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POLLEN MORPHOLOGY OF GENUS *ERITRICHIUM* SCHRAD. (BORAGINACEAE) FROM PAN HIMALAYA AND CHINA (POLLEN MORPHOLOGY OF *ERITRICHIUM* SCHARD.SPECIES)

PARAS MAZARI#*, JIA-CHEN HAO#*, QUAN-RU LIU* AND LATIF AHMAD

College of Life Sciences, Beijing Normal University, 100875, Beijing, China
[*]Corresponding author's email: liuquanru@bnu.edu.cn

Abstract

The genus *Eritrichium* Schrad. comprises about 50 species, mainly distributed in Central Asia and Pan-Himalaya, with a few species in Europe and North America. There are about 43 species in China. The Infrageneric classification of *Eritrichium*has long been controversial and pollen morphology of *Eritrichium* species is still largely unknown. Furthermore, Boraginaceae is a eurapalynous and its complexity is rare in angiosperms especially in regard of pollen apertures. The aim of our study is to analyze the pollen morphology of *Eritrichium*and to evaluate its taxonomic significance. In the present work pollen morphology of 32 taxa belonging to genus *Eritrichium* have been examined by using scanning electron microscope (SEM).

Generally, pollen grains very small to small in size, radially symmetrical, isopolar, 6-heterocolpate, (tricolpate and tricolporate) mostly perprolate, prolate, and rarely subprolate in shape, constricted at the equator rarely without constriction. Exine ornamentation was very uniform among all studied taxa i.e., psilate and perforated at poles only. However, the result showed great diversity within species in regard of the shape of pollen, type of aperture, number and position of pores etc. On the basis of these characters 8 pollen types have been recognized viz. *E. laxum*-type, *E. axilliflorum*-type, *E. deqinense* -type, *E. oligocanthum*-type, *E. minimum*-type, *E. thymifolium*-type, *E.hemisphaericum* type, and *E. sessilifurcatum*-type. All the studied characters provide information for correct identification of the morphologically similar taxa. The pollen data can also be helpful for distinguishing taxa, species-level revisions.

Key words: Pollen morphology, Boraginaceae, Cynoglosseae, *Eritrichium*, SEM, Pan Himalayan.

Introduction

Boraginaceae Juss. (-The Borage or Forget me not family) is a large cosmopolitan family, comprises about 154 genera, 2500 species, however in China there are about 47 genera and 294 species of Boraginaceae, of which four genera and 156 species are endemic to China. *Eritrichium* Schrad. ex Gaudin (Boraginaceae: Cynoglosseae) comprises approximately 50 species around the world, mainly distributed in Central Asia and Pan-Himalaya, with a few species in Europe and North America (Zhu *et al.,* 1995). *Eritrichium* species are annual or perennial herbs with strigose or villous, leaves are alternate, scorpioidcymose inflorescences are branched or not with several blue or white flowers, nutlets are turbinate to ovoid and usually with winged, dentate or glochids.

The infrageneric classification of *Eritrichium* has long been controversial. At least 3 revisions (Popov 1953; Wang *et al.*, 1980; Ovchinnikova, 2003; 2008; 2011) have been proposed based on morphological characters since the establishment of the genus in 1828 (Gaudin, 1828). The most recent and comprehensive classification based on a morphological analysis of 75 *Eritrichium* species, was proposed by Ovchinnikova (2003; 2008; 2011), in which 2 subgenus, 6 sections, 2 subsections and 11 series were recognized within *Eritrichium*.

Wang *et al.*, (1980) suggested placement *Hackelia* within *Eritrichium*, because the morphological characters used to distinguish the two genera were continuous among these different species. Wang *et al.,* (1980) recognized 41 species, 1 subspecies and 2 varieties, and they also divided Chinese *Eritrichium* species into 2 subgenera and 4 sections and 11 series chiefly based on morphological character like leaf shape, the length of pedicel and the shape of fruits. In a recent revision of Chinese *Eritrichium*, Zhu *et al.,* (1995) recognized 39 species, 1 subspecies and 2 varieties, of which 24 species are endemic to China. The infrageneric classification by Wang *et al.,* (1980) was not adopted by Zhu *et al.,* (1995), and no new infrageneric classification was reconstructed. Zhu *et al.,* (1995) considered the gynobase of *Hackelia* was quite distinct from that of *Eritrichium* in shape and height, and separated the genus*Hackelia* from *Eritrichium* but the systematics of Chinese *Eritrichium* species remains controversial.

Boraginaceae was first time studied for pollen morphology by Geoferry (Perveen *et al.,* 1995). Later the family has been studied for pollen morphology by (Erdtman, 1952), Avestisian (1956), Nair (1965), Bou (1968), Marticorena (1968), Gupta, (1971), and Huyunh (1971, 1972), Nowicke & Ridgway (1973), Nowicke & Skvarla (1974), Clarke, (1977), Ben Saad-Liman (1982, 2009), Xi, (1984), Diez (1984), Qureshi & Khan (1985), Ahn & Lee, (1986), Qureshi & Qaiser (1987), Qureshi *et al.,* (1989), Ning *et al.,* (1991, 1992; 1993, 1995), Osaloo & Khatamsaz, (1994), Wang, (1995) El-Ghazaly (1995), Perveen *et al.,* (1995), Scheel *et al.,* (1996), Retief & Van Wyk, (1997), Khatamsaz, (2001), Liu *et al.,* (2001a, 2001b, 2003, 2008, 2010), Hargrove & Simpson, (2003), Limam *et al.,* (2005), Maggi *et al.,* (2008) Binzet*et al.,* (2010), Binzet, (2011), Fukuda & Ikeda (2012), Mehrabian *et al.,* (2012) and Binzet *et al.,* (2014), but only a limited number of studies can be found in the literature regarding pollen morphology of *Eritrichium* species i.e., Qureshi, (1979), Ning, (1991) and Perveen *et al.,* (1995). Palynological data dealing with *Eritrichium* is still scare and pollen morphology of most *Eritrichium* species and newly published species like *Eritrichium serxuense* Wang (Wang 2010) is still unknown. The present study is based

on a comprehensive survey of 32 Chinese *Eritrichium* species by scanning electron microscopy (SEM) from pan Himalayas and China. The palynological data will be helpful to assess the potential significance of pollen characters in the taxonomy of *Eritrichium*.

Material and Methods

Sampling and scanning electron microscopy (SEM): Pollen material was collected by organizing field trips during different flowering seasons in various regions of Pan Himalayan and China. List of species studied for pollen morphology, their voucher numbers and location are presented in (Table 1). Pollen samples were prepared for scanning electronmicroscopy. For this purpose, stubs were prepared by attaching the specimen to stubs with a conductive adhesive and sputter coated with gold-palladium. The prepared specimens were observed under (SEM; Hitachi S-4800). Detailed qualitative characters like pollen unit or type, symmetry, shape in polar view and equatorial view, aperture type, postion of pores and exine sculpturing and quantitative characters like size of pollen (polar and equatorial diameter),P/E ratio, number of colpi, pseudocolpi, pores, length of colpi, pseudocolpi, etc. are studied. For measurements "Image-Pro Plus 6.0" was used. All measurements are in µm and are presented in (Table 2). The terminology is used in accordance to (Punt *et al.,* 2007 & Hesse *et al.,* 2009).

Results

General description of pollen morphology of *Eritrichium* species: Generally, pollen grains usually radially symmetrical, isopolar, 6-heterocolpate, (tricolpate, and tricolporate) perprolate, prolate andsubprolate (Fig. 7) (in only *E. oligocanthum* Lian et J.Q. Wang), mostly constricted at the equator rarely without constriction, very small to small in size less than 25µm. The mean of the polar axis ranged from 8.64 to 16.19 µm while equatorial axis ranged from 3.04 to 8. 29 µm (Fig. 4). The outline in polar view was circular but quadrangular and triangular amb was while equatorial outline was commonly cocoon shaped or dumbbell shaped but also oval and oblong in equatorial view. The mean of length of colpi ranged from 0.50 to 8.36µm (Fig. 6; Table 2). The exine sculpturing was constant i.e., psilate but perforated only at poles. Pores 1-6 pores; either only one pore in the center oron one terminal pole or 2-6 pores located on each pole of aperture of the pollen grain.

Table 1. Voucher and collection localities of *Eritrichium* taxa studied.

