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POLLEN MORPHOLOGY AND ITS SYSTEMATIC SIGNIFICANCE IN *ZANTHOXYLUM* (RUTACEAE) FROM CHINA

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Abstract

Pollen grains of 32 species of *Zanthoxylum* were studied under light microscopy and scanning electron microscopy. The macro- and micro-morphological pollen characters, including shape, pollen size, aperture and exine ornamentation type were examined. The pollen grains are small to medium in size, mostly from subspheroidal to prolate shape in equatorial view and 3–lobed circular in polar view. Three types are recognized based on exine ornamentation, includes macroreticulate, parallel striation, and striate-rugulate. Pollen shape and size are found to have only minor value, but pollen exine ornamentation contains useful information on modify the Englerian classification of *Zanthoxylum* and can be used for identifying different species of *Zanthoxylum* s.l.. Based on the pollen morphological data, the two subgenus, *Fagara* and *Zanthoxylum*, should be combined.

Introduction

Zanthoxylum L., a genus in the family Rutaceae, contains 225 species according to a recent taxonomic revision of the genus (Stevens, 2001). Two subgenera, subgen. *Zanthoxylum* and subgen. *Fagara*, are recognized by the whorl of perianth (Saunders, 1934; Moore 1936). It is widely distributed in pantropical regions including Asia, Africa, Australia, and North America. In China, there are 41 species of *Zanthoxylum* (25 endemic), 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 used in traditional medicine as they are carminative and astringent, or in flavoring food (Xiong *et al*., 1997).

The pollen morphology of Rutaceae has similarly received considerable attention as an important character in interpreting systematic relationships (Morton & Kallunki, 1993; Victor & Van Wyk, 1998, 1999, 2000, 2001; Grant *et al*., 2000; Mou & Zhang, 2009). Barth (1980) examined 17 species of *Zanthoxylum* in Brazil, and concluded that the pollen type is homogeneous in this genus. However, detailed pollen morphology and comparable descriptions of the genus have not been conducted. The pollen morphology of the Chinese *Zanthoxylum* species has received even less attention. Liu (1987) studied the pollen morphology of 12 species and 1 variety of the genus from China, but only gave one illustration, and Wang *et al*., (1997) studied one species, *Z. nitidum* (Roxb.) DC.

The main aim of the present study is to perform a detailed pollen morphological survey of representative species of *Zanthoxylum* distributed in China, in order to evaluate the usefulness of pollen characters for a better understanding of the intergeneric and infrageneric relationship of the genus, and examine the possible use of pollen characters for solving some taxonomical problems in species delimitation in *Zanthoxylum*.

Material and Methods

The material upon which this study is based, including pollen from 32 species of *Zanthoxylum* s.l. from China were studied by light microscopy (LM) and scanning electron microscopy (SEM). The pollen samples of the species were mostly taken from the Herbarium of South China Botanical Garden, the Chinese Academy of Sciences (IBSC), with five pollen samples taken from the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN). All materials were collected from dry specimens. The list of voucher specimens is given in Table 1.

For LM observation, pollen grains were acetolysed using the method of Erdtman (1960), and mounted in glycerol jelly. For SEM observation, pollen grains were fastened on double-sided adhensive tape, coated with gold for 3 min, examined and photographed in a Hitachi S-800 SEM. The measured pollen diameters were based on at least 20 samples and the descriptive terminology followed Erdtman (1969) and Punt *et al*., (2007).

Results

All studied pollen grains are monads, isopolar, and 3-colporate. Pollen grains are medium-sized and their shapes range from spheroidal to prolate. For most species, polar view is triangular, with corners obtuse and sides convex. Equatorial view is rectangular to elliptic, with corners obtuse and sides convex (Fig. I. 1-8). Colpi are usually with margin (Fig. I. 1-12), and grains with a reticulate surface structure, which varies from a simple reticulum to a well marked striation. The detailed information of all the species studied is summarized in Table 2.

In the current study, three broad pollen types were distinguished based on the exine ornamentation of pollen: macroreticulate (type I), parallel striation (type II) and striate-rugulate (type III).

