

## STUDIES ON THE SEED-LIKE FRUITS OF SUB-GENUS *SCLAREA* OF *SALVIA* OF LABIATAE IN CHINA

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### Abstract

The macro-morphology and the ultrastructure ornamentation of seed-like fruits of four Sections, Sect. Drymosphace, Sect. Aethiopis, Sect. Plethiosphace and Sect. Notiosphace (comprising 14 species) of the Sub-genus *Sclarea* of *Salvia* of Labiateae in China were systematically studied by using scanning electron microscope. Their characteristics were recorded as plain, stripes, reticulate, convex meshes to reticulate and convex meshes, and both reticulate and convex meshes appeared on dorsal and ventral surfaces of seed-like fruits of one species at the same time. The plain type was an original type; the stripes, reticulate and convex meshes were intermediate types; and both reticulate and convex meshes appeared on seed-like fruits of one species was regarded as an evolved type. They had intraspecific stabilities, but significant interspecific differences in both the macro-morphological features and the ultrastructure ornamentation, which provided evidences for their classification and identification. Fifty six photographs of the seed-like fruits of 14 species of the subgenus collected by scanning electron microscope have been attached.

### Introduction

The sub-genus *Sclarea* (Moench) Benth. belonging to the genus *Salvia* Linn. of Labiateae of China is comprised of 14 species, which were reported in Flora of China (Wu and Li 1977). *S. miltiorrhiza* Bge. of Sub-genus *Sclarea* is a famous medical plant, its radix and rhizome have remarkable efficacy, such as activating blood and removing stasis, stimulating the menstrual flow during amenorrhea and alleviating pain, easing the disturbed state of mind and so on. In clinic, it was used to treat chest stuffiness and pains, epigastric pain, vexation and anxiety and menoxenia and amenorrhea etc. (National Pharmacopoeia Committee 2010).

In recent years, due to the huge clinical demand, seeking for Danshen medicinal material resources has attracted more and more attention. Some scholars studied plant taxonomy and resource science (Zhou 1993, Li 1993, Xiao 2002), medicinal materials (Xiao 2002, Zhou 1995), chemical constituents (Zhou 1993, Xiao 2002), pollens (Cai and Yu 1987, Xu 1990, Yang *et al.* 2003, Li *et al.* 2013) and DNA (Chen *et al.* 2012, Li *et al.* 2013) of *S. bowleyana* Dunn, *S. miltiorrhiza* f. *alba* C.Y. Wu et H.W. Li, *S. shandongensis* J. X. Li et F. Q. Zhou and *S. trijuga* Diels. Meanwhile, in case of scanning electron microscope (SEM) study on the seed-like fruits of Sub-genus *Sclarea*, Wang *et al.* (2007) reported on *S. miltiorrhiza* only, while such study on other species of this sub-genus were not conducted before.

To fill up the lacking of data on morphological characteristics of seed-like fruits of Sub-genus *Sclarea*, 14 species under four Sections (including) of this sub-genus of *Salvia* of Labiateae in China were systematically studied. Sect. Drymosphace included *S. yunnanensis*, *S. miltiorrhiza*, f. *alba*, *S. shandongensis*, *S. bowleyana*, *S. paramiltiorrhiza*, *S. prionitis*, *S. cavaleriei*, *S. honanica*, *S. plectranthoides* and *S. trijuga*; Sect. Aethiopis included *S. grandifolia*, Sect. Plethiosphace

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included *S. deserta*; and Sect. Notiosphace included *S. plebeia*. Additionally, this study was conducted to explore whether the morphological characteristics of seed-like fruits are significant in the systematics of Sub-genus *Sclarea*, to find the similarities, differences and relationships among the species of the sub-genus, and at the same time, to validate the classifications of Sub-genus *Sclarea*. Micromorphological characteristics of epicarp surface is of certain taxonomical significance for classifying the species within a genus, which is significantly taxonomically valuable when used to study species with similar macroscopic external morphology (Zheng *et al.* 2003). Ornamentation on pericarp reveals conspicuous differences, which can be referred as a basis for the classification between species within a genus in Labiateae (Ma *et al.* 2005). Seed morphology is an important aspect of descriptive taxonomy, as well as a key field of plant systematics and evolution studies. The characteristics of seeds can be a taxonomic basis at the family, genus and species levels (Deng *et al.* 2009).