Taxa	Collectors & Vouchers	Locality
Eritrichium axilliflorum Li Bing Zhang & Yi F. Duan	J.C. Hao & Y. Zhou 161238 (BNU)	China: Qinghai, Chindu
E. borealisinense Kitag.	J.C. Hao 201406001 (BNU)	China: Beijing, Mentougou
E. canum (Benth.) Kitamura	J.C. Hao xz14535	China: Xizang,Gyirong
E. confertiflorum W.T. Wang	J.C. Hao& Y. Zhou 16965 (BNU)	China: Xinjiang,Urumqi
E. deqinense W.T. Wang	J.C. Hao 15584 (BNU)	China: Yunnan, Dêqên
E. deltodentum Y.S. Lian & J.Q. Wang	J.C. Hao& Y. Zhou 16950 (BNU)	China: Xinjiang, Hejing
E. echinocaryum (I.M. Johnst.) Y.S. Lian & J. Q. Wang	J.C. Hao 15578 (BNU)	China: Xizang, Markam
E. hemisphaericum W.T. Wang	J.C. Hao 15183 (BNU)	China: Qinghai, Geermu
E. humillimum W.T. Wang	J.C. Hao 15185 (BNU)	China: Qinghai, Zhiduo
E. kangdingense W.T. Wang	J.C. Hao 15120 (BNU)	China: Sichuan, Kangding
E. latifolium Kar. et Kir.	J.C. Hao& Y. Zhou 16743 (BNU)	China: Xinjiang, Zhaosu
E. laxum I.M. Johnston	J.C. Hao 15195 (BNU)	China: Xizang, Anduo
E. liangyongshanii Li Bing Zhang & Yi F. Duan	L. Wei & J.C. Hao 15330 (BNU)	China: Xizang,Gyirong
E. longifolium Decne.	BNU Exped. LS2015035 (BNU)	China: Xinjiang, Yecheng
E. longipes Y.S. Lian & J.Q. Wang	J.C. Hao 15151 (BNU)	China: Sichuan, Sêrxü
E. medicarpum Y.S. Lian & J.Q. Wang	J.C. Hao & Y. Zhou 161173 (BNU)	China: Qinghai, Gangcha
E. minimum (Brand) H. Hara	J.C. Hao & Y. Zhou 16907 (BNU)	China: Xizang, Zanda
E. oligacanthum Y.S. Lian & J.Q. Wang	J.C. Hao & Y. Zhou 16770 (BNU)	China: Xinjiang, Hejing
E. petiolare W.T. Wang	L. Wei & J.C. Hao 15535 (BNU)	China: Xizang, Gar
E. petiolare var. *subturbinatum* W.T. Wang	L. Wei & J.C. Hao 15290 (BNU)	China: Xizang, Nyalam
E. pauciflorum (Ledeb.) DC.	BNU Exped. LS2015035 (BNU)	China: Ningxia, Wuzhong
E. pseudolatifolium M. Pop.	J.C. Hao & Y. Zhou 16870 (BNU)	China: Xinjiang, Yecheng
E. qofengense Y.S. Lian & J.Q. Wang	J.C. Hao & Y. Zhou 16915 (BNU)	China: Xizang, Tingri
E. serxuense W.T. Wang	J.C. Hao 15158 (BNU)	China: Qinghai, Sêrxü
E. sessilifructum Y.S. Lian & J.Q. Wang	J.C. Hao & Y. Zhou 16797 (BNU)	China: Xinjiang, Taxkorgan
E. sinomicrocarpum W.T. Wang	J.C. Hao xz14378 (BNU)	China: Xizang, Nagarzê
E. subjacquemontii M. Pop.	J.C. Hao & Y. Zhou 16923 (BNU)	China: Xinjiang, Taxkorgan
E. tangkulaense W.T. Wang	Q.R. Liu QH2012092 (BNU)	China: Qinghai, Maoduo
E. thymifolium (DC.) Y.S. Lian & J.Q. Wang	J.C. Hao & Y. Zhou 16951 (BNU)	China: Xinjiang, Hejing
E. thymifolium subsp. *latialatum* Y.S. Lian & J.Q. Wang	L. Wei & J.C. Hao 15531 (BNU)	China: Xizang, Zanda
E. villosum (Ledeb.) Bge.	J.C. Hao & Y. Zhou 16672 (BNU)	China: Xinjiang, Zhaosu
E. wanwencaii Li Bing Zhang & Yi F. Duan	J.C. Hao & Y. Zhou 16902 (BNU)	China: Xizang, Zanda

Table 2. Measurements and pollen characters of *Eritrichium* Schard. species (Scanning electron microscopy).

Name of taxa	(P)	(E)	P/E	Shape	(Amb)	(C)	C/P	No. of pores	Aperture type	Medial constriction
Eritrichium axilliflorum Li Bing Zhang & Yi F. Duan	13.04 (12.48-13.61)	4.80 (3.32-6.29)	2.79	Perprolate	Cir.	7.66 (5.66-9.66)	0.58	2	6-heterocolpate, (3-colporate)	+
E. borealisinense Kitag.	10.88 (7.51-14.26)	3.69 (3.49-8.02)	1.62	Prolate	Cir.	8.36(7.77-8.96)	0.76	2	6-heterocolpate, (3-colporate)	+
E. canum (Benth.) Kitamura	10.01(9.38-10.64)	6.41 (3.33-9.49)	1.56	Prolate	Cir.	6.12 (5.36-6.89)	0.61	1	6-heterocolpate, (3-colporate)	+
E. confertiflorum W.T. Wang	9.92 (8.40-11.45)	3.11(2.75-3.48)	3.18	Perprolate	Cir.	3.98 (6.68-7.30)	0.40	1	6-heterocolpate, (3-colporate)	+
E. deqinense W.T. Wang	12.31 (11.27-13.35)	5.64 (4.91-6.37)	2.18	Perprolate	Quadr.	6.19 (5.57-6.18)	0.50	6	6-heterocolpate, (3-colporate)	+
E. deltodentum Y.S. Lian & J.Q. Wang	11.06 (9.41-10.71)	3.6(3.22-3.98)	3.07	Perprolate	Cir.	5.6 (4.82-6.38)	0.50	1	6-heterocolpate, (3-colporate)	+
E. echinocaryum (Johnst.) Lian et J.Q. Wang	11.44 (10.76-12.13	3.28 (2.98-3.59)	3.48	Perprolate	Cir.	7.20 (5.74-8.67)	0.62	2	6-heterocolpate, (3-colporate)	+
E. hemisphaericum W.T. Wang	10.63 (9.36-11.91	4.67 (5.86-3.48)	2.27	Perprolate	Cir.	7.57 (6.36-8.78)	0.71	2	6-heterocolpate, (3-colporate)	-
E. humillimum W.T. Wang	10.07 (9.50-10.65)	3.04 (2.68-3.40)	3.31	Perprolate	Cir.	6.08 (5.47-6.70)	0.60	2	6-heterocolpate, (3-colporate)	+
E. kangdingense W.T. Wang	10.35 (9.36-11.35	6.08 (5.25-6.92)	1.70	Prolate	Cir.	5.84 (4.98-6.70)	0.56	1	6-heterocolpate, (3-colporate)	-
E. latifolium Kar. et Kir.	10.14(9.04-11.24)	3.46(2.97-3.96)	2.93	Perprolate	Cir.	6.33(5.97-6.70)	0.62	1	6-heterocolpate, (3-colporate)	+
E. laxum I.M. Johnston	9.92 (9.4-10.4)	3.37(3.2-3.55)	2.93	Perprolate	Cir.	5.48 (4.5-6.46)	0.55	-	6-heterocolpate, (3-colpate)	+
E. liangyongshanii Li Bing Zhang & Yi F. Duan	9.40 (8.88-9.93)	3.69 (3.37-4.02)	2.54	Perprolate	Cir.	4.11 (3.35-4.88)	0.43	2	6-heterocolpate, (3-colporate)	+
E. longifolium Decne.	10.94(9.24-12.01)	4.80 (3.42-6.19)	2.27	Perprolate	Cir.	3.50 (4.94-6.51)	0.31	1	6-heterocolpate, (3-colporate)	+
E. longipes Y.S. Lian & J.Q. Wang	11.11(10.03-12.19)	3.85 (2.90-4.81)	2.88	Perprolate	Cir.	5.82 (4.90-6.74)	0.52	1	6-heterocolpate, (3-colporate)	+
E. medicarpum I.M. Johnston	11.00 (10.37-11.64)	3.37(2.96-3.79)	3.37	Perprolate	Cir.	6.5 (5.98-7.02)	0.59	1	6-heterocolpate, (3-colporate)	+
E. minimum (Brand) H. Hara	11.57 (10.31-12.84)	5.86 (4.89-6.83)	1.97	Prolate	Cir.	5.67 (4.17-7.18)	0.49	2	6-heterocolpate, (3-colporate)	+
E. oligacanthum Lian et J.Q. Wang	8.68 (5.74-11.62)	6.82 (3.84-9.81)	1.27	Subprolate	Cir.	5.71(5.19-6.24)	0.65	1	6-heterocolpate, (3-colporate)	+
E. pauciflorum (Ledeb.) DC.	9.96 (8.98-10.94)	3.71 (3.12-4.31)	2.68	Perprolate	Cir.	5.58 (4.09-7.07)	0.56	2	6-heterocolpate, (3-colporate)	+
E .petiolare W.T. Wang	8.64 (3.85-13.43)	3.13 (2.48-3.79)	2.76	Perprolate	Cir.	7.25 (6.42-8.08)	0.83	-	6-heterocolpate, (3-colpate)	+
E. petiolare var. *subturbinatum* W.T. Wang	11.14 (10.21-12.07)	5.00 (2.83-7.17)	2.22	Perprolate	Cir.	6.13 (5.20-7.06)	0.55	1	6-heterocolpate, (3-colporate)	+
E. pseudolatifolium M. Pop.	9.08 (9.3-10.23)	4.35(3.55-5.15)	2.08	Perprolate	Cir.	5.02 (4.35-5.69)	0.55	1	6-heterocolpate, (3-colporate)	+
E. qofengense Lian et J.Q. Wang	10.30 (9.24-11.37)	3.96 (3.38-4.55)	2.60	Perprolate	Cir.	5.72 (4.86-6.58)	0.55	2	6-heterocolpate, (3-colporate)	+
E. serxuense W.T. Wang	10.51 (9.87-11.16)	5.27(3.12-4.31)	1.99	Prolate	Cir.	5.45 (3.54-7.36)	0.51	1	6-heterocolpate, (3-colporate)	+
E. sessilifructum Y.S. Lian & J. Q. Wang	11.22 (12.39-14.48	5.63 (4.49-6.78)	1.99	Prolate	Cir.	10.18 (9.45-10.92)	0.90	1	6-heterocolpate, (3-colporate)	-
E. sinomicrocarpum W.T. Wang	11.41(10.23-12.59)	3.55 (2.43-4.68)	3.55	Perprolate	Cir.	6.82 (5.38-8.27)	0.59	2	6-heterocolpate, (3-colporate)	+
E. subjacquemontii M. Pop.	14.89 (9.03-20.76)	4.28 (2.17-6.40)	3.47	Perprolate	Cir.	5.20 (3.65-6.72)	0.34	1	6-heterocolpate, (3-colporate)	+
E. tangkulaense W.T. Wang	10.78 (9.92-11.65)	4.44 (3.66-5.23)	2.42	Perprolate	Cir.	6.88 (5.98-7.78)	0.63	-	6-heterocolpate, (3-colpate)	+
E. thymifolium (DC.) Y.S. Lian & J.Q. Wang	16.19 (10.07-22.31)	8.29 (4.55-12.03)	1.95	Prolate	Cir.	8.32 (7.64-9.00)	0.15	2	6-heterocolpate, (3-colporate)	+
E. thymifolium subsp. *Latialatum* Y.S. Lian & J.Q. Wang	12.06 (11.32-12.8)	6.26 (4.89-7.63)	1.92	Prolate	Tri.	6.6 (5.42-7.78)	0.54	1	6-heterocolpate, (3-colporate)	+
E. villosum (Ledeb.) Bge.	11.22 (10.40-12.05)	4.04 (3.53-4.56)	2.77	Perprolate	Cir.	5.68 (4.93-6.43)	0.50	1	6-heterocolpate, (3-colporate)	+
E. wangwencaii	10.03 (9.39-10.68)	6.34 (2.86-3.48)	1.58	Prolate	Cir.	5.87 (5.31-6.44)	0.58	1	6-heterocolpate, (3-colporate)	+

