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Pollen morphology and its systematic and ecological significance in Rheum (Polygonaceae) from China

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Pollen morphology of 40 species of *Rheum*, belonging to eight sections, was investigated under LM and SEM. Four new exine patterns were found in the species: a) microechinate-foveolate, b) rugulate, c) verrucate-perforate, and d) verrucate-rugulate ornamentation. In addition, two patterns, the *Rheum*-type pollens with microechinate-perforate and fine-reticulate, as previously described, were also confirmed in the present study. Based on above study the evolution trends of pollen morphology in the taxa involved were discussed phylogenetically as below. As microechinate-perforate exine pattern existed commonly, the pattern is, therefore, regarded as the most primitive among all the six types. The fine-reticulate type was thought as a derivative type, deriving from the basic microechinate-foveolate-perforate pattern, and followed by the rugulate and verrucate-perforate ornamentation. The verrucate-rugulate ornamentation should be the most advanced. More than one pollen type often exist in most of the sections in *Rheum*. The pollen morphology of *Rheum* was strongly correlated with its geographical and ecological distribution. Three medicinally important species *R. officinale, R. palmatum* and *R. tanguticum* can be palynologically distinguished by their ornamentations.

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Introduction

Rheum L. (Polygonaceae), a very diversified genus with about 60 species (Kao & Cheng 1975; Li 1998), is mainly confined to the mountainous and desert regions of NW China and adjacent areas. NW China, where the ecological conditions are diverse and many primitive and endemic species of *Rheum* are found (Li 1998), might be the diversification and origin center of the genus (Chin & Youngken 1947). But few studies have documented the differentiation of pollen morphology associated with its ecological habits in *Rheum*. This genus, commonly known as rhubarb, is medicinally important in the Orient, where most species are used as a source of traditional medicines. Three of the species (*R. officinale, R. palmatum* and *R. tanguticum*) are highly regarded medicines in China, and therefore widely cultivated. Rhubarb is known as the "lord" or "king of herbs". Chinese people have used it for over 2000 years as purgative medicine, although some scientists consider it as a medical enigma.

Nine sections were recognized under *Rheum* by Losina-Losinskaya (1936), and three medicinally

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Table 1. Pollen characteristics of *Rheum* under LM (size and shape) and SEM (ornamentation) from China.

Section	Species	Shape	Size P×E (μm)
Sect. I *Rheum*	*R. undulatum*	subspheroidal-prolate	37.2(31.9-38.8)×31.1(28.1-34.4)
	R. franzenbachii	subspheroidal	32.0(28.1-37.5)×32.8(28.1-34.4)
	R. franzenbachii var. *weichangense*	subspheroidal	28.5(26.9-30.6)×29.5(28.1-31.2)
	R . australe	subspheroidal	26.9(23.8-28.8)×25.5(21.9-28.1)
	R. webbianum	subspheroidal	25.3(23.8-26.9)×25.2(23.8-26.9)
	R. hotaoense	prolate	40.8(34.4-43.8)×32.6(31.2-37.5)
	R. wittrockii	subspheroidal-ellipsoidal	30.7(25-34.4)×31.4(29.4-32.5)
	R. forrestii	subspheroidal	31.6(30-37.5)×29(25.6-31.2)
	R. likiangense	subspheroidal	28(28.1-37.5)×28.9(26.2-33.1)
	R. lhasaense	subspheroidal	33.1(31.2-37.5)×32.5(31.2-36.9)
	R. compactum	subspheroidal	28.2(23.8-31.2)×26.8(25-28.8)
	R. glabricaule	subspheroidal	30.5(28.1-33.1)×26.7(25-28.1)
	R. glabricaule var. *brevilobum*	subspheroidal	30.6(28.1-34.4)×27.8(25-31.2)
	R. ovatum	subspheroidal	28.5(26.9-31.2)×26.8(25-31.2)
Sect. II *Palmata*	*R. officinale*	subspheroidal	30.6(26.9-31.9)×28.7(26.9-31.2)
	R. palmatum	subspheroidal	31.4(26.9-34.4)×29.3(25-31.9)
	R. tanguticum	subspheroidal-prolate	33.8(31.2-38.8)× 22.9(18.8-25.6)
	R. tanguticum var. *liupanshanense*	subspheroidal-prolate	31.6(28.8-34.4)×27.6(25-28.1)
	R. laciniatum	subspheroidal	29.9(26.2-31.9)×28.3(25-31.2)
Sect. III *Acuminata*	*R. acuminatum*	subspheroidal	31.3(25-36.9)×29.8(25-30.6)
	R. maculatum	subspheroidal-prolate	32.4(31.2-34.4)×21.5(18.8-25)
	R. kialense	subspheroidal-prolate	30.9(28.8-34.4)×25.3(20.6-28.1)
Sect. IV *Deserticola*			
Ser.1 *Racemiferae*	*R. racemiferum*	subspheroidal	31.7(30-37.5)×30.9(30-31.2)
2946 (PE)			
	R. sublanceolatum	prolate	34.1(31.2-34.4)×24.7(18.8-28.1)
	R. inopinatum	prolate	34.3(31.2-37.5)×28.1(25.6-31.2)
Ser. 2 *Pumilae*	*R. pumilum*	subspheroidal	31.1(29.7-34.4)×27.8(25-29.7)
	R. delavayi	subspheroidal-prolate	30.6(25-37.5)×23.8(21.9-26.6)

