Pak. J. Bot., 53(5): 1883-1892, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-5(10)

ACHENE MICRO-MORPHOLOGY OF *ANAPHALIS* DC. (GNAPHALIEAE-ASTERACEAE) FROM CHINA

XUEMIN XU, ZHEN WEI, TAO ZHANG AND SHIXIN ZHU*

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

Abstract

Anaphalis DC. is the largest genus in Asian Gnaphalieae, and China, containing 54 known species, is one of the largest centers of *Anaphalis* diversity. In the present study, the achene micro-morphology of 39 Chinese *Anaphalis* taxa were studied in detail using scanning electron microscopy (SEM). The results showed that the Chinese *Anaphalis* could be classified into two groups based on achene surface ornamentation viz. reticulate-claviform (Group I) and ligulate protuberant (Group Ⅱ). Achene micro-morphological characteristics were useful for the delimitation of interspecific and supraspecific classification within *Anaphalis.*

Key words: Achene, Micro-morphology, *Anaphalis*, Gnaphalieae, Scanning Electron Microscopy.

Introduction

Anaphalis DC. (Gnaphalieae, Asteraceae), consisting of ca 110 species, is the largest genus in the Asian Gnaphalieae tribe (Wu *et al.*, 2011). *Anaphalis* is mainly distributed in tropical and subtropical Asia, with only a few species in temperate Asia, Europe, and North America (Wu *et al.*, 2011; Nie *et al.*, 2013). China one of the diversity centers of *Anaphalis* (Nie *et al.*, 2013), with about 54 *Anaphalis* species is intensively distributed in the Himalayas and Hengduan Mountains, and fewer species in North and Central China (Wu *et al.*, 2011). Additionally, four *Anaphalis* species (*A. morrisonicola* Hayata, *A. transnokoensis* Sasaki, *A. nagasawae* Hayata, and *A. horaimontana* Masam.) are insulated in alpine meadows in Taiwan (Lin, 1979; Lin, 1993, 1997; Wu *et al.*, 2003, 2011). The Chinese *Anaphalis* has been classified into two subgenera: Subgen. Ⅰ *Gnaphaliops* and Subgen. Ⅱ *Anaphalis* (Lin, 1979). The subgen. *Anaphalis* was further divided into two sections based on morphological characteristics such as shapes of involucre, color of phyllaries, and shapes of leaf base (Lin, 1979). The nuclear internal and external transcribed spacers (ITS and ETS) of *Anaphalis* species have been sequenced to examine the phyletic position of the genus, with an emphasis on the eastern Himalayan taxa (Nie *et al.*, 2013). Their results suggested that the monophyly of *Anaphalis* was weakly supported. Nie *et al*., (2013) identified two clades in *Anaphalis* based on the shape of the leaf base rather than the morphology of the capitula and phyllaries used by Chen *et al.*, (1966) and Lin (1979).

Achene features have been widely used for taxonomic clarification in Asteraceae (Abid & Qaiser, 2002, 2007a, 2007b, 2007c, 2008a, 2008b; Abid & Zehra, 2007; Blanca & Guardia, 1997; Bruhl & Quinn, 1990; Ghimire *et al.*, 2016; Melahat, 2017; Roque & Funk, 2013; Singh & Pandey, 1984; Zhang *et al.*, 2013). Achene micro-morphological characteristics have played an important role in the systematics of Asteraceae (Abid & Qaiser, 2002, 2007a, 2007b, 2007c, 2008a, 2008b; Abid & Zehra, 2007; Ritter & Miotto, 2006).

Anaphalis was distinguished among all other genera of Gnaphalieae due to the presence of short clavate hairs on the achene surface (Anderberg, 1991; Bremer, 1994; Qaiser & Abid, 2003). Abid & Qaiser (2007a) divided 17 *Anaphalis* taxa of Pakistan into two groups: sparsely or densely papillate-clavate hairy on the achene surface. Zhang & Chen (2008) found that the achene length and width variation coefficients of *Anaphalis* were smaller than the other genera and indicated that *Anaphalis* species was relatively primitive. Achene micro-morphological characteristics of six genera in the tribe Gnaphalieae in Pakistan have been examined, and Gnaphalieae were divided into two groups, achenes monomorphic or dimorphic, based on achene morphology (Abid & Qaiser, 2008b). Therefore, achene features have been useful for assessing the taxonomic delimitation both at the generic and specific levels in Gnaphalieae.

Chinese *Anaphalis* species contain about half of the number of all *Anaphalis* species worldwide and thus play a crucial role in understanding the phylogenetic relationships within this genus. Macro-morphological characteristics, chromosome numbers, karyotypes, and molecular phylogeny based on nrDNA of the Chinese *Anaphalis* have been studied (Chen *et al.*, 1966; Meng *et al.*, 2010, 2014; *Nie et al.*, 2013; Wu *et al.*, 2011). However, achene characteristics of Chinese *Anaphalis* have not received much attention, and the previous studies on the achene morphology of *Anaphalis* were only focused on one or a small group of *Anaphalis* taxa (Chen & Jing, 2007; Zhang & Chen, 2008). For a better understanding of *Anaphalis* taxonomy, more taxa and evidence must be included. Therefore, the current study described, for the first time, the detailed achene micro-morphology of 39 *Anaphalis* taxa distributed in China. Using this achene micro-morphology, we resolve taxonomic relationships in Chinese *Anaphalis* and discuss the relationships between *Anaphalis* and closely related genera.

Materials and Methods

The achenes of 39 *Anaphalis* taxa representing 34 *Anaphalis* species were used in this study. Voucher specimens were deposited at Zhengzhou University (ZZU) and PE Herbarium (Table 1).

Before SEM imaging, achenes were dehydrated in gradient alcohol and fixed onto the sample platform using electric adhesive tape. Then, the achene surfaces were coated with gold (approximately 20 nm thick). SEM imaging was performed using a Phenom Prox scanning electron microscope at 5 kV. The following characteristics of the achene were studied and compared: shape, size, cellular arrangement, cell shape, surface ornamentation, and epicuticular secretion. In most cases, 10 achenes per species were studied. Morphological terms used in this study are from Abid & Qauser (2007a) and Liu *et al.*, (2004).

