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# Histo-anatomical observations on some *Orchis* species (*Orchidaceae*) from the eastern Mediterranean

**Abstract**

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Anatomical and histological observations were carried on leaves and stems of some *Orchis* species collected in the Greek Islands of Rhodes and Crete. Leaves of all species examined showed common morphological aspects which appeared coherently related to Mediterranean environment, where these plants grow, and to their adaptive strategy. Some morphological characteristics always occurred in all *Orchis* stems: a sclerenchymatous sheath was observed in the outer part of cortex; the inner part of ground tissue, the pith, consisted of thin-walled cells; vascular bundles, embedded in parenchyma, were nearly arranged in a circle on the periphery of the ground tissue.

## Introduction

The genus *Orchis* includes about 60 species (cfr. Dressler 1993; Delforge 1994) which mainly occur in a wide range of habitats, i.e. dry grasslands, scrub, abandoned grassland, pastures and limestone slopes. Some species grow in fresh, moist grasslands and on mountainous slopes. *Orchis* is characterized by underground storage organs, two tubers, and true roots; basal leaves are arranged in rosette and cauline leaves are often sheathing along the floral axis.

Relatively few studies, concerning histological and anatomical analysis, have been conducted on vegetative organs of European terrestrial orchids: Camus & Camus (1928); Solereder & Meyer (1930); Borsos (1980); Del Prete & al. (1991); Stern (1997 a, b); Del Prete & Miceli (1999). This paper recounts anatomical and histological observations performed on stem and leaves of six *Orchis* species, sampled on the East Mediterranean islands of Crete and Rhodes. The present study represents a first step within a more extensive project concerning European and Mediterranean wild orchids, carried out to highlight orchid relationships and some ecological aspects.

## Material and Methods

Leaf and stem samples were collected from plants growing in wild sites (Table 1). Pieces of floral axis, considered an extension of the stem, were sampled at about 1.5 cm above the basal rosette of leaves. Fresh material was fixed in FAA (ethanol 70% aqueous solution: formaldehyde 36% in water: glacial acetic acid, ratio 90: 5: 5), dehydrated in ethanol and embedded in Technovit® 7100 (Heraeus Kulzer GmbH & CO. KG, Germany). 10 µm thick sections were obtained with a Reichter Jung Ultracut E ultramicrotome. Transverse stem sections were stained with 1% toluidine blue and entirely reproduced with a Reichert Visopan projection microscope in order to perform anatomical observations. To distinguish chemical nature of cell walls, stem sections were also stained with two fluorescent stains: Coriphosphyne O (0.1% aqueous solution) and Berberine Sulphate (0.1% aqueous solution), observed with a Leitz Orthoplan light microscopy, equipped with a Leica Ploemopak epifluorescence device (excitation filter: 420-490 nm; dichroic mirror: 510 nm; barrier filter: 515 nm). In these conditions, the cellulosic cell walls appear red (stained by Coriphosphyne O) whereas Berberine sulphate causes bright yellow fluorescence of lignified cell walls (Brundrett & al. 1988). Transverse leaf sections were stained with 1% toluidine blue and observed with a Leitz Orthoplan light photomicroscope. Unembedded leaves were stored in absolute ethanol and abaxial and adaxial surfaces were observed and photographed under light microscope. Morphometric measurements were conducted using a micrometric graduated eyepiece.

