



NOTE

Pollen heteromorphism in *Diplarche* Hook. f. & Thomson (Ericaceae) and its taxonomic significance

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ABSTRACT: A comparative pollen morphological study, using both LM and SEM, has been carried out in *D. multiflora* Hook. f. & Thomson and *D. pauciflora* Hook. f. & Thomson belonging to the family Ericaceae. The exine ornamentation supports the conspecific nature of both the species.

KEY WORDS: *Diplarche*; Ericaceae; Pollen heteromorphism; Taxonomic significance.

INTRODUCTION

The genus *Diplarche* includes two species distributed in India, Nepal, Bhutan, China (Tibet Autonomous Region -TAR) and Myanmar (Hanbi & Chamberlain 2005; Panda & Sanjappa 2014). The genus *Diplarche* was erected based on these two species, viz. *D. multiflora* Hook. f. & Thomson and *D. pauciflora* Hook. f. & Thomson (Hooker and Thomson, 1854). Since then the above two species were maintained as independent entities for a long period of time (Hooker & Thomson 1854; Clarke 1882; Airy Shaw 1964; Rae 1991; Kron *et al.* 2002; Hanbi & Chamberlain 2005; Panda & Sanjappa 2014). Recently Maity & Dey (2015) have treated the two taxa as conspecific and recognized *D. multiflora* as the sole species and considered *D. pauciflora* as synonym of the former. They established this fact through extensive morphological evidences. In addition, they have also examined the pollen grains of limited specimens of this species complex. The present study attempts to examine in detail the variability of pollen surface ornamentation in both the species in order to utilize the data for taxonomic purposes.

MATERIALS AND METHODS

Fresh mature flower buds (likely to be opened within following 2–3 days), as well as immature flowers, (just opened or partly opened) were collected during field visits. Samples were preserved in small dry and clean envelopes and labeled properly. Pollen samples, preferably mature flower buds and sometimes immature flowers, were also collected from the herbarium specimens deposited at Central National Herbarium (CAL) and Calcutta University Herbarium (CUH). The

herbarium specimens were properly identified and labeled. For LM studies, pollen samples were acetolysed and mounted with unstained glycerin jelly following Erdtman (1952). Photographs of pollen of all specimens were taken using Leica DM750 Microscope. SEM studies were also carried out. In SEM study, pollen grains were directly transferred to a metallic stub with double-sided adhesive tape and then sputter coated with gold. The specimen stubs were observed under the Scanning Electron Microscope (SEM - FEI, Model Quanta 200) maintaining accelerating voltage of 25 kv. The SEM study was performed at Central National Herbarium (CAL). Descriptions of individual pollen tetrads were done following Erdtman (1952) with necessary modifications, whenever required.

RESULTS

Detailed examination of the pollen tetrads of specimens identified either as *D. multiflora* (*sensu* Hooker & Thomson 1854) or *D. pauciflora* (*sensu* Hooker & Thomson 1854) was carried out. Grains occur in tetrahedral tetrads and joined with distinct viscin threads (Figs. 1A, B, 1D). Individual grain is 3-zonocolporate having faint areolate surface under LM (Fig. 1C). Tetrad size varies from 29–33×24–25 μm. The highly variable surface ornamentation is categorised into seven types. All the types are described in detail with respective specimens.

Psilate type (exine with smooth surface): Pollen tetrads from the specimens *F. Ludlow*, *G. Sherriff* & *H. Elliot* 15126 (4 plants) collected from Eastern Tibet, provisionally identified as *D. multiflora* were mostly with psilate exine (Fig. 1E, F). The psilate nature of the exine was also observed in pollen obtained from the specimen *Maity* 20112.

