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Morphology and micro-morphology of achenes and their taxonomic implications to *Lactuca* species (Cichorieae; Asteraceae)

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ABSTRACT: *Lactuca* L. is the central genus of Lactucinae (Cichorieae; Asteraceae), containing cultivated lettuce and its wild relatives. In this study, we used Scanning Electron Microscope, Plain Stereo Microscope and Automated Digital Microscope to observe, record and discuss the achene characters and surface micro-features of *Lactuca* (including taxa of ex-*Pterocypsela* Shih) and *Notoseris* Shih species. The taxon sampling consisted of fifteen globally distributed *Lactuca* species and Chinese originated *Notoseris* species. The results indicated that the morphological and micro-morphological features of achenes were of great importance to identify *Lactuca* species at the genus and species level. We conclude that two key features, the presence or absence of beak and the arrangement and shape of epidermal cells, can be used to distinguish *Lactuca* from *Notoseris*. The shape and margin of the achene body, the beak length and the number of ribs on either side of achene are key features to classify *Lactuca* species. The ornamentation of epidermal cells can also provide extra evidence to determine closely related *Lactuca* species. The interspecific relationships among the *Lactuca* species based on achene features are consistent with the results of previously molecular systematics of these species.

KEY WORDS: Achene micro-morphology, achene morphology, Lactucinae, *Lactuca*, *Notoseris*, *Pterocypsela*, SEM.

INTRODUCTION

Lactuca L. is the core and typic genus of Lactucinae (Cichorieae; Asteraceae) (Kilian *et al.,* 2009a). Due to the large variations of morphological characters (especially leaf morphology), Lactucinae species had many controversies in the taxonomy and systematics. There had been no less than seventeen genera described in Lactucinae (Shih, 1997; Jeffrey and Beentje, 2000; Shih and Kilian, 2011). *Lactuca*, *Cephalorrhynchus* Boiss., *Cicerbita* Wall., *Faberia* Hemsl. ex F. B. Forbes & Hemsl. and *Prenanthes* L. were considered as '*Prenanthes-Lactuca* line' and treated as Crepidinae species (Stebbins 1953). Jeffrey (1966) determined the *Prenanthes* and *Lactuca* species in East Tropical Africa as PRENANTHES-SERIES in CICHORIUM-GROUP (Jeffrey 1966). Based on key characteristics of achenes, Shih (1987, 1988a, 1988b, 1991, 1997) and Shih and Chen (1996) studied the '*Lactuca-Prenanthes*' complex and established five new genera, *Notoseris* Shih, *Pterocypsela* Shih, *Chaetoseris* Shih, *Stenoseris* Shih and *Faberiopsis* Shih & Chen. The circumscriptions among the '*Lactuca-Prenanthes*' complex, such as *Paraprenanthes* Chang ex Shih, *Mulgedium* Cass. and *Lagedium* Soják, were also revised (Shih, 1997, 1991, 1988a, b, 1987; Shih and Chen, 1996). According to the latest taxonomic and molecular systematic studies, the Lactucinae consists of six core lineages, *Cicerbita*, *Lactuca*, *Notoseris*, *Paraprenanthes*, *Kovalevskiella* Kamelin and *Melanoseris* Decne., and three related lineages, *Prenanthes*, *Astartoseris* Kilian, Hand, Hadjik., Christodoulou & Bou Dagh.-Kharrat and *Faberia* (Wang *et al.,* 2013; Kilian *et al.,* 2017a; Kilian *et al.,* 2017b; Güzel *et al.,* 2021; Wei *et al.,* 2017).

The morphological features of *Lactuca* achene were described with vague concepts: body subcompressed to compressed; winged or not; muricate-scabrid or papillate surface; one to several slender ribs on either side; beak stout, slender, or filiform or absent; pappus white or sometimes with a faint yellow tinge, single, of slender scabrid bristles, or double with an additional outer row of minute hairs (Abid and Qaiser, 2015; Kilian *et al.,* 2009b). Shih (1988a) studied the morphology of *Lactuca* and established *Pterocypsela* as a novel genus, including Asian mainland *Lactuca* speices with achene body strongly compressed and distinctly winged margin (Shih 1988a). *Pterocysela* originally consisted of seven species, namely *Pterocypsela elata* (Hemsl.) Shih, *P. raddeana* (Maxim.) Shih, *P. triangulata* (Maxim.) Shih, *P. indica* (L.) Shih, *P. laciniata* (Houtt.) Shih, *P. sonchus* (H.Lév. & Vaniot) Shih and *P. formosana* (Maxim.) Shih (Shih 1988a). Though these *Pterocypsela* species have distinct achene features from other Asian *Lactuca* species (Shih 1988b, a), the molecular systematics of large taxa samplings, using ITS and chloroplast markers, have shown *Pterocypsela* species are still ingroups of *Lactuca* lineages. Therefore, *Pterocypsela* species was relocated to *Lactuca* (Wang *et al.,* 2013; Shih and Kilian, 2011).

