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SEED MORPHOLOGY OF ZANTHOXYLUM (RUTACEAE) IN CHINA AND ITS SYSTEMATIC SIGNIFICANCE

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Abstract

Seed morphology of 31 species belonging to the genus *Zanthoxylum* in China were examined using light microscopy and scanning electron microscopy. The macro- and micro-morphological characters of seed, including seed surface, colour, shape, size and coat sculpture were examined. Under light microscopy, the results indicate that seed surface is more or less smooth; seed colour is black or brown to red; seed shape is spheroidal or elliptical and size is large. Under scanning electron microscopy, the seed epidermal cellular arrangement is netted and the epidermal cell shape ranges from tetragonal to hexagonal in all taxa. The fine relief of the outer cell wall is rough, smooth and finely folded sculpturing. The systematic significance of these seed characters was evaluated. It was proved that the seed coat sculpture was a valuable character which offers evidence to solve some taxonomical problems of *Zanthoxylum*. Based on the seed morphological data, the present study proved that subgen. *Fagara* and subgen. *Zanthoxylum* shared many common seed characters, suggesting a close relationship between the two subgenera. The value of some characters of seed in identifying species of *Zanthoxylum* is also discussed here.

Key words: Seed Morphology, Systematic Significance, Zanthoxylum, China.

Introduction

Zanthoxylum L., is the largest and the most widespread genus of the family Rutaceae, including 225-250 species, with mostly distribution in pantropical regions extending to temperate latitudes in East Asia and east North America (Stevens, 2011). There are 41 species (25 endemic) in China which are distributed from the Liaodong Peninsula to Hainan Island and from Taiwan to southeastern Tibet (Huang, 1997; Zhang *et al*., 2008). Some species of *Zanthoxylum* are utilized in traditional medicine as they are carminative and astringent, while some species are utilized as flavors in cooking (Xiong *et al*., 1997).

The infrageneric taxonomy of *Zanthoxylum* s.l. has remained controversial since long. Linnaeus (1759) divided *Zanthoxylum* s.l. into *Zanthoxylum* and *Fagara* based on the perianth whorls, including species with one and two perianth whorls as genus *Zanthoxylum* L., those with two perianth whorls as genus *Fagara* L. Linnaeus's circumscription has been followed by some authors (Engler, 1931; Albuquerque, 1968). However, Saunders (1934) comprehensively monographed the genus, treated *Zanthoxylum* in the broad sense, and divided it into two subgenera: *Zanthoxylum* and *Fagara*. Most taxonomists accepted Saunders' system and proposed that it was better to treat *Fagara* as a subgenus of *Zanthoxylum*, and to combine the two taxa under *Zanthoxylum* (Moore, 1936; Fosberg, 1959; Brizicky, 1962; Hartley, 1966; Beurton, 1994; Huang, 1997). *Zanthoxylum* is monophyletic including *Toddalia asiatica* (L.) Lam. according to phylogenetic analysis based on a small number of sequenced species (Poon *et al*., 2007; Groppo *et al*., 2008).

The morphological characters of seed play an important role in solving various taxonomic and evolutionary problems (Barthlott, 1984; Akbar *et al*., 2012; Abid *et al*., 2013; Ather *et al*., 2013). Hamilton *et al*. (2007, 2008) examined the seed morphological characters of 4 *Citrus* (Rutaceae) taxa in Australia to characterise the seed maturity. Despite of so much importance and stability of seed characters, no study on seed characters of Chinese *Zanthoxylum* has been carried out up till now.

The main aims of the present study are: 1) to perform a detailed seed characters survey of representative species of *Zanthoxylum* in China, 2) to elucidate the usefulness of seed micromorphological characters for the systematics of the genus, and 3) to examine the possible significance of these characters for solving certain taxonomical problems.

Materials and Methods

Mature and healthy seeds of 37 samples, representing 31 *Zanthoxylum* species were studied by light microscopy (LM) and scanning electron microcopy (SEM). The seed samples were mostly obtained from specimens in the herbaria of South China Botanical Garden, CAS (IBSC) and Institute of Botany, CAS (PE). The voucher information is presented in Table 1.

For LM, the seed characters including equatorial diameter, polar axis, seed colour, size and external morphology were examined. For SEM observation, dry seeds were directly mounted on stubs coated with gold and palladium, and examined under HITACHIS-4800 SEM for sculpturing observation. Descriptive terminology of seed morphology follows Barthlott (1981, 1984) and Liu *et al*. (2004).

