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ORIGINAL ARTICLE

Taxonomic Implications of Fruit Morphology in Chinese Species of the Genus *Bupleurum* L. (Apiaceae)

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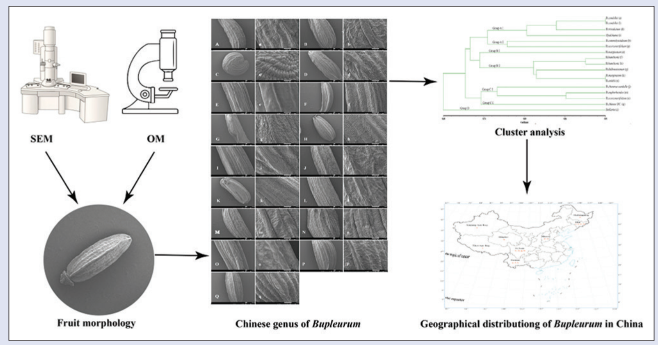
ABSTRACT

Background: Because of the certain overlapping of the genus *Bupleurum*, the identification features are few and the delimitation among species is difficult, *Bupleurum* taxonomy is under frequent revisions. In this research, hierarchical cluster analysis based on morphology characters is done to establish a dendrogram and illustrate the taxonomic relationship among *Bupleurum* species (Apiaceae) from China. **Materials and Methods:** The fruit morphology of 17 *Bupleurum* taxa from China was investigated with a scanning electron microscope (SEM) and optical microscopy (OM). The hierarchical cluster analysis based on 41 morphological character states belonging to 9 characters was done to establish a dendrogram and illustrate the taxonomic relationship among the studied species of *Bupleurum* (Apiaceae) from China. **Results:** Based on fruit morphological characteristics, it is possible to separate the *Bupleurum* species into the following four groups. The research indicates that the classification of *Bupleurum* in China is closely related to geographical distribution, which is divided into Southwestern and Northeastern *Bupleurum*. **Conclusion:** The fruit morphological characteristics are variable enough to distinguish species between *Bupleurum,* such as fruit length, width, shape, colour, the curvature of the outer periclinal and so on in this article. Morphological characters could be probably the taxonomical standard of intraspecific and interspecific delamination, which is significant for understanding the classification of *Bupleurum*.

Key words: *Bupleurum*, cluster analysis, morphology, SEM and OM, taxonomic

SUMMARY

- Morpology of fruit can be used to recognize *Bupleurum* species, primary features like fruit dimension, colour, shape, coefficients of variation even testa structure. The cluster analysis of 41 character states showed the intraspecific and interspecific classification. An identification key of *Bupleurum* species was put forward on cluster analysis. *Bupleurum* species in China is divided into two groups, whose morphological variation may be closely related to their biogeography. From the view of distribution, the first group belongs to southwestern regions of China while the second group is located in northeastern regions of China. From the perspective of biogeography, the Hengduan Mountains are precisely the boundary of the two regions and retained high diversity about *Bupleurum*. Therefore, the origin of *Bupleurum* in China may be associated to Hengduan Mountains.



Abbreviations used: Cv: coefficients of variation; SD: standard deviation, MN: mean values; L/W: length/width.

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INTRODUCTION

Bupleurum L. is one of the largest genera in the Apiaceae family, with approximately 200 species all over the world. They are located mostly in temperate zones of the Northern Hemisphere, Europe, North Africa, North America and Asia.[1] In China, there are 42 species of *Bupleurum* (including 22 endemic species) as typical representatives.[2] It grows in various habitats: from sea level up to 4900 m, from cultivated land margin to rocky slopes, from dense forests to open areas.[3,4] This genus is usually an annual or perennial plants, ranging from small herbs to rare shrubs. *Bupleurum* is also known as traditional Chinese medicine "chai hu", whose roots are extremely rich in medicinal effect and are used in the treatment of coughs, fevers and influenza.[5-7]

Extensive researches on *Bupleurum* are under constant development. Scientists usually studies *Bupleurum* growing in the fields or as herbarium specimens,[8-10] however, the most striking contradictions are the insufficient samples and limitations of endemic species. Furthermore, anatomical characteristics were adopted to evaluate *Bupleurum*, particularly its stem and fruit,[11-13] but the morphological diversity in *Bupleurum* is scarce. Afterwards, several chemical components were

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extracted and metabolites were separated, which have been used in taxonomic research,[14-18] however, the chemical composition changes with the environment. Lastly, interdisciplinary systematic studies were done on the genus *Bupleurum*,[19] such as pollen,[20,21] cytology,[22] biogeography,[23] and molecular research.[24,25] In the past 20 years, a few taxonomic types of research have aimed at *Bupleurum*, especially some was devoted to micromorphological taxonomic studies, but there is no unified answer so far.

