Pak. J. Bot., Special Issue (S.I. Ali Festschrift) 42: 135-154, 2010.

TAXONOMIC AND ECOLOGICAL SIGNIFICANCE OF SEED MICROMORPHOLOGY IN HIMALAYAN BEGONIAS: SEM ANALYSIS

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Abstract

Seed morphology plays an important role for the taxonomic purposes, but inspite of the importance and stability of seed characters in systematics, very little work has been done on seed morphology of *Begonia.* The seeds of *Begonia* are small and not differentiated easily with the naked eye. In fact, they are so small that observation of many of their taxonomically important features is possible only with a scanning electron microscope (SEM). In this paper, the external morphology of the seeds of 23 Nepalese *Begonia* species, belonging to five sections (*Diploclinium, Monopteron, Platycentrum, Putzeysia* and *Sphenanthera*) was studied with SEM techniques. It has been revealed that the differences in seed characteristics were not sufficient to use for sectional delimitation of Nepalese *Begonia,* but helped to separate the *Begonia* at species level.

Introduction

Begonia is the sixth largest genus of flowering plants (Frodin 2004), comprising 1524 species (Govaerts 2009), and divided in to 63 sections (Doorenbos *et al*., 1998), and rapidly approaching the point of exceeding 1600 species as predicted by Sands (2001) and Goodall-Copestake (2005). *Begonia* is a pantropical genus, represented by almost 600 species in Asia, but most abundant in the Far East and Neotropics.

Irmscher (1925) recognized two broad centres of diversity for the genus, the first of which ranges from Mexico to the Andes and Brazil, while the second spans the eastern Himalayas, mountains of Indochina, Malay Archipelago, the Philippines and New Guinea. The genus is characterized by a pervasive asymmetry of leaf form, generally succulent petioles, unisexual flowers that are borne within the same inflorescence, and winged capsules.

The taxonomic history of *Begonia* of Nepal began with the description of *Begonia picta* by Sir J.E. Smith in *Exotic Botany* (1805), based on the specimen collected by Buchanan-Hamilton (1802-1803) from Nepal. Along with *Begonia picta*, he also described two other species *B. cathcartii* and *B. gemmipara* from the Sikkim Himalaya. Later in 1825, D. Don described six species of *Begonia* (*B. dioica, B. hatacoa, B. picta, B. palmata, B. rubella* and *B. tenella* (synonym of *B. dioica*), under Begoniaceae in *Prodromus Flora Nepalensis*, based on the collections of Buchanan-Hamilton (1802-03) and Nathaniel Wallich (1820-21).

Begonia seed characters have long been used as additional important evidence in floristic and taxonomic studies. Especially in bigger seeds the internal architecture is important, but in smaller seeds the external seed structure exhibits a considerable variation as in “dust seeds” found in Orchidaceae and Begoniaceae. Seed coat morphology of *Begonia* sect. *Squamibegonia* Warb. was studied with SEM by Bouman & De Lange (1983) who recently surveyed seed coat structure of a number of *Begonia* species representing several sections (Bouman & De Lange, 1983). Seitner (1972) also examined *Begonia* seeds, describing ranges in size and variations in seed coat pattern, but the value of this latter study is diminished by an apparent lack of voucher specimens.

Recent study on seeds of about 235 Neotropical *Begonia* species, representing almost all recognized American Begonia sections, was conducted using scanning electron microscopy (SEM) by De Lange & Bouman (1999). Seeds of *Begonia* are unique and distinguishable from all other seeds of flowering plants by the presence of a transverse ring specialized, polygonal testa cells and elongated collar cells (De Lange & Bouman 1992), above the collar cells lies the operculum (Fig. 1). In the present study an attempt is made to highlight the taxonomic and ecological significance of seed micromorphology in Himalayan Begonias.

Material and Methods

The fruit was collected from live as well as from herbarium materials. Out of 29 Nepalese *Begonia*, seeds of 23 *Begonia* species have been studied under the light microscope at Central Department of Botany, Tribhuvan University, Nepal and scanning electron microscope (SEM) at the Royal Botanic Garden Edinburgh (RBGE), UK. Healthy dry seed were mounted on Agar Scientific adhesive carbon tabs 12mm in size placed on Aluminium stubs. Due to very small size at least five to eight seeds were mounted on each stub and placed some with the neck of the seed protruding upwards, so that the orientation of the seed could be seen from all angles. The seeds were then sputter-coated with 250 nm platinum particles using an Emitech K575X sputter coater in one run of 2 minutes.

The prepared specimens were examined in a LEO Supra 55VP scanning electron microscope (SEM) at a voltage of 4.5kV for some seed and a working distance at 10 mm. The SEM images were captured at a resolution of 2048 x 1536 pixel and saved in TIF format. The photographs were used to describe the shape and neck (operculum) of the seed, and seed ornamentation was determined from the central regions of the testa and the collar cells. The size of the pollen and seed was measured from the photographs and calculated the size by dividing the length with the scale obtained in the photographs taken at 10 mm.

Results

Nepalese *Begonia* seeds show considerable differences in shape and in the cellular pattern of the seed coat in light-microscopical study. A more detailed study of the micromorphology of the seeds by means of scanning electronic microscope (SEM) revealed a great diversity of characters. The seeds are ellipsoidal or obovoidal (Fig. 2 B-D & A-C), but sometimes oblong to oblong-elliptic or ovate or broadly elliptical to circular in outline in case of smaller seeds. The seeds are normally straight, but slightly curved seeds are found in *B. dioica* (Fig. 2 B & D). Variations in the seed size ranging from 280–842 µm long and 84–457 µm wide. Similarly, there is variation in the form of the operculum, the arrangements and shape of the testa and collar cells, the course of the anticlinal walls, the relief of the cell boundaries and cell ornamentation. The seeds are light brown to dark brown depending on the taxa.

Although in descriptions of the family Begoniaceae and the genus *Begonia* the seeds are generally described as minute or dust, it appears from our study that the seed length varies considerably among the Nepalese species. The seed length and ornamentation appears to be a specific character as the sections of the Nepalese *Begonia* clearly differs in the seed length. *Begonia* Section *Diploclinium* has smallest seeds (*B. tribenensis*: 280 × 155 µm) and the largest seeds (*B. dioica*: 737 × 288 µm), which differ in the shape, ornamentation and anticlinal wall of the testa cells. The seeds of the *Begonia* sections *Platycentrum, Monopteron* and *Sphenanthera* have only slight variation in length between 325–461 µm (Table 1).