Note: All the measurements are in µm. P= Polar length Mean (Min-Max.), E= Equatorial diameter Mean (Min-Max.), C = Colpus length (Min-Max.), P/E= Mean of P/mean of E and C/P= Mean of C/mean of P.

Key to the pollen types

1a) Pollen grains 6-heterocolpate, 3-colpate, pore absent ……*E. laxum*-type(3 spp)
1b) Pollen grains 6-heterocolpate 3-colporate, pore present ……2
2a) Medial constriction absent ……3
2b) Medial constriction present, Amb circular or triangular ……5
3a) Pores 6, Amb quadrangular……*E. deqinense*-type (1 spp)
3b) Pores 1 or 2 ……4
4a) 2 pores at both poles of aperture …… *E. hemisphaericum*-type (1 spp)
4b) 1 pore at one pole of aperture …… *E. sessilifurcatum*-type (1 spp)
5a) Polar view circular …… 6
5b) Polar view triangular …… *E. thymifolium*-type (1 spp)
6a) Pollen shape perprolate or prolate ……7
6b) Pollen shape subprolate …… *E. oligacanthum*-type(1 spp)
7a) Pollen shape prolate …… *E. minimum*-type (7spp)
7b) Pollen shape perprolate ……*E. axilliflorum*-type (17 spp)

1. *Eritrichiumaxilliflorum*Li Bing Zhang & YI F. Duan

Pollen class: Heterocolpate, 3-colporate.
Shape: Perprolate
Measurements: Polar axis (P) 13.04 µm (12.48-13.61 µm), equatorial diameter (E) = 4.80 µm (3.32-6.29 µm), P. E ratio 2.79 µm, colpus length (C) 7.66 µm (5.66-9.66 µm), C/P ratio 00.58 µm
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view- circular, Equatorial view- cocoon shaped rounded ends and constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1A-B).

2. *E. lianyongshanii*Li Bing Zhang & YI F. Duan
Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 9.40 µm (8.88-9.93µm), equatorial diameter (E) 3.69 µm (3.37-4.02 µm), P. E ratio 2.54 µm,colpus length (C) 4.11 µm (3.35-4.88 µm), C/P ratio 0.43 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view-circular, Equatorial view-cocoon shaped rounded ends and constricted at mid region.
Ornamentation: Exine Psilate but perforated at poles (Fig. 1C-D).

3. *E. borealisinense* Kitag.
Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 10.88(7.51-14.26), equatorial diameter (E) 3.69(3.49-8.02), P. E ratio 1.62 µm, colpus length (C) 8.36(7.77-8.96), C/P ratio 0.76 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view-circular, equatorial view- cocoon shaped rounded ends and constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1E-F).

4. *E. canum* (Benth.) Kitamura

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 10.01 µm (9.38-10.64 µm), equatorial diameter (E) 6.41 µm (3.33-9.49 µm), P. E ratio 1.56 µm, colpus length (C) 6.12 µm (5.36-6.89 µm), C/P ratio 0.61 µm.
Apertures: Aperture membrane granular, one pore at one pole of aperture.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1G-H).

5. *E. confertiflorum* W. T. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 9.92 µm (8.40-11.45µm), equatorial diameter (E) 3.11(2.75-3.48 µm), P. E ratio 2.54 µm, colpus length (C) 3.98 µm (6.68-7.30 µm), C/P ratio 0.40 µm.
Apertures: Aperture membrane granular, one pore at one pole of aperture.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1I-J).

6. *E. deqinense* W. T. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 12.31 µm (11.27-13.35 µm), equatorial diameter (E) 5.64 µm (4.91-6.37 µm), P. E ratio 2.18 µm, colpus length (C) 6.19 µm (5.57-6.18 µm), C/P ratio 0.50 µm.
Apertures: Aperture membrane granular with microelements, 6 pores situated at ends of apertures.
Outline: Polar view- quadangular, angulaaperturate, equatorial view-cocoon shaped with rounded ends and constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1 K-L).

7. *E. deltodentum* Y.S. Lian & J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 11.06 µm (9.41-10.71 µm), equatorial diameter (E) 3.60 µm (3.22-3.98 µm), P. E ratio 3.07 µm, colpus length (C) 5.6 µm (4.82-6.38 µm), C/P ratio 0.50 µm.

Apertures: Aperture membrane granular, 1 pore at one ends of aperture.
Outline: Polar view- circular, Equatorial view-cocoon shaped with rounded ends and constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1 M-N).

8. *E. echinocaryum* (Johnst.) Lian et J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 11.44 µm (10.76-12.13 µm), equatorial diameter (E) 3.28 µm (2.98-3.59 µm), P. E ratio 2.18 µm, colpus length (C) 7.20 µm (5.74-8.67 µm), C/P ratio 0.60 µm.
Apertures: Aperture membrane smooth to granular, 2 pores at both ends of apertures.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1O-P).

9. *E. wangwencaii*Li Bing Zhang & YI F. Duan

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 10.03 µm (9.39-10.68 µm), equatorial diameter (E), 6.34 µm (2.86-3.48 µm), P. E ratio1.58 µm, colpus length (C) 5.87µm (5.31-6.44 µm), C/P ratio 0.58 µm.
Apertures: Aperture membrane granular, one pore at one pole of aperture.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1Q-R).

10. *E. hemisphaericum* W.T. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P), 10.63 µm (9.36-11.91 µm), equatorial diameter (E), 6.085 µm (5.86-3.48 µm), P. E ratio 2.79 µm, colpus length (C) 7.57 µm (6.36-8.78 µm), C/P ratio 0.71 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view- circular, Equatorial view-oblong and without constriction at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1S-T).

