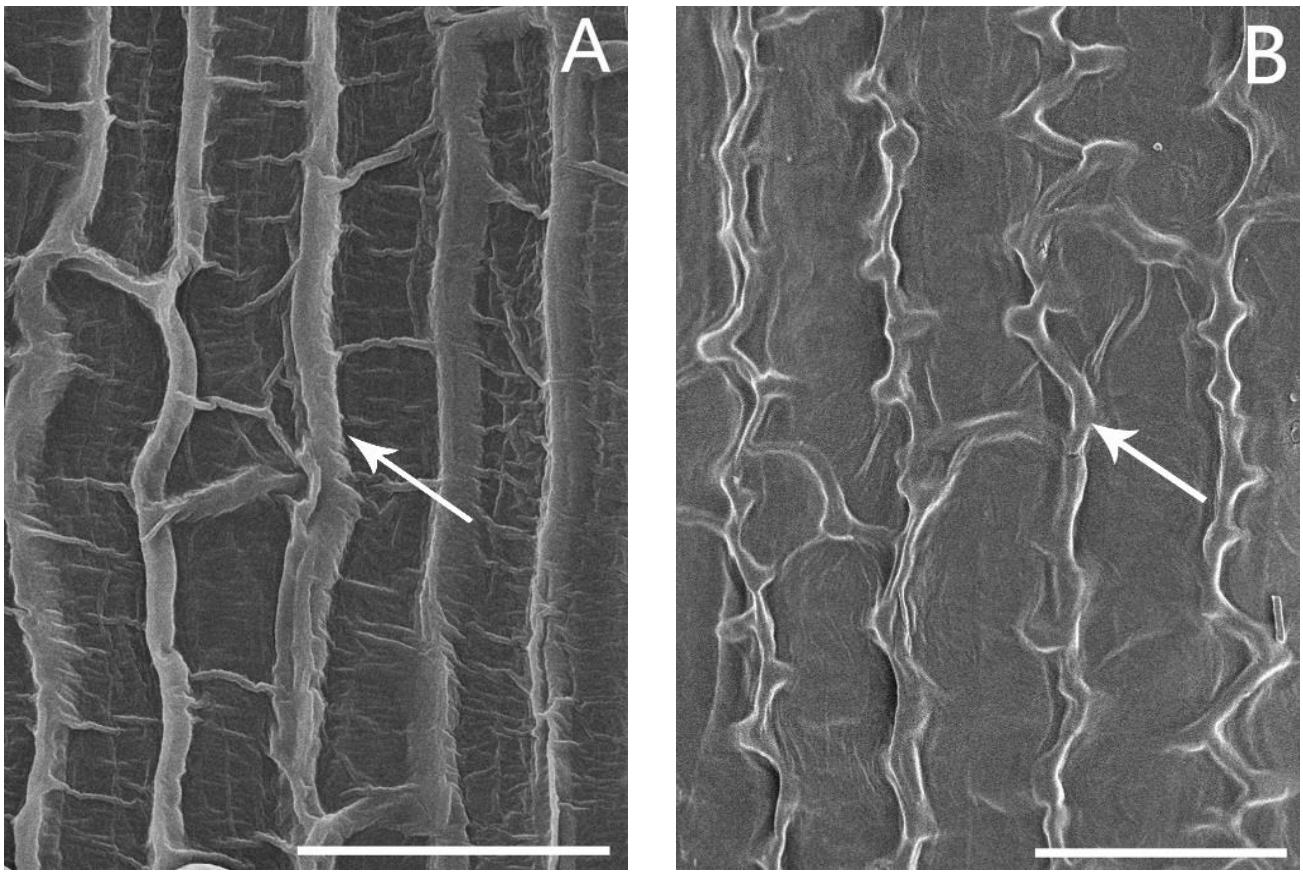


Fig. 2. The two types of surface ornamentation of achene. A, Type I, reticulate type (scale bars = 25 μm); B, Type II, rippled type (scale bars = 25 μm).

