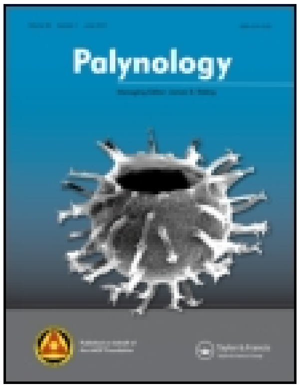

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Pollen analysis in some species of Linaceae-Linoideae from Argentina

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Pollen analysis in some species of Linaceae-Linoideae from Argentina

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The pollen of *Cliococca selaginoides* (Lam.) C. M. Rogers & Mildner and eight *Linum* L. species from Argentina were examined using light and scanning electron microscopy. Both genera share pollen grains which are 3-zonocolpate, isopolar, radiosymmetric, spheroidal and medium to large in size. The sculptural elements of the exine are gemmae or clavae, which allow the genera to be distinguished and the species to be characterised. In *Cliococca* Bad., the pollen is exclusively gemmate whereas in *Linum* the pollen is gemmate and clavate (except in *L. catharticum* L. whose pollen has only gemmae which are apically microechinate). The statistical analyses of quantitative and qualitative morphological characteristics were performed. A key to identify and distinguish the pollen types and sub-types is also provided. A generalized procrustes analysis (GPA) of joint characterisation based on both qualitative and quantitative characters identified four distinct groups.

Keywords: Argentina; *Cliococca*; exine; GPA; *Linum*; sculptural elements

1. Introduction

The Linaceae is a cosmopolitan family comprising about 250 species. in 14 genera and is distributed throughout tropical and temperate regions (McDill et al. 2009). Recent studies based on molecular data recognised two subfamilies: the Linoideae and Hugonoideae, the latter of which is paraphyletic (McDill et al. 2009).

Most species in the family belong to the economically important genus *Linum* L., which has ca. 180 species. The main representative of the genus is *L. usitatissimum* L., a species cultivated worldwide for textiles using phloem fibers and linseed oil (Pengilly 2003; Vaisey-Genser and Morris 2003). The genera of Linaceae found in Argentina are *Cliococca* Bad., a monotypic genus, and *Linum*, which has eight species, mostly concentrated in southern South America (Zuloaga et al. 2008).

An interesting character of the family is the presence of heterostyly in some species of *Linum*, a feature earlier recognised by Darwin in the flowers of *L. perenne* L. (Darwin 1864) but absent in the species studied here.

The first pollen analysis with an optical microscope of the Linaceae was done by Erdtman (1966), who studied the pollen grains of some species including *L. usitatissimum*. This author considered the family to be eurypalynous. Detailed studies of Linaceae pollen were done by Saad (1961a, 1961b, 1962) who studied the apertural mechanisms and stratification of the exine in many species of Linaceae. Rogers and Xavier (1972) analysed the evolution of pollen structure in *Linum*. Xavier et al. (1980) studied the pollen morphology of *Linum*, Sect. *Linastrum*. Rogers (1980) studied pollen dimorphism in two distylous *Linum* species of the section *Linastrum*. Dulberger (1981) evaluated the dimorphism of the exine sculpture in three distylous species of *Linum*. Punt and Den Breejen (1981) studied Linaceae pollen for the Flora of northwest Europe and Rogers (1985) analysed the pollen morphology of the genus *Cliococca*. In addition, Medeiros Carreiras et al. (2005) studied pollen morphology of Amazonian species of the family. Although there are numerous studies on the pollen morphology in Linaceae, none of them characterised species groups according to the variability of the characters. This encouraged us to study the Argentinean species of *Linum* and *Cliococca* by evaluating the morphology of the pollen types via a joint analysis of qualitative and quantitative data by a generalised procrustes analysis (GPA) (Gower 1975; Bramardi 2005).

The present study aims to characterise the pollen morphology of *Cliococca selaginoides* (Lam.) C.M. Rogers & Mildner and Argentinean *Linum* species in order to: (1) provide information on the variability and the characterisation of the pollen morphology of nine species; (2) assess the potential systematic value of the

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pollen features; and (3) provide a key to the species studied based on micromorphological characters of the pollen.

2. Materials and methods

2.1. Plant material

We obtained floral buds for pollen preparations from 2–3 herbarium specimens for each species (specimens deposited at the Córdoba Herbarium (CORD), Corrientes Herbarium (CTES) and the Darwinion Institute Herbarium (SI)). All species studied have homostylous flowers.

2.2. Pollen morphological analyses

Pollen morphology was studied using light microscopy (LM) and scanning electron microscopy (SEM). Pollen grains were acetolyzed according to the procedure of Erdtman (1966) and mounted in glycerine jelly. Pollen samples were deposited in the pollen herbarium of the National University of the Northeast, UNNE (PAL-CTES).

For light microscopy, measurements of 40 grains for each specimen studied were obtained with a Leica DM LB2 light microscope. Different characters were observed in equatorial and polar views (Table 1).

We observed acetolysed grains using the SEM. In order to obtain additional information about sculpture, we obtained microphotographs with a Jeol JSM-580 OLV scanning electron microscope. The pollen grains were mounted on a metal slide and coated in palladium gold. The terminology followed in the description of pollen grains is that of Hesse et al. (2009).

2.3. Statistical analyses

All statistical analyses were performed using the software Infostat (Di Rienzo et al. 2009). We used the Generalized Procrustes Analysis (GPA) to analyse the configurations obtained with Principal Coordinate Analysis (PCoorA) and principal components analysis (PCA), and calculate Euclidean distance and performed the cluster analysis to assess the relative weight of clusters obtained.