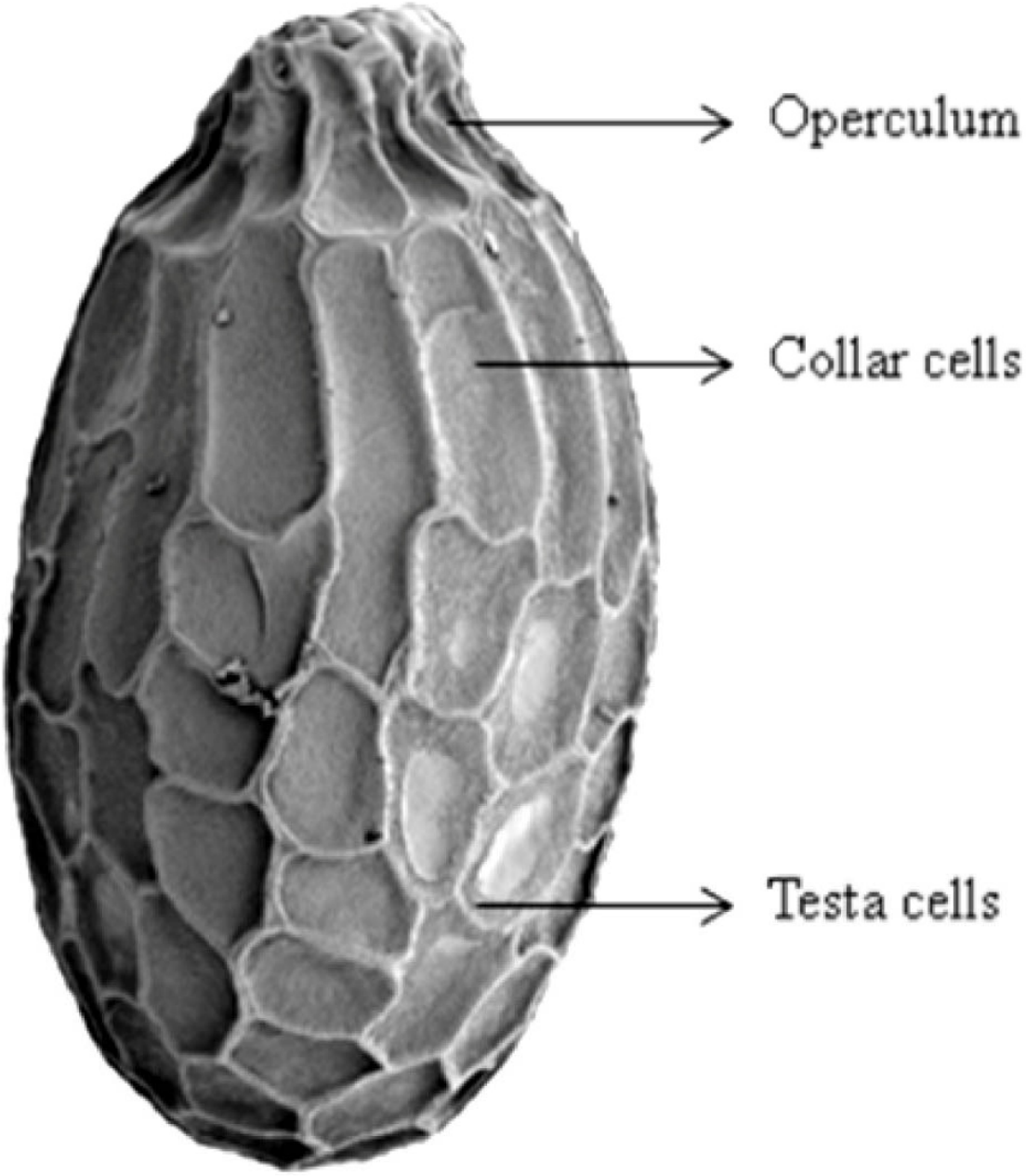

Fig.1. Diagram of the Begonia Seed with Various parts labeled.

Description of Seeds of Himalayan Begonias

Section 1. Diploclinium

Begonia bryophila S. Rajbhandary & K. K. Shrestha

Seeds ellipsoidal, mean size 325-345 × 215-240 µm; collar cells elongated with straight and slightly curved anticlinal walls; testa cells polygonal with straight anticlinal walls; operculum long nipple-shape with layers of cells; anticlinal boundaries broad and flat; the cuticular on the testa consisting of mostly of patch without orientation while in the collar and operculum cuticular consist of long linear loose striate ornamentation (Fig. 9 G-H).

Voucher specimen: Nepal, Kaski, Bamboo to Sinwa, 2080 m, 24.08.2007, *S. Rajbhandary & S.R. Bista* S45 (TUCH).

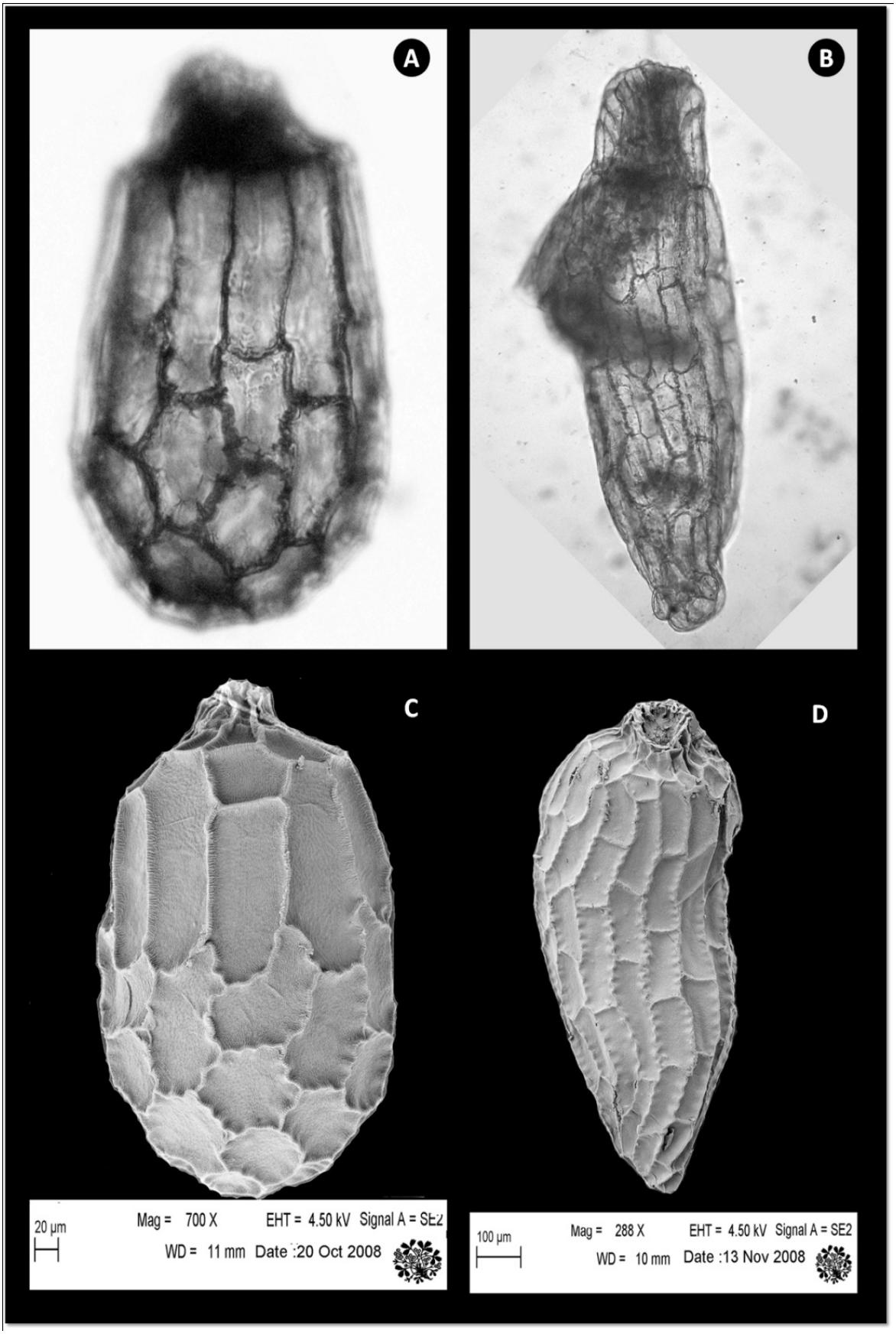

Fig. 2. Different shapes of *Begonia* seeds. A. (LM) & C. (SEM) Obovoidal seed of *B. tribenensis* and B. (LM) & D. (SEM) ellipsoidal seed of *B. dioica*.

Table 1. Variation in seed size, anticlinal wall, ornamentation, testa and collar cells of *Begonia* species.

S. No.	Taxon name	Size of the seed (µm)		Anticlinal wall	Ornamentation	Testa	Collar
		L (µm)	B (µm)				
		Section 1. Diploclinium					
1.	*B. bryophila*	334	210	straight and slightly undulated	long linear loose striate with presence of smooth patch	polygonal	elongated
2.	*B. dioica*	737	288	strongly undulated	Smooth sometimes slightly short striate	elongated	elongated
3.	*B. dolichoptera*	417	250	undulate as well as straight	long linear striate	polygonal	elongated
4.	*B. flagellaris*	379-418	211-243	with straight anti-rugulate	striate and rugulate with smooth patches	polygonal	elongated
5.	*B. josephii*	390	190	straight	compact long linear striate	polygonal	elongated with thin, straight
6.	*B. leptoptera*	280	208-220	straight	short linear striate	polygonal	elongated with thin straight
7.	*B. ovatifolia*	294-311	200-217	straight as well as undulated	very dense, rugulate, long linear striate collar	polygonal	elongated
8.	*B. picta*	292-334	174-184	thick and slightly curved or undulate	very dense regulate, long linear striate	polygonal	elongated
9.	*B. rubella*	284	210	with thick straight and slightly undulate	very dense regulate, long linear striate	polygonal	elongated
10.	*B. tribenensis*	280	155	with straight or undulated	long linear striate	polygonal	elongated

Table 1. (Cont'd.).