Table 1. Collection data for specimens of *Zanthoxylum* sampled for seed study

Taxa	Provenance	Voucher
Subgenus *Fagra*		
Z. ailanthoides Siebold & Zucc.	Hangzhou, Zhejiang, China	Q. G. Zhu 283 (IBSC)
Z. ailanthoides Siebold & Zucc.	Botanical Marunouchi, Japan	Kazuo Terada 14909 (IBSC)
Z. avicennae DC.	Hongkong, China	H. Y. Chen 6406 (IBSC)
Z. calcicola C. C. Huang	Xichou, Yunnan, China	X. R. Luo 1962 (IBSC)
Z. collinsae Craib	Pingbian, Yunnan, China	Sino-Russia Yunnan Exped. 661(IBSC)
Z. dissitum Hemsl. ex Forb. & Hemsl.	Banna, Yunnan, China	K. D. Tao 4892 (IBSC)
Z. echinocarpum Hemsl.	Ruyuan, Guangdong, China	C. Wang 42379 (IBSC)
Z. echinocarpum Hemsl. var. *tomentosum* C. C. Huang	Anlong, Guizhou, China	Guizhou Exp. 3257 (IBSC)
Z. esquirolii H. Lév.	Emei Mt., Sichuan, China	K.H.Yang 56099 (IBSC)
Z. glomeratum C. C. Huang	Rongshui, Guangxi, China	Q. H. Lu 2864 (IBSC)
Z. integrifolium Merr.	Taiwang, China	C. E. Chang 594851 (IBSC)
Z. kwangsiense (Hand.-Mazz.) Chun ex C. C. Huang	Fengjie, Sichuan, China	Sichuan University Exp. 111118 (IBSC)
Z. laetum Drake	Xinyi, Guangdong, China	C. Wang 34650 (IBSC)
Z. leiboicum C. C. Huang	Leibo, Sichuan, China	C. T. Kuan 9452 (IBSC)
Z. micranthum Hemsl.	Fengjie, Sichuan,China	M. Y. Fang 24627 (IBSC)
Z. myriacanthum Wall.	Longquan, Zhejiang, China	S. G. Zhang 3928 (PE)
Z. nitidum (Roxb.) DC.	Yunan, Guangdong, China	H. G. Ye & N. Liu 2979 (IBSC)
Z. oxyphyllum Edgew.	Jingdong, Yunnan, China	M.K.Li 2028 (IBSC)
Z. rhombifoliolatum C.C. Huang	Nanchuan, Sichuan, China	G. F. Li 64042 (IBSC)
Z. schinifolium Siebold & Zucc.	Qingyang, Anhui, China	M. Liu 400069 (PE)
Z. schinifolium Siebold & Zucc.	Korea	89 (IBSC)
Z. stenophyllum Hemsl.	Fengjie, Sichuan, China	Sichuan University Exp. 107923 (IBSC)
Z. tomentellum Hook.f.	Chi-na-tung, Yunnan, China	C.W.Wang 66857 (IBSC)
Z. tomentellum Hook.f.	India	G. Sengupta 2853 (IBSC)
Subgenus *Zanthoxylum*		
Z. acanthopodium DC.	Ching-Tung,Yunnan, China	M.K.Li 0763 (IBSC)
Z. acanthopodium DC.	Thailand	Kailansen S. Larsen 424125 (IBSC)
Z. armatum DC.	Dongkou, Hunan, China	Z.Y.Yang 182 (IBSC)
Z. armatum DC. var. *ferruginum* C. C. Huang	ShanZhi, Hunan, China	Q. Ling 659 (IBSC)
Z. austrosinense C. C. Huang	Yanling, Hunan, China	Central-South Univ. Exp. 77-0319 (IBSC)
Z. bungeanum Maxim.	Nandang, Guangxi, China	C. Wang 41160 (IBSC)
Z. ovalifolium Wight	Baisha, Hainan, China	S. K. Lau 27551 (IBSC)
Z. piasezkii Maxim.	Dajin, Sichuan, China	X. Li 75018 (IBSC)
Z. pilosulum Rehder & H. E. Wilson	Barkam, Sichuan, China	X. Li 72333 (IBSC)
Z. scandens Blume	Taishan, Guangdong, China	Guangdong Exp. 73-02744 (IBSC)
Z. simulans Hance	Nanxiong, Guangdong, China	Guangdong Exp. 123 6(IBSC)
Z. stipitatum C. C. Huang	Ruyuan, Guangdong, China	S. P. Ko 52739 (IBSC)
Z. undulatifolium Hemsl.	Ping-an, Shanxi, China	T. N. Liou & P.C. Tsoong 1220 (IBSC)

