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Pollen morphology and its taxonomic significance in the genus *Bomarea* Mirb. (Alstroemeriaceae) – II. Subgenus *Bomarea*

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ABSTRACT

Pollen morphology of 52 species (out of *c.* 79) of the *Bomarea* subgenus *Bomarea* was examined using light microscopy and scanning electron microscopy (SEM), or using SEM alone. The studied species of *Bomarea* were stenopalynous, characterized by large, oblate, monosulcate monads with reticulate exine sculpture in most species. Wide variation was observed in quantitative palynological features. The studied taxa were divided into four major groups based on exine ornamentation observed under SEM: microreticulate, reticulate, coarsely rugulate, and psilate-perforate. The reticulate exine sculpture may be a plesiomorphic character state for the genus *Bomarea*, and the coarsely rugulate and finely rugulate-perforate or psilate-perforate exine sculptures may have evolved independently more than once. In agglomerative hierarchical clustering (AHC) analyses of the genus *Bomarea* using quantitative pollen data, the studied species were distributed in either two (similarity-based) or four (dissimilarity-based) major clusters. Neither the recent molecular phylogenetic analyses nor the AHC analyses of *Bomarea* have recovered clades/clusters that represent traditionally recognized subgeneric taxa for the genus. Therefore, the most reliable infrageneric classification of *Bomarea* can be achieved by combining morphological, palynological, and molecular data from more extentive sampling of all the species.

Keywords: agglomerative hierarchical clustering, *Bomarea*, exine sculpture, infrageneric classification, scanning electron microscopy

Introduction

Bomarea is the most diverse genus of Alstroemeriaceae, and it includes 100-120 species with a Neotropical distribution from Mexico in the north to Argentina/Chile in the south (Neuendorf 1977; Alzate 2005). The genus is largely restricted to the Andean range and its continuation in Central America (Hofreiter & Tillich 2002). Based on morphological features, the genus *Bomarea* is divided into four subgenera: *Baccata* (five spp.), *Bomarea s. str.* (*c.* 79 spp.), *Sphaerine* (12 spp.), and *Wichuraea* (18 spp.; Hofreiter & Tillich 2002). The subgenus *Bomarea* is characterized by a generally twining habit, with flowers pendulous and actinomorphic or horizontally oriented and zygomorphic, tepals deciduous, ovary inferior and dehiscent, and fruit leathery. Baker (1888) divided this subgenus into four main groups according to the structure of the inflorescence and the relation of the inner and outer tepals, and Killip (1935) later named these four groups *Multiflorae*, *Caldasianae*, *Edules*, and *Vitellinae*. Presently, four sections are recognized within the subgenus *Bomarea*: *Multiflorae*, *Edules*, *Goniocaulon*, and *Pardinae* (Harling & Neuendorf 2003; for details see Hofreiter 2008).

As part of a comprehensive survey of pollen morphology in the genus *Bomarea*, pollen studies of three subgenera, *viz.* *Baccata*, *Sphaerine*, and *Wichuraea*, have already been published (Sarwar *et al.* 2015 and references therein). Light microscopy (LM) was mainly employed in previous palynological studies of this genus, and scanning electron microscopy (SEM) was employed in a few cases (for details see Sarwar *et al.* 2015). We describe here, using both LM and SEM, the pollen morphological features of the subgenus *Bomarea*, and evaluate their usefulness for the infrageneric classification of this genus.

Materials and Methods

Pollen morphology of 52 species (out of *c.* 79) of the *Bomarea* subgenus *Bomarea* and of one species each of the subgenera *Baccata* and *Wichuraea* was examined by light microscopy (LM) and scanning electron microscopy (SEM), or by SEM alone (Tab. 1). Polliniferous materials used in this investigation were taken from dried specimens from the herbaria K, MO, MOL, NY, and USM. Herbarium abbreviations follow the Index Herbariorum (Thiers 2007).