Table 1. Voucher specimen of taxa included in this study.

Taxon	Provenance	Voucher
Subgenus *Fagara*		
Z. ailanthoides Siebold & Zucc.	China: Fujian , Jianyang	Wuyishan Pl. Exp. 820105 (IBSC)
Z. avicennae DC.	China: Guangdong, Gaoyao	S.Wang 162298 (IBSC)
Z. calcicola C.C. Huang	China: Yunnan, Guangnan	C. W. Wang 87861(IBSC)
Z. dissitum Hemsl. ex Forb. & Hemsl.	China: Guangxi, Donglan	C. C. Chang 11399 (IBSC)
Z. echinocarpum Hemsl.	China: Guangdong, Ruyuan	S. B. Guo 80300 (IBSC)
Z. esquirolii H. Lév.	China: Guizhou, Guiyang	S.W.Deng 90040 (IBSC)
Z. khasianum Hook. f.	China: Yunan, Longling	H. T. Tsai 55610 (KUN)
Z. kwangsiense (Hand.-Mazz.) Chun ex C.C. Huang	China: Guangxi, Tianyang	Z. Y. Chen 53962 (KUN)
Z. laetum Drake	China: Guangdong, Xinyi	C. Wang 31888 (IBSC)
Z. leiboicum C.C. Huang	China: Sichuan, Leibo	Lichuan Exp. 0007(KUN)
Z. macranthum (Hand.-Mazz.) C.C. Huang	China: Sichuan, Emei	J. H. Xiong 30489 (IBSC)
Z. micranthum Hemsl.	China: Sichuan, Fengjie	M.Y.Fang 24883 (IBSC)
Z. molle Rehder	China: Jiangxi, Wuning	J. H. Zhang 99905 (IBSC)
Z. multijugum Franch.	China: Yunnan, Chengjiang	X. Wang 41511(IBSC)
Z. myriacanthum Wall.	China: Guangxi, Ronshui	S.H.Chun 16168 IBSC
Z. nitidum (Roxb.) DC.	China:Guangdong, Dinghushan	G. Q. Ding 165 (IBSC)
Z. oxyphyllum Edgew.	China: Yunan, Jingdong	M. K. Li 2028 (IBSC)
Z. scandens Blume	China: Hunan, Chengbu	Z. T. Li 1811 (IBSC)
Z. schinifolium Siebold & Zucc.	China: Zhejiang, Tianmu	H. Q. Zhu 000060 (IBSC)
Z. stenophyllum Hemsl.	China: Sichuan, Fengjie	Sichuan University Exp. 107923 (IBSC)
Z. tomentellum Hook. f.	China: Yunnan, Judian	Y. Z. Zhao 20480 (KUN)
Z. yuanjiangensis C.C. Huang	China: Yunnan, Yuanyang	Luchun Exp. 1401 (KUN)
Subgenus *Zanthoxylum*		
Z. acanthopodium DC.	China: Guizhou, Luodian	Z. S. Zhang 0288 (IBSC)
Z. armatum DC.	China: Hunan, Yizhang	S. H. Chun 346 (IBSC)
Z. austrosinense C.C. Huang	China: Guangxi, Guilin	J. Y. Liang 284 (IBSC)
Z. bungeanum Maxim.	China: Sichuan, Nanchuan	J. H. Xiong 90889 (IBSC)
Z. ovalifolium Wight	China: Hainan, Baisha	S. K. Lau 27551 (IBSC)
Z. piasezkii Maxim.	China: Sichuan, Dajin	X. Li 75445 (IBSC)
Z. pilosulum Rehder & H.E. Wilson	China: Sichuan, Barkam	X. Li 70428 (IBSC)
Z. simulans Hance	China: Zhejiang, Tianmu	X. Y. He 21573 (IBSC)
Z. stipitatum C.C. Huang	China: Guangdong, Ruyuan	Guangdong Exp. 7182 (IBSC)
Z. undulatifolium Hemsl.	China: Hubei, Wuhan	H. J. Li 738 (IBSC)

Pollen type I: macroreticulate (Fig. I. 9-20, Fig. II. 21-22). Pollen grains are prolate sphaeroidal in shape with sculpturing elements form an open network with curved muri. The lumina are polygon, and its equatorial diameter is more than 1-3µm. The type I pattern occurs in 14 species which is the most common one.