Discussion

Taxonomic significance of differences in achene characteristics between *Leontopodium* and related genera: In Asteraceae, achene micromorphological characteristics show taxonomic value at the genus level (Zhu *et al*., 2006; Hussein & Eldemerdash, 2016; Tahir *et al*., 2019). It was confirmed that the achene shape and size could be used as an important taxonomic tool in the Gnaphalieae tribe (Abid & Qaiser, 2008a; Koekemoer, 2016). The achenes of *Filago* were 0.5–1.0-mm long and approximately cylindrical (Andrés-Sánchez *et al*., 2015); those of *Antennaria* were approximately 0.8–1.2-mm long and oblong (Freire *et al*., 2014); and those of *Gamochaeta* were 0.5–1.5-mm long and cylindrical with ribs (Chen & Bayer, 2011; Urtubey *et al*., 2016). In *Gnaphalium*, the achenes were oblong or oblong-ellipsoid, 0.5–2.0-mm long, and 0.25–0.75-mm wide, without ribs, and the carpopodium was narrow, circular, and ring-shaped (Abid & Qaiser, 2008b). In the present study, the achenes of *Leontopodium* were approximately 0.7–1.6-mm long, elliptical or narrow elliptical, without ribs. The *Leontopodium* achene shape and size and the presence or absence of ribs are significantly different from those of related genera (FLAG).

Previous studies focused primarily on the morphology of achene trichomes. Anderberg (1991) identified five types of achene trichomes within the Gnaphalieae: elongated, short-clavate, globose, spirally coiled, and glabrous. The achenes of *Filago* showed clavate or baculate twin hairs on the surface (Andrés-Sánchez *et al*., 2015). Federico *et al*., (2017) reported that the achenes of *Antennaria* had tiny papillary twin hairs or were glabrous and those of *Gamochaeta* showed globose twin trichomes. The results of the present study showed that there was only one type of trichome in *Leontopodium*. The achenes were covered with clavate twin hairs (20–90 μm), and the trichomes were similar in shape to the clavate trichomes of *Filago*. The clavate twin hairs of *Filago* were 5–60 μm (Andrés-Sánchez *et al*., 2015), and the trichomes of the two genera differed in length. Additionally, the trichome shape was different between *Leontopodium* and two other related genera: *Antennaria* and *Gamochaeta* (Federico *et al*., 2017).

Taxonomic significance of achene characteristics in *Leontopodium*: Achene micromorphological characteristics have been found to be useful in classification under the genus level (Abid & Zehra, 2007). Chen *et al*., (2007) found that the achene surface ornamentation of four *Leontopodium* species was rippled. This study examined the achenes of 29 *Leontopodium* taxa, and the results showed that achene surface ornamentation could be divided into two types: reticulate (Type I) and rippled (Type II). According to the macromorphological characteristics, Lin (1965) and Zhuang (2003) divided the Chinese *Leontopodium* taxa into two subgenera: *Paragnaphalium* (monotypic subgenus containing only *L. forrestianum*) and *Leontopodium* (containing sections *Nobilia* and *Leontopodium*). The achenes of subgenus *Paragnaphalium* were Type I-2, whereas those of sections *Nobilia* (belonging to subgenus

Leontopodium) and *Leontopodium* (belonging to subgenus *Leontopodium*) were mainly Type I and Type II, respectively. The achenes of *L. conglobatum*, *L. microphyllum*, and *L. japonicum* var. *microcephalum* were different from the traditional classification of Handel-Mazzetti (1928) and Lin (1979). However, they were consistent with the molecular phylogenetic results reported by Zhao (2020). In short, the results of the achene micromorphological characteristics generally support the traditional taxonomic system, and the achene surface ornamentation is valuable for taxonomic classification at the section level within *Leontopodium*.

The carpopodium shape and diameter have been reported to be useful for specific demarcation in Asteraceae (Haque & Godward, 1984; Mukherjee & Nordenstam, 2004; Abid & Zehra, 2007; Abid & Qaiser, 2008a, 2008b). In subsection *Pseudantennaria* (belonging to section *Leontopodium*), the carpopodium diameter was <0.12 mm and the height was <0.05 mm. However, the carpopodium diameter and height in subsection *Chromotricha* (belonging to section *Leontopodium*) were greater (diameter >0.12 mm, height >0.05 mm). Similarly, in subsections *Subulata*, *Glandulosa*, and *Haastata* (belonging to section *Nobilia*), the carpopodium diameter was <0.12 mm. The carpopodium diameter of subsection *Japonica* (belonging to section *Nobilia*) was >0.12 mm. This study found that the carpopodium diameter and height were of great importance in taxonomic classification at the subsection level in *Leontopodium*.