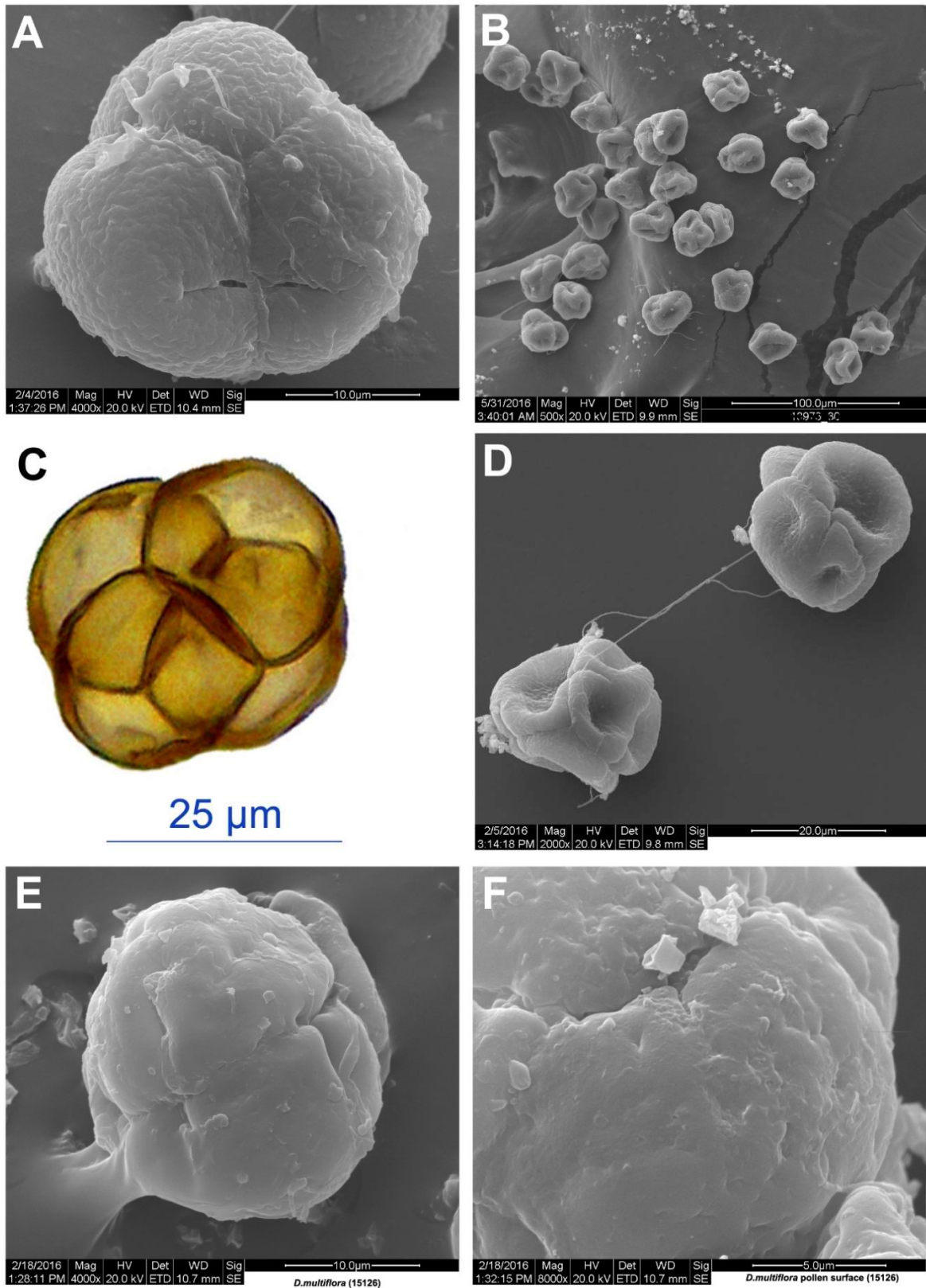


Fig.1. **A.** Single pollen tetrad, **B.** Panoramic view of pollen tetrads, **C.** LM image of pollen tetrad, **D.** Pollen tetrads joined by viscin threads, **E.** Psilate type pollen from specimens Ludlow, G. Sherriff & H. H. Elliot 15126-*D. multiflora*, **F.** Enlarged surface view of Ludlow, G. Sherriff & H. H. Elliot 15126-*D. multiflora*.

