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COMPARATIVE PALYNOLOGY AND ANATOMY OF *PINUS HENRYI*, *PINUS MASSONIANA* AND *PINUS TABULAEFORMIS* (PINACEAE) AND THEIR TAXONOMIC IMPLICATIONS

ZHI HONG LIU, QING XIE AND ZHOU QI LI*

College of Forestry, Northwest A & F University, Yangling, Shaanxi, 712100, China
**Corresponding author's e-mail: lizhouqi@nwsuaf.edu.cn; Tel (fax): 86-29-87082992*

Abstract

In the present study, micromorphological and anatomic characteristics of 3 *Pinus* L. taxa (*Pinus henryi*, *P. massoniana* and *P. tabulaeformis*) were compared. These taxa have both endemic and limited distribution in China. For the micromorphological studies, the pollens were examined using light microscopy (LM) and scanning electron microscope (SEM). For the anatomical studies, needle anatomy characteristics of the 3 taxa were studied. After the studies, new characteristics of corpus exine ornamentation had been found. Spiny on the regulate of corpus exine is presented in *P . henryi* while baculate on the rugulate of corpus exine is existed in *P. tabuliformishave*. However, there is not ornamentation on rugulate in *P. massoniana*. The features of pollen size, corpus exine ornamentation, number of microperforations, cappula ridges are significant to distinguish the 3 taxa. Number of resin canals, stomatal rows, epithelial cells and sheath cells, dimensions of cross section, dimensions of resin canals and width of needle are also important in separating these taxa. These studies give taxonomic support for recognizing *P. henryi* as an independent species closely related to *P. massoniana*.

Key words: *Pinus henri*; *Pinus massoniana*; *Pinus tabulaeformis*; Pollen grain; Leaf anatomy.

Introduction

P. henryi investigated in this study is endemic in China. There is controversy regarding the taxonomic status of *P. henryi* from 19th century to the present. *P. henryi* is either considered synonymous to *P. tabulaeformis* (Shaw, 1948) or a variety of *P. massoniana* (Wu, 1956), a variety of *P. tabulaeformis* (Guan, 1982), or an independent species (Zheng, 1975, 1983; Niu, 1990; Li & Xu, 1989; Zhang *et al*., 1995; Zhao & Liu, 2010). According to the comparative studies on morphology (Mao & Liu, 1989) and wood anatomy (An & Zhao, 1992) of *P. henryi*, *P. massoniana* and *P. tabulaeformis*, these 3 taxa are similar, however, the differentiation still have been found. Qu (1990) recognized that the genetic relationship between *P. henryi* and *P. tabulaeformis* are closer than *P. henryi* and *P. massoniana* based on chemical composition.

There are plenty of studies concerning pollen morphology and needle anatomy based on LM or SEM (Zhang, 1989; Nakagawa *et al*., 1996, 2000; Sun, 2002; Huysmans *et al*., 2003; Wang *et al*., 2005; Wu *et al*., 2007; Fukuda *et al*., 2008; He *et al*., 2008; Fan *et al*., 2010; Liu *et al*., 2010; Yurdanur *et al*., 2012). However, palynological structures of *P. henryi* have not been studied before. Comparative account of needle anatomical structures of *P. henryi* and other 4 species of *Pinus* L. have been studied by Zhao & Liu (2010), but studies at the species level are less readily available. In this paper, *P. henryi*, *P. massoniana* and *P. tabulaeformis* are selected to observe their pollen morphological characteristics and needle anatomical characteristics by LM and SEM, to clarify the taxonomic position of *P. henryi*.

Materials and Methods

The pollen grains and two-year old needles were collected from different localities in China (Table 1).

Fresh pollen grains derived from at least 10 different mother trees, mounted in 0.6% agar, more than 20 fresh pollen grains were studied under Olympus-BX51 LM with a Moticam2306 camera. The grains were treated according to Nakagawa's (2000) and observed with (HITACHI, S-4800) SEM, the representatives were photographed. Most of the descriptive terms were taken from Erdtman (1952), Kremp (1965), Bagnell (1975), Nakagawa (2000), Zanni and Ravazzi (2007). The terminology is explained in Fig. 1.

Needles were preserved in FAA fixative and then freehand section was applied (Wang *et al*., 2007). Photographs were taken using Olympus-BX51 LM with a Moticam2306 camera. Measurements were carried out by Motic Images Advanced 3.2. The squared euclidean distance and the average Linkage (Between taxa) was adopted for H-cluster analysis by SPSS statistic 17.0.

Results and Discussion

Pollen morphological analysis by LM and cluster analysis: Detailed measurements of palynological features of *P. henryi*, *P. massoniana* and *P. tabulaeformis* are shown in Table 2. There are differences on the size of E_1t, E_1c, E_2c, E_1c, SpE_1s, E_1g, PC, Pt, d_1s, d_2s, E_1s, E_2s, Bs, A_1 and on the ratio of E2s/E2c, E1c/E1t, E1c/E2c, E1s/E2s, d1s/d2s. Pollen size of *P. henryi* is the smallest while the angular between cappa and sacci is the biggest. E1c<E2c in *P. massoniana*, while E1c>E2c in *P. tabulaeformis* and *P. henryi*. Outline is even, or slightly undulate only in the region of saccus attachment. Sacci results narrower than the corpus (E2s<E2c) of all taxa in polar view (Fig. 2).