Pollen type II: parallel striation (Fig. II. 23-29). The form varies from prolate to prolate-spheroidal, and the lumina are small and more or less rounded. The pollen muri of grains are cross-linked to form a reticulum in the grooved, and the connections between the muri lie on a single level or different levels. The type II pattern occurs in 7 species.

Pollen type III: striate-rugulate (Fig. II. 30-40). The form varies from prolate to prolate-spheroidal, and the lumina are small and more or less rounded, in which the length of lumina is longer than type II. Pollen grains with parallel or subparallel muri are cross-linked to form a reticulum in the grooved. The connections between the muri lie on a single level or different levels. The type III pattern occurs in 11 species.

Discussion

Intrageneric relationships: Historically, the circumscription of *Zanthoxylum* has been highly controversial. Linnaeus (1759) suggested that it is sufficiently heterogeneous to be divided into two genera: *Zanthoxylum* and *Fagara*, and defined the taxon with one and two perianth whorls as genus *Zanthoxylum* L., those taxon with only two perianth whorls as genus *Fagara* L. The recognition of the generic status of *Fagara* has been followed by some authors (Engler, 1931; Reeder & Cheo, 1951; Albuquerque, 1968). Saunders (1934) comprehensively monographed the genus, treated it in its broad sense, and divided it into two subgenera: *Zanthoxylum* and *Fagara*. Most taxonomist accepted Saunders' circumscription and suggested that the simple perianth of *Zanthoxylum* s.s. was most likely a secondary condition derived from *Fagara* by abortion of some or all of the sepals, and proposed that it was better to treat *Fagara* as a subgenus under *Zanthoxylum* and to combine the two taxa under *Zanthoxylum* (Moore, 1936; Fosberg, 1959; Brizicky, 1962; Hartley, 1966; Beurton, 1994; Huang, 1997).