The intraspecific level in *Bupleurum* is notoriously hard to evaluate, but morphological features are useful for distinguishing species. It is reported that the classification of *Bupleurm* depends severely on characteristics of fruit morphology, anatomy and testa structure.[13,26] Researchers discussed relationships among *Bupleurum* given 55 fruit features of 17 species,[27] soon after others,[28] conducted similar research about *Bupleurum* utilizing 38 seed features. Vaughan,[29] (1968) also indicated that epidermal construction may sustain taxonomy at the family or genus levels, which was subsequently verified by other scholars.[30-35] In addition, embryos of *Vicia* species[36] could reveal the morphological characters and their taxonomic significance. Thus characteristics description seems particularly significant. The early terms were critically summarized,[37] which is the most comprehensive classification of the family up to now. A refined classification and terminology of seed surface characters were proposed.[38] The terminology and research methods of fruit morphology in common use were collected[39] and an attempt was made to propose standardized terms for considerably morphological features in the Apiaceae.[40]

Because of the certain overlapping of the genus *Bupleurum*, the identification features are few and the delimitation among species is difficult, which is easy to cause source confusion. In this research, hierarchical cluster analysis based on morphology characters is done to establish a dendrogram and illustrate the taxonomic relationship among *Bupleurum* species (Apiaceae) from China. This work reveals the significance of morphology to identify and evaluate intraspecies and interspecies classification to provide a set of useful taxonomic basis.

MATERIALS AND METHODS

Fruit source

Fruits of *Bupleurum* species were provided from the Germplasm Bank of Wild Species, Southwest China and the Chinese herbal medicine planting cooperative. The information of origins of samples are shown in Table 1.

Morphological examination

A light stereo microscope (SAGA) was used to examine fruit morphology. Ten randomly chosen fruits were examined and recorded for each sample. A JME-7500F, cold field emission scanning electron microscope (SEM) was used to investigate epidermal characteristics. Three fruits per sample were examined by a double-sided tape mounted on a metal stub. The dried fruits were coated with a gold sputter coater using Auto Fine Coater JEC-3000FC.

Physical characterization

We observed the following fruit characters: (1) fruit shape; (2) fruit dimension: fruit length (the axial length between the fruit end of the fruit umbilical and the opposite end), fruit width (the maximum linear distance of the fruit perpendicular to the length axis), length/width (L/W) index; (3) colour; (4) fruit testa morphology: the curvature of the outer periclinal wall, epidermal secretion, the surface of the cuticle in Table 2. Based on previous research,[41-43] the describing terminology has been modified.

Statistical analysis

According to morphological observations, the extracted characters of *Bupleurum* were coded as 0 and 1 for numerical analysis: 41 characters were recorded for each taxon in Table 3. Phenograms illustrating the relationship between the studied taxa were constructed by the average taxonomic distance by using NTsys 2.1. Using those qualitative characters, the cluster analysis depending on the fruit morphology of 17 specimens was made in Figure 1. Given this morphological characteristic, we prepare an identification key. Draw a geographic map of our *Bupleurum* distribution according to the classification results.

RESULTS

A summary of various morphological characteristics of 17 species of *Bupleurum* is given in Table 2. Variation in 30 surface character states manifested mainly includes the following aspects: size, shape, colour, fruit testa morphology. Seeds range from 1.60 ~ 2.21 × 0.35 ~ 0.55 mm in size (*B. marginatum*) to 4.88 ~ 6.97 × 1.42 ~ 1.91 mm in size (*B. candollei*). The colour varies from light brown and tan brown to grey–brown. Fruit shape showed six types: oval, ovate, nearly round, oblong, long oval, short cylindrical. SEM observation revealed that fruit coat micromorphology can be divided into five

Table 1: Source of *Bupleurum* fruit used in this study

Latin name	Serial number	Acquisition number	Collection place
B. candollei Wallich ex Candolle (a)	868710000363	SCSB-A-000075	Yunnan Province, China
B. commelynoideum var. *flaviflorum* R. H. Shan and Yin Li (b)	868710086664	SCU-20080522	Sichuan Province, China
Stellaria dichotoma var. lanceolata Bunge (c)	868710109581	Yanglm0138	Jilin Province, China
B. triradiatum Adams ex Hoffmann (d)	868710068715	ZhangCB0208	Sichuan Province, China
B. marginatum Wallich ex Candolle (e)	868710035895	SunH-07ZX-2030	Jilin Province, China
B. hamiltonii N. P. Balakrishnan (f)	868710049686	YNS0383	Yunnan Province, China
B. dalhousieanum (C. B. Clarke) Koso-Poljansky (g)	868710085371	08cs827	Sichuan Province, China
B. hamiltonii N. P. Balakrishnan (h)	868710124635	MY450	Sichuan Province, China
Hyalolaena bupleuroides Pimenov and Kljuykov (i)	868710145830	Liujq-fjj-0108	Xinjiang Uygur Autonomous Region, China
B. chinense Candolle (j)	868710063777	Yanglm0050	Jilin Province, China
B. marginatum Wallich ex Candolle (k)	868710011037	ygp-033	Yunnan Province, China
B. candollei Wallich ex Candolle (l)	868710119736	09CS1601	Tibet Autonomous Region, China
B. euphorbioides Nakai (m)	868710167115	ZhouHC008	Jilin Province, China
B. smithii var. *parvifolium* R. H. Shan and Yin Li (n)	868710182787	Chensl1776	Qinghai Province, China
B. scorzonerifolium Willd (o)	201807150001	Heilongijang daqing	Heilongjiang Province, China
B. scorzonerifolium Willd (p)	201807150003	Shanxi Province	Shanxi Province, China
B. chinense DC. (q)	201807150004	Shanxi Province	Shanxi Province, China

Table 2: Fruit and testa micromorphological characteristics of *Bupleurum* species