**Table 1.** Comparison of major characters of pollen grains of *D. multiflora* and *D. pauciflora*

Features	<i>D. multiflora</i>	<i>D. pauciflora</i>
Pollen tetrad shape	sub-prolate	sub-prolate
Pollen tetrad size	29–33×24–25 µm	29–33×24–25 µm
Pollen tetrad type	tetrahedral	tetrahedral
Pollen surface ornamentation	psilate, rugose, irregular granular, micro-verrucate, microverrucate-gemmate, flattened verrucate-gemmate, microverrucate-gemmate-grannulate	psilate, rugose, microrverrucate

Rugose type (exine with wrinkled surface): The pollen tetrads from the specimens *G. H. Cave*134 (2 plants, identified as *D. multiflora*) collected from Peigu, Sikkim showed more or less rugose surface (Fig. 2A, B). Similar observation was also noted in pollen obtained from specimens of *D. pauciflora* collected from Sikkim (*H&B* 164, 2 plants; and *Maity* 20112, 3 plants).

Irregular granular type (exine with irregularly scattered granules or small rounded elements): Pollen tetrads from the specimens *Dungboo s.n.*, acc. no. 267994 (4 plants, identified as *D. multiflora*) collected from Chumbi and Phari showed rugose exine with few scattered irregular granules (Fig. 2C, D).

Micro-verrucate type (exine with minute warts with broad base): The specimens of *D. pauciflora* collected from Sikkim (*H&B* 164, 2 plants) showed finely verrucate exine ornamentation. However, at some places adjacent verrucae were found to coalesce to form compound verrucae. At some places low plate-like structure with trapezoid endings were also observed. Apocolpial region were found to be apparently smooth (Fig. 2E, F).

Microverrucate-gemmate type (exine with irregularly scattered ± rounded elements with constricted base): The specimens *F. Ludlow, G. Sherriff & H. H. Elliot* 15126, (with 4 plants), collected from Deyang La, Kongbo, South East Tibet and identified as *D. multiflora* show microverrucate exine with several scattered large gemmae (Fig. 3A, B).

Flattened verrucate-gemmate type (exine with flattened verrucae and scattered gemmae): In the specimens *F. Ludlow, G. Sherriff & H. H. Elliot* 13772, (with 4 plants), collected from Lisum, NunkhuPhu Chu Valley, Near Tongyuk, Pome, South East Tibet and identified as *D. multiflora*, the pollen surface ornamentation was found to vary to a greater extent. Some pollen tetrads were found to bear typical verrucae. Some of the verrucae seemed quite larger whereas others were small. The large and the small verrucae were irregularly arranged on the pollen surface. At some places pollen surfaces were also found to bear gemmae. On some pollen tetrads low plate-like structures were observed and in other places units were found to be arranged in a somewhat jig-saw puzzle-like manner (Fig. 3C, D).

Microverrucate-gemmate-grannulate type (exine surface with microverrucae, scattered gemmae-like and granule-like structures): Pollen tetrads from the specimen *F. Ludlow, G. Sherriff, & H. H. Elliot* 13973 *D. multiflora* from Ba La, Pasum Chu, Kongbo, collected from South East Tibet were found to have a

more or less verrucate surface. At some places the verrucae were flattened and plate-like trapezoid structures were present. Gemmae or sometimes even compound gemmae were found to be prevalently associated with the junctional region of the pollen grains in the tetrad. Grannules are also found among gemmae (Fig. 3E, F).