Pollen type	Ornamentation	Voucher (PE or BMU)
3	microechinate, tectum uneven, smooth sparsely foveolate to perforate	Longde Ningxia 91-02-017 (PU)
1	microechinate tectum smooth and densely perforrated	Wutaishan Shanxi 01506 (PU)
2	microechinate, microreticulate, relatively narrow muri, small lumina	Weichang Hebei J. H. Zheng Y. X. Qing 5 (PU)
3	microechinate, tectum uneven, smooth sparsely foveolate to perforate	Qingzang Group 751366 (PU)
3	microechinate-foveolate, relatively sparser foveole and denser microechinus	Pulan Xizang Tibet Exped. 4128 (PU)
3	microechinate, tectum uneven, smooth sparsely foveolate	Ledu Qinghai M. H. Yang L. Xiang W. Wang Q99130 (PU)
1	microechinate, tectum smooth and densely perforated	Nanshan Xinjiang 814386 (PU)
3	tectum uneven, smooth sparsely perforate	Lijiang Yunnan Z. J. Gao 1298 (PU)
3	microechinate, tectum even to uneven, smooth sparsely foveolate to perforate	Yushu Qinghai S. W. Liu 2339 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate	Lasa Xizang F. Xue A. M. Shang 011 (PU)
1	microechinate, tectum smooth and densely perforated	FuYun Xinjiang 11575 (PU)
1	microechinate, tectum smooth and densely perforated	Zhuoni Gansu F. Xue A. M. Shang 019 (PU)
3	microechinate, tectum even to uneven, smooth sparsely foveolate to perforate	Zhuoni Gansu F. Xue A. M. Shang 020 (PU)
1	microechinate, tectum smooth and densely perforated	Chengduo Qinghai S. W. Liu 2548 (PU)
2	microechinate, microreticulate, relatively wide muri, large lumina	Sichuan X. B. Zhang 168 (PU)
1	microechinate, tectum smooth and densely perforated	Changdu Xizang J. S. Yang 91-604 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate to foveolate	Xiahe Gansu K. T. Fu 1007 (PE)
2	microechinate, microreticulate, relatively wide muri, large lumina	Longde Ningxia H. B. Zhang 9101 (PU)
2	microechinate, microreticulate, relatively wide muri, large lumina	Longde Ningxia H. B. Zhang 9102 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate	Lijiang Yunnan Q. W. Wang 71148 (PE)
3	microechinate, tectum even to uneven, smooth sparsely perforate	Maoxian Sichuan T. He Z. L. Zhou 12644 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate	Daocheng Sichuan X. Wang 86023(PU)
3	microechinate, tectum even to uneven, smooth sparsely foveolate	Alashanqi Neimeng Y. Q. He
3	microechinate, tectum even to uneven, smooth sparsely foveolate to perforate	Xiahe Gansu L. H. Zhou K. Z. Zhang 1505 (PU)
1	microechinate, tectum smooth and densely perforated	Xizang J. S. Yang 90-177 (PU)
1	microechinate, tectum smooth and densely perforated	Maduo Qinghai M. H. Yang L. Xiang W. Wang Q99140 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate	Lijiang Yunnan Y. Z. Zhao 21276 (PU)

