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## **Seed micromorphology in the Iberian *Orchidaceae*. I. Subfamily *Cypripedioideae***

### **Abstract**

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The seed micromorphology of *Cypripedium calceolus* L. from Spanish populations is described. Morphology of the seed and the cells, measures (length, width) and characteristics of the periclinal and anticlinal walls are analyzed. The resulting data are compared with others from Russian and North American populations.

### **Introduction**

The family *Orchidaceae* comprises 25 genera and 114 taxa in the Iberian Peninsula and Balearic islands, according to Galán Cela & Gamarra (2002, 2003). Following the taxonomic scheme of Dressler (1993), these genera belong to four subfamilies (Table 1).

In our research about this family, one of the aims is the analysis of the micromorphology of the seed coat of each one taxa presented in the territory indicated above.

Previous works on orchid seeds have demonstrated the diagnostic and phylogenetic value of some quantitative and qualitative characters (Clifford & Smith 1969; Barthlott 1976; Barthlott & Ziegler 1981; Chase & Pippen 1988; Molvray & Kores 1995). However, studies on European orchids, and in particular, about taxa occurring in the Iberian Peninsula are scarce (Alarcón & Aedo 2002).

This report describes the seed micromorphology of *Cypripedium calceolus* L., the only one species belonging to the subfamily *Cypripedioideae* which occurs in the Iberian Peninsula, exclusively in Pyrenees.

### **Material and methods**

Seeds were obtained from mature capsules collected in the field (Spain, Huesca, Valle de Tena), air dried for two weeks, and stored in small envelopes. With the aim to compare disjunct

Table 1. Taxonomic scheme of the *Orchidaceae*, according to Dressler (1993), present in the Iberian Peninsula and Balearic islands.

subfamily	tribe	subtribe	genus
<i>Cypripedioideae</i>	<i>Cypripedieae</i>	<i>Cypripediinae</i>	<i>Cypripedium</i>
<i>Epidendroideae</i>	<i>Gastrodieae</i>	<i>Epipogoninae</i>	<i>Epipogium</i>
	<i>Neottieae</i>	<i>Limodorinae</i>	<i>Cephalanthera, Epipactis, Limodorum</i>
		<i>Listerinae</i>	<i>Listera, Neottia</i>
	<i>Calypsoeae</i>	<i>Corallorhizinae</i>	<i>Corallorhiza</i>
<i>Orchidoideae</i>	<i>Orchideae</i>	<i>Habenariinae</i>	<i>Gemma</i>
		<i>Orchidinae</i>	<i>Aceras, Anacamptis, Barlia, Coeloglossum, Dactylorhiza, Gymnadenia, Himantoglossum, Neotinea, Nigritella, Ophrys, Orchis, Platanthera, Pseudorchis, Serapias</i>
<i>Spiranθοideae</i>	<i>Cranichidae</i>	<i>Goodyerinae</i>	<i>Goodyera</i>
		<i>Spiranθinae</i>	<i>Spiranthes</i>

populations, we also study materials from Murmansk Region, Kola Peninsula (Russia).

