

Pollen aperture heteromorphism in *Centaurium pulchellum* (Gentianaceae)

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Abstract

Pollen variations involving the number and position of the apertures are relatively frequent in certain members of Gentianaceae. In this paper the high incidence of pollen variation in *Centaurium pulchellum* (Sw.) Druce is described. Two other species of *Centaurium*: *C. ameghinoi* (Speg.) Druce and *C. cachanlahuen* (Molina) B. L. Rob were also examined. These two species have 3-colporate or, less frequently, 4-colporate pollen grains, which are the two basic aperture arrangements. However, there are also a variable proportion of atypical grains. Apart from typical 3-zonacolporate grains, *C. ameghinoi* sometimes has 3-syncolpate or parasyncolpate pollen, while *C. cachanlahuen* occasionally has 4-colporate grains with short colpi that converge in pairs. *C. pulchellum* also has 3 or 4-colporate grains, but a high proportion of the pollen grains show a range of different aperture patterns. A total of 13 aperture configurations, including the typical 3-colporate and 4-colporate types, were recorded. The proportion of the different pollen types varied widely between and within individuals. However, the production of atypical grains does not seem to have any notable effect on pollen viability within the species. It is suggested that the wide variation in aperture pattern observed in *C. pulchellum* may be partially induced by environmental factors.

Keywords: Variation, configuration, trizonacolporate, tetrazonacolporate, pollen fertility, syncolpate, colpoid furrows

Pollen morphological variability is a surprisingly frequent phenomenon in angiosperms. Variation in the number and arrangement of apertures appears to be the most common type of variation, especially within the eudicots, as for example in the Fabaceae (Ferguson, 1980; Pardo, 1990), Gentianaceae (Nilsson, 1975; Punt & Nienhuis, 1976; Rao & Chinnappa, 1983a), Clusiaceae (Clarke, 1975), Krameriaceae (Pozhidaev, 2002), Solanaceae (Till-Bottraud et al., 1995; Ressayre et al., 2002) and Violaceae (Dajoz et al., 1993; Till-Bottraud et al., 1999). Pollen diversity can occur between individuals of a population (polymorphism) or within the same plant (heteromorphism), although the latter constitutes the most frequent type of variation (Mignot et al., 1994).

Aperture heteromorphism is common in some species of the genus *Centaurium* Hill. (Gentianaceae), where it has been described in eight

of the 12 species for which pollen has been studied (Erdtman et al., 1961; Nilsson, 1975; Punt & Nienhuis, 1976; Rao & Chinnappa, 1983a; Díez, 1987; Makino, 1991). *Centaurium* comprises approximately 40 species, mostly distributed in the Northern Hemisphere, but a few are endemic to Australia or South America. The basic pollen type for the genus is 3-(4) zonacolporate, although pollen grains showing variation in the number and disposition of the apertures are also frequent (Erdtman et al., 1961). Punt and Nienhuis (1976) found 4-pantocolporate and, occasionally, syncolporate grains in three *Centaurium* species, which have the same basic pollen type as *C. pulchellum*. Similarly, Díez (1987) determined the occurrence of 6-pantocolporate (3 × 3) pollen forms in *C. erythraea* Rafin., *C. maritimum* (L.) Fritsch. and *C. spicatum* (L.) Fritsch., which also have the same basic pollen type as *C. pulchellum*. A detailed analysis of the

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apertural variation in the pollen of *Centaurium* was carried out by Rao and Chinnappa (1983a). They described the aperture configurations in the pollen of *C. exaltatum* (Griseb.) Wright, *C. littorale* (Turner) Gilmour and *C. pulchellum* (Sw.) Druce. All three species showed pantocolporate grains apparently derived from 3-zonacolate forms in highly variable frequencies ranging from 5 to 69%. In addition to the 3-zonacolate and pantocolporate pollen grains, these species had various intermediate forms although in lesser proportion (Rao & Chinnappa, 1983a).

During a general analysis of Gentianaceae pollen (Pire & Dematteis, 2006), we found higher aperture heteromorphism in *C. pulchellum* than previously reported for this species. This Old World species is a common weed in South America and is widely distributed in Argentina, where it is found in low moist soils of disturbed habitats. The specimens we studied from north-west and central Argentina have, in addition to the two basic pollen types, a notable proportion of less common aperture configurations which are described. The pollen of *Centaurium ameghinoi* and of *C. cachanlahuen*, two species endemic to Patagonia, has not been described previously.

Material and methods

Eight collections selected from different populations of *Centaurium pulchellum* were examined to determine the pollen variation within the species. In addition, two other *Centaurium* species from Argentina were analysed for comparison.

Pollen samples were obtained from flower buds of herbarium specimens taken from the Instituto de Botánica del Nordeste (CTES) and Instituto de Botánica Darwinion (SI). For light microscopy (LM), the pollen was acetolysed according to Erdtman (1966) and mounted in glycerol-jelly. Pollen observations and measurements were carried out using a Leitz Ortholux microscope. Permanent light microscope slides were deposited at the Palynological Laboratory of the Universidad Nacional del Nordeste (PAL-CTES) and are available for consultation.

For scanning electron microscope (SEM) observations, the acetolysed material was sputter coated with gold-palladium, although in some specimens

the pollen grains of each anther were also examined unacetolysed to determine possible modification in the pollen size. The SEM preparations were examined using the Jeol JSM-5800 LV microscope in the Universidad Nacional del Nordeste.

For each specimen the polar axis (P), equatorial diameter (E), exine thickness, length and width of the apertures (ectoapertures and endoapertures) and apocolpium diameter were measured. These measurements were based on at least 30 pollen grains for each sample.

To evaluate the possible occurrence of differences in size between 3-4 colporate and pantocolporate grains we made supplementary measurements of the diameter of each pollen grain. Pollen fertility was established by observation of 800–1 000 grains of each sample using the acetic carmine-glycerol technique of Marks (1954).

Terminology

The general terminology applied for the pollen descriptions is mainly based on Erdtman et al. (1961), with modifications of Kremp (1965) and Punt et al. (1994). The term colpoid is used in reference to apertures similar to colpi, but less clearly defined in outline (Erdtman, 1952).

Results

Pollen morphology

The grains are isopolar and radially symmetrical; 3-zonacolate or, less frequently, 4-zonacolate (*C. pulchellum*); oblate spheroidal to prolate, small to average (Table I). The colpi are relatively long in relation to the polar length of grain, meridional, with acute apices; endoapertures almost circular, 1.5–3.0 μm in diameter or, in some cases, with H-shaped endoapertures which have short lateral extensions in *C. pulchellum*. Exine 1.5–2.5 μm thick; sexine similar in thickness to twice as thick as the nexine; sexine notably attenuate towards the colpus margo, while in the same region the nexine thickens to form costate endocolpi, ranging from 1.5 to 2.8 μm wide in the equatorial zone. Tectum perforate, striate, striae narrow; infractum with uniformly distributed columellae.