S. No.	Taxon name	Size of the seed (µm)		Anticlinal wall	Ornamentation	Testa	Collar
		L (µm)	B (µm)				
		Section 2. Platycentrum					
11.	*B. annulata*	335-345	195-215	straight or thin slightly curved or slightly undulated	Smooth or short linear loose striate	polygonal	elongated
12.	*B. cathcatii*	394	232	thin, straight or slightly curved	long linear striate	elongated and polygonal	elongated
13.	*B. flaviflora*	334	224	thin or thick straight or slightly curved	Striate	polygonal	elongated
14.	*B. hatacoa*	388-461	228-244	thin and straight	short linear striate	polygonal	elongated
15.	*B. megaptera*	325	165	thin straight or thick undulated	long linear striate	elongated	elongated
16.	*B. nuwakotensis*	325	240	straight	rugulate	polygonal	Elongate
17.	*B. palmata*	340	225	with straight	short linear striate and regulate and dimples	pentagonal to hexagonal	elongated
18.	*B. panchtharensis*	325-400	230-240	straight	Striate and smooth	polygonal	elongated
19.	*B. sikkimensis*	300-325	200-220	slightly undulated	long linear striate	polygonal	elongated
20.	*B. taligera*	392	228	straight or slightly curved	long linear striate	polygonal	elongated
		Section 3. Monopteron					
21.	*B. nepalensis*	354-368	84-70	slightly undulated	long linear striate	elongated	elongated
		Section 4. Sphenanthera					
22.	*B. roxburghii*	345-380	220	thin and undulated	loose short striate and rugulate with dimples	elongated	elongated
		Section 5. Putzeysia					
23.	*B. gemmipara*	822, 840 - 842	420, 444-457	irregular thickness and elevated	short linear striate and zigzag	elongated as well as polygonal	elongated

Begonia dioica Buch.-Ham ex D. Don

Seeds narrow ellipsoidal, mean size 737 x 288 µm; collar and testa cells short elongated, with thin strongly undulated anticlinal wall; operculum flat nipple-shaped; anticlinal wall slightly elevated, cuticular mostly without ornamentation but in some there is presence of short linear striate ornamentation (Fig. 2).

Voucher specimen: Nepal, Dolakha, Charikot, 2330 m, 16.09.2007, *S. Rajbhandary & S.R. Bista* 55 (TUCH).

Begonia dolichoptera S. Rajbhandary & K. K. Shrestha

Seeds ellipsoidal, mean size 417 × 250 µm; collar cells elongated with straight anticlinal walls; testa cells polygonal with undulate as well as straight anticlinal walls; operculum very short, flat nipple-shape with a layers of flat elongated cells; anticlinal boundaries thick and elevated; the cuticular consisting of long linear striate ornamentation (Fig. 8 M-N).

Voucher specimen: Nepal, Taplejung, SW of Amjilassa, Ghunsa Khola, 2500 m, 05.09.1989, *M. Crawford, S., Grey-Wilson, C., Long, D., McBeath, R. Noltie, H. Zmarzty, S. Sinnott, M. Subedi* 248 (E).

Begonia flagellaris H**.** Hara

Seeds ellipsoidal, mean size 379-418 × 211-243 µm; collar cells elongated with slightly curved anticlinal walls; testa cells polygonal with straight and curved anticlinal walls; operculum flat nipple-shape with single layer of cells; anticlinal boundaries thin and elevated; the cuticular consisting of short zigzag striate ornamentation along with smooth patches on collar as well as testa cells (Fig. 7 A-B).

Voucher specimen: Nepal, Rasuwa, Khamjim, 03.10.1977, *N.P. Manandhar et al.* 442 (KATH).

Begonia josephii A.DC.

Seeds oblong, mean size 388-461 x 228-244 µm; collar cells elongated with thin, straight anticlinal wall; testa cells polygonal, with thin straight anticlinal walls; operculum short, broad, flat nipple-shape, composed of single layer of undefined cells; anticlinal boundaries flat, cuticular pattern consisting of mainly of compact long linear striate ornamentation (Fig. 1).

Voucher specimen: Nepal, Sankhuwa Sabha, Arun Valley; ridge between Bhotebas and Chichila, 1950 m, 20.09.1991, *D. Long, R.J.D. McBeath, D.R. McKean, D.A.H. Rae & N.K. Bhattarai* 85 (E).

Begonia leptoptera H. Hara

Seeds ellipsoidal and obovoid, mean size 280-380 x 208-220 µm; collar cells elongated with thin straight anticlinal walls; testa cells polygonal, with straight anticlinal walls; operculum broad, flat nipple-shaped, short with a layers of polygonal elongated

cells; anticlinal boundaries flat, cuticular pattern consisting of mainly of short linear striate ornamentation (Fig. 9 I-J).

Voucher specimen: Nepal, Dolakha, Deurali (Charikot), 2340 m, 16.08.2006, *S. Rajbhandary, K. Humagain, J. Gajurel & S.R. Bista* 20 (TUCH).

Begonia ovatifolia A. DC.

Seeds obovoidal, mean size 294-311 x 200-217 µm; collar cells elongated with thick slightly curved anticlinal walls; testa cells polygonal, with straight as well as undulated anticlinal walls; operculum short, flat nipple-shaped with single layers of elongated cells; anticlinal boundaries broad and flat; the cuticular pattern is quite characteristic, consisting of very dense, short zigzag striate ornamentation on the testa cells and long linear striate ornamentation on the collar cells with some smooth patches in testa and collar cells (Fig. 7 C-D).

Voucher specimen: Nepal, Sunsari, Dharan, 1900 m, 02.09.1967, *L.H. Williams & A. Stainton* 8317 (K).

Begonia picta Sm.

Seeds obovoidal or oblong, mean size 292-334 × 174-184 µm; collar cells elongated with thick slightly curved and slightly undulate anticlinal walls; testa cells polygonal, with slightly curved anticlinal walls; operculum short, flat nipple-shaped with single layers of elongated cells; anticlinal boundaries thick and elevated; the cuticular consisting of very dense, short zigzag striate ornamentation near the anticlinal walls on the testa cells and long linear striate ornamentation in the centre and on the collar cells (Fig. 9 E-F).

Voucher specimen: Nepal, Dolakha, Charikot Deurali on the way to Jiri, 1950 m, 17.10.2006, *S. Rajbhandary, K. Humagain, J. Gajurel & S.R. Bista* 24 (TUCH).

Begonia rubella Buch.-Ham. ex D. Don

Seeds obovoidal, mean size 284 x 210 µm; collar cells elongated with thick straight anticlinal walls; testa cells polygonal, with slightly undulate anticlinal walls; operculum very short, flat nipple-shape with single layers of flat elongated cells; anticlinal boundaries thick and elevated; the cuticular consisting of very dense, short zigzag striate ornamentation as well as linear striate ornamentation (Fig. 7 G-H).

Voucher specimen: Nepal, Kaverepalanchok, Namobudha, 1640 m, 05.10.2006, *S. Rajbhandary, K. Humagain, J. Gajurel & S.R. Bista* 15 (TUCH).

Begonia tribenensis C. R. Rao

Seeds ellipsoidal, mean size 280 × 155 µm; collar cells elongated with straight anticlinal walls; testa cells polygonal with undulate anticlinal walls; operculum small nipple-shape with a layer of elongated cells; anticlinal boundaries thick and elevated; the cuticular consisting of long linear striate ornamentation (Fig. 8 A-B).

Voucher specimen: Nepal, Tanahue, Damauli, 380 m, 16.07.2006, *S. Rajbhandary, S. Adhikari & Sudeep* S1 (TUCH).

Section 2. Platycentrum

Begonia annulata K. Koch.

Seeds ellipsoidal, mean size 335-345 × 195-215 µm; collar cells elongated with thin, straight anticlinal wall; testa cells elongated as well as polygonal, with thin slightly curved and slight undulated anticlinal walls; operculum short flat nipple-shape composed of multilayer cells of lower layer elongated and upper layer of polygonal cells; anticlinal boundaries flat, cuticular pattern consisting of short linear loose striate ornamentation, and some patch without orientation in the testa cells (Fig. 9 A-B).

Voucher specimen: East Himalaya, Bootan ("Bhutan") *Griffith* 2505 (K).

Begonia cathcartii Hook. f. & Thomson

Seeds ellipsoidal, mean size 394 x 232 µm; collar cells elongated and short, with thin straight anticlinal wall; testa cells elongated and polygonal, with straight or slightly curved anticlinal walls; operculum flat nipple-shaped, broad and short; anticlinal boundaries flat, cuticular pattern consisting of long linear striate ornamentation (Fig. 9. Q-R).

Voucher specimen: Nepal, Panchthar, Chamling Danda, Chyangtharpu, 1950 m, 28.09.2007, *K.K. Shrestha, R. Kunwar, K. Humagain, J. Pandey & N.B. Ksettri* 282 (TUCH).

Begonia flaviflora H. Hara

Seeds ellipsoidal, mean size 334 × 224 µm; collar cells elongated with thick straight or slightly curved anticlinal wall; testa cells polygonal, with thin straight or slightly curved anticlinal walls; operculum short nipple-shape composed of many irregularly arranged polygonal cells; anticlinal boundaries flat, cuticular pattern consisting of short linear striate ornamentation and some pimples on the collar cells (Fig. 9 C-D).

Voucher specimen: India, Darjeeling, Senchal, 2400 m, 05.08.1972, *H. Kanai, H. Ohashi, H. Hara, K. Iwatsuki & H. Obha* 723692 (E).