Results

General achene characteristics of Chinese *Anaphalis*: *Anaphalis* achenes were elliptic or oblong, and several achenes were subcylindrical, 0.6–1.5 mm long, and 0.25–0.6 mm wide. Epidermal cells were neatly arranged and parallel to their longitudinal axis. The achene surfaces, with clear cellular outlines, were reticulate-claviform or ligulate protuberant. The hilum was circular, 0.05–0.15 mm in diameter, and constricted at the base. The density of the wax layer varied in different species. Individual achene morphological parameters and their features are presented in Table 2. Based on surface ornamentation, the Chinese *Anaphalis* were divided into two groups. Group 2 contained those taxa whose achene surface ornamentation were reticulate-claviform (Table 2, Fig. 1), including *A. acutifolia*; *A. adnata*; *A. busua*; *A. contorta*; *A. margaritacea*; *A. nepalensis*, and *A. triplinervis*. In Group Ⅱ, the surface ornamentation of the achenes was ligulate protuberant (Table 2, Figs. 2, 3, 4) and included the remaining studied Chinese *Anaphalis* species studied in this work. A key to the species of Chinese *Anaphalis* also was generated based on the morphology, size, and micro-characteristics of achenes.

Table 1. Voucher details of taxa used in this study.

Taxa	Locality	Voucher specimen
A. acutifolia	Sichuan, Muli	*S. X. Zhu et al. DS15043* (ZZU)
A. adnata	Yunnan, Kunming	*T. N. Liou 14340* (PE)
A. aureopunctata	Shanxi, Liuba	*S. X. Zhu et al. DS13459* (ZZU)
A. bicolor	Yunnan, Lijiang	*S. X. Zhu et al. DS11457* (ZZU)
A. bulleyana	Sichuan, Muli	*S. X. Zhu et al. DS13580* (ZZU)
A. busua	Yunnan, Lijiang	*S. X. Zhu et al. DS11458* (ZZU)
A. chlamydophylla	Sichuan, Daocheng	*D. E. Boufford et al. 37391* (ZZU)
A. cinerascens	Yunnan, Zhongdian	*K. M. Feng 02394* (PE)
A. contorta	Yunnan, Chuxiong	*S. X. Zhu et al. DS11462* (ZZU)
A. contortiformis	Yunnan, Xinping	*S. X. Zhu et al. DS11523* (ZZU)
A. corymbifera	Sichuan, Jiuzhaigou	*S. X. Zhu et al. DS13504* (ZZU)
A. delavayi	Sichuan, Xinlong	*D. E. Boufford et al. 37286* (ZZU)
A. deserti	Sichuan, Litang	*S. X. Zhu et al. DS13590* (ZZU)
A. elegans	Yunnan, Xianggelila	*S. X. Zhu et al. DS11438* (ZZU)
A. flaccida	Yunnan, Xianggelila	*S. X. Zhu et al. DS13599* (ZZU)
A. flavescens	Sichuan, Litang	*S. X. Zhu et al. DS13592* (ZZU)
A. gracilis	Sichuan, Rangtang	*D. E. Boufford et al. 38867* (ZZU)
A. gracilis var. *ulophylla*	Sichuan, Dajin	*X. Li 78308* (PE)
A. lactea	Sichuan, Rangtang	*D. E. Boufford et al. 39074* (ZZU)
A. larium	Yunnan, Deqin	*S. X. Zhu et al. DS11434* (ZZU)
A. latialata	Sichuan, Baoxing	*S. X. Zhu et al. DS13533* (ZZU)
A. likiangensis	Yunnan, Xianggelila	*S. X. Zhu et al. DS13597* (ZZU)
A. margaritacea	Sichuan, Chengkou	*S. X. Zhu et al. DS11456* (ZZU)
A. margaritacea var. *angustifolia*	Unknown	*Anonymous 3859* (PE)
A. nepalensis	Yunnan, Deqin	*S. X. Zhu et al. DS11429* (ZZU)
A. nepalensis var. *corymbosa*	Sichuan, Xiaojin	*S. S. Chang et al. 6837* (PE)
A. nepalensis var. *monocephala*	Xizang	*Y. T. Zhang et al. 2731* (PE)
A. nepalensis var. *nepalensis*	Yunnan, Gongshan	*S. X. Zhu et al. DS13598* (ZZU)
A. pachylaena	Sichuan, Muli	*S. X. Zhu et al. DS13575.* (ZZU)
A. pannosa	Yunnan, Deqin	*S. X. Zhu et al. DS11433* (ZZU)
A. rhododactyla	Sichuan, Litang	*S. X. Zhu et al. DS13591* (ZZU)
A. sinica var. *lanata*	Unknown	*Anonymous* 4608 (PE)
A. souliei	Sichuan, Muli	*S. X. Zhu et al. DS13576* (ZZU)
A. spodiophylla	Sichuan, Muli	*S. X. Zhu et al. DS13574* (ZZU)
A. surculosa	Sichuan, Baoxing	*S. X. Zhu et al. DS13533* (ZZU)
A. triplinervis	Sichuan, Yajiang	*D. E. Boufford et al. 35925* (ZZU)
A. virens	Sichuan, Xinlong	*D. E. Boufford et al. 37279* (ZZU)
A. xylorhiza	Xizang, Changdu	*D. E. Boufford et al. 41132* (ZZU)
A. yunnanensis	Yunnan, Zhongdian	*D. E. Boufford et al. 42019* (ZZU)

Table 2. Achene micro-morphological characteristics of Chinese *Anaphalis*.

