Bangladesh J. Plant Taxon. **19**(2): 191-200, 2012 (December)

POLLEN MORPHOLOGY OF *BEGONIA* L. (BEGONIACEAE) IN NEPAL

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Keywords: *Begonia* L.; Nepal; Pollen morphology; SEM.

Abstract

The pollen morphology of 28 *Begonia* species of Nepal has been examined by scanning electron microscopy (SEM). Comparative pollen analysis was made based on size, shape in polar and equatorial views, P/E ratio, aperture, and exine ornamentation. In this study, four types of pollen ornamentation morphology have been identified. Among Nepalese *Begonia*, *B. roxburghii* (section *Sphenanthera*) has the smallest pollen (11.2-12.8 × 6.4-7.0 µm) and *B. flagellaris* (section *Diploclinium*) has the largest pollen (24.3-30.6 × 11.4-12.0 µm). Presence of margo in the pollen ornamentation is a distinguishing character that separates *Begonia* section *Platycentrum* from all other sections of Nepalese *Begonia*.

Introduction

The genus *Begonia* L. (Begoniaceae) is the sixth largest genus of flowering plants (Frodin, 2004). The genus was previously represented by 18 species in Nepal (Hara *et al*., 1978; Doorenbos *et al*., 1998; Press *et al*., 2000), one new record (Rajbhandary and Shrestha, 2009) and three new species (Rajbhandary *et al.*, 2010) bring the current number of *Begonia* species known from the country to 22. There are seven endemic species in Nepal: *B. tribenensis* C.R. Rao, *B. minicarpa* H. Hara, *B. flagellaris* H. Hara, *B. leptoptera* H. Hara, *B. nuwakotensis* S. Rajbhandary, *B. panchtharensis* S. Rajbhandary and *B. taligera* S. Rajbhandary. Nepalese *Begonia* are placed within five different sections: *Diploclinium* (Lindl.) A.DC., *Monopteron* (A.DC.) Warb., *Platycentrum* (Klotzsch) A.DC., *Putzeysia* (Klotzsch) A.DC. and *Sphenanthera* (Hassk.) Warb. (Smith *et. al*., 1986; Doorenbos *et al*., 1998).

The variation in ultrastructural characteristics and surface morphology of pollen grains is often of valuable assistance in delimiting taxonomic relationships, particularly at higher taxonomic ranks for family level: Euphorbiaceae (Perveen and Qaiser, 2005), Tiliaceae, Sterculiaceae and Malvaceae (Husseini, 2006), Cucurbitaceae (Perveen and Qaiser, 2008), Campanulaceae (Erkara *et al.*, 2008), for generic level in *Sambucus* (Tamas *et al.*, 2009), but also between species as in *Pedicularis* (Bano *et al.*, 2012). Scanning electron microscopy (SEM) is a valuable tool which can reveal many of these taxonomically useful characters, and have the potential to reveal relationships that are obscure and difficult to demonstrate by other means.

Although pollen morphology is important in taxonomic research, very few studies have been carried out in this regards on *Begonia* species. Erdtman (1966) noted the small size, the prolate and perprolate shape of the *Begonia* pollen and a very thin exine which did not show much stratification. Van Den Berg (1985) carried out an SEM study of the pollen morphology of African *Begonia* to shed light on sectional delimitation in the genus. There has been no study regarding both American and Asian species, including Nepalese *Begonia*. In some works on pollen morphology (Wodehouse, 1935; Erdtman, 1966) a few of the indigenous plants of Southeast Asia

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have been described, but none from mainland Asia or Nepal. Recognition of pollen types may lead to a better understanding of the relationships of the taxa within the genus (Van Den Berg, 1984). The objective of this paper is to provide a detailed account of the pollen morphology of Nepalese *Begonia* as highlighted through SEM.

Materials and Methods

Pollen of 28 *Begonia* species in Nepal belonging to four sections, namely *Diploclinium*, *Monopteron*, *Platycentrum* and *Sphenanthera* were examined from the herbarium specimens collected in Nepal and deposited in TUCH, KATH, E, and K herbaria. Due to lack of male flowers on specimens of *Begonia gemmipara* (section *Putzeysia*), the pollen morphology of this species could not be studied. Morphological variation in pollen includes the size, P/E ratio, aperture and ornamentation (Table 1).