Begonia hatacoa Buch.-Ham. ex D. Don

Seeds ellipsoidal, mean size 388-461 × 228-244 µm; collar cells elongated with thin, straight anticlinal wall; testa cells polygonal, with thin straight and slightly curved anticlinal walls; operculum short flat nipple-shape composed of single layer of elongated cells; anticlinal boundaries flat, cuticular pattern consist short linear striate ornamentation (Fig. 8 K-L).

Voucher specimen: Nepal, Kathmandu, Dashinkali, 1350 m, 29.07.2006, *S. Rajbhandary & S.R. Bista 4* (TUCH).

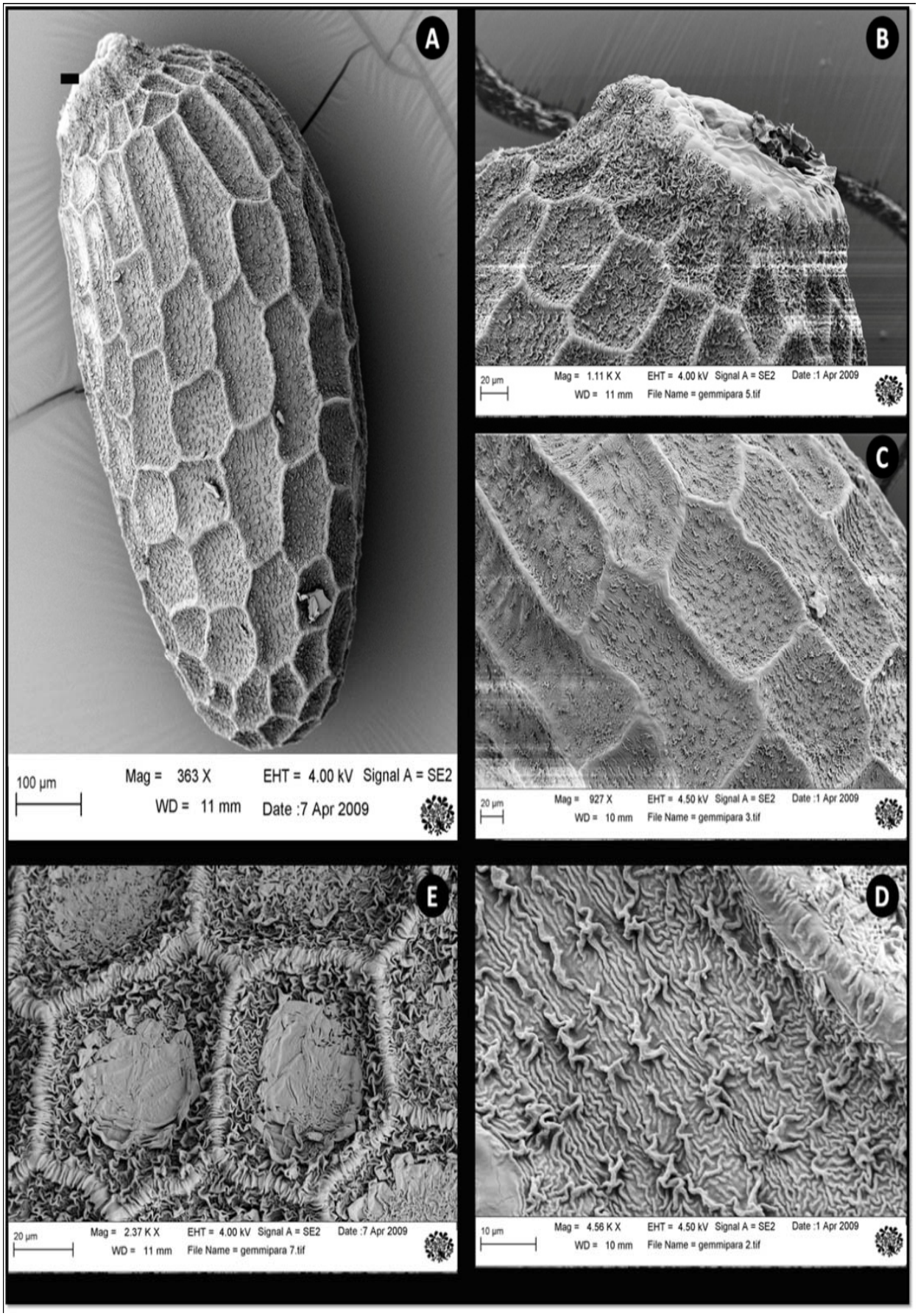

Fig. 3. *Begonia gemmipara*: A. seed; B. operculum; C. Collar cells; D. detail of ornamentation of collar and lower layers of testa cells; E. ornamentation of testa cells (KEKE 901).

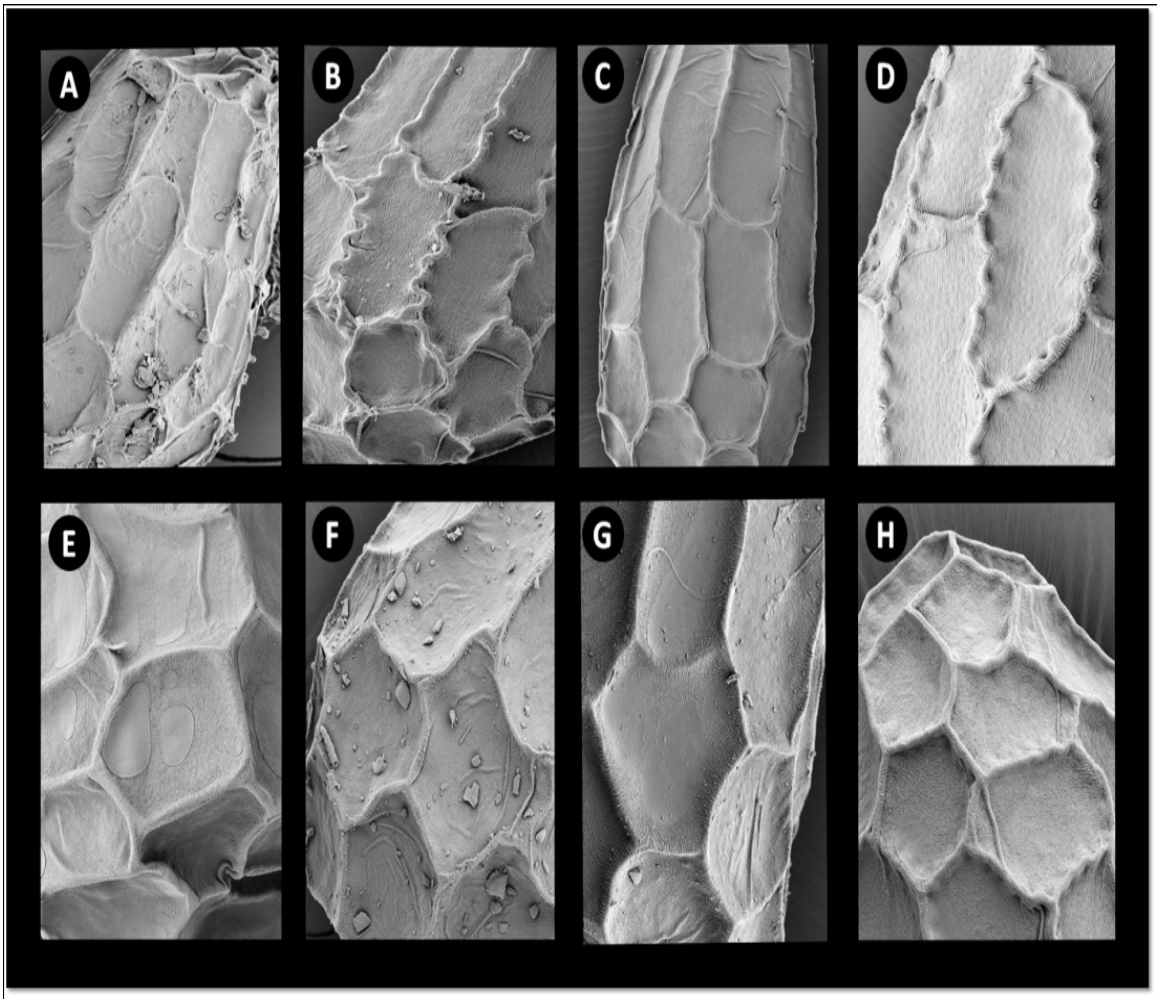

Fig. 4 Testa cell variation in *Begonia* seeds. A-D. Elongated testa cells (*B. cathcartii; B. megaptera; B. nepalensis*; *B. dioica*); E-H. polygonal testa cells (*B. flagellaris, B. hatacoa, B. nuwakotensis, B. bryophila*).

Begonia megaptera A. DC.

Seeds ellipsoid to ovoid, mean size 325 × 165 µm; collar cells elongated with thin straight anticlinal walls toward the lower side and undulated on the upper half; testa cells polygonal, with undulated anticlinal walls; operculum broad, flat nipple-shaped, short with single layers cells; anticlinal boundaries broad and flat, cuticular pattern consisting of long linear striate ornamentation (Fig. 8 O-P).