### Materials and Methods

The voucher specimens of 14 species of Sub-genus *Sclarea* were collected from different localities of China and preserved in traditional Chinese medicine resource center laboratory of the resources college of Beijing Normal University (Table 1).

**Table 1. List of voucher specimens.**

Taxon	Specimen	Locality
<i>S. miltiorrhiza</i> Bge.	X. J. Li 201201	Jinan, Shandong
<i>S. bowleyana</i> Dunn	X. J. Li 201204	Yingtan, Jiangxi
	M. H. Li 965	Nanyue, Hunan
<i>S. paramiltiorrhiza</i> H. W. Li et X. L. Huang	E China Exped.4472	Shucheng, Anhui
<i>S. shandongensis</i> J. X. Li et F. Q. Zhou	X. J. Li 201203	Jinan, Shandong
<i>S. prionitis</i> Hance	B. Z. Xiao 4321	Yizhang, Hunan
<i>S. yunnanensis</i> C. H. Wright	Guizhou Exped.7028	Xingyi, Guizhou
<i>S. sinealba</i> (C. Y. Wu et H. W. Li) J. X. Li and X. J. Li	X. J. Li 201202	Jinan, Shandong
<i>S. cavaleriei</i> Levl.	Anshun Exped.61	Anlong, Guizhou
<i>S. honaria</i> L. H. Bailey	J. X. Li 201511	Xinyang, Henan
<i>S. plectranthoides</i> Griff.	Q. Wang 200705	Wenxian, Gansu
<i>S. trijuga</i> Diels	W Sichuan Econ. Pl. Exped.3662	W Sichuan
<i>S. grandifolia</i> W. W. Smith	S. G. Wu 3570	Muli Sichuan
<i>S. deserta</i> Schang.	G. F. Sun X010	Tacheng, Xinjiang
<i>S. plebeia</i> R. Br.	J. X. Li 201511	Jinan, Shandong

The well-developed, fully-matured fruits were collected and then placed at a dust proof site for air drying over 96 hrs. Then, the dried seeds were examined under a dissection microscope. Meanwhile, the average size of the fruits was measured and ten fruits for each species with three repetitions to obtain the data. Subsequently, the fruits were evenly spread on a wood-free printing paper on specimen holder. After spraying gold particles for 30s, the fruits were placed under SUPRATM55 thermal field emission scanning electron microscope (SEM) to observe the dorsum and venter, respectively. Typical and representative fruits were selected and observed first at magnification of 1000 $\times$  and then of 30 $\times$ . When the voltage was stable, focal length was adjusted to collect the pictures. Adobe Photoshop was used for processing the photos and charting.

### Results and Discussion

The results of study on morphology and epidermal micromorphology of seed-like fruits of Sub-genus *Sclarea* are shown in Table 2.

#### Taxonomic key to the Sections of Sub-genus *Sclarea*

- |    |   |               |
|----|---|---------------|
| 1. | Fruits medium, 2.00-2.80 mm long; with reticulate, convex meshes and both them  | Drymosphace   |
| +  | Fruits large, 2.90 - 4.00 mm long; or small and less than 2.00 mm long; without reticulate and convex meshes  | (2)           |
| 2. | Fruits large, 2.90 - 4.00 mm long; not-rough surface with sparse papillae and irregular long elliptical convex  | Aethiopis     |
| +  | Fruits small, less than 2.00 mm long  | (3)           |
| 3. | Fruits oblong, with obvious convave veining of dorso, uneven surface between veining and short hairy appendage; with rough surface vente and obvious convex hilum | Plethiosphace |
| +  | Fruits obround, without obvious veining, with sparse irregular even convex, irregular granular appendages among them  | Notiosphace   |

Four nutlets are one of the significant features of Labiate, and Sub-genus *Sclarea* have these typical nutlets (Xiao 2002), which are also called as ‘seeds’ by some scholars (Wang 2007, Zhang and Wang 2008). As their fruits look like seeds in appearance the term ‘seed-like fruit’, presented by Liu *et al.* (2004), has been used in this study for describing the Sub-genus *Sclarea* fruits. In this study on the macro-morphological and ultrastructural features of fruits of 14 species of Sub-genus *Sclarea*, it was found to be difficult to identify different species of the Sub-genus based only of the macro-morphology of fruits due to its similarity between the species. However, when the features of macro-morphology are combined with micro-morphological features, these can be useful in identification of the species of Sub-genus *Sclarea*. Therefore, the ultrastructures of seed-like fruits can be considered as important characters for taxonomic identification.