The concept of *Notoseris* was firstly proposed by Shih and included twelve species mainly distributed in south of

Table 1. The information of plant materials

Species	Collector and Collection Number/Barcode	Herbarium code	Year	Country
Lactuca canadensis	*Brummitt et al., 20741*	K	2002	U.S.A
L. formosana	*Zhu 2011-1575*	ZZU	2011	China
L. georgica	*Akhani 11926*	K	1995	Iran
L. indica	*CF023571*	ZZU*	-	China
L. lasiorhiza	*Jenik & Hall 1120*	K	1965	Ghana
L. longispicata	*Richards 4694*	K	1983	Zambia
L. ludoviciana	*Palmer 447*	K	1907	Mexico
L. raddeana	*Zhu 2012-1813*	ZZU	2012	China
L. schweinfurthii	*Daramola 62749*	K	1969	Nigeria
L. serriola	*Zhu 2018-0718*	ZZU	2018	China
L. setosa	*Pawek 4354*	K	1971	Malawi
L. virosa	*Ross-Craig &Sealy*	K	1960	England
L. zambesiaca	*Brummitt et al., 14115*	K	-	Zambia
Notoseris henryi	*Zhu 2012-1805*	ZZU	2012	China
N. macilenta	*Zhu 2012-1804*	ZZU	2012	China

The herbarium code follows the Index Herbariorum (Thiers, 2016), K - Royal Botanic Gardens, Kew, ZZU - Zhengzhou University.
*: The plant seeds were from National Animal Husbandry Station of China (NAHS) and the herbarium sheet was stored at ZZU.

Yangtze River and Qinling Mountains in mainland China (Shih, 1987). The achenes of *Notoseris* are usually purplish to brownish red, apex truncate, cylindric to subfusiform and compressed with 6–9 ribs on each side (Shih, 1997; Kilian *et al.,* 2009a, 2009b). Despite of minor revisions of the species number, the independent taxonomic position of *Notoseris* was admitted by other taxonomists (Kilian *et al.,* 2009a, 2009b) and confirmed by molecular phylogenetic analyses (Wang *et al.,* 2013; Kilian *et al.,* 2017b; Güzel *et al.,* 2021).

The achene morphology and micro-morphology characters of achene epidermis under Scanning Electron Microscopy (SEM) provided additional evidence to evaluate the systematics of Cichorieae and Asteraceae species at the genus level and species level (Sikder and Moktan, 2021; Abid and Qaiser, 2015; Ayaz *et al.,* 2020; Ozcan and Demir, 2021; Bona, 2020). Zhu *et al.,* (2006) investigated the achene wall anatomy and surface sculpturing of *Lactuca* and its allied genera, *Pterocypsela*, *Cicerbita*, *Chaetoseris*, *Stenoseris*, *Notoseris* and *Paraprenanthes*, and concluded that the achene wall type, the ornamentation and the shape of epidermal cells were essential features to define the intergeneric and interspecific relationships among these genera (Zhu *et al.,* 2006). Zhang *et al.,* (2013) observed achene morphology of thirteen species from *Soroseris*, *Syncalathium* and *Parasyncalathium* using SEM and evaluated the systematic significances of achene morphology (Zhang *et al.,* 2013). The anatomy of achene wall and achene morphology of *Notoseris*, *Pterocypsela*, *Prenanthes* and *Paraprenanthes* were compared and considered as important features with taxonomic significance at the genus level (Liu *et al.,* 2015).

The previous studies of achene morphology and micro-morphology of *Lactuca* were mainly involved in regional species, such as China, Pakistan, and Kashmir (Zhu *et al.,* 2006; Abid and Qaiser, 2015; Liu *et al.,* 2015). In this study, we aimed to observe the achene body and surface micro-features of *Lactuca, Notoseris* and *Pterocypsela* from different continents, including Africa, Asia, and North America. The parameters of achenes, such as body shape, color, size, beak, carpopodium, the number of ribs on each side, surface sculpturing, and so on, were carefully investigated and recorded using SEM, Plain Stereo Microscope (PSM) and Automated Digital Microscope (ADM). The key characters of taxonomic significances for *Lactuca* species were determined to delimit taxa at the species level.

MATERIALS AND METHODS

In the study, we observed the achene body and surface micro-features of the *Lactuca* species from Africa, Asia (China), and North America. The achene materials were from authors' personal collection (ZZU), National Animal Husbandry Station of China (NAHS) and voucher specimens stored in international herbariums (K) (Table 1; herbarium code referring to Index Herbariorum, http://sweetgum.nybg.org/science/ih/) (Thiers, 2016). All the taxa were identified by Dr. Shixin Zhu and Dr. Yang Lu. The taxa sampling included thirteen *Lactuca* (including three former *Pterocypsela* species) and two *Notoseris* species (Table 1).

Note: We accepted the taxonomic treatment of transferring *Pterocypsela* back into *Lactuca*, but still use the genus name '*Pterocypsela*' for easier comparison and discussion in the main text.

Plain Stereo Microscope (PSM) and Automated Digital Microscope (ADM)

The morphological characters of the achenes were observed and recorded mainly using PSM (Nikon SMZ645 Microscope Model C-PS, Nikon, Japan) and ADM (Zeiss Smartzoom 5, Zeiss, Germany). We also

Table 2. The achene features of the ***Lactuca*** species.