Voucher specimen: India, Assam, Meghalaya, Garo Hills, Nokrek, 07.03.1950, *T.R. Chand* 2753 (MICH).

Begonia nuwakotensis S. Rajbhandary

Seeds ellipsoidal to obovoid, mean size 325 × 240 µm; collar cells elongated, testa cells polygonal with straight or slightly curved anticlinal walls; anticlinal walls of testa cells thin and slightly raised; operculum short, flat and nipple-shaped; cuticular pattern consisting of short linear zigzag striate ornamentation (Fig. 7 E-F).

Voucher specimen: Nepal, Nuwakot, Ranipauwa, 1700 m, 09.08.2007, *S. Rajbhandary, S. Ranjitkar & S.R. Bista* S31 (TUCH).

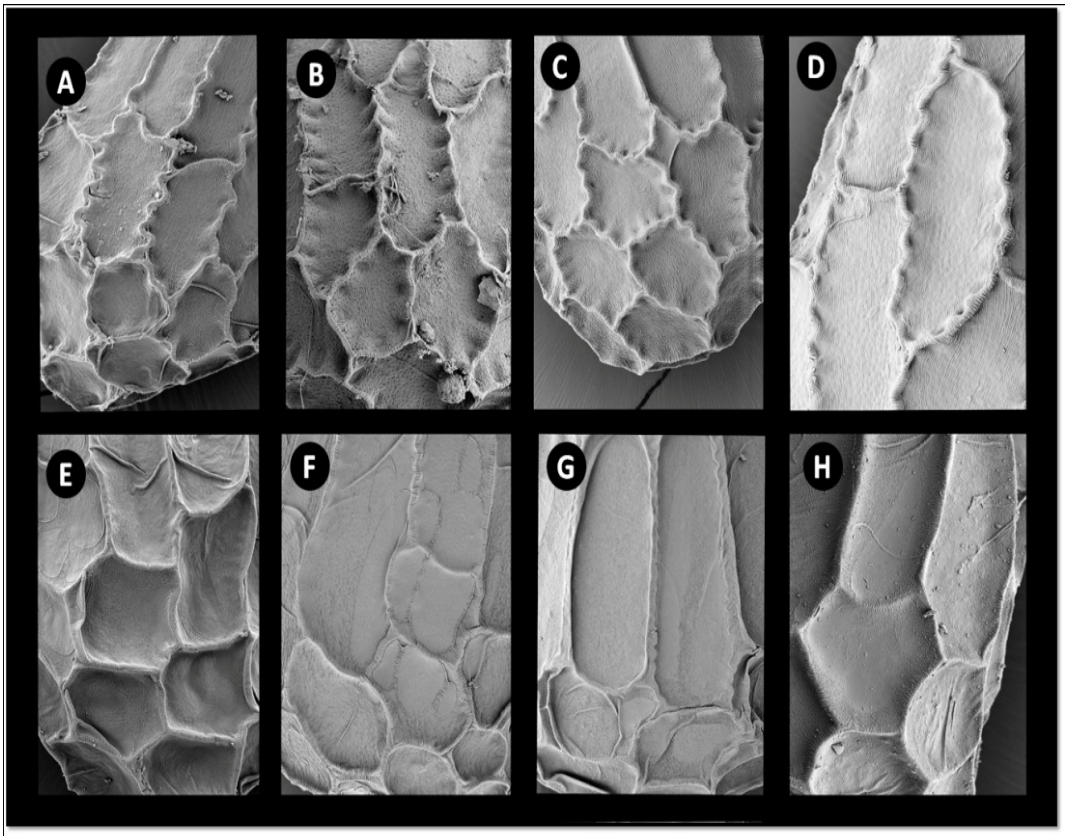

Fig. 5. Variation in anticlinal wall in *Begonia* seeds. A-D. Undulated anticlinal wall (*B. megaptera, B. roxburghii, B. tribenensis and B. dioica*); E-H. simple straight anticlinal wall (*B. flagellaris, B. panchtharensis, B. leptoptera, B. nuwakotensis*).

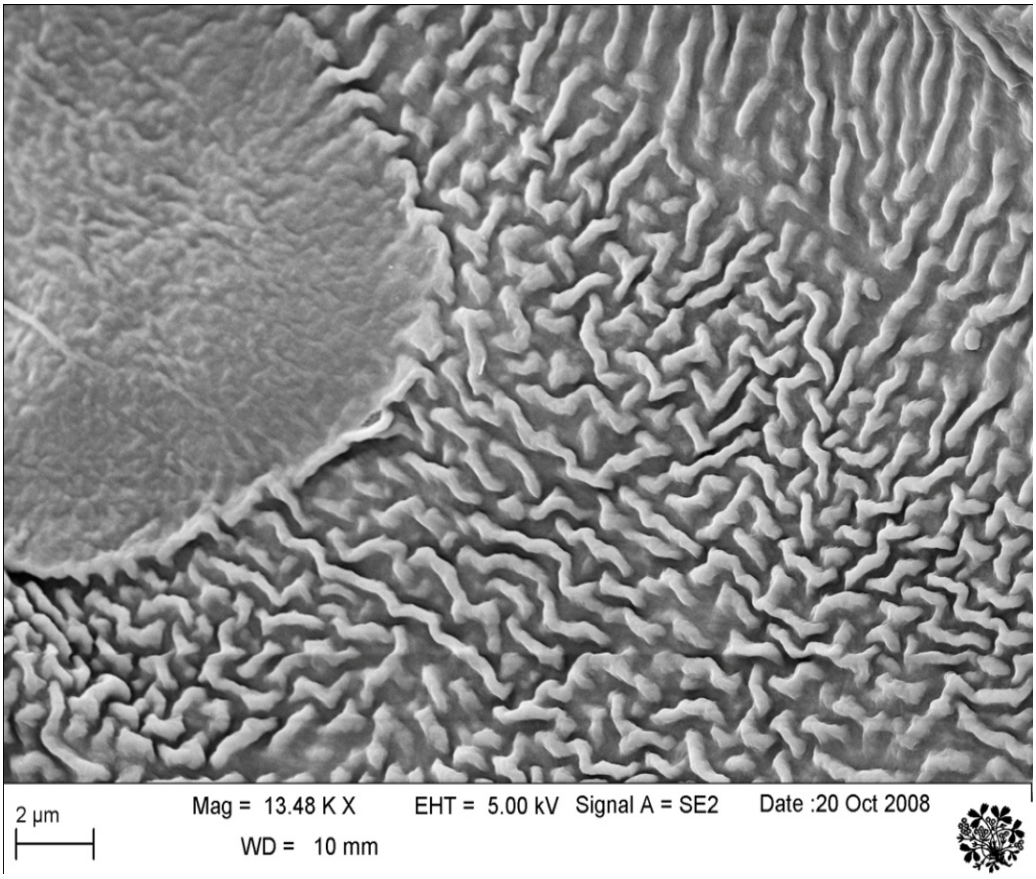

Fig. 6. Ornamentation with distinct smooth patches in collar cells of *B. leptoptera*.

Begonia palmata D. Don

Seeds ellipsoidal to obovoid, mean size 340 × 225 µm; collar cells elongated, other testa cells pentagonal to hexagonal with straight or slightly curved anticlinal walls; anticlinal walls of testa cells thin and slightly undulated; operculum nipple-shaped and slightly elongated; anticlinal boundaries flat, cuticular pattern consist short linear striate ornamentation with dimples on the collar and testa cells (Fig. 9. S-T).

Voucher specimen: Nepal, Kathmandu, Shivapuri, 1640 m, 07.07.2007, *S. Rajbhandary, K. Humagain, J. Gajurel & S.R. Bista* 27 (TUCH).

Begonia panchtharensis S. Rajbhandary

Seeds ellipsoidal, 325-345 × 215-240 µm; collar cells elongated with straight and slightly curved anticlinal walls; testa cells polygonal with straight anticlinal walls; operculum long nipple-shaped with layers of cells; anticlinal boundaries broad and flat; the cuticle on the testa consist smooth patches while long linear loose striate ornamentation is present in the collar and operculum cuticule (Fig. 8 E-F).

Voucher specimen: Cultivated plant grown from Eastern Nepal, Panchthar, Tinubote, Sisire, Prangbung, VDC, 2250 m, 02.10.2007, *U. Thamsuhang s.n.*, vouchered as *S. Rajbhandary* S74 (TUCH).

Begonia sikkimensis A. DC.

Seeds ellipsoidal, mean size 325 × 220 µm; collar cells elongated with straight anticlinal walls; testa cells polygonal, with slightly undulate anticlinal walls; operculum nipple-shape with two layers of cells; anticlinal boundaries thick and elevated; the cuticular consisting of long linear striate ornamentation (Fig. 8 C-D).