Table 1. **Location of studied pollen and needle samples.**

Taxa	Localities	Position	Altitude (m)
P. henryi	Nanzheng, Shaanxi	106°6′E/32°9′N	1254.1
P. massoniana	Yangxian, Shaanxi	107°6′E/33°3′N	735.0
P. tabulaeformis	Huanglong, Shaanxi	108°7′E/36°0′N	1327.1

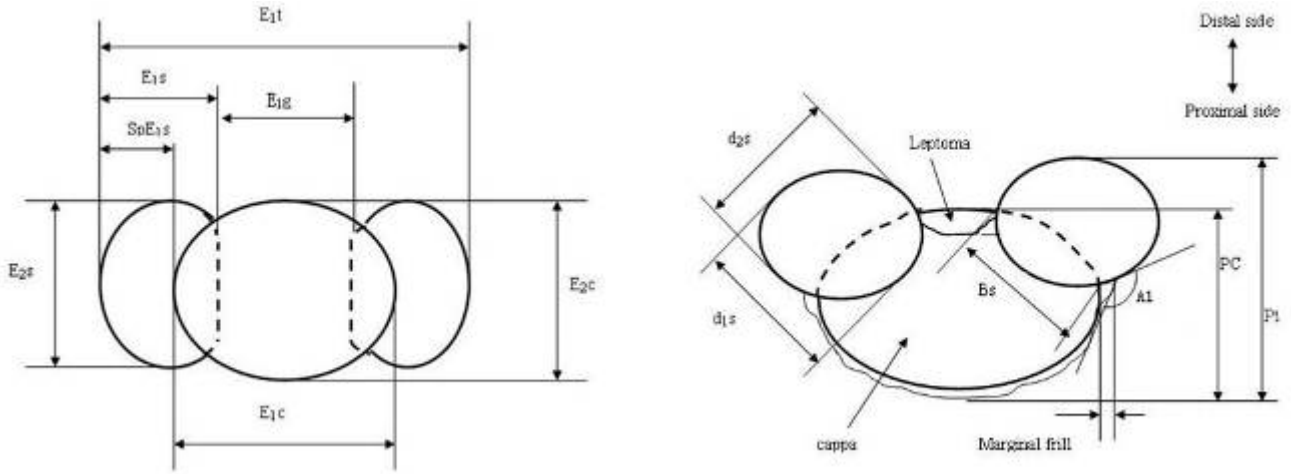

Fig. 1. Terminology for the LM and SEM description of the pollen grains.

Table 2. Results of the pollen morphological analysis.

Characteristics [a]	*P. henryi*	*P. massoniana*	*P. tabulaeformis*
E_1t (μm)	46.66-(68.53)-79.07	64.61-(71.05)-79.08	54.68-(74.01)-88.92
E_1c (μm)	41.77-(50.70)-64.99	40.80-(48.51)-55.57	45.65-(56.65)-71.71
E_2c (μm)	40.08-(49.96)-63.21	39.91-(50.45)-58.85	35.43-(52.95)-64.31
SpE_1s (μm)	6.60-(10.66)-14.08	6.30-(10.89)-14.90	5.30-(9.35)-13.25
E_1g (μm)	6.79-(13.13)-18.92	7.39-(13.59)-19.33	9.20-(15.65)-22.29
PC (μm)	34.93-(43.95)-52.65	33.85-(45.13)-52.13	44.75-(49.98)-60.53
Pt (μm)	42.55-(48.51)-55.55	36.43-(48.35)-53.93	47.23-(54.22)-64.93
d_1s (μm)	22.35-(26.79)-34.35	22.23-(28.48)-37.65	26.05-(30.00)-36.13
d_2s (μm)	26.10-(31.76)-37.53	26.73-(34.30)-41.10	27.73-(36.06)-43.65
E_1s (μm)	31.61-(41.48)-66.57	35.81-(42.47)-48.58	28.01-(43.81)-52.53
E_2s (μm)	23.55-(27.4)-34.15	22.90-(27.85)-32.18	22.70-(30.74)-39.38
Bs (μm)	22.06-(31.34)-43.67	25.17-(32.32)-40.70	29.58-(35.19)-42.78
A_1 (°)	101.31-(136.46)-173.57	112.10-(134.19)-155.16	104.20-(123.38)-143.40
E2s/E2c	0.41-(0.55)-0.76	0.46-(0.56)-0.71	0.36-(0.58)-0.84
E1c/E1t	0.62-(0.74)-0.96	0.62-(0.68)-0.77	0.64-(0.77)-0.98
E1c/E2c	0.84-(1.02)-1.16	0.83-(0.97)-1.11	0.85-(1.09)-1.67
E1s/E2s	1.28-(1.54)-1.28	1.24-(1.53)-2.03	1.03-(1.48)-2.08
d1s/d2s	0.70-(0.88)-1.01	0.68-(0.84)-1.32	0.69-(0.84)-1.07