Table I. Morphological data for 3-4-colporate pollen in *Centaurium ameghinoi*, *C. cachanlahuen* and *C. pulchellum*.

| Species | Colpi number | P (μm) | E (μm) | P/E ratio | Exine thickness (μm) | Margin (μm) |
|------------------------|--------------|---------------------|---------------------|------------------|-----------------------------------|--------------------------|
| <i>C. ameghinoi</i> | 3 | 29 (31.0) 35 | 18 (22) 23 | 1.14 (1.40) 1.80 | 2.0–2.5 | 2.8 |
| <i>C. cachanlahuen</i> | 3 | 28 (30.5) 34 | 19.8 (25) 28 | 1.12 (1.28) 1.40 | 2.0–2.5 | 2.5 |
| <i>C. pulchellum</i> | 3 (4) | 17 (21.7) 28 | 16 (20) 23 | 0.90 (1.08) 1.34 | 1.5–2.0 | 1.5 |

In *C. ameghinoi* (Figure 2A) and *C. cachanlahuen* (Figure 2B, C) the pollen grains are prolate spherical to prolate, the endoapertures are lalongate and lack lateral extensions, the muri (lirae) are short, varying in length between 2–10 μm , disposed in different directions but frequently inter-crossed. While in *C. pulchellum* (Figure 2D) the muri are longer and extending almost from one pole to the other, frequently the muri are grouped at differing angles, but each group shows an almost parallel and ordered disposition.

Variants to apertural system (Figures 1–4)

Centaurium pulchellum has isopolar, 3- or 4-zonacolporate grains (Figures 2D, 3A, 4A, G) typical for the genus, but notable variation in aperture configuration between other pollen grains also occurs (Figures 2E–F, 3A–D). Within single anthers we observed different proportions of irregular forms representing several modifications of the basic aperture system (Figures 2E–1F). The most frequent modifications were syncolpy, and an increase in aperture number, with a tendency towards a more global distribution of the apertures, this is also associated with shorter ectoapertures (Figures 2E, 3E). Some grains show displacement of the endoapertures towards one end of the colpus while others reveal S-shaped or obliquely disposed ectoapertures. Modification of the apertures often results in heteropolarity, and occasionally, asymmetry of the grains.

Taking into account the number and disposition of the ectoapertures we recognised 13 variants in aperture configuration (Figure 4Ai–F, H–O), which were ordered according to the increasing complexity of the apertural system in the following categories:

Typical tricolporate (Figures 2D, 4A)

Pollen isopolar, 3-colporate; colpi long, perpendicularly disposed relative to the equator, apices of colpi free; P=17 (22) 28 μm , E=16 (20) 23 μm ; shape oblate spheroidal – prolate (P/E=0.91 (1.10) 1.37).

Variants on typical tricolporate

Type 1 (Figure 4Ai). Grains 3-colporate, one colpus disposed diagonally relative to the equator; colpi similar in length or having the oblique colpus of lesser size; P=20 (22.5) 26 μm , E=16.8 (20) 24.7 μm ; prolate spheroidal – subprolate [P/E=1.05 (1.12) 1.29].

Type 2a (Figures 2E, 4Aiiia). Grains 3-syncolporate, colpi merged at one or both poles; P=18.5 (21)

22.5 μm ; E=16 (19.8) 23 μm ; oblate spheroidal – subprolate (P/E=0.97 (1.06) 1.15).

Type 2b (Figure 4Aiiib). 3-syncolporate grains, with oblique colpi joined at the apices (mesocolpia curved in S-shape); P=18 (20.3) 22 μm , E=16 (20.1) 24 μm ; oblate spheroidal – subprolate (P/E=0.91 (1.00) 1.20)

Type 3 (Figures 2E, 4Aiii). 6-pantocolp(or)ate grains [3+3]; the three meridional colpi provided with endoapertures, sometimes totally or partially merged at one pole; opposite pole connected to three short colpi or colpoid furrows (with or without endoaperture), which are connected, and form a triangular area; P=21 (22.5) 26 μm , E=19 (21.9) 27 μm ; oblate spheroidal – prolate-spheroidal (P/E=0.96 (1.02) 1.14).

Type 4 (Figures 4Aiv). 9-pantosyncolpate grains [3+3+3]; the three meridional colpi joined at each pole to three colpoid furrows which are connected and form triangular areas; P=22 (24.2) 26 μm , E=20 (25) 25.6 μm ; oblate spheroidal – prolate spheroidal (P/E=0.91 (0.97) 1.01).

Typical tetracolporate (Figures 3A, 4B)

Pollen 4-colporate; colpi long, disposed perpendicular to the equator, apices free; P=21 (22.8) 25.2 μm , E=15.5 (20.3) 25.8 μm ; oblate spheroidal – prolate (P/E=0.92 (1.12) 1.35).

Variants on typical tetracolporate

Type 5 (Figure 4Bi). 4-colporate grains with two oblique colpi; usually with two long colpi disposed perpendicular to the equator and two short colpi disposed diagonal to the equator; apices free; P=19 (21.6) 28 μm , E=15.5 (19) 23 μm ; oblate spherical – prolate (P/E=0.99 (1.13) 1.35).

Type 6 (Figure 4Bii). 4-syncolporate grains, with four colpi joined at their apices to one or both poles; P=19.6 (20.8) 22.4 μm , E=17.5 (18.6) 19.2 μm ; prolate – spheroidal – subprolate (P/E=1.03 (1.12) 1.20).

Type 7a (Figures 3B, D, 4Biiia). 4-syncolporate grains [2+2], adjacent pairs of colpi fused, delimiting a tennis ball-like figure around the grain, which avoids the endoapertures; P=22 (23.9) 25.6 μm , E=21 (23.6) 25 μm ; oblate spheroidal – prolate spheroidal (P/E=0.96 (1.01) 1.05).