Our results showed that the macro-morphological features of Sub-genus *Sclarea* had intra-specific stabilities, but significant interspecific differences that provid evidences for classification and identification of four sections of Sub-genus *Sclarea*.

In Sect. Drymosphace, the ultrastructure of seed-like fruits of species showed that they had reticulate and convex meshes; *S. grandifolia* of Sect. Aethiopis were large with non-reticulate surface; *S. deserta* of Sect. Plethiosphace and *S. plebeia* of Sect. Notiosphace were small and ovoid, with hilum located at the base of ventral top and directed to the venter and non-reticulate surface. These features provided the basis for recognizing these four Sections of Sub-genus *Sclarea*, which is supported by the treatment of Sub-genus *Sclarea* in Flora of China.

There is a distinct feature in the micromorphology of Sect. Drymosphace, the reticulate and convex meshes were appeared on dorsal and ventral surfaces of seed-like fruits in the *S. shandongensis* J. X. Li et F. Q. Zhou. Previously we studied on the plant taxonomy, pollen (Li *et al.* 2013), seed-like fruits (Li *et al.* 2013) and DNA genetic molecules (Li *et al.* 2013) of *S. shandongensis* and its relatives, and it was evident that *S. shandongensis* is a new member of Sect. Drymosphace.

Table 2. The morphology and epidermal micro-morphology of the seed-like fruits of Sub-genus *Scarea*.

Name of the species	Shape	Size (mm)	Hilum	Ventral view of the epidermis	Dorsal view of the epidermis	Plate
<b>Sect. Aethiopis</b> <i>S. grandifolia</i>	Ovoid, base dull and apex blunt	Large 3.70 - 4.00 × 2.90 - 3.00	Rectangle, oblique at the base of the fruit	With irregular papillae, without reticulate, few short hairy appendage of papillae	With irregular papillae, without reticulate, uneven surface and have granular or short hairy appendage	II: 17-20
<b>Sect. Drymosphace</b> <i>Salvia militorrhiza</i>	Oblong, base truncate and apex blunt	Medium 2.42 - 2.64 × 1.20 - 1.50	Similar round, located at the base of the fruit	Reticulate, with similar square lumina, narrow muri and rough retina	Reticulate, with similar square lumina, narrow muri and mesh shape ornamentation of convave retina	I:1-4
<b>Sect. Drymosphace</b> <i>S. howleyana</i>	Fusiform, both ends pointed	Medium 2.37 - 2.52 × 1.20 - 1.32	Long elliptic, oblique at the base of the fruit	Intensive verrucose protrusions, reticulate, with rough surface, similar square lumina, narrow muri and convex retina	Intensive verrucose protrusions, reticulate, with similar square lumina, wide muri and fine particles retina	I:5-8
<b>Sect. Drymosphace</b> <i>S. paramilitorrhiza</i>	Oblong, both ends blunts	Medium 2.23 - 2.59 × 1.21 - 1.30	Similar square, oblique at the base of the fruit	Intermittent flake reticulate, with irregular polygon lumina, wide muri, convave lumina and flat retina	Flat surface, intermittent flake reticulate, with polygon lumina, wide double stripe muri and flat retina, convave	I:9-12
<b>Sect. Drymosphace</b> <i>S. shandongensis</i>	Oblong, both ends blunts	Medium 2.40 - 2.68 × 1.25 - 1.43	Long elliptic, oblique at the base of the fruit	Convex meshes, with rhombic lumina, narrow network ditch, uneven retina and stripe ornamentation	Round convex, convex meshes, with narrow network ditch, uneven retina	I:13-16
<b>Sect. Drymosphace</b> <i>S. prioritis</i>	Long oval, base flat and apex dull	Medium 1.90 - 2.10 × 1.02 - 1.14	Elliptical, located at the base of the fruit	Convex meshes, with square lumina, wide network ditch, less convex retina, unevenness	Reticulate, with similar square lumina, convex muri and convave retina	I:17-20
<b>Sect. Drymosphace</b> <i>S. yunnanensis</i>	Long oval, both ends blunts	Medium 2.41 - 2.53 × 1.20 - 1.32	Elliptical, oblique at the base of the fruit	Convex meshes, with polygon lumina, narrow network ditch, rough retina	With not obvious verrucose protrusions, intermittent flake reticulate, polygon lumina, convave and uneven retina	I:21-24

(Contd.)