The classification of some species in *Zanthoxylum* has been disputed for a long time. However, based to the seed coat feature, it possible it to classify them at species level. For example, Hartley (1966) placed *Z. kwangsiense* in synonymy of *Z. scandens*, *Z. collinsae* in synonymy of *Z. nitidum*. But Zhang *et al*. (2008) separated them on the basis of leaf, hairiness, flower size and capsule characters. In the present study, it is found that *Z. kwangsiense* has a channeled anticlinal cell wall boundaries and a flat, smooth to rough periclinal cell wall (Fig. 28), while *Z. scandens* has a raised anticlinal cell wall boundaries and a concave, smooth periclinal cell wall (Fig. 38); *Z. collinsae* has a channeled anticlinal cell wall boundaries and a sculptural striate periclinal cell wall (Fig. 24), while *Z. nitidum* has a raised, anticlinal cell wall boundaries and a rough periclinal cell wall (Fig. 33). Hence, the results of seed morphology study support the treatment of Zhang *et al*. (2008).

Results

In all studied samples, the outer surface of the testa is more or less smooth when dry. Most of the seeds are black in colour, and a few are brown to red. The seed shapes are spheroidal or elliptical (Figs. 1-15). The seeds are large-sized, and the size of seed vary greatly among the examined species, from ca 5.2×5.7 mm in *Zanthoxylum integrifolium* Merr. to ca 2.2×2.6 mm in *Z. armatum* var. *ferruginum.* The detailed seed characters of all investigated species in *Zanthoxylum* are summarized in Table 2.

The sculptures of the epidermal cell wall of most examined taxa vary considerably. The organization and sculpture of the epidermal cell wall were observed in detail.

The pattern of the epidermal cell is netted, and the outlines of epidermal cells are usually polygonal, varying from tetragonal to hexagonal in all examined species (Table 2).

The organization of the anticlinal cell boundaries can be categorized into two main types: 1) raised type, occurs in 20 species, e.g. *Zanthoxylum ailanthoides* (Figs. 18, 19), *Z. avicennae* (Fig. 21) and *Z. esquirolii* (Fig. 26) *et al*.; 2) channeled type, occurs in 17species, e. g. *Z. acanthopodium* (Figs 16, 17), *Z. bungeanum* (Fig. 22) and Z. *calcicola* (Fig. 23) *et al*. The sculpture of the anticlinal walls ranges from smooth to finely folded in the species examined which can be divided: 1) curved type, occurs in 5 species, viz., *Z. bungeanum*, *Z. integrifolium*, *Z. laetum* (Fig. 29), *Z. leiboicum* (Fig. 30) and *Z. oxyphyllum* (Fig. 35); 2) straight to slightly curved type, occurs in 31 species, e. g., *Z. collinsae* (Fig. 24), *Z. echinocarpum* (Fig. 25) and *Z. kwangsiense* (Fig. 28) *et al*.

The outer periclinal walls show considerable variation. The curvature of outer periclinal walls can be divided into four main types: 1) concave type, occurs in 2 species: *Zanthoxylum esquirolii* and *Z. scandens* (Fig. 38); 2) flat type, occurs in 7 species, e. g., *Z. integrifolium* (Fig. 27), *Z. kwangsiense* and *Z. micranthum* (Fig. 31) *et al*.; 3) flat to slightly convex type, occurs in 8 species, e.g., *Z. armatum* (Fig. 20), *Z. avicennae* and Z. *schinifolium* (Figs. 39, 40) *et al*.; 4) convex type, occurs in 20 species, e. g., *Z. bungeanum*, *Z. simulans* (Fig. 41) and *Z. undulatifolium* (Fig. 45) *et al*. The sculture of periclinal wall can be divided into three main types: 1) rough type, occurs in 9 species, e. g., *Z. nitidum* (Fig. 33), *Z. simulans* and *Z. tomentellum* (Figs. 43, 44) *et al*.; 2) finely folded sculpturing type, occurs in 12 species, e. g. *Z. integrifolium*, *Z. myriacanthum* (Fig. 32) and *Z. stenophyllum* (Fig. 42) *et al*.; 3) smooth type occurs in 16 species, such as Z. *micranthum*, Z. *pilosulum* (Fig. 37) and Z. *scandens*.