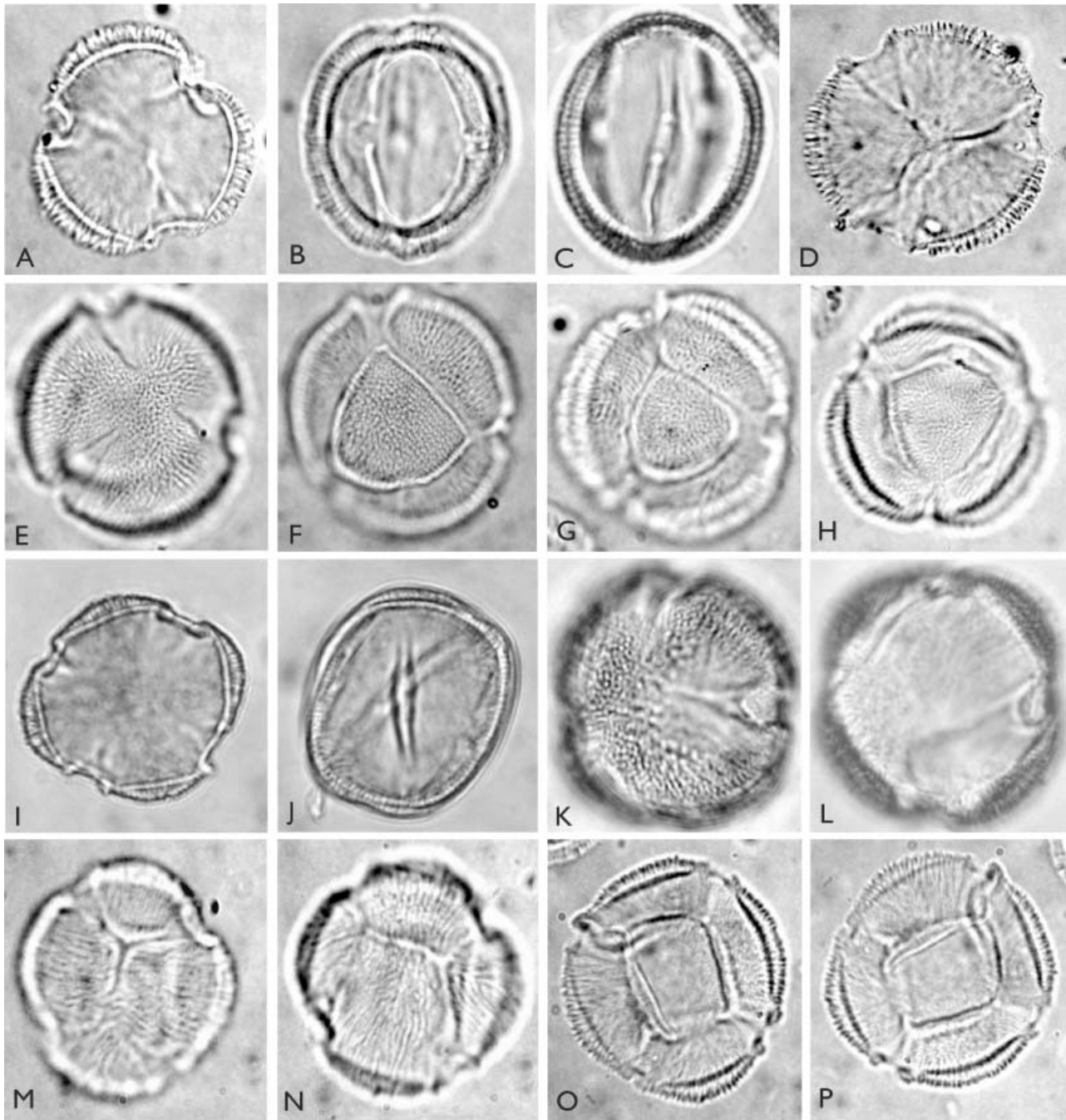


Figure 1. LM of typical and atypical pollen grains of *Centaurium pulchellum*: (A) typical 3-colporate grain, polar view; (B) typical 3-colporate grain, equatorial view; (C) type 1 grain, equatorial view; (D) type 2 grain, polar view; (E–F) type 3 grain, polar view, upper (E) and lower foci (F); (G–H) type 4 grain, polar view, upper (G) and lower foci (H); (I) typical 4-colporate grain, cross section; (J) type 5 grain, equatorial view; (K–L) type 7 grains, polar view, upper (K) and lower foci (L); (M–N) type 10 grains, pantocolporate, upper (M) and lower foci (N); (O–P) type 11 grains, pantocolporate, upper (O) and lower foci (P). Scale=10 μm .

Type 7b (Figure 4Biiib). The four colpi are combined 2+2 on one pole, while on the other pole the apices are free; P=22 (24.3) 26 μm , E=21 (23.8) 26.6 μm ; oblate spheroidal – subprolate (P/E=0.92 (1.02) 1.19).

Type 8 (Figure 4Biv). 4-hemiparasyncolpate grains; close to one pole the apices of colpi are linked by

arcs, and a small quadrangular apocolpial field is delimited; on the opposing pole the apices are free; P=21 (21.9) 25 μm , E=17.5 (19.2) 23 μm ; prolate spheroidal – subprolate (P/E=1.01 (1.14) 1.25).

Type 9 (Figure 4Bv). 5-syncolporate grains [4+1], the four colpi are joined on either pole in pairs, but