Latin name	Fruit length (mm)	Cv	Fruit width (mm)	Cv	L/W	Cv	Fruit shape	Colour	Mericarp surface	Ornamentation type	Epidermal secretion	Ridge
B. candollei (a)	(3.48) 3.93 (4.54)	8%	(1.30) 1.61 (1.85)	10%	(1.88) 2.47 (2.96)	13%	Long oval	Brown	Concave or convex	Rugose	Granules	Prominent
B. commelynoideum (b)	(3.61) 3.91 (4.28)	10%	(1.2) 1.55 (1.70)	8%	(2.29) 2.53 (2.96)	7%	Oval	Red brown	Immersed	Shallow rugose	Granules	Inconspicuous
Stellaria dichotoma (c)	(2.24) 2.47 (2.90)	7%	(1.79) 2.09 (2.37)	7%	(1.07) 1.19 (1.38)	8%	Nearly round	Gray black	Protuberant	Not obvious	Ovoido –rectangular elevations	Not obvious
B. triradiatum (d)	(3.62) 3.97 (4.66)	7%	(1.42) 1.55 (1.82)	7%	(2.05) 2.57 (3.11)	10%	Oval	Red brown	Concave or convex	Reticulate	Mass squama	Inconspicuous
B. marginatum (e)	(1.60) 1.93 (2.21)	10%	(0.35) 0.47 (0.55)	14%	(2.92) 4.24 (5.92)	23%	Long oval	Light brown	Smooth	Undulate	Not obvious	Prominent
B. hamiltonii (f)	(3.04) 3.45 (3.70)	7%	(0.99) 1.22 (1.47)	15%	(2.77) 2.89 (3.74)	17%	Oval	Brown	Protuberant	Verrucate	Colliculate	Prominent
B. dalhousieanum (g)	(3.25) 3.71 (4.32)	8%	(0.96) 1.37 (1.54)	16%	(2.15) 2.78 (3.05)	21%	Long oval	Brown	Protuberant	Verrucate	Mass squama and colliculate	Inconspicuous
B. hamiltonii (h)	(2.58) 2.78 (2.99)	6%	(0.98) 1.19 (1.32)	11%	(1.93) 2.37 (2.65)	15%	Short oval	Brown	Protuberant	Verrucate	Colliculate	Prominent
Hyalolaena bupleuroides (i)	(5.06) 5.59 (6.79)	11%	(1.59) 1.75 (1.92)	7%	(2.54) 3.21 (3.79)	13%	Long oval	Light brown	Immersed	Shallow rugose	Mass squamaand granules	Inconspicuous
B. chinense Candolle (j)	(4.21) 5.08 (5.88)	10%	(1.77) 1.96 (2.60)	12%	(1.79) 2.63 (3.35)	16%	Long oval	Light brown	Immersed	Rugose	Platelets and granules	Prominent
B. marginatum (k)	(2.04) 2.86 (3.09)	5%	(1.01) 1.21 (1.45)	13%	(1.82) 2.39 (2.70)	12%	Oval	Brown	Protuberant	Shallow rugose	Mass squama	Inconspicuous
B. candollei (l)	(4.88) 5.91 (6.97)	10%	(1.42) 1.70 (1.91)	8%	(3.18) 3.49 (4.32)	14%	Long oval	Dark brown	Immersed	Rugose	Granules	Prominent
B. euphorbioides (m)	(4.10) 4.90 (5.75)	10%	(1.11) 1.47 (1.75)	10%	(2.49) 3.34 (3.70)	10%	Long oval	Dark brown	Concave or convex	Rugose	Colliculate	Prominent
B. smithii (n)	(4.32) 4.56 (5.31)	6%	(1.37) 1.99 (2.46)	19%	(1.83) 2.38 (3.38)	20%	Oval	Brown	Concave or convex	Reticulate	Mass squama and granules	Inconspicuous
B. scorzonerifolium willd. (o)	(3.71) 4.26 (4.82)	7%	(1.37) 1.57 (1.96)	12%	(2.30) 2.75 (3.15)	10%	Short oval	Dark brown	Immersed	Undulate	Platelets	Prominent
B. scorzonerifolium willd. (p)	(2.90) 3.25 (3.57)	6%	(1.29) 1.50 (1.75)	10%	(1.82) 2.19 (2.63)	12%	Oval	Dark brown	Concave or convex	Rugose	Granules	Prominent
B. chinense DC. (q)	(3.06) 3.68 (4.58)	14%	(1.34) 1.68 (1.94)	10%	(1.73) 2.20 (2.75)	16%	Oval	Light brown	Immersed	Rugose	Granules	Prominent

Table 3: Codes 0 and 1 are used for data analysis of research on *Bupleurum* species

Fruit coat morphology	Character	0 and 1 codes																
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q
Fruit length	<2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	2~3	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
	3~4	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1
	4~5	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
	>5	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0
Fruit width	<1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	1~1.5	0	1	0	0	0	1	1	1	0	0	1	0	1	0	0	1	0
	1.5~2	1	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	1
	>2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L/W index	1~2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2~3	1	1	0	1	0	1	1	1	0	1	1	0	0	1	1	1	1
	3~4	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
	>4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Fruit shape	Oval	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	1	1
	Long oval	1	0	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0
	Short oval	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
	Nearly round	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit colour	Brown	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0
	Red brown	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Gray black	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Light brown	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1
	Dark brown	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0
Curvature of outer periclinal	Concave or convex	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0
	Immersed	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1
	Protuberant	0	0	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0
	Smooth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Surface of the cuticle	Reticulate	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
	Rugose	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1
	Verrucose	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
	Not obvious	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Undulate	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
	Shallow rugose	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Epidermal secretion	Granules	1	1	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
	Mass squama	0	0	0	1	0	0	1	0	1	0	1	0	0	1	0	0	0
	Platelets	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
	Ovoido-retangular	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Colliculate	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0
	Not obvious	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ridge	Prominent	1	0	0	0	1	1	0	1	0	1	0	1	1	0	1	1	1
	Inconspicuous	0	1	0	1	0	0	1	0	1	0	1	0	0	1	0	0	0
	Not obvious	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