[a]Minimum – (average) – Maximum

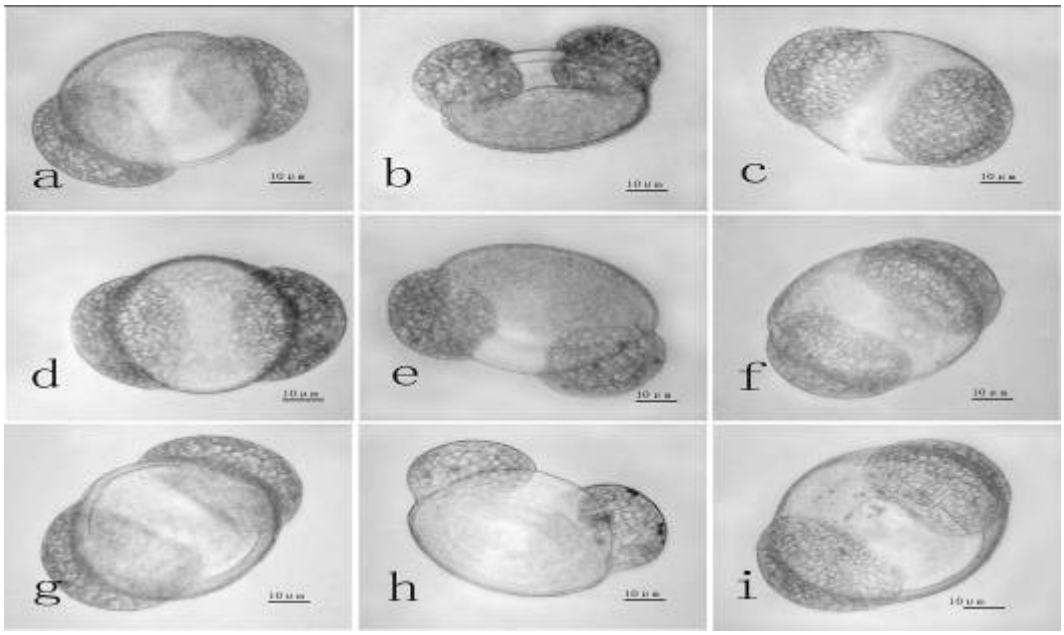

Fig. 2. LM pictures of pollens of *P. henryi*(a-c), *P. massoniana*(d-f) and *P. tabulaeformis*(g-i). a-i. ×40.

The genetic relationships among the 3 taxa are analyzed by the H-cluster analysis (Fig. 3). In the dendrogram, *P. henryi* is clustered with *P. massoniana*, and they are clustered further with *P. tabulaeformis*. The squared euclidean distance value between *P. henryi* and *P. massoniana*, *P. henryi* and *P. tabulaeformis* are 11.064, 56.542, respectively, which indicates that *P. henryi* has a closer genetic relationship with *P. massoniana*.

Pollen characteristics by SEM: On a descriptive level, we focused on the following common features of the 3 taxa. The pollen grains are bisaccate (Fig. 4a-c, Fig. 5a-c, Fig. 6a-c). Saccus/cappa attachment is sharp in proximal view (Fig. 4a, Fig. 5a, Fig. 6a). In equatorial view, marginal frill is absent in some cases (Fig. 4d, Fig. 5d, Fig. 6d). cappa/leptoma transition is faint, characterized by the reduction of the undulations (Fig. 4e, f, Fig. 5e, f, Fig. 6e, f).

Saccus surface is smooth and microperforated in its "apical" area, the perforations are sometimes ornamentation on the rugulate in *P. massoniana* (Fig. 5j). The distal surface of the leptoma region is wrinkled normally. In some cases, cappula ridges (Fig. 5k) appear in leptoma region in *P. massoniana*, which could not be found in *P. henryi* and *P. tabulaeformis* in this study. The perforations are sometimes connected by furrows (Fig. 4g, h, Fig. 5g, h, Fig. 6g, h). The number of microperforations is the least in *P. massoniana*.

Corpus exine ornamentation is another distinguishing character. Corpus exine on cappa rugulate, deeply sculptured (Fig. 4i, Fig. 5i, Fig. 6i). Spiny on the regulate of corpus exine is present in *P. henryi* while baculate on the rugulate of corpus exine exist in *P. tabuliformishave*. However, there is no ornamentation about rugulate in *P. massoniana*.

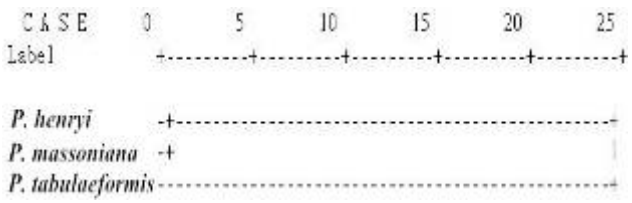

Fig. 3. Dendrogram of 3 taxa based on pollen characteristics.

Needle anatomical characteristics by LM and cluster analysis: Needle anatomy characteristics of *P. henryi*, *P. massoniana* and *P. tabulaeformis* are shown in Table 3 and Fig. 7. Number of stomatal rows on convex side less than 7 in *P. henryi*, while more than 7 in *P. massoniana* and *P. tabulaeformis*. Sheath cells number of *P. massoniana* less than 9, while more than 10 in *P. henryi* and *P. tabulaeformis*. Epithelial cells number of *P. tabulaeformis* are more than 9, while less than 7 in *P. henryi* and *P. massoniana*. Width of needle is shorter than 1mm in *P. massoniana*, while more than 1 mm in *P. henryi* and *P. tabulaeformis*. *P. tabulaeformis* has more resin canals and stomatal rows on flat side than *P. henryi* and *P. massoniana*. The dimensions of cross section, average dimensions of resin canals and total dimensions of resin canals are the largerest in *P. tabulaeformis*.