11. *E. humillimum* W.T. Wang

Pollen class: Heterocolpate, 3-colporate.
Shape: Perprolate
Measurements: Polar axis (P), 10.07 µm (9.50-10.65 µm), equatorial diameter (E), 3.04 µm (2.68-3.40 µm), P. E ratio 3.31 µm, colpus length (C) 6.08 µm (5.47-6.70 µm), C/P ratio 0.60 µm.
Apertures: Aperture membrane tuberculated, 2 pores at both ends of apertures.
Outline: Polar view-circular, Equatorial view-cocoon shaped with rounded ends and deeply constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 1U-V).

12. *E. kangdingense* W.T. Wang

Pollen class: Heterocolpate, 3-colporate.
Shape: Prolate
Measurements: Polar axis (P), 10.35 µm (9.36-11.35 µm), equatorial diameter (E), 6.08 µm (5.25-6.92 µm), P. E ratio1.70 µm, colpus length (C) 5.84 µm (4.98-6.70 µm), C/P ratio 0.56 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view-circular, Equatorial view-oval with rounded ends and without constriction at mid region.
Ornamentation:Psilate but perforated at poles (Fig. 1W-X).

13. *E. latifolium* Kar. et Kir.

Pollen class: Heterocolpate, 3-colporate.
Shape: Perprolate
Measurements: Polar axis (P) 10.14 µm (9.04-11.24 µm), equatorial diameter (E) 3.46 µm (2.97-3.96 µm), P. E ratio 2.93 µm,colpus length (C) 6.33 µm (5.97-6.70 µm), C/P ratio 0.62 µm.
Apertures: Aperture membrane granular, 1 pore at one pole of aperture.
Outline: Polar view- circular, Equatorial view-cocoon shaped with rounded ends and deeply constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2A-B).

14. *E. laxum* I.M. Johnst.

Pollen class: Heterocolpate, 3-colpate
Shape: Perprolate
Measurements: Polar axis (P) 9.9 µm (9.4-10.4 µm), equatorial diameter (E) 3.37 µm (3.2-3.55 µm), P. E ratio 2.93 µm, colpus length (C) 5.48 µm (4.5-6.46 µm), C/P ratio 0.55 µm.
Apertures: Aperture membrane granular, without any pore.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2C-D).

15. *E. longipes* Y.S. Lian & J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 11.11 µm (10.03-12.19 µm), equatorial diameter (E) 3.85 µm (2.90-4.81 µm) P. E ratio 2.88 µm, colpus length (C) 5.82 µm (4.90-6.74) µm, C/P ratio 0.52 µm.
Apertures: Aperture membrane granular, 1 pore at one pole of aperture.
Outline: Polar view- circular, Equatorial view-cocoon shaped with rounded ends and deeply constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2E-F).

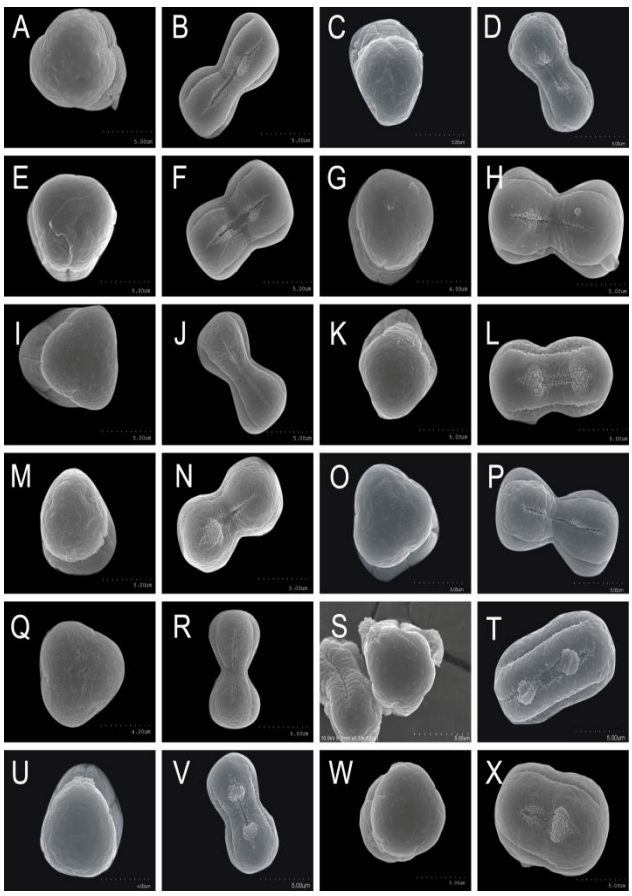

Fig. 1. Scanning electron micrographs (S.E.M.) of pollen grains: polar and equatorial view. A-B *Eritrichium axilliflorum*, C-D *E. liangyongshanii*, E-F *E. borealisinense*, G-H *E. canum*, I-J *E. confertiflorum*, K-L *E. deqinense*, M-N *E. deltodentum*, O-P *E. echinocaryum*, Q-R *E. wangwencaii*, S-T *E. hemisphaericum*, U-V *E. humillimum* ,W-X *E. kangdingense.*

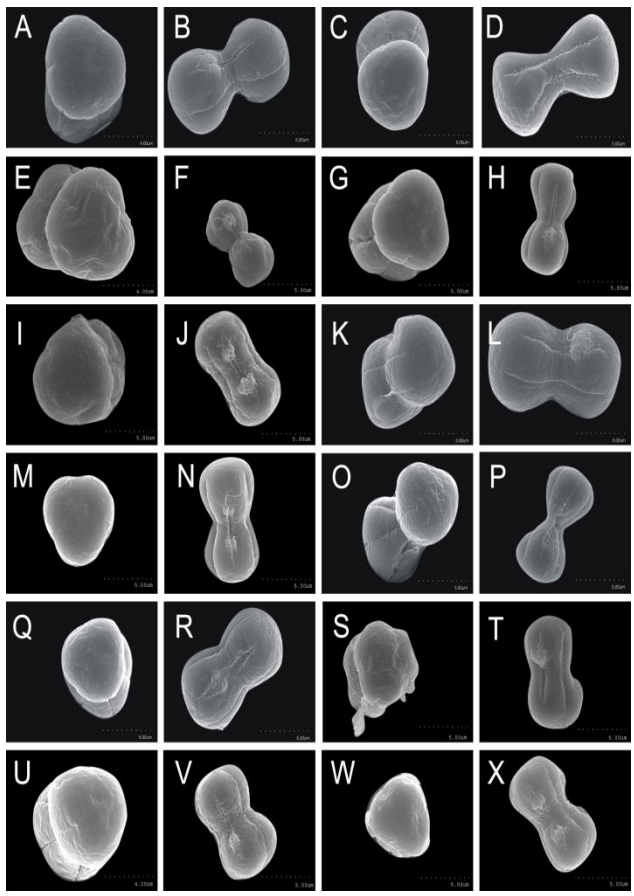

Fig. 2. Scanning electron micrographs (S.E.M.) of pollen grains: polar and equatorial view. A-B *Eritrichium latifolium*, C-D *E. laxum*, E-F. *E. longipes*, G-H *E. medicarpum*, I-J *E. minimum*, K-L *E. oligacanthum*, M-N *E. pauciflorum*, O-P *E. petiolare*, Q-R *E. petiolare* var. *subturbinatum*, S-T *E. longifolium*, U-V *E. pseudolatifolium*, W-X *E. qofengense.*

16. *E. medicarpum* I.M. Johnst.

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 11.00 µm (10.37-11.64 µm), equatorial diameter (E) 3.37 µm (2.96-3.79 µm), P. E ratio 3. 37 µm, colpus length (C) 6.5 µm, (5.98-7.02 µm), C/P ratio 0.59 µm.
Aperture: Aperture membrane granular, 1 pore at one pole of aperture.
Outline: Polar view- circular, Equatorial view- cocoon shaped in with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2G-H).

17. *E. minimum* (Brand) H. Hara

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 11.57 µm (10.31-12.84 µm), equatorial diameter (E) 5.86 µm (4.89-6.83 µm), P. E ratio 1.97 µm, colpus length (C) 5.67 µm (4.17-7.18 µm), C/P ratio 0.49 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view- circular, Equatorial view- cocoon shaped view with rounded ends and slightly constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2I-J).

18. *E. oligacanthum* Lian et J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Subprolate
Measurements: Polar axis (P) 8.68 µm (5.74-11.62 µm) equatorial diameter (E) 6.82 µm (3.84-9.81 µm), P. E ratio 1.27 µm, colpus length (C) 5.71 µm (5.19-6.24 µm), C/P ratio 0.65 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2K-L).

19. *E. pauciflorum* (Ledeb.) D.C.