Table 1. List of the palynological characters observed in equatorial and polar views with LM.

Code	Character
A	Polar axis (μm)
B	Equatorial axis (μm)
C	Polar/equatorial ratio
D	Exine thickness (μm)
E	Aperture
F	Structure 0: gemmae 1: gemmae and clavae
G	Surface 0: scrabrous or scabrate 1: scrabrous to microgranular 2: fractured

2.4. Specimens examined

Cliococca selaginoides (Lam.) C.M. Rogers & Mildner: ARGENTINA. Córdoba, Dept. Punilla, *A. Hunziker 816* (CORD), PAL-CTES 2519; Dept. Río Cuarto, Punta sur de Sierra de Comechingones, *A. Hunziker* et al. *24409* (CORD), PAL-CTES 2521.

Linum burkartii Mildner: ARGENTINA. Entre Ríos: Dept. Federación, Santa Ana, 8 km north of Federación, *A. Burkart 22615* (SI), PAL-CTES 2520; Dept. La Paz, La Paz, 30° 45′37.2″S, 59°39′27.2″W, *E. Lattar* et al. *1* (CTES) PAL-CTES 2553.

Linum carneum A. St.-Hil.: ARGENTINA. Entre Ríos: Dept. Paraná, Paraná, *M. Muñoz 4255* (SI), PAL-CTES 2548.

Linum catharticum L.: ARGENTINA. Tierra del Fuego: Dept. Ushuaia, *J. Goodall, N. s.n.* (BAB), PAL-CTES 2555.

Linum erigeroides A. St.-Hil.: ARGENTINA. Entre Ríos: Dept. Paraná, Paraná, Cerro Hospice, *A. Burkart 23973* (SI), PAL-CTES 2515; *A. Burkart* et al. *27981* (SI), PAL-CTES 2521.

Linum junceum A.St.-Hil.: ARGENTINA. Corrientes: Dept. Esquina, along the Paraná river, *H. Keller* et al. *6157* (CTES), PAL-CTES 2549.

Linum littorale A. St.-Hil.: ARGENTINA. Corrientes: Dept. Sauce, along the river, *A. Ahumada* et al. *1214* (CTES), PAL-CTES 2550; Dept. Mercedes, on route 123, 50 km of Mercedes, *J. Fernández 1074* (CTES), PAL-CTES 2508; Dept. Curuzú Cuatiá, Ea. Las Marías, *T. M. Pedersen 12539* (CTES), PAL-CTES 2509.

Linum scoparium Griseb. ARGENTINA. Jujuy: Dept. Tumbaya, Tumbaya, *N. Deginani* et al. *378* (SI), PAL-CTES 2551.

Linum usitatissimum L. ARGENTINA. Corrientes: Dept. Mercedes, 3 km north of route 123, *S. Cáceres 38* (CTES), PAL-CTES 2523; on route 123, 40 km southeast of Mercedes, *S. Cáceres 56* (CTES), PAL-CTES 2552; Chaco: Dept. Primero de Mayo, blue flowers grown for the oil industry, *A. G. Schulz 3678* (CTES) 2510. ESPAÑA, Ciudad Real, 10 km east of Tornello, *A. Schinini* et al. *32519* (CTES), PAL-CTES 2522.

3. Results

We present first the general features and then the specific features of the pollen of each species studied.

3.1. General features

Pollen grains 3-zonocolpate, rarely pantoaperturate, released as monads. Isopolar, radiosymmetric, spheroidal, amb circular. Size variable, 25–115 μm in diameter. Colpus short to long, 22.5–100 μm long. Exine 1.5–5.0 μm, sexine thicker than nexine; sculptural elements of gemmae or clavae. Basal pollen surface granulate, scabrous or smooth, sometimes cracked at the base of the elements.

3.2. *Specific features of each species*

Cliococca selaginoides (Plate 1, figures 1, 2)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, suboblate–oblate spheroidal, medium size, P = 24.5 (37.5) 47.5 μm, E = 25 (33.75) 42.5 μm and P/E = 0.86 (1.00) 1.15. Amb circular. Colpus 25–32.5 μm long. Exine 1.5–3.5 μm thick, sexine 1–3 μm and nexine 0.5 μm. With LM, the sculpture consists almost exclusively of gemmae. With SEM, the gemmae range between 0.8 and 2.0 μm in height and between 0.5 and 2.0 μm in diameter. The distal part of this element presents a conical head. The basal surface is very irregular, scabrous to microgranular (Plate 2, figure 1).

Linum burkartii (Plate 1, figures 3, 4)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, prolate–spheroidal, very large, P = 75 (95) 115 μm, E = 75 (95) 115 μm and P/E = 0.69 (0.91) 1.13. Amb circular. Colpus 62.5–100 μm long, with irregular borders. Exine 3–5 μm thick, sexine 2–4 μm and nexine 0.5–1 μm. With LM, gemmate and baculate surface.- With SEM, gemmae 1.08 μm in height and 1.2–1.84 μm in diameter; clavae 0.5–0.8 μm in height and 0.5–0.7 μm in diameter. The distal part of both elements ends in a tip. In some grains, there are connections between the elements. The basal surface is finely scratched (Plate 2, figures 2, 3).