In the dendrogram (Fig. 8), as the similar result as cluster analysis based on pollen morphological analysis, *P. henryi* is clustered with *P. massoniana*, and they are clustered further with *P. tabulaeformis*. The squared euclidean distance value between *P. henryi* and *P. massoniana*, *P. henryi* and *P. tabulaeformis* are 16.509, 30.745, respectively. *P. henryi* has a closer genetic relationship with *P. massoniana*.

The remarkable differences among the 3 taxa are corpus exine ornamentation, pollen size, number of microperforations, cappula ridges present or not. Number of resin canals, stomatal rows, epithelial cells and sheath cells, the dimensions of cross section, dimensions of resin canals and width of needle are also important to distinguish each other. These characteristics could be used for classification. Though *P. henryi* and *P. massoniana* have similar structural characteristics and closer relationship, *P. henryi* can be still identified by comparing characteristics and measurements datum of palynology and needle anatomy. Therefore, *P. henryi* should be considered as an independent species closely related to *P. massoniana*.

Table 3. Results of the needle anatomical analysis.

Characteristics [a,b]	*P. henryi*	*P. massoniana*	*P. tabulaeformis*
Perimeter of cross section (mm)	2.85-(3.13)-3.42	2.15-(2.72)-3.92	3.32-(3.75)-4.12
Dimensions of cross section (mm^2)	0.46-(0.57)-0.72	0.27-(0.45)-0.93	0.65-(0.81)-0.92
Number of resin canals	3-(6.80)-9	3-(4.50)-8	3-(7.80)-12
Average dimensions of resin canals (μm^2)	622-(1112)-1624	578 -(1271)-2696	1087- (1578)-2813
Total dimensions of resin canals (μm^2)	3595-(7587)-11366	2157-(5151)-8087	5739-(10952)-15092
Number of epithelial cells	4-(6.84)-10	4-(6.20)-9	7-(9.83)-13
Number of sheath cells	7-(10.43)-13	7-(8.66)-12	7-(11.74)-16
Thickness of needle(mm)	0.60-(0.66)-0.80	0.47-(0.61)-0.91	0.71 -(0.81)-0.85
Width of needle(mm)	0.93-(1.09)-1.33	0.68-(0.86)-1.25	1.09-(1.25)-1.42
Needle thickness/ Width ratio	0.58-(0.61)-0.69	0.67-(0.71)-0.74	0.58-(0.65)-0.68
Number of stomatal rows on convex side	3-(6.58)-9	4-(7.20)-11	4-(7.52)-10
Number of stomatal rows on flat side	3-(4.31)-7	3-(3.93)-6	4-(6.05)-8
Minimum distance of vascular bundles (μm)	24.40-(39.90)-65.30	10.90-(22.40)-36.50	24.50-(35.70)-56.00