Discussion

Present study revealed that the seed coat characters of the same species from different geographical areas (*Zanthoxylum ailanthoides*, *Z. schinifolium*, *Z. tomentellum*, *Z. acanthopodium*, cf. Table 2) are quite constant, and the seed coat characters of different species in the same country exhibit variations. Therefore, it supports the hypothesis that the seed characters are only slightly influenced by environmental conditions (Barthlott, 1984) and some characters of the seed can be of considerable diagnostic value.

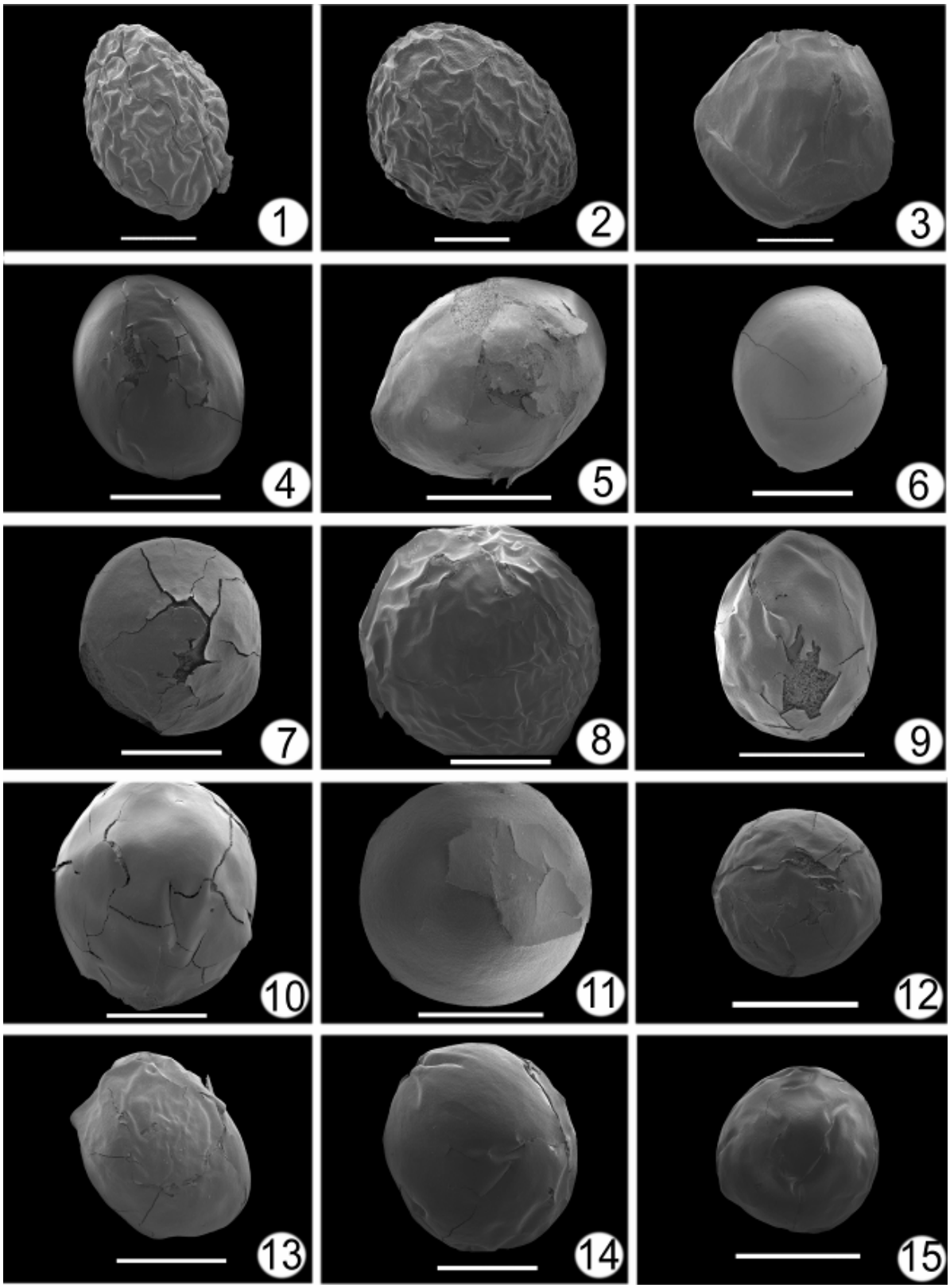
Similar to the case of Australian *Citrus* species (Hamilton *et al*., 2007, 2008), the seeds of the Chinese *Zanthoxylum* exhibit appreciable diversities in surface, colour, shape, size, and structure. It was found that seed surface, shape and colour only have a minor diagnostic value at generic level. On the other hand, the seed coat shape, size and structure are often of valuable assistance in specific delimitation and establishing taxonomic relationships in this genus.