Name of the species	Shape	Size (mm)	Hilum	Ventral view of the epidermis	Dorsal view of the epidermis	Plate
<b>Sect. Drymosphae</b> <i>S. sinealba</i>	Ovoid, both ends blunts	Medium 2.33 - 2.50 × 1.23 - 1.36	Round, located at the base of the fruit	Uneven surface, reticulate, with polygon lumina, irregular muri and uneven retina	Sparse cavernous convave, convex meshes, with polygon lumina, narrow network ditch, uneven retina	I:25-28
<b>Sect. Drymosphae</b> <i>S. cavailleriei</i>	Long oval both ends blunts	Medium 2.35 - 2.47 × 1.13 - 1.21	Similar round, oblique at the base of the fruit	Reticulate, with irregular polygon lumina, ridge muri, convave retina and stripe ornamentation	Convex meshes, with irregular polygon lumina, wide network ditch and even retina	II:1-4
<b>Sect. Drymosphae</b> <i>S. homanii</i>	Long oval, both ends rounds	Small 1.80 - 2.00 × 1.13 - 1.21	Elliptical, located at the base of the fruit	Reticulate, with polygon convave lumina uneven	Surface of verrucous protruded, reticulate, with polygon rough lumina, wide muri and convave or convex retina	II:5-8
<b>Sect. Drymosphae</b> <i>S. plectranthoides</i>	Long oval, both ends rounds or blunts	Medium 2.10 - 2.30 × 0.70 - 0.80	Round, located at the base of the fruit	Reticulate, with polygon lumina and even stripe ornamentation retina	Convex meshes, with similar square lumina, wide network ditch and even stripe ornamentation retina	II:9-12
<b>Sect. Drymosphae</b> <i>S. trifuga</i>	Oblong, both ends blunts or rounds	Large 2.90 - 3.14 × 2.04 - 2.76	Round, oblique at the base of the fruit	Without reticulate, with uneven surface, cavernous convave	Without reticulate, with uneven surface	II:13-16
<b>Sect. Notiosphae</b> <i>S. plebeia</i>	Long ovoid, both ends rounds	Small 0.45 - 0.60 × 0.38 - 0.40	Convex round, located at the base of the fruit	Sparse irregular convex, without reticulate, with rough surface, slight convave	Irregular convex, without reticulate, with uneven surface, slight convave and short hairy appendage	II:25-28
<b>Sect. Plethiosphae</b> <i>S. deserta</i>	Ovoid, base tip and apex round	Small 1.12 - 1.20 × 0.70 - 0.76	Convex round, located at the base of the fruit	Without reticulate, rough surface, slight convave	Without reticulate, evident rough convave veining, round shallow cave and intensive short hairy appendage	II:21-24