Pollen class: Heterocolpate, 3-colporate.
Shape: Perprolate
Measurements: Polar axis (P) 9.96 µm (8.98-10.94µm), equatorial diameter (E) 3.71 µm (3.12-4.31 µm), P. E ratio 2.68 µm, colpus length (C) 5.58 µm (4.09-7.07-6.73 µm), C/P ratio 0.56 µm.

Apertures: Aperture membrane granular,2 pores present at both poles of apertures.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2M-N).

20. *E. petiolare* W.T. Wang

Pollen class: Heterocolpate, 3-tricolpate.
Shape: Perprolate
Measurements: Polar axis (P) 8.64 µm (3.85-13.43 µm), equatorial diameter (E), 3.13 µm (2.48-3.79 µm), P. E ratio 2.76 µm, colpus length (C) 7. 25 µm (6.42-8.08 µm), C/P ratio 0.83 µm.
Apertures: Aperture membrane granular, without any pore.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2O-P).

21. *E. petiolare* var. *subturbinatum* W.T. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 11.14 µm (10.21-12.07 µm), equatorial diameter (E) 5.00 µm (2.83-7.17 µm), P. E ratio2.22µm, colpus length (C) 6.13 µm (5.20-7.06 µm), C/P ratio 0.55 µm.
Apertures: Aperture membrane granular, 1 pore present at one pole.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2Q-R).

22. *E. longifolium* Decne.

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 10.94 µm (9.24-12.01 µm), equatorial diameter (E) 4.80 µm (3.42-6.19 µm), P. E ratio 2.27 µm, colpus length (C) 3.50 µm (4.94-6.51 µm), C/P ratio 0.31 µm.
Apertures: Aperture membrane granular, 2 pores at both ends of apertures.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2S-T).

23. *E. pseudolatifolium* M. Pop.

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 9.08 µm (9.00-10.23 µm), equatorial diameter (E) 4.35 µm (3.55-5.15 µm), P. E ratio 2.08µm, colpus length (C) 5.02 µm (4.35-5.69 µm), C/P ratio 0.55 µm.
Apertures: Aperture membrane granular, 1 pore present at one pole.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2U-V).

24. *E. qofengense* Lian et J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 10.30 µm (9.24-11.37 µm), equatorial diameter (E) 3.96 µm (3.38-4.55 µm), P. E ratio 2.60 µm, colpus length (C) 5.72 µm (4.86-6.58 µm), C/P ratio 0.55 µm.
Apertures: Aperture membrane granular, 2 pores present at both ends of apertures.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 2W-X).

25. *E. serxuense* W.T. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 10.51 µm (9.87-11.16 µm), equatorial diameter (E) 3.96 µm (3.38-4.55 µm), P. E ratio1.99 µm, colpus length (C) 5.45 µm (3.54-7.36 µm), C/P ratio 0.51 µm.
Apertures: Aperture membrane granular, 1 pore present at one pole of the aperture.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3A-B).

26. *E. sessilifructum* Y.S. Lian & J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 11.22 µm (12.39-14.48 µm), equatorial diameter (E) 5.63 µm (4.49-6.78 µm), P. E ratio1.99 µm, colpus length (C) 10.18 µm (9.45-10.92 µm), C/P ratio 0.90 µm.
Apertures: Aperture membrane granular, 1 pore present at one pole of the aperture.
Outline: Polar view-circular, Equatorial view- oblong with rounded ends and slightly without constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3C-D).

27. *E. sinomicrocarpum* W.T. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Perprolate
Measurements: Polar axis (P) 11.41 µm (10.23-12.59 µm), equatorial diameter (E) 3.55 µm (2.43-4.68 µm) P. E ratio 3.55 µm, colpus length (C) 6.82 µm (5.38-8.27 µm), C/P ratio 0.59 µm.
Apertures: Aperture membrane granular, 2 pores present at both ends of apertures.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3E-F).

28. *E. subjacquemontii* M. Pop.

Pollen class: Heterocolpate, 3-colporate.
Shape: Perprolate
Measurements: Polar axis (P) 14.89 µm (9.03-20.76 µm), equatorial diameter (E) 4.28 µm (2.17-6.40 µm), P. E ratio 3.47µm, colpus length (C) 5.20 µm (3.65-6.73 µm), C/P ratio 0.34 µm.
Apertures: Aperture membrane granular,1 pore present at one pole of aperture.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3G-H).

29. *E. tangkulaense* W. T. Wang

Pollen class: Heterocolpate, 3-colpate.
Shape: Perprolate
Measurements: Polar axis (P) 10.78 µm (9.92-11.65 µm), equatorial diameter (E) 4.44 µm (3.66-5.23 µm) P. E ratio2.42 µm, colpus length (C) 6.88 µm (5.98-7.78 µm), C/P ratio 0.63 µm.
Apertures: Aperture membrane granular,without any pore.
Outline: Polar view-circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3I-J).

30. *E. thymifolium* (DC.) Y.S. Lian & J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 16.19 µm (10.07-22.31 µm), equatorial diameter (E) 8.29 µm (4.55-12.03 µm) P. E ratio1.95µm,colpus length (C) 8.32 µm (7.64-9.00 µm), C/P ratio 0.15 µm.
Apertures: Aperture membrane granular, 2 pores present at both ends of apertures.
Outline: Polar view- triangular, Equatorial view- oval with rounded ends and very slightly constricted at mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3K-L).

31. *E. thymifolium* subsp. *Latialatum* Y.S. Lian & J.Q. Wang

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 12.06 µm (11.32-12.8 µm), equatorial diameter (E) 6.26 µm (4.89-7.63 µm), P. E ratio1.95µm, colpus length (C) 6.6 µm (5.42-7.78 µm), C/P ratio 0.54 µm.
Apertures: Aperture membrane granular, 1 pore at one end of aperture.
Outline: Polar view- circular, Equatorial view- oval with rounded ends and constricted at mid region.
Ornamentation: Psilate (Fig. 3M-N).

32. *E. villosum* (Ledeb.) Bge.

Pollen class: Heterocolpate, 3-colporate
Shape: Prolate
Measurements: Polar axis (P) 11.22 µm (10.40-12.05 µm), equatorial diameter (E) 4.04 µm (3.53-4.56 µm) P. E ratio 1.95µm, colpus length (C) 5.68 µm (4.93-6.43 µm), C/P ratio 0.54 µm.
Apertures: Aperture membrane granular, 1 pore at one end of aperture.
Outline: Polar view- circular, Equatorial view- cocoon shaped with rounded ends and deeply constricted mid region.
Ornamentation: Psilate but perforated at poles (Fig. 3O-P).

Discussion

Pollen type, size and shape: Boraginaceae is exclusively eurypalynous and their pollen characters (Clark 1977; Diez 1984 and Diez & Valdes 1991) can recognize large number of species. Previous palynotaxonomic studies on the Boraginaceae were shown to be valuable in delimiting genera and in the study of the evolutionary trends within the whole family (Bigazzi & Selvi, 1998). The two basic pollen types have been recognized within the family viz. homoaperturate (isocolpate) and heteroaperturate (heterocolpate). The isocolpate pollen grains is a fundamental character of Boraginaea and Lithospemeae whereas heterocolpate pollen grains is of tribe Eritrichaeae and Cynoglosseae (Diez & Valdes, 1991; Perveen *et al.,* 1995; Bigazzi & Selvi, 1998; Hagroove& Simpson (2003). The heterocolpate pollen grains have been also discussed in previous palynological studies on *Eritrchium* species by Qureshi, (1979) Ning, (1991), and Perveen *et al.,* (1995). Our study confirmed the presence of heterocoplate pollen gains in *Eritrichium.* Moreover, Perveen *et al.,* (1995) discussed the heterocolpate pollen grains of Boraginaceae are usually dumb-bell shaped or rectangular and with or without constriction at the equator and mostly forming endocingulum. On the basis of presence or absences constriction at equator and andendocingulum they separated the pollen grains of *Heliotropium* from *Lindelofia*types. The *Heliotropioum subulatum*-type was recognized without constriction and endocingulum while the later was constricted and forming endocingulum. The pollen grains of the *Eritrichium* species were included in type *Heliotropioum subulatum*-type. Our results are in agreement with Perveen *et al.,* (1995) that in the pollen grains of *Eritrichium* or a is not forming endocingulum but in current study the pollen grains are found constricted as well as unconstructed at the equator.