Pollen data obtained in this study are based on observations using SEM. Pollen grains were mounted on Agar Scientific adhesive carbon tabs 12 mm in size placed on Aluminium stubs. The pollen on the stubs was then sputter-coated with 250 nm platinum particles using an Emitech K575X sputter coater at one runs of 2 min. The prepared specimens were examined in a LEO Supra 55VP Scanning Electron Microscope at a voltage of 5kV and a working distance 6 mm. The SEM images were captured at a resolution of 2048 × 1536 pixel and saved in TIF format. The SEM was carried out at the Royal Botanic Garden Edinburgh, UK.

Results and Discussion

The pollen grains in *Begonia* are single, isopolar and 3-zono-colporate (Fig. 1a-b). The shape ranges from prolate to perprolate and the polar axis measures from 11.2-28.9 µm and the equatorial diameter from 1.7-3.1 µm. The outline of *Begonia* pollen is mostly somewhat elliptic. The long sides though usually convex, can be straight or even concave, the poles can be rounded or somewhat pointed. The outline in the polar view can be either circular (*B. flagellaris* Fig. 2 A-B; *B. minicarpa* Fig 4. M-N; *B. flaviflora* Fig. 5 C-D) or triangular with interaxillary apertures in majority of species. The ecto-apertures are very long and closed by a granular colpus membrane. The striate ornamentation continues up to the margin of the colpus and in some species a margo of deviating not-striate ornamentation is present along the colpus.

The pollen of *B. anisanthera ined., B. manangiensis ined., B. cathcartii, B. flaviflora* and *B. roxburghii* are prolate while rest of the taxa studied have perprolate pollen (Table 1). Among Nepalese *Begonia, B. roxburghii* (section *Sphenanthera*) has the smallest pollen (11.2-12.8 × 6.4-7.0 µm) and *B. flagellaris* (section *Diploclinium*) has the largest pollen (24.3-30.6 × 11.4-12.0 µm). The shape and type of aperture in the pollen grains in the *Begonia* species studied proved to be of little systematic significance, as most of the species have perprolate pollen with only a few species having prolate pollen. Even so, within the taxa and even within the samples a certain amount of variability is still encountered, especially in size, shape, and apertures.

The nature of surface ornamentation is the most important feature which is of systematic significance. The sculpture on the surface of the grains is formed by a pattern of exine elements separated by spaces. The exine elements are called muri. The striate pattern shown by *Begonia* pollen can be defined as a regular pattern of approximately parallel muri. More in particular in striate grains or the ridges of exineous material, is termed as lirae and the grooves in-between the ridges as striate (Van Den Berg, 1984). Depending on the width of the lirae and striae, the striate pattern can be designated as finely or coarsely striate. A number of minute perforations are present

Table 1. Comparison in pollen size, aperture and ornamentation of 28 *Begonia* taxa in Nepal.