Taxa	Shape	Size (mm)	Surface ornamentation	Hilum		Epidermal cells		Wax layer
				Diameter (μm)	Shape	Shape	Size (μm)	
A. acutifolia	Oblong	1.2 × 0.3	reticulate-claviform	120	Base constricted	Narrowly oblong	65 × 10	Sparse
A. adnata	Subcylindrical	0.6 × 0.25	reticulate-claviform	80	Base constricted	Oblong	50 × 15–20	Dense
A. aureopunctata	Oblong, slightly curved	0.8 × 0.3	ligulate protuberant	80	Base slightly constricted	Ovate	20–25 × 5	Without
A. bicolor	Subcylindrical	0.5 × 0.2	ligulate protuberant	50	Base constricted	Broadly ovate	25 × 10	Dense
A. bulleyana	Elliptic	0.8 × 0.35	ligulate protuberant	100	Base constricted	Broadly ovate	30 × 15–20	Dense
A. busua	Subcylindrical	0.6 × 0.28	reticulate-claviform	80	Base constricted	Oblong	50 × 5–10	Sparse
A. chlamydophylla	Elliptic	1.3 × 0.6	ligulate protuberant	50	Base constricted	Narrowly ovate	10–15 × 5	Sparse
A. cinerascens	Elliptic	1.2 × 0.6	ligulate protuberant	50	Slant, base constricted	Narrowly ovate	20–25 × 5	Sparse
A. contorta	Subcylindrical	0.5 × 0.15	reticulate-claviform	75	Base constricted	Ovate	20–25 × 10	Dense
A. contortiformis	Elliptic	0.8 × 0.38	ligulate protuberant	80	Base constricted	Ovate	50 × 20–25	Dense
A. corymbifera	Subcylindrical	0.8 × 0.3	ligulate protuberant	100	Base constricted	Narrowly ovate	25–30 × 20	Sparse
A. delavayi	Oblong, slightly curved	1.5 × 0.5	ligulate protuberant	150	Curved, base constricted	Broadly ovate	20–30 × 20	Sparse
A. deserti	Oblong	1.5 × 0.3	ligulate protuberant	120	Slightly curved, base constricted	Narrowly ovate	30–35 × 10–15	Dense
A. elegans	Elliptic, slightly curved	0.9 × 0.35	ligulate protuberant	80	Base constricted	Narrowly ovate	25–30 × 20	Sparse
A. flaccida	Elliptic	0.75 × 0.4	ligulate protuberant	110	Base constricted	Ovate	25–35 × 10	Dense
A. flavescens	Oblong, slightly curved	1.0 × 0.3	ligulate protuberant	60	Slightly curved, base constricted	Narrowly ovate	15–20 × 5	Dense
A. gracilis	Oblong, slightly curved	1.0 × 0.5	ligulate protuberant	150	Slightly curved, base constricted	Ovate	25–35 × 15–20	Dense
A. gracilis var. *ulophylla*	Oblong	0.9 × 0.35	ligulate protuberant	100	Base constricted	Ovate	20–25 × 15–20	Dense
A. lactea	Oblong, slightly curved	1.2 × 0.4	ligulate protuberant	80	Elongate, base constricted	Narrowly ovate	25–30 × 10–15	Sparse
A. larium	Elliptic	1.0 × 0.5	ligulate protuberant	110	Slightly curved, base constricted	Ovate	25–30 × 20–23	Dense
A. latialata	Elliptic, slightly curved	0.75 × 0.3	ligulate protuberant	90	Curved, base constricted	Narrowly ovate	40–50 × 20	Dense
A. likiangensis	Oblong	0.9 × 0.3	ligulate protuberant	100	Slightly curved, base constricted	Narrowly ovate	30–40 × 15–20	Dense
A. margaritacea	Oblong	0.7 × 0.25	reticulate-claviform	40	Curved, base constricted	Ovate	10 × 5	Dense
A. margaritacea var. *angustifolia*	Oblong	0.9 × 0.25	reticulate-claviform	100	Curved, base constricted	Oblong	45–50 × 10–15	Without
A. nepalensis	Obovate	0.8 × 0.3	reticulate-claviform	80	Base constricted	Oblong	40–45 × 10–15	Without
A. nepalensis var. *corymbosa*	Oblong	0.8 × 0.3	reticulate-claviform	80	Slightly curved, base constricted	Oblong	55–75 × 15–20	Without
A. nepalensis var. *monocephala*	Oblong	0.8 × 0.3	reticulate-claviform	80	Slightly curved, base constricted	Oblong	45–50 × 5–10	Sparse
A. nepalensis var. *nepalensis*	Oblong	0.9 × 0.3	reticulate-claviform	50	Base constricted	Oblong	20–25 × 5–10	Without
A. pachylaena	Elliptic	0.6 × 0.3	ligulate protuberant	100	Curved, base constricted	Ovate	25–30 × 15–20	Without
A. pannosa	Oblong	1.2 × 0.5	ligulate protuberant	120	Curved, base constricted	Ovate	30–40 × 10–15	Without
A. rhododactyla	Elliptic	0.9 × 0.45	ligulate protuberant	100	Curved, base constricted	Ovate	40–50 × 15–20	Dense
A. sinica var. *lanata*	Elliptic	0.8 × 0.4	ligulate protuberant	150	Base constricted	Ovate	30–40 × 15	Dense
A. souliei	Oblong	1.0 × 0.5	ligulate protuberant	130	Elongate, base constricted	Ovate	30–40 × 10–15	Dense
A. spodiophylla	Elliptic	0.8 × 0.4	ligulate protuberant	100	Slightly curved, base constricted	Ovate	35–45 × 20	Dense
A. surculosa	Elliptic	0.8 × 0.3	ligulate protuberant	120	Slightly curved, base constricted	Ovate	30–40 × 15–20	Dense
A. triplinervis	Elliptic	0.65 × 0.4	reticulate-claviform	80	Slightly curved, base constricted	Oblong	25–30 × 15	Without
A. virens	Oblong	0.8 × 0.35	ligulate protuberant	150	Base constricted	Narrowly ovate	35–45 × 20–23	Dense
A. xylorhiza	Elliptic	1.0 × 0.45	ligulate protuberant	80	Slightly curved, base constricted	Narrowly ovate	35–45 × 15–20	Sparse
A. yunnanensis	Elliptic	0.9 × 0.5	ligulate protuberant	120	Base constricted	Ovate	25–30 × 15–20	Dense