Molecular markers have provided new insights into the evaluation of genetic relationships and taxonomic positions and have been widely used to study *Leontopodium* (Blöch *et al*., 2010; Safer *et al*., 2011; Zhao, 2020). Zhao (2020) studied the molecular phylogeny of Chinese *Leontopodium* and divided the species into three clades. The surface ornamentation of two clades was Type I and of the other clade was Type II. Safer (2011) reported that the achenes of nine Chinese *Leontopodium* taxa belonged to two types: those of eight species belonged to Type I and that of one species belonged to Type II. This result was consistent with the clade grouping of the species. Our micromorphological results are consistent with those of Blöch (2010). *L. dedekensii* and *L. sinense* belonging to Type I were grouped in one clade. *L. leontopodioides*, *L. ochroleucum* and *L. souliei* belonging to Type II were classified in a different clade. In general, the achene micromorphological characteristics support the molecular phylogenetic results of *Leontopodium*.

Chen *et al*., (2007) revealed that the achene surface ornamentation showed an evolutionary trend within each genus of Asteraceae. They speculated that the reticulate surface of the achene in *Anaphalis* was of a more primitive type, and the surface ornamentation evolved from reticulate to ligule bulge (Chen & Jing, 2007). In a systematic molecular research study on Chinese *Leontopodium* (Zhao, 2020), section *Nobilia* was more primitive than section *Leontopodium*, which was consistent with the macromorphological characteristics (Lin, 1979). This study indicated that the surface ornamentation of sections *Nobilia* and *Leontopodium* was Type I and II, respectively. The achene surface ornamentation was also consistent with the molecular phylogenetic results (Zhao, 2020). Therefore, the reticulate surface is more primitive than the rippled surface.

Conclusion

In this study we investigated the achene micromorphological characteristics of 29 *Leontopodium* taxa from China. We complied a new key based on the achene characteristics and discussed the taxonomic significance of inter and intra-genera achene characteristics. The results showed that the achene micromorphological characteristics provided taxonomically useful information that was helpful in the identification and delimitation of species in *Leontopodium* and can be summarized as follows: 1. The achene shape, size, and trichome shape are valuable in the circumscription of *Leontopodium* and related genera. 2. The achene surface ornamentation can be classified into two types and could be used as a key taxonomic feature at the section level in *Leontopodium*. 3. The carpopodium diameter and height, shape of the carpopodium base, and trichome density are important for taxonomic classification under the section level.

Acknowledgements

The author thanks herbarium PE for providing plant materials. This work was supported by [grant 31670188; 31700175] of The National Natural Science Foundation of China [to SXZ and ZW].

References

Abid, R. and N. Zehra. 2007. Micromorphology of cypsela and its taxonomic significance of some genera in the tribe Inuleae (Asteraceae) from Pakistan. *Pak. J. Bot*., 39(5): 1407-1416.

Abid, R. and M. Qaiser. 2008a. Cypsela morphology of some genera in the tribe Gnaphalieae (Asteraceae) from Pakistan. *Pak. J. Bot*., 40(2): 473-485.

Abid, R. and M. Qaiser. 2008b. Cypsela morphology of *Gnaphalium* L. and its allied genera (Gnaphalieae-Asteraceae) from Pakistan. *Pak. J. Bot*., 40(1): 25-31.

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

Anderberg, A.A. 1994. Tribe Gnaphalieae. *In: Bremer, K. (Ed.), Asteraceae: Cladistics and classification*. Timber Press, Portland, pp. 304-364.