Sl. No	Species	Pollen size (µm)		P/E Ratio	Aperture	Ornamentation
		P (µm)	E (µm)			
	Section *Diploclinium*					
1.	*B. anisanthera* S. Rajbhandary *ined.*	13.6-13.8	7.2-7.4	1.8-1.9	prolate	coarsely striate
2.	*B. bryophila* S. Rajbhandary *ined.*	21.6-22.8	9.3-10	2.3-2.5	perprolate	coarsely striate
3.	*B. dioica* Buch.-Ham ex D. Don	24.2-26.2	9.4	2.6-2.8	perprolate	fine faint striate ornamentation with pores
4.	*B. dolichoptera* S. Rajbhandary *ined.*	23.8-24.8	10.2-10.4	2.3-2.4	perprolate	fine faint striate ornamentation with pores
5.	*B. flagellaris* H. Hara	24.3-30.6	11.4-12	2.0-2.6	perprolate	coarsely striate
6.	*B. josephii* A. DC.	24.8	9.8	2.5	perprolate	fine faint striate ornamentation with pores
7.	*B. leptoptera* H. Hara	26.0	8.6-9.4	2.7-3.0	perprolate	fine faint striate ornamentation with pores
8.	*B. manangiensis* S. Rajbhandary *ined.*	19.6-20.8	9.4-10.4	2.1	prolate	coarsely striate
9.	*B. minicarpa* H. Hara	17.4-18.2	7.6-8.0	2.2-2.3	perprolate	fine faint striate ornamentation with pores
10	*B. oedotheca* S. Rajbhandary & K.K. Shrestha *ined.*	22.0-23.0	11.0	2.0-2.1	perprolate	coarsely striate
11.	*B. ovatifolia* A. DC.	18.4	6.8-7.2	2.5-2.7	perprolate	fine striate
12.	*B. picta* Sm.	22.0-23.2	7.4	2.9-3.1	perprolate	fine faint striate ornamentation with pores
13.	*B. rubella* Buch.-Ham. ex D. Don	17.6-21.6	7.4-8.6	2.3-2.5	perprolate	fine striate
14.	*B. sinwaensis* S. Rajbhandary *ined.*	20.8-21.9	8.2-8.8	2.4-2.7	perprolate	coarsely striate
15.	*B. staintonii* S. Rajbhandary & K.K. Shrestha *ined.*	25-26	11.0	2.3-2.4	perprolate	fine faint striate ornamentation with pores
16.	*B. tribenensis* C.R. Roa	19.6-21.4	7.5-8.9	2.4-2.6	perprolate	fine striate
	Section *Platycentrum*					
17.	*B. annulata* K. Koch.	19.4-23.0	8.6-9.75	2.2-2.3	perprolate	fine striate with margo
18.	*B. cathcartii* Hook. f. & Thomson	17.4-17.6	9.2	1.8-1.9	prolate	coarsely striate with margo
19.	*B. flaviflora* H. Hara	14.4-19.5	8.4-10.0	1.7-1.9	prolate	coarsely striate with margo
20.	*B. hatacoa* Buch.-Ham. ex D. Don	16.0-18.6	8.0	2.0-2.3	perprolate	fine striate with margo

(Table contd.)

Table 1 Contd.

Sl No	Species	Pollen size (µm)		P/E Ratio	Aperture	Ornamentation
		P (µm)	E (µm)			
	Section *Platycentrum*					
21.	*B. megaptera* A. DC.	23.0-23.4	8.4	2.7-2.8	perprolate	fine striate with margo
22.	*B. nuwakotensis* S. Rajbhandary	22.0-22.5	7.6-8.4	2.7-2.8	perprolate	fine striate with margo
23.	*B. palmata* D. Don	21.6-25.1	8.4-8.9	2.6-2.8	perprolate	fine striate with margo
24.	*B. panchtharensis* S. Rajbhandary	16.8-21	8.0-10.0	2.1	perprolate	fine striate with margo
25.	*B. sikkimensis* A. DC.	24.6-28.9	8.8-10	2.6-2.8	perprolate	fine striate with margo
26.	*B. taligera* S. Rajbhandary	17.4-20	8.0	2.1-2.5	perprolate	coarsely striate with margo
	Section *Monopteron*					
27.	*B. nepalensis* (A. DC.) Warb.	19.6-20.0	8.2-8.6	2.3-2.4	perprolate	fine striate
	Section *Sphenanthera*					
28.	*B. roxburghii* (Miq.) A. DC.	11.2-12.8	6.4-7.0	1.7-1.8	prolate	fine striate