Key of Chinese *Anaphalis* species based on achene characteristics

1a. Surfaces of achenes reticulate-claviform 2
1b. Surfaces of achenes ligulate protuberant 8
2a. Achenes subcylindrical 3
2b. Achenes oblong, obovate or elliptic 5
3a. Achenes 0.5 × 0.15 mm, hilum 75 μm in diameter, epidermal cells ovate, 20–25 × 10 μm ……..….. *A. contorta*
3b. Achenes 0.6 × 0.25–0.28 mm, hilum 80 μm in diameter, epidermal cells oblong, 50 × 5–20 μm ……..…… 4
4a. Epidermal cells 50 × 15–20 μm, with dense wax layer ..... *A. adnata*
4b. Epidermal cells 50 × 5–10 μm, with sparse wax layer ..... *A. busua*
5a. Achenes 1.2 × 0.3 mm, hilum 120 μm in diameter ..... *A. acutifolia*
5b. Achenes 0.65–0.9 × 0.25–0.4 mm, hilum 40–100 μm in diameter 6
6a. Achenes elliptic, 0.65 × 0.4 mm ..... *A. triplinervis*
6b. Achenes oblong or obovate, 0.7–0.9 × 0.25–0.3 mm 7
7a. Achenes 0.7–0.9 × 0.25 mm, hilum curved ..... *A. margaritacea*
7b. Achenes 0.8–0.9 × 0.3 mm, hilum slightly curved or not ..... *A. nepalensis*
8a. Achenes surfaces without wax layer 9
8b. Achenes surfaces with sparse or dense wax layer 11
9a. Hilum 80 μm in diameter, base slightly constricted; achenes slightly curved; epidermal cells 20–25 × 5 μm ……. …… *A. aureopunctata*
9b. Hilum 100–120 μm in diameter, base constricted; achenes not curved; epidermal cells 25–40 × 10–20 μm …..….. 10
10a. Achenes elliptic, 0.6 × 0.3 mm, hilum 100 μm in diameter, epidermal cells 25–30 × 15–20 μm …….. *A. pachylaena*
10b. Achenes oblong, 1.2 × 0.5 mm, hilum 120 μm in diameter, epidermal cells 30–40 × 10–15 μm ...…….... *A. pannosa*
11a. With sparse wax layer 12
11b. With dense wax layer 18
12a. Achenes subcylindrica ..... *A. corymbifera*
12b. Achenes elliptic or oblong 13
13a. Hilum 150 μm in diameter, epidermal cells broadly ovate ..... *A. delavayi*
13b. Hilum 50–80 μm in diameter, epidermal cells narrowly ovate 14
14a. Achenes oblong, hilum elongate ..... *A. lactea*
14b. Achenes elliptic, hilum not elongate 15
15a. Hilum 50 μm in diameter 16
15b. Hilum 80 μm in diameter 17
16a. Hilum slant, epidermal cells 20–25 × 5 μm ..... *A. cinerascens*
16b. Hilum not slant, epidermal cells 10–15 × 5 μm ..... *A. chlamydophylla*
17a. Achenes slightly curved, 0.9 × 0.35 mm, hilum not curved, epidermal cells 25–30 × 20 μm ……..... *A. elegans*
17b. Achenes not curved, 1.0 × 0.45 mm, hilum slightly curved, epidermal cells 35–45 × 15–20 μm ..... *A. xylorhiza*
18a. Achenes subcylindrical, 0.5 × 0.2 mm, hilum 50 μm in diameter ..... *A. bicolor*
18b. Achenes elliptic or oblong, 0.75–1.5 × 0.3–0.5 mm, hilum 60–150 μm in diameter 19
19a. Hilum elongate ..... *A. souliei*
19b. Hilum not elongate 20
20a. Achenes oblong 21
20b. Achenes elliptic 25
21a. Epidermal cells ovate ..... *A. gracilis*
21b. Epidermal cells narrowly ovate 22
22a. Achenes slightly curved, hilum 60 μm in diameter, epidermal cells 15–20 × 5 μm ..... *A. flavescen*
22b. Achenes not curved, hilum 100–150 μm in diameter, epidermal cells 30–45 × 10–23 μm 23
23a. Hilum slightly curved 24
23b. Hilum not curved ..... *A. virens*
24a. Achenes 1.5 × 0.3 mm, 120 μm in diameter, epidermal cells 30–35 × 10–15 μm ..... *A. deserti*
24b. Achenes 0.9 × 0.3 mm, 100 μm in diameter, epidermal cells 30–40 × 15–20 μm ..... *A. likiangensis*
25a. Achenes slightly curved, epidermal cells narrowly ovate ..... *A. latialata*
25b. Achenes not curved, epidermal cells broadly ovate or ovate 26
26a. Hilum curved or slightly curved 27
26b. Hilum not curved 30
27a. Achenes 1.0 × 0.5 mm, epidermal cells 25–30 μm long ..... *A. larium*
27b. Achenes 0.8–0.9 × 0.3–0.45 mm, epidermal cells 30–50 μm long 28
28a. Achenes 0.9 × 0.45 mm, hilum curved, epidermal cells 40–50 μm long ..... *A. rhododactyla*

28b. Achenes 0.8 × 0.3–0.4 mm, hilum slightly curved, epidermal cells 30–45 μm long 29
29a. Hilum 100 μm in diameter ..... *A. spodiophylla*
29b. Hilum 120 μm in diameter ..... *A. surculosa*
30a. Epidermal cells broadly ovate ..... *A. bulleyana*
30b. Epidermal cells ovate 31
31a. Hilum 80 μm in diameter, epidermal cells 50 μm × 20–25 μm ..... *A. contortiformis*
31b. Hilum 110–150 μm in diameter, epidermal cells 25–40 × 10–20 μm 32
32a. Achenes 0.75 × 0.4 mm, hilum 110 μm in diameter, epidermal cells 25–35 × 10 μm ..... *A. flaccida*
32b. Achenes 0.8–0.9 × 0.4–0.5 mm, hilum 120–150 μm in diameter, epidermal cells 25–40 × 15–20 μm 33
33a. Achenes 0.8 × 0.4 mm, hilum 150 μm in diameter, epidermal cells 30–40 × 15 μm ..... *A. sinica* var. *lanata*
33b. Achenes 0.9 × 0.5 mm, hilum 120 μm in diameter, epidermal cells 25–30 × 15–20 μm ..... *A. yunnanensis*

Discussion

The genus *Anaphalis* is distinguished among all genera of Gnaphalieae because of short clavate hairs on the achene surface (Anderberg, 1991, Bremer, 1994; Qaiser & Abid, 2003). However, Abid & Qaiser (2007a) indicated that the achene surface of *Anaphalis* was covered by papillate-clavate hair, not just the clavate hair, and they were the first to consider the density of hair as crucial characteristic. Based on the density of achene surface hairs, Abid & Qaiser (2007a) divided all *Anaphalis* species in Pakistan into two groups: sparsely or densely papillate-clavate hairs. Our results demonstrated two types of surface ornamentation of Chinese *Anaphalis* achene: reticulate-claviform and ligulate protuberant. The micro-morphological characteristics of the achene surfaces were distinct among species in Chinese *Anaphalis*. In the study based on nuclear DNA sequences (Nie *et al.*, 2013), *A. adnata*, *A. margaritacea*, *A. nepalensis*, and *A. triplinervis* had a close relationship with well support, and the surfaces of these four species had reticulate-claviform achene surfaces. Species in Group Ⅱ are assembled into a well-supported clade in the study of molecular systematics (Nie *et al.*, 2013).