Taxa Name	Achene Body					
	Shape	Surface	Color	Length × Breadth	No of ribs on each side	Margin
Lactuca canadensis	Ellipsoid and compressed	Muricate-hispid	Pale and blackish brown	3–4 × 1.5–2 mm	1	Broadly winged
L. formosana	Ellipsoid and compressed	Muricate-hispid	Reddish, dark, or blackish brown	4.5–6.5 × 2–2.5 mm	1–3	Broadly winged
L. georgica	Ellipsoid and compressed	Scabrid-hispidulous	Blackish brown	4–4.5 × 1.5–2 mm	7	Narrowly winged
L. indica	Ellipsoid and compressed	Muricate-hispid	Reddish, dark, or blackish brown	3–5 × 1.5–2 mm	1	Broadly winged
L. lasiorhiza	Ellipsoid and flattened	Muricate-hispid	Reddish or blackish brown	6–7 × 1.5–2.5 mm	3–5	Thickened
L. longispicata	Ellipsoid and flattened	Muricate-hispid	Dark to blackish brown	4.5–5.5 × 2–2.5 mm	1	Thickened and broadly winged
L. ludoviciana	Ellipsoid and flattened	Hispidulous	Pale and blackish brown	4–6 × 1.5–3 mm	1–2	Thickened and broadly winged
L. raddeana	Ellipsoid and compressed	Muricate-hispid	Reddish to dark brown	3–4 × 1–2 mm	3–5	Broadly winged
L. schweinfurthii	Flattened ellipsoid	Hispidulous	Pale to dark brown	4.5–5 × 1.5–2.5 mm	1–2	Thickened and broadly winged
L. serriola	Obovoid to fusiform and strongly compressed	Scabrid-hispidulous	Pale and dark brown	2.8–4.2 × 1.2–1.5 mm	5–8	Normal
L. setosa	Fusiform	Muricate-hispid	Reddish, or blackish brown	4–5.5 × 1–1.5 mm	3–4	Thickened
L. virosa	Ellipsoid and compressed	Hispid	Reddish, or blackish brown	4–4.7 × 1–1.5 mm	6–7	Narrowly winged
L. zambesiaca	Compressed-ellipsoid	Hispidulous	Dark–brown to black	4–5.5 × 1.5–2 mm	1–3	Thickened and broadly winged
Notoseris henryi	Oblanceolate long and compressed	Muricate-hispid	Brownish red	5–5.5 × 1–1.5 mm	6–8	Normal
N. macilenta	Oblong lanceolate and compressed	Muricate-hispid	Dark purple	6–6.5 × 0.6–1 mm	6–7	Normal

took into account of the descriptions of achene characters in previous publications and books. The achene characters studied in this work included body shape, size (length × breadth), color, surface, margin, the absence or presence of beak, carpopodium size (diameter × height), the number of ribs on each side, and pappus (if present and remained on the achene body). The terminologies of achene referred to previous publications (Zhu *et al.,* 2006; Abid and Qaiser, 2015).

Scanning Electron Microscope (SEM)

Mature and well-developed achenes were selected for surface micro-morphology study, prepared as previous publications (Xu *et al.,* 2021; Zhu *et al.,* 2006) and then directly mounted on aluminum stubs with double-sided adhesive tape and sputter-coated with gold to a maximum thickness of 20 μm. The achene body and surface micro-morphology were observed, examined and photographed with SEM (KYKY Scanning Electron Microscope, Beijing, China). The terminologies for the micro-morphology of achenes were described followed previous research (Barthlott, 1981; Zhu *et al.,* 2006). The cellular arrangement and the shape of epidermal cells, the ornamentation of outer cell walls, and the secretions of epicuticular waxes were compared, recorded and described in details.

RESULTS AND DISCUSSION

Achene morphology of *Lactuca* species

We examined and measured the achene features of *Lacutca*, *Pterocypsela*, and *Notoseris* species (Table 2; Table 3; Figure 1). In our sampling, the presence of beak is the most important feature of *Lactcua* species (Figure 1A to 1M), distinguishing them from *Notoseris* species. The achene beaks of *Lactcua* species are usually present, stout, slender, or filiform (Figure 1A to 1M). The achene beaks of *Pterocypsela* species are generally stout (Figure 1E and 1F) or filiform (Figure 1D), while the beaks of other *Lactuca* species are generally slender or filiform. In contrast, the achenes of *Notoseris* (Figure 1N and 1O) have no beaks.

The achene body shape is also a key feature for *Lactuca* species at the genus level (Figure 1A to 1M; Table 2). The achene body of *Lactuca* is generally narrowly or broadly ellipsoid or fusiform or oblong and subcompressed to compressed (including *Pterocypsela*; Figure 1A to 1M; Table 2). The achenes of *Notoseris* are cylindric to subfusiform and compressed, basally more attenuate than apically (Table 2; Figure 1N and 1O).

The margin of achene is an essential character for identifying *Lactuca* species. The *Pterocypsela* species were established based on the broadly winged type of achenes (Shih, 1988a) and this treatment was accepted in

Table 3. The achene surface micro–features of the ***Lactuca*** species

Name	Beak				Carpopodium	Pappus	
	Shape	Surface	Color	Length (mm)	Diameter × Length (mm)	Color	Length
Lactuca canadensis	Filiform	Smooth	Concolorous to greenish	2–3	0.26–0.34 × 0.15–0.2	white	4–7
L. formosana	Filiform	Smooth	greenish	2–3.5	0.25–0.3 × 0.05–0.1	white	7–8
L. georgica	Filiform	Smooth	pale	2–2.5	0.24–0.33 × 0.08–0.12	white	4–7
L. indica	Stout	Smooth	pale to greenish	0.4–1.6	0.18–0.28 × 0.09–0.13	white	7–8
L. lasiorhiza	Stout	Smooth	pale	1–3	0.48–0.52 × 0.12–0.17	white	8–10
L. longispicata	Stout-slender	Smooth	pale	0.5–1	0.26–0.36 × 0.12–0.2	white	5–6
L. ludoviciana	Slender	Hispid	greenish	2–3	0.27–0.32 × 0.12–0.15	white	8–10
L. raddeana	Stout	Hispid	Concolorous or apically pale	0.2–0.4	0.22–0.24 × 0.06–0.12	white	6–7
L. schweinfurthii	Filiform	Smooth	Concolorous-greenish	2	0.22–0.26 × 0.14–0.2	white	3–6
L. serriola	Filiform	Smooth	white	3–4.5	0.21–0.35 × 0.1–0.18	white	3–7
L. setosa	Not present				0.16–0.17 × 0.06–0.09	white	4–5
L. virosa	Filiform	Smooth	white	3–3.5	0.25–0.36 × 0.08–0.14	white	5–6
L. zambesiaca	Filiform	Smooth	pale brown or greenish	1–1.5	0.34–0.4 × 0.23–0.28	white	4–6
Notoseris henryi	Not present				0.26–0.33 × 0.12–0.15	white	8–9
N. macilenta	Not present				0.25–0.38 × 0.1–0.19	white	8–9