Observations. Although the presence of heterostyly in *L. burkartii* was mentioned by Bacigalupo (2005), in the specimen Lattar et al. 1, all individuals of the population were homostilylous and the relationship between styles and stamens in flowers or floral buds matches plate 24 of *L. burkartii* of Bacigalupo (2005). The presence of heterostyly in this species is thus doubtful.

Linum carneum (Plate 1, figures 5, 6)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, suboblate–spheroidal, medium–large size, P = 55 (60.25) 65.5 μm, E = 60 (63.75) 67.5 μm and P/E = 0.88 (0.94) 1.00. Amb circular. Colpus 30–42.5 μm long, with irregular borders. Exine 2.5–4 μm thick, sexine 2–3 μm and nexine 0.5–1 μm. With LM, gemmate and baculate surface. With SEM, gemmae 1.05–1.08 μm in height and 1.63–2.37 μm in diameter; clavae 0.8–0.9 in height and 0.51–0.68 in diameter. The distal part of both elements ends in a tip. The basal surface is finely scratched (Plate 2, figures 4, 5).

Linum catharticum (Plate 1, figures 7, 8)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, suboblate–oblate-spheroidal, large size, P = 37.5 (43.75) 50 μm, E = 40 (45) 50 μm and P/E = 0.85 (0.92) 1.00. Amb circular. Colpus 17.5–32.5 μm long, with irregular borders. Exine 1.5–2 μm thick, sexine 1 μm and nexine 0.5–1 μm. In LM, gemmate surface. In SEM, gemmae 1.13 μm in height and 0.8–0.9 μm in diameter, these ones apically microechinate (0.2 μm in height). The basal surface is scratched (Plate 2, figures 6, 7).

Linum erigeroides (Plate 1, figures 9, 11)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, prolate–spheroidal, very large size, P = 67.5 (86.25) 105 μm, E = 67.5 (86.25) 105 μm and P/E = 1.00 (1.04) 1.08. Amb circular. Colpus 47.5–87.5 μm long, with irregular borders. Exine 3–4 μm thick, sexine 2–3 μm and nexine 1 μm. With LM, gemmate and baculate surface. With SEM, gemmae 1.05–1.8 μm in height and 1.63–2.37 μm in diameter. The distal part of both elements ends in a tip (Plate 2, figures 8–10).

Linum junceum (Plate 1, figures 12, 13)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, oblate-spheroidal, large size, P = 62.5 (71.25) 80 μm, E = 57.5 (68.5) 80 μm and P/E = 1.00 (1.08) 1.16. Amb circular. Colpus 40–50 μm long, with irregular borders. Exine 1.5–3 μm thick, sexine 1–2 μm and nexine 0.5–1 μm. With LM, gemmate and baculate surface. With SEM, gemmae 1.3–2.08 μm in height and 0.8–2.04 μm in diameter; clavae 0.6–0.8 μm in height and 0.6–0.8 μm in diameter. The distal part of both elements ends in a tip. The basal surface around each element is broken (Plate 2, figures 11, 12).

Linum littorale (Plate 1, figures 14, 15)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, prolate–spheroidal, large size, P = 62.5 (72.5) 82.5 μm, E = 62.5 (71.25) 80 μm and P/E = 0.88 (0.97) 1.06. Amb circular. Colpus long 37.5–62.5 μm long, with irregular borders. Exine 2.5–5 μm thick, sexine 2–5 μm and nexine 0.5–1 μm. With LM, gemmate and baculate surface. With SEM, gemmae 1.3–1.98 μm in height and 0.7–1.94 μm in diameter; clavae 0.6 μm in height and 0.6–0.7 μm in diameter. The distal part of both elements ends in a tip. In some pollen grains there are connections between the elements. The basal surface is fractured around each element (Plate 3, figures 1–3).

Linum scoparium (Plate 1, figures 16–18)
Pollen grains 3-zonocolpate, isopolar, radiosymmetric, oblate–spheroidal, medium size, P = 50 (57.5) 65 μm, E = 55 (61.25) 67.5 μm and P/E = 0.86 (0.93) 1.00. Amb circular. Colpus 42.5–50 μm long, with irregular borders. Exine 3–3.5 μm thick, sexine 2–3 μm and nexine 0.5–1 μm. With LM, gemmate and baculate surface. With SEM, gemmae 1.05–-1.42 μm in height and 0.97–1.26 μm in diameter; clavae 0.4–0.6 μm in diameter. The basal surface is fractured around each element (Plate 3, figures 4, 5).

Linum usitatissimum (Plate 1, figures 19, 20)

Pollen grains 3-zonocolpate, isopolar, radiosymmetric, oblate–spheroidal, medium size, P = 50 (56.25) 62.5 μm, E = 47.5 (55) 62.5 μm and P/E = 0.47 (0.78) 1.09. Amb circular. Colpus 22.5–47.5 μm long, with irregular borders. Exine 3–3.5 μm thick, sexine 2–3 μm and nexine 1–2 μm. With LM, gemmate surface. With SEM, exine with sculptural processes partially fused. Gemmae 0.8–1.17 μm in height and 0.9–1.05 μm in diameter; clavae 0.4–0.5 μm in height and 0.4–0.6 μm in diameter. The distal part of both elements are microechinate, radially striated. The basal surface is fractured around each element (Plate 3, figures 6–8).