Andrés-Sánchez, S., M. Galbany-Casals, E. Bergmeier, E. Rico and M.M. Martínez-Ortega. 2015. Systematic significance and evolutionary dynamics of the achene twin hairs in *Filago* (Asteraceae, Gnaphalieae) and related genera: further evidence of morphological homoplasy. *Plant Syst. Evol*., 301(6): 1653-1668.

Barthlott, W. 1984. Microstructural features of seed surfaces. In: (Eds.): Heywood, V. H. and D.M. Moore. *Current concepts in plant taxonomy*. Academic Press, London, pp. 95-105.

Barthlott, W. 1990. Scanning electron microscopy of the epidermal surface in plants. In: (Ed.): Claugher, D. *Application of the scanning electron microscopy in taxonomy and functional morphology. Systematics association special volume*. Clarendon Press, Oxford, pp. 69-94.

Bayer, R.J, I. Breitweiser, J. Ward and C.F. Puttock. 2007. Tribe Gnaphalieae. In*:* (Eds.): Kadereit, J.W. and C. Jeffrey. *The families and genera of vascular plants. Flowering plants, Eudicots, Asterales, vol. VIII*. Springer, Berlin, Germany, pp. 246-283.

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

Blöch, C., W.B. Dickore, R. Samuel and T.F. Stuessy. 2010. Molecular Phylogeny of the edelweiss (*Leontopodium*, Asteraceae-Gnaphalieae). *Edinburgh J. Bot*., 67(2): 235-264.

Brown, R. and Cassini. 1819. Bulletin des Sciences, par la Societe Philomatique. *Bull. Sci. Soc. Philom. Paris*.: 144.

Chen, X.L. and G.H. Jing. 2007. A study on fruit coats micro-morphological characters of thirty-six species of Compositae from alpine meadow in eastern Qinghai–Tibet Plateau. *Pratacultural Science*., 24(5): 9-15.

Chen, Y.S. and R.J. Bayer. 2011. *Leontopodium*. *In: Flora of China*. Science Press, Beijing, 20: 778-788.

Dobner, M.J., S. Sosa, S. Schwaiger, G. Altinier, R.D. Loggia, N.C. Kaneider and H. Stuppner. 2004. Anti-inflammatory activity of *Leontopodium alpinum* and its constituents. *Planta Med*., 70: 502-508.

Federico, L., M.M. Andrés, W. Katharina and O.D. Michael. 2017. Phylogeny and evolution of achenial trichomes in the *Lucilia*-group (Asteraceae: Gnaphalieae) and their systematic significance. *Taxon*, 66(5): 1184-1199.

Flann, C., W. Greuter and D.J.N. Hind. 2010. Cassini's Compositae genera: A nomenclatural and taxonomic assessment. *Taxon.*, 59(4): 1206-1244.

Freire, S.E., M.A. Chemisquy, A.A. Anderberg, S.G. Beck, R.I. Meneses, B. Loeuille and E. Urtubey. 2014. The *Lucilia* group (Asteraceae, Gnaphalieae): phylogenetic and taxonomic considerations based on molecular and morphological evidence. *Plant Syst. Evol*., 301(4): 1227-1248.

Galbany-Casals, M., S. Andrés-Sánchez, N. Garcia-Jacas, A. Susanna, E. Rico and M.M. Martínez-Ortega. 2010. How many of Cassini anagrams should there be? Molecular systematics and phylogenetic relationships in the "*Filago* group" (Asteraceae, Gnaphalieae), with special focus on the genus *Filago*. *Taxon*., 59: 1671-1689.

Ghimire, B., M.J. Jeong and K.M. Lee. 2016. Achene morphology of *Saussurea* species (Asteraceae, Cardueae) in Korea and its systematic implications. *Bot. J. Linn. Soc*., 181: 692-710.

Guan, L. 2014. Primary Systematics of *Notoseris* (Asteraceae-Cichorieae). Zhengzhou: Zhengzhou University.

Handel-Mazzetti, H. 1928. Systemarische monographie der gattung *Leontopodium*. *Beih. Bot. Centralbl*., 44: 1-178.

Haque, M.Z. and M.B.E. Godward. 1984. New records of the carpopodium in Compositae and its taxonomic use. *Bot. J. Linn. Soc*., 89: 321-340.

