HOKKAIDO UNIVERSITY

Title	Pollen morphology and its taxonomic significance in the genus Bomarea Mirb. (Alstroemeriaceae) - I. Subgenera Baccata, Sphaerine, and Wichuraea
Author(s)	Sarwar, Abul Khayer Mohammad Golam; Hoshino, Yoichiro; Araki, Hajime
Citation	Acta botanica brasilica, 29(3), 425-432 https://doi.org/10.1590/0102-33062015abb0077
Issue Date	2015
Doc URL	https://hdl.handle.net/2115/60249
Rights(URL)	https://creativecommons.org/licenses/by-nc/4.0/
Type	journal article
File Information	0102-3306-abb-29-03-00425.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Acta Botanica Brasilica 29(3): 425-432. 2015.
doi: 10.1590/0102-33062015abb0077

Pollen morphology and its taxonomic significance in the genus *Bomarea* Mirb. (Alstroemeriaceae) – I. Subgenera *Baccata*, *Sphaerine*, and *Wichuraea*

Abul Khayer Mohammad Golam Sarwar[1, 2*], Yoichiro Hoshino[1] and Hajime Araki[1]

Received: April 7, 2015. Accepted: May 19, 2015

ABSTRACT

Pollen morphology of 24 of the 33 species of three *Bomarea* subgenera, *Baccata*, *Sphaerine,* and *Wichuraea*, was examined by light microscopy (LM) and scanning electron microscopy (SEM), or SEM alone. The studied species of *Bomarea* were stenopalynous, characterized by large, monosulcate monads with reticulate exine sculpture in most species. Opercula-like structures were present on the sulcus in *B. huanuco* and *B. involucrosa*. Differences in pollen size, exine thickness, and exine sculpture were observed. The studied taxa were divided into four major groups based on exine ornamentation observed under SEM: microreticulate, reticulate, coarsely rugulate, or finely rugulate-perforate. Pollen characters alone did not appear to correlate clearly with the current subgeneric classification of *Bomarea*, but they may have some taxonomic utility below the subgeneric level. The most reliable infrageneric classification of *Bomarea* can be achieved through combined analyses of morphological, palynological, and molecular data from larger samples of specimens of all the species.

Keywords: *Bomarea*, exine sculpture, infrageneric classification, opercula-like structures, scanning electron microscopy

Introduction

Bomarea is the most diverse genus of Alstroemeriaceae, with 100–120 species (Neuendrof 1977; Alzate 2005) distributed primarily in the Andean and Austroamerican regions, but found from Mexico and the Caribbean to Chile and Argentina (Sanso & Xifreda 1995). The genus includes climbing, erect, or prostrate herbs with persistent, often resupinate leaves and rhizomes, umbellate inflorescences, free perianth segments, six stamens, an inferior ovary, and elongate and anacolpate pollen (Alzate 2007). The characteristic large inflorescences of *Bomarea* represent an important food source for birds and insects in the Andean highlands (Sanso & Xifreda 1995); the starchy storage roots of several species of *Bomarea* are also used as food (Bayer 1998).

The circumscription and taxonomic relationships between the two largest and closely related genera of the family Alstroemeriaceae, *Alstroemeria* and *Bomarea*, have also been controversial, as some species have been published under both generic names with different epithets (Sanso & Xifreda 1995; Hofreiter & Tillich 2002). Along with other characters, the differences in pollen grain wall structure and exine ornamentation (sculpture) are found to be valuable characteristics for distinguishing between *Alstroemeria* and *Bomarea* (Sanso & Xifreda 2001; Sarwar *et al.* 2010), and the close relationship between these two genera has also been confirmed by molecular data (Aagesen & Sanso 2003). Based on morphological features, the genus *Bomarea* is traditionally divided into three to four subgenera: *Baccata* (five spp.), *Bomarea s.str.* (*c.* 79 spp.), *Sphaerine* (12 spp.), and *Wichuraea* (18 spp.) (Hofreiter & Tillich 2002). Recently, the subgeneric circumscriptions of *Baccata* (Hofreiter 2008), *Sphaerine* (Hofreiter 2006), and *Wichuraea* (Hofreiter & Tillich 2003) have been revised. However, three clades within *Bomarea* have been identified by Alzate *et al.* (2008), using the genomic regions nrDNA ITS, *psbA-trnH*, *rpoB-trnC,* and *matK*, which conflicts with the traditional subgeneric classification of *Bomarea* based on morphology and biogeography.

Erdtman (1952, p. 44) described the pollen grains of *Bomarea* as "1-sulcate, large (longest axis 75–100 μm), usually distinctly plane-convex with sulcus on the convex part of grains, sexine scrobiculoidate; sexine is thicker than nexine." Pollen morphology of *Bomarea* has also been included in some taxonomic papers and/or as a part of regional flora (Elsik & Thanikaimoni 1970; Heusser 1971; Neuendorf

[1] Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0811, Japan
[2] Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
* Corresponding author: drsarwar@bau.edu.bd

1977; Schulze 1978; Kosenko 1994; Rudall *et al*. 2000; Rojas & Gutiérrez 2001; Sanso & Xifreda 2001; Hofreiter 2006; Alzate 2007). As a part of the comprehensive pollen morphological survey on the family Alstroemeriaceae, pollen morphology of the genus *Alstroemeria* has already been published (Sarwar *et al*. 2010). We present herein pollen morphological features of three subgenera, *viz*. *Baccata*, *Sphaerine*, and *Wichuraea*, of the genus *Bomarea* to search for new characters that could add information pertinent to infrageneric classification of this genus. The evolutionary trend in palynological features has also been discussed in light of the molecular phylogenetic relationships of *Bomarea* (Alzate *et al*. 2008).