Table 1. List of specimens examined and their provenance.

| **Taxon** | **Locality** |
|---|---|
| *Orchis italica* Poir. | Near the Heraklion – Festos road, Crete<br>Kolibia, Rhodes |
| *Orchis laxiflora* Lam. | Agios Nikolaos, Rhodes |
| *Orchis morio* L. | Mt. Prophitis Ilias, Rhodes |
| *Orchis papilionacea* var. *heroica* (Clarke) Delforge | Mt. Prophitis Ilias, Rhodes |
| *Orchis quadripunctata* Cyr. ex Ten. | Moclos, Crete |
| *Orchis sancta* L. | Nea Kamiros, Rhodes<br>Kalithea thermae, Rhodes |

## Results and Discussion

### Stem anatomy

Transversal sections of stem showed lack of hairs and reduced thickness of cuticle. Epidermal cells appeared isodiametric with some stomata apparatuses. The external part of cortex contained few layers of chlorenchyma, while the inner one, observed under fluorescent light at the conditions reported above, showed a bright yellow fluorescence, localized in lignified cellular walls (Fig. 1). These last were arranged in a mechanical-like tissue formed by a number of layers variable from species to species. Similar occurrence of sclerenchymatous layers in the cortex of the inflorescence axis, was reported by Möbius (1886) and Borsos (1980), too. The pith, the inner part of ground tissue, was always observable and composed of parenchyma with large, rounded, thin-walled cells.

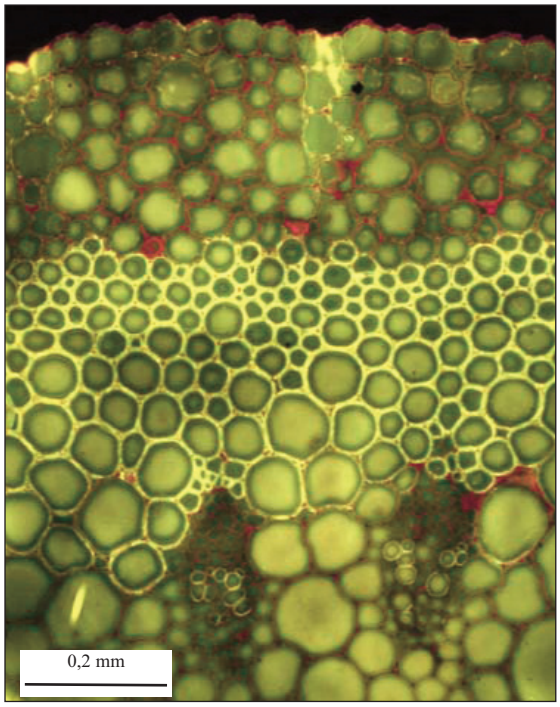


Fig. 1. *Orchis italica*. Fluorescence micrograph of stem (floral axis) cross section. The outer cortical layers show red fluorescence due to cellulosic cell walls of chlorenchyma. The inner cortical parenchyma shows bright yellow fluorescence due to the presence of sclerenchyma, consisting of lignified cell walls.

The vascular tissue consisted in collateral bundles nearly arranged in a circle, just inside the cortical sclerenchymatous sheath (Figs 1-2). As such, these can be considered an unusual stele for the monocotyledons, which generally show vascular bundles scattered throughout ground parenchyma. The same atypical arrangement was observed in some *Ophrys* species (Sgarbi & al. 2001), in *Orchis provincialis* Balb. ex Lam. et DC., *O. pauciflora* Ten., *O. colemanii* Cortesi, *O. mascula* (L.) L. (Del Prete & Miceli 1999), *O. morio* L., *Dactylorhiza maculata* (L.) Soó [sub *Orchis*], *Dactylorhiza majalis* (Rchb.) P.F. Hunt & Summerh. [sub *Orchis latifolia*], *Anacamptis pyramidalis* (L.) Rich., *Gymnadenia conopsea* (L.) R. Br., *Platanthera bifolia* (L.) Rchb. and *P. chlorantha* (Custer) Rchb. (Möbius 1886). On the other hand, an "orthodox" arrangement of vascular bundles widely dispersed, i.e. a typical atactostele, was observed in different orchids *taxa*, namely *Barlia robertiana* (Loisel.) Greuter (Sgarbi & al. 2001), *Anacamptis urvilleana* Sommier Car.-Gatto (Del Prete & al. 1991), *Platanthera cristata* (Michx.) Lindl. and in the subtribe *Habenariinae* Dressler (Stern 1997 a, b). At present the "eustele-like" organization of the vascular system seems to represent a peculiar character in the *Orchis* genus, even if it is not exclusive of this *taxon*.