3.3. Statistical analyses

The consensus configuration based on both qualitative and quantitative characters is presented in Figure 1, in which the first two components are responsible for 62.7.0% and 37.3% of the total variation, respectively. The GPA clearly identified four distinct groups: Group 1: conformed by *Linum burkartii* (LB), *Linum carneum* (LC), *Linum erigeroides* (LE) and *Linum scoparium* (LS); Group 2: conformed by *Linum junceum* (LJ), *Linum littorale* (LL) and *Linum usitatissimum* (LU); Group 3: conformed by *Linum catharticum* (LCA); and Group 4: conformed by *Cliococca selaginoides* (CS). The two types of characters, qualitative and quantitative, make a different contribution to the consensus configuration. As regards the qualitative characters, the aperture type and exine surface were the most important. Regarding the quantitative characters, the grain size and the thickness of the exine were the most significant variables.

To study the behavior of the first two axes obtained from the consensus configuration, an Unweighted Pair Group Method using Arithmetic averages (UPGMA) dendrogram was determined using Euclidean distance between points of the two-dimensional space (Figure 1). The dendrogram clearly showed the grouping of the species with similar behavior, according to the previous description with the GPA (Figure 2).

4. Discussion

4.1. Pollen morphology

The pollen morphology of nine species belonging to the genera *Cliococca* and *Linum* of Argentina is presented here. In addition, *L. burkartii*, *L. junceum* and *L. littorale* are analysed for the first time. The analysis allowed us to create a key to the identification of the species of *Cliococca* and *Linum*, based on the exine structure and the presence of sculptural elements. The exine type, the main palynological characters used in this key, allowed us to identify the following pollen types: *Cliococca selaginoides* and *Linum burkartii* (Table 2). The first type includes only *C. selaginoides*, in which the exine consists exclusively of gemma and the basal surface is densely granulate. Our results are in accord with those of Rogers (1985) who described the pollen of this species as 3-zonocolpate, a common character in some species of *Linum*, although the exine ornamentation is very different from the known species of this genus. Rogers (1985) interpreted the exine as more or less papillate with various-sized processes, the larger irregularly conical, without defining them with a precise term. Moreover, *C. selaginoides* differs from the species of the genus *Linum* by presenting the following morphological and reproductive characters: perennial herb with dense foliage, solitary flowers (which occur rarely in the rest of the species of Linoideae) and fruit with the shape of an indehiscent capsule (Rogers and Mildner 1971; Rogers and Smith 1975; Bacigalupo 2005). The South American monotypic genus *Cliococca* (Linaceae, Linoideae) was separated from *Linum* based on morphological and palynological features, but recent molecular phylogenetic analyses of the subfamily Linoideae placed it as a sister of a clade of South American *Linum* species (McDill et al. 2009). Our results indicate that *Cliococca* should be separated from the South American species of *Linum*.

The *Linum* type includes the remaining species studied here, with an exine of gemmae and clavae with the basal surface smooth, scabrous or sometimes perforate. Based on the sculptural elements, four pollen subtypes are distinguished within this type. *L. junceum*, *L. scoparium*, *L. catharticum* and *L. usitatissimum* subtypes are different in terms of morphology, spatial density and differentiation grade of sculptural elements. *Linum burkartii*, *L. carneum*, *L. erigeroides*, *L. junceum*, *L. littorale*, *L. scoparium* and *L. usitatissimum* have heteromorphic sculptural processes, gemmae and clavae. For *L. carneum*, *L. erigeroides* and *L. junceum*, these processes are clearly differentiated, whereas for *L. burkartii*, *L. littorale* and *L. scoparium*, these processes are poorly differentiated (although in *L. scoparium* the variation between them is continuous).

Punt and Den Breejen (1981) analysed pollen of the species of Linaceae for the flora of northwest Europe