Materials and Methods

Pollen morphology of a total of 24 species of three subgenera of the genus *Bomarea*, i.e., *Baccata* Hofreiter (three spp. out of five, Hofreiter 2008), *Sphaerine* (Herb.) Baker (nine spp. out of 12, Hofreiter 2006), and *Wichuraea* (M. Roemer) Baker (12 spp. out of 16, Hofreiter & Tillich 2003), was examined by means of light microscopy (LM) and scanning electron microscopy (SEM), or SEM alone (Tab. 1). Polliniferous materials used in this investigation were taken from dried specimens from the herbaria MO, USM, K, and MOL. Abbreviations of the herbarium names are according to the Index Herbariorum (Thiers 2007).

The preparation of pollen grains for LM and SEM, and pollen parameters studied follow Sarwar *et al*. (2010). Pollen slides of all collection are deposited at the Hokkaido University Museum, Sapporo, Japan. The measurements are based on at least 30 randomly selected grains from each specimen (Tab.2). Pollen size and shape classes were made following Erdtman (1952) and descriptive terminology follows Punt *et al*. (2007).

Results

The pollen grains of *Bomarea* species investigated were monad, large, ellipsoid (boat-shaped), heteropolar; monosulcate, sulcus on the convex part of the grains, distinct, long, straight, wide at the equator, narrow near the poles, sometimes extended to the proximal pole (Fig. 1A); auricula-like structures at the end of sulcus were observed in *B. brachysepala*, *B. bracteolata*, *B. glaucescens,* and *B. huanuco* (Fig. 1B; Tab. 2), opercula-like structures were present on the sulcus in *B. huanuco* and *B. involucrosa* (Fig. 2A-B). Symmetry was bilateral. Sizes ranged from 27.88–52.70 µm (polar length P) × 44.46–79.19 µm (equatorial diameter E), P/E 0.51–0.68, oblate in shape, exine thickness 1.08–2.39 µm (Tab. 2).

Table 1. List of *Bomarea* taxa used in this study along with their voucher specimens.

No.	Taxa	Voucher specimens
	Bomarea* subg. *Baccata (5 spp.)	
1.	*B. bracteolata* Gereau	Panama: Ridge of the Cadillera de Tute, above Escuela Alto de Piedra, 05.06.1982. S. Knapp & R.L. Dressler 5456 (MO3042031) (LM, SEM)
2.	*B. carderi* Mast.	Panama: Panamá, 27.01.1966. E.L. Tyson, J.D. Dwyer & K.E. Blum 3311 (MO2011164) (LM, SEM)
3	*B. diffracta* Baker	Colombia: Chocó, 06.01.1979. A. Gentry & E. Renteria A. 23717 (MO2717115) (LM, SEM)
	Bomarea* subg. *Sphaerine (12 spp.)	
4.	*B. brachysepala* Benth.	Peru: Depto. Piura, Prov. Huancabamba. 07.27.2006. A. Cano, W. Mendoza & N. Valencia 16761 (USM212472) (LM, SEM)
5.	*B. brevis* (Herb.) Baker	Bolivia: La Paz, Sud Yungas, 07.11. 1982. J.C. Solmom 8745 (MO3148123) (SEM)
6.	*B. coccinea* (R. & P.) Baker	Peru: Depto. Junin, Prov. Tarma. 13.01.1987. S.C. Dias & S. Baldeon 2217 (USM163830) (SEM)
7.	*B. distichifolia* (R. & P.) Baker	Peru: Dep. De Cajamarca, Prov. Jaen. Colasay, 7.3 – 9.5.1998. No name (USM202018) (LM, SEM)
8.	*B. foertheriana* Hofreiter	Peru: Depto. De Pasco, Prov. Oxapampa. 21.11.2002. A. Monteagudo, C. Mateo & G. Ortiz 4360 (USM198515) (LM, SEM)
9.	*B. huanuco* Hofreiter	Peru: Huanuco, 15.01.1987. C. Diaz S. & S. Baldeon 2249 (MO3518930) (LM, SEM)
10.	*B. linifolia* (H.B.K.) Baker	Colombia: Cauca Paramo de las Barbillas, near Guachicono 19.07.1944. E.L. Core 973 (MO1801230) (LM, SEM)
11.	*B. nervosa* (Herb.) Baker	Peru: San Martín, 12.04.1984. T.B. Croat 58201A (MO3186188) (LM); Amazonas, Chachapoyas. 09.07.1962. J.J. Wurdack 1270 (USM161711) (SEM)
12.	*B. pauciflora* (H.B.K.) Herb.	Colombia: Cundinamarca, no day.08 – 12.1990. A. Repizzo & Z. Calle 264 (MO4330152) (LM, SEM)
	Bomarea* subg. *Wichuraea (16 spp.)	
13.	*B. albimontana* Smith & Gereau	Peru: Ancash, Huaraz, 13.07.1985. D.N. Smith & M. Buddensiek 11148 (MO3312498) (LM, SEM)
14.	*B. ampayesana* Vargas	Peru: Cusco, La Convención, 25.07.1944. C. Vargas C. 4439 (MO1633770) (LM, SEM)
15.	*B. andimarcana* (Herb.) Baker	Bolivia: La Paz Inquisiri, 19.12.1999. J.R.I. Wood and D.J. Goyder 15546 (K) (LM)
16.	*B. chimboracensis* Baker	Ecuador: Azuray, 15.8.1996. G.P. Lewis 2484 (K) (LM)

Continues

Table 1. Continuation.