For the examined species, the seeds of subgen. *Zanthoxylum* closely resemble to those of subgen. *Fagara.* There are no differences in anticlinal walls, periclinal cell walls, and fine relief of the outer cell wall between the two subgenera. Therefore, the Chinese taxa do not have a characteristic seed structure at the generic level, which indicates that seed coat type in *Fagara* and *Zanthoxylum* is homogeneous and suggests a close relationship of them. The seed morphological similarities of *Fagara* and *Zanthoxylum* support the idea of combining the two subgenera previously suggested by some botanists based on a combination of morphological, chemical, leaf epidermal and pollen characters (Fish & Waterman, 1973; Cao & Zhang, 2008; Cao *et al*., 2014). Based on seed morphological evidence and other data, it is suggested that *Fagara* should be treated as a subgenus of *Zanthoxylum*, s.l., thus the Englerian classification of *Zanthoxylum* appears to be unsatisfactory.

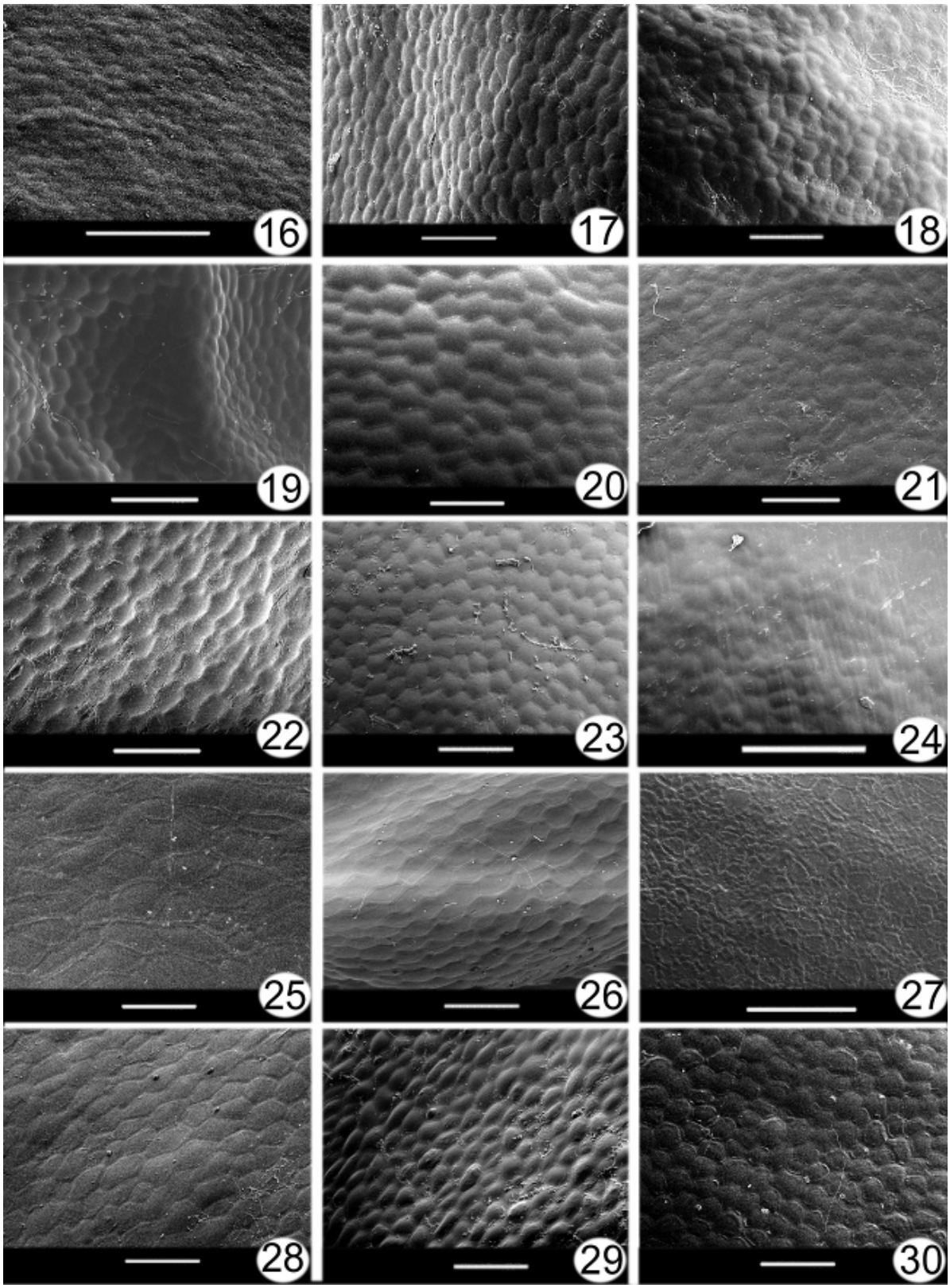
It is difficult to classify some species of *Zanthoxylum* by macromorphological characters. The results show that seed characters are useful in indentifying some closely related species of *Zanthoxylum.* For example, two resembling species, *Z. esquirolii* and *Z. oxyphyllum*, are separated quite well based on seed characters; the former is characterized by the presence of raised, straight to slightly curved anticlinal cell wall boundaries and a concave, smooth periclinal cell wall (Fig. 26), while the latter is characterized by the presence of channeled, curved anticlinal cell wall boundaries and a convex, rough periclinal cell wall (Fig. 35). *Z. piasezkii* and Z. *pilosulum* are further distinguished by the seed characters: the former is characterized by the presence of folded anticlinal cell wall boundaries and a finely sculpturing periclinal cell wall (Fig. 36), while the latter is characterized by the presence of smooth anticlinal cell wall boundaries and a smooth periclinal cell wall (Fig. 37).