[a]Minimum – (average) – Maximum, [b] on the cross section

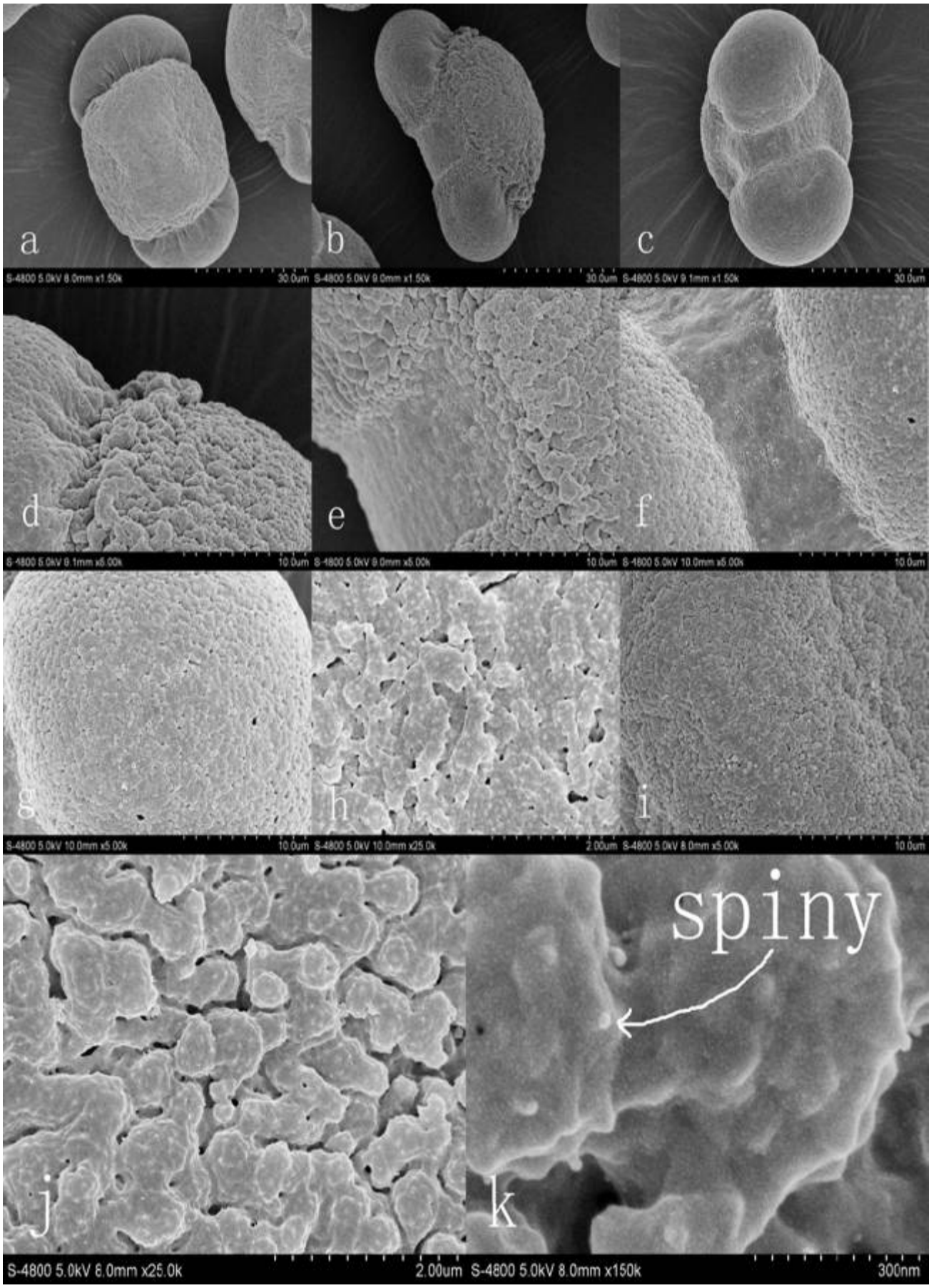

Fig. 4. SEM pictures of pollens of *P. henryi.* a. Proximal view×1500, b. Equatorial view×1500, c. Distal view×1500, d. Marginal frill×5000, e. Cappa/leptoma transition×5000, f. Leptoma×5000, g. Saccus surface×5000, h. Saccus surface×25000, i. Corpus exine ornamentation×5000, j. Corpus exine ornamentation×25000, k. Corpus exine ornamentation×150000.