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

No.	Taxa	Voucher specimens
1.	*B. acutifolia* (Link & Otto) Herb.	Costa Rica: Prov. San Jose-Cartago, Cordillera de Talamanca, Davidse 24682 (MO3135888)
2.	*B. amazonica* Hofr. & Rod.*	Peru: Prov. Bagua, La Peca, Humid cloud forest, 12.08.1978. Barbour 2859 (MO2796435)
3.	*B. anceps* (R. & P.) Herb.*	Peru: (MOL2940370)
4.	*B. andreana* Baker*	Costa Rica: Prov. San Jose, northern Cordillera Talamanca, Cerro da la Muerte, 04.04.1978. Davidson 7239 (MO2660647) Panama: Chiriqui, Boquete, 18.05.1976. Croats 34872 (MO2698093)
5.	*B. angulata* Benth.*	Peru: Lambayeque, Ferrenafe, 11.09.1985. Sagastegui 12759 (MO3328919)
6.	*B. aurantiaca* Herb.*	Bolivia: Cochabamba, Chapare, 17.02.1971. Hawkes *et al.* 4438 (MO2888570) Peru: Depto. Cusco, Prov. Paucartambo. 03.15.1991. Cano 4679 (USM107269)
7.	*B. boliviensis* Baker	Bolivia: Chuguisaca, Sucre, 2.1.1994. Wood 7818 (K); La Paz, Murillo, 09.01.1982. Solmon 6654 (MO3148134)
8.	*B. bredemeyerana* (Schlecht.) Herb.*	Venezuela: Lara, 24.06.1979. Liesner *et al.* 7935 (MO2994103)
9.	*B. campanularia* Harl. & Neuend.	Ecuador: Pichincha, Quito, 08.02.1990. Cerón & Iguago 8599 (MO04778566)
10.	*B. campylophylla* Killip*	Bolivia: La Paz, Franz Tamayo, Senda Apolo-San Jose de Uchupiamonas, 05.12.2002. Miranda *et al.* 588 (MO5836245)
11.	*B. carderi* Mast.	Panama: Cocle, 24.11.1983. Churchill 3904 (MO3198551)
12.	*B. chiriquina* Killip*	Panama: Chiriquí, 20.04.1975. Mori & Kullunki 5651 (MO3155731)
13.	*B. cochabambensis* Killip*	Bolivia: Cerveceria Taquina, N-W of Cochabamba, 28.01.1943. Cutler 7678 (NY)
14.	*B. cordifolia* (R. & P.) Herbert.*	Peru: Depto. Pasco, Prov. Oxapampa. 9.10.1982. Foster & Smith 9076 (USM60834)
15.	*B. cornigera* Herb.*	Peru: Cajamarca, Jaén, 20.02.1985. Stein & Todzia 2230 (MO3294282)
16.	*B. crassifolia* Baker*	Peru: Cajamarca, Jaén, 10.06.1947. Fosberg 27845 (MO2603296)
17.	*B. crocea* (R. & P.) Herb.	Peru: Cusco, Cusco, 23.10.2004. Galiano *et al.* 6987 (MO04840135)
18.	*B. cornuta* Herb.*	Peru: Huánuco, Huánuco, 02.03.1985. Stein & Todzia 2275 (MO3294279)
19.	*B. costaricensis* Kranz.*	Costa Rica: Cartago, El Guarco, 02.02.1985. Chavarria 663 (MO5850981)
20.	*B. densiflora* Herb.*	Peru: Amazonas, Chachapoyas, no day.12.1962. Woytkowski 7829 (MO1793882) Junín, 06.11.1962. Vigo 6229 (MO5384465)
21.	*B. dolichocarpa* Killip*	Peru: Madre de Dias, 25.1.1989. Smith *et al.* 1579 (K)
22.	*B. dispar* Herb.*	Peru: Depto. Pasco, Prov. Oxapampa. 30.03.1984. Smith 6582 (USM155639).
23.	*B. edulis* (Tussac) Herb.	Japan: Hokkaido, Sapporo, Hokkaido univ., cult. FSC, 15.04.2009. Sarwar & Hoshino s.n. (SAPS) (LM, SEM) Mexico: Oaxaca, Ixtlan, 03.07.1988. Luna & Martin 0302 (MO4059183)
24.	*B. endotrachys* Kraenzl.*	Peru: Depto. de Pasco, Prov. Oxapampa. 10.12.2002. Vasquez & Rojas 27762 (USM185591)
25.	*B. euryphylla* Harl. & Neuend.*	Ecuador: Napo Quijos, Valle del Rio Cosanga, 19.10.1990. Palacios 6420 (MO04833022)
26.	*B. formosissima* (R. & P.) Herb.*	Peru: Cusco, Paucartambo, 30.10.1987. Nunez *et al.* 8511 (MO3677097)
27.	*B. goniocaulon* Baker*	Peru: Depto San Martin, 1.9.1965. Hamilton & Holligan 522 (K)
28.	*B. hartwegii* Baker*	Peru: Depto. Cajamarca, Prov. San Ignacio. 18.11.1998. Campos *et al.* 5735 (USM165038)
29.	*B. hirsuta* (Kunth) Herb.	Costa Rica: San José, Dota, 09.03.1995. Chavarria 756 (MO5850982)
30.	*B. hirtella* (Kunth) Herb.	Mexico: Chiapas, La Concordia, 15.07.2005. Chagala 68 (MO04854601)
31.	*B. lopezii* Hofreiter & E. Rodr.*	Peru: Cajamarca, Contumaza, 20.04.1984. Sagastegui 11402 (MO3226570)
32.	*B. lutea* Herb.*	Ecuador: Azuay, 10.12.1984. Scolnik 1448 (MO3155643)
33.	*B. macusanii* Hofreiter & E. Rodr.*	Peru: Cusco, Paucartambo, 30.10.1987. Nunez *et al.* 8477 (MO3677094)
34.	*B. martiana* Schen.*	Argentina: Prov. Jujuy, 25.02.1936. West 6114 (MO5257197)
35.	*B. multiflora* (L.f.) Mirb.*	Costa Rica: Cartago, Paraiso, 18.03.1994. Lepiz *et al.* 217 (MO5593431) Ecuador: 28.09.1959. Barclay 9394 (MO3189902) Costa Rica: Prov. De Puntarenus, Monteverde, Monteverde Cloud Forest Reserve, 11.04.1983. Feinsinger *et al.* 667 (MO3108601) Ecuador: Imbabura, Cotacachi, 25.05.1991. Panafiel *et al.* 90 (MO4065292) Colombia: 30.08.1957. H.G. Barclay 5295 (MO04923181)
36.	*B. multipes* Benth.*	Ecuador: Loja, 03.10.1981. Besse *et al.* 751 (MO4310504)
37.	*B. nematocaulon* Killip	Peru: Depto. Huanuco, Prov. Huanuco. Dto. Chinchao, Loc. San de Carpish. 03.11.2001. Salinas 260 (USM193748).
38.	*B. obovata* Herb.*	Nicaragua: Atlántico Sur, 13-14.02.1978. Vincelli 209 (MO2698155)
39.	*B. ovata* (Cov.) Mirb.	Peru: Depto. de Lima, Entre Matucana & San Mateo, 17.01.1949. Ferreyra 5300 (MOL00007277)

Continues.

Table 1. Continuation.

No.	Taxa	Voucher specimens
40.	*B. pardina* Herb.	Ecuador: Carchi, 31.07.1989. Werff & Gudino 10794 (MO3805857)
41.	*B. patacocensis* Herb.	Ecuador: Imbabura, 08.11.1990. Luteyn *et al.* 14078 (MO5829477)
42.	*B. patinii* Baker*	Colombia: Antioquia, Salgar, 29.09.1987. J.L. Zarucchi *et al.* 5934 (MO3592494)
43.	*B. perglabra* Harling & Neuendorf*	Ecuador: Cotopaxi, 26.04.1979 Løjtnant & Ulf Molau 13708 (MO2982422)
44.	*B. peruviana* Hofreiter*	Peru: Cajamarca, Contumaza, 31.01.1985. Stein & Todzia 2033B (MO2384243)
45.	*B. purpurea* (R. & P.) Herb.	Peru: Amazonas, Chachapoyas, 21.02.1984. Smith 6118 (MO3473926)
46.	*B. rosea* (R. & P.) Herb.*	Bolivia: Santa Cruz, Manuel Maria Caballero, 10.04.2004. Ledezma *et al.* 710 (MO04829598)
47.	*B. salsilla* (L.) Mirb.	Chile: no. date.1914. Calvert s.n. (MO1612096)
48.	*B. setacea* (R. & P.) Herb.	Ecuador: Loja, 09.12.1994. Jorgensen *et al.* 1373 (MO04833026) Peru: Depto. Amazonas, Prov. Chachapoyas, 5.2 – 2.4.97. Weigend *et al.* 97/405 (USM971405)
49.	*B. suberecta* Gereau*	Costa Rica: Limon, Cordillera de Talamanca, 11.09.1984. Devidse *et al.* 28957 (MO3211739)
50.	*B. superba* Herb.*	Peru: Depto. Cuzco, Prov. Calca, Dto. Lares, Rayampata, Bosque Primario. 20.02.2005. Farfan *et al.* 564 (USM00221035)
51.	*B. tarmensis* Kraenzl.	Peru: Junín, Chanchamayo, 08.03.1985. Stein & Todzia 2350 (MO3294277)
52.	*B. tribachiata* Kraenzl.	Peru: Depto. La Libertad, Prov. Otuzco, Road Otuzco-Usquil, 05.02 - 02.04.97. Weigend *et al.* 97/211b (USM178661).
53.	*B. trichophylla* Killip*	Venezuela: Lara, 03.01.1979. Berry 3273 (MO2725720)
54.	*B. weigendii* Hofreiter & E. Rodr.*	Peru: Depto. Ayacucho, Loc. Yanamonte, Aina. 20.12.1966. Tovar 5780 (USM161735)

The pollen parameters studied and the LM and SEM preparation methods used follow Sarwar *et al.* (2010). Measurements were taken from at least 30 randomly selected grains from each specimen (Tab. 2). To visualize the relationships among the studied species of the subgenera *Baccata*, *Bomarea*, *Sphaerine*, and *Wichuraea*, agglomerative hierarchical clustering (AHC) analyses were conducted using the XLSTAT 2009.3 program, based on the quantitative characters polar length (P), equatorial diameter (E), P/E ratio, and exine thickness. The palynological data for the subgenera *Baccata*, *Sphaerine*, and *Wichuraea* were taken from Sarwar *et al.* (2015), and dendrograms were built using AHC. Slides of all specimens have been deposited in the Hokkaido University Museum, Sapporo, Japan. Pollen size and shape classes were defined using the criteria of Erdtman (1952), and our descriptive terminology follows Punt *et al.* (2007) and Hesse *et al.* (2009). The sectional classification of subgenus *Bomarea* follows Harling & Neuendorf (2003) and Hofreiter (2008).