Table 2. Seed characters of the investigated species of *Zanthoxylum*.

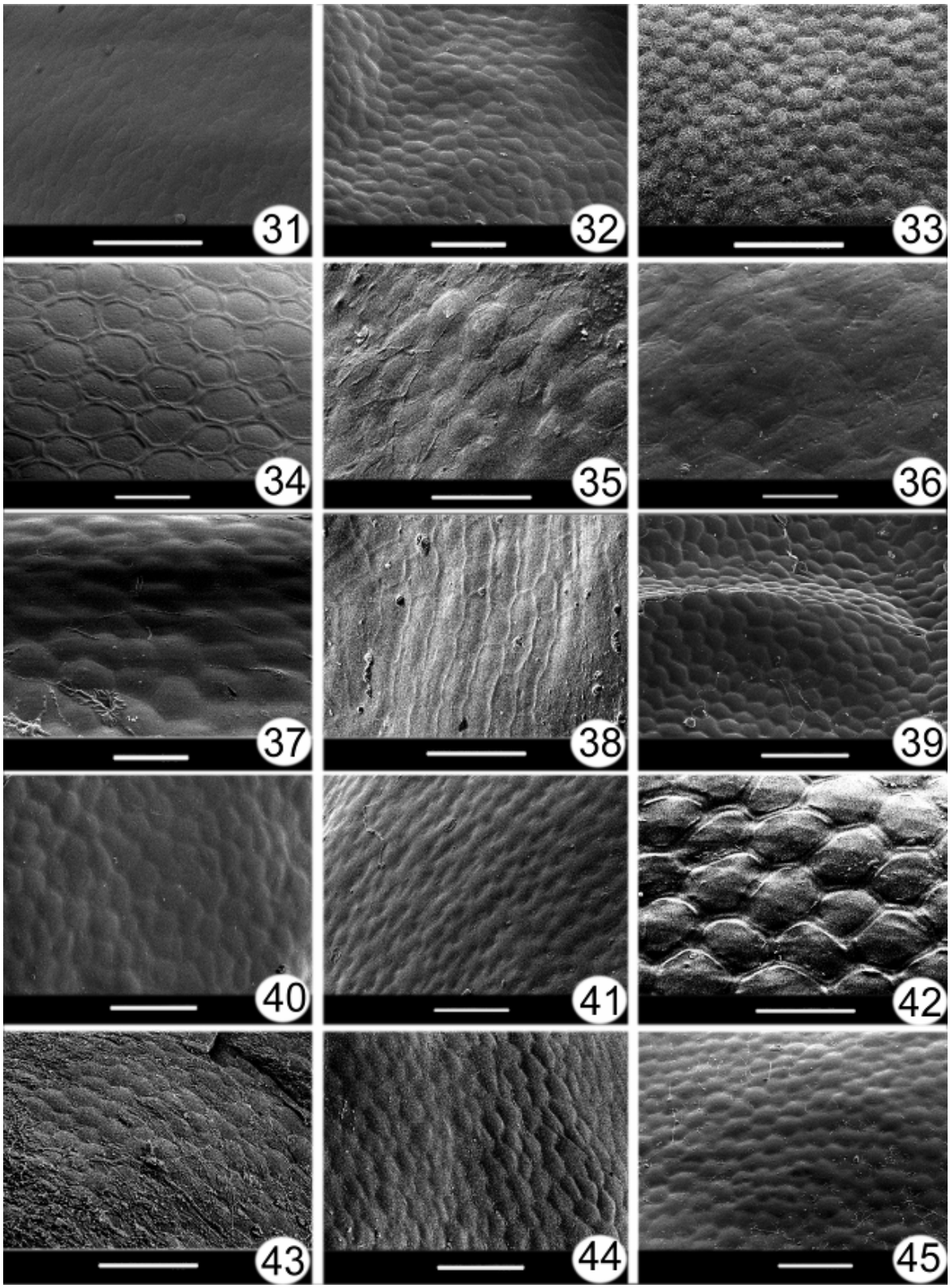
Taxon	Seed shape	Seed size (mm)	Epidermal cell shape	Anticlinal cell wall boundaries	Periclinal cell wall
Subgenus *Fagara*					
Z. ailanthoides (China)	spheroidal	2.5×3.0	5–6 gonals	Raised, straight to slightly curved, smooth	Convex; smooth
Z. ailanthoides(Japan)	ellipse	2.5×3.0	5–6 gonals	Raised, straight to slightly curved, smooth	Convex; rough
Z. avicennae	spheroidal	3.0×3.6	4–5 gonals to indistinct	Raised, straight to slightly curved, smooth to fine folded	Flat to slightly convex; sculpturing
Z. calcicola	spheroidal	3.6×3.8	4–5 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; sculpturing
Z. collinsae	ellipse	2.6–3.2	4–5 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; sculpturing
Z. dissitum	spheroidal	7.1×8.3	4–5 gonals to indistinct	Raised to indistinct, straight to slightly curved, smooth	Convex; sculpturing
Z. echinocarpum	ellipse	5.5×5.9	4–5 gonals to indistinct	Channeled, straight to slightly curved, smooth	Flat; smooth to rough
Z. echinocarpum var. *tomentosum*	spheroidal	4.7×6.1	4–6 gonals	Channeled, straight to slightly curved, smooth	Flat; smooth
Z. esquirolii	spheroidal	2.9×3.5	5–6 gonals to indistinct	Raised, straight to slightly curved, smooth	Concave; smooth
Z. glomeratum	spheroidal	3.6–4.3	4–5 gonals to indistinct	Raised, straight to slightly curved, smooth	Convex; rough
Z. integrifolium	ellipse	6.4–8.3	5–6 gonals to indistinct	Channeled, curved, smooth to folded	Flat; finely sculpturing
Z. kwangsiense	ellipse	3.5×3.8	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Flat; smooth to rough
Z. laetum	spheroidal	5.0×5.5	4–5 gonals to indistinct	Raised to indistinct, curved, folded	Convex; finely sculpturing
Z. leiboicum	ellipse	5.2×6.2	4–5 gonals	Channeled, curved, folded	Convex; finely sculpturing
Z. micranthum	spheroidal	2.5×3.4	5–6 gonals	Channeled, straight to slightly curved, smooth	Flat; smooth
Z. myriacanthum	spheroidal	3.5×3.8	4–5 gonals to indistinct	Raised, straight to slightly curved, smooth to fine folded	Convex; finely sculpturing
Z. nitidum	ellipse	4.8×5.6	5–6 gonals to indistinct	Raised, straight to slightly curved, smooth	Convex; rough
Z. oxyphyllum	spheroidal	3.0×3.5	5–6 gonals to indistinct	Channeled, curved , smooth	Convex; rough