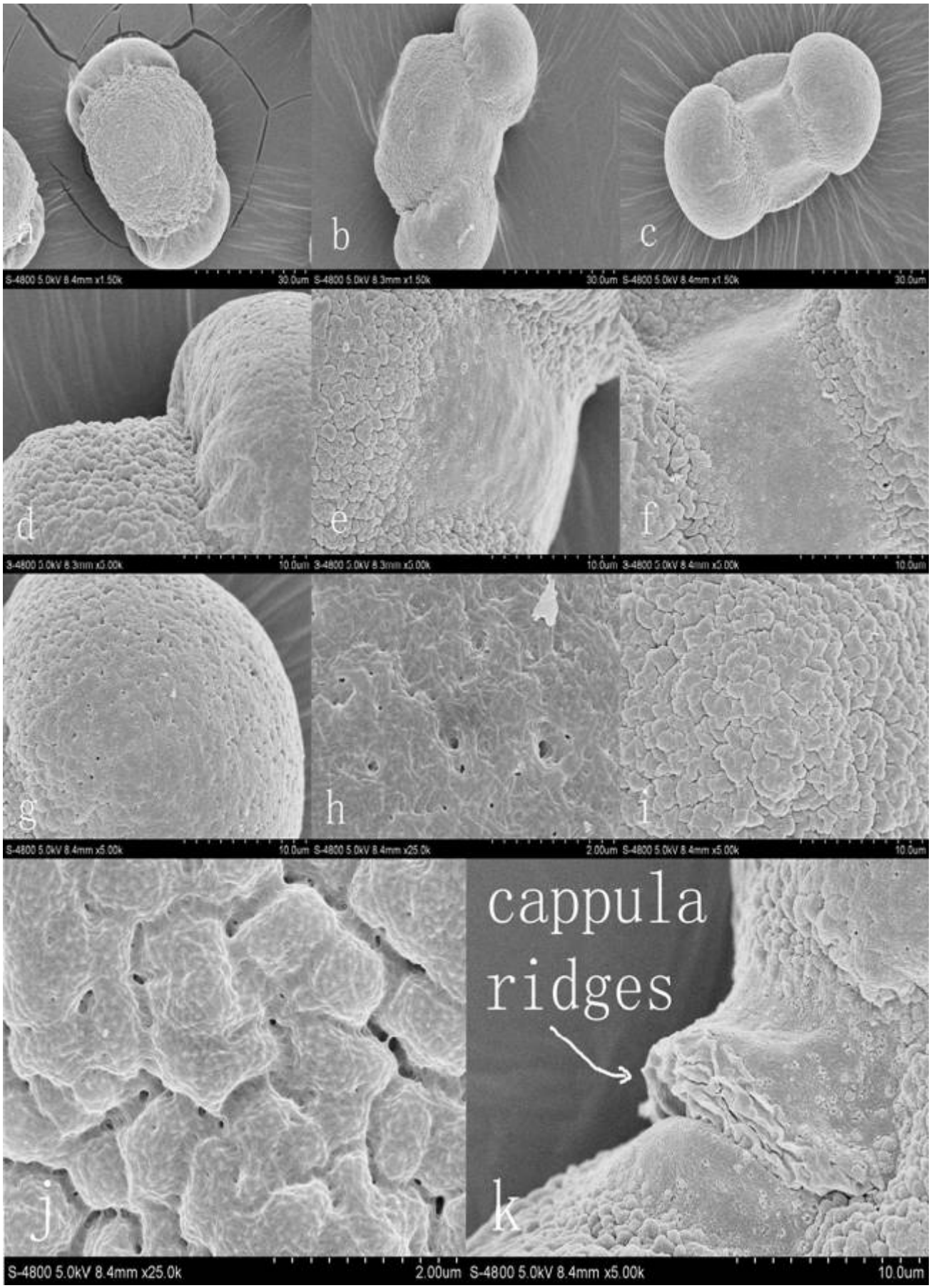

Fig. 5. SEM pictures of pollens of *P. massoniana*. a. Proximal view×1500, b. Equatorial view×1500, c. Distal view×1500, d. Marginal frill×5000, e. Cappa/leptoma transition×5000, f. Leptoma×5000, g. Saccus surface×5000, h. Saccus surface×25000, i. Corpus exine ornamentation×5000, j. Corpus exine ornamentation×25000, k. Cappula ridges×5000.

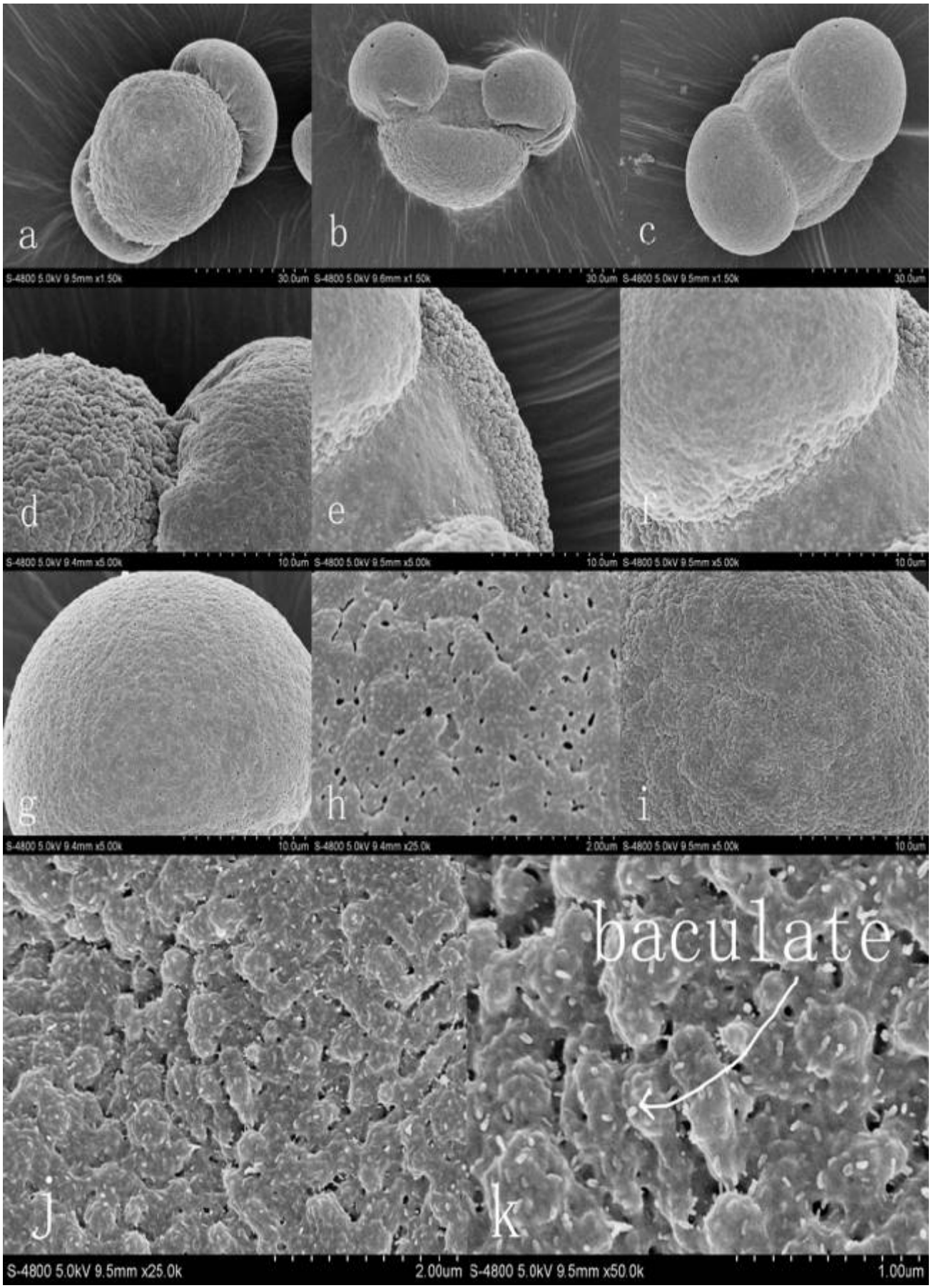

Fig. 6. SEM pictures of pollens of *P. tabulaeformis*. a. Proximal view×1500, b. Equatorial view×1500, c. Distal view×1500, d. Marginal frill×5000, e. Cappa/leptoma transition×5000, f. Leptoma×5000, g. Saccus surface×5000, h. Saccus surface×25000, i. Corpus exine ornamentation×5000, j. Corpus exine ornamentation×25000, k. Corpus exine ornamentation×50000.