Results

Based on LM and SEM observations, the *Bomarea* pollen grains studied were monad, large, ellipsoid (boat-shaped), heteropolar; monosulcate, sulcus on the convex part of the grain, distinct, long, straight, wide at the equator, narrow near the poles, sometimes extended to the proximal pole (Fig. 1A-D); auriculae-like structures at the end of the sulcus in *B. acutifolia*, *B. amazonica*, *B. andreana* (Croats 34872), *B. bredemeyerana*, *B. cornigera*, *B. crocea, B. dispar*, *B. euryphylla*, *B. formosissima*, *B. hirsuta*, *B. lutea*, *B. macusanii*, *B. multiflora* (Barclay 5295), *B. suberecta*, *B. multipes*, *B. martiana*, *B. nematocaulon*, *B. pardina*, and *B. tarmensis* (Fig. 1E-F; Tab. 2); opercula-like structures present on the sulcus in *B. ovata* (Fig. 1D). Symmetry is bilateral. Size ranges from 25.26-38.82 μm (polar length P) × 47.18-69.30 μm (equatorial diameter E), P/E 0.43-0.62, peroblate to oblate in shape, exine thickness 1.11-2.76 μm (Tab. 2).

The pollen grains of *Bomarea* species were characterized by a semitectate exine sculpture. Four different exine sculpture types were observed:

Type I – Microreticulate (Figs. 1G-O, 2A-F; Tab. 2), with perforate muri, heterobrochate; lumina less than or equal to 1 μm in length or diameter; observed in *B. amazonica*, *B. andreana* (Davidson 7239), *B. aurantiaca* (Hawkes *et al.* 4438), *B. boliviensis*, *B. cornigera*, *B. crassifolia*, *B. dispar*, *B. edulis*, *B. endotrachys*, *B. hartwegii*, *B. hirtella*, *B. martiana*, *B. multiflora* (Feinsinger *et al.* 667), *B. multipes*, *B. rosea*, *B. setacea* (Jorgensen *et al.* 1373), *B. suberecta,* and *B. peruviana*.

Type II – Reticulate (Figs. 2G-O, 3A-O, 4A-F; Tab. 2), with perforate muri, heterobrochate; lumina larger than 1 μm in length or diameter; observed in *B. acutifolia*, *B. anceps*, *B. andreana* (Croats 34872), *B. angulata*, *B. bredemeyerana*, *B. campylophylla*, *B. carderi*, *B. chiriquina*, *B. cochabambensis*, *B. cordifolia*, *B. cornuta*, *B. costaricensis*, *B. crocea*, *B. euryphylla*, *B. formosissima, B. goniocaulon*, *B. hirsuta*, *B. lopezii*, *B. lutea*, *B. macusanii*, *B. multiflora* (Barclay 9394; Lepiz 217; Panafiel *et al.* 90; Barclay 5295), *B. nematocaulon*, *B. ovata*, *B. patacocensis*, *B. patinii*, *B. perglabra*, *B. purpurea,* and *B. trichophylla*.

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

Name of Taxa		Polar length (P)	Equatorial diameter (E)	P/E	Exine thickness	Exine sculpture*	Fig. No.
Bomarea* subg. *Bomarea (c. 79 spp.)							
Section *Multiflorae*							
B. acutifolia (A)		32.41±1.81 (28.29-37.30)	56.48±1.99 (53.08-60.33)	0.57	1.43±0.11 (1.20-1.60)	Type II	2G
B. amazonica (A)		28.64±2.37 (24.67-34.92)	58.01±2.94 (49.96-63.56)	0.49	1.11±0.12 (0.95-1.34)	Type I	1G
B. andreana	Davidson 7239	33.26±3.92 (27.92-39.65)	59.89±5.06 (48.83-63.95)	0.56	1.16±0.29 (0.85-1.69)	Type I	1H
	Croats 34872 (A)	38.35±2.88 (33.14-43.39)	66.65±3.51 (55.71-73.56)	0.58	1.40±0.10 (1.20-1.57)	Type II	2H
B. aurantiaca	Hawkes *et al.* 4438	37.59±2.91 (31.38-42.03)	67.32±3.03 (60.79-73.21)	0.56	1.50±0.21 (0.95-1.90)	Type III	-
	Cano 4679	n.d.	n.d.	n.d.	n.d.	Type III	4G
B. bredemeyerana (A)		31.58±1.61 (28.28-34.94)	59.05±2.53 (54.57-65.30)	0.53	1.40±0.19 (0.93-1.60)	Type II	2I
B. chiriquina		36.22±2.29 (31.41-40.45)	58.41±3.21 (52.68-63.40)	0.62	1.26±0.14 (0.95-1.53)	Type II	2J
B. costaricensis		n.d.	n.d.	n.d.	n.d.	Type II	2K
B. crassifolia		34.51±2.50 (29.87-40.43)	59.74±3.20 (53.07-66.35)	0.58	1.59±0.09 (1.45-1.80)	Type I	1I
B. crocea (A)		32.26±1.26 (30.22-35.57)	52.89±3.19 (41.17-56.90)	0.61	1.18±0.08 (1.04-1.35)	Type II	2L
B. densiflora	Woytkowski 7829	33.60±4.05 (31.12-38.27)	60.00±1.83 (57.89-61.10)	0.56	1.79±0.04 (1.75-1.82)	-	-
	Vigo 6229	25.26±3.06 (21.90-32.81)	47.18±3.70 (39.83-53.85)	0.54	1.37±0.18 (1.15-1.80)	Type IV	4J
B. endotrachys		30.87±2.42 (26.48-35.42)	49.83±2.93 (44.84-56.65)	0.62	1.34±0.13 (1.10-1.62)	Type I	-
B. euryphylla (A)		32.86±2.83 (26.86-37.46)	59.61±2.91 (53.47-66.76)	0.55	1.80±0.15 (1.53-2.12)	Type II	2M
B. formosissima (A)		31.53±1.84 (28.77-35.64)	58.32±2.29 (53.75-63.66)	0.54	1.67±0.14 (1.50-2.05)	Type II	2N
B. hartwegii		31.69±2.57 (26.54-37.95)	58.60±2.77 (52.77-62.97)	0.54	1.64±0.14 (1.43-1.94)	Type I	1J
B. hirsuta (A)		31.53±2.65 (26.48-37.95)	59.05±2.54 (50.90-63.67)	0.53	1.57±0.13 (1.30-1.75)	Type II	2O
B. lutea (A)		35.84±2.28 (31.80-41.12)	69.30±3.41 (61.10-76.48)	0.52	1.89±0.15 (1.53-2.18)	Type II	3A
B. macusanii (A)		35.59±1.86 (31.86-39.75)	59.02±2.53 (51.15-61.82)	0.60	1.55±0.11 (1.34-1.75)	Type II	3B
B. multiflora	Lepiz 217	34.99±2.60 (30.99-40.57)	63.22±3.21 (55.63-72.46)	0.55	1.73±0.15 (1.53-2.16)	Type II	3C
	Barclay 9394	31.83±3.24 (28.08- 43.47)	58.39±3.02 (52.44-65.11)	0.55	1.26±0.15 (0.95-1.60)	Type II	3D
	Feinsinger *et al.* 667	34.08±2.02 (30.47-38.91)	58.28±2.70 (53.27-63.18)	0.58	1.54±0.07 (1.43-1.75)	Type I	1K
	Panafiel *et al.* 90	n.d.	n.d.	n.d.	n.d.	Type II	3E
	Barclay 5295 (A)	33.72±2.63 (29.23-40.15)	62.43±3.15 (57.50-68.51)	0.54	1.80±0.18 (1.53-2.16)	Type II	3F
B. patacocensis		32.15±3.70 (26.12-40.47)	63.26±2.99 (56.76-68.00)	0.51	1.63±0.17 (1.27-1.92)	Type II	3G
B. patinii		31.78±2.11 (27.71-35.95)	59.84±3.59 (51.66-65.92)	0.53	1.82±0.13 (1.53-2.12)	Type II	3H
B. purpurea		29.51±2.49 (26.37-34.23)	57.82±2.41 (54.31-60.71)	0.51	1.31±0.21 (1.02-1.57)	-	-
B. setacea	Jorgensen *et al.* 1373	n.d.	n.d.	n.d.	n.d.	Type I	-
	Weigend *et al.* 97/405	31.01±2.07 (27.61-36.04)	51.35±2.60 (46.96-57.05)	0.60	1.64±0.17 (1.43-2.16)	Type IV	4K
B. suberecta (A)		27.80±1.51 (24.25-30.87)	53.31±1.84 (49.36-56.62)	0.52	1.51±0.18 (1.25-1.80)	Type I	1L
B. superba		38.03±3.35 (33.44-43.39)	65.18±3.76 (60.80-71.94)	0.58	1.72±0.20 (1.53-2.12)	Type IV	4L
Section *Edules*							
B. campylophylla		38.82±3.06 (32.26-45.50)	67.55±3.19 (61.54-74.27)	0.57	1.55±0.09 (1.43-1.75)	Type II	3I
B. cordifolia		34.30±2.67 (28.17-41.03)	61.48±2.39 (57.10-67.62)	0.56	1.65±0.11 (1.45-1.83)	Type II	3J
B. cornigera (A)		30.64±1.84 (26.54-34.06)	55.17±2.30 (51.52-60.60)	0.56	1.52±0.18 (1.15-1.80)	Type I	1M
B. cornuta		29.15±2.08 (24.78-32.63)	54.29±2.04 (49.96-57.37)	0.54	1.25±0.10 (1.04-1.53)	Type II	3K
B. dolichocarpa		28.05±3.08 (24.41-33.10)	65.35±5.76 (55.47-74.06)	0.43	2.76±0.48 (2.12-3.66)	-	
B. edulis		n.d.	n.d.	n.d.	n.d.	Type I	1N
B. lopezii		35.94±2.33 (31.78-39.79)	59.56±2.83 (52.72-65.39)	0.60	1.37±0.19 (1.07-1.75)	Type II	3L
B. obovata		n.d.	n.d.	n.d.	n.d.	Type IV	4M
B. ovata		30.14±3.67 (22.39-35.87)	59.66±4.09 (52.84-66.87)	0.51	1.42±0.19 (1.20-1.90)	Type II	3M