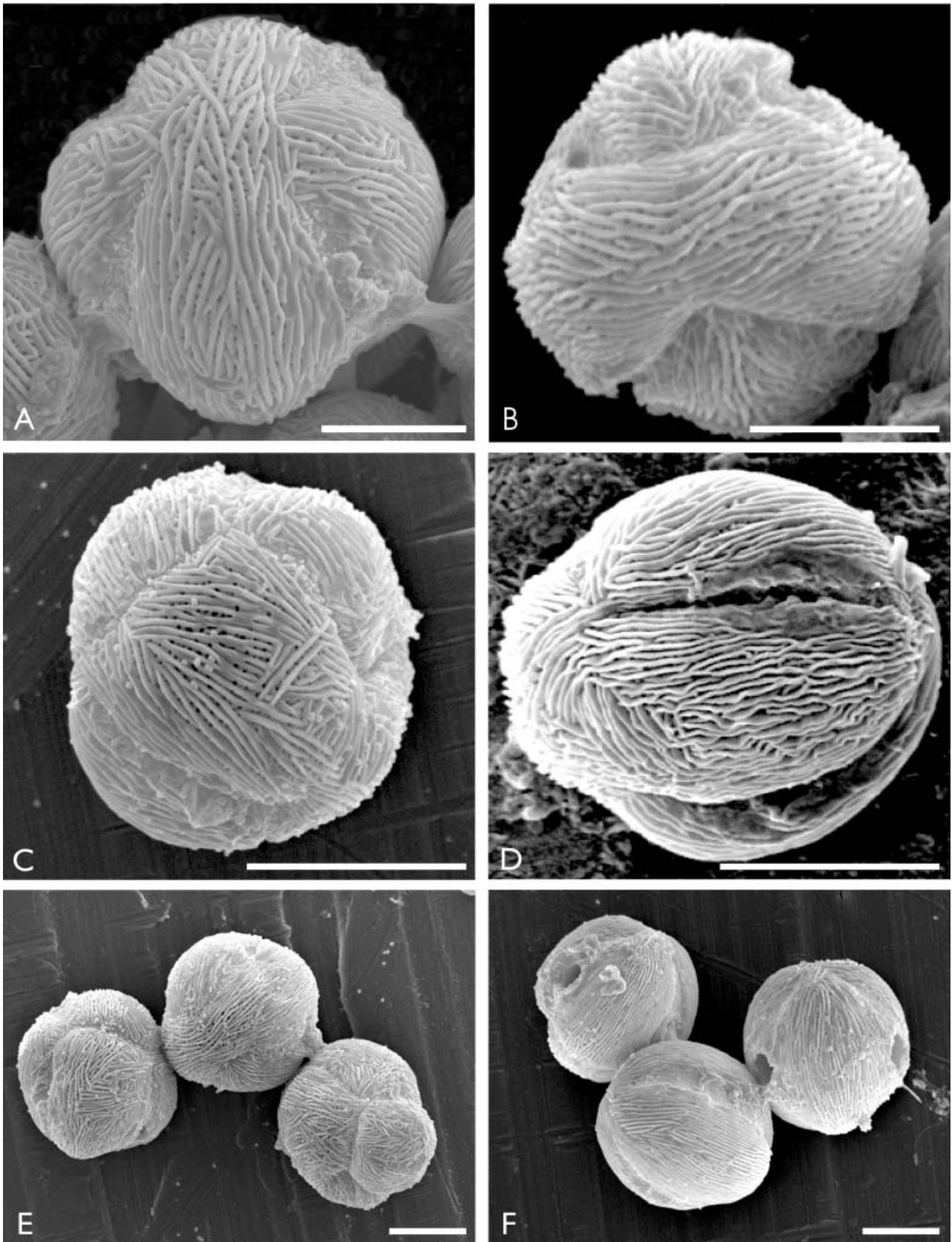


Figure 2. SEM of pollen grains of the three species of *Centaurium* which were examined: (A) *C. ameghinoi*; (B–C) *C. cachenlahuen*; (D–F) *C. pulchellum*: (D) typical 3-colporate grains; (E) 6-pantocolporate grain (left) and 3-syncolporate grains; (F) 3-syncolporate grain with oblique colpi. Scale – 10 μm.

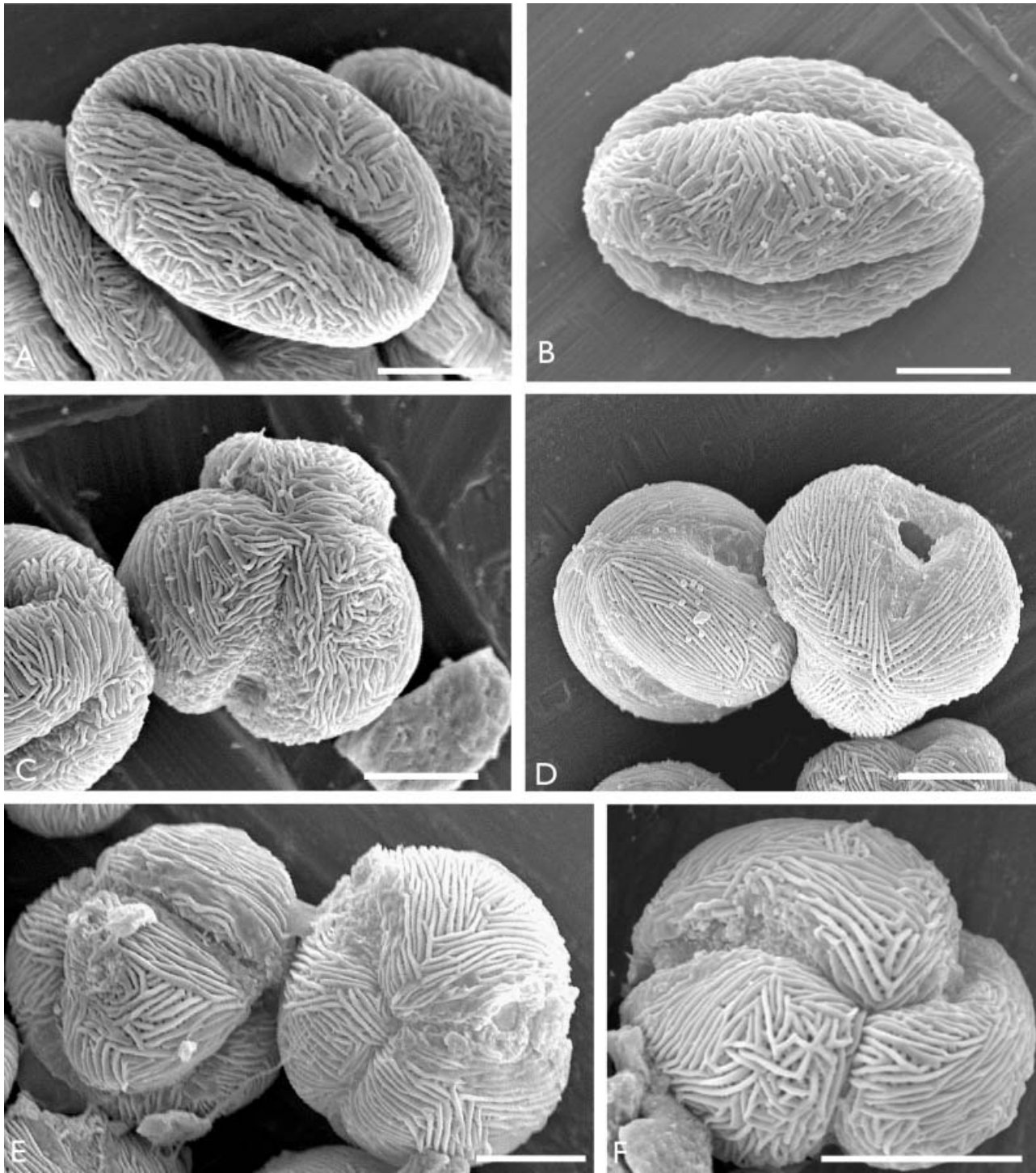


Figure 3. SEM of typical and atypical pollen grains of *Centaurium pulchellum*: (A) typical 4-colporate grain; (B) 4-syncolporate grain; (C) 12-pantocolporate grain; (D) 4-syncolporate grain; (E–F) Different pollen types within same anther. Scale – 10 μm .

on one pole the apices of the four colpi are connected by a colpoid (2+2+1), while on the other pole they are not (2+2); E=21 (22.8) 26.6 μm ; apparently oblate spheroidal – subprolate, although there were no pollen grains in equatorial view from which to establish polar length accurately.

Type 10 (Figure 4Bvi). 6-syncolpate grains [4+2]; four large meridional colpi, apically connected at both poles to two short colpi or colpoid furrows (without endoapertures); the two short colpi are arranged perpendicular to each other; E=23 (24) 25.2 μm ; apparently prolate spheroidal – subprolate,