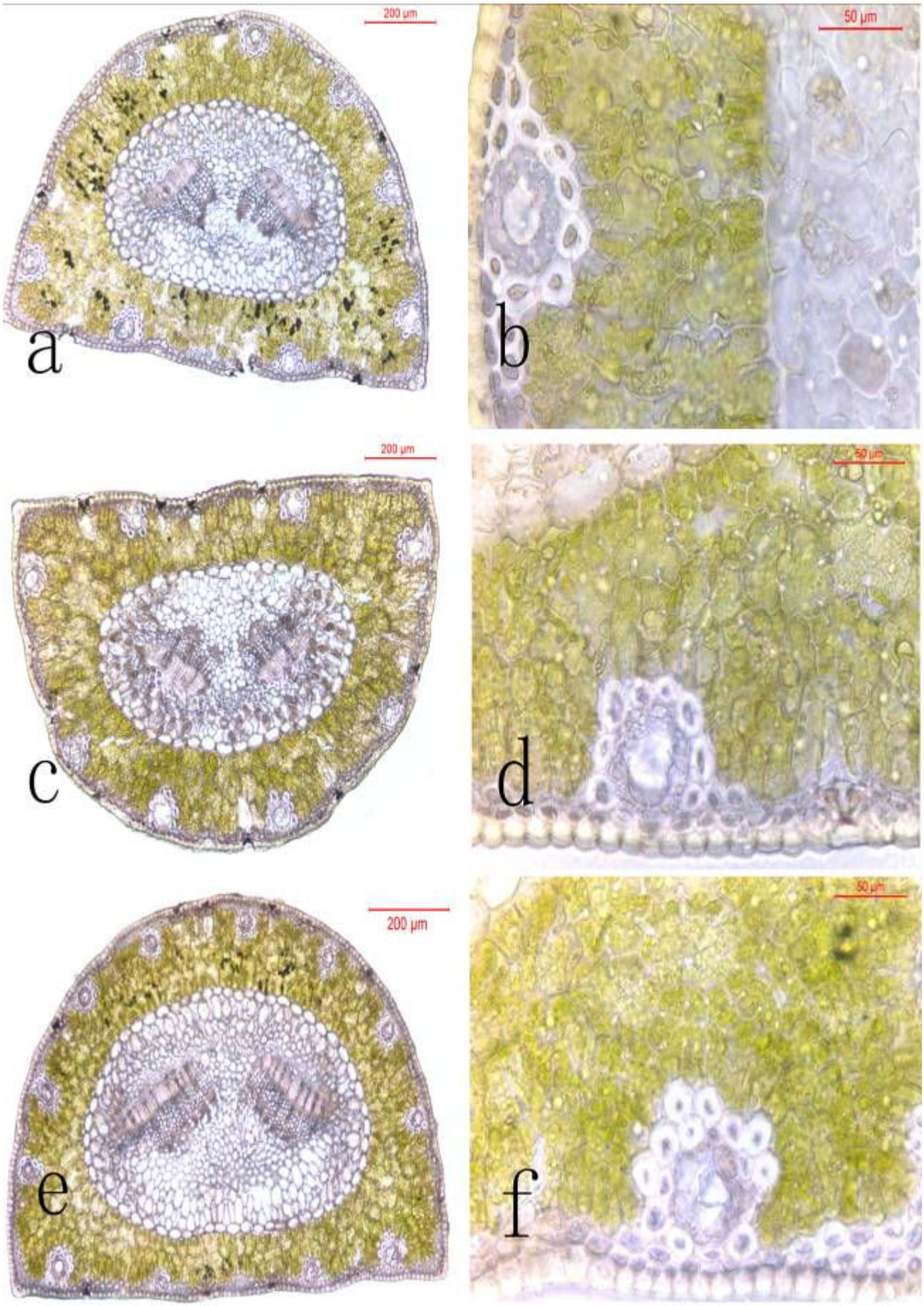

Fig. 7. Needle anatomical pictures of *P. henryi* (a, b), *P. massoniana* (c, d) and *P. tabulaeformis* (e, f). a, c, e ×40, b ×100, d, f ×200.

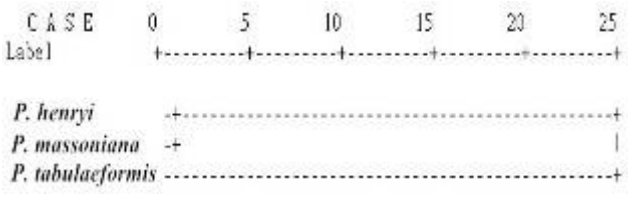

Fig. 8. Dendrogram of 3 taxa based on needle anatomical characteristics.

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References

An, P.J. and L. Zhao. 1992. Identification and wood anatomical features of *Pinus henryi* Mast. *J. Northwest Forest. Univ.*, 7(2): 1-5.

Bagnell, Jr. C.R. 1975. Species distinction among pollen grains of *Abies, Picea*, and *Pinus* in the Rocky Mountain Area (a scanning electon microscope study). *Rev. Palaeobot. Palyno.*, 19: 203-220.

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy Angiosperms*. Almqvist and Wiksell, Stockholm.