Continues.

Table 2. Continuation.

Name of Taxa	Polar length (P)	Equatorial diameter (E)	P/E	Exine thickness	Exine sculpture*	Fig. No.
Section *Goniocaulon*						
B. angulata	n.d.	n.d.	n.d.	n.d.	Type II	3N
B. goniocaulon	32.26±2.61 (27.99-38.27)	59.31±3.22 (51.81-66.50)	0.54	2.12±0.31 (1.75-2.80)	Type II	3O
B. multiples (A)	32.69±1.63 (28.89-36.28)	61.23±2.37 (55.84-66.18)	0.53	1.81±0.18 (1.53-2.18)	Type I	1O
B. perglabra	35.11±3.17 (31.16-40.67)	60.11±3.75 (54.52-64.25)	0.58	1.40±0.15 (1.20-1.56)	Type II	4A
Section *Pardinae*						
B. pardina (A)	n.d.	n.d.	n.d.	n.d.	Type III	4H
Don't fit into any section						
B. anceps	30.87±2.00 (27.61-33.96)	57.45±2.21 (52.20-61.34)	0.54	1.63±0.16 (1.34-2.09)	Type II	4B
B. boliviensis	n.d.	n.d.	n.d.	n.d.	Type I	2A
B. cochabambensis	29.40±3.60 (24.73-37.61)	55.16±3.62 (48.78-61.77)	0.53	1.12±0.11 (0.95-1.27)	Type II	4C
B. dispar (A)	32.56±2.43 (28.30-38.40)	58.76±3.08 (51.15-65.13)	0.54	1.63±0.13 (1.43-1.90)	Type I	2B
B. hirtella	36.03±2.78 (30.97-41.79)	67.80±3.07 (62.30-73.63)	0.53	1.85±0.20 (1.57-2.40)	Type I	2C
B. martiana (A)	32.43±3.29 (28.17-38.63)	56.39±4.52 (48.51-68.87)	0.58	1.38±0.19 (1.20-1.90)	Type I	2D
B. nematocaulon (A)	27.88±2.23 (24.25-34.51)	51.18±2.97 (44.42-57.64)	0.55	1.58±0.13 (1.27-1.80)	Type II	4D
B. rosea	34.66±1.84 (31.57-38.25)	67.60±2.95 (61.99-75.14)	0.51	2.13±0.22 (1.75-2.58)	Type I	2E
B. tarmensis (A)	27.93±2.98 (23.59-31.40)	56.37±3.16 (53.17-59.76)	0.50	1.82±0.19 (1.64-2.12)	Type IV	4N-O
B. trichophylla	32.13±1.80 (28.74-35.41)	54.11±2.44 (51.21-59.00)	0.59	1.76±0.16 (1.53-2.12)	Type II	4E
B. weigendii	n.d.	n.d.	n.d.	n.d.	Type III	4I
Bomarea* subg. *Baccata (5 spp.)						
B. carderi	36.39±2.63 (31.57-42.80)	60.77±2.50 (55.96-66.14)	0.60	1.34±0.14 (0.95-1.57)	Type II	4F
Bomarea* subg. *Wichurea (16 spp.)						
B. peruviana	30.00±3.74 (25.88-38.07)	61.29±4.45 (54.15-68.71)	0.49	1.82±0.20 (1.53-2.12)	Type I	2F

* 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, psilate-perforate. For details see text.

Type III – Coarsely rugulate (Fig. 4G-I; Tab. 2); observed in *B. aurantiaca* (Cano 4679), *B. pardina,* and *B. weigendii*.
Type IV – Psilate-perforate (Fig. 4J-O; Tab. 2); observed in *B. densiflora* (Vigo 6229), *B. obovata*, *B. setacea* (Weigend *et al*. 97/405), *B. superba,* and *B. tarmensis*.