No.	Taxa	Voucher specimens
17.	*B. dulcis* (Hook.) Beauvard	Peru: Ancash, Cordillera blanca encima de Yungay, no date, A. Weberbauer 3268 (MOL00007284) (LM, SEM)
18.	*B. glaucescens* (H.B.K.) Baker	Peru: Depto. San Martin, Prov. Mariscal Caseres. 25.06.96. A. Cano, K. Young, B. Leon & J. Roque 7307 (USM167984) (LM, SEM)
19.	*B. involucrosa* (Herb.) Baker	Peru: Lima, Huarochiri, 21.09.1982. D.N. Smith & J. Alban C. 2357 (MO4398129) (LM, SEM)
20.	*B. longistyla* Vargas	Peru: Lima, Canta, 16.05.1992. G. Vilcapoma 1730 (MO4395443) (LM, SEM)
21.	*B. parvifolia* Baker	Peru: Huaraz, Anacash, Yungay, 31.9.1975. S.G. Baunders 1335 (K) (LM); Ancash, Yungay, 28.01.1985. B.A. Stein 2019 (MO3294289) (SEM)
22.	*B. porrecta* Killip	Peru: Depto. La Libertad, Prov. Sanchez Carrion, Senal Huayllides. 21.08.1982. D. Smith 2267 (USM154048) (LM)
23.	*B. torta* (H.B.K.) Herb.	Ecuador: Loza, 15.8.1996. G.P. Lewis 2473 (K) (LM); Peru: Depto. Cajamarca, El Pargo. 18.09.1991. Al. Gentry, C. Diaz & R. Ortiz 74904 (USM133439) (SEM)
24.	*B. vargasii* Hofreiter	Peru: Cumbemayo, Hill west and S of Cumbemayo, 5.2–2.4.97. M. Weigend, Dostert & K. Drieble (USM971343) (SEM)

Table 2. Variation in pollen characters of *Bomarea* showing mean value in micrometer and standard deviation. Maximum – minimum values in micrometer in parenthesis; Taxa are arranged alphabetically within the group. (A) Pollen grains with auricula-like structure; n.d. Not discern.

Name of Taxa	Polar length (P)	Equatorial diameter (E)	P/E	Exine thickness	Exine sculpture*	Fig. No.
Subg. *Baccata*						
B. bracteolata (A)	37.29±2.94 (42.04 – 31.30)	62.37±4.18 (69.30 – 51.60)	0.60	1.87±0.23 (2.85 – 1.57)	Type III	3K
B. carderi	37.91±2.78 (42.47 – 33.42)	63.93±2.64 (69.55 – 59.77)	0.59	1.80±0.12 (1.96 – 1.53)	Type I	2E
B. diffracta	34.74±1.87 (37.54 – 30.44)	59.96±2.48 (65.27 – 56.44)	0.58	1.76	Type II	2J
Subg. *Sphaerine* *Distichifolia*-group						
B. brevis	n.d.	n.d.	n.d.	n.d.	Type II	2K
B. distichifolia	30.13±2.97 (34.30 – 24.76)	44.46±4.16 (52.83 – 39.23)	0.68	1.52±0.08 (1.70 – 1.43)	Type IV	3L
B. foertheriana	39.84±3.05 (45.95 – 35.32)	69.39±3.11 (78.71 – 64.51)	0.57	1.63±0.11 (1.90 – 1.50)	Type II	2L
B. huanuco (A)	30.30±1.99 (36.92 – 26.54)	52.67±2.87 (59.00 – 46.63)	0.51	1.67±0.11 (1.90 – 1.50)	Type II	3A
B. nervosa	35.30±3.79 (42.06 – 29.41)	53.88±1.46 (56.39 – 50.47)	0.66	1.66±0.13 (1.90 – 1.53)	Type IV	3M–N
***Linifolia*-group**						
B. brachysepala (A)	33.04±2.50 (38.90 – 29.30)	56.60±2.53 (61.23 – 51.68)	0.58	1.67±0.11 (1.9 – 1.5)	Type I	2F
B. coccinea	n.d.	n.d.	n.d.	n.d.	Type I	2G
B. linifolia	34.34±2.61 (38.58 – 29.89)	56.71±4.86 (63.82 – 47.38)	0.61	1.61±0.10 (1.80 – 1.43)	Type III	3O
***Pauciflora*-group**						
B. pauciflora	34.15±2.04 (38.95 – 30.97)	59.60±3.43 (66.44 – 53.86)	0.57	1.67±0.10 (1.80 – 1.53)	Type I	2H
Subg.*Wichuraea* Northern-group						
B. albimontana	32.51±2.80 (39.40 – 27.26)	56.34±3.14 (62.57 – 49.88)	0.58	1.65±0.11 (1.85 – 1.50)	Type II	3B
B. chimboracensis	30.12±4.91 (42.16 – 24.67)	53.66±5.34 (64.25 – 46.03)	0.56	2.32±0.22 (2.72 – 1.90)	Type I	-
B. glaucescens (A)	31.61±2.15 (35.86 – 27.89)	58.26±2.84 (64.64 – 51.41)	0.58	1.56±0.10 (1.80 – 1.35)	Type II	3C
B. porrecta	33.45±1.68 (36.97 – 31.10)	59.80±2.72 (63.40 – 54.52)	0.56	1.31±0.07 (1.50 – 1.20)	Type I	-
B. vargasii	n.d.	n.d.	n.d.	n.d.	Type II	3D
B. torta	30.46±3.50 (40.11 – 25.33)	55.40±4.33 (65.12 – 45.73)	0.60	2.39±0.24 (2.85 – 1.75)	Type I	2I
Southern-group						
B. ampayesana	52.70±4.36 (52.70 – 34.09)	79.19±3.84 (86.60 – 73.62)	0.67	1.80±0.10 (2.00 – 1.55)	Type II	3E
B. andimarcana	39.85±3.95 (53.10 – 33.27)	65.89±4.59 (78.33 – 54.53)	0.60	2.31±0.27 (2.72 – 1.80)	Type II	3F
B. dulcis	30.99±2.25 (35.81 – 27.38)	55.48±3.43 (66.20 – 49.80)	0.56	1.08±0.13 (1.35 – 0.85)	Type II	3G
B. involucrosa	36.49±2.13 (41.82 – 33.97)	68.40±2.55 (72.30 – 62.45)	0.56	1.60±0.10 (1.75 –1.43)	Type II	3H
B. longistyla	40.39±2.37 (44.68 – 34.63)	68.90±2.48 (75.32 – 64.20)	0.59	1.45±0.11 (1.75 – 1.20)	Type II	3I
B. parvifolia	27.88±2.76 (32.81 – 20.49)	52.50±3.36 (63.42 – 47.23)	0.53	1.83±0.31 (2.57 – 1.27)	Type II	3J

* Exine ornamentation type by SEM. Type I, microreticulate, lumina less than or equal 1 μm in size; Type II, reticulate, lumina larger than 1 μm in size; Type III, coarsely rugulate; Type IV, finely rugulate-perforate. For details see text.