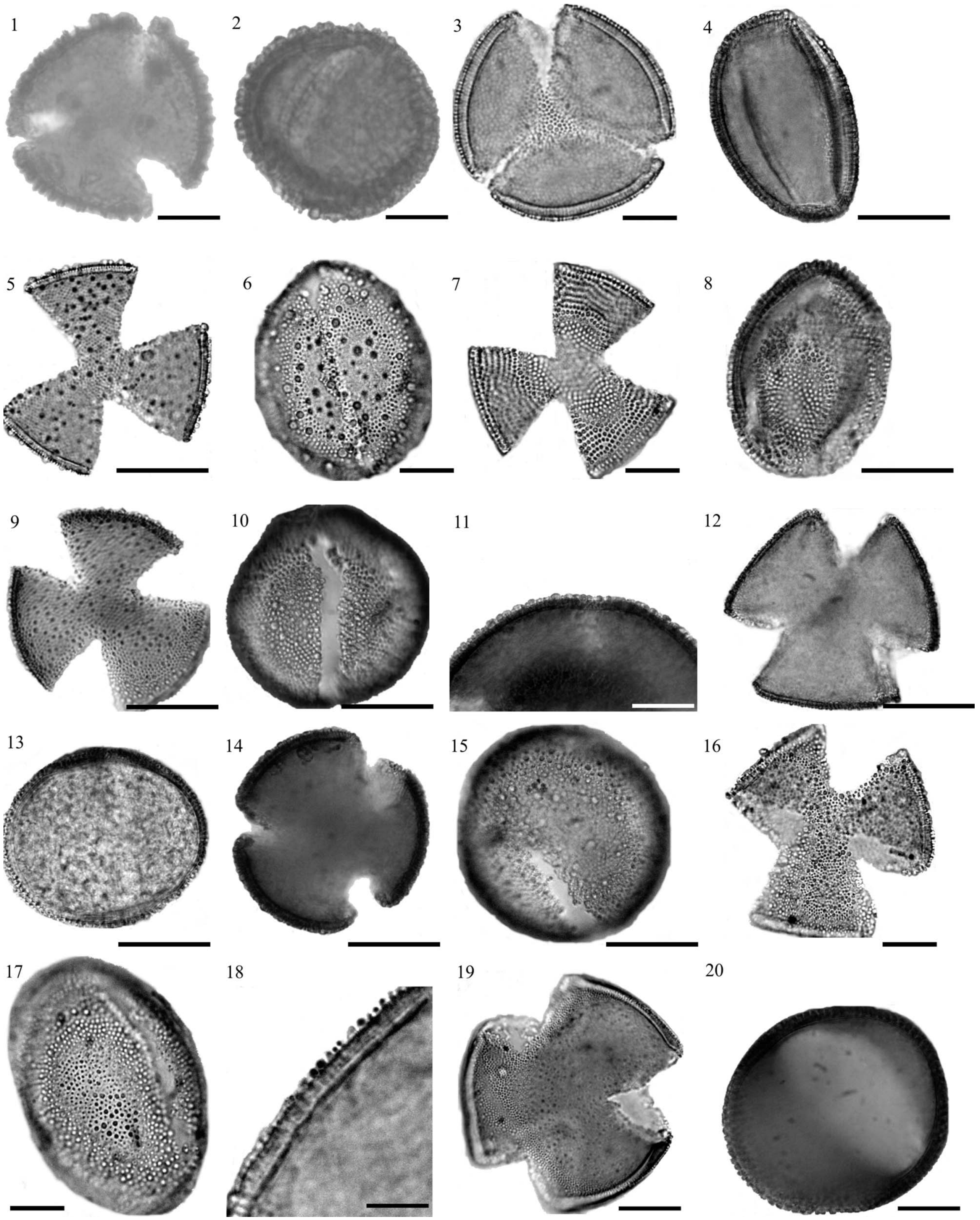

Table 2. Key to pollen types and subtypes of *Cliococca* and *Linum* species from Argentina.

Exine with gemmae and basal surface densely granulate	Type Cliococca: *Cliococca selaginoides*
Exine with gemmae and clavae, and basal surface smooth or scabrous, sometimes perforated	Type Linum
Homomorphic sculptural elements (gemmae)	Subtype Linum catharticum: *L. catharticum*
Sexine with heteromorphic sculptural elements (gemmae and clavae)	
Sculptural elements often fused together	Subtype Linum usitatissimum: *L. usitatissimum*
Sculptural elements never fused together	
Elements clearly differentiated	Subtype Linum junceum: *L. junceum, L. carneum* and *L. erigeroides*
Elements little differentiated, but the variation is continuos in both elements	Subtype Linum scoparium: *L. scoparium, L. burkartii* and *L. littorale*

and recognised five types: *Linum austriacum*, *L. catharticum*, *L. perenne*, *L. usitatissimum* and *Radiola linoides* based on the pollen aperture type, space between the sexine elements, grain size, colpus margin and whether the endocolpus was distinctly visible or indistinct. These authors observed that the pollen of *L. catharticum* is 3-zonocolpate, the sexine varies from thinner than the nexine to distinctly thicker than the nexine and the sculpture is represented by gemmae of different sizes. In this contribution, the presence of homomorphic sculptural processes consisting of small gemmae which are apically microechinate is described for the first time. This species presents a unique sculpture pattern, clearly distinguished from other species of *Linum* studied here. These features support the taxonomic position of *L. catharticum*, which belongs to Sect. *Cathartolinum*, within the clade of yellow flowers, as the only monotypic section of *Linum* (McDill et al. 2009).

The *L. usitatissimum* subtype is characterised by a discontinuous tectum and the colpus with irregular borders. The sexine consists of densely arranged gemmae often fused together. Our observations agree with the description given by Erdtman (1966), who described it as 3-zonocolpate. In relation to the exine, our results disagree with the interpretation of Punt and Den Breejen (1981) and Candau (1987). Based on European populations of *L. usitatissimum*, the first authors described the sexine as '*tectate scabrate*' and the tectum with the heads of the sculptural elements was very close, and possibly overlapped or fused. These authors recognised two types of sculptural elements depending on their size, and they considered the tectum as continuous. On the other hand, Candau (1987) discovered that the *L. usitatissimum* type has a well-developed tectum, with more or less regular elements densely arranged. In the specimens of *L. usitatissimum* studied here, the heads of the sculptural elements are fused only occasionally.

Regarding the stratification of the exine in *Linum*, we observed that it is thicker than the nexine in the species studied. This result agrees with those obtained by Saad (1961a, 1961b, 1962), although in colpate grains this author distinguished a wall composed of three layers: the exine comprising the sexine and the nexine and a middle layer or medine between the exine and the intine. Our observations suggest that the exine of *Linum burkartii* and *L. usitatissimum* is constituted by a sexine composed of sculptural elements (gemmae and clavae), a loose basal layer and a thin nexine.