Hussein, H.A. and M.M. Eldemerdash. 2016. Comparative morphology and surface microsculpture of cypsela in some taxa of the Asteraceae and their taxonomic significance. *Egypt. J. Bot*., 56: 409-422.

Inceer, H., N.A. Kalmuk, K.V. Imamoglu, G. Arslan, O. Duman, S. Hayırlıoglu-Ayaz and G. Guner. 2018. Achene anatomy and stomatal characteristics of eighteen *Crepis* L. (Asteraceae) taxa from Turkey with notes on their systematic significance. *Nord. J. Bot*., 36.

Jing, G.H. 2007. A Study on micro-morphological features of diaspores from the alpine meadow on the eastern Qinghai–Tibet Plateau. Lanzhou: Northwest Normal University.

Koekemoer, M. 2016. A monograph of *Stoebe* and *Seriphium* (Gnaphalieae, Asteraceae) in southern Africa and neighbouring islands. *Phytotaxa*., 242(1): 1179-3155.

Lee, D.H., J.H. Lee and W.B. Cho. 2016a. The establishment history of alpine *Leontopodium japonicum* (Asteraceae) resembles that of warm-temperate plants on the Korean Peninsula. *Plant Syst. Evol*., 302(10): 1483-1494.

Lee, D.H., J.S. Park and B.H. Choi. 2016b. A taxonomic review of Korean *Leontopodium* R. Br. ex Cassini (Asteraceae). *Taxon*., 46(2): 149-162.

Lin, R. 1965. Notulae de nonnulis generibus tribus Inulearum faliliae Compositarum florae sinicae. *Acta Phytotaxon. Sin.*, 10(2): 167-181.

Lin, R. 1979. *Leontopodium*. *In: Flora Reipublicae Popularis Sinicae*. Science Press, Beijing, 75: 72-141.

Liu, C.J., Q. Lin and J.X. He. 2004. Methods and terminology of study on seed morphology from China. *Acta Bot. Boreal. - Occident. Sin*., 24(1): 178-188.

Lu, Y. 2013. Primary systematics of *Paraprenanthes* (Asteraceae-Cichorieae). Zhengzhou: Zhengzhou University.

Ma, X.L., Q. Chen, T.G.L. Xia, P. Gou, Y. Zhang and H.Y. Wei. 2018. Isolation and identification of chemical constituents from *Leontopodium leontopodioides* (Willd.) Beauv. *Journal of Shenyang Pharmaceutical University*., 35(9): 729-732.

Meng, Y., Z.L. Nie, H. Sun and Y.P. Yang. 2012. Chromosome numbers and polyploidy in *Leontopodium* (Asteraceae: Gnaphalieae) from the Qinghai–Tibet Plateau of S.W. China. *Caryologia*., 65(2): 87-93.

Monti, C., N.D. Bayón, D.A. Giuliano and S.E. Freire. 2013. New combinations, new synonyms, and typifications in *Pseudognaphalium* (Asteraceae:Gnaphalieae) from south America. *J. Bot. Res. Inst. Texas*., 7(1): 195-202.

Mukherjee, S.K. and B. Nordenstam. 2004. Diversity of carpopodial structure in the Asteraceae and its taxonomic significance. *Comp. Newsl*., 41: 29-50.

Nie, Z.L., V.A. Funk, Y. Meng, T. Deng, H. Sun and J. Wen. 2016. Recent assembly of the global herbaceous flora: evidence from the paper daisies (Asteraceae: Gnaphalieae). *New Phytol*., 209: 1795-1806.

Pak, J.H. and S. Kawano. 1990a. Biosystematic studies on the genus *Ixeris* (Compositae-Lactueeae) I. Fruit wall anatomy and its taxonomic implications. *Acta. Phytotax. Geobot*., 41: 43-60.

Pak, J.H. and S. Kawano. 1990b. Biosystematic studies on the genus *Ixeris* and its allied genera (Compositae-Lactuceae) III. Fruit wall anatomy and karyology of *Crepidiastrum* and *Paraixeris*, and their taxonomic implications. *Acta Phytotax. Geobot*., 41: 109-128.