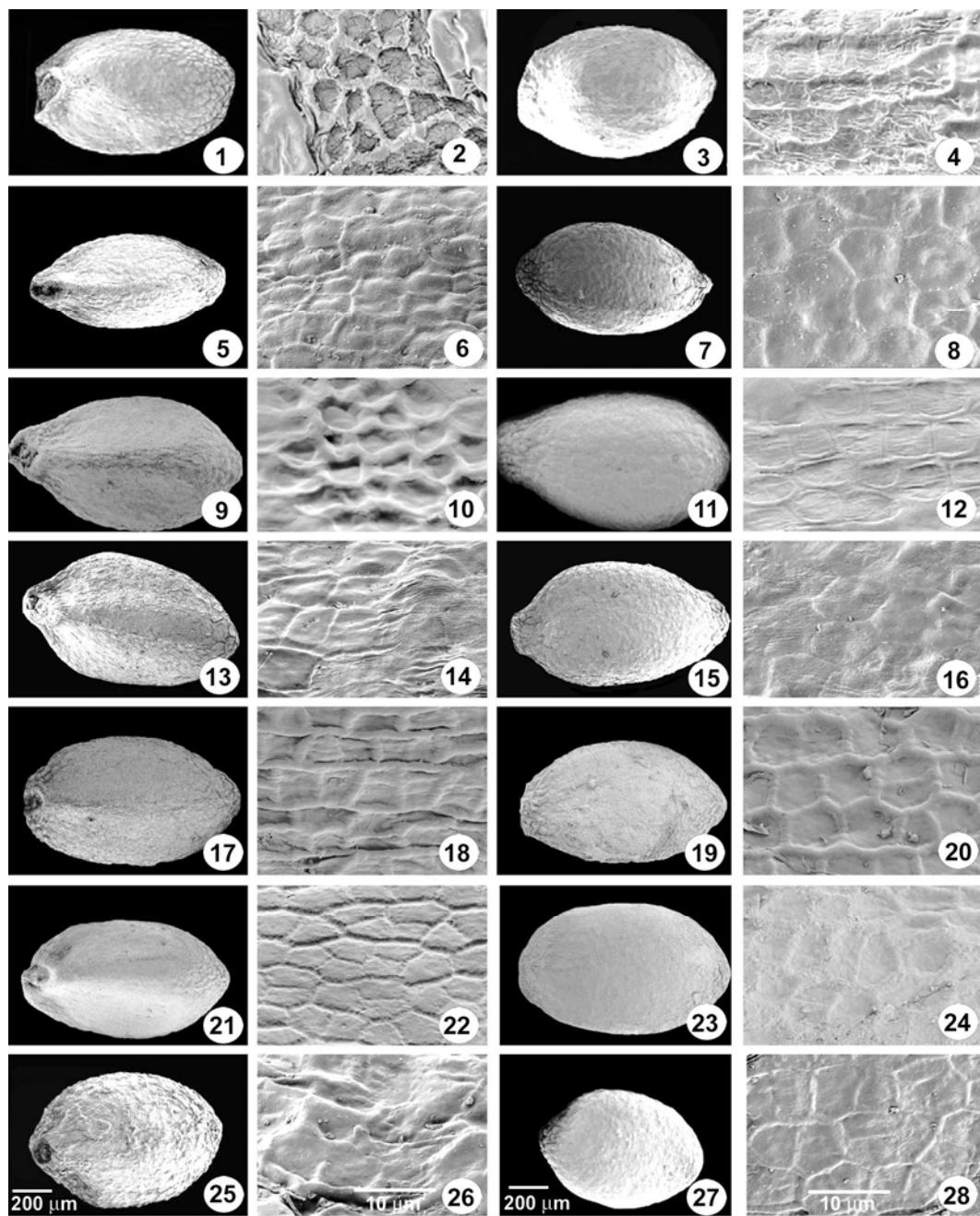


Plate I. SEM images of the seed-like fruits of seven species of Sect. Drymophace (Sub-genus *Sclarea*) 1-4.  
*S. miltiorrhiza*; 5-8. *S. bowleyana*; 9-12. *S. paramiltiorrhiza*; 13-16. *S. shandongensis*; 17-20.  
*S. prionitis*; 21-24. *S. yunnanensis*; 25-28. *S. sinealba*.