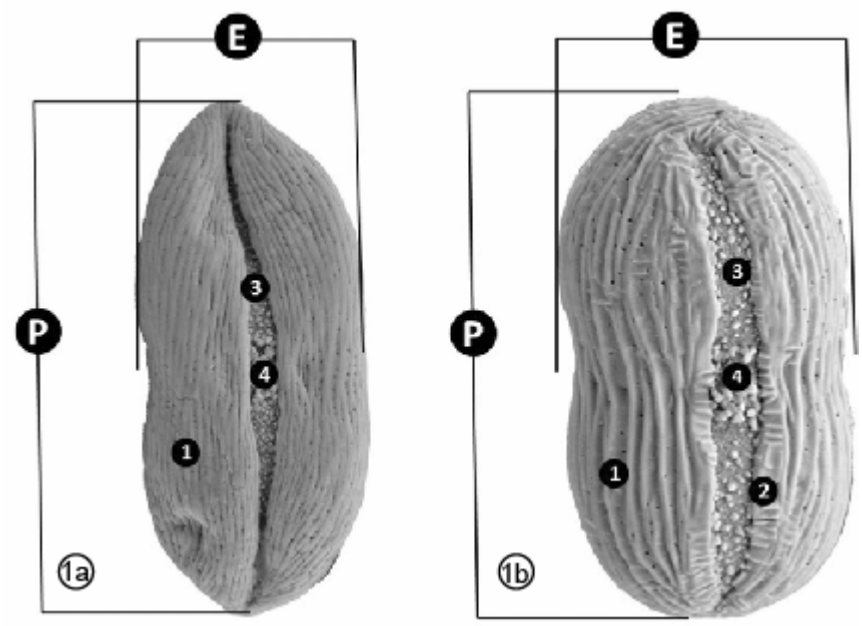

Fig. 1. General morphology of *Begonia* pollen equatorial view. 1a. Pollen without margo; 1b. Pollen with margo. P = Polar axia; E = equatorial diameter; 1 = striate ornamentation; 2 = margo; 3 = colpus membrane; 4 = endoaperture.

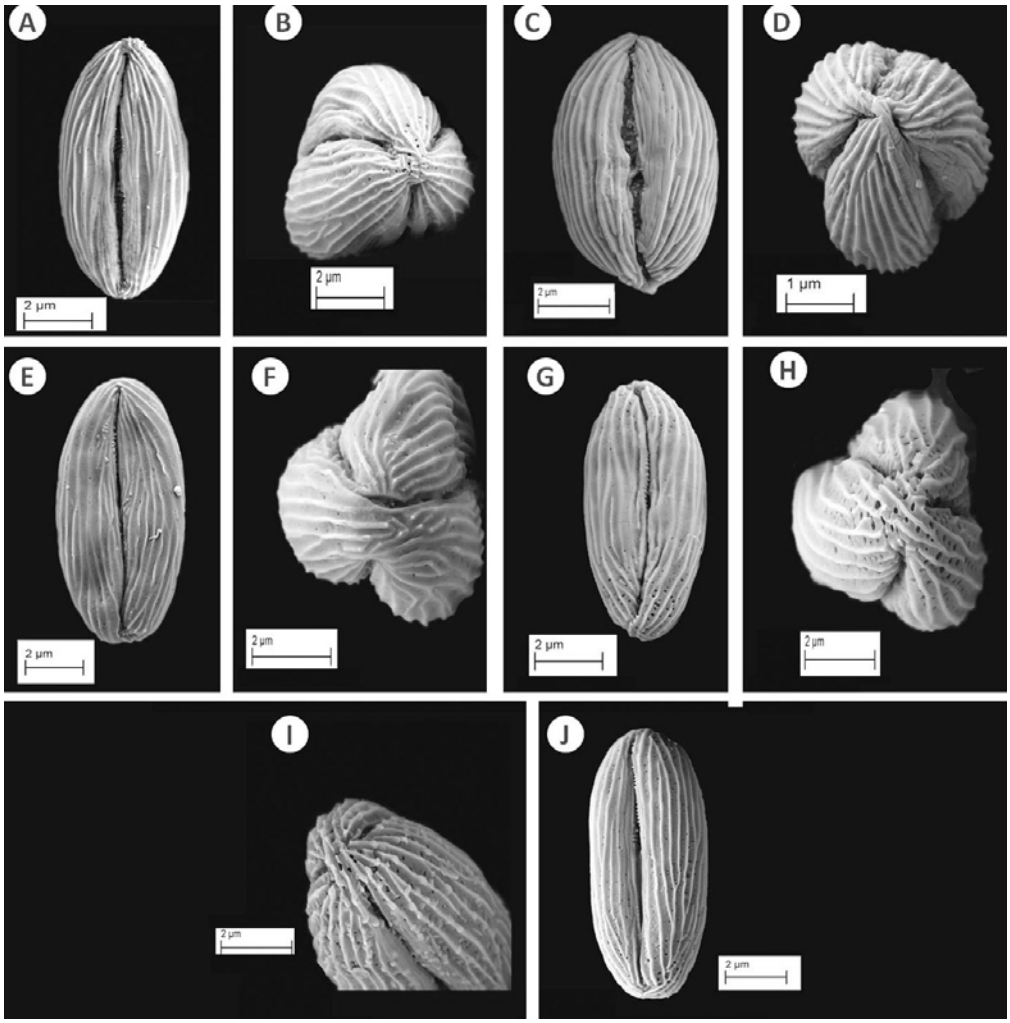

Fig. 2. Pollen with fine striate ornamentation with or without pores and absence of margo. A-B. *Begonia tribenensis* (Rajbhandary & Poudyal S1); C-D. *B. roxburghii* (Chand 5998); E-F. *B. nepalensis* (Stainton 8906); G-H. *B. rubella* (Rajbhandary *et al.* S34); I-J. *B. ovatifolia* (Williams & Stainton 8317).