DISCUSSION

Pollen character usually play an important role in diagnosis of a particular taxon (Erdtman 1952). In the course of present study, we examined all specimens of the genus *Diplarche* housed at CAL, ASSAM and CUH, collected not only from India but also from neighbouring countries, viz. Bhutan, China (TAR), Yunnan (see Appendix). As in most Ericaceae, the pollen grains of both the taxa shed as tetrahedral tetrads and were often found to be associated with viscin threads (Fig. 1 A, B & D,) (Erdtman 1952). The size of the tetrad is more or less same in all the studied specimens. SEM study of the sporoderm from both the species revealed that the pollen are widely heteromorphic in this species complex with regard to the exine ornamentation. There were no obvious correlations between the pollen tetrads (surface architecture) and either the geography or the ecology of the plants from which it was collected. Notably, the pollen surface ornamentation does not correlate with the size of the plants or the attachment of stamens on the corolla tube and thus fails to justify the species status, either *D. pauciflora* or *D. multiflora*, of the studied specimens.

The heteromorphic nature of pollen grains are limited in expression and rare often found in few angiosperm species or species complexes (Pire & Dematteis 2007; Yasmin *et al.* 2010; Bhowmik & Datta 2012; Siddhanta *et al.* 2018), also in the family Ericaceae (Rosatti 1988; Reunova *et al.* 2007). The present study supports the conspecific nature of both species and well corroborated with the view of Maity & Dey (2015). However, the grouping of specimens based on pollen morphology as predicted by them is not acceptable. Present study reveals that pollen ornamentation is highly variable in both the species provisionally identified either as *D. pauciflora* or *D. multiflora*. Thus, a comparison of major pollen morphological characters of both the species has also been provided (Table 1). Pollen morphology not only varies between specimens but also within the same anther. For example, pollen grains of different species have the same exine ornamentation