In relation to the exine processes, our results agree with the evolutionary tendency of 'the sexinous processes tending to be dimorphic' proposed by Saad (1961b). However, we disagree with the tendency that 'the sexine shows a transition from a primitive granular state to the formation of a tegillum supported by bacula' proposed by Saad (1961b, 1962). In the species studied here, the exine can be formed of gemmae and clavae, or only gemmae. Recently, Medeiros Carreiras et al. (2005) analysed the pollen of other species of Linaceae belonging to the genera *Hebepetalum* Benth., *Ochthocosmus* Benth. and *Roucheria* L. and found differences in the aperture type and the exine surface. These species of *Roucheria* and *Hebepetalum* have 3-zonocolpate grains, but differ in the exine surface

Plate 1. Micrographs of acetolysed pollen grains (LM). Figure 1, 2. Pollen type Cliococca. Figure 1. *Cliococca selaginoides*: pollen grain polar view. Figure 2. *Cliococca selaginoides*: pollen grain equatorial view. Figure 3–20. Pollen type Linum. Figure 3. *Linum burkartii*: pollen grain polar view. Figure 4. *L. burkartii*: pollen grain equatorial view. Figure 5. *L. carneum*: pollen grain polar view. Figure 6. *L. carneum*: pollen grain equatorial view. Figure 7. *L. catharticum*: pollen grain polar view. Figure 8. *L. catharticum*: pollen grain equatorial view. Figure 9. *L. erigeroides*: pollen grain polar view. Figure 10. *L. erigeroides*: pollen grain equatorial view. Figure 11. *L. erigeroides*: polar view, cross-section, detail of gemmae and clavae. Figure 12. *L. junceum*: pollen grain polar view. Figure 13. *L. junceum*: pollen grain equatorial view. Figure 14. *L. littorale*: pollen grain polar view. Figure 15. *L. littorale*: pollen grain equatorial view. Figure 16. *L. scoparium*: pollen grain polar view. Figure 17. *L. scoparium*: pollen grain equatorial view. Figure 18. *L. scoparium*: polar view, cross-section, detail of gemmae and clavae. Figure 19. *L. usitatissimum*: pollen grain polar view. Figure 20. *L. usitatissimum*: pollen grain equatorial view. Scale bars: 10 μm (1, 2, 11, 18–20); 20 μm (3, 6, 7, 17); 50 μm (5, 8–10, 12–15).