in some species. A striking feature that was found in the pollen is the presence of a margo, which was very useful to separate *Begonia* sections *Platycentrum* from *Diploclinium*, *Sphenanthera* and *Monopteron*.

Within *Begonia* sections *Diploclinium*, *Sphenanthera*, *Monopteron* and *Platycentrum* exine ornamentation varies among species and four types of ornamentation morphology have been identified: (a) pollen with fine striate ornamentation with or without pores and absence of margo (Fig. 2); (b) pollen with coarsely striate ornamentation with few scattered pores and absence of margo (Fig. 3); (c) pollen with faint fine striate ornamentation with pores and absence of margo (Fig. 4) and (d) pollen with striate ornamentation with or without pores and presence of margo (Fig. 5).

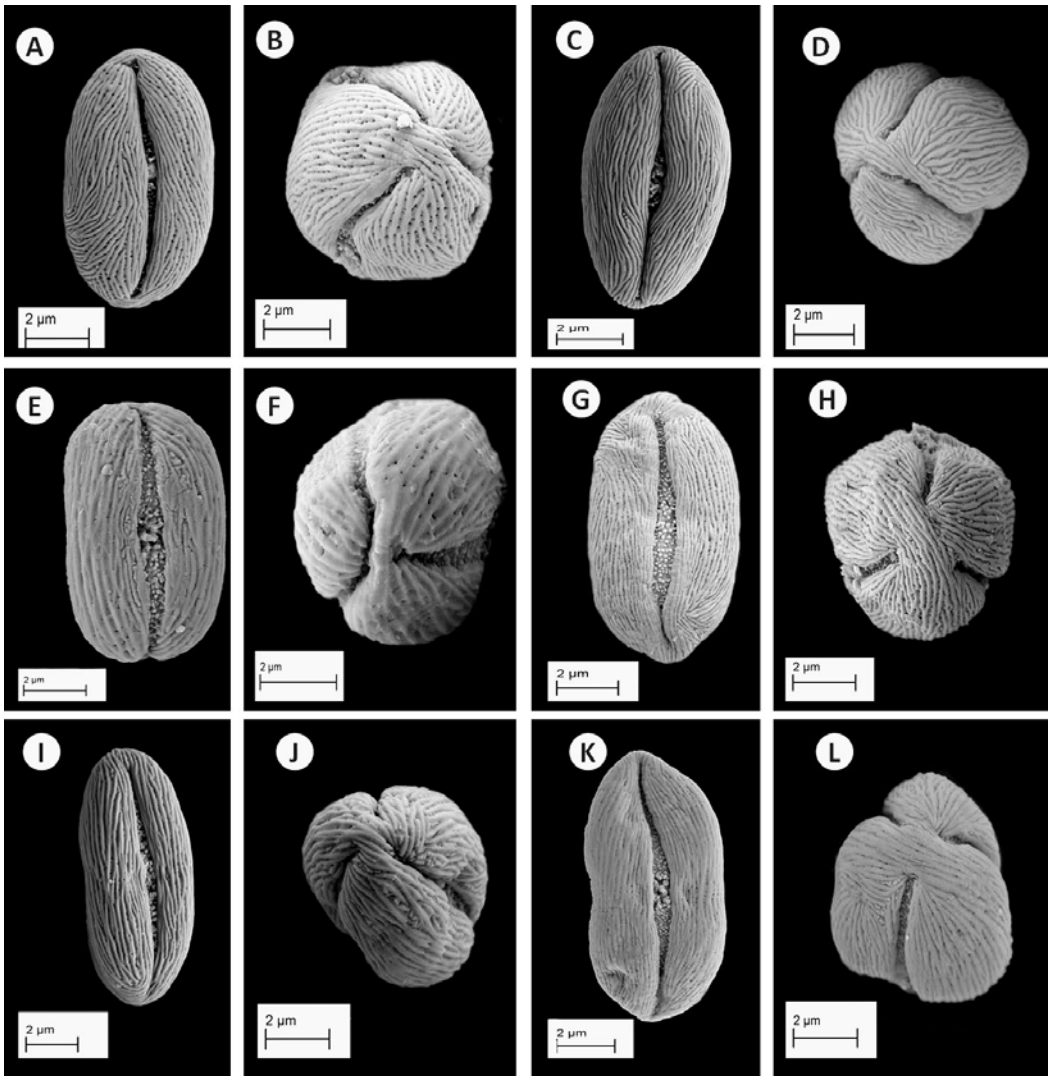

Fig. 3. Pollen with coarsely striate ornamentation with few scattered pores and absence of margo. A-B. *Begonia flagellaris* (Rajbhandary & Bista 10); C-D. *B. oedotheca ined.* (Stainton, Sykes & Williams 8419); E-F. *B. anisanthera ined.* (EMAK 6); G-H. *B. manangiensis ined.* (M. Mikage *et al.* 9470411); I-J. *B. sinwaensis ined.* (Rajbhandary & Bista S43); K-L. *B. bryophila ined.* (Rajbhandary & Bista S45).

There is complete absence of pores and margo (Fig. 2) in *B. roxburghii* (section *Sphenanthera*) and *B. nepalensis* (section *Monopteron*). *B. tribenensis*, *B. rubella* and *B. ovatifolia* have more pores near the polar region and on the poles (Fig. 2). The pores in *B. dolichoptera ined.* (Fig. 4 A-B) and *B. josephii* (Fig. 4 C-D) of section *Diploclinium* and in *B. annulata* (Fig. 5 A-B) and in *B. megaptera* (Fig. 5 G-H) of section *Platycentrum* are larger in size and very distinct at the polar region.

