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Seeds in subtribe Orchidinae (Orchidaceae): the best morphological tool to support molecular analyses

Roberto Gamarra, Emma Ortúñez, Ernesto Sanz,
Iris Esparza, Pablo Galán

Abstract — Seeds of the genera belonging to the subtribe Orchidinae (Orchidaceae) have been studied using scanning electron microscopy. Qualitative data concerning the general morphology of the seed and testa cells, and ornamentation of periclinal and anticlinal walls, are characters that allow to recognize genera and taxa at infrageneric levels. The results show that seed micromorphology is the best tool to support the molecular analyses published in recent years for this taxonomic group.

Index Terms — qualitative data, seed micromorphology, SEM, subtribe Orchidinae.

1 Introduction

The family Orchidaceae is probably the largest among flowering plants, with 25.158 species [1]. The systematics has undergone many changes along the last few decades. The latter taxonomic proposals were published by Dressler [2] and Szlachetko [3]. In the subfamily Orchidoideae, Dressler [2] divided the tribe Orchideae into two subtribes: Orchidinae with 34 genera and 370 species, and Habenariinae with 23 genera and 930 species. Within this tribe, Philip Cribb [4] recognizes 62 genera and near 1800 species. This is the principal orchid group of the north temperate area, also with considerable diversity in Africa, South Asia and Australia; only the genus *Habenaria* occurs in South America.

Flower morphology (lip, anther, stigma, rostellum) and vegetative characters (habitus, inflorescence, tuberoids) have been used to elucidate the systematics of this tribe. According to Dressler [2], the morphology of tuberoids is essential

R. Gamarra, E. Ortúñez, E. Sanz and I. Esparza are with the Departamento de Biología, Universidad Autónoma de Madrid, C/ Darwin, 2, E-28049 Madrid, Spain. E-mail: roberto.gamarra@uam.es. P. Galán is with the Departamento de Producción Vegetal: Botánica y Protección Vegetal, E.U.I.T. Forestal, Universidad Politécnica, E-28040 Madrid, Spain, E-mail: pablo.galan@uam.es.

to recognise the genera in the subtribe Orchidinae, while in Habenariinae the stigmas are more important. Szlachetko [3] proposes 6 subtribes (Orchidinae, Herminiinae, Bartholininae, Androcorytinae, Platantherinae, Habenariinae) based on stigma and rostellum morphology.

Recently, molecular analyses have changed the taxonomy of several genera and species in this tribe [5], [6], e.g., the monotypic genus *Coeloglossum* Hartm. is integrated into *Dactylorhiza* Neck. ex Nevski, while some species attributed to the genus *Orchis* L. are included into *Anacamptis* Rich. and *Neotinea* Rchb. fil.

Beer [7] published the first study about the seed morphology in Orchidaceae. In his book, the figures show the great diversity in genera belonging to different subfamilies.

Clifford & Smith [8] proposed the first methodology to analyze the testa morphology studying 49 species of the subfamily Epidendroideae. They showed a strong correlation between qualitative characters of seeds and taxa above genus level.

Later, Barthlott [9] studied 58 genera using SEM, demonstrating the great diagnostic value of seed morphology and its phylogenetic significance, principally at tribe and subtribe level. He also indicated that this is a useful taxonomic tool to recognise the genera. In 1979, Joseph Arditti and colleagues established the methodology for quantitative analyses, related to the sizes and volumes of seeds and embryos [10]. Several authors published different papers about seed morphology in the family Orchidaceae [11], [12], [13]. All these papers show the great diagnostic value of the qualitative and quantitative characters of the seed, to approach phylogenetic studies in this family. Arditti & Al-Ghani [14] published an overview of previous works, and they conclude the importance to continue this research.

More recently. Krishna Swamy et al. [15] described seeds of the genus *Cymbidium* using SEM and morphometric data, and Tsutsumi et al. [16] compared the phylogenetic proposal using molecular markers with seed morphology of the japanese species of the genus *Liparis.*

Different authors have obtained similar results in other flowering plants, as in the genus *Phyllocladus* (Phyllocladaceae) [17], in the tribe Massonieae (Hyacinthaceae) [18], in the genus Veronica (Plantaginaceae) [19], and in the genus Moehringia (Caryophyllaceae) [20]. These papers show that seed morphology is an important tool to elucidate the taxonomy of these genera.

Since 2003, our research group initiated a study of seed micromorphology of iberian orchids using SEM and the methodology proposed by former authors. We have published data about genera of subfamilies Cypripedioideae [21] and Orchidoideae [22], [23], some of them previously presented in the 17th International Botanical Congress, held in Wien in 2005. Our studies show that qualitative and quantitative characters strongly support the results using molecular markers. These characters are of good diagnostic value to recognize many taxa, principally above the species level.

Presently, the main aim of our research is the study of the seed micromorphology in all groups within the genera of the tribe Orchideae.

2 Results

In the studied genera, the seed morphology varies from elongate fusiform (*Dactylorhiza, Platanthera, Neotinea, Anacamptis)*, to shortly fusiform and almost ovoid (*Gymnadenia, Pseudorchis, Amitostigma)*. Generally, in the elongate fusiform, the medial cells are longer than apical and basal cells; in the other morphologycal type, are similar or slightly longer.

The apical pole mainly consist of short and polygonal cells. Only the genus *Platanthera* finished in a truncated cell.

The chalazal end is opened, with short and polygonal cells. Exclusively, in the genus *Ophrys*, a distinct asymmetry is showed.

In several genera (*Orchis, Pseudorchis, Gymnadenia)*, the periclinal walls are unsculptured. However, many genera present a type of ornamentation, with prominent and spaced ridges *(Ophrys)*, to slight ondulations *(Serapias, Amerorchis, Comperia)*. The distribution of ridges and ondulations varies from lax *(Platanthera, Himantoglossum)* to dense *(Serapias, Steveniella, Comperia)*, and from transversal *(Neotinea, Steveniella*) to oblique *(Anacamptis, Himantoglossum, Aorchis)*. Only the genus *Dactylorhiza* shows a great variation, from unsculptured periclinal walls *(D. incarnata* group) to different types of ornamentation *(D. maculata* group, *D. majalis* group).

The morphology of the anticlinal walls varies from straight *(Platanthera, Himantoglossum, Aorchis*) to undulate *(Gymnadenia, Orchis* p.p., *Anacamptis* p.p.) The las type is more typical in the cells of apical pole. Also, a distinct type of lamella can be found in these walls *(Ophrys)*.

3 Conclusion

The morphological study, including qualitative and quantitative characters, of the seed coat in the genera of the subtribe Orchidinae has showed that each genus and each subgroup within this, have its own morphological type. Each type fully agrees with the clades obtained in the recently published molecular analyses [5], [6]. For example, it is consistent with the inclusion of the genus *Coeloglossum* with the species of the *Dactylorhiza incarnata* group, *Nigritella* into *Gymnadenia* or *Barlia* into *Himantoglossum*. Also, within genera with many species as *Anacamptis, Orchis* or *Dactylorhiza*, each clade is consistent with each morphological seed type. Likewise, it also supports the monophyly of genera such as *Ophrys* and *Serapias*.

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