Based on macro-morphological characteristics, Chen *et al*., (1966) divided *Anaphalis* into two subgenera: Subgen. Ⅰ *Gnaphaliops* Ling, with only one species (*A. bulleyana*), and Subgen. Ⅱ *Anaphalis*. *A. bulleyana* is closely related to *Gnaphalium* due to the ratio of male flowers to female flowers and the characteristics of the involucre and pappus (Chen *et al.*, 1966). Our current study suggests that *A. bulleyana*, belonging to Group Ⅱ, should not be treated as a separate species in Subgen. *Gnaphaliops*. Furthermore, the results of molecular phylogeny also support this view (Nie *et al.*, 2013). According to Chen *et al*., (1966), *A. busua* belongs to Ser. *Busuae* Ling; *A. contorta* and *A. margaritacea* are in Ser. *Margaripes* (DC.) Boiss.; and *A. acutifolia*, *A. nepalensis*, and *A. triplinervis* belong to Ser. *Nepalenses* Ling. In the present study, all species mentioned in Chen *et al*., (1966) fall into our Group Ⅰ. Species in our Group Ⅱ belonged to Ser. *Flavescentes* Ling, Ser. *Margaripes* (DC.) Boiss., Ser. *Pannosae* Ling, Ser. *Sinicae* Ling, Ser. *Suffruticosae* Ling and Ser. *Xylorhizae* Ling (Chen *et al.*, 1966). Therefore, the traditional partition of subgenus, section, and series in *Anaphalis* was not supported from the view of achene micro-mophological characteristics in this study.

Wu *et al*., (2011) indicated that the middle flowers in the capitulum of *A. adnata* were bisexual, so they transferred *A. adnata* to *Pseudognaphalium* as *Pseudognaphalium adnatum* (DC.) Y.S. Chen. However, based on achene micro-morphology in our study and molecular phylogeny (Nie *et al.*, 2013), *A. adnata* has a close relationship with *A. margaritacea*, *A. nepalensis*, and *A. triplinervis*. Therefore, transferring *A. adnata* to *Pseudognaphalium* is not supported in the present study.

Anaphalis was closely related to *Gnaphalium*, *Pseudognaphalium*, and *Helichrysum*. Abid & Qaiser (2007a) described, the achene surface ornamentation of *Anaphalis* was papillate-clavate hairy. Additionally, Abid & Qaiser (2008a) also indicated that achene of *Gnaphalium* and *Pseudognaphalium* were oblong or oblong-oblanceolate, sparsely papillose, or papillose-clavate hairy. Based on achene features, *Pseudognaphalium* could not be distinguished from *Gnaphalium* (Abid & Qaiser, 2008a). The papillate-clavate hairs were similar to the characteristics of Group Ⅰ in this study. Therefore, *Anaphalis* could not be separated from *Gnaphalium* and *Pseudognaphalium* due to their similar achene characteristics. However, to date, there have been no reports about the achene morphological characteristics of *Helichrysum*. The previous studies on achene morphological characteristics of *Anaphalis* and closely related genera have focused only on Pakistani species (Abid & Zehra, 2007; Abid & Qaiser, 2008a, 2008b). Thus, to understand the relationships of these related genera, it is necessary to include more taxa in further studies.

Conclusions

Based on achene surface ornamentation, Chinese *Anaphalis* can be divided into two groups: Group Ⅰ with reticulate-claviform surfaces, and Group Ⅱ with ligulate protuberant achene surface ornamentation. Based on these criteria, *A. adnata*, *A. margaritacea*, *A. nepalensis*, and *A. triplinervis* had a close relationship, with good support from micro-morphology. Therefore, *A. adnata* should be retained in *Anaphalis*. Micro-morphological characteristics of achene do not support the traditional classifications of subgenus, section, and series of Chinese *Anaphalis.*