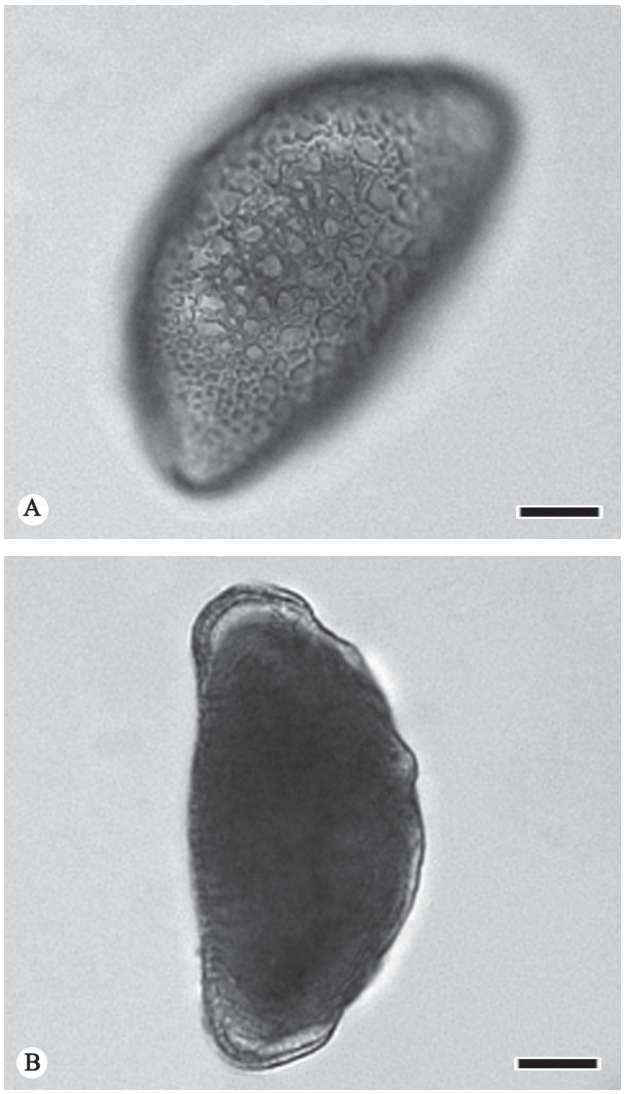


Figure 1. Light micrographs of *Bomarea* pollen grains (A–B). A. *B. involucrosa* (Smith & Alban 2357); B. *B. bracteolata* (Knapp & Dressler 5456). Scale bars: 10 μm.

The pollen grains of *Bomarea* species were characterized by a semitectate exine sculpture. Four different exine sculptures were observed among the species of *Bomarea* studied.

Type I – Microreticulate (Fig. 2E-I; Tab. 2), with perforate muri, heterobrochate; lumina are less than or equal 1 μm in length or diameter, observed in *B. brachysepala*, *B. carderi*, *B. coccinea*, *B. pauciflora,* and *B. torta*.

Type II – Reticulate (Figs. 2J-L, 3A-J; Tab. 2), with perforate muri, heterobrochate; lumina are larger than 1 μm in length or diameter, observed in *B. albimontana*, *B. ampayesana*, *B. andimarcana*, *B. brevis*, *B. diffracta*, *B. dulcis*, *B. foertheriana, B. glaucescens*, *B. huanuco*, *B. involucrosa*, *B. longistyla*, *B. parvifolia* and *B. vargasii*.

Type III – Coarsely rugulate (Fig. 3K, O; Tab. 2), observed in *B. bracteolata* and *B. linifolia*.

Type IV – Finely rugulate-perforate (Fig. 3L-N; Tab. 2), observed in *B. distichifolia* and *B. nervosa*.

Sometimes granula were visible at the bottom of the lumina; these correspond to reduced or modified columellae (Fig. 2J). The exine sculpture along with sulci was similar to that appearing at the equatorial position, but had relatively smaller lumina.

Discussion

Variations in pollen morphology

Most of the *Bomarea* species examined were characterized by monad, monosulcate, and large pollen indicating that the species of genus *Bomarea* are closely related (Figs. 1-3; Tab. 2). Thus far, the pollen morphology of only five species, *viz. B. distichifolia*, *B. dulcis*, *B. glaucescens*, *B. involucrosa,* and *B. linifolia*, of three subgenera has been studied with either LM or SEM. The results of present study are in agreement with previous reports (Erdtman 1952; Heusser 1971; Neuendorf 1977; Schulze 1978; Kosenko 1994; Sanso & Xifreda 2001; Alzate 2007), and monophyly of the genus *Bomarea* is also supported by molecular data (Aagesen & Sanso 2003; Alzate *et al.* 2008). However, there are significant differences in the value of quantitative palynological characters that may, to some extent, be related to differences in the preparation methods of pollen grains as well as the mounting media. Similar phenomena have also been observed and discussed for *Alstroemeria* (Sarwar *et al.* 2010 for detail). The pollen length (equatorial diameter) of studied *Bomarea* species varies from 44.46–79.19 μm (Tab. 2). This wide variation in pollen size might be correlated with floral size (Sarwar *et al.* 2010). *Bomarea ampayesana* produces the largest pollen (Tab. 2) and produces large showy flowers 10–12 cm in size (Hofreiter & Tillich 2003).