Ser. 3 *Nana*	*R. uninerve*	subspheroidal-prolate	34.2(31.2-37.5)×29.5(28.1-31.2)
	R. nanum	subspheroidal	29.4(26.9-31.2)×30.2(28.1-31.9)
	R. tibeticum	subspheroidal	28.2(25-31.2)×25.7(23.4-28.1)
Sect. V *Orbicularia*	*R. tataricum*	prolate	35.2(31.2-37.5)×31.1(28.1-34.4)
Sect. VI *Spiciformia*	*R. spiciforme*	subspheroidal-prolate	28.1‘(26.2-30)×28.6(26.2-30.6)
	R. moocroftianum	subspheroidal	28.6(25.6-30)×27.38(25-30)
	R. rhomboideum	subspheroidal-prolate	27.1(25-28.1)×28.2(26.9-29.4)
	R. przewalskyi	subspheroidal-prolate	29.6(26.2-31.2)×26.9(25-31.2)
	R. rhizostachyum	subspheroidal-prolate	28.2(26.9-30)×26.4(25-28.1)
	R. reticulatum	subspheroidal	28.9(26.2-31.2)×27.8(21.9-30)
Sect. VII *Globulosa*	*R. globulosum*	subspheroidal	32.3(29.4-37.5)×30.3(28.1-31.9)
Sect. VIII *Nobilia*	*R. nobile*	subspheroidal	23.3(20.6-25)×24.5(23.1-30)
	R. alexandrae	prolate	31.3(28.1-34.4)×26(23.1-31.2)

PE: The Herbarium of the Institute of Botany, the Chinese Academy of Sciences

important species were ascribed to the sect. *Palmata*. Sect. *Palmata* was considered to be closely related to sect. *Rheum*, and both sections were the primitive groups of the genus. Kao & Cheng (1975) acknowledged only 5 sections of Losina-Losinskaya's (1936) and erected two new sections. Li (1998) added another section in Flora of China. Up to now, there have been 8 sections under *Rheum*, according to Li (1998). He further accepted Losina-Losinskaya's (1936) phylogenetic hypothesis of *Rheum*, although no new data was given to support it. Furthermore, our field expedition found that three medicinally important species are morphologically interwoven. The anatomical research also indicated that the three species were similar to and can not be distinguished from one another (Li & Zhang 1983).

Pollen morphology is one line of important evidence for phylogenetic and evolutionary analyses (Erdtman 1952; Walker 1974). Nowicke & Skvarla (1977) first reported only one species of the genus *Rheum*, *R. delavayi*. Later, Leeuwen et al. (1988) and Wang (1995) described another species, *R. officinale*. Zhang & Zhou (1998) added the pollen data of *R. palmatum* in their monograph on pollen morphology of Polygonaceae from China. They further thought that the pollen morphology of *R. palmatum* and *R. officinale* is similar and named as the *Rheum*-type pollen of Polygonaceae. In an effort to provide more data to understand the phylogeny and evolution of *Rheum*, especially referring to its diversity in NW China, we examined the pollen morphology of 40 species, representing all the 8 sections of the whole genus, most of them occuring in the highlands of NW China. In addition to the microechineate-perforate and fine-reticulate pattern named as Rheum-type, four new pollen patterns were found. The present paper reports the results of the observations and gives a comprehensive analysis of the pollen morphology with special reference to its evolutionary and ecological significance.