Flora Reipublicae Popularis Sinnicae (Shih, 1997). Later on, the molecular markers had been used as tools to resolve complex phylogenetic relationships among the Lactucinae species and denied the independent position of *Pterocypsela* (Shih and Kilian, 2011; Wang *et al.,* 2013; Kilian *et al.,* 2017b; Wei *et al.,* 2017; Güzel *et al.,* 2021). Indeed, in terms of winged types, the achenes of *Pterocypsela* are clearly different from other *Lactuca* species in East and South Asia. However, when the taxa sampling extended to other geographic groups of *Lactuca*, the winged achenes are not unique. The observations of endemic North American species using SEM showed *L. canadensis* L. and *L. ludoviciana* (Nutt.) Riddell had broadly winged achenes. The two mentioned North American species, *L. serriola* L., *L. virosa* L., and *L. georgica* Grossh. were reported to be capable of hybriding with the cultivated lettuce, *L. sativa* L. (Zohary, 1983, 1991; Wei *et al.,* 2014, 2021; Uwimana *et al.,* 2012; Jones *et al.,* 2018; Lebeda *et al.,* 2019a, 2019b). However, the achene body of *L. serriola* turned to have normal margin whereas the other four species had winged type of achene (Figure 1A to 1C, 1G and 1H).

The African endemic *Lactuca* species, *Lactuca lasiorhiza* (Hoffm.) Jeffrey, *L. longispicata* de Wild., *L. schweinfurthii* Oliv. et Hiern, *L. setosa* Stebbins ex Jeffrey, and *L. zambesiaca* Jeffrey (Lebeda *et al.,* 2004a, 2004b), were studied using SEM for the first time. The observations using PSM and ADM revealed that *L. longispicata*, *L. schweinfurthii*, and *L. zambesiaca* have winged type of achene with much thicker margins, which is different from other *Lactuca* species with winged type of achene. Compared to other *Lactuca* species in this study, the *L. lasiorhiza* achene is hardly beaked and apically attenuate with very obvious thick main ribs and margins. The achene characters of *L. lasiorhiza* are very close to species of *Melanoseris* Decaisne (Shih and Kilian, 2011), another complex genus in Lactucinae. The achenes of *L. setosa* are cylindric with truncate apex and thick ribs.

Wei *et al.,* (2017) first studied the molecular phylogeny of endemic African *Lactuca* species, including *L. lasiorhiza*, *L. setosa*, *L. schweinfurthii*, and *L. zambesiaca*, and found they were sister groups to *Melanoseris* instead of *Lactuca* ingroups. Later phylogenetic studies using ITS and plastid markers also confirmed that the endemic African species had a different origin from the core of *Lactuca* species and indicated close relationships between endemic African *Lactuca* species and *Melanoseris* species (Kilian *et al.,* 2017b; Güzel *et al.,* 2021). Our observations of achene morphology supported the close relationships between endemic African *Lactuca* and *Melanoseris* species. The achene morphology of former *Pterocypsela* species, *L. indica* L, *L. raddeana* Maxim. and *L. formosana* Maxim., was in accordance with molecular systematics of *Lactuca* speices and supported that all *Pterocypsela* should be transferred back into *Lactuca* (Wang *et al.,* 2013; Kilian *et al.,* 2017b; Wei *et al.,* 2017). In our study, the *Lactuca* species with winged achenes can be divided into three groups based on achene morphology, the ex-*Pterocypsela* species (*L. indica*, *L. raddeana* and *L. formosana*), the endemic North American species (*L. canadensis* and *L. ludoviciana*), and the close relatives of cultivated lettuce (*L. virosa* and *L. georgica*). The three groups had been revealed different systematic positions in phylogenetic trees constructed using ITS and plastid DNA regions (Kilian *et al.,* 2017b; Jones *et al.,* 2018). In general, the results of our achene morphology supported previous molecular phylogeny of *Lactuca* species.

Other achene features observed in this study, such as body size, color, beak length, surface, carpopodium size, the number of ribs on each surface, and pappus length could be utilized for identifying *Lactuca* species at the species level. These characters were summarized in the following sections (Table 2 & 3).