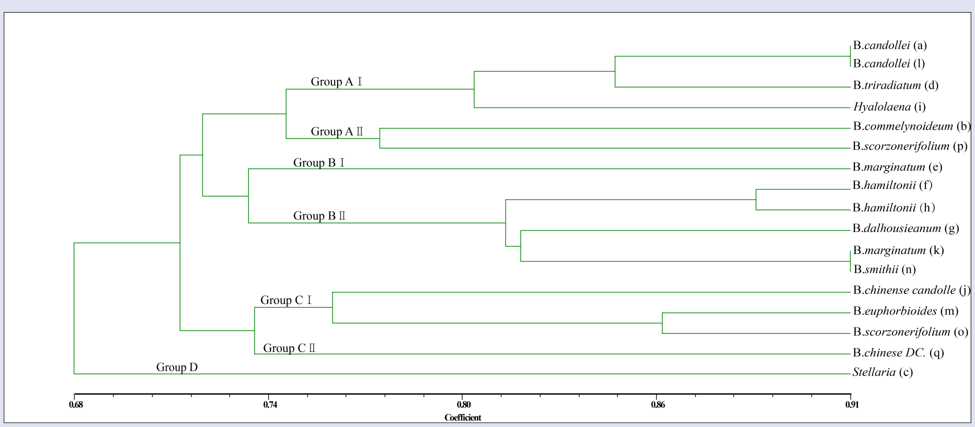

Figure 1: UPGMA phenogram of *Bupleurum* genera clustered given morphological characteristics

types: concave or convex type, convex, concave, flat, and papillate. Given the decoration of epidermal cells, morphological types can be distinguished as reticulate, rugose, rugulose, colliculate, verrucate, and not obvious. The fruit morphological characteristics are variable