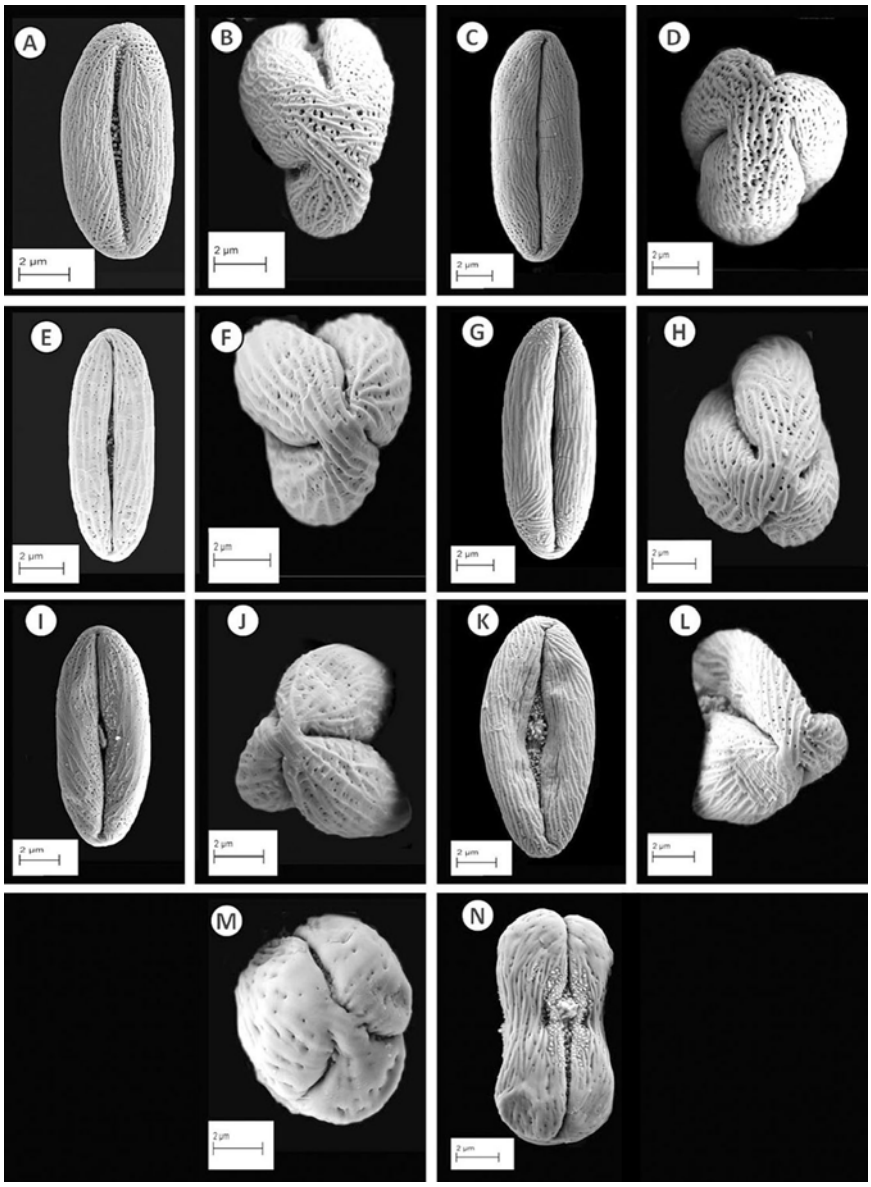

Fig. 4. Pollen with fine faint striate ornamentation with pores presented more towards the pole and absence of margo. A-B. *Begonia dolichoptera ined.* (KEKE 248); C-D. *B. josephii* (Rajbhandary & Bista S56); E-F. *B. picta* (Rajbhandary & Bista S02); G-H. *B. leptoptera* (Rajbhandary *et al.* S19).; I-J. *B. dioica* (Rajbhandary & Bista S42); K-L. *B. staintonii ined.* (Stainton 1414); M-N. *B. minicarpa* (Williams & Stainton 8319).

The pollen types sharing coarsely striate ornamentation differ in other characters such as size, shape and aperture (Fig. 3). In most of the species in this category, the ectoaperatures are very long with wide colpi and straight margin and closed by a granular colpus membrane which becomes coarser in the endo-apertural area (Fig. 3).