Voucher specimen: Nepal, Kaverepalanchok, Khopasi, 1510 m, 04.10.2006, *S. Rajbhandary, K. Humagain, J. Gajurel & S.R. Bista* 14 (TUCH).

Begonia taligera S. Rajbhandary

Seeds ellipsoidal, mean size 392 × 228 µm; collar cells elongated with straight anticlinal walls; testa cells polygonal with straight and slightly curved anticlinal walls; operculum very short, flat nipple-shaped with a layer of flat elongated cells; anticlinal boundaries thin and slightly elevated; the cuticule with long linear striate ornamentation (Fig. 8 I-J).

Voucher specimen: Nepal, Kaski, Bharat Pokhari, near Pokhara 720 m, 05.09.2007, *S. Rajbhandary and S Adhikari S52* (E).

Section 3. Monopteron

Begonia nepalensis (A. DC.) Warb.

Seeds narrow ellipsoidal, mean size 354-368 × 84-170 µm; collar cells elongated with thin straight anticlinal walls; testa cells polygonal, with slightly undulated anticlinal walls; operculum nipple-shaped and short with two layers of cells; anticlinal boundaries flat, cuticular pattern consisting of long linear striate ornamentation (Fig. 8 G-H).

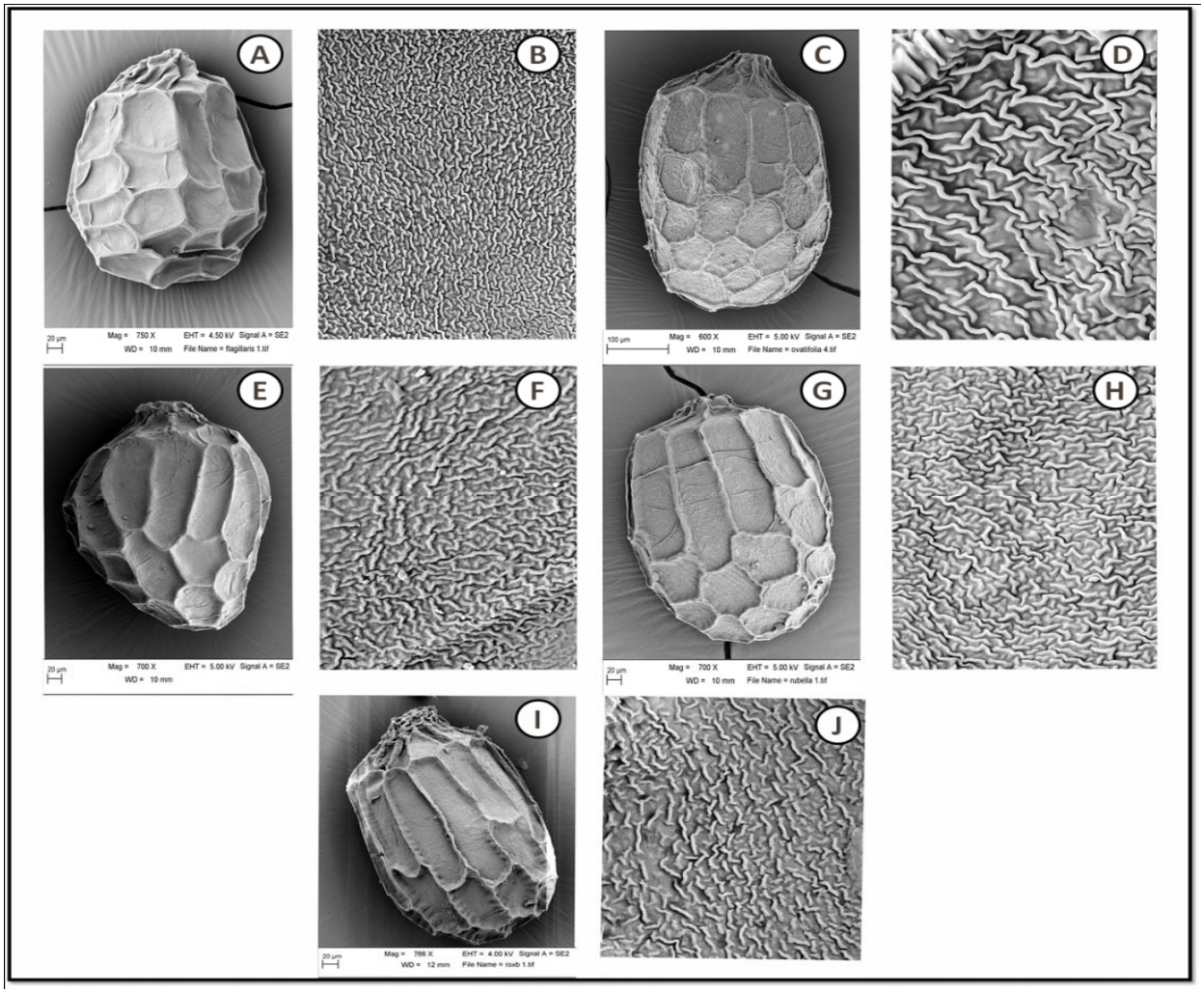

Fig. 7. Seeds with dense short zigzag bifurcated labyrinth striate ornamentation. A-B. *B. flagellaris*; C-D. *B. ovatifolia;* E-F. *B. nuwakotensis*; G-H. *B. rubella*; I-J. *B. roxburghii.*

Voucher specimen: Nepal, Jhapa, Chula Chuli, 300 m, 04.02.1985, *J.D.A. Stainton* 8906 (E).

Section 4. Sphenanthera

Begonia roxburghii (Miq.) A. DC.

Seeds obovoidal or oblong, mean size 345-380 × 220 µm; collar cells elongated with thick slightly curved and slightly undulate anticlinal walls; testa cells near the collar cells are elongated and polygonal towards the base, anticlinal walls undulate; operculum short, flat nipple-shaped with single layers of elongated cells; anticlinal boundaries thick and elevated; the cuticular consisting of very dense, short zigzag striate ornamentation near the anticlinal walls on the testa cells and long linear striate ornamentation in the centre and on the collar cells with dimples present all over the seed (Fig. 8 I-J).

Voucher specimen: India, Assam, Meghalaya, Khasi Hills, Cherrapunjee, 1220 m, 04.08.1952, *T.R. Chand* 6293 (MICH).