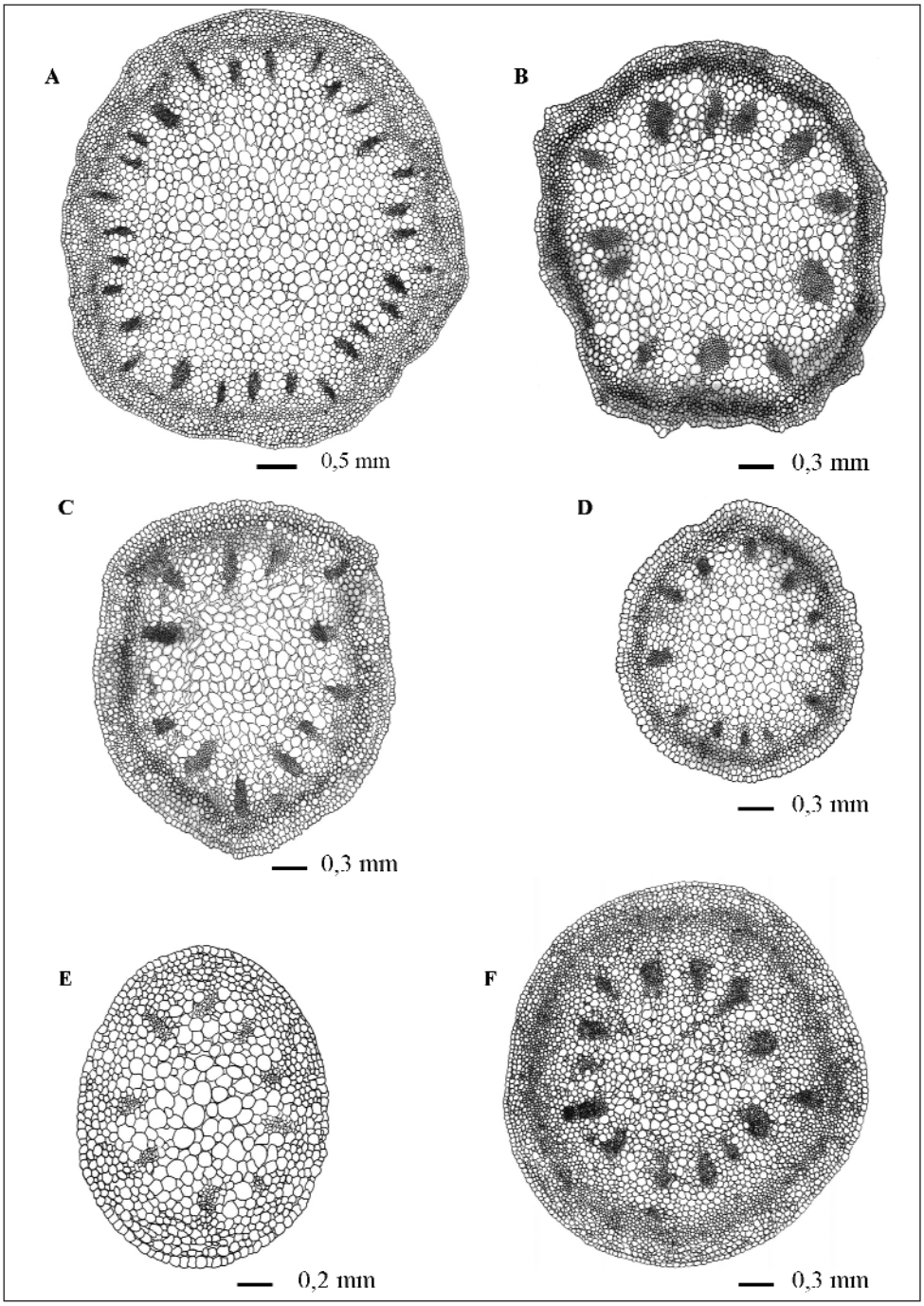


Fig. 2. Drawings of stem (floral axis) cross sections. **A,** *Orchis italica*; **B,** *O. laxiflora*; **C,** *O. morio*; **D,** *O. papilionacea* var. *heroica*; **E,** *O. quadripunctata*; **F,** *O. sancta*.