Previously, the presence of possible opercula was only observed in the SEM illustration of *B. salsilla* (Kosenko 1994). Here, we have reported opercula-like structures present on the sulcus in two *Bomarea* spp., *B. huanuco,* and *B. involucrosa* (Fig. 2A-B). Although operculate pollen is fairly common in some families of the order Liliales, the presence of operculate pollen in these three *Bomarea* species as well as in Alstroemeriaceae requires confirmation (Furness & Rudall 2003). However, it has been shown that insulae are present in the sulcus of some *Alstroemeria* pollen (Furness & Rudall 2003; AKM Golam Sarwar unpubl. res.).

Among the studied species of these three subgenera, auricula-like structures in pollen grains were observed only in *B. brachysepala*, *B. bracteolata*, *B. glaucescens,* and *B. huanuco* (Fig. 1B; Tab. 2). Pollen with auricula-like structures has previously been reported for only two species of *Bomarea* (subgenus *Bomarea*), *B. pardina* (*B. lyncina*), and *B. ceratophora* (Elsik & Thanikaimoni 1970; Neuendorf 1977; Schulze 1978). Elsik & Thanikaimoni (1970) considered auriculae as equatorial modifications of the exine, and auriculae sometimes get detached from the pollen effectively creating a circular opening that resembles a pore. However,

the auricula-like structures are absent in most of the species studied (Tab. 2; Elsik & Thanikaimoni 1970; Sanso & Xifreda 2001; AKM Golam Sarwar unpubl. res.).

Although pollen grains of *Bomarea* species are characterized by the (micro)reticulate exine sculptures (Type I, II; Figs. 2E-L, 3A-J), this study is the first to report the coarsely rugulate (Type III) and finely rugulate-perforate (Type IV) exine sculptures in *Bomarea* (Fig. 3K-O). The reticulate exine sculpture might be a plesiomorphic character state for *Bomarea* and the coarsely rugulate and the finely rugulate-perforate exine sculptures might have evolved independently more than once (Alzate *et al.* 2008). The coarsely rugulate(-psilate) exine sculpture, observed both in *Bomarea* and *Alstroemeria* (Sarwar *et al.* 2010), might make a morphological (connection) bridge between striate-reticulate and reticulate exine sculptures. The major evolutionary trend of exine sculpture is postulated to be from reticulate through rugulate to striate-reticulate or vice-versa, in Alstroemeriaceae (Aagesen & Sanso 2003). Alternately, the rugulate (or a different) pollen sculpturing could be plesiomorphic within the family and then both striate-reticulate and reticulate pollen sculpturing would be synapomorphies for *Alstroemeria* and *Bomarea*, respectively. With TEM thin-sections, it has been shown that the infra-