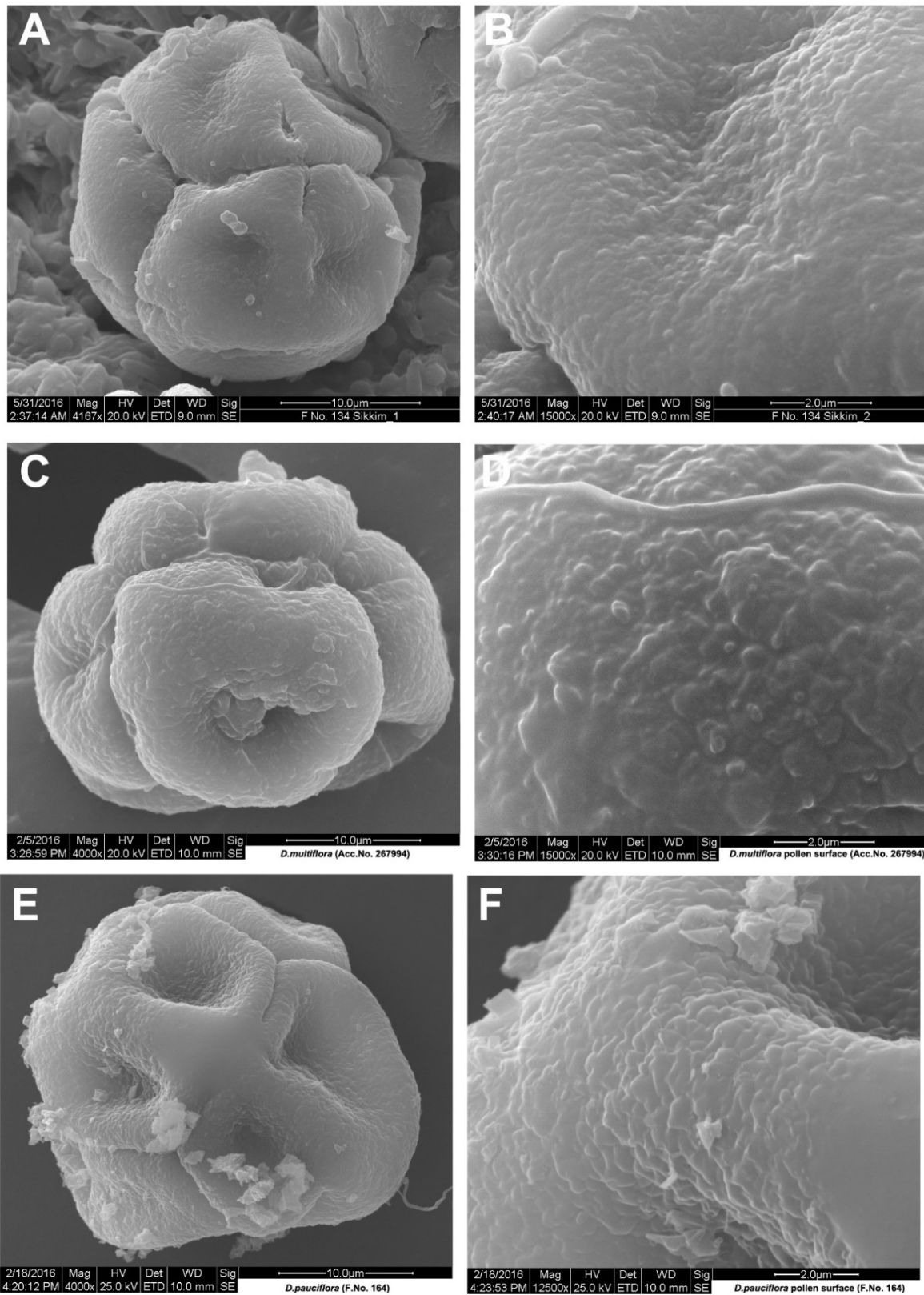


Fig. 2. A. Rugose type pollen from specimens G. H. Cave 134-*D. multiflora*, B. Enlarged surface view of G. H. Cave 134-*D. multiflora*, C. Irregular granular type pollen from *Dungboos.n.*, acc. no. 267994-*D. multiflora*, D.Enlarged surface view of *Dungboos.n.*, acc. no. 267994-*D. multiflora*,E. Micro-verrucate type pollen from H&B 164 *D.pauciflora*, F.Enlarged surface view of H&B 164 *D.pauciflora*.