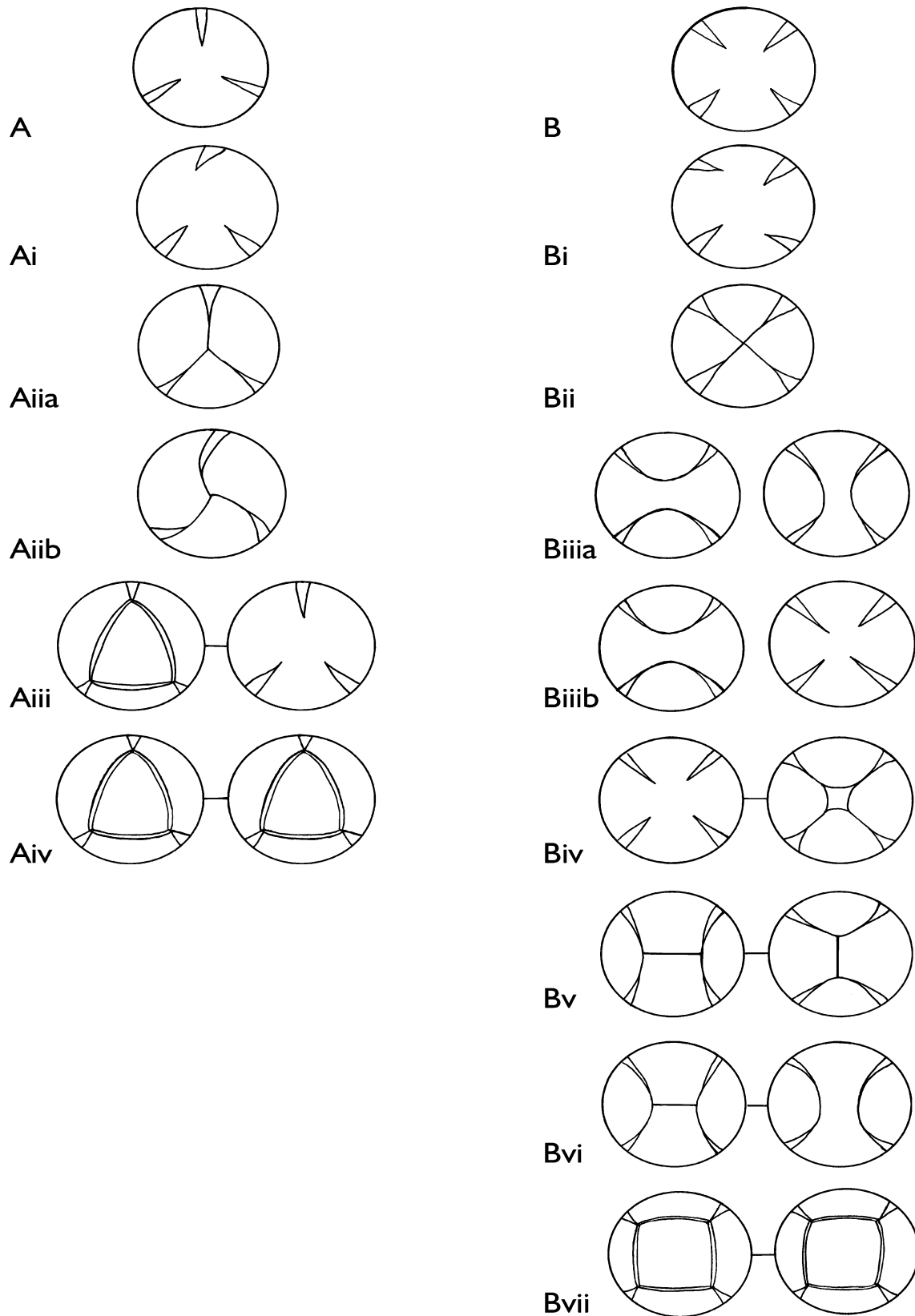


Figure 4. Diagrams in polar view of the two typical and 13 atypical aperture configurations observed in the pollen of *Centaurium pulchellum*. (A) typical 3-zonacolporate grain; (Ai) type 1; (Aii) type 2a; (Aiiib) type 2b; (Aiii) type 3; (Aiv) type 4 [Aiii–Aiv show high and low foci]; (B) typical 4-colporate grain; (Bi) type 5; (Bii) type 6; (Biiia) type 7a; (Biiib) type 7b; (Biv) type 8; (Bv) type 9; (Bvi) type 10; (Bvii) type 11 [Biiia – Bvii show high and low foci].

although there were no pollen grains in equatorial view from which to establish polar length accurately.

Type 11 (Figures 3C, 4Bvii). 12-pantosyncolpate grains [4+4+4]; four meridional colpi are connected on both poles to four colpoid furrows, the connected colpi define six quadrangular faces; P=25 (25.5) 26 μm , E=21 (23.3) 25.5 μm ; prolate spherical – subprolate (P/E=1.02 (1.09) 1.19).

These differing aperture configurations occur in variable proportions within each specimen, and the irregular grains may be a higher proportion than the typical 3- or 4-colporate forms (Table II). The endoapertures may be disposed in the centre of the colpi or they may be asymmetrically displaced towards one end. The colpoid furrows commonly lack of endoapertures.

The number and disposition of the apertures has a considerable influence on the ornamentation of the grains. In atypical forms, the striae are frequently unordered; some have a parallel disposition, while others show an indeterminate pattern.

In the specimens of *C. ameghinoi*, which were studied, the pollen is 3-zonacolporate grains with a variable proportion of 3-syncolpate or parasyncolpate grains (10–23%), while in *C. cachanlahuen* a low percentage of 4-colporate grains have short colpi converging in pairs (3%).

Pollen fertility

The proportion of fertile and sterile pollen grains within each of the eight specimens of *C. pulchellum* which were studied is detailed in Table III. The percentages of infertile grains are subdivided to show which grains were from the typical types and which were from the atypical types. However, in almost all cases, the pollen fertility was high, ranging from 81.22 to 95.65%.

It is noteworthy that the percentage of sterile pollen is not related to the proportion of the atypical grains. Although the greater percentage of sterile grains belongs to the irregular forms (79.26%), pollen sterility is not related to any particular grain type. Both the typical three or four colporate and the irregular pollen forms can be fertile or sterile.

Discussion

The pollen grains of *C. ameghinoi* and *C. cachanlahuen* are described here for the first time. Their characteristics are similar and it is not possible to distinguish these two species from their pollen morphology. However, based on pollen size, shape and exine sculpturing these two species can be clearly separated from *C. pulchellum*. In *Centaurium*

Table II. Distribution and percentages of typical (basic) and atypical pollen types in *Centaurium pulchellum*.

| Sample | Typical forms | | | | Atypical pollen forms | | | | | | | | | | | |
|----------------|---------------|-------|-------|-------|-----------------------|-------|------|-------|------|-------|------|------|------|------|------|-------|
| | 3-Cp | 4-Cp | T1 | T2a | T2b | T3 | T4 | T5 | T6 | T7a | T7b | T8 | T9 | T10 | T11 | Other |
| Pedersen 15576 | 46.00 | 18.50 | 7.50 | 5.00 | 0.50 | 1.00 | – | 13.50 | – | 6.50 | – | 0.50 | – | 1.00 | – | – |
| Franceschi 245 | 34.00 | 10.00 | 1.00 | 8.00 | 1.00 | 33.00 | – | 0.50 | 0.50 | 8.50 | 1.30 | 0.50 | 0.40 | 1.30 | – | – |
| Schulz 11661 | 21.50 | 13.50 | 2.50 | 1.00 | 5.00 | 23.00 | 3.00 | 4.00 | 0.50 | 3.50 | 5.00 | 3.00 | 2.00 | 0.50 | 7.00 | 5.00 |
| Muñoz 1781 | 22.80 | 14.50 | 13.0 | 6.00 | 0.50 | 6.00 | – | 14.80 | 0.50 | 12.50 | 0.40 | – | 1.00 | 6.00 | 0.50 | 1.40 |
| Burkart 10057 | 38.00 | 1.50 | 18.50 | 12.40 | 3.30 | 20.50 | – | 2.80 | – | – | – | – | 1.50 | 1.50 | – | – |
| Gamero 46 | 50.00 | 6.00 | 14.50 | – | 3.00 | 15.50 | – | 9.50 | – | 0.50 | 0.50 | – | – | – | 0.50 | – |
| Kiesling 4358 | 31.00 | 2.50 | 10.00 | 4.50 | 3.00 | 21.00 | – | 8.00 | – | 5.50 | 3.00 | – | 2.50 | 8.00 | 0.50 | 0.50 |
| Kiesling 6131 | 44.50 | 0.50 | 14.10 | 10.00 | 5.00 | 17.00 | – | 5.00 | – | 1.50 | – | – | – | 2.40 | – | – |
| Average | 36.68 | 9.22 | 10.15 | 6.05 | 2.63 | 16.57 | 0.43 | 7.16 | 0.21 | 4.72 | 1.03 | 0.57 | 0.71 | 1.81 | 1.15 | 0.91 |

[3-Cp = zonatricolporate, 4-Cp = zonatetracolporate, T = type].