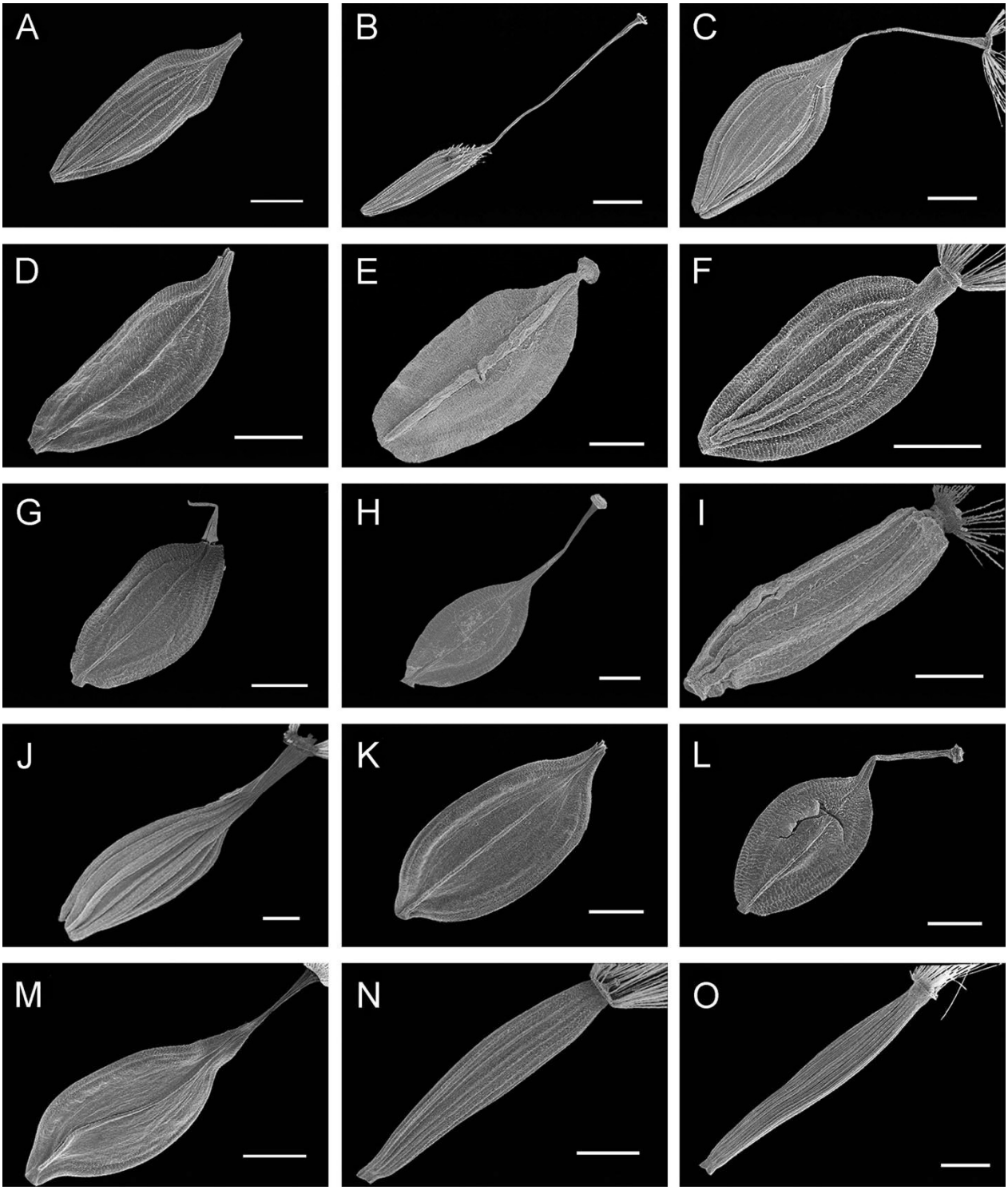

Fig. 1. Scanning electron micrographs of achenes. The micrographs were arranged in the following orders. **A**: ***Lactuca georgica;*** **B**: ***Lactuca serriola;*** **C**: ***Lactuca virosa;*** **D**: ***Lactuca formosana;*** **E**: ***Lactuca indica;*** **F**: ***Lactuca raddeana***; **G**: ***Lactuca canadensis***; **H**: ***Lactuca ludoviciana***; **I**: ***Lactuca setosa***; **J**: ***Lactuca lasiorhiza***; **K**: ***Lactuca longispicata***; **L**: ***Lactuca schweinfurthii***; **M**: ***Lactuca zambesiaca***; **N**: ***Notoseris henryi***; **O**: ***Notoseris macilenta***; Bar = 1 mm.