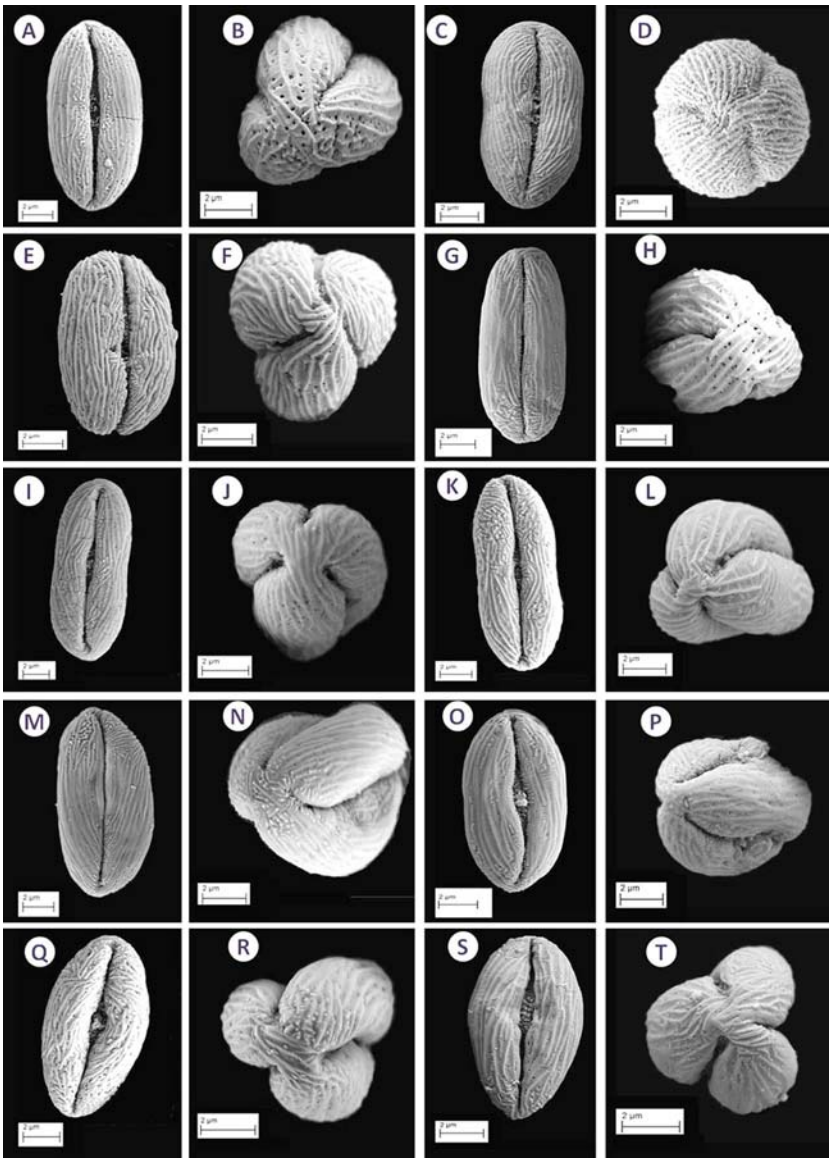

Fig. 5. Pollen in section *Platycentrum*, with striate ornamentation with or without pores and presence of margo. All pollens are photographes at same scale (2 µm but in different magnification). A-B. *Begonia annulata* (Shrestha S75); C-D. *B. flaviflora* (Adhikari *et al.* 146) ; E-F. *B. cathcartii* (Noshiro 9241006); G-H. *B. megaptera* (Kshretri 76); I.-J. *B. palmata* (Rajbhandary *et al.* S27); K-L. *B. nuwakotensis* (Rajbhandary *et al.* S31); M-N. *B. sikkimensis* (Rajbhandary *et al.* S14); O-P. *B. panchtharensis* (Rajbhandary S74); Q-R. *B. taligera* (Rajbhandary & Adhikari S52); S-T. *B. hatacoa* (Rajbhandary S4).

The poles can be rounded, or somewhat pointed. The outline in polar view can be circular or rounded (*B. flagellaris, B. anisanthera ined.*, *B. flaviflora, B. manangiensis ined.* and *B. minicarpa*), but most of the *Begonia* species have a somewhat triangular profile with interaxillary

apertures (Figs 2-5). In all the taxa the ending of the colpi are acute and approach each other closely at the polar axis. Therefore, the invaginating colpi often gives a lobate impression in the polar view. Pollen with faint fine striate ornamentation shows large pores on the polar region, very clearly seen in *B. dolichoptera ined.* and *B. josephii* (Fig. 4). The pollen of *B. minicarpa* is totally different (Fig. 4 M-N), with smooth spherical polar region and scattered pores and very different in shape, which separates it from other species.

Pollen grains in sections *Diploclinium*, *Sphenanthera* and *Monopteron* of *Begonia* lack margo, but margo is present in all the species in section *Platycentrum* (Fig. 5). From this it can be pointed out that the exine ornamentation is a more significant character than the apertures and shape of pollen to separate the sections of Nepalese *Begonia*.