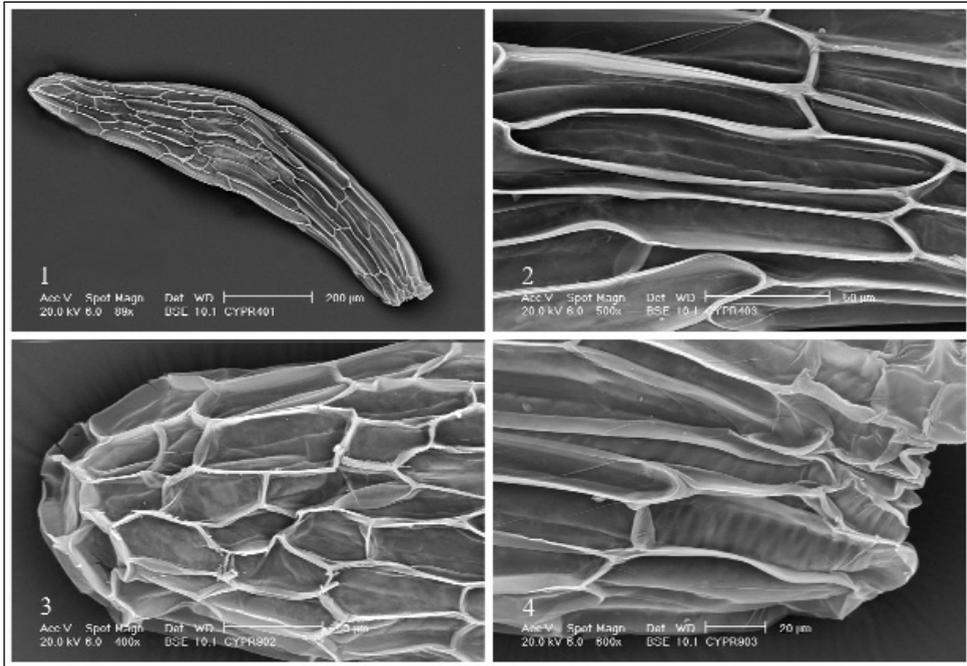
The samples were mounted on SEM (scanning electron microscope) stubs and coated with gold in a sputter (Sem Coating System, Bio-Rad SC 502). The seeds were examined on a Philips XL30, with a filament voltage of 20 kv.

Length and width were measured (average of 30 seeds for each sample) under a light microscope at the longest and widest axes of the seed. Number of cells along the longitudinal axis, the morphology of the total seed, the apical, medial and chalazal cells, and the colour were also analyzed. Samples examined under light microscope were mounted on lactic acid.

The terminology and methods adopted follow Arditti & al. (1979) and Molvray & Kores (1995).

## Results

All seeds examined are fusiform (Fig. 1) and the coat is brown in colour. Cells are similar in morphology along the longitudinal axis, regularly rectangular (Fig. 2), but they show differences in the size, because the medial cells are lightly longer than the apical and chalazal ones (Fig. 3, 4). Anticlinal walls are high and smooth, with adhesion zones lightly sunken at the cell vertices (Fig. 2). Periclinal walls are generally unsculptured, but in some cells of the chalazal pole transversal ridges can be observed (Fig. 4). This last character is also visible under light microscope.



Figures 1-4. Seed micromorphology of *Cypripedium calceolus*: **1.** Seed shape; **2.** Medial cells, with smooth periclinal walls and the anticlinal ones with adhesion zones slightly sunken at the cell vertices; **3.** Apical cells; **4.** Sculpturing of cells of the chalazal pole showing transversal ridges.

A set of 30 seeds were measured. They vary in length from 0.930 mm to 1.270 mm, and in width from 0,220 to 0,310 mm. The number of cells at longest axis of testa vary from 9 to 12. Average of quantitative characters and comparative data from Russia and North American populations (Arditti & al. 1979) are showed in Table 2.

**Discussion**

Morphological aspects showed some details not registered in previous works, like the occurrence of sculpturing in cells next to the chalazal end, and the greater length of the medial cells

Table 2. Quantitative characters of the seeds of *Cypripedium calceolus* from Spain, Russia and North America.

Characters	Spain, Pyrenees	Russia, Murmansk Region	North America (Arditti & al., 1979: 1134)
Average length (mm)	1.150	1.135	0.91-1.27
Average width (mm)	0.258	0.253	0.25-0.27
Average length/width	4.457	4.524	3.8-4.7
Number of cells at longest axis of testa	9-12	9-13	7-11

forehead to the apical and chalazal ones.

Numerical data are similar to those of Russian populations, and also with Arditti & al. (1979), concerning to length and width of the seed, and the length-to-width ratio. However, the number of cells at longest axis of testa is lower in the North American populations (7 to 11).

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