Granula were sometimes visible at the bottom of the lumina; these may correspond to "free standing columellae" (e.g. Fig. 1G; Hesse *et al*. 2009). The exine sculpture along with sulci was similar to that appearing at the equatorial position, but had relatively smaller lumina.

In agglomerative hierarchical clustering (AHC) analyses of the genus *Bomarea* using quantitative pollen data, the species studied were distributed in either two (similarity-based) or four (dissimilarity-based) major clusters (Fig. 5). Among these clusters, three include at least one species from each subgenus; cluster 1 (blue) comprises most of the taxa studied (41), cluster 2 (pink) comprises 12 taxa, cluster 3 (brown) comprises 16 taxa, and cluster 4 (black) comprises the single species *B. ampayesana* from the subgenus *Wichuraea*.

Discussion

The *Bomarea* species studied were stenopalynous and characterized by monad, monosulcate, large pollen grains (Figs. 1-4; Tab. 2; Sarwar *et al*. 2015). The pollen morphology of 36 of these species, indicated by asterisks in Tab. 1, was studied for the first time using either LM or SEM. Our results are in agreement with previous reports (Erdtman 1952; Heusser 1971; Neuendorf 1977; Schulze 1978; Kosenko 1994; Sanso & Xifreda 2001; Alzate 2007; Sarwar *et al*. 2015). The monophyly of the genus *Bomarea* is supported by molecular data (Aagesen & Sanso 2003; Alzate *et al*. 2008) and by the palynological data as well (Tab. 2; Sarwar *et al*. 2015). However, there were significant differences in the values of the quantitative palynological characters, which may to some extent be related to differences in the mounting media (Meltsov *et al*. 2008) and the methods of preparation of the pollen grains (Schols *et al*. 2004), as well as the species' geographical distribution, floral size, etc. (AKM Golam Sarwar pers. obs.). For example, there were

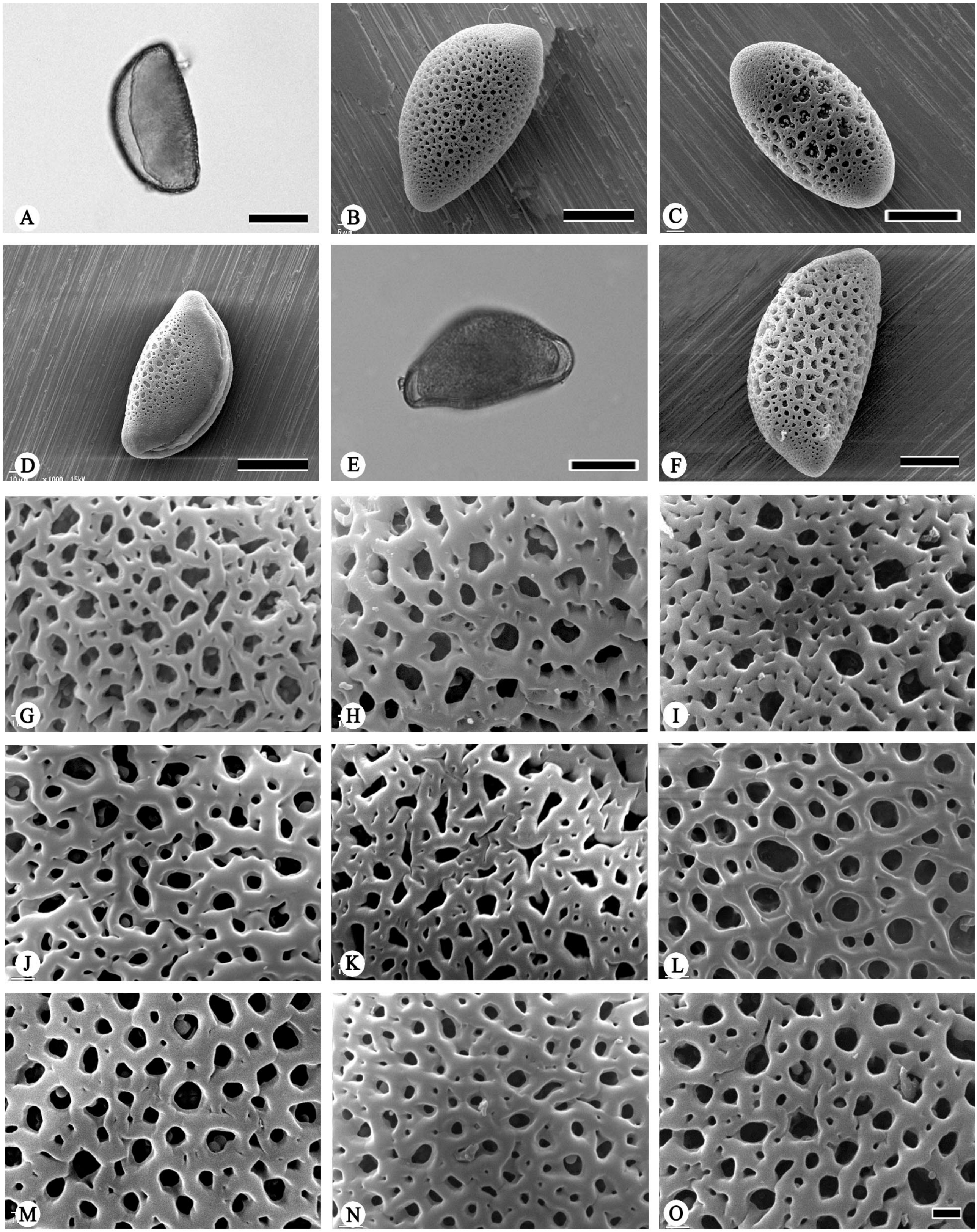

Figure 1. Light and scanning electron micrographs of *Bomarea* pollen. Pollen grains (A-F); exine sculpture (G-O). A. *B. rosea* (Ledezma *et al.* 710); B. *B. crocea* (Galiano *et al.* 6987); C. *B. cochabambensis* (Cutler 7678); D. *B. ovata* (Ferreyra 5300); E. *B. multiflora* (Barclay 5295); F. *B. andreana* (Croats 34872); G. *B. amazonica* (Barbour 2859); H. *B. andreana* (Davidson 7239); I. *B. crassifolia* (Fosberg 27845); J. *B. hartwegii* (Campos *et al.* 5735); K. *B. multiflora* (Feinsinger *et al.* 667); L. *B. suberecta* (Devidse *et al.* 28957); M. *B. cornigera* (Stein & Todzia 2230); N. *B. edulis* (Sarwar & Hoshino *s. n.*); O. *B. multipes* (Besse *et al.* 751). Scale bars: A-F, 10 µm; G-O, 1 µm. Specimen numbers are detailed in Tab. 1.