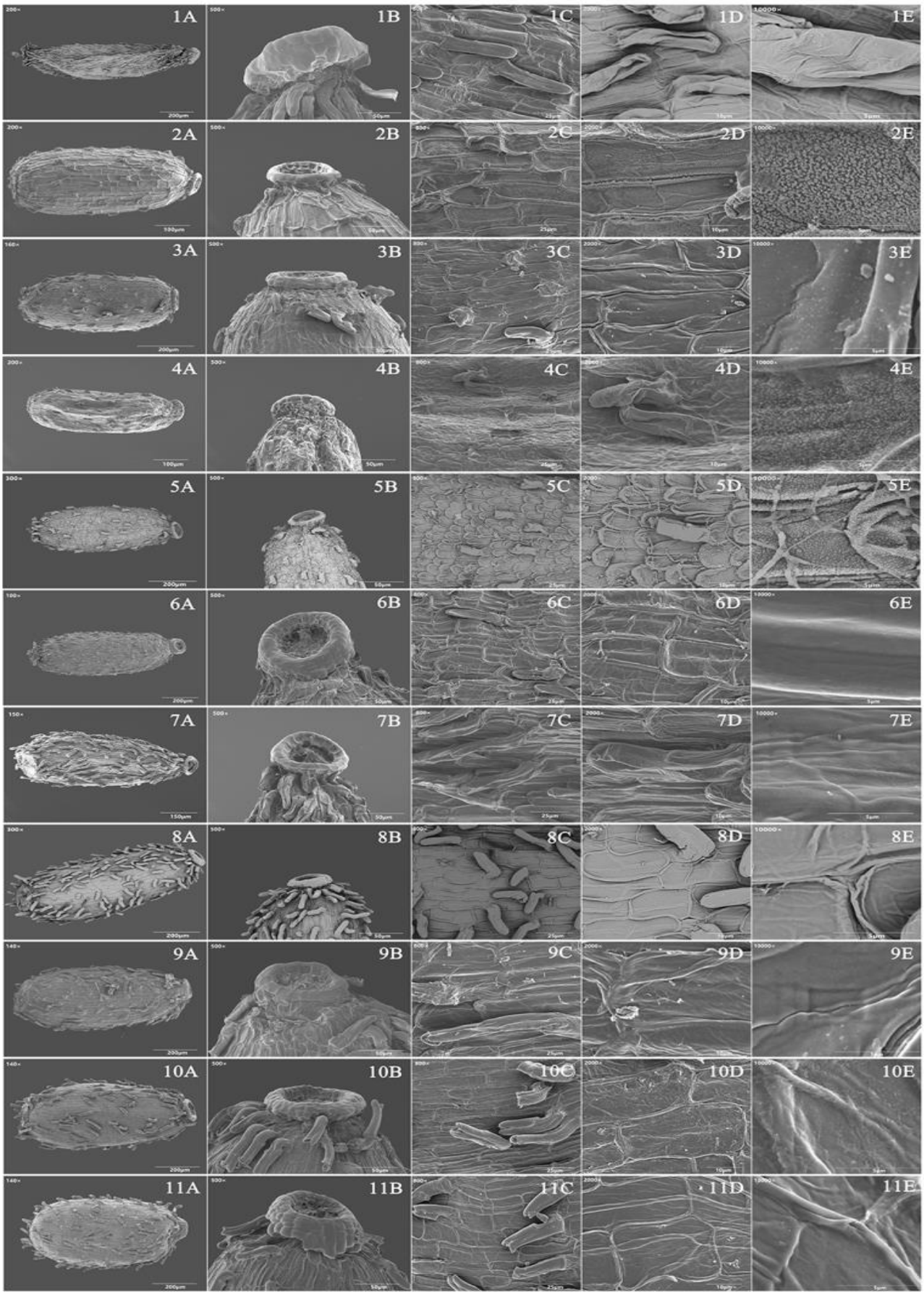

Fig. 1. Scanning Electron micrographs of Group Ⅰ: *A. acutifolia*: 1A-E; *A. adnata*: 2A-E; *A. busua*: 3A-E; *A. contorta*: 4A-E; *A. margaritacea*: 5A-E; *A. margaritacea* var. *angustifolia*: 6A-E; *A. nepalensis*: 7A-E; *A. nepalensis* var. *nepalensis*: 8A-E; *A. nepalensis* var. *corymbosa*: 9A-E; *A. nepalensis* var. *monocephala*: 10A-E; *A. triplinervis*: 11A-E.

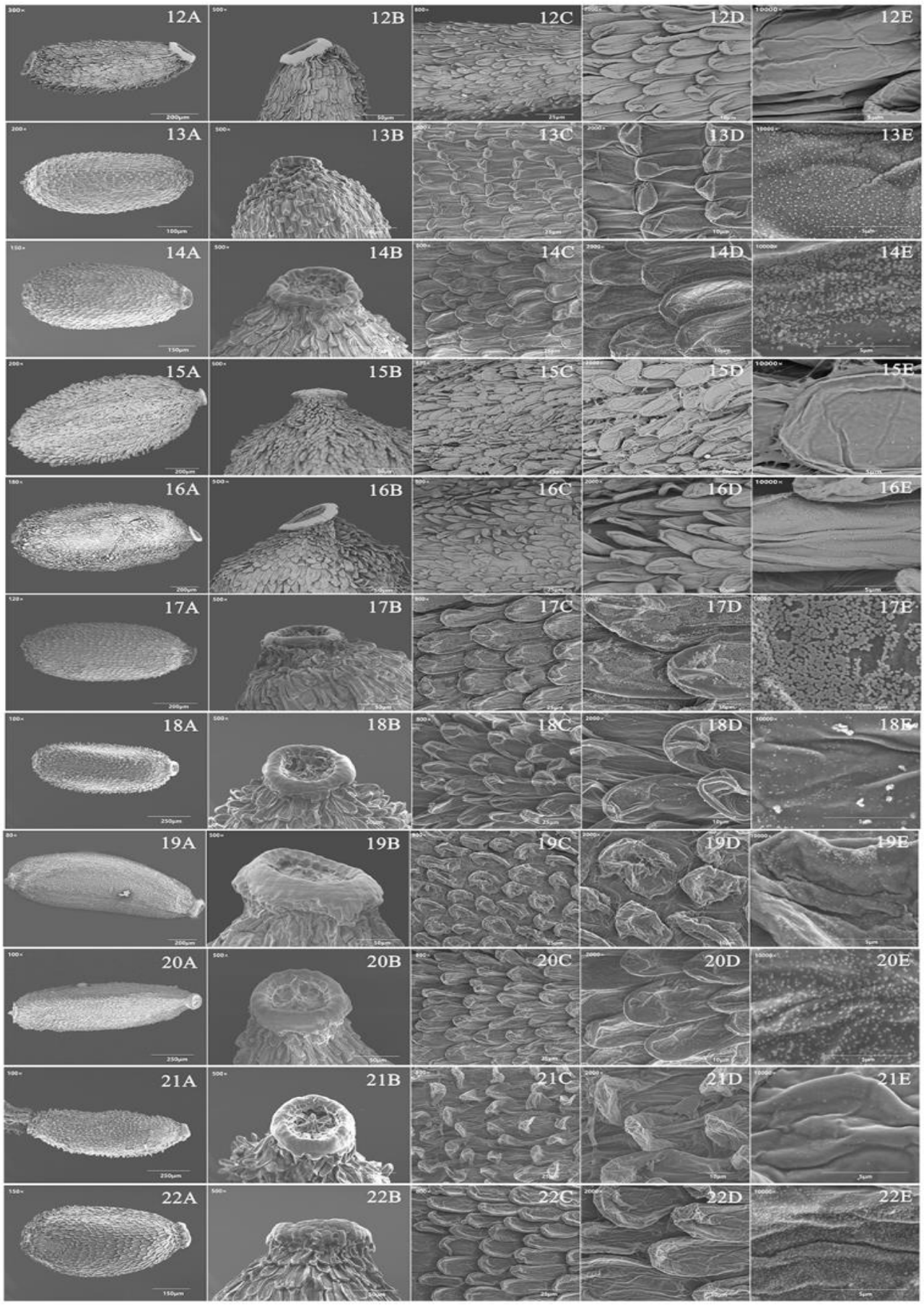

Fig. 2. Scanning Electron micrographs of Group Ⅱ: *A. aureopunctata*: 12A-E; *A. bicolor*: 13A-E; *A. bulleyana*: 14A-E; *A. chlamydophylla*: 15A-E; *A. cinerascens*: 16A-E; *A. contortiformis*: 17A-E; *A. corymbifera*: 18A-E; *A. delavayi*: 19A-E; *A. deserti*: 20A-E; *A. elegans*: 21A-E; *A. flaccida*: 22A-E.