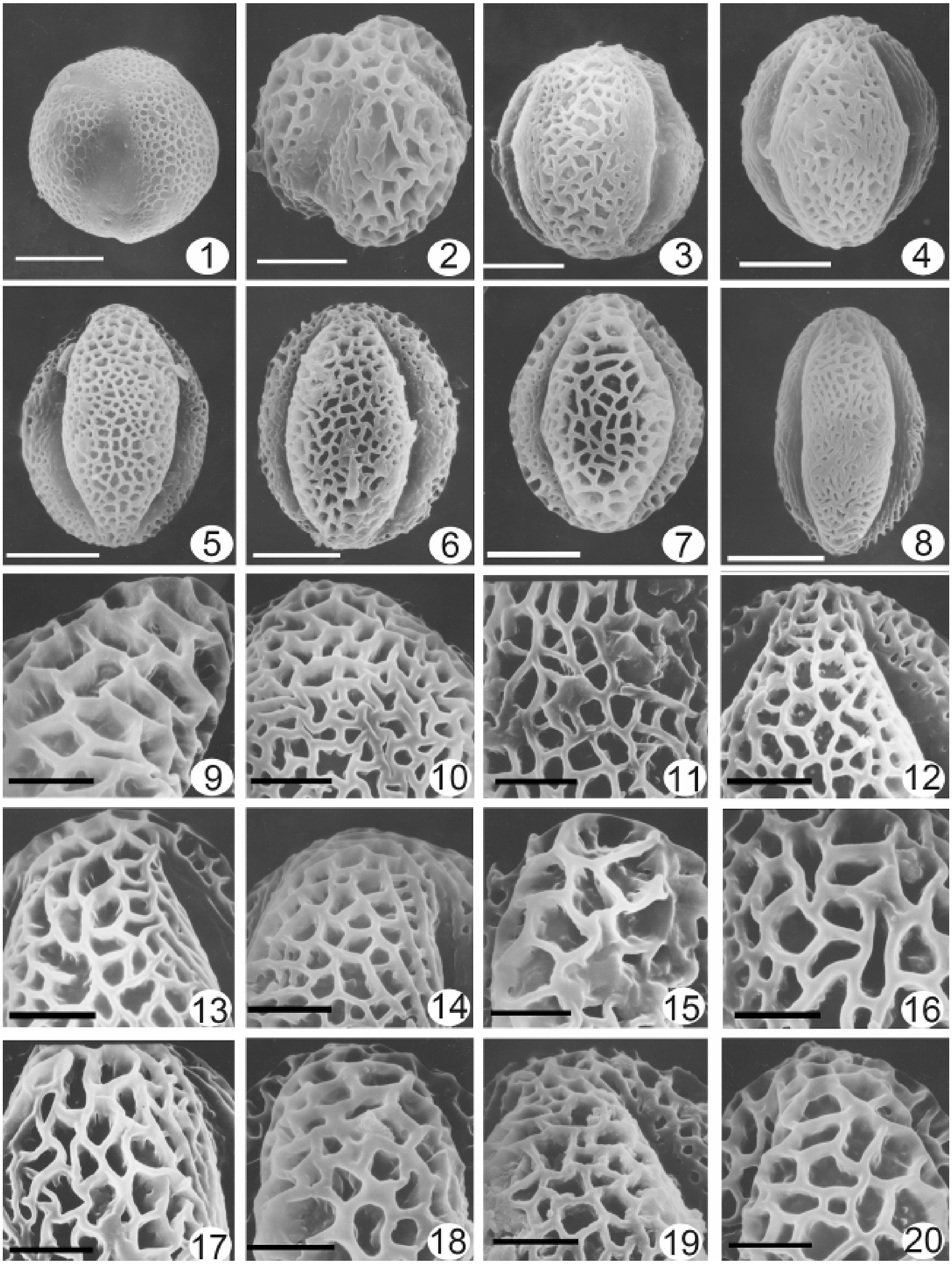

Fig. I. SEM micrographs of pollen grains in genus *Zanthoxylum*. 1, *Z. kwangsiense;* 2, *Z. myriacanthum*; 3, *Z. khasianum*; 4, *Z. scandens*; 5, *Z. echinocarpum*; 6, *Z. nitidum*; 7, *Z. avicennae*; 8, *Z. piasezkii*; 9, *Z. ailanthoides*; 10, *Z. avicennae*; 11, *Z. echinocarpum*; 12, *Z. esquirolii*; 13, *Z. leiboicum*; 14, *Z. macranthum*; 15, *Z. micranthum*; 16, *Z. molle*; 17, *Z. multijugum*; 18 *Z. myriacanthum*; 19, *Z. nitidum*; 20, *Z. schinifolium*. (Scale bars 1= 8.6µm; 2,4=6.0µm; 3=5.0µm; 5,6,7,8=7.5µm; 9,10,11,12,13,14,15,16,17,19,20=3.0µm; 18=3.8µm).