Table III. Pollen fertility in the studied specimens of *Centaurium pulchellum*.

| Sample | Morphology of fertile grains | | | Morphology of the sterile grains | | |
|----------------|------------------------------|-------------------------|---------------------------|----------------------------------|-------------------------|---------------------------|
| | Fertile grains (Total %) | Typical pollen type (%) | Atypical pollen forms (%) | Sterile grains (Total %) | Typical pollen type (%) | Atypical pollen forms (%) |
| Pedersen 15576 | 89.42 | 72.76 | 27.24 | 10.58 | 21.85 | 78.15 |
| Franceschi 245 | 93.91 | 38.54 | 61.46 | 6.09 | 25.50 | 74.50 |
| Schulz 11661 | 87.62 | 44.85 | 55.15 | 12.38 | 20.37 | 79.63 |
| Muñoz 1781 | 81.22 | 51.58 | 48.42 | 18.78 | 23.98 | 76.02 |
| Gamerro 46 | 90.90 | 63.15 | 36.85 | 9.10 | 21.32 | 78.68 |
| Burkart 10057 | 95.65 | 43.07 | 56.93 | 4.35 | 17.94 | 82.06 |
| Kiesling 4358 | 91.63 | 40.21 | 59.79 | 8.37 | 19.86 | 80.14 |
| Kiesling 6131 | 90.56 | 53.01 | 46.99 | 9.44 | 15.16 | 84.84 |
| Average | 90.11 | 50.89 | 49.11 | 9.89 | 20.74 | 79.26 |

ameghinoi and *C. cachanlahuen* the pollen grains are larger (P: 28–34 μm) and usually prolate-spheroidal to prolate, the muri or lirae are short and slightly curved, with a disordered arrangement. *Centaurium pulchellum* has smaller (P: 17–28 μm) and generally oblate spheroidal to prolate spherical pollen grains, the muri tend to be longer, extending almost from one pole to another, the groups of lirae show a more ordered disposition but frequently change direction abruptly. Furthermore, *C. ameghinoi* and *C. cachanlahuen* have longitudinal endoapertures which lack lateral extensions, whereas, *C. pulchellum* has H-shaped endoapertures with small lateral extensions. The basic number of apertures in the pollen of *C. ameghinoi* and *C. cachanlahuen* is more or less constant in both species. Tetracolporate grains occur only occasionally while pollen of *C. pulchellum* has a wide range of aperture configurations which include a number of irregular pollen types.

Variations to the apertural system of pollen at species level are known in a number of genera in

Gentianaceae (Table IV) where, in addition to basic 3- or 4-colporate grains, there are several atypical forms which show a considerable heterogeneity in the number and disposition of the apertures. The occurrence of aperture variation in *Centaurium* species has been noted by Punt and Nienhuis (1976), Rao and Chinnappa (1983a) and Díez (1987). In most of these studies 3-zonacolporate grains are recognised as the basic aperture arrangement. Punt and Nienhuis (1976) reported the presence of 4-pantocolporate and syncolporate pollen grains in variable proportions; Díez (1987) described 6-pantocolporate (3 \times 3) grains as having a relatively low frequency, in addition to the basic 3-colporate and 4-colporate forms. For *C. pulchellum* and two other *Centaurium* species, *C. exaltatum* and *C. littorale*, Rao and Chinnappa (1983a) found a high percentage of pantocolporate grains accompanied by other “transitional” aperture arrangements in lesser proportions. In their study the percentage of pantocolporate grains observed in *C. pulchellum*

Table IV. Variation in number and disposition of the apertures in some genera of Gentianaceae.

| Genus | Pollen Types | References |
|---------------------|--|-------------------------------|
| <i>Bartonia</i> | 3-colporate or 4-colporate | Rao & Chinnappa (1983b) |
| <i>Blackstonia</i> | (3) 4-zonacolporate or 4-pantocolporate, rarely syncolporate or 5-pantocolporate | Punt & Nienhuis (1976) |
| | 3- (4) zonacolporate, rarely 4-syncolporate, colpi joined 2+2, composing a W figure around the grain | Pire & Dematteis (2006) |
| <i>Centaurium</i> | 3-(4) zonacolporate | Nilsson (1975), Makino (1991) |
| | 3-zonacolporate, frequently 4-pantocolporate, rarely syncolporate | Punt & Nienhuis (1976) |
| | 3-colporate to pantocolporate, sometimes syncolporate | Rao & Chinnappa (1983b) |
| <i>Exacum</i> | 3-colporate or 4-colporate | Rao & Chinnappa (1983b) |
| <i>Gentiana</i> | 3- (4) colporate, rarely 6-colporate; exceptionally 4-colporate with colpi converging in pairs or well 6-aperturate having 3 oriferous colpi+3 colpoid furrows alternate | Nilsson (1967) |
| <i>Gentianella</i> | 3-5 colporate (colporoidate), zonacolporate or pantocolporate, rarely 4-colporate, colpi crossed 2 \times 2 | Nilsson (1967) |
| <i>Leiphaimos</i> | 1 to 6-porate | Punt & Nienhuis (1976) |
| <i>Lomatogonium</i> | 3-colporate (colporoidate), rarely 4-colporate | Nilsson & Skvarla (1969) |
| <i>Voyria</i> | 1 to 6 porate | Nilsson (1967) |
| <i>Voyriella</i> | 3-zonacolporate or 4-colporate, colpi converging obliquely in pairs | Nilsson & Skvarla (1969) |

was approximately 30%, while the other two taxa showed, respectively, 23% and 69% pantocolporate grains.

The current study shows that pollen morphological variation in *C. pulchellum* is much greater than previously thought. In addition to the basic 3-zonacolporate type *C. pulchellum* also has 4-zonacolporate pollen grains, although within collections 3-colporate grains tend to be more frequent than 4-colporate grains. Nevertheless the proportions of each of these types vary between the specimens examined. Apart from these two basic types, there is a high percentage, from 35.5–65.0%, of irregular grains present between collections. In some instances, the atypical pollen grains represent a higher proportion than the 3- and 4-colporate grains (see Table II). The frequency of the different pollen types not only varied from one specimen to another but also between anthers in the same flower. A pollen form present in a low percentage in one specimen may be the dominant type in another individual; for example, in Pedersen 5576 there is 1% of type 3, while in Franceschi 245 the same form represents 33% of the pollen sample.