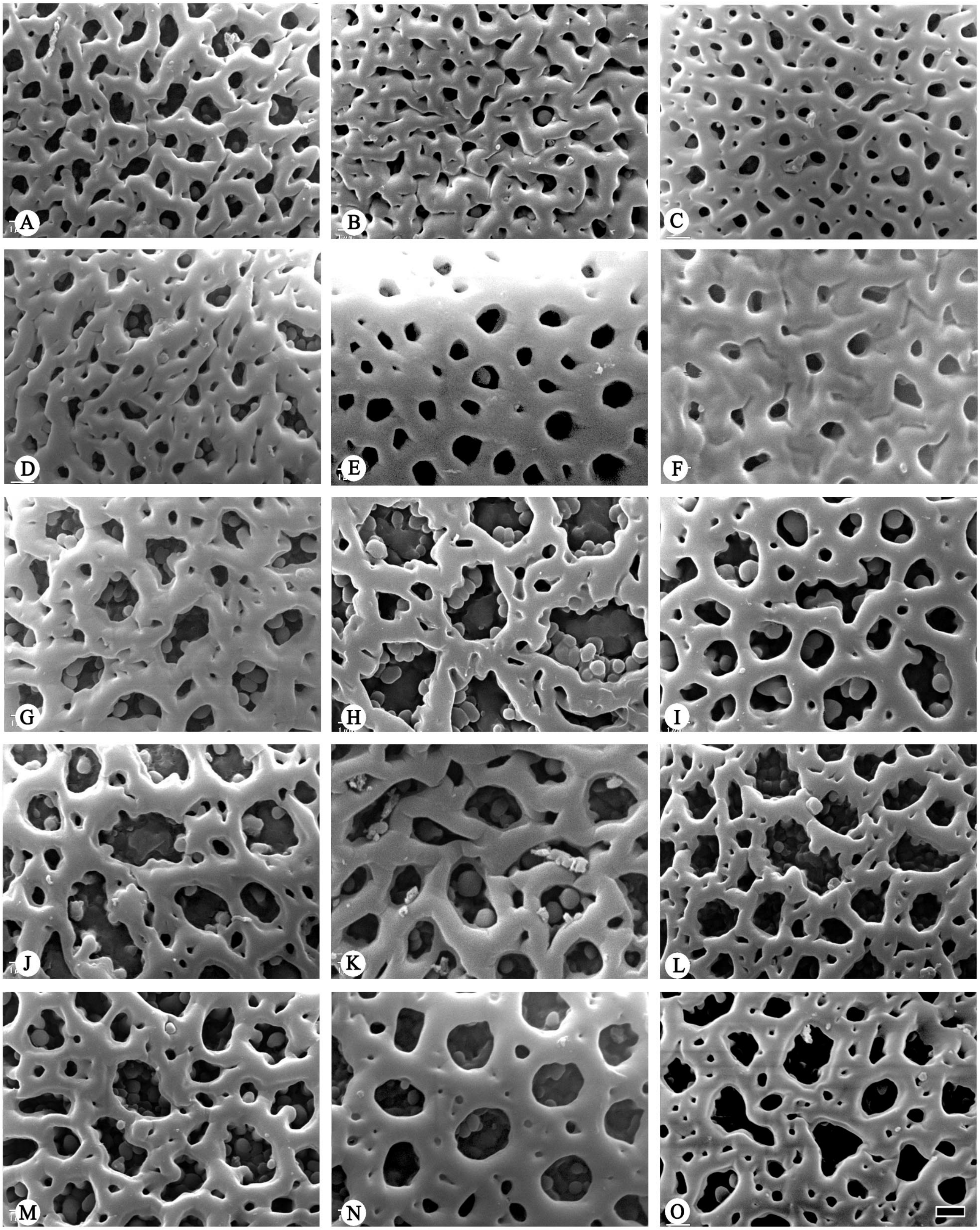

Figure 2. Scanning electron micrographs of *Bomarea* pollen. Exine sculpture (A-O). A. *B. boliviensis* (Wood 7818); B. *B. dispar* (Smith 6582); C. *B. hirtella* (Chagala 68); D. *B. martiana* (West 6114); E. *B. rosea* (Ledezma *et al.* 710); F. *B. peruviana* (Stein & Todzia 2033B); G. *B. acutifolia* (Davidse 24682); H. *B. andreana* (Croats 34872); I. *B. bredemeyerana* (Liesner *et al.* 7935); J. *B. chiriquina* (Mori & Kullunki 5651); K. *B. costaricensis* (Chavarria 663); L. *B. crocea* (Galiano *et al.* 6987); M. *B. euryphylla* (Palacios 6420); N. *B. formosissima* (Nunez *et al.* 8511); O. *B. hirsuta* (Chavarria 756). Scale bars: A-O, 1 μm. Specimen numbers are detailed in Tab. 1.