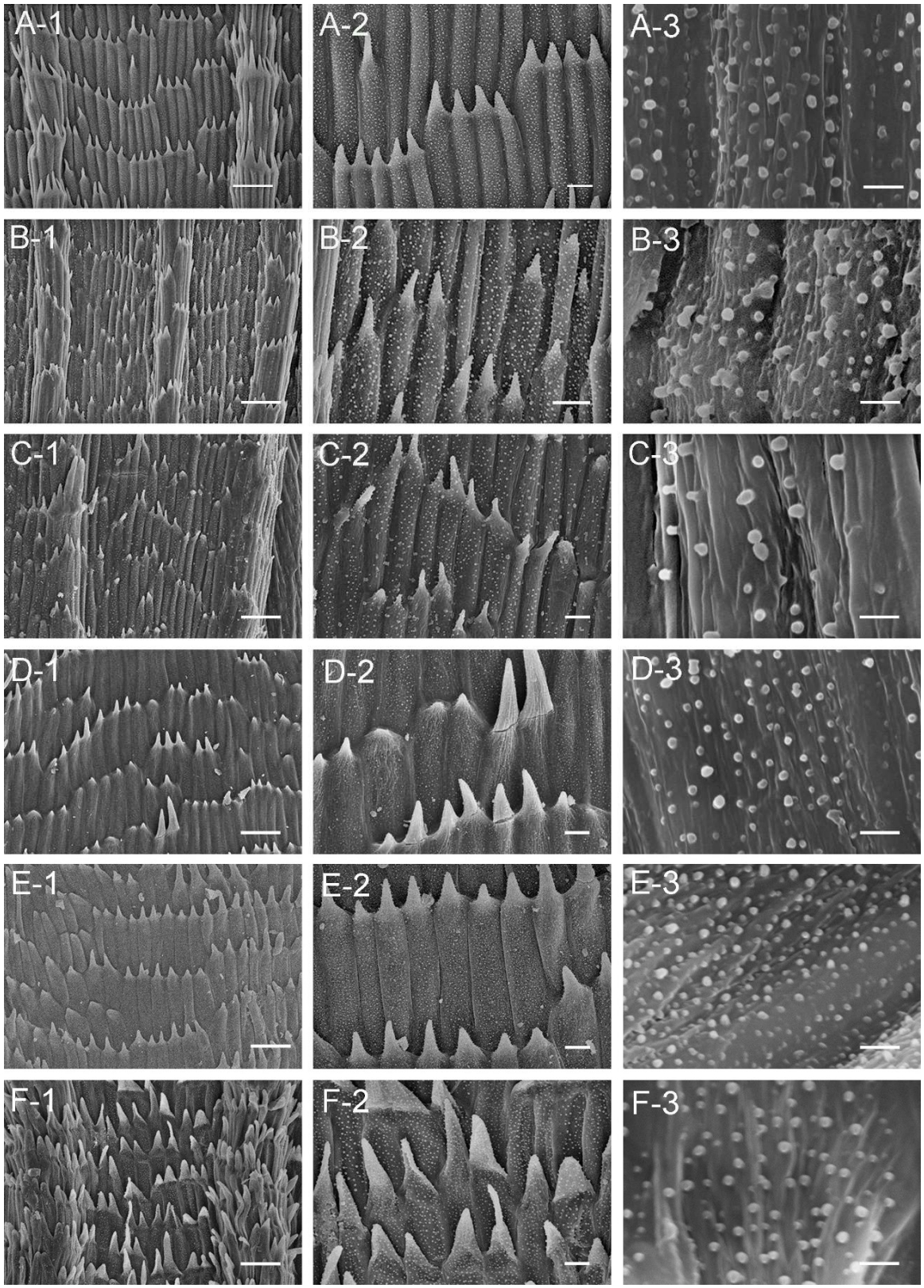

Fig. 2. Scanning electron micrographs of micro-features of achenes. The micrographs illustrated (1) the arrangement of epidermal cells, bar = 40 μm (2) the apical part of epidermal cells, bar = 10 μm and (3) the ornamentation of epidermal cells, bar = 2 μm. The species were arranged in the following orders. **A**: ***Lactuca georgica;*** **B**: ***Lactuca serriola;*** **C**: ***Lactuca virosa;*** **D**: ***Lactuca formosana;*** **E**: ***Lactuca indica;*** **F**: ***Lactuca raddeana***.