The patterns of variation described for the pollen of *C. pulchellum* are noteworthy within the eudicotyledons, where the proportion of families which have been recorded with irregular pollen variants is low, probably not exceeding 10–15% (Pozhidaev, 2000a).

The different pollen forms can be arranged in a continuous series that presents a gradual increase in complexity of the apertural system, showing certain correspondence with the successiform series described by Van Campo (1967). However, the increase in complexity of the apertural system is not related to a significant increase of the pollen grain size, which has been observed in some previous studies (Van Campo, 1967; Pozhidaev, 2000a).

Our data indicate that the production of irregular pollen aperture patterns does not affect pollen fertility, which is more than 80% in all analysed specimens. Overall the irregular pollen types have a lower fertility than the typical forms, although a considerable proportion of typical grains were also sterile. Low fertility is often associated with the production of irregular grains (Clarke, 1975), but this association is not apparent in *Centaurium pulchellum*. The high frequency of fertile atypical pollen grains combined with a size similar to that of 3- and 4-colporate pollen suggests that atypical pollen is probably part of the normal condition. This further suggests that atypical forms are probably not caused by aberration during meiosis.

Pollen aperture variability is encountered in species from a number of angiosperm families,

although the factors that determine such differences are not the same in all cases (Mignot et al., 1994; Pozhidaev, 2000b). Pollen aperture variability is frequently associated with heterostyly or zygomorphy (Till-Bottraud et al., 2005) and is related to breeding systems or pollination strategy (Punt et al., 1974; Mignot et al., 1994; Barrett, 2002). Nevertheless, the development of pollen heteromorphism is not clearly understood, and remains a subject of discussion. In many angiosperm species a major cause of pollen heteromorphism is polyploidy, which usually results in a notable increase in pollen size (Maurizio, 1956; Bronckers, 1963; Erdtman, 1966; Thomas, 1970; Punt et al., 1974; Pfahler et al., 1992). It is also suggested that it might influence variation in aperture number (Clarke, 1975; Ferguson, 1980; Mignot et al., 1994). In *Centaurium pulchellum* we have not observed differences in pollen size between the different aperture variants. Therefore, in this particular case polyploidy could probably be excluded as the possible cause of the variation in aperture number. However, this remains to be tested. Another hypothesis is that pollen heteromorphism probably provides some form of advantage to individuals and consequently may be preserved in natural populations (Mignot et al., 1994). In *Viola diversifolia* it was observed that pollen with 4-aperturate grains germinate more rapidly than pollen with 3-aperturate grains (Dajoz et al., 1991). Consequently, the production of pollen grains with varying aperture number in the same plant has been interpreted as an ecological strategy. Pollen aperture variation observed in *Viola calcarata* shows that pollen heteromorphism is characteristic of the species, and can be determined genetically (Till-Bottraud et al., 1999). Although variation in aperture number may be genetically determined in some cases, part of this variation is probably due to external environmental conditions (Wodehouse, 1935; Thanikaimoni, 1986; Pardo, 1990) or internal factors, such as interactions between meiotic poles during meiosis or disturbances of cytokinesis (Ressayre et al., 1998). In tobacco cultivars the proportions of the different pollen types varied during the season, among branches and even flowers of the same genotype (Till-Bottraud et al., 1995; Ressayre et al., 2002).

Different pollen forms within unrelated taxa can be arranged in similar morphological series (Van Campo, 1967, 1976; Clarke, 1975; Pozhidaev, 1998, 2000a, b, 2002), suggesting that it could result from ontogenetic control mechanisms common to all dicotyledons (Pozhidaev, 2000a). On the basis of this fact, it has been suggested that the successive series (Van Campo, 1967) might be a result of volume changes in the pollen grains, which

might induce additional apertures (Wodehouse, 1935). In some cases, these volume changes could be produced either as a response to occupation of new habitats (Thanikaimoni, 1986) or by variation in the humidity of the existing environment (Pardo, 1990). Taking into account that *Centaurium pulchellum* is an invasive weed, the aperture variation is perhaps more easily explained by the first of these two hypotheses. Further studies will be needed to improve our understanding of the causes and reproductive consequences of pollen aperture variation in *C. pulchellum*. However, the variation described is sufficiently interesting to justify a detailed study of the underlying cause(s) of the aperture variation in this species.

Conclusions

Centaurium pulchellum presents 3-colporate or 4-colporate grains, but has also a high percentage of pollen grains with aperture patterns which differ from the two basic types. These aperture variants can be arranged in a continuous series with a gradual increase in complexity of the aperture system; the proportion of each pollen type varies widely between, and within, individuals. However, no adverse influence on pollen fertility has been observed in the individuals which were analysed. The high fertility of the atypical grains, and the similar size of typical and atypical pollen, suggest that the occurrence of atypical morphs constitutes the normal condition for *C. pulchellum*. It is possible that the atypical forms may not be caused by irregularities at the meiosis, but rather in response to volume changes to the pollen grains induced by occupation of new habitats and/or by humidity changes in the natural environment.

Specimens examined

Centaurium ameghinoi. ARGENTINA. Prov. Neuquén: Minas, Boelcke et al. 14429 (SI) [PAL-CTES 6446]. Prov. Río Negro: Río Negro. Scala 106 (SI) [PAL-CTES 6445].
C. cachanlahuen. ARGENTINA. Prov. Río Negro: El Bolsón, Martínez Crovetto 3232 (SI) [PAL-CTES 6447].
C. pulchellum. ARGENTINA. Prov. Buenos Aires: La Balandra, Gamarro 46 (SI) [PAL-CTES 6449]; Cañuelas, Burkart 10057 (SI) [PAL-CTES 6448]. Prov. Corrientes: Curuzú-Cuatiá, Puente sobre arroyo Timbó, Pedersen 15576 (CTES) [PAL-CTES 6383]. Prov. Chaco: Colonia Benítez, Schulz 11661 (CTES) [PAL-CTES 6443]. Prov. Entre Ríos: Concordia, Muñoz 1781 (CTES) [PAL-CTES 6444]. Prov. Santa Fe: Isla Campo Rico, río Paraná, Franceschi 245 (CTES) [PAL-CTES 6385]. Prov. San Juan: Calingasta, Castaño Viejo, Kiesling

et al. 6131 (SI) [PAL-CTES 6452]; Ullúm, Kiesling et al. 4358 (SI) [PAL-CTES 6451].