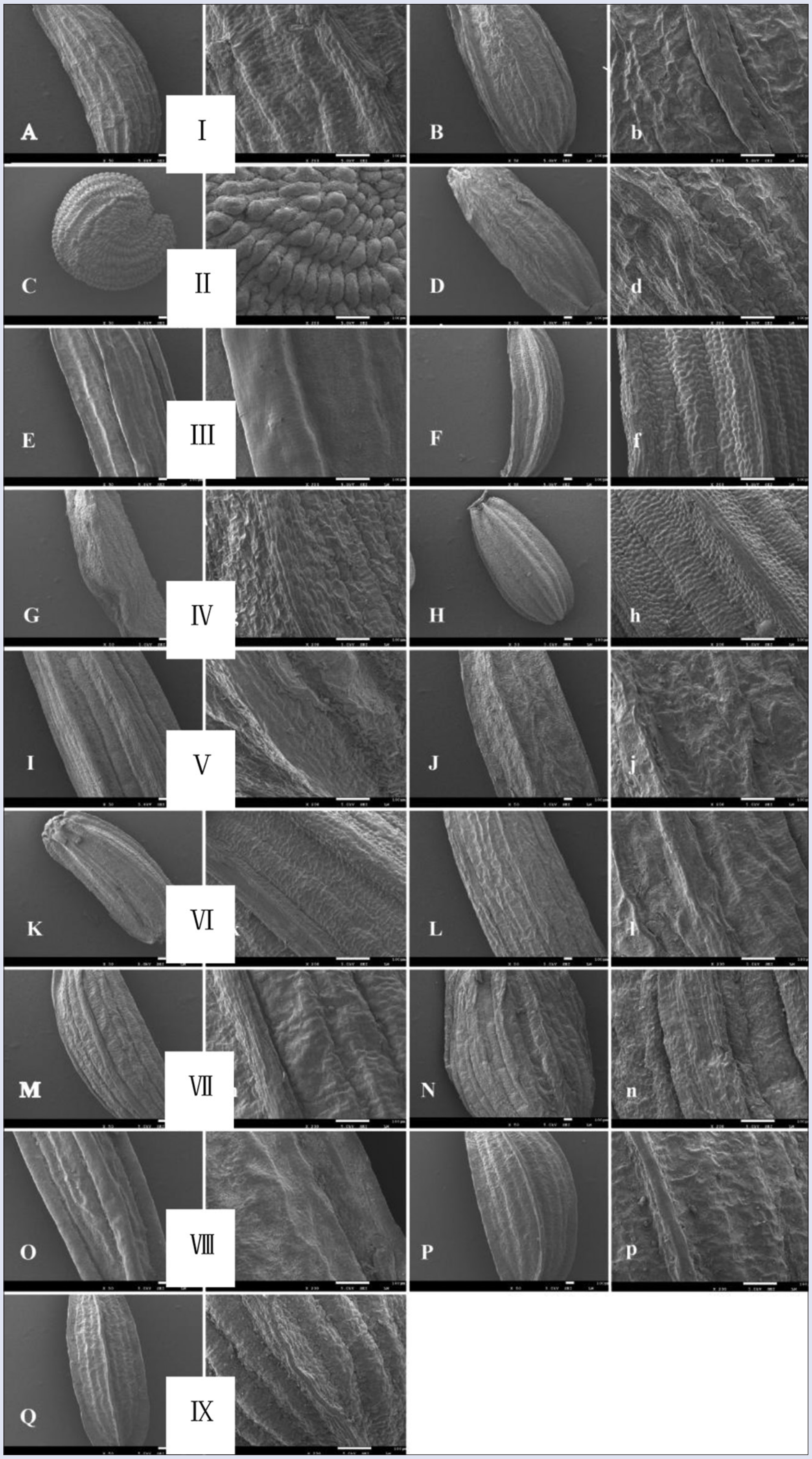

Figure 2: Surface sculptures of the fruits of *Bupleurum* by SEM

enough to distinguish species, which is significant for understanding the classification of *Bupleurum*. Based on fruit morphological characters, it is possible to separate the studied species into the following groups:

Group AI: The conspicuously raised structures are arranged parallel longitudinally, elevations with dense folding, obvious in ribs. The pattern of the rugose type, with dense obvious folding of irregular wrinkles and a few conspicuous secretions, such as granules and mass squama, evenly cover the entire surface. Taxa of this type of fruits are: *B. candollei* [Figure 2a 2l], *B. triradiatum* [Figure 2d] and *Hyalolaena bupleuroides* [Figure 2i].

Group AII: These species are comparatively large, 5.06 ~ 6.79 × 1.59 ~ 1.92 mm, long oval, light brown. L/W is 2.54 ~ 3.79, coefficients of variation (Cv) is 13%. The pattern of the shallow is rugose type. The elevated epidermal cells are arranged in slight loose longitudinal ripple, with some mass squama and granules. Rugose-like sculpturing shows slightly raised foldings wrinkles arrangement, light ribs. Taxa with this type of fruit are: *B. commelynoideum* [Figure 2b] and *B. scorzonerifolium* will [Figure 2p].

Group BI: Fruits are 1.60 ~ 2.21 × 0.35 ~ 0.55 mm, long oval in shape, brown in colour, L/W is 2.92 ~ 5.92. Found only in *B. marginatum*, this type of fruit coat is characterized by an almost smooth epidermal surface without epicuticular secretions. The pericarp surface is prominent ribs parallel longitudinal arrangement on the surface. Among the above species, only *B. marginatum* [Figure 2e], has this type of fruit.

Group BII: The surface is protuberant with dense epicuticular secretions between ridges, which are evenly distributed across the entire fruit coat. The conspicuously large foldings are arranged longitudinal, ribs thick and prominent. Occasionally some loose large foldings and calculates in fruit coat. Sometimes the inconspicuously scale-like epidermal secretions, the loose covered platelet-like thin secretions, but small particles are found mostly on the fold depression. Taxa with this type of fruit are *B. hamiltonii* [Figure 2f and 2h], *B. dalhousieanum* [Figure 2g], *B. smithii* [Figure 2n] and *B. marginatum* [Figure 2k].

Group CI: Testa is densely colliculate. The pericarp surface is concave or convex with irregular wrinkles folding. And cuticular granules and mass squama are seen on the pits of the epidermal surface. The epicuticular secretions are located densely in the depressions. The protrusions are more complex and crest-like. Taxa with this type of fruit are *B. euphorbioides* [Figure 2m], *B. scorzonerifolium* willd [Figure 2o] and *B. chinense* Candolle [Figure 2j].

Group CII: Fruits are 3.06 ~ 4.58 × 1.34 ~ 1.94 mm, and oval, dark brown in colour. L/W is 1.73 ~ 2.75, Cv is 16%. The parallel longitudinal striations show slightly raised with foldings wrinkles, light in ribs. The hillock and large particles cover the entire surface. Most large granules come from the base of ridges. Testa cells appear concave or convex. Taxa with this type of seed is: *B. chinense* DC [Figure 2q].

Group D: Fruits are 2.24 ~ 2.90 × 1.79 ~ 2.37 mm, nearly round, L/W is 1.19, Cv is 8%. Seen only in *Stellaria dichotoma*, the surface is not smooth, gray black or tan in colour. This type of fruitcoat is characterized by many ovoido–rectangular elevations uniformly covering the entire surface, one end of the seed hole has a curved cone-shaped structure. When the surface of fruit is partially enlarged by 50 ~200 times, ovoido–rectangular elevations tightly are arranged in a circle to point to the center. Among the above species, only *Stellaria dichotoma* [Figure 2c], has this type of fruit.

The uppercase English letters indicate surface sculptures of the fruits of Bupleurum at (50×) magnifications by SEM, Scale bar = 100 μm; The lowercase English letters indicate surface sculptures of the fruits of *Bupleurum* at (200×) magnifications by SEM, Scale bar = 100 μm; Aa *B. candollei*, Bb *B. commelynoideum*, Cc *Stellaria dichotoma*, Dd *B. triradiatum*, Ee *B. marginatum*, Ff *B. hamiltonii*, Gg *B*. dalhousieanum, Hh *B. hamiltonii*, Ii *Hyalolaena bupleuroides*, Jj *B. chinense* Candolle, Kk *B. marginatum*, Ll *B. candollei*, Mm *B. euphorbioides* Nakai, Nn *B. smithii*, Oo *B. scorzonerifolium* willd, Pp *B. scorzonerifolium* willd, Qq *B. chinese*. DC.