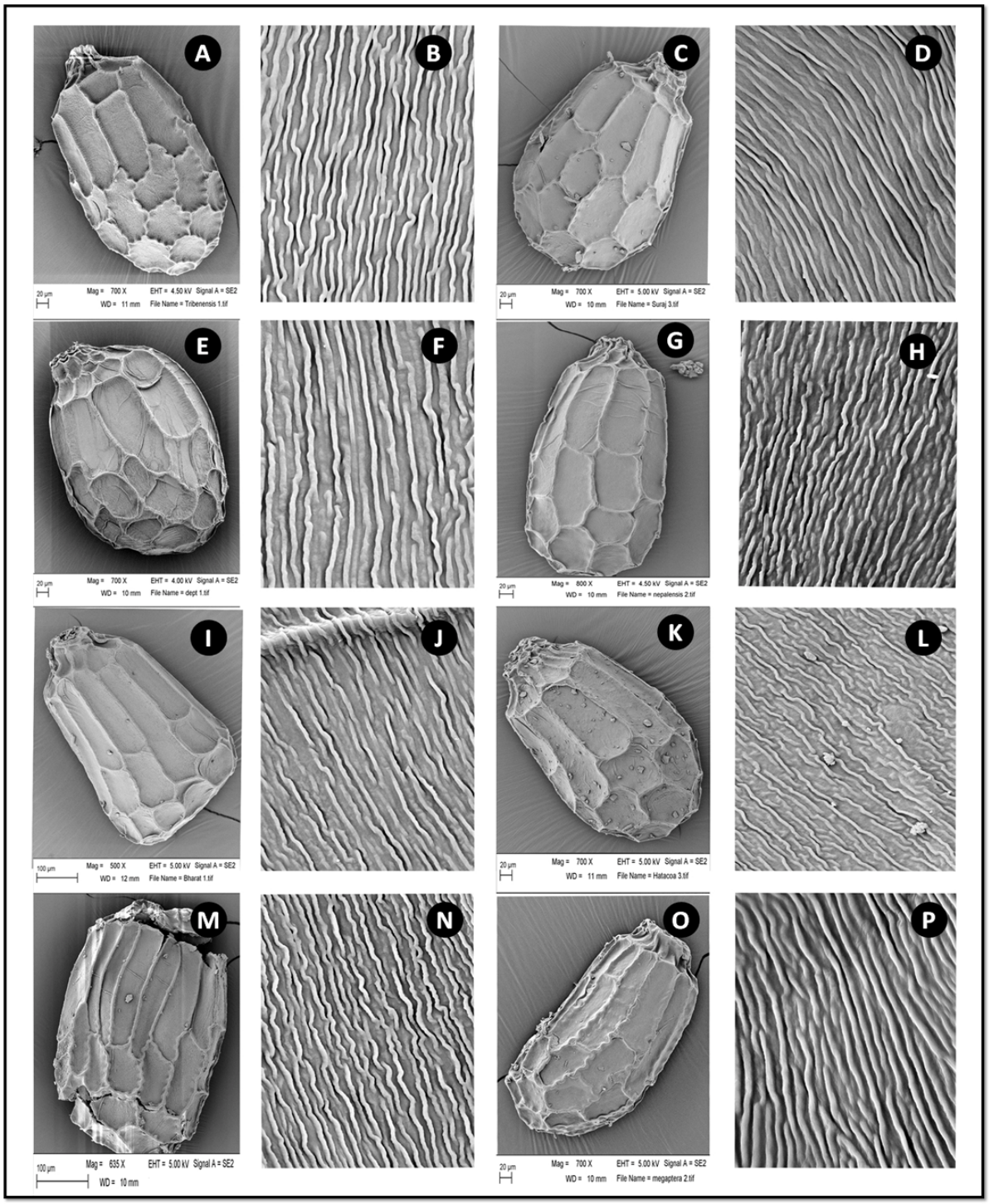

Fig. 8. Seeds with long parallel running striate ornamentation. **A-B**. *B. tribenensis*; **C-D**. *B. sikkimensis;* **E-F.** *B. panchtharensis*; **G-H.** *B. nepalensis*; **I-J**. *B. taligera*; **K-L.** *B. hatacoa*; **M-N** *B. dolichoptera*; and **O-P** *B. megaptera*.

Section 5. Putzeysia

Begonia gemmipara Hook. f. & Thomson

Seeds ellipsoidal, mean size 822-842 × 420-457 µm; collar cells elongated and short and irregular with thin, straight, slightly elevated anticlinal wall; testa cells elongated as well as polygonal, with thin slightly curved and undulated anticlinal walls; operculum

short flat nipple-shape composed of single layer of polygonal cells and smooth layer; anticlinal boundaries flat, cuticular pattern consisting of short linear striate ornamentation leaving central field of randomly orientated, small zigzag cuticular folding of irregular thickness and elevation (Fig. 3).

Voucher specimen: India, Darjeeling, Senchal, 07.09.1917, *G.H. Cave* s.n. (E).

One of the striking characteristics of the *Begonia* seeds is the presence of specialised collar cells. These cells are longitudinally stretched and form a transverse ring around one end of the seed which vary greatly in length. In the most commonly occurring type of seed, they occupy almost half of the total seed, but in some they occupy one-third and in some around two thirds (Fig. 1). The collar cells bound the operculum or the lid of the seed. The shape of the seed lid varies considerably. It may be almost flat as in *B. rubella* (Fig. 7 G), *B. dolichoptera* (Fig. 8 M), *B. megaptera* (Fig. 8 O), *B. bryophila* (Fig. 9 G), and *B. managensis* (Fig. 9 O), more commonly nipple shaped.

The variation of shape of the testa cells is rather small. They are mostly polygonal (Fig 4 E-H) and isodiametric, sometimes those adjacent to the collar cells are elongated as in *B. cathcartii, B. megaptera, B. nepalensis, B. roxburghii,* and *B. dioica* (Fig. 4 A-D). The boundary between the collar cells and the adjacent testa cells is usually rather obscure. Quite often the testa cells line in line with the collar cells but in bigger cells they forms rows of cells as in *B. gemmipara* (Fig. 3) and *B. josephii* (Fig. 1). In smaller seeds the testa cells are few. The anticlinal walls of the testa cells in *B. dioica, B. dolichoptera, B. megaptera, B. roxburghii, B. sikkimensis, B. tribenensis* are undulated (Fig. 5 A-D), and in *B. nepalensis, B. ovatifolia* and *B. rubella* slightly undulated, while in rest of the taxa they are straight or slightly curved (Fig. 5 E-H). These characters are of taxonomic value and help in the delimitation of species.

Dimples in both testa and collar cells are present in *B. flaviflora, B. palmata, B. roxburghii*. Mostly the ornamentation is uniformly distributed in the testa and collar cells but sometimes they contain one or more distinct smooth patches (Fig. 6). In those areas the cuticular sculpture has flattened appearance and sometimes a contrasting orientation as seen in *B. annulata, B. bryophila, B. leptoptera* (Fig. 6), and *B. panchtharensis.*

The surface of the testa and collar cells are covered by a cuticle, which shows interesting pattern in *Begonia* seeds. The linear striate orientation is especially found in the collar but varies from a weak to a strongly pronounced pattern in testa cells. Completely smooth seeds are rare, among the Nepalese species *B. dioica* has smooth ornamentation but sometimes faint striate ornamentation is also seen. In majority of the species the ornamentation of the collar cells are striate but differ in having more dense short zigzag bifurcated labyrinth striate ornamentation as in *B. flagellaris, B. nuwakotensis, B. ovatifolia, B. rubella,* and *B. roxburghii* (Fig. 7), long parallel running striations (Fig. 8), or shorter slightly undulated striations (Fig. 9).

Discussion and Conclusion

Morphological characters of seeds and their ultrastructural characteristics often exhibit large variations in the surface of seeds. These surface characteristics are often of valuable assistance in delimiting generic and taxonomic relationships. In the modern context, SEM has been used to emphasize the taxonomic significance of seed characters as a criterion for the separation of family, genera and species. Seed micromorphology provides an additional character set which appears very helpful in the description and delimitation of taxa.

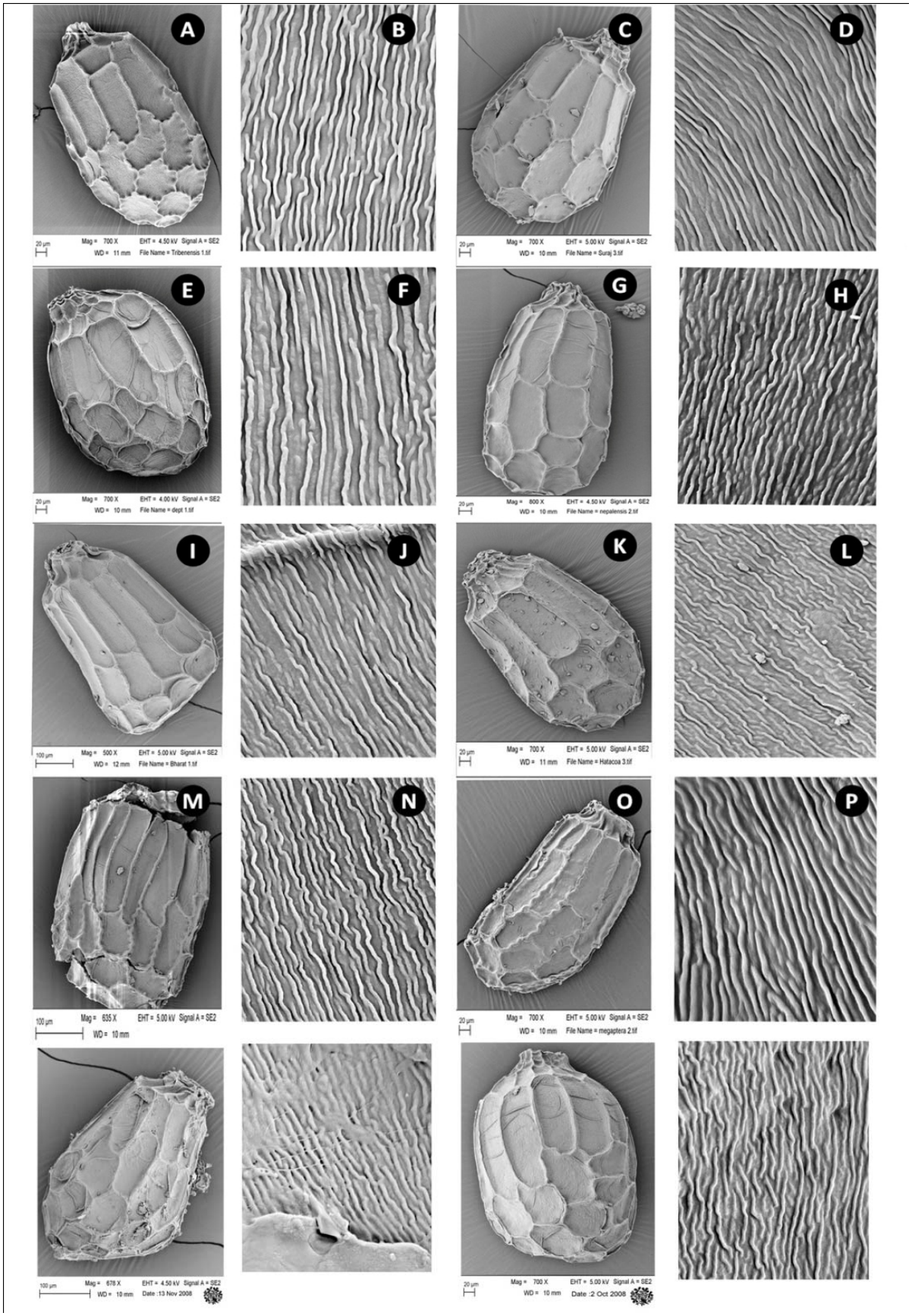

Fig. 9. Seeds with shorter slightly undulated striate orientation. **A-B**. *B. annulata*; **C-D**. *B. flaviflora;* **E-F.** *B. picta*; **G-H.** *B. bryophila*; **I-J**. *B. leptoptera*; **K-L.** *B. josephii*; **M-N** *B. flagellaris*; **O-P** *B. managensis*; **Q-R** *B. cathcartii*; and **S-T** *B. palmata*.