Z. rhombifoliolatum	spheroidal	3.6×3.9	4–5 gonals to indistinct	Channeled, straight to slightly curved, smooth	Flat to slightly convex; rough
Z. schinifolium (China)	ellipse	2.5×3.0	5–6 gonals to indistinct	Raised, straight to slightly curved, smooth	Flat to slightly convex; smooth to rough
Z. schinifolium (Korea)	ellipse	2.5×3.0	5–6 gonals	Raised, straight to slightly curved, smooth	Flat to slightly convex; smooth to rough
Z. stenophyllum	ellipse	2.9×3.7	4–5 gonals to indistinct	Raised, straight to slightly curved, smooth	Convex; finely sculpturing
Z. tomentellum (China)	ellipse	3.6×4.5	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; rough
Z. tomentellum (India)	ellipse	3.6×4.7	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; rough
Subgenus *Zanthoxylum*					
Z. acanthopodium (China)	spheroidal	2.8×3.2	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; smooth
Z. acanthopodium (Thailand)	ellipse	2.8×3.2	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; smooth
Z. armatum	ellipse	3.5×3.7	5–6 gonals	Raised, straight to slightly curved, smooth	Flat to slightly convex; smooth
Z. armatum var. *ferruginum*	ellipse	2.2×2.6	5–6 gonals	Raised, straight to slightly curved, smooth	Flat to slightly convex; smooth
Z. austrosinense	spheroidal	2.9×4.1	5–6 gonals to indistinct	Raised, straight to slightly curved, smooth to folded	Flat to slightly convex; rough
Z. bungeanum	spheroidal	3.4×4.5	5–6 gonals to indistinct	Channeled, curved, smooth	Convex; smooth
Z. ovalifolium	ellipse	3.6×3.8	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Flat; finely sculpturing
Z. piasezkii	ellipse	2.6×3.4	5–6 gonals	Raised, straight to slightly curved, folded	Flat; finely sculpturing
Z. pilosulum	spheroidal	2.6×3.4	4–5 gonals to indistinct	Raised to indistinct, straight to slightly curved, smooth	Flat to slightly convex; smooth
Z. scandens	spheroidal	3.6×3.7	4–5 gonals	Raised, straight to slightly curved, folded	Concave; smooth
Z. simulans	ellipse	3.1×3.9	4–5 gonals to indistinct	Raised to indistinct, straight to slightly curved, folded	Convex; rough
Z. stipitatum	spheroidal	3.3×3.8	5–6 gonals	Raised to indistinct, straight to slightly curved, smooth	Convex; finely sculpturing
Z. undulatifolium	spheroidal	3.3×4.2	5–6 gonals to indistinct	Channeled, straight to slightly curved, smooth	Convex; smooth to rough