Mean values (with minimum and maximum values in brackets); Cv = SD/MN * 100%, (Cv: coefficients of variation, SD: standard deviation, MN: mean values, L/W: length/width) 0 and 1 codes used for data analysis of research on *Bupleurum* species: a *B*. candollei, b *B. commelynoideum*, c *Stellaria dichotoma*, d *B.triradiatum*, e *B. marginatum*, f *B. hamiltonii*, g *B. dalhousieanum*, h *B. hamiltonii*, i *Hyalolaena bupleuroides*, j *B. chinense Candolle*, k *B. marginatum*, l *B.candollei*, m *B. euphorbioides Nakai*, n *B. smithii*, o *B. scorzonerifolium* willd, *P B. scorzonerifolium willd*, q *B. chinense DC.*

DISCUSSION

Morphological study

Observations on SEM and OM showed considerable variation of 17 *Bupleurum* L. from China in fruit micromorphology [Table 2]. Species within *Bupleurum* are notoriously difficult to identify, but morphological characters have been useful in delimitating species in traditional taxonomic studies. It is reported that classification of *Bupleurm* relies heavily on characters of fruit morphology, anatomy and surface structure.[13,26] Therefore, morphologyical characters of the fruit provided a set of useful taxonomic basis.

Each species has a characteristic that cannot be confused with other taxa. From the size point of view, *Bupleurum* L. fruits range from 2 ~ 3 × 1 ~ 1.5 mm (length × width) in size. However, larger fruit such as Hyalolaena bupleuroides and *B. candollei* Wallich ex Candolle, the general is 4.88 ~ 6.97 × 1.42 ~ 1.91 mm in size. Smaller fruit such as *Stellaria dichotoma* var. lanceolata Bunge and *B. marginatum* Wallich ex Candolle, the general length is 1.60 ~ 2.21 × 0.35 ~ 0.55 mm in size. In fact, the bigger sizes could probably reflect a greater vigor than the smaller ones. It is reported positive correlations between sizes of the fruit and the vigor of the sporophytes.[20]

From the shape point of view, there are oval, ovate, nearly round, oblong, long oval, short cylindrical in shape. Oblong: this fruit type of L/W ratio is generally 1.5 ~ 2, such as *B. marginatum* Wallich ex Candolle. Oval: this L/W ratio is close to 2 ~ 3, such as *B. smithii* var. parvifolium R. H. Shan and Yin Li. The fruit shape of the genus *Bupleurum* is elliptical to ovate, except in *Stellaria dichotoma* var. lanceolata Bunge and Hyalolaena bupleuroides with respectively nearly round and long oval. The colour varies from light brown and tan brown to gray brown. The fruit color of *Bupleurum* is related to the pigment component of the skin cells.[10]

SEM observation revealed that fruitcoat micromorphology can be divided into five types: concave or convex type, convex, concave, flat and papillate. Based on the structure and ornamentation of the epidermal cells, morphological types can be distinguished: reticulate, rugose, rugulose, colliculate, verrucate, not obvious. The relief of outer cell walls (the secondary sculpture) is usually caused by shrinkage and cracking of dry cells.[13] The tertiary sculpture superimposed on the secondary sculpture is formed by epicuticular secretions such as waxy and viscous lipophilic substances, the wax always hides the epidermis, so the secondary sculpture and the tertiary sculpture superimposed on the secondary sculpture are mutually exclusive.[43] Only *B. marginatum* Wallich ex Candolle belongs to not obvious type, the surface decoration is not obvious when it is 200 times. Moreover, according to fruit morphological characters used, a key to identification have been observed among the species.

Fruit morphology can be used as taxonomic basis for plant families, genera and species, the latest Europeanchecklist online,[44] and the most recent Crimean checklist,[45] also accepted this occurrence. Considerably different types of sculpturing at species and similarities between related taxa were observed. In the angiosperm order Apiales, traditional classification has relied heavily on fruit characters.[46] 20 *Bupleurum* taxa were investigated for micromorphological typology of the fruit surfaces with SEM.[43] In the classification system of Chinese *Bupleurum*, Su,[27] was first proposed that 14 species of Chinese *Bupleurum* were divided two subgenus. The eight species of the genus *Bupleurum* in northeastern China were studied for the first time, which were divided into two large groups based on fruit anatomy.[12] The six morphobiometric characters of pollen grains of *Bupleurum* have been analysed and formulate a key of identification.[26] However, Vaughan,[29] pointed out that testa structure may support taxonomic distinctiveness at the family and genus levels, which was later confirmed by others. Özcan,[13] examined seven of the endemic *Bupleurum* species in Turkey for petal and fruit surface features using the SEM. Embryos of Vicia L species could reveal the micromorphological character and taxonomic significance,[36] Identification of species of *Bupleurum* is difficult due to subtle differences in morphology and conflicting opinions about the taxonomic rank of most taxa. Nevertheless, there are still many gaps in our knowledge of this genus, especially of those species endemic to China that have often been excluded from previous studies.