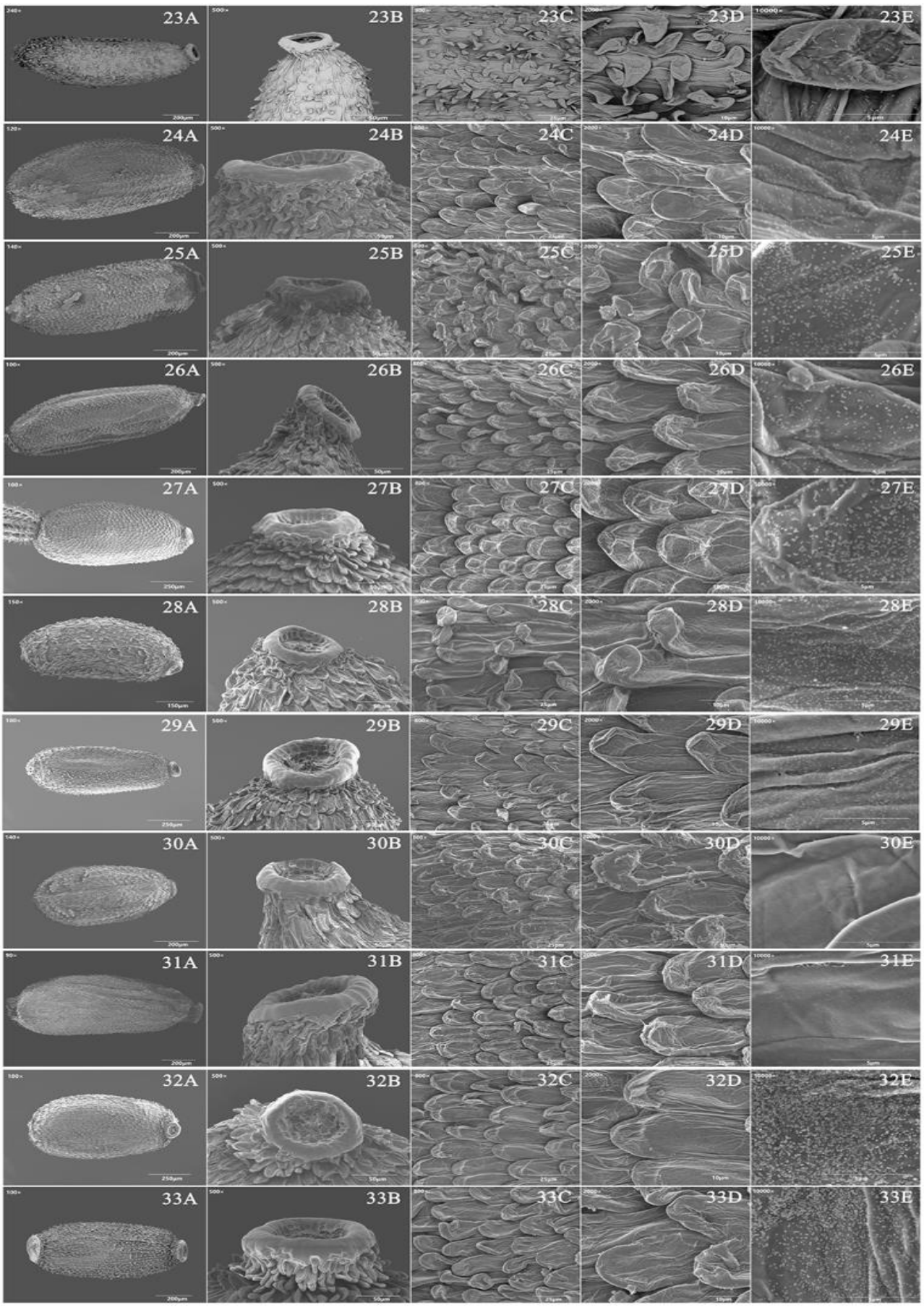

Fig. 3. Scanning Electron micrographs of Group Ⅱ: *A. flavescens*: 23A-E; *A. gracilis*: 24A-E; *A. gracilis* var. *ulophylla*: 25A-E; *A. lactea*: 26A-E; *A. larium*: 27A-E; *A. latialata*: 28A-E; *A. likiangensis*: 29A-E; *A. pachylaena*: 30A-E; *A. pannosa*: 31A-E; *A. rhododactyla*: 32A-E; *A. sinica* var. *lanata*: 33A-E.

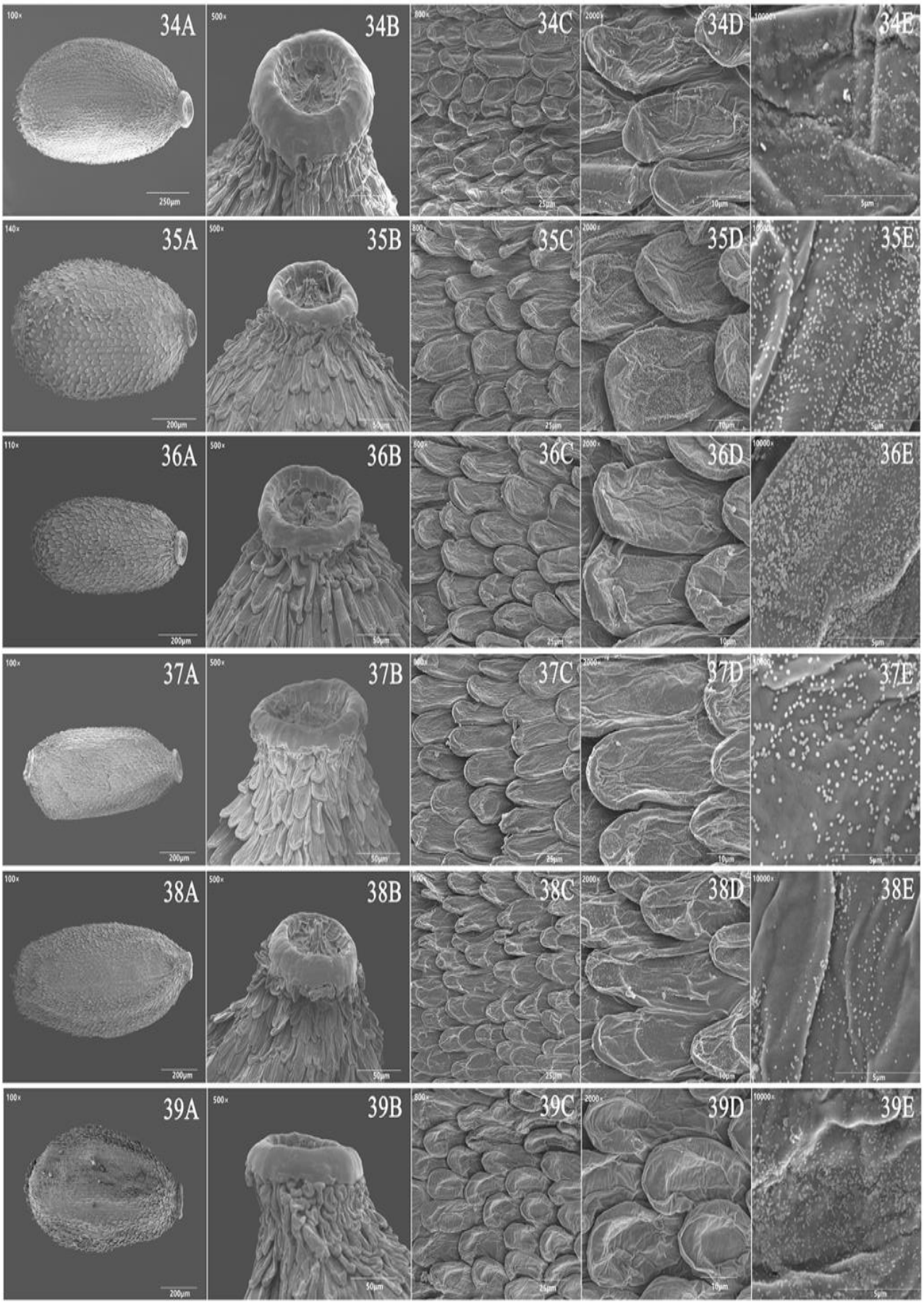

Fig. 4. Scanning Electron micrographs of Group Ⅱ: *A. souliei*: 34A-E; *A. spodiophylla*: 35A-E; *A. surculosa*: 36A-E; *A. virens*: 37A-E; *A. xylorhiza*: 38A-E; *A. yunnanensis*: 39A-E.