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References

- Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nat. Rev. Genet.*, 3, 274–284.
- Bronckers, F. (1963). Variations polliniques dans une série d'autopolyploïdes artificiels d'*Arabidopsis thaliana* (L.) Heynh. *Pollen Spores*, 5, 233–238.
- Clarke, G. C. S. (1975). Irregular pollen grains in some *Hypericum* species. *Grana*, 15, 117–125.
- Dajoz, I., Till-Bottraud, I. & Gouyon, P. H. (1991). Evolution of the pollen morphology. *Science*, 253, 66–68.
- Dajoz, I., Till-Bottraud, I. & Gouyon, P. H. (1993). Pollen aperture polymorphism and gametophyte performance in *Viola diversifolia*. *Evolution*, 47, 1080–1093.
- Díez, M. J. (1987). Gentianaceae. In B. Valdés, M. J. Díez & I. Fernández (Eds), *Atlas Polínico de Andalucía Occidental* (pp. 246–248). Sevilla: Grafites-Utrera.
- Erdtman, G. (1952). *Pollen morphology and taxonomy. Angiosperms*. Stockholm: Almqvist & Wiksell.
- Erdtman, G. (1966). *Pollen morphology and plant taxonomy. Angiosperms*. New York/London: Hafner Publ. Co.
- Erdtman, G., Berglund, B. & Pragłowski, J. (1961). An introduction to Scandinavian pollen flora. *Grana*, 2, 3–92.
- Ferguson, I. K. (1980). The pollen morphology of *Ceratonia* (Leguminosae: Caesalpinoideae). *Kew Bull.*, 35, 273–277.
- Kremp, G. O. W. (1965). *Morphologic encyclopedia of palynology*. Tucson: Univ. Arizona Press.
- Makino, H. (1991). Flora polínica da Reserva do Parque Estadual das Fontes do Ipiranga (São Paulo, Brasil). *Hoehnea*, 18, 43–48.
- Marks, G. E. (1954). An acetic-carminic glycerol jelly for use in pollen fertility counts. *Stain Technol.*, 29, 277.
- Maurizio, A. (1956). Pollengestaltung bei einigen polyploiden Kulturpflanzen. *Grana*, 1, 59–69.
- Mignot, A., Hoss, C., Dajoz, I., Leuret, C., Henry, J. P., Dreuillaux, J. M., Heberle-Bors, E. & Till-Bottraud, I. (1994). Pollen aperture polymorphism in the Angiosperms: importance, possible causes and consequences. *Acta Bot. Gall.*, 141, 109–122.
- Nilsson, S. (1967). Pollen morphological studies in the Gentianaceae-Gentianinae. *Grana*, 7, 46–143.
- Nilsson, S. (1975). Gentianaceae. Pollen morphology. In R. E. Woodson & R. W. Schery (Eds), *Flora of Panama. Ann. Mo. Bot. Gard.*, 62, 61–101.
- Nilsson, S. & Skvarla, J. (1969). Pollen morphology of saprophytic taxa in the Gentianaceae. *Ann. Mo. Bot. Gard.*, 56, 420–438.

- Pardo, C. (1990). Tipos harmomérgatas en *Gleditschia triacanthos* (Papilionaceae, Caesalpinoideae). *Polen, Esporas Aplicac.*, 1, 211–216.
- Pfahler, P. L., Barnett, R. D. & Blazey, E. B. (1992). Genotype and ploidy effects on maize pollen and in vitro germination characteristics. In E. Ottaviano, D. L. Mulcahy, M. Sari-Gorla & G. Bergamini Mulcahy (Eds), *Angiosperm pollen and ovules* (pp. 285–290). New York: Springer.
- Pire, S. M. & Dematteis, M. (2006). Gentianaceae. In S. M. Pire et coll. (Eds), *Flora Polínica del Nordeste Argentino*, vol. 3 (pp. 71–80). Corrientes: Eudene.
- Pozhidaev, A. E. (1998). Hypothetical way of pollen aperture patterning. 1: formation of 3-colpate patterns and endoaperture geometry. *Rev. Palaeobot. Palynol.*, 104, 67–83.
- Pozhidaev, A. E. (2000a). Hypothetical way of pollen aperture patterning. 2. Formation of polycolpate patterns and pseudoaperture geometry. *Rev. Palaeobot. Palynol.*, 109, 235–254.
- Pozhidaev, A. E. (2000b). Pollen variety and aperture patterning. In M. M. Harley, C. M. Morton & S. Blackmore (Eds), *Pollen and spores: Morphology and biology* (pp. 205–225). Kew: R. Bot. Gards.
- Pozhidaev, A. E. (2002). Hypothetical way of pollen aperture patterning. 3. A family-based study of Krameriaceae. *Rev. Palaeobot. Palynol.*, 127, 1–23.
- Punt, W., van Weenen, J. S. & van Oostrum, W. A. P. (1974). The Northwest European Pollen Flora. 3. Primulaceae. *Rev. Palaeobot. Palynol.*, 17, 31–70.
- Punt, W. & Nienhuis, W. (1976). The Northwest European Pollen Flora. 6. Gentianaceae. *Rev. Palaeobot. Palynol.*, 31, 89–123.
- Punt, W., Blackmore, S., Nilsson, S. & Le Thomas, A. (1994). *Glossary of pollen and spore terminology*. Utrecht: LPP Found. Utrecht Univ. LPP Ser. 1.
- Rao, K. S. & Chinnappa, C. C. (1983a). Pericolporate pollen in Gentianaceae. *Can. J. Bot.*, 61, 174–178.
- Rao, K. S. & Chinnappa, C. C. (1983b). Studies in Gentianaceae. Microsporangium and pollen. *Can. J. Bot.*, 61, 324–336.
- Ressayre, A., Raquin, C., Mignot, A., Godelle, B. & Gouyon, P. H. (2002). Correlated variation in microtubule distribution, callose deposition during male post-meiotic cytokinesis, and pollen aperture number across *Nicotiana* species (Solanaceae). *Am. J. Bot.*, 89, 393–400.
- Thanikaimoni, G. (1986). Pollen apertures: Form and function. In S. Blackmore & I. K. Ferguson (Eds), *Pollen and spores: Form and function* (pp. 119–136). London/New York: Acad. Press.
- Thomas, J. L. (1970). Haploid and diploid pollen in *Hypericum patulum*. *J. Arnold Arbor.*, 51, 247–250.
- Till-Bottraud, I., De Paepe, R., Mignot, A. & Dajoz, I. (1995). Pollen heteromorphism in *Nicotiana tabacum* (Solanaceae). *Am. J. Bot.*, 82, 1040–1048.
- Till-Bottraud, I., Vincent, C., Dajoz, I. & Mignot, A. (1999). Pollen aperture heteromorphism. Variation in pollen type proportions along altitudinal transects in *Viola calcarata*. *C. R. Acad. Sci. (Paris) Sci. Vie*, 322, 579–589.
- Till-Bottraud, I., Joly, D., Lachaise, D. & Snook, R. R. (2005). Pollen and sperm heteromorphism: Convergence across kingdoms? *J. Evol. Biol.*, 18, 1–18.
- Van Campo, M. (1967). Pollen et classification. *Rev. Palaeobot. Palynol.*, 3, 65–71.
- Van Campo, M. (1976). Patterns of pollen morphological variations within taxa. In I. K. Ferguson & J. Muller (Eds), *Pollen and spores: The evolutionary significance of the exine* (pp. 163–183). New York: Acad. Press.
- Wodehouse, R. P. (1935). *Pollen grains: Their structure, identification and significance in science and medicine*. New York: McGraw-Hill.