Interspecific classification

Bupleurum revealed high diversity in fruit coat and fruit morphology. Due to the wide distribution of *Bupleurum*, and because climatic conditions and geographical locations, this results in the quality in difference. The dendrogram of *Bupleurum* fruit morphological characters [Figure 1] clustered the studied taxa into four distinct groups A, B, C and D. The group D includes only *Stellaria dichotoma* (an out-group), a species from family Caryophyllaceae which according to our knowledge was never treated taxonomically as a *Bupleurum* species, however, according to the Encyclopedia of Chinese Materia Medica, *S. dichotoma* was used as a substitute for *B. scorzonerifolium* and *B. chinense* because of its similar morphology and name. Later *S. dichotoma* was used as a new herb because of its different medical effect from *B.chinense*. However, in our dendogram, the unique round shape and grey black colour of *Stellaria* fruit separate it under group D away from all *Bupleurum* species that have short to long oval fruit with light to dark brown colour. Also fruit coat of *S. dichotoma* is characterized by many ovoido–rectangular elevations uniformly covering the entire surface while in the fruit coat of *Bupleurum*, the conspicuously raised structures are arranged parallel longitudinally, elevations with dense folding, obvious in ribs as shown in Figure 2, this indicates that the uniform fruit shape of *Bupleurum* species could distinguish them from other morphologically similar species belonging to other families.

Both *Hyalolaena bupleuroides* (an out-group) and *Bupleurum* species are in the Apiaceae family, they also belong to the same subfamily Apioideae but belong to different genera: *Hyalolaena* genus and *Bupleurum* genus. However, according to the fruit morphology, *Hyalolaena bupleuroides* was nested within *Bupleurum* species, indicating the similarity of *Bupleurum* fruit morphology with other members of the same subfamily. From the other three groups, the two major close groups were A and B with about 72% of similarity. Most species belonging to these two groups are located in the southwest part of China while all species from the other group C are located in the northeast part of China, which indicates the effect of the geographical distribution on the fruit morphological characteristics.

Considering the dendrogram of group A, it is separated into two major subgroups (AI and AII) with 74% similarity. Group AI belongs to reticulate type, with dense obvious folding of irregular wrinkles, evenly covering the entire surface. The *B. candollei* and *B. triradiatum* show a higher similarity (85%), this may be because their geographical locations are probably close (Tibet Autonomous Region, Yunnan and Si Chuan). However, the two accessions of the same species *B.candollei* showed some variation in fruit morphology from brown colour with concave or convex mericarp surface to dark brown colour with immersed mericarp surfaces, because of the difference in climatic conditions and geographical locations between the origin of the two accessions (Tibet Autonomous Region and Yunnan). Group AII is ribbed-rugose type. The inconspicuously scale-like epidermal secretion are arranged longitudinally and the ridges with loose epicutic secretions. This group also displays such unusual characters, which make it isolated taxonomically. Group AII is formed by two subgroups with 76% similarity, and most of them come from northeast china. The variation in fruit coat morphology between the two accessions of the same species *B.scorzonerifolium* was expected due to the difference in climatic conditions and geographical locations between the origin of the two accessions (Heilongjiang and Shanxi). However, the variation exceeded our expectations that they have only 72% similarity and were separated under two different groups A and C.

Group B is separated into two major subgroups (BI and BII) with about 73% of similarity. Group BI is characterized by fruit coat with a smooth epidermal surface without epicuticular secretions while group BII is characterized by fruit coat with the protuberant surface with dense epicuticular secretions between ridges. With about 89% similarity, little variation in fruit shape between the two accessions of the same species *B. hamiltonii* was observed from oval to short oval shape due to the geographical distance between their origin (Sichuanand Yunnan). Another wide variation in fruit coat morphology between the two accessions of the same species *B. marginatum* was observed due to the big difference in climatic conditions and geographical locations between the origin of the two accessions (Jilin from the Northeast of China and Yunnan from the southwest of China). However, the variation also exceeded our expectations that they have only 73% similarity that they were separated under the different subgroups of group B, at the same time, the accession from Yunnan show 91% similarity with other *Bupleurum* species *B. smithii* from the same geographical location (Qinghai from the southwest part of China). This indicates that the climatic conditions and geographical locations not only affect the genus at the interspecific level but also at the intraspecific level.

Taxonomic and biogeography

According to the above discussion, we pointed out that *Bupleurum* in China was divided into two groups, whose morphological variation may be closely related to their biogeography as shown in Figure 3. From the view of distribution, the first group consists of ten taxa from Sichuan, Yunnan, Xinjang, Qinghai and Tibet Autonomous Region (southwestern regions of China), most of these taxa were separated depending on the fruit morphology under groups C and D. Whereas the second group is composed of seven taxa from Heilongjiang, Jilin and Shanxi (northeastern regions of China), most of these taxa were separated depending on the fruit morphology under groups A and B. Thus, the distribution of *Bupleurum* in China is split into southwest and northeast regions, which correspond to the south *Bupleurum* and north *Bupleurum* mentioned in Flora of China. From the perspective of biogeography, the Hengduan Mountains are precisely the boundary of the two regions and retained high diversity about *Bupleurum*. Therefore, the origin of *Bupleurum* in China may be associated with Hengduan Mountains. Furthermore, *Hyalolaena bupleuroides* is distributed in Xinjiang Uygur Autonomous Region which is located in northwest China. Considering the dendrogram, *Hyalolaena bupleuroides* was nested within group AI with 81% similarity with other *Bupleurum* species belonging also to northwest of China which could give the probability that geographical distribution also has an effect on the fruit morphology of different genera of the same family.

