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Vegetative anatomy of some *Ophrys*, *Orchis* and *Dactylorhiza* (Orchidaceae) taxa in Trakya region of Turkey

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ABSTRACT

The anatomical properties of leaves, stems, roots and root tubers of 27 taxa belonging to three orchid genera (*Ophrys*, *Orchis* and *Dactylorhiza*) were investigated to determine their diagnostic characteristics and assess interspecific and intergeneric relationships by means of cladistic analyses. Anatomical sections were done using different staining methods, such as Sartur, Phloroglucin–HCl, Delafield's Hematoxylin–Safranin, and Alcian Blue–Periodic Acid–Schiff. Additionally, lengths of raphides (in all the organs), cuticle thickness, cell size and stomata dimensions (in the leaf epidermes), as well as dimensions of starch granules (only in root tubers) were measured and statistically compared. During root tuber investigations, for every taxon the types of meristele were outlined and diagrammed. All morphometric data except starch grain dimensions, are generally not significantly different between the investigated taxa. The anatomical sections showed the following basic anatomical characteristics: Leaf surface was glabrous, stomata were abaxially located and mostly anomocytic. Cuticle was mostly striate. The chlorenchyma was homogeneous. The stem had a uniseriate epidermis with several rows of thick- or thin-walled, sclerenchymatous (or collenchymatous) cortical parenchymas, a pericycle, collateral vascular bundles within a central ground tissue. *Orchis purpurea* lacks a pericycle. The roots generally had a uniseriate epidermis, an exodermis, a parenchymatous cortex with occasional inclusions of fungal pelotons and hyphae, an endodermis bearing casparian strips, pericycle, and vascular cylinders. Root tubers, from the outside to the centre, had orderly velamen, exodermis, and ground tissue, which were scattered with meristeles, mucilage cells and assimilatory cells bearing cruciate starch grains. Based on the anatomical results, tables were created based on the important primary (species-specific) and secondary (common-specific, used only in some taxa) descriptive characteristics. Some of the primary descriptive characters include the following: Leaf-epidermal wall properties (thin/thick), chlorenchyma cell shape, chloroplast distribution, presence or absence of large lacunae; stem-presence/absence of pericycle, cortical thickness; root-cell shape of exodermis, presence/absence of tilosome passage cells, shapes of endodermal and/or pericycle cells, structures and distributions of archs; root tuber forms of starch grains and mucilage cells and meristele types. In general, leaf chlorenchyma and stem pericycle were found to be thicker and mucilage-water storage cells were more frequent in the *Orchis* and *Dactylorhiza* taxa. Cladistically analysed anatomical data support the hypothesis that *Ophrys* and *Dactylorhiza* are monophyletic, but contrast the triphyletic states of *Orchis*, which is very likely to be the reason for uncovering the other *Orchis* taxa. Finally, we strongly emphasize the importance of this kind of detailed anatomical study to solve identification problems of orchid taxonomy, which have wrongly been disregarded in recent works.

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Introduction

Ophrys, *Orchis* and *Dactylorhiza* taxa are terrestrial orchid plants characterised by a basal rosette and a terminal, unbranched inflorescence that is composed of small to moderately large

resupinate flowers. Plants also typically bear storage root tubers and “normal” absorbing roots. These genera belong to the Orchidinae tribe, and are mainly distributed in the Northern Hemisphere (Dressler, 1993). *Ophrys*, *Orchis*, *Dactylorhiza* are represented by 388, 265, 176 taxa, respectively, in the world and 46, 32, 25 taxa in Turkey (Delforge, 1995; Renz and Taubenheim, 1984; Sezik, 1984). Kreutz (2000), using different synonyms and new descriptions, added 49 taxa to the genera in Turkey. Generally, identification of some taxa of these genera can be very problematic due to morphological variations in labellum so that

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flower characters are problematical. Quite a number of systematical problems remain unresolved in these genus (Delforge, 2005; Sezik, 1984). Closely related orchids were preliminary grouped in the comprehensive overview of by Delforge (2005), and their morphological problems are presented separately in his “Review of Investigated Orchid Groups”. In Turkey, the genera have been subjected to many chorological (Arditti et al., 1980; Baumann and Künkele, 1982; Borsos, 1980; Dressler, 1981; Möbius, 1887; Sezik, 1984), caryological, palynological, and embryological investigations (Aybeke, 2000, 2002), but have not been examined anatomically. In a recent study, Del Prete and Miceli (1999) succeeded in identifying anatomical variations leading to better systematic positions of some closely related *Orchis* taxa such as *O. provincialis*, *O. pauciflora*, *O. colemannii*, *O. mascula* subsp. *mascula*, *O. mascula* subsp. *ichnusae*, and *O. mascula* subsp. *olbiensis*. But a detailed and comprehensive examination of the anatomical properties of the taxa was not attempted previously. Therefore, in this study, we describe the anatomy of 26 *Ophrys*, *Orchis*, and *Dactylorhiza* species growing in Turkey in order to provide data for subsequent comprehensive analyses of the anatomical relationships of the Orchidoideae.