Ning, (1991), examined 7 species of *Eritrichium* and found cocoon-shaped and tri-colporate and tri-pseudocolpate pollen grains within *Eritrchium* species. Similarly, Perveen *et al.,* (1995) described prolate and 6-hetrocolpate pollen grains in *E. nanum.* Prolate and perprolate shape of the pollen grains also reported by Qureshi, (1979). In the present investigation of *Eritrichium* species three pollen shapes have been recognized; the common shape of pollen grains was prolate and perprolate (Fig. 7) similar to previous studies by Qureshi, (1979) and Perveen *et al.,* (1995) but subprolate pollen shape was only observed in only *E. oligacanthum* Lian et J.Q. Wang (Fig. 2K-L; Fig. 7). The pollen grains were found very small to small pollen size less than 25 µm in accordance with the study of Qureshi, (1979).The largest pollen grain was found in *E.*

thymifolium (DC.) Y. S. Lian & J.Q. Wang with 16.19 µm long polar axis while the smallest polar axis was observed in *E. petiolare* W.T. Wang 8.64 µm (Fig. 4). The equatorial diameter ranged from 3.04 µm (*E. humillimum* W.T. Wang) to 8.29 µm (*E. thymifolium* (DC.) Y.S. Lian & J.Q. Wang). The mean P/E ratio ranged from 1.56 µm to 3.55 µm in *E. canum* (Benth.) Kitamura and *E. sinomicrocarpum* W.T. Wang respectively (Fig. 5, Table 2). According to Walker, (1976) the large pollen grains belong to the more primitive type, however the pollen grains of this genus are very small to small in size less than 10 µm and occupy an advance position in the family.

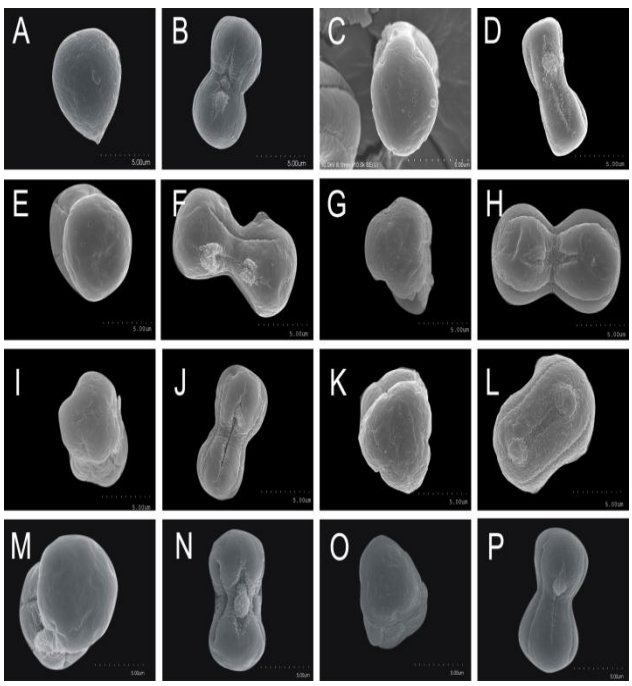

Fig. 3. Scanning electron micrographs (S.E.M.) of pollen grains: polar and equatorial view. A-B *Eritrichium serxuense*, C-D *E. sessilifructum*, E-F *E. sinomicrocarpum*, G-H *E. subjacquemontii*, I-J *E. tangkulaense*, K-L *E. thymifolium*, M-N *E. thymifolium* subsp. *latialatum*, O-P *E. villosum.*

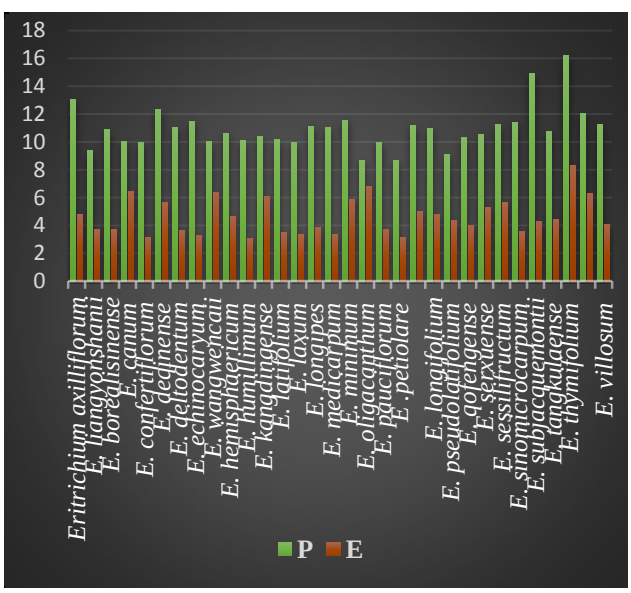

Fig. 4. Mean of polar axis and equatorial diameter of taxa investigated.

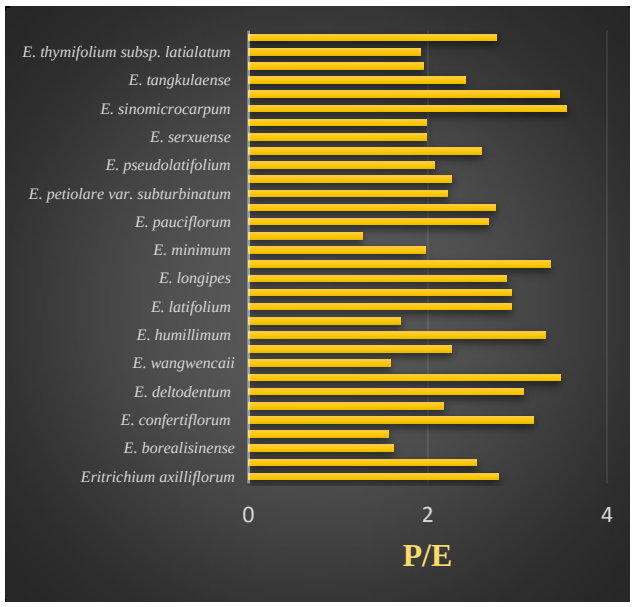

Fig. 5. Mean of P/E ratio of taxa investigated.

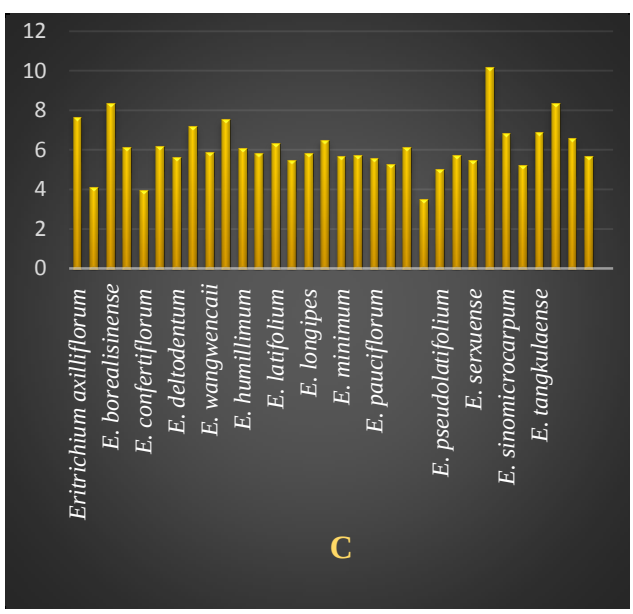

Fig. 6. Colpus length of the investigated *Eritrichium* species.

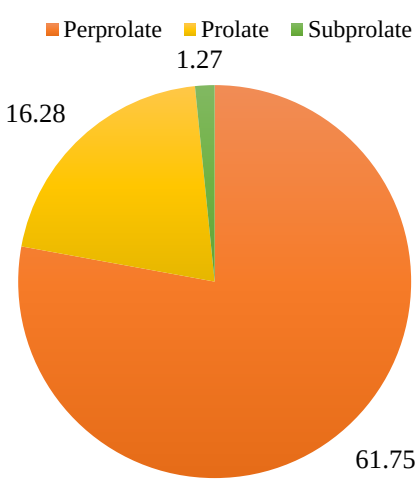

Fig. 7. Sum of pollen shapes based on P/E ratio.

Apertures and pores: According to Willis, (1975) pollen grains of the family Boraginaceae are characterized by having large differences in aperture morphology. Majority of Boraginaceae taxa has 3–4-aperturate grains. Within *Eritrichium* species, the number and position of apertures and pores as well as length of were also significantly different. The mean of length of colpi (C) ranged from 0.50 to 8.36µm in *E. liangyongshanii* and *E. borealisinense* respectively (Fig. 6). Similarly, the C/P ratio was also minimum *E. liangyongshanii* i.e., 0.31µm while maximum in *E. sessilifructum* i.e., 0.90µm (Table 2). In some species like *E. longipes* single pore was located on one terminal pole (Fig. 2E-F). However, *E. hemisphaericum* had two pores located on each pole of the pollen grain (Fig. 1S-T).Generally, pollen pore number in Boraginaceae has increased from three to six or more and this increase in number of pores in plants may be advantageous for germination by providing greater number of germination sites (Dajos *et al.,* 1991; Furness & Rudall, 2004; Cohen, 2014). In the present study, greater number of pores were found in *E. deqinense* W. T. Wang (Fig. 1K-L; Table 2).