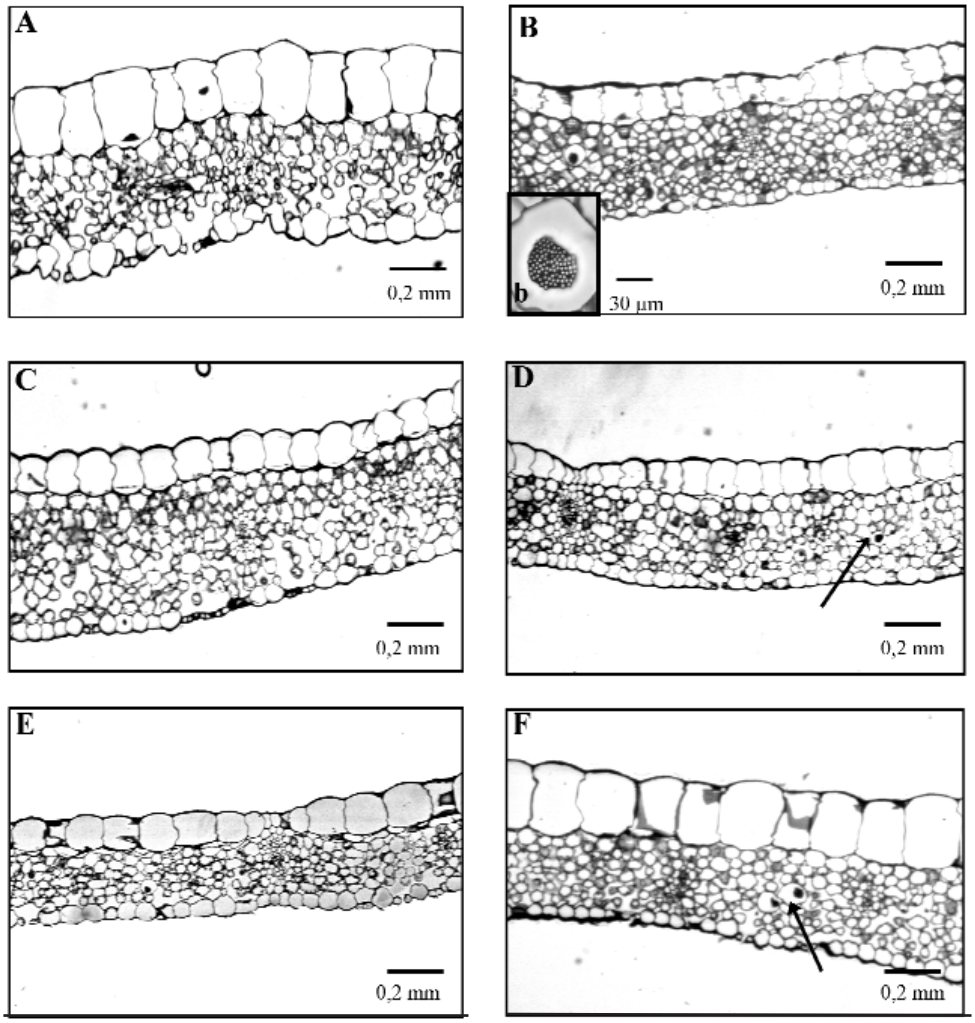


Fig. 3. Micrographs showing leaves in cross section. The arrows indicate some of the idioblasts containing raphides. A detail of one of these cells is observable in the micrograph **b**. **A,** *Orchis italica*; **B,** *O. laxiflora*; **C,** *O. morio*; **D,** *O. papilionacea* var. *heroica*; **E,** *O. quadripunctata*; **F,** *O. sancta*.