Materials and methods

Pollen grains were examined under light microscope (LM) and scan electronic microscope (SEM). All materials were collected from dry specimens in the Herbarium (PE) of the Institute of Botany, the Chinese Academy of Sciences and the Herbarium of School of Pharmaceutical Sciences, Beijing Medical University. For LM observation, pollen grains were acetolysed by the standard method (Erdtman 1960), and mounted in glycerine jelly. For SEM observation, they were fastened on double-sided adhensive tape, coated with gold for 3 min, and examined and photographed in a Hitachi S-800 SEM. Equatorial diameter and polar axis were measured for 20 grains for each species under LM, and means and ranges are presented. Descriptions of aperture, ornamentation and other characters are based on SEM observation. Terminology follows Leeuwen et al. (1988), Zhang & Zhou (1998) and Punt et al. (1994). The species examined for this study are listed in Table 1.

3	microechinate, tectum even to uneven, smooth sparsely foveolate to perforate	Ningxia Y. Q. He 2172(PU)
1	microechinate, tectum smooth and densely perforated	Balikun Xinjiang M. H. Yang L. Xiang W. Wang 99120 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate	Kangding Sichuan F. Xue A. M. Shang 024 (PU)
3	microechinate, tectum even, smooth sparsely foveolate	Hejing Xinjiang C830036 (PU)
3	tectum uneven, smooth sparsely perforate	Changdu Xizang J. S. Yang 91-603 (PU)
1	microechinate, tectum smooth and densely perforated	84-A-176 (PU)
3	microechinate, tectum uneven, smooth sparsely perforate	Kekexili Qinghai R. F. Huang K-101 (PU)
4	indestinctly rugulate, low relief	Yushu Qinghai W. Y. Wang 177 (PU)
1	microechinate, tectum smooth and densely perforated	Hetian Xinjiang (PU)
3	microechinate, tectum uneven, smooth sparsely perforate to foveolate	Tashikuergan Xinjiang Tibet Exped. 3206 (PU)
5	distinctly and densely microechinate, sparsely perforate	Xizang Exped. Team 208 (PU)
6	distinctly rugulate, high relief, verrucate	Xizang Tibet Exped. 1404 (PU)
3	microechinate, tectum even to uneven, smooth sparsely perforate to foveolate	Kangding Sichuan B. Z. Guo W. Y. Wang 20664 (PU)

PU: The Herbarium of School of Pharmaceutical Sciences, Beijing Medical University