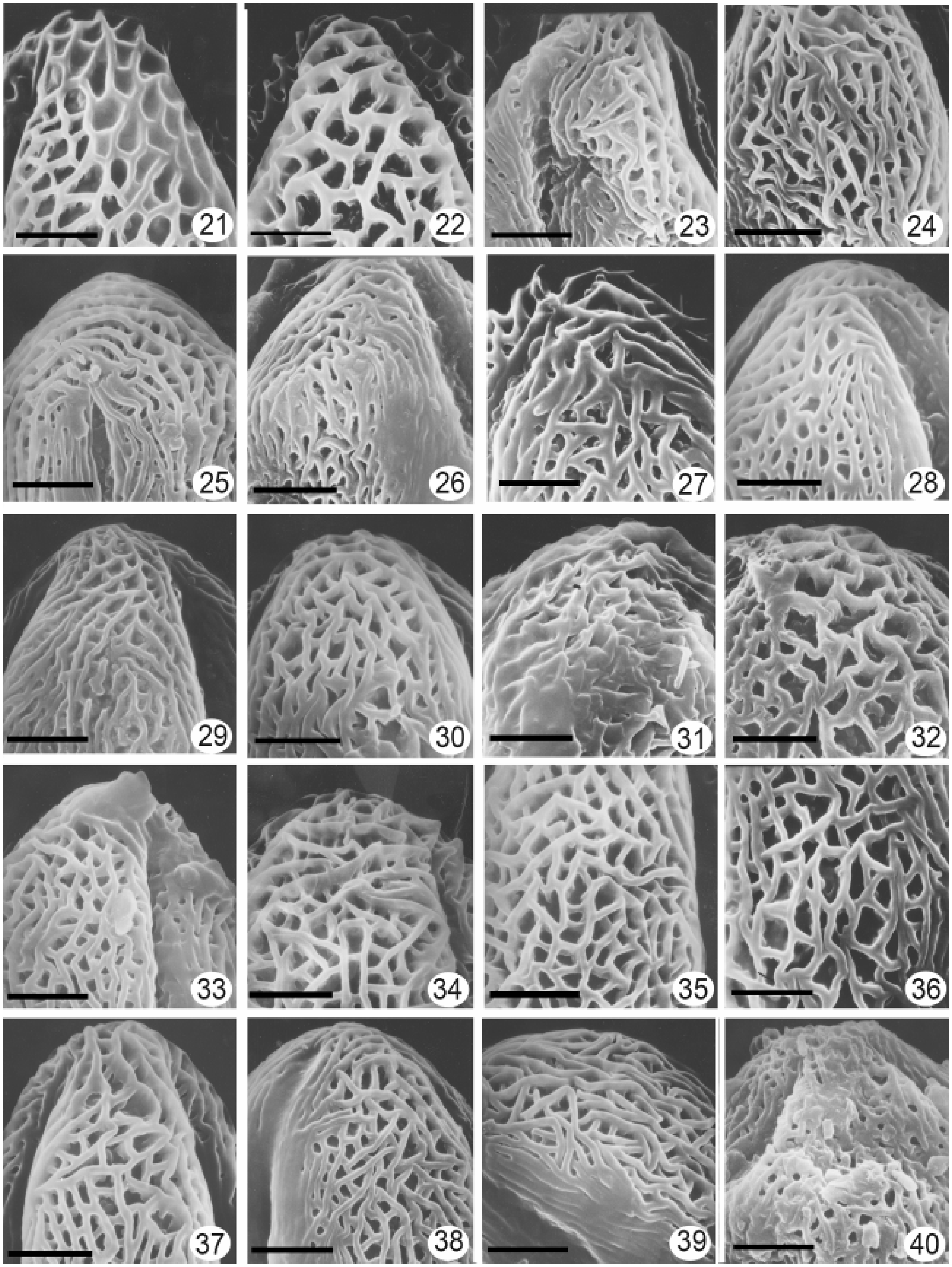

Fig. II. SEM micrographs of pollen grains in genus *Zanthoxylum*. 21,*Z. tomentellum*; 22, *Z. austrosinense*; 23, *Z. calcicola*; 24, *Z. acanthopodium*; 25, *Z. armatum*; 26, *Z. bungeanum*; 27, *Z. ovalifolium*; 28, *Z. pilosulum*; 29, *Z. stipitatum*; 30, *Z. scandens*; 31, *Z. dissitum*; 32, *Z. khasianum*; 33, *Z. kwangsiense*; 34, *Z. oxyphyllum*; 35, *Z. stenophyllum*; 36, *Z. yuanjiangense*; 37, *Z. piasezkii*; 38, *Z. simulans*; 39, *Z. undulatifolium*; 40, *Z. laetum*. (Scale bars: 21,22,23,24,25,26,27,28,29,30,31,33,34,35,36,37,38,39,40= 3.0µm; 32=2.31µm).

Table 2. Characters of the pollen morphology of studied taxa.