Fan, L.K., Y.M. Lu, G.J. Yan and Q.X. Zhang. 2010. Classification of Chinese wintersweet (*Chimonanthus praecox*) cultivars supported by pollen morphology. *Chin. Agri. Sci.*, 9(7): 958-964.

Fukuda, T., A. Naiki and H. Nagamasu. 2008. Pollen morphology of the genus *Skimmia* (Rutaceae) and its taxonomic implications. *J. Plant Res.*, 121: 463-471.

Guan, Z.T. 1982. *Geography of Pinaceae and Taxodiaceaein in SiChuan*. Chengdu People Publishing House, Chengdu.

He, Y.Y., G.F. Qin, A.X. Gao and Z.L. Wang. 2008. Study on the pollen morphology of *Masson Pine* and other Pine species and varieties. *Forest. Res*., 21(4): 456-463.

Huysmans, S., S. Dessein, E. Smets and E. Robbrecht. 2003. Pollen morphology of NW European representatives confirms monophyly of Rubieae (Rubiaceae). *Rev. Palaeobot. Palyno.*, 127: 219-240.

Kremp, G.O.W. 1965. *Morphologic Encyclopedia of Palynology*. The University of Arizona Press, Tucson.

Li, C.X. and H.C. Xu. 1989. A numerical analysis on the dividing line of geographic distribution and taxonomy relation between *Pinus henryi* Mast. and *P. tabulaeformis* Carr. *Sci. Silvae Sin.*, 25(1): 14-21.

Liu, J.X., J.Y. Li, Y.L. Zhang and J.C. Ning. 2010. Pollen morphology of the tribe Lithospermeae of Boraginoideae in China and its taxonomic significance. *Plant Syst. Evol*., 290: 75-83.

Mao, S.X. and Y.C. Liu. 1989. A numerical analysis on the dividing line of geographic distribution and taxonomy relation between *Pinus henryi* Mast. and *P. tabulaeformis* Carr. *J. Northwest Forest. Univ.*, 4(2): 94-99.

Nakagawa, T., Y. Yasuda and H. Tabata. 1996. Pollen morphology of Himalayan *Pinus* and *Quercus* and its importance in palynological studies in Himalayan area. *Rev. Palaeobot. Palyno.*, 91: 317-329.

Nakagawa, T., J.L. Edouard and J.L. Beaulieu. 2000. A scanning electron microscopy (SEM) study of sediments from Lake Cristol, southern French Alps, with special reference to the identification of *Pinus cembra* and other Alpine *Pinus* species based on SEM pollen morphology. *Rev. Palaeobot. Palyno.*, 108: 1-15.

Niu, C.S. 1990. *Woody flora of Shaanxi*. Chinese Forestry Publishing House, Beijing.

Qu, S.Z., F.S. Zhang and Y.D. Chen. 1990. Chemical composition of essential oils from wood and needles of several *Pinus* species, and discussion on chemotaxonomy of *Pinus henryi*. *J. Northwest Forest. Univ*., 5(2): 1-9.

Shaw. 1948. Florin in acta hort. *Berg.*, 14: 348.

Sun, J.T. 2002. Study on pollen morphology characteristics of *Pinus* L. and its taxonomic significance. *Shandong Sci.*, 15(4): 35-40.

Wang, A.H., J.L. He, C.G. Wang and Z.H. Peng. 2007. Anatomical research on resin canal in leaves of *Cedrus deodara*. *J. Anhui Agr. Univ.*, 34(1): 93-96.

Wang, C., B.Z. Hu and L.X. Ma. 2005. The observation the pollen of 3 medicine *Gentiana* and the analysis of classification. *Natur. Sci. J. Harbin Normal Univ*., 21(5): 406-412.

Wu, Z.H., J.S. Shi, M.L. Xi and G.X. Liu. 2007. Pollen characteristics and relationships of 12 species and 6 cultivars of *Lilium*. *J. of Zhejiang Forest. Univ*., 24(4): 406-412.

Wu, Z.L. 1956. The Classification and distribution of Pinaceae in China. *Acta Phytotax. Sin*., 5(3): 153-154.

Yurdanur, A., A. Emine and A.Ö. Canan. 2012. The morphological, anatomical and palynological properties of ebdemic *Haplophyllum megalanthum* Bornm. (Rutaceae). *Pak. J. Bot.*, 44(3): 1121-1126.

Zanni, M. and C. Ravazzi. 2007. Description and differentiation of *Pseudolarix amabilis* pollen Palaeoecological implications and new identification key to fresh bisaccate pollen. *Rev. Palaeobot. Palyno.*, 145: 35-75.

Zhang, C.X., F.Q. Zhang and G.M. Qiu. 1995. A study on the taxonomy position of *Pinus henryi* in Nanzheng of Shaanxi. *J. Northwest Forest. Univ.*, 10(1): 38-42.

Zhang, J.T. 1989. Study on the pollen morphology of *Pinaceae* in Chnia. *Bull. Bot. Res.*, 9(3): 87-98.

Zhao, Y. and Z.L. Liu. 2010. Numerical analysis to anatomical structures of needles between five species of Pines. *J. Northwest Forest. Univ.*, 25(2): 19-24.

Zheng, W.J. 1975. Chinese gymnosperms. *Acta Phytotax. Sin*., 13(4): 56-89.

Zheng, W.J. 1983. *Woody flora of China*. Chinese Forestry Publishing House, Beijing.

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