Result

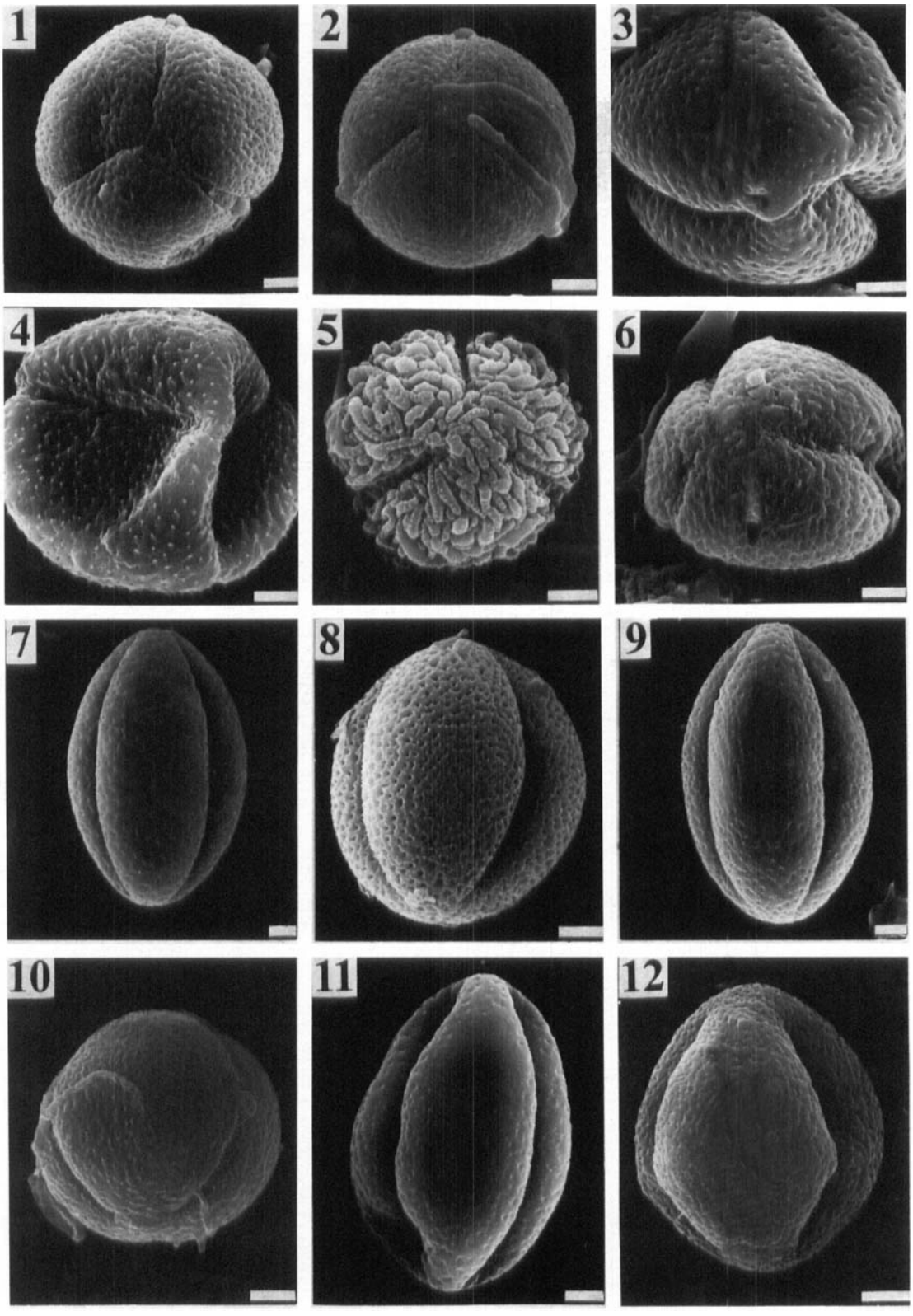
Pollen morphology of 40 species representing 8 sections of *Rheum* was examined under LM and SEM. The pollen morphology of each species studied is listed in Table 1 and partly shown in Figs 1-24.

The polar axis of the pollen grains measured 20.6-43.8 μm long, equatorial diameter 18.8-37.5 μm. The grains are spheroidal to subspheroidal, elliptic in equatorial view, more or less triangular or circular in polar view. The pollen grains are 3-colporate, colpi long and wide with acute ends, ora usually distinct, rarely indistinct. The sexine is thicker than the nexine. The exine ornamentation shows a great variation, and can be distributed on six main types: (1) microechinate-perforate type (Figs 1, 7, 9, 16, 18), with micro-echinate, tectum smooth and densely perforated ornamentation, occurring mainly in sect. *Rheum*, but seldom in sect. *Palmata*, sect. *Desertico-la* and sect. *Spiciformia;* (2) finely-reticulate type (Figs 8 and 17), with microechinate, microreticolate ornamentation, occurring mainly in sect. *Palmata*, and sparsely in sect. *Rheum;* (3) microechinate-foveolate type (Figs 2, 3, 6, 10, 11, 15, 19, 20, 24), with microechinate, tectum even to uneven, smooth sparsely foveolate/perforate ornamentation, occurring in sect. *Rheum*, sect. *Palmata*, sect *Acuminata*, sect. *Deserticola*, sect. *Orbicularia*, sect. *Spiciformia* and sect. *Nobilia*; (4) rugulate type (Figs 12 and 21), with indestinctly rugulate, low relief ornamentation, only occurring in Sect. Spiciformia; (5) verrucate – perforate type (Fig. 22), only occurring in the sect. *Globulosa* and (6) verrucate-rugulate type (Figs 5, 14, 23), with distinctly rugulate, high relief, verrucate ornamentation, only occurring in sect. *Nobilia.*