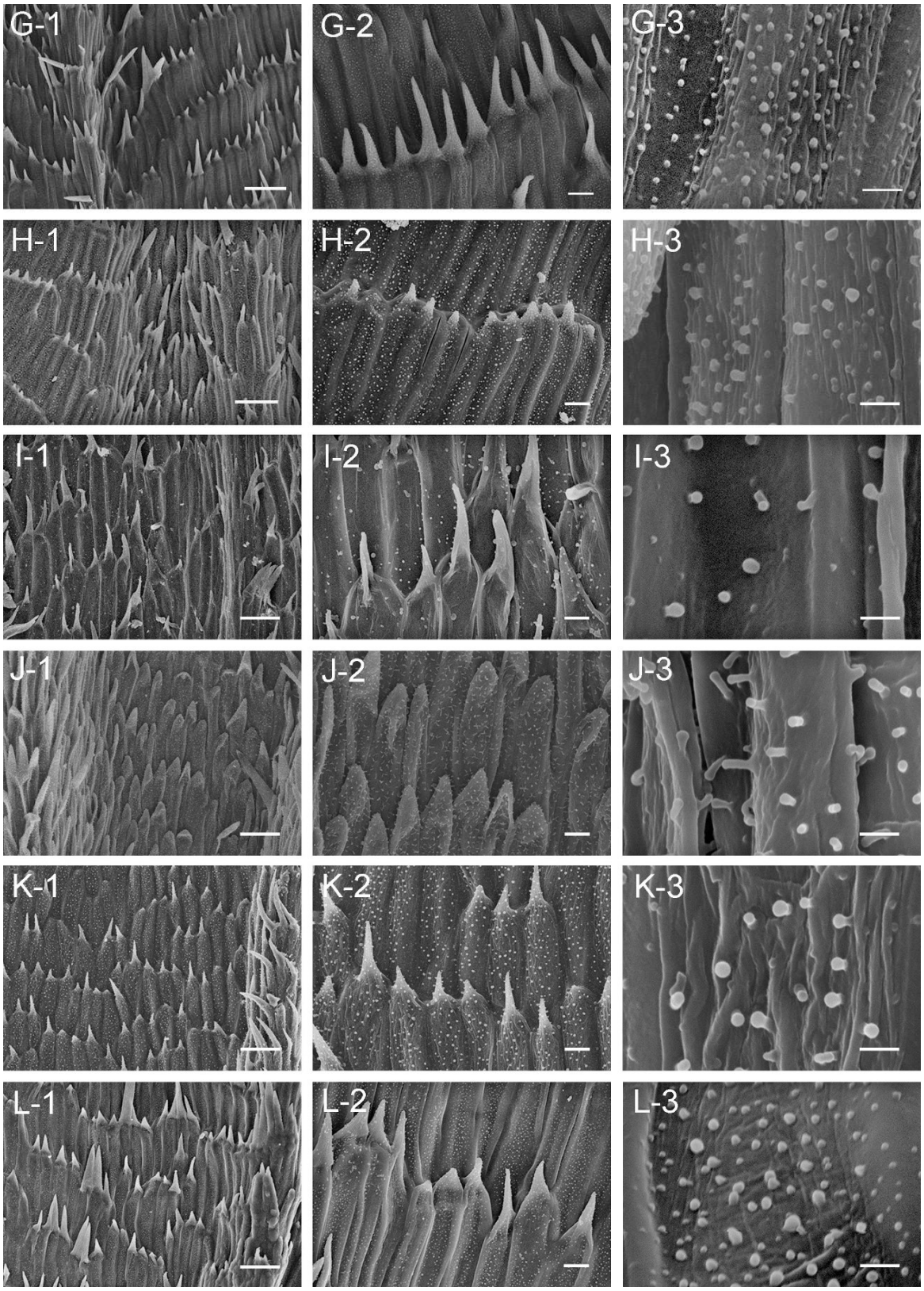

Fig. 3. Scanning electron micrographs of micro-features of achenes. The micrographs illustrated (1) the arrangement of epidermal cells, bar = 40 μm (2) the apical part of epidermal cells, bar = 10 μm and (3) the ornamentation of epidermal cells, bar = 2 μm. The species were arranged in the following orders. **G**: ***Lactuca canadensis***; **H**: ***Lactuca ludoviciana***; **I**: ***Lactuca setosa***; **J**: ***Lactuca lasiorhiza***; **K**: ***Lactuca longispicata***; **L**: ***Lactuca schweinfurthii***.

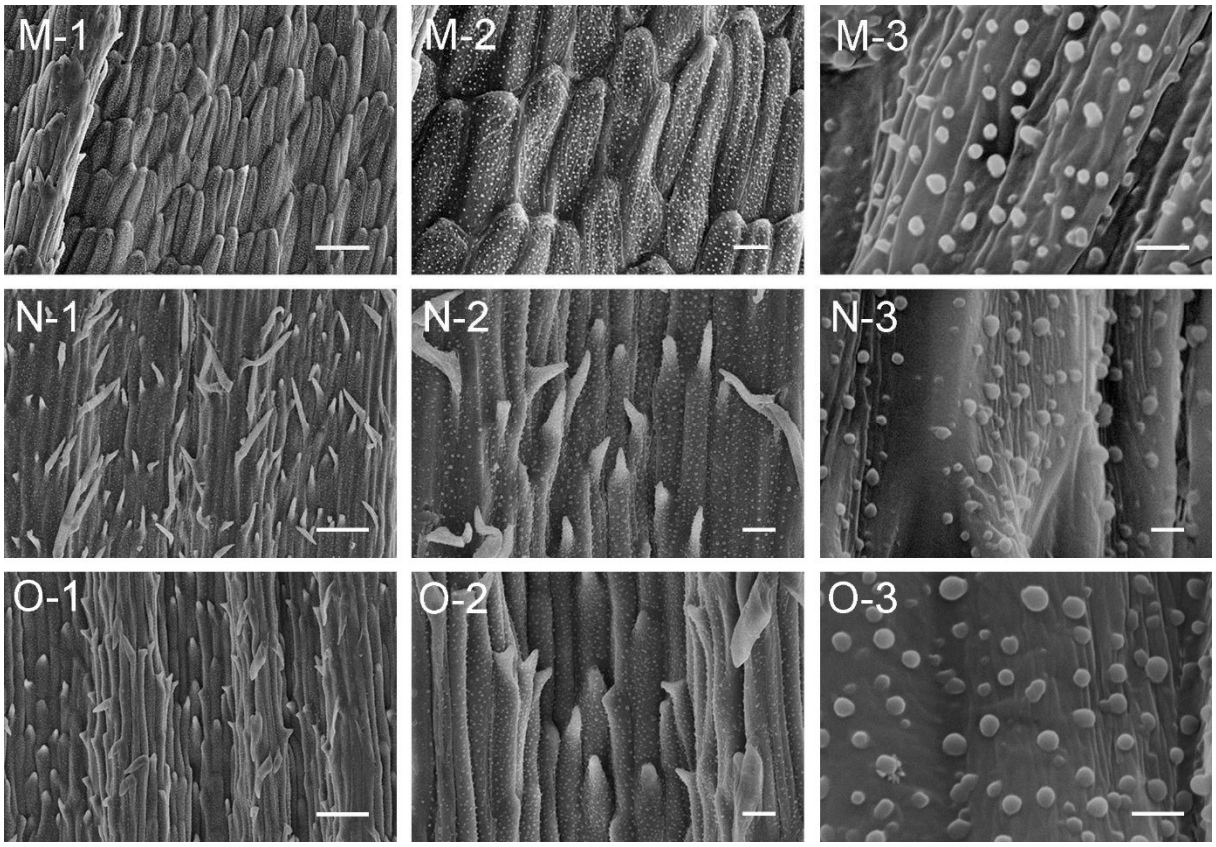

Fig. 4. Scanning electron micrographs of micro-features of achenes.The micrographs illustrated (1) the arrangement of epidermal cells, bar = 40 μm (2) the apical part of epidermal cells, bar = 10 μm and (3) the ornamentation of epidermal cells, bar = 2 μm. The species were arranged in the following orders. **M**: ***Lactuca zambesiaca***; **N**: ***Notoseris henryi***; **O**: ***Notoseris macilenta***.

Lactuca: Achene size 2.8–7 mm × 1–3 mm, beak length 0.2–4.5 mm, carpopodium size 0.16–0.52 mm × 0.05–0.28 mm, achene surface scabrid, muricate, or hispid, with 1–8 ribs on either side, pappus white, 3–10 mm, of slender scabrid bristles. The achene color is mostly blackish brown and has variations from dark brown or pale brown or reddish brown to purplish brown. The achenes of *L. canadensis*, *L. ludoviciana*, and *L. serriola* have uneven colors with dark or blackish and pale brown.