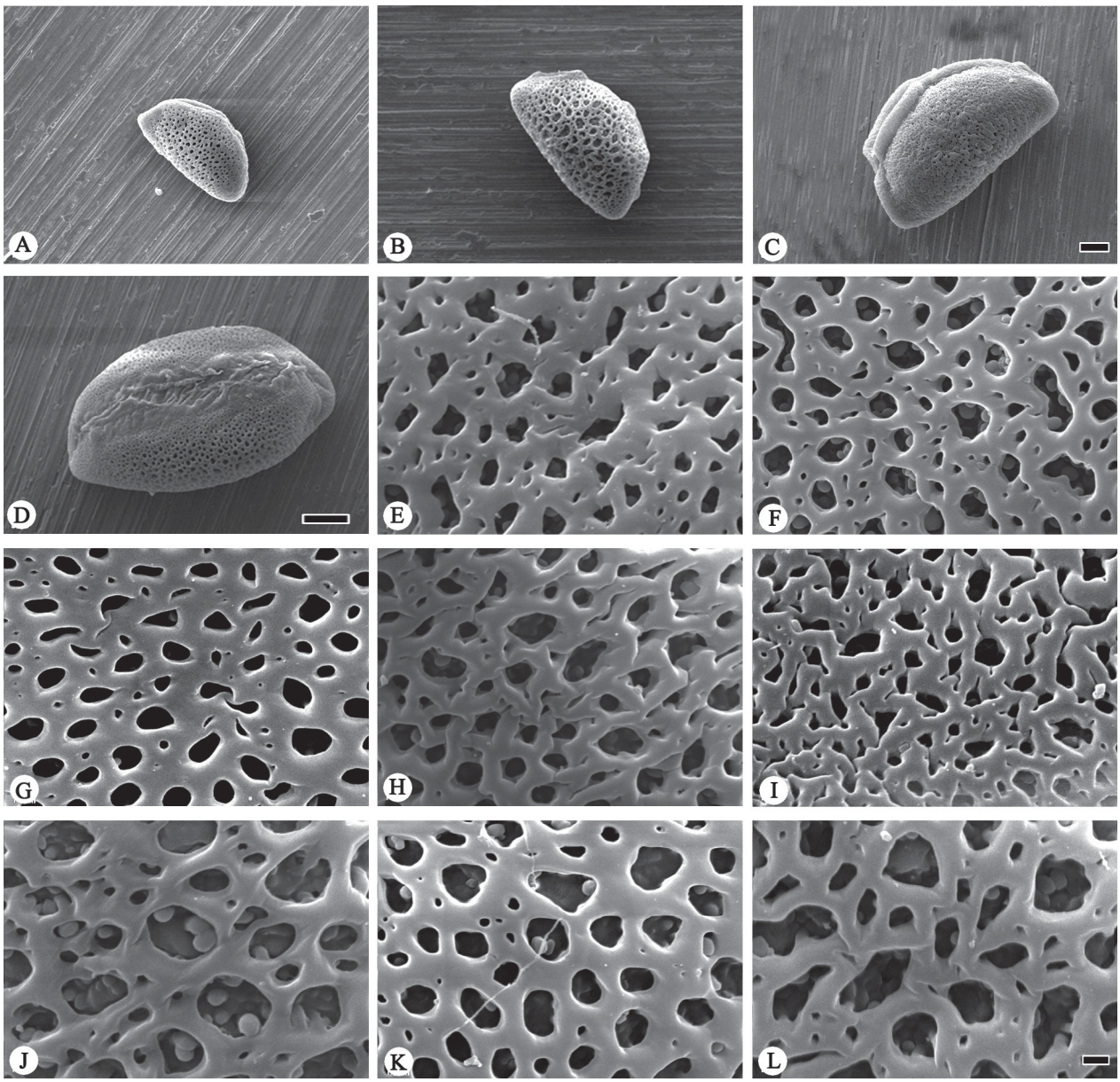


Figure 2. Scanning electron micrographs of *Bomarea* pollen. Pollen grain (A–D); exine sculpture (E–L). A. *B. huanuco* (Diaz & Baldeon 2249); B. *B. involucrosa* (Smith & Alban 2357); C. *B. linifolia* (Core 973); D. *B. longistyla* (Vilcapoma 1730); E. *B. carderi* (Tyson *et al.* 3311); F. *B. brachysepala* (Cano *et al.* 16761); G. *B. coccinea* (Camilo & Baldeon 2217); H. *B. pauciflora* (Repizzo & Calle 264); I. *B. torta* (Gentry *et al.* 74904); J. *B. diffracta* (Gentry & Renteria 23717); K. *B. brevis* (Solmom 8745); L. *B. foertheriana* (Monteagudo *et al.* 4360). Scale bars A–D: 10μm, E–L: 1μm.