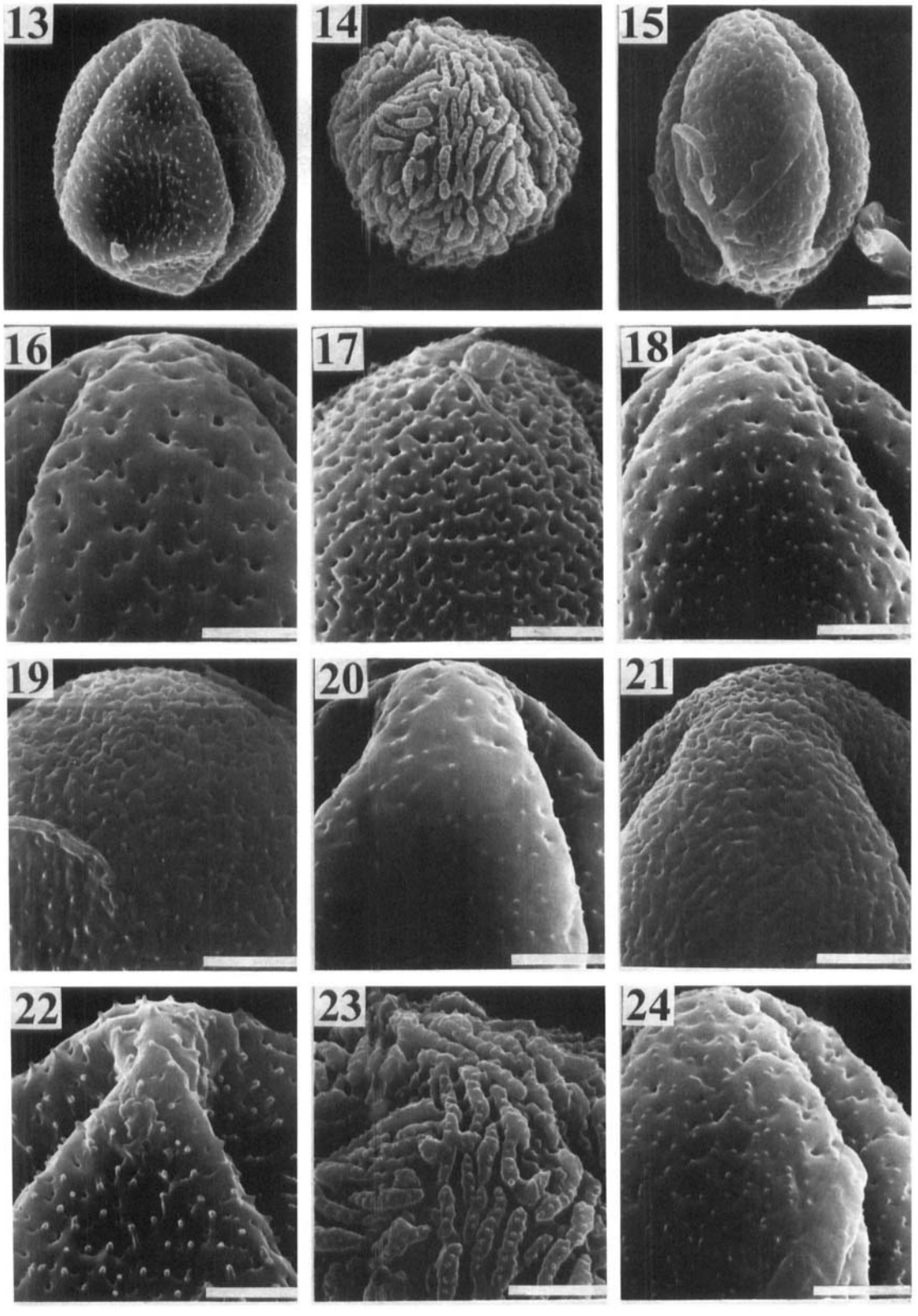
Discussion

The evolutionary trend of the pollen types

It is generally believed that the pollen grains of *Rheum* are similar in their sculpture on the exine with microechinate-perforate exine sculpture of *Rheum*-type (Zhang & Zhou 1998). Our observations reveal that the pollen morphology of this genus is diverse. At least six pollen types can be distinguished. The microechinate-foveolate/perforate type predominates in the the whole genus and it should be considered as the basic or primitive type of *Rheum*. According to Walker (1974), reduction of exine, from tectate to semitectate to intectate, could be regarded as the main trend in the pollen evolution of the angiosperms. Investigations on the pollen morphology of the whole Polygonaceae confirmed this evolutionary trend (Leeuwen et al. 1988; Zhang & Zhou 1998; and the present results). The evolutionary trend of the pollen morphological variation in *Rheum* is therefore considered as follows: (a) the common microechinate-foveolate/perforate exine pattern be the most primitive due to its universal existence in the genus; (b) the fine-reticulate (microreticulate) type be derived from the primitive micro-



Figs 1-12. SEM Photographs of pollen grains of *Rheum*. 1 and 9. *R. palmatum*; 2 and 10. *R. tanguticum*; 3 and 11. *R. tataricum*; 4. *R. globulosum*; 5. *R. nobile*; 6. *R. alexandrae*; 7. *R. wittrockii*; 8. *R. officinale*; 12. *R. przewalskyi* – Bar: 2μm.



Figs 13-24. SEM Photographs of pollen grains of *Rheum*. 13 and 22. *R. globulosum*; 14 and 23. *R. nobile*; 15 and 24. *R. alexandrae*; 16. *R. wittrockii*; 17. *R. officinale*; 18. *R. palmatum*; 19. *R. tanguticum*; 20. *R. tataricum*; 21. *R. przewalskyi* – Bar: 2μm.

echineate-foveolate/perforate pattern, followed by the rugulate and verrucate-perforate ornamentations and (c) the verrucate-rugulate ornamentation be the most advanced. This evolutionary trend of the pollen types in *Rheum* is tentative and needs confirmed with further evidence.

The systematic and ecological significance

As stated above, the principle center of diversity of *Rheum* is NW China or the Himalaya Mountains, where eight sections and about 40 species, many of them being endemic, occur, according to Li (1998). The morphologically primitive groups, e.g., sect. *Rheum* and sect. *Palmata*, are also distributed in this area. Thus the area could be as well considered as the origin center of *Rheum*. Pollen data are positively correlated with the morphological data, which also indicate that this area might be both of the diversity and origin centers of *Rheum*. All five pollen types, from the most primitive type to the most advanced one, were found in NW China and its adjacent areas.