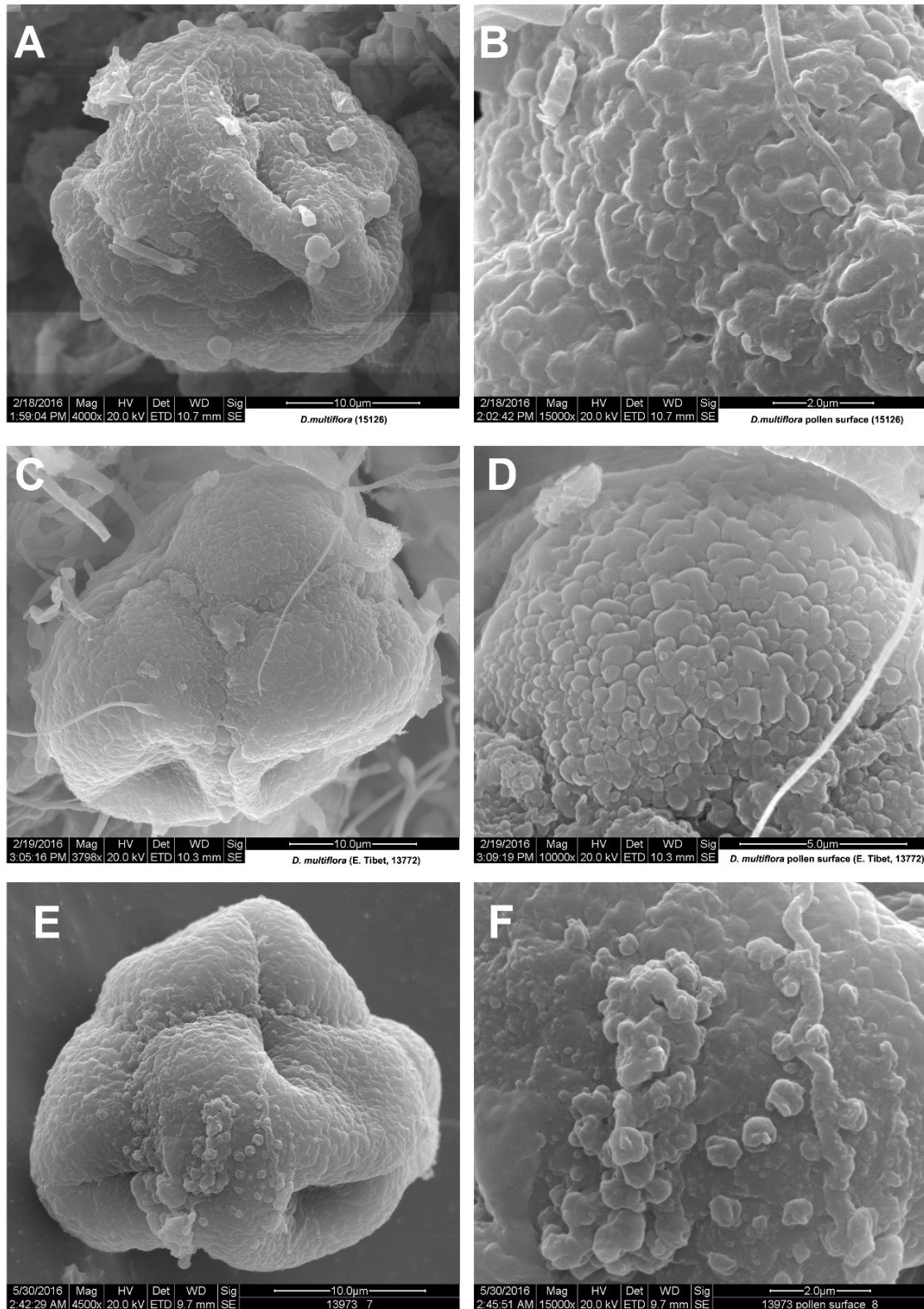


Fig. 3. **A.** Microverrucate-gemmata type pollen from specimen of *F. Ludlow, G. Sherriff & H. H. Elliot* 15126 *D. multiflora*, **B.** Enlarged surface view of *F. Ludlow, G. Sherriff & H. H. Elliot* 15126 *D. multiflora*, **C.** Flattened verrucate-gemmata type pollen from specimen of *F. Ludlow, G. Sherriff & H. H. Elliot* 13772 *D. multiflora*, **D.** Enlarged surface view of *F. Ludlow, G. Sherriff & H. H. Elliot* 13772 *D. multiflora*, **E.** Microverrucate-gemmata-granulate type pollen from *F. Ludlow, G. Sherriff & H. H. Elliot* 13973 *D. multiflora*, **F.** Enlarged surface view *F. Ludlow, G. Sherriff & H. H. Elliot* 13973 *D. multiflora*.



(rugose type) as has been noted in case of *G. H. Cave* 134 and *Maity* 20112 (identified as *D. multiflora*) and *H&B* 164 (identified as *D. pauciflora*), whereas pollen from same specimens (*H&B* 164 and *F. Ludlow*, *G. Sherriff* & *H. H. Elliot* 15126) have different exine ornamentation patterns. Thus, the view of Maity & Dey (2015), i.e. pollen grains with warty exine are associated with *D. pauciflora*, whereas psilate to scabrate exine ornamentation is always associated with *D. multiflora*, contradicts the present observation. Heteromorphism in exine ornamentations are often in perception with some external factor like environment (Pire & Dematteis 2007) and/or an internal factor such as change in ploidy levels (Mignot *et al.* 1994; Dajoz *et al.* 1995; Nadot *et al.* 2000). Though the cytological report of *Diplarche* is not available till date, however, being a Himalayan high altitude taxa, both species, viz. *D. multiflora* and *D. pauciflora* experience diverse and harsh climatic as well as ecological conditions which could be a possible reason for such great exine heteromorphism.