Notoseris: Achene size 5–6.5 mm × 0.6–1.5 mm, apex truncate, carpopodium size 0.25–0.38 mm × 0.1–0.19 mm, surface muricate or hispidulous, with 6–8 ribs on either side, color purplish to brownish red or pale to dark purple, pappus white, sometimes brown, 8–9 mm, of slender scabrid bristles.

Achene surface micro-features of the *Lactuca* species

The results showed that the surface micro-features of achenes could be used to identify the *Lactuca* alliance at the species level. The surface micro-morphology of each genus was described in the followings.

Lactuca: In all observed *Lactuca* plants, the epidermal cells are parallel to or slightly deviated from the long axis of the achenes (Figure 2, Figure 3, and Figure 4M). The epidermal cells are palisade-like and arranged regularly with a long steeple end or a short acute end. The endemic African species *L. lasiorhiza* (Figure 3J) and *L. zambesiaca* (Figure 4M) are exceptional as their epidermal cells mostly having blunt tips. The outer cell walls of epidermal cells are covered with wax of verrucose or fingerlike papillae. The waxy secretions of the upper epidermis are well developed in all *Lactuca* species. However, the density of epicuticular wax of *L. lasiorhiza* (Figure 3J), *L. longispicata* (Figure 3K), *L. setosa* (Figure 3I), and *L. virosa* (Figure 2C) are much lower than other *Lactuca* species, implying similarities among these species. From the aspects of epidermal cells shape and epicuticular wax density, *L. lasiorhiza*, *L. zambesiaca*, *L. longispicata* and *L. setosa* showed distant relationships from other *Lactuca* species and this result was consistent with molecular studies (Kilian *et al.,* 2017b; Wei *et al.,* 2017).

Notoseris: The achenes of *Notoseris* species were observed to have epidermal cells arranged mostly parallel to the long axis of achene with twisted cell apexes (Figure 4N and 4O). The ornamentations of *Notoseris* achene surface are mostly verrucose papillaes of well-developed wax with similar density. There is no clue to taxonomy in the surface micro-morphology of *Notoseris* species.

Key to the *Lactuca* species

1a. Achene not winged ……..……...……..…… 2
1b Achene winged ……..……..……..…….. 4
2a. Achene beak filiform, length 3–4.5 mm, achene body obovoid to fusiform and strongly compressed, 5–8 ribs on either side . ***L. serriola***
2b. Achene apically truncate or attenuate, 3–5 ribs on each side ……. 3
3a. Achene apically truncate, without beak, body size 4–5.5 mm × 1–1.5 mm, achene body margin thickened ….……..…… ***L. setosa***
3b. Achene with apex attenuate, body size 6–7 mm × 1.5–2.5 mm, beak length 1–3 mm ……..……..…… ***L. lasiorhiza***
4a. Achene with margin flatten ……..……..…… 5
4b. Achene with margin thickened ……..…….... 11
5a. Achene beak stout and short ……..…… 6
5b. Achene beak filiform ……..……..…… 7
6a. Beak length 0.2–0.4 mm, 3–5 thick ribs on either side .. ***L. raddeana***
6b. Beak length 0.4–1.6 mm, one thick rib on either side ….… ***L. indica***
7a. Achene with 1–3 main rib on either side ……...…… 8
7b. Achene with 6–7 ribs on either side ….…… 10
8a. Achene color reddish, dark, or blackish brown, body size 4.5–6.5 mm × 2–2.5 mm …… ***L. formosana***
8b. Achene color pale and blackish brown, body size 3–6 mm × 1.5–3 mm ……..… 9
9a. Achene pappus length 4–7 mm, body size 3–4 mm × 1.5–2 mm ……..... ***L. canadensis***
9b. Achene pappus length 8–10 mm, body size 4–6 mm × 1.5–3 mm ……..…… ***L. ludoviciana***
10a. The density of epicuticular wax sparse ……..……...… ***L. virosa***
10b. The epicuticular wax developed well and dense …..… ***L. georgica***
11a. Achene beak stout and short, beak length 0.5–1 mm, body color dark brown to blackish brown ……..……..…… ***L. longispicata***
11b. Achene beak filiform, beak length 1–2 mm …… 12
12a. Achene beak length 2 mm, body color pale brown to dark brown, epidermal cells with a long steeple end …..…… ***L. schweinfurthii***
12b. Achene beak length 1–1.5 mm, body color dark-brown to black, epidermal cells with a blunt end ……..….. ***L. zambesiaca***

CONCLUSIONS

This work studied the achenes morphology and micro-morphology of thirteen *Lactuca* and two *Notoseris* species using SEM, PSM and ADM. Based on the results and discussion, we conclude that the achene features are of great importance in the taxonomy of *Lactuca* species. The presence or absence of beak, the arrangement and shape of epidermal cells can be used as key features for classification at genus level. For *Lactuca* species, the shape and margin of achene body, the beak length and the number of ribs on either side are key features for interspecific relationships. Additionally, the development of wax and the ornamentation of outer cell walls of epidermis cells can distinguish two closely related species. Within *Notoseris*, the shape and size of achene body, and the number of ribs on each side can be used as useful characters for species identification.

AUTHOR CONTRIBUTIONS

Z.W. and S.Z. conceived the study, R.C., M.L., Z.L., X.L. and Y.M. conducted the experiments and analyzed the data, Z.W. and R.C. wrote the manuscript, Z.L., Y.M., Y.L., X.X. and S.Z. reviewed and edited the manuscript, Z.W. and S.Z. supervised the students, Y.M., Y.L., X.X. and S.Z. field investigation and material collection, all authors have read and approved the final version.

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