On the basis of pollen shapes, apertures and pores (as well as other pollen characters like constriction at equator and pollen shapes etc); 8 pollen types have been recognized within genus *Eritrichium* viz., *E. laxum*-type, *E. axilliflorum*-type, *E. deqinense*-type, *E. oligocanthum*-type, *E. minimum*-type, *E. thymifolium*-type, *E. hemisphaericum*-type, and *E. sessiliufurcatum*-type. *Eritrichiumlaxum*–type can be characterized by having 3-colporate pollen grains and by absence of any pore. This type included three species i.e., *E. laxum* I.M. Johnston, *E. petiolare* W.T. Wang, *E. tangkulaense* W.T. Wang (Fig. 2C-D; O-P; Fig. 3I-J). *E. axilliflorum* -type can be easily recognized by 3-colporate, porate, and prolate shape of pollen grains with circular amb and medial constriction at the equator in equatorial view. This type was observed in maximum number of species and comprised of 17 species i.e., *E. axilliflorum* Li Bing Zhang & Yi F. Duan, *E. liangyongshanii*, *E. confertiflorum* W.T. Wang, *E. deltodentum* Y.S. Lian & J.Q. Wang, *E. echinocaryum* (I.M. Johnst.) Y.S. Lian& J.Q. Wang (Fig. 1A-B, C-D, I-J, M-N, O-P, U-V), *E. humillimum* W.T. Wang, *E. latifolium* Kar. et Kir., *E. longipes* Y.S. Lian & J.Q. Wang, *E. medicarpum* (Brand) H. Hara, *E. pauciflorum* (Ledeb.) DC., *E. petiolare* var. *subturbinatum* W.T. Wang, *E. longifolium* Decne., *E. pseudolatifolium* M. Pop., *E. qofengense*Lian et J.Q. Wang (Fig. 2 A-B, E-F, G-H, M-N, Q-R, S-T, U-V, W-X), *E. sinomicrocarpum* W.T. Wang, *E. subjacquemontii* M. Pop**.,** and *E. villosum* (Ledeb.) Bge. (Fig. 3 E-F, G-H, O-P). *E. minimum* -type is similar to *E. axilliflorum* -type in having 3-colporate, porate, pollen grains with medial constriction and circular amb but the latter type is different in pollen shape i.e., prolate instead of perprolate. Seven species i.e., *E. minimum* (Brand) H. Hara, *E. borealisinense* Kitag., *E. canum* (Benth.) Kitamura, *E. gracile* W.T. Wang, *E. kangdingense* W.T. Wang (Fig.1 E-F, G-H, Q-R, W-X,), *E. minimum* (Brand) H. Hara (Fig. 2J-E). *E. serxuense* W.T. Wang and *E. thymifolium* subsp. *Latialatum* Y.S. Lian & J.Q. Wang (Fig. 3A-B, M-N) have been recognized within *E. minimum*–type.

A single species, *E. oligacanthum* Lian et J.Q. Wang having subprolate pollen grains (Fig. 2K-L) and *E. deqinense* W.T. Wang with maximum number of pores i.e., 6 (Fig. 1K-L) were recognized as *E. oligacanthum*-type (Fig. 2 K-L) and *E. deqinense*–type respectively. Similarly, *E. thymifolium*-type also represented by single species *E. thymifolium* (DC.) Y.S. Lian & J.Q. Wangand can be distinguished from rest of the species by having triangular amb (Fig. 3K-L).

E. sessilifurcatum-type and *E. hemisphaericum*-type can be easily recognized mainly by the un-constricted pollen grains but both types can be distinguished on the basis of number of pores. *E. hemisphaericum*-type *is* characterized by having two pores at each pole of aperture (Fig. 1S-T), while *E. sessilifurcatum* -type by having1 pore at one pole of aperture (Fig. 3C-D).

The differences and similarities in pollen morphology of investigated species are obviously of high systematic significance and can be used identify species on the basis of pollen characters, for comparative studies with morphological and/or molecular and other biosystematics purposes.

Exine sculpturing: Exine sculpturing or ornamentation has been proved very diverse in Boraginaceae. Ahn & Lee, (1986) found psilate sculpturing and some variations like foveolate to coarsely regulate in *Ehretia*, scrabate in *Amsinckia*, *Bracybotrys* and *Omphalodes*, and gemmate in *Symphytum*. The pollen morphology and its surface ornamentation were found useful in separation of *Maharanga* taxa from *Onosma* (Ning *et al.,* 1995). Although the exine sculpturing in all the investigated species was psilate but perforated at poles only but other pollen traits like shape, apertures and pores have significant taxonomic differences and can be in identification of taxa.

Conclusion

In the current study detailed pollen characters of *Eritrichium* investigated for the first time from China especially Pan-Himalayan regions. The present palynological data provides new information about pollen morphology of all *Eritrichium* species; especially the newly published species *E. serxuense* W.T. Wang (2010). Interesting pollen characters were examined in *Eritrichium* species especially in regard of shape (prolate, perprolate and subprolate), the number and arrangement of apertures (3-colpate and 3-colporate), number and position of pores (1, 2 or 6 pores found at one pole or both poles of apertures), and presence or absence of constriction at the equator and other palynological characters. Based on these characters palynological key to the types is developed (and 8 pollen types have been recognized) which can serve as an aid for correct identification of species. The present palynological data can be used taxonomically for correlation of characters as well as for any further research (molecular and cytological) on this group in future.

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References

Ahn, Y.M. and S. Lee. 1986. A palynotaxonomic study of the Korean Boraginaceae. *Korean J. Pl. Tax.*, 16: 199-215.

Avetisian, E.M. 1956. Morphologie des microspores de Boraginaceae.*Tran. Inst. Bot. Akad. Arm.*, 10: 7-66.

Ben Saad-Limam, S. and M.A. Nabli. 1982. Ultrastructure de Exine de I *Arnebia decumbens* (Vent.) Coss et Karl. Et du *Cerinthe major* L. (Boraginaceae).*Pollen Spores*, 24: 9-19.

Ben Saad-Limam, S. and M.A. Nabli. 2009. Ultrastructure of the exine of *Borago officinalis* (Boraginaceae). *Grana*, 23(1): 1-10.

Bigazzi, M. and F. Selvi. 1998. Pollen morphology in the Boragineae (Boraginaceae) in relation to the taxonomy of the tribe. *Pl. Syst. Evol.*, 213: 121-151.

Binzet, R. 2011. Pollen Morphology of some *Onosma* species (Boraginaceae) from Turkey. *Pak. J. Bot.*, 43(2): 731-741.

Binzet, R., I. Kandemair and F. Orcan. 2010. Palynological classification of *Onosma* L. (Boraginaceae) species from east Mediteranean region in Turkey. *Acta Bot. Croat*., 692: 259-274.

Binzet, R., I. Potoğlu Erkara, H.Özler and S.Pehlivan. 2014. Pollen morphology and systematical contribution of some *Onosma* (Boraginaceae) taxa distribution in Turkey. *J.Pl. Syst. Evol*., 300(10): 2135-2146.

Bou, G. 1968. *Caraceres pollinniques de quelques Boraginacees mediterranees et Saharoindiennes.* Moontpellier: Rapport de Stag,D.E.A. Biologie Vegetale.

Clarke, G.C.S. 1977. *The Northwest European pollen flora*, 10.Boraginaceae. *Rev. Paleobot. Palynol.*, 24(2): 59-101.

Cohen, J.I. 2014. A phylogenetic analysis of morphological and molecular characters of Boraginaceae: evolutionary relationships, taxonomy, and patterns of evolution character. *J. Cladistics.*, 30: 139-169.

Dajoz, I., I. Till-Bottraud and P.H. Gouyon. 1991. Evolution of pollen morphology. *Sci*., 253: 66-68.

Díez, M.J. 1984. Contribucian al Atlas Polinico de Andalucia Occidental, I. Boraginaceae. *Lagascalia*, 13: 147-171.

Díez, M.J. 1994. A general survey of pollen types in *Anchusa* L. (Boraginaceae) in relation to taxonomy. *Acta Bot. Gal.*, 141(2): 233-242.

Díez, M.J. and B. Valdés. 1991. Pollen morphology of the tribe Eritrichieae and *Cynoglosseae* (Boraginaceae) in the Iberian Peninsula and its taxonomic significance. *Bot. J. Linn. Soc.*, 107: 49-66.

El Ghazaly, G. 1995. Pollen morphology of the family Boraginaceae in Qatar.*Qatar Univ. Sci. J.*, 15(10): 65-75.

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy. Angiosperms* (*An introduction to palynology)* Vol. 1. Almquist and Wiksell, Stockhol.

Fukuda, T. and H. Ikeda. 2012. Palynological analysis and taxonomic position of the genus *Mertensia* (Boraginaceae). *Bot.*, 90: 722-730.