Taxon	Aperture	Polar axis (µm.)	Equatorial axis (µm.)	P/E-ratio	Shape	Exine sculpture
Subgenus *Fagara*						
Z. ailanthoides	3-colporate	(16.2)15.91 ± 1.47(14.7)	(21.3)18.6 ± 3.17(15.6)	1.17	subspheroidal	macroreticulate
Z. avicennae	3-colporate	(17.6)16.22 ± 1.63(15.2)	(21.6)20.3 ± 1.81(18.8)	1.25	prolate	macroreticulate
Z. calcicola	3-colporate	(12.3)10.33 ± 2.38(9.60)	(20.2)18.1 ± 2.13(16.8)	1.75	prolate	parallel striation
Z. dissitum	3-colporate	(22.9)21.71 ± 1.85(19.5)	(28.3)26.4 ± 2.43(22.2)	1.22	subspheroidal	striate-rugulate
Z. echinocarpum	3-colporate	(19.7)16.72 ± 3.83(13.8)	(29.9)28.4 ± 1.93(26.6)	1.70	prolate	macroreticulate
Z. esquirolii	3-colporate	(18.1)16.12 ± 2.28(14.3)	(23.1)21.9 ± 1.83(20.3)	1.36	prolate	macroreticulate
Z. khasianum	3-colporate	(14.0)13.05 ± 1.01(12.6)	(15.3)13.2 ± 2.33(11.2)	1.02	subspheroidal	striate-rugulate
Z. kwangsiense	3-colporate	(15.8)14.31 ± 1.75(13.2)	(22.1)20.9 ± 2.10(18.3)	1.46	prolate	striate-rugulate
Z. laetum	3-colporate	(18.1)16.15 ± 2.13(14.7)	(22.2)20.2 ± 2.33(16.9)	1.25	subspheroidal	striate-rugulate
Z. leiboicum	3-colporate	(16.5)14.51 ± 2.21(13.1)	(24.9)23.4 ± 1.75(21.6)	1.61	prolate	macroreticulate
Z. macranthum	3-colporate	(17.5)16.46 ± 2.37(14.3)	(25.1)23.4 ± 2.12(20.8)	1.43	prolate	macroreticulate
Z. micranthum	3-colporate	(18.5)15.17 ± 3.65(14.6)	(24.6)22.9 ± 2.05(20.7)	1.52	prolate	macroreticulate
Z. molle	3-colporate	(19.8)15.95 ± 3.83(12.8)	(21.2)18.6 ± 3.02(15.3)	1.17	subspheroidal	macroreticulate
Z. multijugum	3-colporate	(17.6)14.71 ± 3.75(11.8)	(22.6)20.1 ± 2.76(19.1)	1.36	subspheroidal	macroreticulate
Z. myriacanthum	3-colporate	(17.6)16.73 ± 1.18(15.9)	(20.9)19.0 ± 2.13(17.0)	1.13	prolate	macroreticulate
Z. nitidum	3-colporate	(16.5)17.91 ± 2.33(20.1)	(20.1)22.7 ± 1.75(24.3)	1.26	prolate	macroreticulate
Z. oxyphyllum	3-colporate	(18.8)15.65 ± 2.12(14.1)	(25.6)22.7 ± 3.13(19.5)	1.45	prolate	striate-rugulate
Z. scandens	3-colporate	(15.9)13.92 ± 2.27(12.0)	(17.8)16.3 ± 1.87(15.2)	1.17	subspheroidal	striate-rugulate
Z. schinifolium	3-colporate	(18.0)14.87 ± 3.26(12.5)	(24.9)23.3 ± 1.86(20.8)	1.57	prolate	macroreticulate
Z. stenophyllum	3-colporate	(16.9)15.03 ± 2.17(13.3)	(23.7)22.4 ± 1.75(20.9)	1.49	prolate	striate-rugulate
Z. tomentellum	3-colporate	(17.8)14.93 ± 3.66(14.3)	(27.9)26.3 ± 1.53(24.8)	1.76	prolate	macroreticulate
Z. yuanjiangense	3-colporate	(18.3)16.22 ± 2.37(14.3)	(24.1)23.1 ± 1.37(20.8)	1.43	prolate	striate-rugulate
Subgenus *Zanthoxylum*						
Z. acanthopodium	3-colporate	(15.9)12.91 ± 3.17(10.8)	(18.2)15.8 ± 2.58(13.7)	1.22	subspheroidal	parallel striation
Z. armatum	3-colporate	(16.8)14.51 ± 2.65(12.2)	(19.6)17.7 ± 2.27(15.9)	1.22	subspheroidal	parallel striation
Z. austrosinense	3-colporate	(15.5)13.35 ± 2.37(11.5)	(22.2)19.4 ± 3.17(16.7)	1.46	prolate	macroreticulate
Z. bungeanum	3-colporate	(13.7)11.52 ± 2.52(9.10)	(17.3)15.2 ± 2.33(13.0)	1.32	prolate	parallel striation
Z. ovalifolium	3-colporate	(16.3)15.21 ± 1.33(13.1)	(19.6)17.9 ± 1.91(16.2)	1.18	subspheroidal	parallel striation
Z. piasezkii	3-colporate	(13.6)12.34 ± 1.55(11.3)	(22.6)21.3 ± 1.65(19.8)	1.73	prolate	striate-rugulate
Z. pilosulum	3-colporate	(16.3)14.31 ± 2.33(12.1)	(20.8)19.4 ± 1.78(17.9)	1.36	prolate	parallel striation
Z. simulans	3-colporate	(15.1)13.43 ± 1.86(11.8)	(20.6)18.4 ± 2.53(16.1)	1.37	prolate	striate-rugulate
Z. stipitatum	3-colporate	(15.9)13.55 ± 2.52(11.9)	(21.8)20.4 ± 1.96(18.6)	1.51	prolate	parallel striation
Z. undulatifolium	3-colporate	(15.3)13.50 ± 2.28(11.3)	(16.3)14.7 ± 1.95(12.9)	1.09	subspheroidal	striate-rugulate