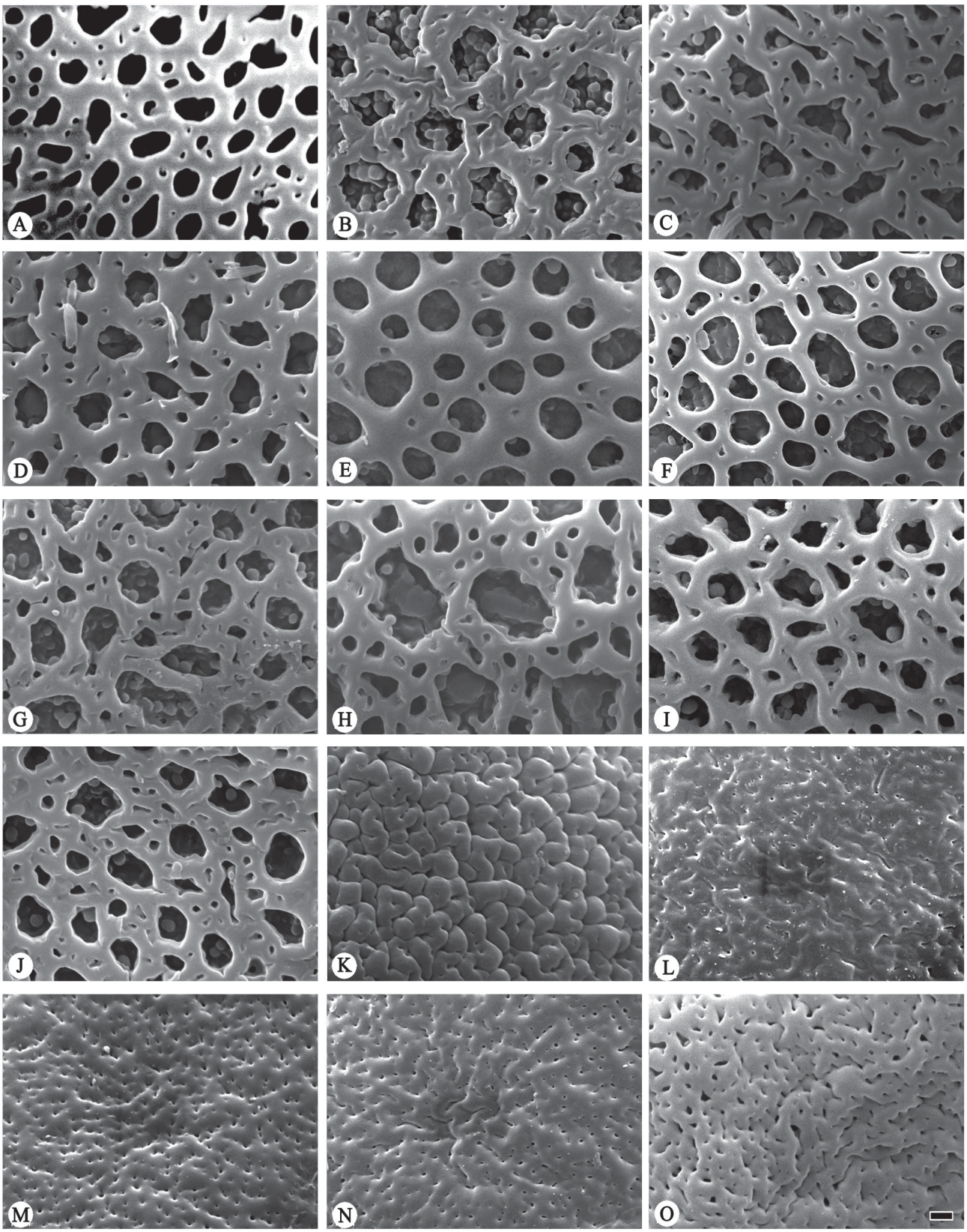


Figure 3. Scanning electron micrographs of *Bomarea* pollen. Exine sculpture (A–O). A. *B. huanuco* (Diaz & Baldeon 2249); B. *B. albimontana* (Smith & Buddensiek 11148); C. *B. glaucescens* (Cano *et al.* 7307); D. *B. vargasii* (Rodriguez & Arroyo 2932); E. *B. ampayesana* (Vargas 4439); F. *B. andimarcana* (Wood and Goyder 15546); G. *B. dulcis* (Weberbauer 3268); H. *B. involucrosa* (Smith & Alban 2357); I. *B. longistyla* (Vilcapoma 1730); J. *B. parvifolia* (Stein 2019); K. *B. bracteolata* (Knapp & Dressler 5456); L. *B. distichifolia* (USM 202018); M–N. *B. nervosa* (Wurdack 1270); O. *B. linifolia* (Core 973). Scale bars: 1 μm.

tectum corresponding with a reticulate exine in *Bomarea* has more widely spaced columellae (Fig. 5G in Sanso & Xifreda 2001). We used the lumina diameter as a character of taxonomic importance; however, the arrangement (and thickness) of muri are sometimes found to be useful characters at the subgeneric level (Sanso & Xifreda 2001).

The existence of a general relationship between pollen morphology and pollen vectors was suggested by Woodhouse (1935). The (micro)reticulate exine sculpture might represent a hummingbird mode of pollination in *Bomarea*, as all species of subgenus *Wichuraea*, including those with green flowers, are likely to be hummingbird pollinated (Hofreiter & Tillich 2003). In contrast, coarsely rugulate and finely rugulate-perforate exine sculptures might be related to pollination by butterflies (Hofreiter 2006).

Taxonomic significance of palynological features

According to our results, pollen characters alone fail to distinguish between any subgenera of *Bomarea* (Hofreiter & Tillich 2002), although pollen size (equatorial diameter) shows some variation among the subgenera *Baccata* (59.96–63.93 μm), *Sphaerine* (44.46–59.60 μm) except *B. foertheriana*, and *Wichuraea* (55.50–79.19 μm) except *B. parvifolia* (Tab. 2). In an agglomerative hierarchical clustering (analysis) of *Bomarea* using quantitative pollen data, the studied species are distributed in either two (similarity based) or four (dissimilarity based) major clusters (AKM Golam Sarwar unpubl. res.). The recent molecular phylogenetic analyses of *Bomarea* (Alzate *et al.* 2008) have also failed to recover clades that represent traditionally recognized subgeneric taxa for the genus (Hofreiter & Tillich 2002), but recovered three major clades. Only five of the studied species were included in this molecular study and these are positioned in two different clades (Fig. 2 in Alzate *et al.* 2008): *Bomarea glaucescens* is sister to the *B. cumbrensis*-*B. diffracta* subclade in clade A; *B. linifolia* is sister to the *Leontochir ovallei* (as *B. ovallei*)-*B. involucrosa* subclade in clade B; *B. pauciflora* is resolved as sister to clades B and C (Alzate *et al.* 2008). The sister relationship between *B. glaucescens* and *B. diffracta* is likely supported by pollen morphological features, but the relationship between *B. linifolia* and *B. involucrosa* is not (Tab. 2). However, pollen morphology may have some taxonomic utility below the subgeneric level.

The species of subgenus *Sphaerine* were arranged in three informal groups, *viz*. *Distichifolia*–, *Linifolia*–, and *Pauciflora*–groups (Hofreiter 2006). Palynological features may give additional support to this supposition. The pollen grains of the *Distichifolia*–group are comparatively smaller in size (44.46–53.88 μm) except *B. foertheriana* (69.39 μm), followed by the *Linifolia*–group (56.60–56.71 μm), and the monospecific *Pauciflora*–group (59.60 μm; Tab. 2). The exceptionally large pollen of *B. foertheriana* might be an adaptation to its specific habitat as it only occurs in the shadows of continually cool (without frost at night), wet, humid mist forests in Peru at 1300–3500 m; it also produces much wider leaves compared with its thickness (Hofreiter & Lyshede 2006).

Based on the geographical distribution, the species of subgenus *Wichuraea* were arranged in two groups: northern and southern (Hofreiter & Tillich 2003). Morphologically, the species of the northern group are characterized by unguiculate inner tepals, i.e., these are divided into blade and claw. One-third to one-half of the ripe fruit originates from the part above the insertion level of the petals and stamens. The species of the southern group are characterized by their spatulate inner tepals. The gynoecium is semi-inferior, one-half to two-thirds of the ripe fruit originate from the part above the insertion level of the tepals and stamina (Hofreiter & Tillich 2003). This pattern is also supported by palynological characteristics. The pollen grains of the northern group are comparatively smaller in size (53.66–59.80 μm) than those of the southern group (65.89–78.94 μm) except *B. parvifolia* (52.50 μm) and *B. dulcis* (55.48 μm; Tab. 2). The flowers of the northern group are 2–3 cm long, i.e., they are also small compared to the flowers of some species of the southern group (Hofreiter & Tillich 2003). The smaller pollen of *B. parvifolia* and *B. dulcis* are an indication of their limited distribution to a drier region and/or small flowers, and suggests similarities in other (external) morphological characters (Hofreiter & Tillich 2003). In the southern group, *B. parvifolia* is restricted to the dry western cordilleras, whereas the widespread *B. dulcis* exclusively occurs above the fog forest region. *Bomarea parvifolia* grows in central Peru at altitudes between 3500 m and 4300 m, almost exclusively on steep slopes and between rocks. *Bomarea dulcis* can occur up to 5200 m, and has small leaves closely appressed to the stem (Hofreiter & Tillich 2003). Pollen grains of members of the southern group are consistently characterized by Type II exine sculpture (Fig. 3E–J; Tab. 2).