Figs. 1-15. SEM micrographs of seeds in genus *Zanthoxylum*. 1,*Z. armatum* var. *ferruginum*; 2, *Z. avicennae*; 3, *Z. myriacanthum*; 4, *Z. armatum*; 5, *Z. austrosinense*; 6, *Z. calcicola*; 7, *Z. collinsae*; 8, *Z. echinocarpum* var. *tomentosum*; 9, *Z. esquirolii*; 10, *Z. leiboicum*; 11, *Z. ovalifolium*; 12, *Z. piasezkii*; 13, *Z. pilosulum*; 14, *Z. scandens*; 15, *Z. stenophyllum*. (Scale bars: 1, 2, 3 = 1.0 mm，4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 = 2.0 mm).



Figs. 16-30. SEM micrographs of seeds in genus *Zanthoxylum*. 16, *Z. acanthopodium* (Tailand); 17, *Z. acanthopodium* (China); 18, *Z. ailanthoides* (Japan); 19, *Z. ailanthoides* (China); 20, *Z. armatum*; 21, *Z. avicennae*; 22, *Z. bungeanum*; 23, *Z. calcicola*; 24, *Z. collinsae*; 25, *Z. echinocarpum*; 26, *Z. esquirolii*; 27, *Z. integrifolium*; 28, *Z. kwangsiense*; 29, *Z. laetum*; 30, *Z. leiboicum*. (Scale bars：16, 24, 27 = 200μm, 17, 18, 19, 20, 21, 22, 23, 25, 26, 28, 29, 30 = 100μm).



Figs. 31-45. SEM micrographs of seeds in genus *Zanthoxylum*. 31, *Z. micracanthum*; 32, *Z. myriacanthum*; 33, *Z. nitidum*; 34, *Z. ovalifolium*; 35, *Z. oxyphyllum*; 36, *Z. piasezkii*; 37, *Z. pilosulum*; 38, *Z. scandens*; 39, *Z. schinifolium* (Korea); 40, *Z. schinifoltum* (China); 41, *Z. simulans*; 42, *Z. stenophyllum*; 43, *Z. tomentellum* (India); 44, *Z. tomentellum* (China); 45, *Z. undulatifolium*. (Scale bars: 31, 33 = 200μm, 32, 34, 35, 38, 39, 40, 41, 43, 44, 45 = 100μm, 36, 37, 42, = 50μm).

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