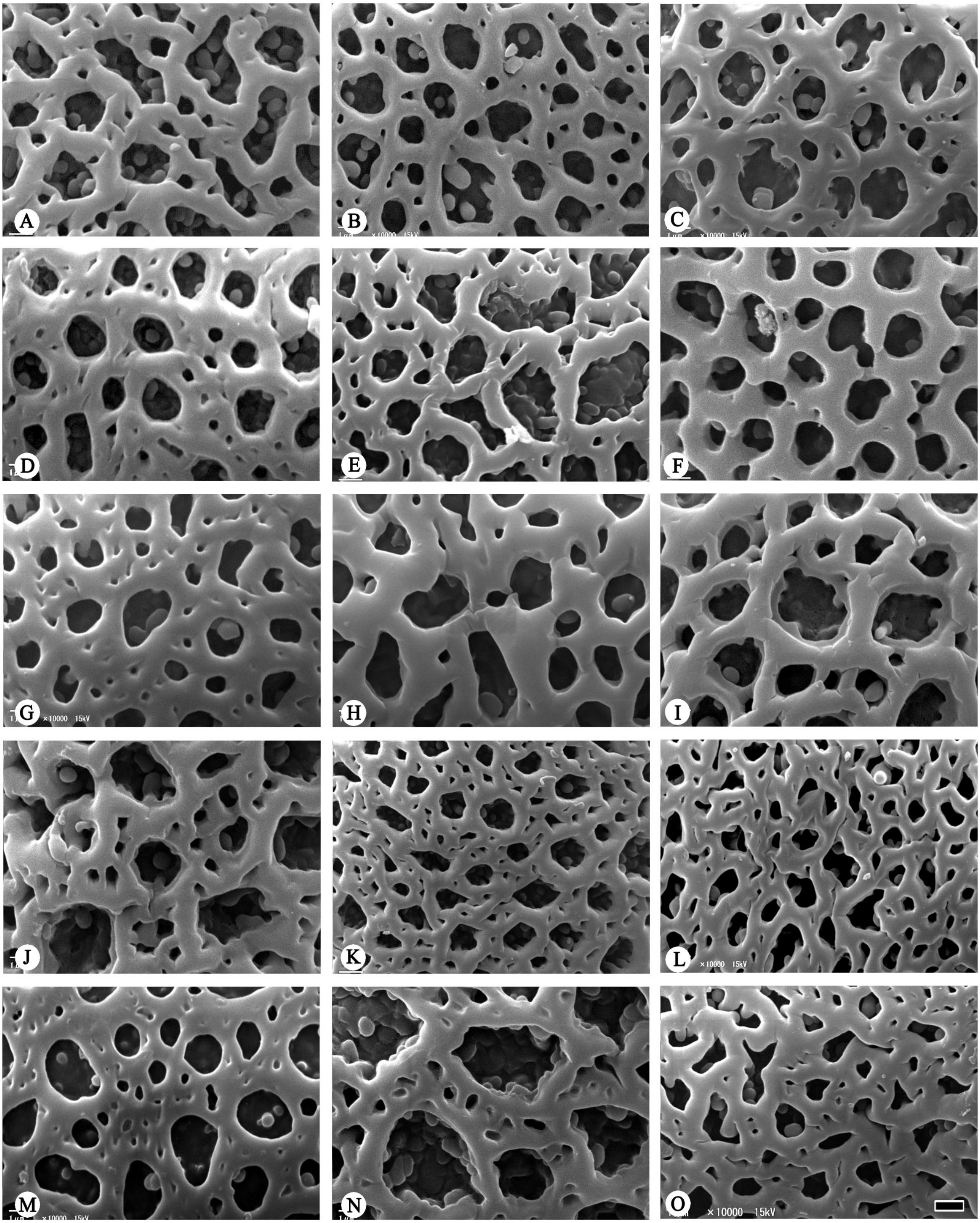

Figure 3. Scanning electron micrographs of *Bomarea* pollen. Exine sculpture (A-O). A. *B. lutea* (Scolnik 1448); B. *B. macusanii* (Nunez *et al.* 8477); C. *B. multiflora* (Lepiz 217); D. *B. multiflora* (Barclay 9394); E. *B. multiflora* (Panafiel *et al.* 90); F. *B. multiflora* (Barclay 5295); G. *B. patacocensis* (Luteyn *et al.* 14078); H. *B. patinii* (Zarucchi *et al.* 5934); I. *B. campylophylla* (Miranda *et al.* 588); J. *B. cordifolia* (Foster & Smith 9076); K. *B. cornuta* (Stein & Todzia 2275); L. *B. lopezii* (Sagastegui 11402); M. *B. ovata* (Ferreyra 5300); N. *B. angulata* (Sagastegui 12759); O. *B. goniocaulon* (Hamilton & Holligan 522). Scale bars: A-O, 1 μm. Specimen numbers are detailed in Tab. 1.

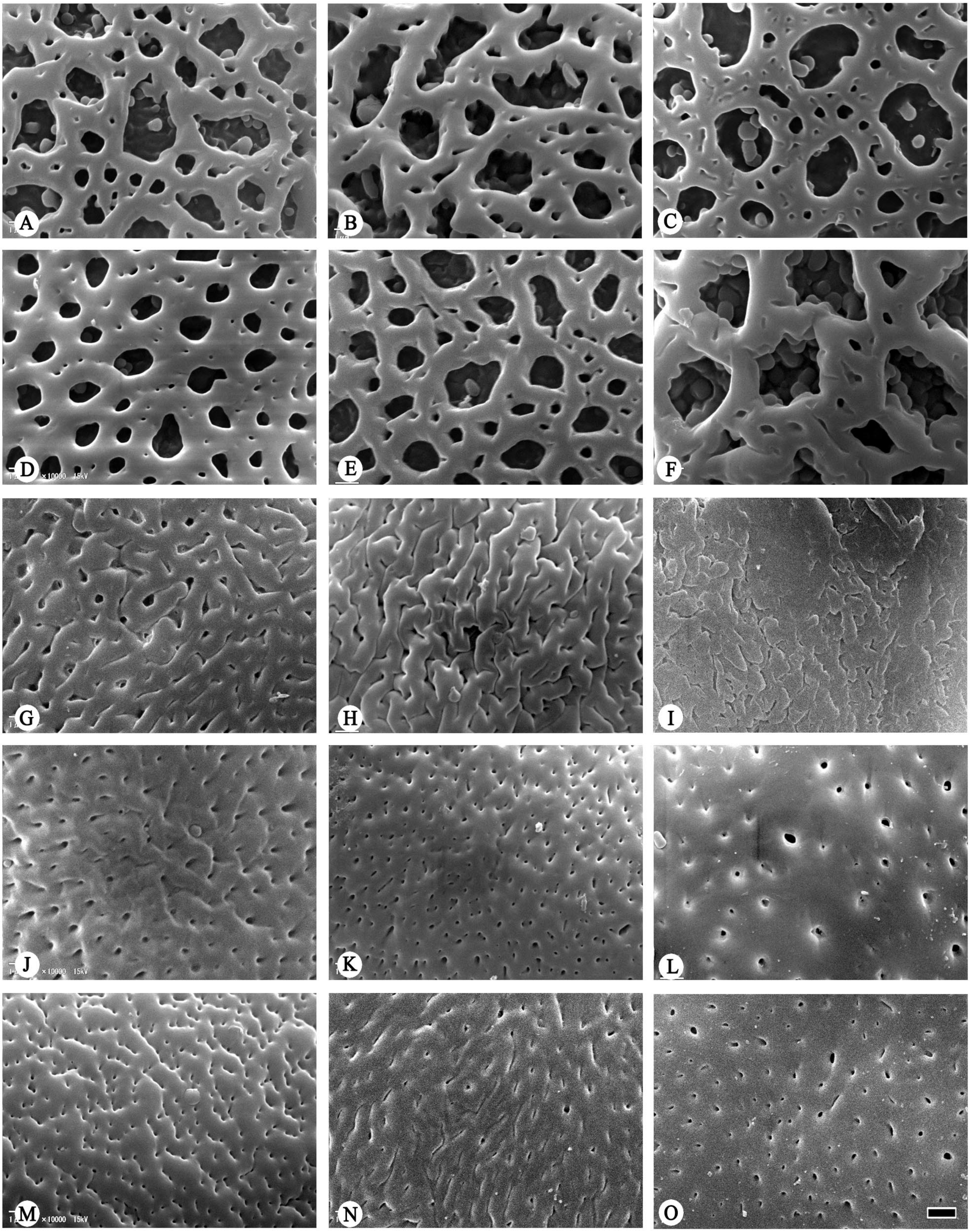

Figure 4. Scanning electron micrographs of *Bomarea* pollen. Exine sculpture (A-O). A. *B. perglabra* (Løjtnant & Ulf Molau 13708); B. *B. anceps* (MOL2940370); C. *B. cochabambensis* (Cutler 7678); D. *B. nematocaulon* (Salinas 260); E. *B. trichophylla* (Berry 3273); F. *B. carderi* (Churchill 3904); G. *B. aurantiaca* (Cano 4679); H. *B. pardina* (Werff & Gudino 10794); I. *B. weigendii* (Tovar 5780); J. *B. densiflora* (Vigo 6229); K. *B. setacea* (Weigend *et al*. 97/405); L. *B. superba* (Farfan *et al*. 564); M. *B. obovata* (Vincelli 209); N-O. *B. tarmensis* (Stein & Todzia 2350). Scale bars: A-O, 1 µm. Specimen numbers are detailed in Tab. 1.