Acknowledgments

The authors thank the National Natural Science Foundation of China (31670188) and the project "Flora of Pan-Himalayas" (2013FY112100) for financial support. We appreciate Professor Boufford *et al*., from Harvard University for collecting the plant materials. Thanks to PE herbarium for providing material for study. Finally, we greatly appreciate the reviewers for their helpful comments and suggestions.

References

Abid, D.R and M. Qaiser. 2002. Achene morphology of *Inula* L. (s.str.) and its allied genera (Inuleae-Compositae) from Pakistan and Kashmir. *Pak. J. Bot.*, 34: 207-223.

Abid, R. and M. Qaiser. 2007a. Achene morphology of the genus *Anaphalis* DC. (Gnaphalieae-Asteraceae) from Pakistan. *Pak. J. Bot.*, 39: 1897-1906.

Abid, R. and M. Qaiser. 2007b. Micromorphology of achene in the Tribe Plucheeae from Pakistan. *Pak. J. Bot.*, 39: 671-677.

Abid, R. and M. Qaiser. 2007c. Achene Morphology of the Genus *Pulicaria* Gaertn., (Inuleae-Asteraceae) from Pakistan. *Pak. J. Bot.*, 39: 991-997.

Abid, R. and N. Zehra. 2007. Micromorphology of achene and its Taxonomic Significance of some Genera in the Tribe Inuleae (Asteraceae) from Pakistan. *Pak. J. Bot.*, 39: 1407-1416.

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

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

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

Blanca, G. and C.D.D.L. Guardia. 1997. Fruit morphology in *Tragopogon* L. (Compositae: Lactuceae) from the Iberian Peninsula. *Bot. J. Linn. Soc.*, 125: 319-329.

Bremer, K. 1994. *Asteraceae Cladistics & classification*. Timer Press, Portland.

Bruhl, J.J. and C.J. Quinn. 1990. achene anatomy in the 'Cotuleae' (Asteraceae-Anthemideae). *Bot. J. Linn. Soc.*, 102: 37-59.

Chen, F.H., Y. Ling and Y.L. Chen. 1966. De genere *Anaphalis* DC. familiae compositarum e florae sinicae. *Acta Phytotax. Sin.*, 11: 91-113.

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. *Pratacul. Sci*., 24: 9-15.

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

Lin, R. 1979. *Flora Reipublicae Popularis Sinicae, Compositae Ⅱ*, Vol. 75. Science Press, Beijing.

Lin, Y.R. 1993. On the primary study of the systematics and floristics of compositae from china. *Bull. Bot. Res.*, 13: 151-201.

Lin, Y.R. 1997. On the primary study of the systematics and floristics of compositae from china. *Bull. Bot. Res.*, 17: 6-20.

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: 178-188.

Melahat, O. 2017. achene micromorphology and anatomy in *Cirsium* sect. *Epitrachys* (Asteraceae, Carduoideae) and its taxonomic implications. *Nord. J. Bot.*, 35: 653-668.

Meng, Y., H. Sun, Y.P. Yang and Z.L. Nie. 2010. Polyploidy and new chromosome counts in *Anaphalis* (Asteraceae: Gnaphalieae) from the Qinghai-Tibet Plateau of China. *J. Syst. Evol.*, 48: 58-64.

Meng, Y., Y.P. Yang, H. Sun, T. Deng and Z.L. Nie. 2014. Chromosome numbers, karyotypes, and polyploidy evolution of *Anaphalis* species (Asteraceae: Gnaphalieae) from the Hengduan Mountains, SW China. *Caryologia*, 67: 238-249.

Nie, Z.L., V. Funk, H. Sun, T. Deng, Y. Meng and J. Wen. 2013. Molecular phylogeny of *Anaphalis* (Asteraceae, Gnaphalieae) with biogeographic implications in the Northern Hemisphere. *J. Plant Res.*, 126: 17-32.

Qaiser, M. and R. Abid. 2003. Asteraceae (II) Inuleae, Plucheeae and Gnaphalieae. *In* Ali, S.I. & M. Qaiser. (Eds). *Flora of Pakistan*. Department of Botany, University of Karachi and Missouri Botanical Press, Missouri, pp. 1-215.

Ritter, M.R. and S.T.S. Miotto. 2006. Micromorphology of fruit surfaces in species of *Mikania* Willd. (Asteraceae) occurring in Rio Grande do Sul State, Brazil. *Acta. Bot. Bras.*, 20: 241-247.

Roque, N. and V.A. Funk. 2013. Morphological characters add support for some members of the basal grade of Asteraceae. *Bot. J. Linn. Soc.*, 171: 568-586.

Singh, R.P. and A.K. Pandey. 1984. Development and structure of seeds and fruits in Compositae-Cynareae. *Phytomorphology*, 34: 1-10.

Wu, Z.Y., A.M. Lu, Y.C. Tang, Z.D. Chen and D.Z. Li. 2003. *The Fami. & Gen. Angiosp. China*. Science Press, Beijing.

Wu, Z.Y., P.H. Raven and D.Y. Hong. 2011. *Flora of China*. Vol. 20-21 (Asteraceae). Science Press & Missouri Botanical Garden Press, Beijing & St. Louis.

Zhang, D.C., S.Z. Li, X.H. Hou and M.Q. Zhang. 2013. Morphology and germination features of achenes of 12 species of the genus *Saussurea* (Asteraceae) in Western Sichuan Plateau. *Pl. Diver. Res.*, 35: 273-279.

Zhang, X.C. and X.L. Chen. 2008. Studies on achene size and variations' evolutionary ecology of six genera of Compositae from eastern Qinghai-Tibetan Plateau. *J. NW A & F Univ. (Nat. Sci. Ed.)*. 36: 207-214.

(Received for publication 21 October 2019)