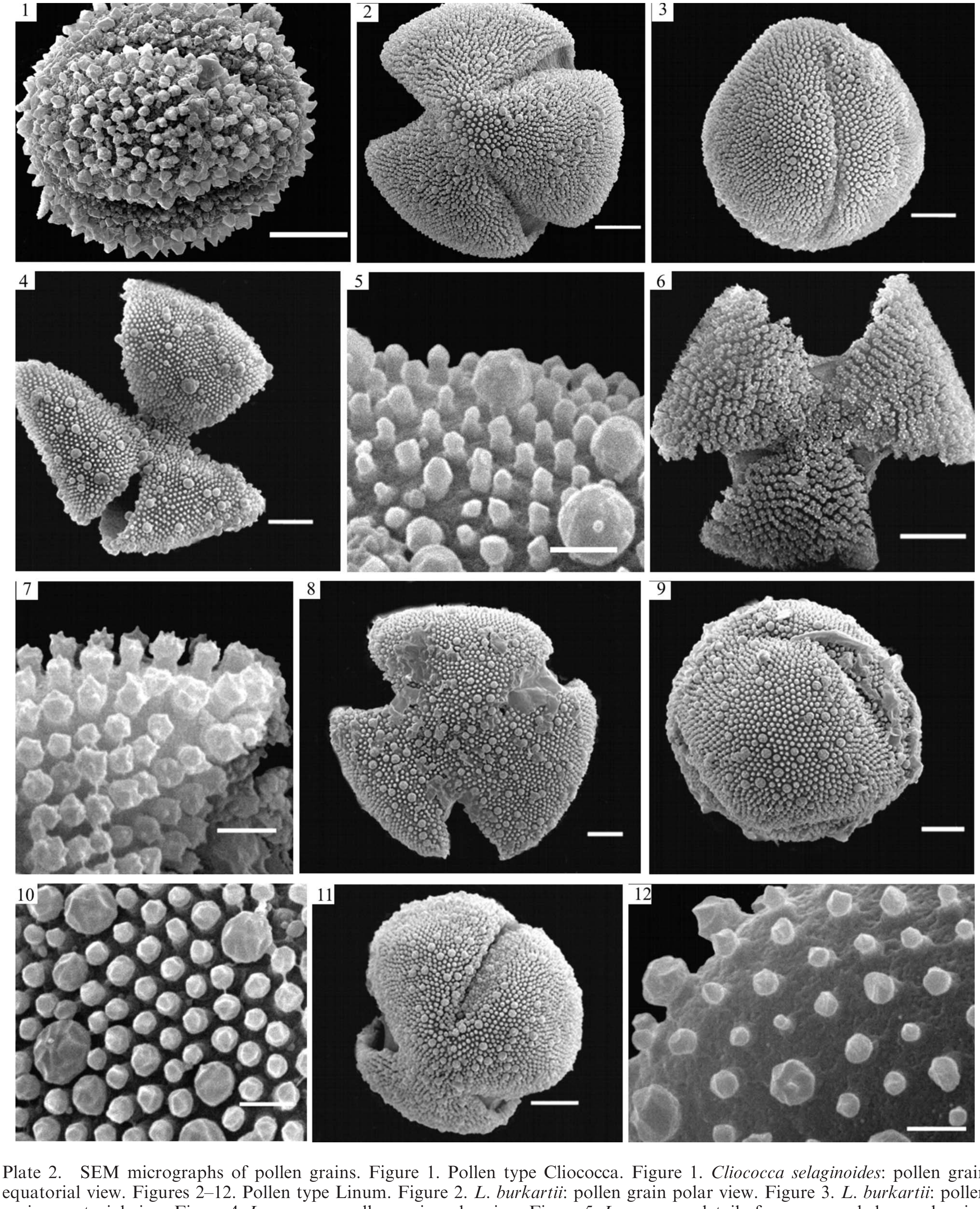

Plate 2. SEM micrographs of pollen grains. Figure 1. Pollen type Cliococca. Figure 1. *Cliococca selaginoides*: pollen grain equatorial view. Figures 2–12. Pollen type Linum. Figure 2. *L. burkartii*: pollen grain polar view. Figure 3. *L. burkartii*: pollen grain equatorial view. Figure 4. *L. carneum*: pollen grain polar view. Figure 5. *L. carneum*: detail of gemmae and clavae, showing the difference in their size. Figure 6. *L. catharticum*: pollen grain polar view. Figure 7. *L. catharticum*: detail of small gemmae apically microechinate. Figure 8. *L. erigeroides*: pollen grain polar view. Figure 9. *L. erigeroides*: pollen grain equatorial view. Figure 10. *L. erigeroides*: detail of sculpture. Figure 11. *L. junceum*: pollen grain polar view. Figure 12. *L. junceum*: gemmae and clavae ornamentation clearly differentiated. Scale bars: 15 μm (1); 10 μm (2–4, 6, 8, 9); 5 μm (5, 7, 10, 12).

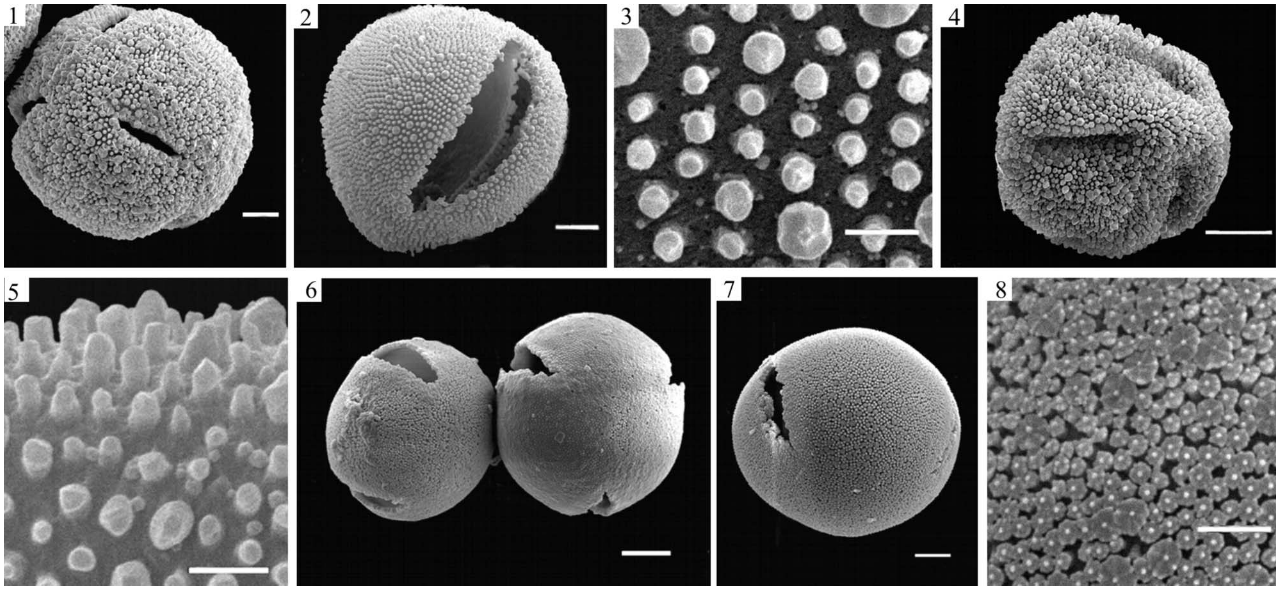

Plate 3. SEM micrographs of pollen grains. Pollen type Linum. Figure 1. *Linum littorale*: pollen grain in subpolar view. Figure 2. *L. littorale*: pollen grain in equatorial view. Figure 3. *L. littorale*: gemmae and clavae ornamentation. Figure 4. *L. scoparium*: pollen grain in subpolar view. Figure 5. *L. scoparium*: detail of gemmae and clavae. Figure 6. *L. usitatissimum*: two pollen grains in polar view. Figure 7. *L. usitatissimum*: pollen grain in equatorial view. Figure 8. *L. usitatissimum*: detail of gemmae and clavae ornamentation. Scale bars: 10 μm (1, 2, 6–7); 5 μm (3, 8) or 15 μm (4).

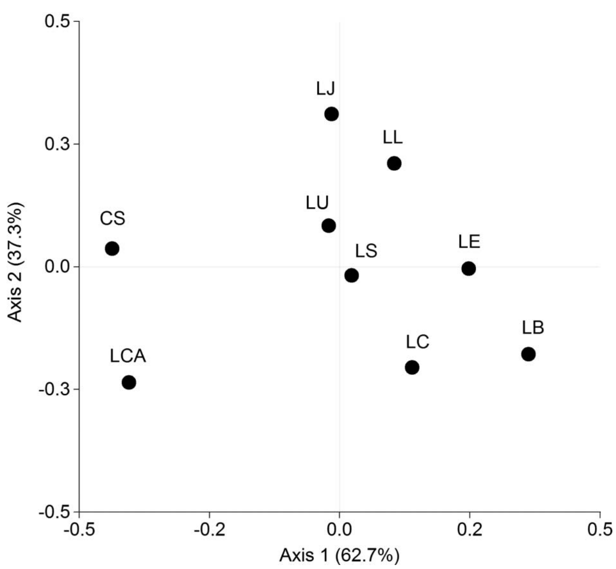

Figure 1. Consensus configuration

which is spinulose in the former and psilate in the latter. On the other hand, the species of *Ochthocosmus* have 3-zonocolporate grains with a microreticulate surface. These authors made no reference to the presence of a tectum, but we can infer through the photomicrographs that *Hebepetalum* shares the presence of homomorphic elements with *C. selaginonoides* and *L. catharticum*, while the other two genera would be tectate in *Roucheria* or semitectate in *Ochthocosmus*.