The majority of authors are congruent with the above view. Shu and Sheh,[47] posed the origin centre of *Bupleurum* maybe Western China, because about 40% of *Bupleurum* in China is distributed in western China. Subsequently, Wang,[48] indicated that Hengduan Mountains could become probably one of current centres of biodiversity about *Bupleurum*, furthermore widespread distribution and origin of *Bupleurum* in China maybe extend to other countries. After that, Wang,[28] emphasized the first Chinese group D, a group that probably with extending distribution to the countries near the Himalayan, such as Nepal, Pakistan, India, Bhutan and southern Asia. Group II also shows a

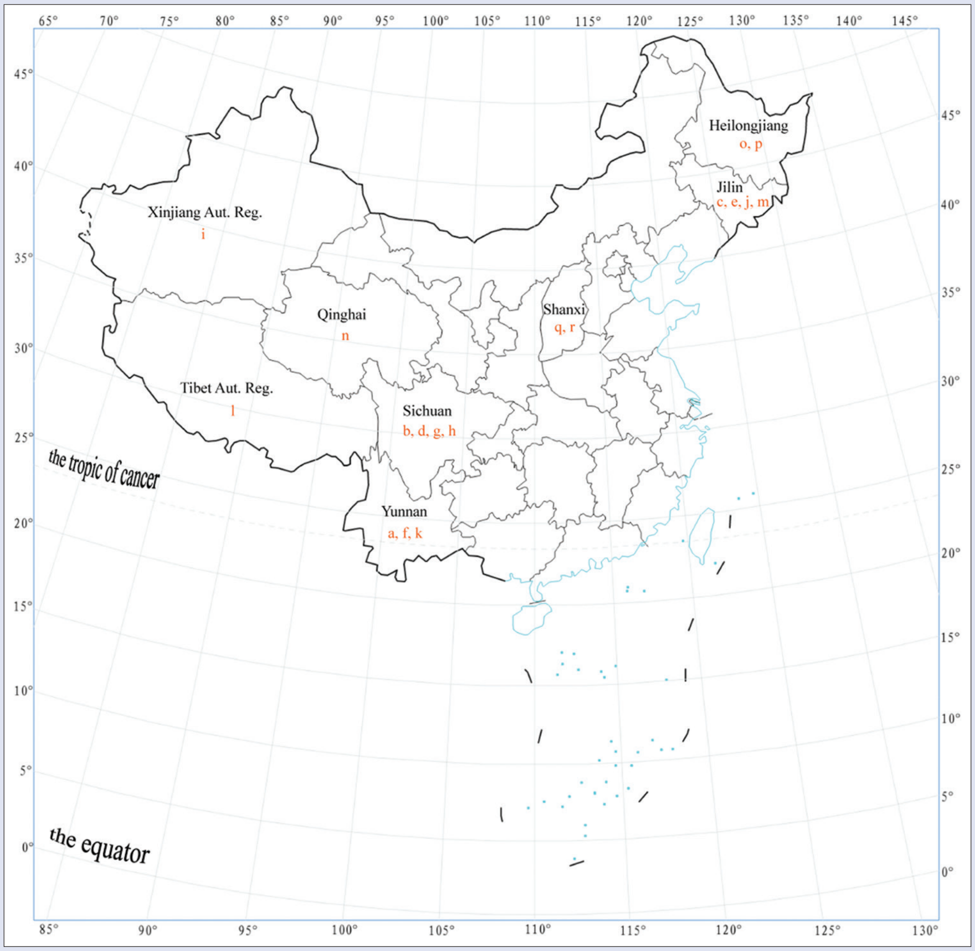

Figure 3: Phyto-geographical region of *Bupleurum*

close relationship with Korea, Russia and Japan. Plunkett,[49] put forward that *Bupleurum* originated from Africa and spread northward through the Middle East to Eurasia. Subsequently, Neves and Watson,[50] proposed that *Bupleurum* originated somewhere in the western Mediterranean region. In order to verify this biogeographic hypothesis, more species should be included in the biogeographic analysis, in particular from Asia and the Mediterranean region.

Fruit characteristics have always been one of the most important signs of Apiaceae. Due to the wide distribution of *Bupleurum*, as well as climatic conditions and geographical locations are very distinct. To further reveal the diversity of *Bupleurum*, we should expand our research methods, especially the complete chloroplast genomes sequencing inferred from their evolutionary implications and the phylogenetic relationship.[51-53] Meanwhile, it is possible to adopt nontargeted metabolic profiling,[54,55] to analyze the chemical differences in *Bupleurum*. Therefore, studies may provide a better building of taxonomical standards.

CONCLUSION

Morpology of fruit can be used to recognize *Bupleurum* species, primary features such as fruit dimension, colour, shape, Cv even testa structure. It is shown to be useful for taxonomic identification. The cluster analysis of 41 character states showed the intraspecific and interspecific classification. An identification key of *Bupleurum* species was put forward on cluster analysis and fruit morphology characters. *Bupleurum* species in China is divided into two groups, whose morphological variation may be closely related to their biogeography. From the view of distribution, the first group belongs to the southwestern regions of China while the second group is in the northeastern regions of China. Thus, the distribution of *Bupleurum* in China is split into southwest and northeast regions, which correspond to the south *Bupleurum* and north *Bupleurum* mentioned in Flora of China. From the perspective of biogeography, the Hengduan Mountains are precisely the boundary of the two regions and retained high diversity about *Bupleurum*. Therefore, the origin of *Bupleurum* in China may be associated with Hengduan Mountains. It is recommended to expand the research scope, especially to Africa, Asia and Europe. Meanwhile, it is possible to establish a more comprehensive terminology and classification. Therefore, studies on fruit morphology might supply the better building of taxonomical standards.

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Conflicts of interest

There are no conflicts of interest.

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