Furness, C.A. and P.J. Rudall. 2004. Pollen aperture evolution-A crucial factor for eudicot success. *Trends in Pl. Sci.*, 9 (3): 154-8.

Gaudin, J. 1828. Flora Helvetica sive historia stirpium hucusque cognitarum in Helvetia et in tractibus counterminis aut sponte nascentium aut in hominis animaliumque usus vulgo cultarum continuata. Vol. 2. Orelli, Fuessli & Co., Zürich, 57 pp. Translate.

Gupta, H.P. 1971. Studies of Indian Pollen Grains-IV. Boraginaceae. *Geophytology*, 1: 127-134.

Hargrove, L. and M.G. Simpson. 2003. Ultrastructure of Heterocolpate Pollen in *Cryptantha* (Boraginaceae). *Int. J. Pl. Sci*., 164(1): 137-151.

Hesse, M., H.R. Halbritter, M. Zetter, R. Weber, A. Buchner, A. Frosch-Radivo and S. Ulrich. 2009. *Pollen terminology: An Illustrated Handbook* Springer. New York.

Huyunh, K.L. 1971. *Le pollen du genera Arnebia* Frossk.et du genre. Macrdomia DC. (Boraginaceae), *et al* Position taxonomiquue Partcullariere du M. echioides (L.) Boiss. *Candollea*, 26: 165-171.

Huyunh, K.L. 1972. The original position of the generative nucleus in the pollen tetrads of *Agropyron*, *Jtea, Linnanthes* and *Onosma*, and its phylogenetic significance in the Angiosperms. *Grana*, 12: 105-112.

Khatamsaz, M. 2001. Pollen morphology of Iranian Boraginaceae family and its taxonomic significance. *Iran J. Bot.*, 9: 27-40.

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. *J.Pl. Syst. & Evol*., 290: 75-83.

Liu, J.X., Y.L. Zhang, J.C., Ning, Y.Y. Zhao, Y.X. Li, J.M. Zhang and X.H. Sun. 2001b. Pollen morphology of the Tribe Cynoglosseae of Boraginoideae (Boraginaceae) in China.*Acta Bot. Sin.*, 39(6): 515-522.

Liu, J.X., Y.Z. Xi, J.C. Ning, J.M. Zhang, Y.X. Li, Y.Y. Zhao and X.H. Sun. 2001a. Pollen morphology and Exine Ultrastructure of genus *Cordia* in Boraginaceae in China. *Acta Bot. Sin.*, 43(9): 893-899.

Liu, J.X., Y.Z. Xi, J.C. Ning, X.H. Sun, J.M. Zhang and Q. Li. 2003.Pollen morphology of the subfamily Ehretiodeae in the Boraginaceae from China and its systematic significance. *Acta Bot. Sin.*, 41(3): 209-219.

Liu, J.X., Y.Z. Xi, Y.L. Zhang, J.C., Ning and X.R. Liu. 2008. Pollen morphology of Heliotropiodeae in Boraginaceae from China and its systematic significance. *J. Syst. & Evol*., 46(6): 891-898.

Maggi, F., V. Kolarchik and P. Martonfi, 2008. Palynological analysis of five selected *Onosma* taxa: *Biologia*, 63(2) 183-186.

Marticorena, P.C. 1968. *Granos de polen de plantas chilenas. Gayana: Instituto Central de Biologia Botanica*, 17: 36-60.

Mehrabian, A.R., M. Sheidai, Z. Noormohammadi, V. Mozaffarian and Y. Asri. 2012. Palynological diversity in the genus *Onosma* L. (Boraginaceae) of Iran. *Ann. Bio. Res.*, 3 (8): 3885-3893.

Nair, P.K.K. 1964. *Advances in Palynology*. National Botanical Garden, Lukhnow, India.

Ning, J.C. 1991. A study of Pollen morphology of Boraginaceae in China. *Inst. Bot. Chinese Acad. Scie.*, pp. 60.

Ning, J.C., X.Y. Zhen and Y.L. Zhang. 1995. A comparative palynological study on the *Maharanga* and *Onosma* (Boraginaceae). *Acta Phytotax. Sin*., 33(1): 52-57.

Ning, J.C., Y.Z. Xi and Y.L. Zhang. 1992. Pollen morphology of the tribe Anchusae DC (Boraginaceae) and its taxonomic significance. *Cathaya*, 4: 79-90.

Ning, J.C., Y.Z. Xi and Y.L. Zhang. 1993. A study on pollen morphology of *Microula* Benth. and allied genera (Boraginaceae). *Acta Phytotax. Sin*., 31: 318-328.

Nowicke, J.W. and J.E. Ridgway. 1973. Pollen studies in the genus *Cordia* (Boraginaceae). *Amer. J. Bot.*, 60: 584-591.

Nowicke, J.W. and J.J. Skvarla. 1974. A palynological investigation of the genus *Tournefortia* (Boraginaceae). *Amer. J. Bot.*, 61: 1021-1031.

Osaloo, K.S. and M. Khatamsaz. 1994. Pollen morphology of the genus *Echiochilon* (Boraginaceae) in Iran. *Iran J. Bot.*, 6(2): 247-249.

Ovchinnikova, S.V. 2003.The system of *Eritrichium* (Boraginaceae). *J. Botanicheskii Zhurnal*, 7: 77-88.

Ovchinnikova, S.V. 2008.Conspectus of the genus *Eritrichium* (Boraginaceae) species in North *Asia. J. Pl. Life of Asian Russia*, 1: 17-36.

Ovchinnikova, S.V. 2011. Conspectus of the subgenus Pseudohackelia of genus *Eritrichium* (Boraginaceae) species. *J. Pl. Life of Asian Russia*, 1: 41-45.

Perveen, A., U.S. Qureshi and M. Qaiser. 1995. Pollen flora of Boraginaceae Pakistan. *Pak. J. Bot*., 27(2): 327-360.

Popov, M.G. 1953. Boraginaceae. In: (Ed.): Shishkin, B K. *Flora of the U.S.S.R Izdatel'stvo Akademii Nauk* SSSR. 19: 359-388.

Popov, N.G. 1953. Anchusae DC. In: (Ed.): Komarov, V.L. *Flora of U.S.S.R*, vol. 19. Keter Press, Jerusalem, 207-263.

Punt, W., P.P. Hoen, S. Blackmore, S. Nilson and A. Le-Thomas. 2007. *Glossary of Pollen and Spore Terminology. Rev. Paleobot. Palynol.*, 143: 1-81.

Qureshi, U.S. 1979. *Pollen Morphology of Boraginaceae of Pakistan and Kashmir*.Doctoral Thesis University of Sind, Pakistan.

Qureshi, U.S. and K.M. Khan. 1985. Anomaly of pollen apertures of *Coldenia procumbens* (Boraginaceae). *Pak. J. Bot.*, 17(1): 115-117.

Qureshi, U.S. and M. Qaiser. 1987. Palynological study of *Onosma* (Boraginaceae) from Pakistan. *Pak. J. Bot.*, 19(1): 99-105.

Qureshi, U.S., M. Qaiser and S. Shaukat. 1989. Studies in pollen morphology of *Arnebia* species (Boraginaceae) from Pakistan. *Pak. J. Bot.*, 21(1): 148-156.

Retief, E. and A.E. Vanwyk. 1997. Palynology of southern African Boraginaceae: the genera *Lobostemon, Echiostachys* and *Echium. Grana*, 36(5): 271-278.

Scheel, R., J.P. Ybert and O.M. Barth. 1996. Pollen morphology of the Boraginaceae from Santa Catarina State (southern Brazil), with comments on the taxonomy of the family. *Grana*, 35(3): 138-153.

Walker, J.W. and J.A. Doyle. 1975. The Bases of angiosperm Phylogeny: palynology. *Ann. Mo. Bot. Gard.*, 62: 664-723.

Wang, F.X., N.F. Qian and Y.L. Zhang. 1995. *Pollen flora of China*, 2nd edn, Science Press Beijing, pp. 103-110.

Wang, W.T. 2010. New taxa of Boraginaceae from China *J. Guihaia*, 30 (4): 437-438.

Wang, W.T., Y.S. Lian and J.Q. Wang. 1980. A study of the genus *Eritrichium* from China. Bulletin of Botanical Laboratory of North-Eastern Forestry Institute, 18 (4): 31-52.

Willis, J.C. 1973. *A Dictionary of Flowering Plantsand Ferns.* Univ. Press, Cambridge.

Xi, Y.Z. 1984. Pollen Morphology of Trigonotideae. *Bulletin of Botanical Research*, 4(3): 69-81.

Zhu, G.L. 1995. Boraginaceae. In: (Eds.): Wu, Z.Y.&P.H. Raven. *Flora of China*, Beijing: Science Press & St. Louis: *Mo. Bot. Gard.* 16: 329-427.

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