**Leaf anatomy**

Both leaf surfaces appeared glabrous and both outer walls covered by cuticle (Fig. 3). Stomata were prevalently anomocytic, sometimes tetracytic, disposed on lower epidermis only at epidermis level (Fig. 4). All specimens showed low stomata density, always below 100 stomata per square mm and very large stomata apparatuses. Morphometric parameters of stomata apparatuses are reported in Table 2. In cross section, adaxial epidermis cells appeared approximately isodiametric and always noticeably larger than abaxial cells; this

feature was particularly marked in *Orchis italica* and *O. sancta*. Anticlinal walls, both in upper and in lower epidermis appeared wavy. Hypodermis was never observed. Mesophyll, as seen in transversal section, appeared relatively homogeneous, prevalently compact in the upper part of the leaf, with few intercellular spaces and in position with substomatal chambers. Palisade, and likewise spongy parenchyma, were not distinguishable. Collateral vascular bundles were disposed in a single row in the middle part of the mesophyll, never surrounded by sclerenchymatous sheaths and never extended between upper and lower epidermis. Neither sclereids nor fibres were visible in mesophyll. Idioblasts containing raphides were frequently observed in the middle part of mesophyll (Fig.3).

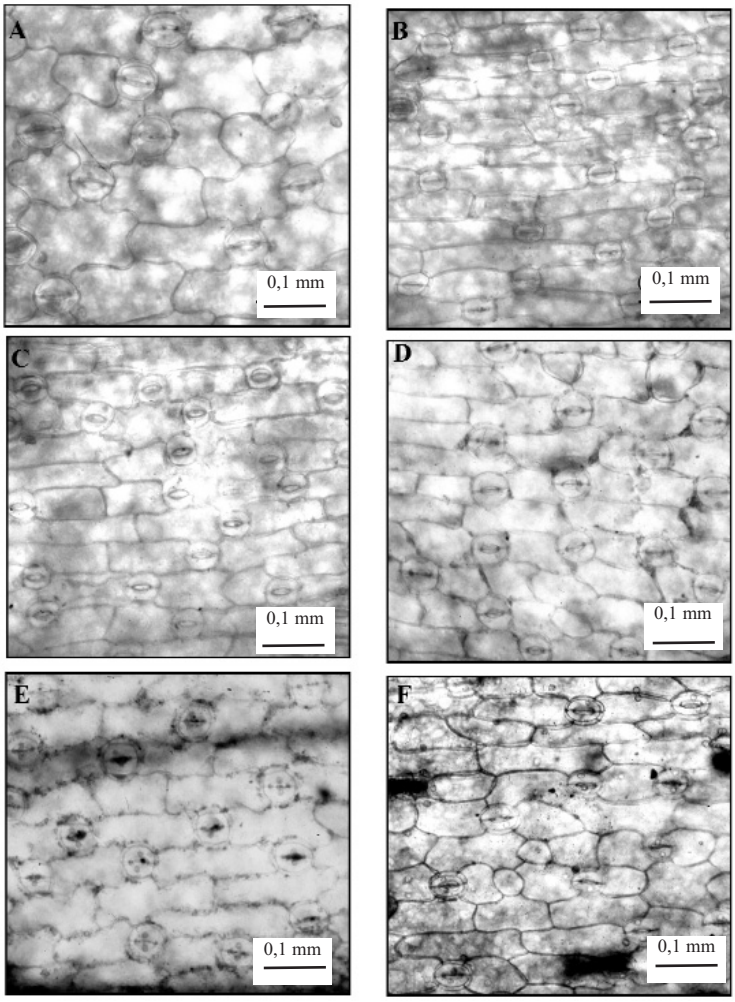


Fig. 4. Abaxial epidermis of leaves showing anomocytic and tetracytic stomatal apparatuses. **A,** *Orchis italica*; **B,** *O. laxiflora*; **C,** *O. morio*; **D,** *O. papilionacea* var. *heroica*; **E,** *O. quadripunctata*; **F,** *O. sancta*.