More than one pollen types were found in the same section (see Table 1), which makes it new difficulty to compare the pollen data with the morphology-based traditional classification and phylogenetic hypothesis. However, once a taxon bears both plesiomorphic and apomorphic characters, the characters are usually considered as plesiomorphy (Farris 1982). If this rule is applied to *Rheum*, we find that most sections have the basic and primitive microechinate-foveolate/perforate exine pattern. The result indicates that *Rheum* is a natural group from a common ancestry. Both the above-mentioned evolutionary trend of the pollen type and the fact of occurrence of more than one pollen type in the same section indicate that there exists an obvious parallel-evolution or, on the other hand, some types within a section might be extincted in *Rheum*.

The parallel evolution of the pollen types in *Rheum* is correlated with geographical distribution and with ecological environment. The more primitive types usually appear at low altitudes regions while the more advanced pollen types are found at high altitude regions (e.g. sect. *Globulosa* and sect. *Nobilia*, which usually grow at 3000-5000 m in the Himalaya, have the most advanced pollen type). This correlation occurs within the same section. For example, sect. *Rheum* and sect. *Palmata*, the primitive groups of *Rheum*, are widely distributed in China. Pollen grains of the species in these two sections at low altitude regions have the microechinate-foveolate/perforate exine pattern while the species at high altitude regions have fine-reticulate type.

Zhang & Zhou (1998) misplaced the pollen type of *R. officinate*, described by Leeuwen et al. (1988) and Wang (1995), into the microechinate-perforate type. In fact, both the legends and the figures of Leeuwen et al. (1988) and Wang (1995) showed that the pollen of this species has finely-reticulate ornamentation, which is further confirmed by the present research. The three medicinally important species, *R. officinale, R. palmatum* and *R. tanguticum* can be palynologically distinguished from their ornamentations.

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