Based on the present study, it appears that there is considerable variation in pollen morphology, especially in the exine and its sculpturing as revealed under SEM, which is of systematic value in the delimitation of some species and also sectional delimitation. Presence of margo in the pollen ornamentation is a distinguishing character that separates *Begonia* section *Platycentrum* from all other sections of Nepalese *Begonia*, as it is completely lacking in other sections. The observations presented here on the utility of pollen characters in section delimitation match that found in studies of African *Begonia* (Van Den Berg, 1984). The characters agree well with those reported earlier for Begoniaceae (Erdtman, 1966; Van Den Berg, 1984; Ma and Li, 2006).

Pollen morphology, especially the exine ornamentation, has supported placing seven undescribed species within *Begonia* section *Diploclinium*: *B. anisanthera ined.*, *B. bryophila ined. B. manangiensis ined.*, *B. oedotheca ined.*, *B. sinwaensis ined.* (Fig. 3), *B. dolichoptera ined.* and *B. staintonii ined.* (Fig. 4) with absence of margo. This sectional placement is consistent with other macro-morphological characters of these species, especially those of the fruit and tepals.

Acknowledgements

The authors thank the curators of E, K, KATH and TUCH for allowing access to herbarium materials. We sincerely thank the staff of the Royal Botanic Garden Edinburgh (RBGE) and Frieda Christie (RBGE) especially for SEM technical support. The M.L. MacIntyre *Begonia* Trust, The Sibbald Trust and Fergusson Bequest and their trustees are thanked for providing financial support for this work.

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(Manuscript received on 8 October 2012; revised on 31 October 2012)