Appendix: Details of studied specimens of *Diplarche* deposited at CAL, CUH and ASSAM.

D. multiflora Hook. f. & Thomson: **India:** Chumbi & Phari; July 1879, *Dungboo s.n.* (CAL acc. no. 267991-267995); Tankra Mt, Sikkim, 15,000ft (4500m); 04 Aug. 1892, *G. A. Gammie 554* (CAL acc. no. 267996-267997); Paigu, Sikkim, 12,300ft. (3700m); 17 Jul. 1906, *G. H. Cave 134* (CAL); Eumtso La Sikkim, 15,000ft. (4500m); 12 Jul. 1909, *W.W. Smith & G. H. Cave 1272* (CAL acc. no. 267990); On the way to Sadiya from Lohit valley, Arunachal Pradesh; 1950, *Kingdon-Ward s.n.* (ASSAM); Upper Thangu valley, Sikkim, 4200 m; 13 Jul. 2013, *Maity 20112 & 20113* (CUH); Upper Thangu valley, Sikkim, 4200 m; 15 Jul. 2013, *Maity 20309* (CUH); Upper Thangu Valley, Sikkim, 4100 m; 26 Jul. 2014, *Dey & Maity 21556* (CUH); Upper Thangu valley, Sikkim, 4200 m; 04 Aug. 2015, *Dey & Maity 21727* (CUH); Upper Thangu valley, Sikkim, 4200 m; 04 Aug. 2015, *Dey & Maity 21728* (CUH); Upper Thangu valley, Sikkim, 4100 m; 04 Aug. 2015, *Dey & Maity 21726* (CUH); Upper Thangu Valley, Sikkim, 4200 m; 05 Aug. 2015, *Dey & Maity 21732* (CUH); Upper Thangu Valley, Sikkim, 4200 m; 05 Aug. 2015, *Dey & Maity 21733* (CUH); Upper Thangu Valley, Sikkim, 4400 m; 05 Aug. 2015, *Dey & Maity 21729* (CUH); Upper Thangu Valley, Sikkim, 4400 m; 05 Aug. 2015, *Dey & Maity 21730* (CUH). **China:** Tibet, South Eastern Tibet; 1917–1919, *George Forrest 18923* (CAL acc. no. 268002); Chubumu, La, Langong, Takpo Province, South-Eastern Tibet, 12,500–13,500 ft (3800–4100 m); 06 Jun. 1938, *F. Ludlow, G. Sherriff & G. Taylor 3979* (CAL acc. no. 562039); Lusha Chu, Kongbo Province, South-Eastern Tibet, 12,500 ft. (3800 m); 10 Jun. 1938, *F. Ludlow, G. Sherriff & G. Taylor 4653* (CAL); Deyang La, Kongbo, South East Tibet, 12,500 ft. (c.3800 m); 4 Jun. 1947, *F. Ludlow, G. Sherriff & H. H. Elliot 15126* (CAL); Ba La, Pasum Chu, Kongbo, South East Tibet, 14,500 ft. (c.4400m); 221947 *F. Ludlow, G. Sherriff & H. H. Elliot 13973* (CAL); Nambu La, Kongbo, 14,500 ft. (c. 4400m); 12 Jul. 1947, *F. Ludlow, G. Sherriff & H. H. Elliot 15409* (CAL); Yunnan; 1917–1919, *George Forrest 14179* (CAL acc. no. 268003); **Bhutan:** Me La, South side, Bhutan, 13,000 ft (3900 m); 16 Jun. 1949, *F. Ludlow, G. Sherriff & J. H. Hicks 20368* (CAL).

D. pauciflora Hook. f. & Thomson: **India:** Eumtso La (pass), Sikkim, 14,600ft. (4400m); 20 Jul. 1906, *H & B 164* (CAL).

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