Table 2. Morphometric parameters of stomatal apparatuses.

| **Taxon** | **Stomata density** (n° stomata /mm$^2$ ± S.D.) | **Stomata length** (μm ± S.D.) |
|---|---|---|
| *Orchis italica* | 29,54 ± 6,7 | 83,5 ± 7,0 |
| *Orchis laxiflora* | 76,25 ± 8,9 | 60,5 ± 4,5 |
| *Orchis morio* | 65,16 ± 9,4 | 54,5 ± 4,7 |
| *Orchis papilionacea* var. *heroica* | 54,43 ± 9,0 | 60,0 ± 3,7 |
| *Orchis quadripunctata* | 48,50 ± 2,1 | 57,5 ± 4,7 |
| *Orchis sancta* | 51,87 ± 5,3 | 61,0 ± 7,0 |

Table 3. Distinctive characters of leaf structure (listed according to Roth 1984, with slight modi cations).

| **Taxon** | ***Orchis italica*** | ***Orchis laxiflora*** | ***Orchis morio*** | ***O. papil.*** var. ***heroica*** | ***O. quadri-punctata*** | ***Orchis sancta*** |
|---|---|---|---|---|---|---|
| **Leaf blade thickness *** | thin 440 μm | thin 460 μm | thin 520 μm | thin 400 μm | thin 360 μm | thin 390 μm |
| **Upper epidermis** -cuticle | thick; 6-8 μm | thick; 4-13 μm | thick; 8-16 μm | thick; 8-12 μm | thick; 6-12 μm | thick; 6-12 μm |
| -cell size (in t.s.) | large | large | large | large | large | large |
| -water-storing | yes | yes | yes | yes | yes | yes |
| -anticlinal walls | wavy | wavy | wavy | wavy | wavy | wavy |
| **Hypodermal layer** | absent | absent | absent | absent | absent | absent |
| **Lower epidermis** -cell size (in t.s.) | small | small | small | small | small | small |
| -anticlinal walls | wavy | wavy | wavy | wavy | wavy | wavy |
| Stomata density | very low | low | low | low | low | low |
| Stomata size | very large | large | large | large | large | large |
| Level of stomata | epidermal | epidermal | epidermal | epidermal | epidermal | epidermal |
| Hairs | absent | absent | absent | absent | absent | absent |
| Vascular bundles density | low 0.7/mm | low 0.6/mm | low 0.8/mm | low 0.66/mm | low 0.7/mm | low 0.9/mm |
| -type | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. |
| Sclerenchymatous sheat | absent | absent | absent | absent | absent | absent |
| Fibers or sclereids | absent | absent | absent | absent | absent | absent |

*The leaf blade thickness was measured in the middle part of each semi-leaf.