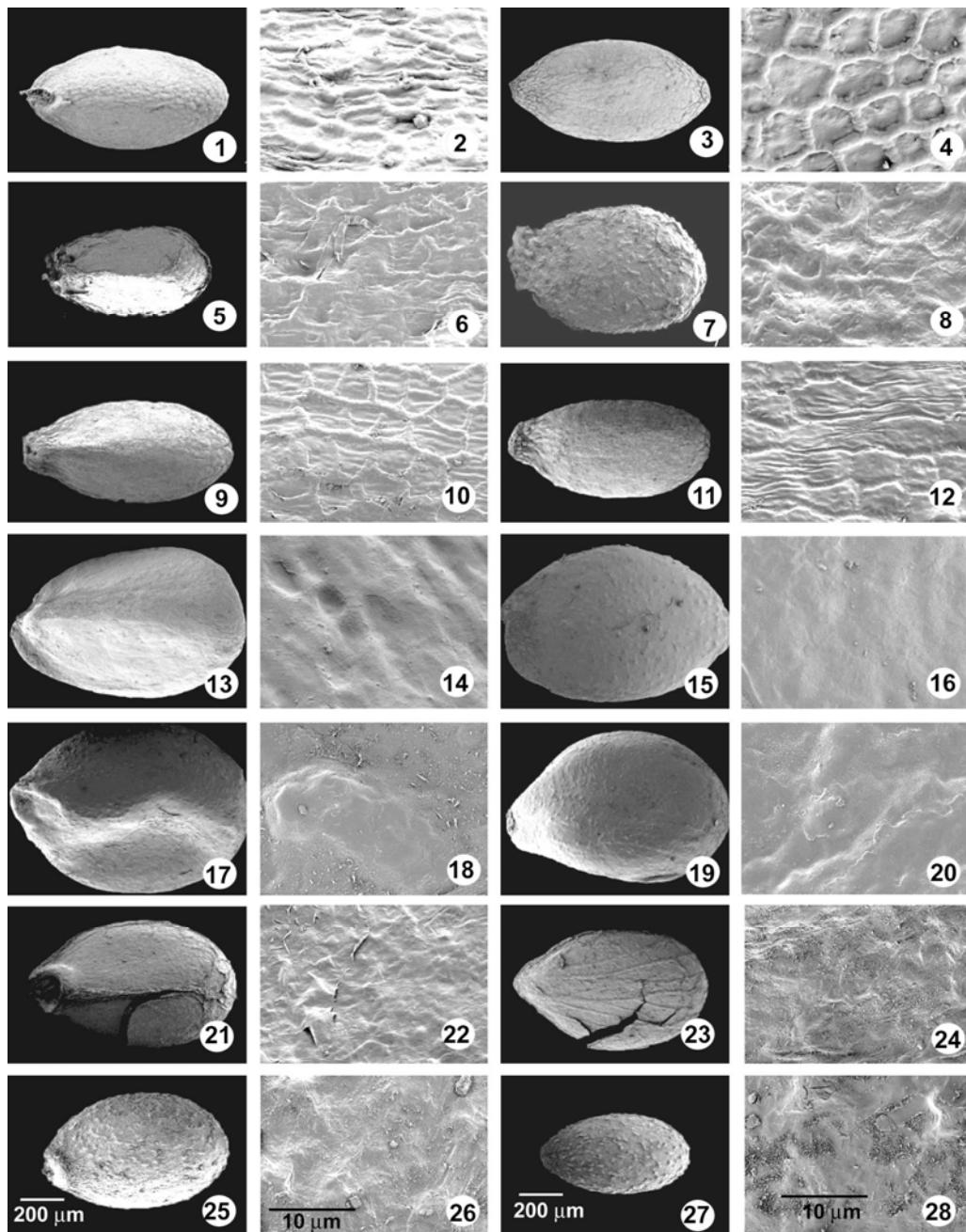


Plate II. SEM images of the seed-like fruits of seven species of Sect. Drymosphace, Sect. Aethiopis, Sect. Plethiosphace and Sect. Notiosphace (Sub-genus *Sclarea*). 1-4. *S. cavaleriei* (Sect. Drymosphace); 5-8. *S. honanica* (Sect. Drymosphace); 9-12. *S. plectranthoides* (Sect. Drymosphace); 13-16. *S. trijuga* (Sect. Drymosphace); 17-20. *S. grandifolia* (Sect. Aethiopis); 21-24. *S. deserta* (Sect. Plethiosphace); 25-28. *S. plebeia* (Sect. Notiosphace).

*S. miltiorrhiza* f. *alba* was a variant of *S. miltiorrhiza* on the basis of traditional plant taxonomy. Our studies showed the significant differences between *S. miltiorrhiza* f. *alba* and *S. miltiorrhiza* (Wu and Li 1977, Li et al. 2013, Zhou 1993, Li et al. 2013). We have reviewed these differences and found that *S. miltiorrhiza* f. *alba* differs from *S. miltiorrhiza* in corolla color, pollen, ultrastructure ornamentation of leaf epidermis, seed-like fruits, and activate complements, especially in *psbA-trnH* intergenic region barcoding identification of both DNA genetic molecules, with 22 variation sites between them. It differs from its close relatives (*S. miltiorrhiza*) by its above characteristics. This indicates that *S. miltiorrhiza* f. *alba* should not be categorized as an intraspecific variant, rather it should be recognized as a species. Therefore, we have recombined *S. miltiorrhiza* f. *alba* as *Salvia sinealba* (C. Y. Wu et H. W. Li) J. X. Li and X. J. Li, stat. nov. under the Sect. Drymophace.

It is concluded that there are intraspecific stabilities but significant interspecific differences in both the macro-morphological features and the ultrastructure ornamentations of the seed-like fruits of 14 species of Sub-genus *Sclarea*, which provide evidences for the sectional divisions and classification of this sub-genus, consistent with its traditional taxonomy, and the identification of its species.

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