According to Fishbein *et al.* (2001), morphology can be very useful in resolving the relationships within and among groups with rapid radiation events. Often minimal genetic changes are required to produce morphological variation, and these changes can be easily observed and quantified for use in phylogenetic inferences. Although pollen characters alone do not appear to correlate clearly with the current subgeneric classification of *Bomarea* (Hofreiter & Tillich 2002), it would be useful to develop a morphological and anatomical data set for future systematic analyses of *Bomarea* (Hofreiter & Lyshede 2006). The infrageneric classification of *Bomarea* can be reliably achieved by combined analyses of morphological, palynological, and molecular data from larger numbers of specimens of all species.

Acknowledgements

We thank the directors and curators of the consulted herbaria for allowing us to examine and/or for sending specimens on loan to sample polliniferous material. We

appreciate the help of two anonymous reviewers for their valuable comments in improving this manuscript. The first author is especially grateful to the Japan Society for the Promotion of Science (JSPS) for the Postdoctoral Fellowship for Foreign Researchers during the period of this study.

References

Aagesen L, Sanso M. 2003. The phylogeny of the Alstroemeriaceae based on morphology, *rps* 16 intron and *rbcL* sequence data. Systematic Botany 28: 47-69.

Alzate F. 2005. Three new species of *Bomarea* (Alstroemeriaceae) from the Andean region of Colombia. Novon 15: 253-258.

Alzate F. 2007. Two new species of *Bomarea* (Alstroemeriaceae) from Colombia. Novon 17: 141-144.

Alzate F, Mort ME, Ramirez M. 2008. Phylogenetic analyses of *Bomarea* (Alstroemeriaceae) based on combined analyses of nrDNA ITS, *psbA-trnH*, *rpoB-trnC* and *matK* sequences. Taxon 57: 853-862.

Bayer E. 1998. Alstroemeriaceae. In: Kubitzki K. (ed.) Families and genera of vascular plants 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae). Berlin, Springer-Verlag. p. 79-82.

Elsik WC, Thanikaimoni G. 1970. *Bomarea lyncina* Herb. (Amaryllidaceae) and *Auriculiidites* Elsik. Pollen *et* Spores 12: 177-180.

Erdtman G. 1952. Pollen morphology and plant taxonomy – Angiosperms. Leiden, E. J. Brill.

Fishbein M, Hibsch-Jetter C, Soltis DE, Hufford L. 2001. Phylogeny of Saxifragales (Angiosperms, Eudicots): analysis of a rapid, ancient radiation. Systematic Biology 50: 817-847.

Furness CA, Rudall PJ. 2003. Apertures with lids: Distribution and significance of operculate pollen in monocotyledons. International Journal of Plant Science 164: 835-854.

Heusser CJ. 1971. Pollen and spores of Chile. Tucson, Univ. Arizona Press.

Hofreiter A. 2006. Revision of *Bomarea* Mirb. Subgenus *Sphaerine* (Herb.) Baker (Alstroemeriaceae). Nordic Journal of Botany 24: 117-141.

Hofreiter A. 2008. Revision of *Bomarea* Mirb. Subgenus *Baccata* Hofr. (Alstroemeriaceae). Feddes Repertorium 119: 1-12.

Hofreiter A, Lyshede O. 2006. Functional leaf anatomy of *Bomarea* Mirb. (Alstroemeriaceae). Botanical Journal of the Linnean Society 152: 73-90.

Hofreiter A, Tillich HJ. 2002. The delimitation, ecology, distribution and infrageneric subdivision of *Bomarea* Mirbel (Alstroemeriaceae). Feddes Repertorium 113: 528-544.

Hofreiter A, Tillich HJ. 2003. Revision of the subgenus *Wichuraea* (M. Roemer) Baker of *Bomarea* Mirbel (Alstroemeriaceae). Feddes Repertorium 114: 208-239.

Kosenko VN. 1994. Pollen morphology of the family Alstroemeriaceae. Botanischesky Zhurnal 79: 1-8.

Neuendorf M. 1977. *Pardiniae*, a new section of *Bomarea* (Alstroemeriaceae). Botanical Notiser 130: 55-60.

Punt W, Hoen PP, Blackmore S, Nilsson S, Thomas A. 2007. Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143: 1-81.

Rojas S, Gutiérrez S. 2001. Atlas palinológico de las plantas visitadas por Colibríes en el sff Galeras. Bogotá, Universidad Nacional de Colombia.

Rudall PJ, Stobart KL, Hong WP, *et al*. 2000. Consider the Lilies: Systematics of Liliales. In: Wilson KL, Morrison DA. (eds.) Monocots: Systematics and evolution. Melbourne, CSIRO. p. 347-358.

Sanso AM, Xifreda CC. 1995. El genero *Bomarea* (Alstroemeriaceae) en Argentina. Darwiniana 33: 315-336.

Sanso AM, Xifreda CC. 2001. Generic delimitation between *Alstroemeria* and *Bomarea* (Alstroemeriaceae). Annals of Botany 88: 1057-1069.

Sarwar AKM Golam, Hoshino Y, Araki H. 2010. Pollen morphology and infrageneric classification of *Alstroemeria* L. (Alstroemeriaceae). Grana 49: 227-242.

Schulze W. 1978. Beitragezur Taxonomie der Liliifioren III. Alstroemeriaceae. Wissenschaftliche Zeitschrift der Friedrich-Schiller-Universitat Jena/Thüringen. Mathematisch-naturwissenschaftliche Reihe 27: 79-85.

Thiers B. 2007. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/. 27 Jan. 2014.

Wodehouse RP. 1935. Pollen grains. Their structure, identification and significance in science and medicine. New York, McGraw Hill.