t.s.: transversal section

n.t.: not trascurrent

Leaves are very plastic organs and leaf morphology shows adaptive responses to environmental and climate conditions, like water availability and light conditions. A number of leaf morphological characters are considered criteria of xero- meso- or hygromorphy. Some of these were taken into account to estimate ecological aspects of the plants studied here (Table. 3). Leaf structures, such as comparatively thick cuticle, large-celled upper epidermis (observed in transversal section) with probable significance of water-storing tissue and relatively compact mesophyll, with few and small intercellular spaces, were referable to xeromorphic characters. All the others features, however, were chiefly referable to hygromorphic leaves: reduced blade thickness, low stomatal density together with large stomata apparatuses size, lack of hypodermis, hairs, crypts with stomata, sclereids and fibers. Moreover, the low vascular system frequency is also characteristic of hygromorphic leaves because they do not need a high vascularization frequency to compensate for large water loss through transpiration and evaporation (Roth 1984; Dickison 2000). Bundle sheaths and other sclerenchyma were lacking in the mesophyll. These observations led to remark that in *Orchis* leaves few xeromorphic characteristics coexist with several hygromorphic ones. Mediterranean ecosystems are characterized by hot temperate climate, variable rainfall (between 300 and 1000 mm) and dry summer (Pignatti 1995). In these environments the plant species show many adaptative strategies in order to minimize unfavourable seasons. Some Mediterranean *Orchis* species produce roots and wintergreen rosette of leaves in autumn, whereas in other species leaves and floral axis sprout in spring (Rasmussen 1995). Their life history is characterized by a perennation by means of a tuber; this underground organ emerges from an axillary bud at the base of the leafy shoot, is perennial and its overriding function is to accumulate storage nutrients. During the unfavourable dry summer season, when the fruits ripe and the seeds are dispersed, the leaves wither while only the underground tuber survives. The epigeous parts of these plants grow only when there is a relative water availability, since they develop in autumn and spring. This appears consistent with the leaves' predominantly hygromorphic characters, in spite of these plants grow in a Mediterranean environment.

**References**

Borsos, O. 1980: Anatomy of wild orchids in Hungary. I. Tissue structure of leaf and floral axis. — Acta Agron. Acad. Sci. Hung. **29**: 369-389.

Brundrett, M. C., Enstone, D. E., & Peterson, C. A. 1988: A Berberine-Aniline Blue fluorescent staining procedure for suberin, lignin, and callose in plant tissue. — Protoplasma **146**: 133-142.

Camus, E. G. & Camus, A. 1928: Iconographie des Orchidées d'Europe et du bassin Méditerranéen. — Paris.

Delforge, P. 1994: Guide des Orchidées d'Europe, d'Afrique du Nord et du Proche-Orient. Lausanne & Paris.

Del Prete, C., Mazzola, P. & Miceli, P. 1991: Karyological differentiation and speciation in C. Mediterranean *Anacamptis* (*Orchidaceae*) — Pl. Syst. Evol. **174**: 115-123.

— & Miceli, P. 1999: Osservazioni istoanatomiche e tassonomiche su alcune entità centro-medi-

terranee di *Orchis*, sect. *Labellotrilobatae* P. Vermeul. subsect. *Masculae* Nevski e Provinciales Nevski (*Orchidaceae*). — Caesiana **12**: 21-44.
Dickinson, W. C. 2000: Integrative plant anatomy. — USA.
Dressler, R. L. 1993: Phylogeny and classification of the Orchid family. — Cambridge.
Möbius, M. 1886: Untersuchungen über die Stammanatomie einiger einheimischer Orchideen. — Berichte der Deutschen Bot. Gesellsch. **4**: 284-292.
Pignatti, S. (ed.) 1995: Ecologia vegetale. — Torino.
Rasmussen, H. N. 1995: Terrestrial orchids. — Cambridge.
Roth, I. 1984: Stratification of tropical forests as seen as in leaf structure. — The Hague, The Netherlands.
Sgarbi, E., Ferrari, C., Del Prete, C. 2001: Histo-anatomical observation on some eastern Mediterranean wild *Orchids*. — Pp. 134 in X Optima Meeting abstracts. — Palermo.
Solereder, H. & Meyer, F. J. 1930: *Orchidaceae*. In: *Scitaminae-Microspermae*. Systematische Anatomie der Monokotyledonen. Heft 6 — Berlin.
Stern, W. L. 1997a: Vegetative anatomy of subtribe *Orchidinae* (*Orchidaceae*). — Bot. J. Linn. Soc. **124**: 121-136.
Stern, W. L. 1997b: Vegetative anatomy of subtribe *Habenariinae* (*Orchidaceae*). — Bot. J. Linn. Soc. **125**: 211-227.

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