Russell, A., S. Safer, E. Temsch, H. Stuppner, T.F. Stuessy and R. Samuel. 2013. Chromosome counts and genome size of *Leontopodium* species (Asteraceae: Gnaphalieae) from south-western China. *Bot. J. Linn. Soc*., 171: 627-636.

Ru, J. 2018. Morphological and systematic studies of Asteraceae taxa of four subfamilies in northeast China. Harbin: Harbin Normal University.

Safer, S., K. Tremetsberger, Y.P. Guo, G. Kohl, M.R. Samuel, T.F. Stuessy and H. Stuppner. 2011. Phylogenetic relationships in the genus *Leontopodium* (Asteraceae: Gnaphalieae) based on AFLP data. *Bot. J. Linn. Soc*., 165: 364-377.

Smissen, R.D., R.J. Bayer, N.G. Bergh, I. Breitwieser, S.E. Freire, M. Galbany-Casals, A.N. Schmidt-Lebuhn and J.M. Ward. 2020. A revised subtribal classification of Gnaphalieae (Asteraceae). *Taxon*., 69(4): 778-806.

Stille, J.S., T.F. Stuessy, W.B. Dickoré, M. Jaeger, B. Gemeinholzer and V. Wissemann. 2016. Comparative pappus micromorphology of edelweiss (*Leontopodium*, Gnaphalieae, Asteraceae) with implications for taxonomy, ecology and evolution. *Bot. J. Linn. Soc*., 182(3): 612-636.

Tahir, S.S., M.T. Rajput, A.L. Khokhar and N. Shaikh. 2019. Microstructural features of achene of sibbaldia species (Rosaceae). *Pak. J. Bot*., 51(1): 1793-1796.

Urtubey, E., A. López, M.A. Chemisquy, A.A. Anderberg, C.M. Baeza, N.D. Bayón, L.P. Deble, A. Moreira-Muñoz, G.L. Nesom, M.H. Alford, L. Salomón and S.E. Freire. 2016. New circumscription of the genus *Gamochaeta* (Asteraceae, Gnaphalieae) inferred from nuclear and plastid DNA sequences. *Plant Syst. Evol*., 302: 1047-1066.

Ward, J., R.J. Bayer, I. Breitwieser, R. Smissen, M. Galbany-Casals and M. Unwin. 2009. Gnaphalieae. In: (Eds.): Funk, V.A., A. Susanna, T.F. Stuessy and R.J. Bayer. *Systematics, evolution, and biogeography of Compositae*. International Association for Plant Taxonomy, Vienna, pp. 539-588.

Xu, X.M. 2017. Systematics of *Anaphalis* from Pan-Himalaya. Zhengzhou: Zhengzhou University.

Xu, X.M., Z. Wei, T. Zhang and S.X. Zhu. 2021. Achene micro-morphology of *Anaphalis* DC. (Gnaphalieae-Asteraceae) from China. *Pak. J. Bot*., 53(5): 1883-1892.

Xu, Y.L., W.Y. Zhu, J.J. Tan and H. Zhang. 2019. Chemical constituents of *Leontopodium haplophylloides* and their anti-bacterial activities. *Nat. Prod. Res. Dev*., (3): 450-454.

Zhao, Q.F. 2020. A Study of *Leontopodium* in China based on morphology and molecular phylogeny. Zhengzhou: Zhengzhou University.

Zhang, X.C. and X.L. Chen. 2008. Studies on cypsela size and variations' evolutionary ecology of six genera of Compositae from eastern Qinghai–Tibetan Plateau. *Journal of Northwest A&F University (Nat. Sci. Ed.)*., 36(10): 207-214.

Zhu, S.X., H.N. Qin and C. Shih. 2006. Achene wall anatomy and surface sculpturing of *Lactuca* L. and related genera (Compositae: Lactuceae) with notes on their systematic significance. *J. Integ. Plant Biol*., 48(4): 390-399.

Zhuang, X.X. 2003. *Leontopodium*. *In: Flora of Yunnan*. Science Press, Beijing, 13: 232-247.

(Received for publication 30 November 2020)