SEM studies on the seed coat pattern have demonstrated the existence of genetic diversity at various levels of Taxonomic hierarchy (Linskens, *et al.* 1977; Caroline 1980; Rejdali 1990). Seed coat pattern have been used for various purposes: to solve classificatory problems (*Tantawy et al*., 2004), to establish evolutionary relationships (Suilaman 1995, Akbari and Azizian 2006) and to elucidate the adaptive significance of seed coat (Alcitepe, 2010).

All these studies emphasise seed morphology as an important tool for the taxonomic work. Inspite of the importance and stability of seed characters in systematics, very little work seems to have been done on seed morphology of *Begonia*, especially no studies have been conducted so far regarding the seeds of Nepalese *Begonia.* Apart from few studies on seed coat morphology (Bouman & De Lange 1983) and sections of *Begonia* (Bouman & De Lange, 1983), the study on seeds of about 235 Neotropical *Begonia* species, representing almost all recognized American *Begonia* sections, using scanning electron microscopy (SEM) by De Lange & Bouman (1999) are most important as mention above. The seeds showed an appreciable diversity in size, shape, and micro-morphology, which was helpful in the delimitation of sections and sometimes also of species.

As is the case in African and Neotropical *Begonia* species, the seeds of the Nepalese begonias exhibit an appreciable diversity in seed size and structure. This diversity is not as wide as in the African begonias (De Lange & Bouman, 1992), in which all sections are distinguishable from one another on the basis of seed characters. The seeds of Nepalese begonias vary in shape from almost globular in most of the species to narrowly ellipsoidal in *B. dioica* (Fig. 2).

The *Begonia* Section *Putzeysia* is quite diverse in seed morphology among Nepalese *Begonia*. The largest seeds are found in *B. gemmipara* (section *Putzeysia*) measuring 822-842 × 420-457 µm, and differ from all other species and section in having short flat nipple-shaped operculum, with short elongated collar cells and layers of testa cells (Fig. 3). Except for the anticlinal wall of the collar cells the walls are undulated. Cuticular pattern consisting mainly of short linear striate ornamentation leaving central field of randomly orientated, small zigzag cuticular folding of irregular thickness and elevation is a characteristic feature which separate this taxa from all other Nepalese species and other sections. But the shape and arrangement of the testa cells on the seed of *B. gemmipara* (Fig. 3) resembles with the seed of *B. josephii* (Fig. 1) does supporting the relationship between *Begonia* Sections section *Diploclinium* and *Putzeysia* as commented by Doorenbos *et al.,* (1998). Doorenbos *et al.,* (1998) have commented that Putzeysia characterized by the clusters of tubercles which arise in some axils instead of inflorescences and the peculiar seeds and in other respects the species could be a member of Begonia section *Diploclinium* group II. The seed shape and structure resembles *B. raimondi* from Peru (De Lange & Bouman 1999) but belong to different section. Apart from other morphological characters, seed character is also important to separate the *Begonia* section *Putzeysia*.

In *Begonia* sections *Diploclinium* and *Platycentrum* the largest seeds are found in *B. dioica* (section *Diploclinium*) measuring 737 x 288 µm and also differ in having narrow ellipsoidal shape (Fig. 2) and pointed chalaza1 ends. In all other species the chalaza1 ends of the seed are rounded. Smallest seeds are found in *B. tribenensis* (280 × 155 µm) and *B. rubella* (284 × 210 µm) of *Begonia* section *Diploclinium*. Seed shape may be determined by flattened, extended, or swollen chalaza1 ends. A flat chalazal end is

caused due to contact with the ovary or fruit wall, which is also similar in Neotropical *Begonia* species (De Lange & Bouman 1999).

The comparative seed micromorphology does not provide cogent arguments for the separation of *Begonia* section *Diploclinium*, *Platycentrum, Monopteron* and *Sphenanthera.* The seeds of all specimens observed closely resemble each other and been separated on the basis of ornamentation of testa cells but there are differences in anticlinal wall, shape of the testa cells and size. Therefore, mainly Nepalese sections do not have a characteristic seed structure at the sectional level, and most of their species have seeds that conform to the ordinary *Begonia*.

The differences in size and morphology of the Nepalese *Begonia* seeds are nicely correlated with the differences in their type of fruit and reflect different adaptation to seed dispersal. The seeds are dispersed mostly by wind in *Begonia* section *Diploclinium, Putzeysia* and *Monopteron*, by water in section *Platycentrum* and by animal in section *Sphenanthera*. The pendulous fruit in *Begonia* section *Diploclinium, Putzeysia* and *Monopteron* are dry and dehisce by basal pores or slits, between the wings. The seeds are liberated gradually and may be dispersed further by the same gust of wind that shakes the fruit. In *Begonia* section *Platycentrum*, most of the species have recurved fruit which supports water dispersal as two small wings on the back of the inverted capsule trap falling raindrops; when enough water has accumulated it causes the capsule to nod up and down thereby shaking out the seeds.

According to Tebbitt (2005) wind-dispersed seeds are usually small and either have buoyant air-filled cells or pronounced surface ornamentation that helps catch air currents. This is true in case of Nepalese *Begonia*, except for *B. dioica* all other species in *Begonia* section *Diploclinium* are smaller in size. As the seeds in *Begonia* section *Diploclinium* are wind dispersed. In contrast, the seed in *Begonia* section *Platycentrum* are water dispersal as in most of the species the size measures between 300 × 400 µm and are larger than in *Begonia* section *Diploclinium*. It shows that seed dispersal affect the size of the seeds.

In section *Casparya* the seeds are recognizable by a combination of characters, especially the roughness of the testal surface, the mostly undulated anticlinals, the flat operculum, and sometimes the double structure of the cuticle (De Lange and Bouman 1999). These characters are very similar with *B. roxburghii* of section *Sphenanthera* previously identified as *Casparya oligocarpa.* But these sections have a relatively restricted geographical distribution *Begonia* section *Sphenanthera* is confined to Asia, while the section *Casparya* is restricted to the Andean and Guianan regions.

Along with *B. roxburghii*, majority of the seeds of all these five sections of Nepalese *Begonia* confirms to have ordinary seed type and resembles more common seed type as found in sections *Augustia-Rostrobegonia* of South African, many American section and Madagascan begonias but differ totally in the morphology of the plant. Our study therefore, clearly supports Doorenbos *et al.*, (1998) delimitation of section according to distribution pattern. Seed micromorphology generally provides restricted arguments for delimitation of sections or relationships between the sections.

In the present study, the differences we have found in the size, and the ornamentation of testa and collar cells helped us to separate the *Begonia* at species level. We agree with De Lange and Bouman (1999) that the differences in the ornamentation of testa and collar cells are significantly great to justify placing them into different species. But due to lack of seed material for all the species, it can't be exactly used for sectional delimitation of Nepalese *Begonia*. One main future work may be to focus on more seed materials to get additional characters to be used in sectional delimitation of Nepalese *Begonia*.

Acknowledgements

The authors thank the curators of BM, E, K, KATH, MICH and TUCH for allowing access to herbarium material. Prof. Steve Blackmore, Dr. Mark Watson and Dr. Colin Pendry of the Royal Botanic Garden Edinburgh (RBGE) for their support and encouragement and Frieda Christie (RBGE) for SEM technical support. The M.L. MacIntyre Trust, Edinburgh Botanic Garden (Simbald) Trust and Fergusson Bequest and their trustees are thanked for providing financial support for this work. Finally, especial thanks goes to the students, friends and S. R. Bista Plant collector, Central Department of Botany, T.U. for their help during the field trips.

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