4.2. Heterostyly evolution in Linum

Historically, the genus *Linum* has been studied because of its mechanism of heterostyly in many species (Darwin 1864). In a macroevolutionary context, the presence of heterostyly has been considered as an ancestral condition in this genus (Rogers 1982). The heterostylous species are confined to the Old World and are prevalent in the Mediterranean region, although they are also found in Western Asia. Heterostylous species have been observed in other genera of the subfamilies Hugonioideae and Linoideae (Xavier et al. 1980; Rogers 1982; McDill et al. 2009). In this paper, the species analysed are homostylous. These data support the observations of Rogers (1982), who found that homostylous species have a cosmopolitan distribution but are mainly present in the New World.

4.3. Statistical analyses

The joint characterisation provided by the GPA, based on both qualitative and quantitative variables, revealed the importance of the similarity between the different types of variables analysed to group individuals with similar characteristics (Bramardi et al. 2005). This analysis allowed us to define four groups of species, based on the size of pollen grains and exine thickness.

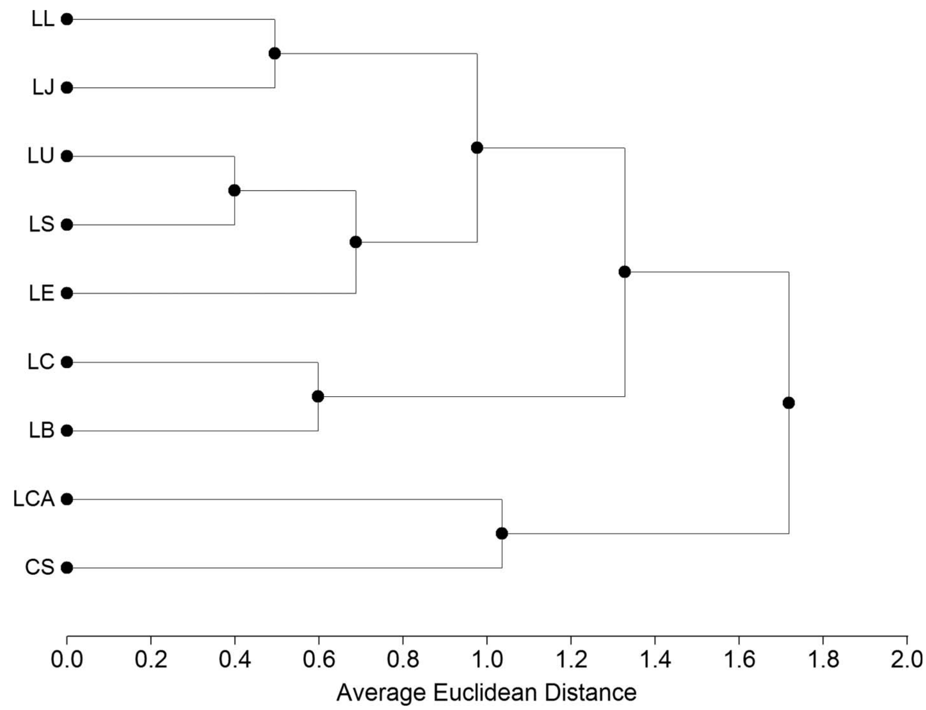

Figure 2. Phenogram showing the similarity between the taxa of *Cliococca* and *Linum* and its allies (Cophenetic Correlation Coefficient = 0.783. Taxa acronyms: CL: *Cliococca selaginoides*; LB: *L. burkartii*; LC: *L. carneum*; LCA: *L. catharticum*; LE: *L. erigeroides*; LJ: *L. junceum*; LL: *L. littorale*; LS: *L. scoparium*; LU: *L. usitatissimum*.

4.4. Taxonomy

The characters mentioned above, along with the aperture type, sculpture type and degree of distribution of the sculptural elements used in the pollen key, are considered the main palynological characters of taxonomic value for Linaceae, in agreement with the contributions of Saad (1961a, 1961b), Xavier et al. (1980) and Dulberger (1981) for the family. Together with the palynological descriptions, our statistical results are a contribution to a better understanding of the relationships between the species within Linaceae.

5. Conclusions

The exine type allowed us to identify two pollen types: Type Cliococca selaginoides and Type Linum. The first type includes only *C. selaginoides*, in which the exine consists exclusively of gemmae and the basal surface is densely granulate. The second type includes all species of *Linum*; the exine is composed of gemmae and clavae with the basal surface smooth, scabrous or sometimes perforate. The Generalized Procustes Analysis (GPA) allowed us to define groups of species according to the joint variability of the size of pollen grains and exine thickness. These results support the view that pollen characters are important for understanding interspecific relationships within the Linaceae.

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