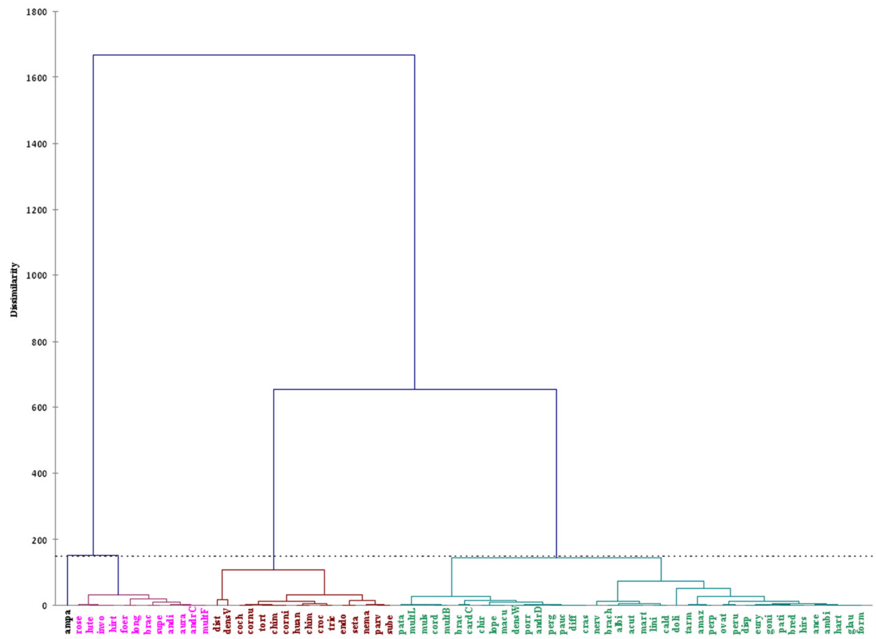

Figure 5. Dendrogram obtained from quantitative data by agglomerative hierarchical clustering analysis. Names of species are abbreviated to the first four to six letters of the specific epithets.

significant differences between two *B. andreana* specimens, one of which had smaller pollen grains with a thinner exine (Davidson 7239, collected from the warmer region of Costa Rica), while the other had larger pollen grains with a thicker exine (Croats 34872, collected from the cooler region of Panama) (Tabs. 1, 2).

A wide and generally continuous pattern of variation in P, E, P/E ratio, and exine thickness was observed at both the infra- and inter-species level (Tab. 2). Among the sections of *Bomarea* subgenus *Bomarea*, section *Goniocaulon* produces pollen grains with relatively consistent P/E ratios (0.53-0.58) (Tab. 2). Pollen grain size within a genus is influenced by internal (chromosome number and floral character) and external (temperature, mineral nutrition, and water conditions) factors (Stanley & Linskens 1974). However, no correlation between ploidy level and palynological features was observed in *Bomarea*. The only tetraploid species of *Bomarea*, *B. hirtella*, produced pollen grains similar in size to or slightly smaller than the diploid species (Tab. 2; Cave 1967).

Auricula-like structures in pollen grains have been previously reported from only four *Bomarea* species, namely, *B. brachysepala*, *B. bracteolata*, *B. glaucescens,* and *B. huanuco* (Sarwar *et al.* 2015). In the present study, pollen with auricula-like structures was observed in 19 taxa (Fig. 1E; Tab. 2), including in *B. ceratophora* (Neuendorf 1977) in the subgenus *Bomarea*. These auricula-like structures may have some taxonomic importance for the genus *Bomarea* since they are relatively common in the subgenus *Bomarea*, but are completely absent in members of the southern group of the subgenus *Wichuraea* (Tab. 2; Sarwar *et al.* 2015). Auriculate pollen grains are not common among extant plant taxa, and diverse Cretaceous pollen-bearing auriculate appendages have been variously described as gymnospermous and monocotyledonous (Elsik 1974). As the term "auriculae" is only applicable to the spores' structure (Punt *et al.* 2007), the term "apex" has sometimes been used for these auricula-like pollen structures (see Martín *et al.* 2012 for detailed terminological discussion).

A recent molecular phylogenetic analysis of *Bomarea* (Alzate *et al.* 2008) identified three major clades, but none of them correspond to traditionally recognized subgeneric taxa (Hofreiter & Tillich 2002). Only seven of the species we studied were included in that molecular analysis, and these seven are positioned in two different clades (Fig. 2 in Alzate *et al.* 2008). Exine sculpture was found to be the most important palynological character possessing phylogenetic importance. In the molecular phylogenetic analysis, four members of section *Multiflorae* (*B. vestita* syn. *B. multiflora*; Hofreiter 2008) were included in clade C; this was also supported and confirmed by palynological characters, especially exine sculpture Type II (Figs. 2I, O, 3C-F; Tab. 2). The other species of section *Multiflorae* (*B. setacea*) are sister to clade B and are characterized by exine sculpture Type IV (Fig. 4K; Tab. 2), although wide variation was observed in the exine sculpture of two specimens of *B. setacea* (Type I *vs.* Type IV; Tab. 2), which may be due to polymorphism. *Bomarea setacea* is polymorphous taxon, especially in its general stature and in the shape and size of the leaves and inflorescence (Harling & Neuendorf 2003). Of the two other species of the subgenus *Bomarea*, *B. edulis* was included in clade A, and *B. salsilla* was sister to *B. straminea* (Fig. 2 in Alzate *et al.* 2008).

Pollen grains of *Bomarea* species are characterized by their (micro-)reticulate exine sculptures (Figs. 1G-O, 2, 3, 4A-F; Sarwar *et al*. 2015), although coarsely rugulate and finely rugulate-perforate or psilate-perforate exine sculptures are also observed in a few *Bomarea* species (Fig. 4G-O; Hofreiter 2008; Sarwar *et al*. 2015). This may give additional support to the supposition that (micro-)reticulate exine sculptures might be a plesiomorphic character state for *Bomarea* and the coarsely rugulate and finely rugulate-perforate or psilate-perforate exine sculptures might have evolved independently more than once (Alzate *et al*. 2008; Sarwar *et al*. 2015). These results may also confirm the major evolutionary trend in exine sculpture in the family Alstroemeriaceae from reticulate through rugulate-psilate to striate-reticulate, or vice-versa (Aagesen & Sanso 2003; Sarwar *et al*. 2010; 2015).

Among the quantitative pollen characters analyzed, equatorial diameter had the greatest influence on the position of taxa in the AHC cladogram (Fig. 5; Tab. 2; Sarwar *et al*. 2015). The members of cluster 1 commonly produced pollen grains 52.67-62.93 μm in size; cluster 2 produced relatively larger pollen grains (63.93-69.30 μm); cluster 3 produced the smallest pollen grains (44.46-55.30 μm). The large pollen grains of *B. ampayesana* (subgenus *Wichuraea*) might be one of the reasons for its very distinct position in cluster 4 (black) of the AHC (Fig. 5; Tab. 2; Sarwar *et al*. 2015). The greatest variation in pollen morphological features was observed in the subgenus *Wichuraea* (Fig. 5; Hofreiter & Tillich 2002), which also exhibited the greatest variation in other morphological features.

There were significant differences in the species composition of the different subclades/clusters recovered from both the molecular phylogenetic analysis (based on a few species; Alzate *et al*. 2008) and the AHC (based on quantitative pollen features; Fig. 5). Moreover, analyses based on different genomes (nuclear *vs*. plastid) not only lead to disagreements in the evolutionary relationships of taxa, but also greatly affected other phylogeny-based inferences and interpretations relating to taxonomy, morphological evolution, historical biogeography, and phylogenetic diversity (Zhang *et al*. 2015). Therefore, the most reliable infrageneric classification of *Bomarea* will be achieved through combined analyses of morphological, palynological, and molecular data from more extentive sampling- of all the species in the genus.

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