Overview of the investigated orchid groups (Delforge, 1995, 2005)

Ophrys speculum group

The speculum of the flower lip may be more or less reduced; it is very rarely completely absent. Also occasional hybrids and transition forms with *O. erinxia* Brotero are found.

Ophrys fusca, *O. lutea* and *O. iricolor* (*Ophrys fusca*–*lutea* group)

Ophrys fusca has been (and is still often) considered as one polytypical species, but the taxon actually covers a heterogeneous collection. In fact all the flowers are quite similar morphologically. *O. lutea* is a fairly variable species, distinguished by size and shape of the labellum. Owing to its variability, *O. lutea* is pollinated by several species of large bees from the subgenus *Chlorandrena* (Hymenoptera, Andrenidae), making possible even intergeneric hybridization. *Ophrys iricolor* has the most stable morphology compared with the other members of the group.

Ophrys sphegodes group

These taxa are sometimes confused with *Ophrys aymonii* owing to the labellum colour. *Ophrys sphegodes*, *Ophrys mammosa* and *Ophrys pseudomammosa* are very variable species and mostly confused with each other due to their labellum colour and speculum shape, which makes identifications very difficult. They are heterogeneous taxa and sometimes form large populations with variable characteristics intermediate between each other. Sometimes, depending on specific pollinators, they form more stable populations that than better can be circumscribed as independent taxa.

Ophrys tenthredinifera and *O. bombyliflora* (*O. tenthredinifera* group)

These species are sometimes confused with *O. fusca*, variable due to their ability to attract different pollinators. Also similarities exist with *O. fuciflora* (F.W.Schmidt) Moench owing to the stigmatic cavity, oblique pseudo-eyes and tuft of hairs over the appendages. *O. tenthredinifera* is a very variable species in size, shape and colour of labellum and bears some primitive characters,

e.g., long thick hairs of indumentum covering the labellum. In addition, *O. tenthredinifera* can hybridize with *O. speculum* (*O. × heraultii* Keller ex Schrenk.) and *O. bombyliflora* (*O. × sommieri* Sommier ex E.G.Camus). *O. bombyliflora* is close to members of the *O. scolopax* group (e.g., *O. cornuta*, *O. heldreichii*), to *O. umbilicata*, and especially to *O. apifera* which is quite close to *O. bombyliflora* and has probably been isolated from it since long times due to its frequent self-pollination.

Ophrys cornuta group

These *Ophrys* species vary quite widely in colour of labellum and speculum; they are distinguished by the small labellum together with the elongate lateral lobes. Morphological similarities are seen with several members of the *O. scolopax* group, viz. *O. scolopax* Cavanilles, *O. brevipifera* Steven in M.Bieb., *O. rhodia* (H.Baumann & Künkele) Delforge, and *O. heldreichii*. Anatolian specimens have the dorsal sepal bending over the gynostemium, possibly a sign of *O. abchasica* (Kümpel) Delforge influence. The specific status of the various Eastern, Balcan and Greek populations is still problematic. *O. heldreichii* is generally not well known due to confusion with the neighbor taxon *O. cornuta*, as described above.

Ophrys umbilicata–*bucephala* group

Members of the *O. scolopax* group (e.g., *O. cornuta*, *O. heldreichii*) and both *O. umbilicata* and *O. bucephala* attract the same pollinators, owing to their rather similar labellum morphology in which lateral lobes are conical. *O. bucephala* is a rather variable species; it can be distinguished by its larger flowers and a relatively shallow stigmatic cavity. Transition forms with *O. umbilicata* were found.

Ophrys apifera group

Frequent self-pollination leads to many hypochromic individuals and aberrant forms of *O. apifera*.

Orchis coriophora group

Orchis coriophora shows several similarities with *O. fragrans*, concerning habit, sepal form, colour and consistency of the labellum. The southern limits of its distribution are not well known due to morphological confusion in the literature. The distinction between the two taxa is rather problematic, because the populations show a mosaic of characters from both types. In particular *O. fragrans* is a polymorphic species and more variable than *O. coriophora*.

Orchis tridentata group

Orchis tridentata, found in Anatolia and Greece, is often confused with *O. commutata* Todaro in terms of plant habit, distribution zone and flower properties.

Orchis punctulata, *O. purpurea* and *O. simia* (*Orchis militaris* group)

Orchis purpurea is not seldom confused with *O. caucasica*. Additionally, hybrids between *O. simia* and *O. purpurea* are frequent and sometimes too difficult identifying. *O. simia* produces interspecific hybrids with *O. purpurea*, and intergeneric ones with *A. anthropophorum* (L.) W.T.Aiton. *O. punctulata* is quite

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