In order to understand the relationship between the two closely related taxa better, we compared the pollen character in detail. Three exine ornamentation types of *Zanthoxylum* are found, namely macroreticulate (type I), parallel striation (type II) and striate-rugulate (type III). The type I and type II are found in most species of subgenus *Fagara* and subgenus *Zanthoxylum*, which indicate that pollen type in the two subgenera is homogeneous and suggest a close relationship between them. Based on our studies, pollen data support the combination of the two subgenera, which is provided by morphological traits (Brizicky, 1962; Cao & Zhang, 2008; Cao *et al*., 2009) and chemical characters (Fish & Waterman, 1973; Da Silva *et al*., 1988). According to palynological evidence and other data, we suggest that it is better to treat *Fagara* as a subgenus under *Zanthoxylum*, which constitutes the *Zanthoxylum* s. l. Therefore the Englerian classification of *Zanthoxylum* appears to be unsatisfactory.

Taxonomic significance and species identification: The classification of some species in *Zanthoxylum* has been disputed for a long time. For example, Hartley (1966) treated *Z. kwangsiense* (Hand.-Mazz.) Chun ex C.C. Huang and *Z. khasianum* Hook. f. as synonym of *Z. scandens* Blume. But Huang (1997) and Zhang *et al*., (2008) treated them as three different species on the basis of the habit, leaf, hairiness, flower size and capsule characters. In our study, *Z. kwangsiense* (Fig. **II**. 33), *Z. khasianum* (Fig. **II.** 32) and *Z. scandens* (Fig **II**.30) share the same exine ornamentation by having striate-rugulate. This feature reflects the close relationship between them and supports the treatment of Hartley (1966).

There are some difficulties in classifying species of *Zanthoxylum* based on the habit, leaf, hairiness, flower size and capsule characters. The results of this study show that pollen morphological characters can help identify species of *Zanthoxylum*. For example, *Z. oxyphyllum* Edgew. and *Z. esquirolii* H. Lév. are difficult to distinguish based on gloss morphology, but it is possible to separate the two species by the pollen characters: the former is characterized by the presence of striate-rugulate (Fig. II.34), while the latter is characterized by the presence of macroreticulations (Fig. I. 12). Also, *Z. piasezkii* Maxim. and Z. *pilosulum* Rehder & E. H. Wilson can be distinguished by the pollen characters: the former is characterized by the presence of striate-rugulate (Fig. II. 37), while the latter is characterized by the presence of parallel striation (Fig. II. 28).

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