

Review

Description and morphometric analysis of a novel species of *Ophrys* (Orchidaceae) from Hymettus, Greece

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An orchid from Mt. Hymettus in Athens, Greece was isolated and described as new in science. The plant, *Ophrys dicipulus* (sp. nova), a scolopaxoid orchid, morphologically related to the *Ophrys heldreichii* group was encountered in three distinct locations within an enclosed and previously uncharacterized area of 259000 m² on the slopes of Mt. Hymettus. The study to taxonomically define the plant was mediated through its morphometric analysis and geographical distribution. The plant was found to be of greater size with mature specimens displaying an average 10 cm difference in height than the tallest specimens of its closest morphological relative, *Ophrys schlechteriana* and displaying novel characteristics for the genus (lack of pseudoeye formation on its stigmatic cavity, smaller stigmatic cavity relative to its size as well as lateral lobe differentiations). Furthermore, the plants distribution was not shared with any other species of the *Ophrys* genus revealing its distinctive geography and ecology.

Key words: Ecosystem, morphology, Mt. Hymettus, new species, *Ophrys dicipulus*, taxonomy.

INTRODUCTION

Following a project for the characterization of the campus ecosystem of the Hymettus chapter of the American College of Greece (ACG), a new species of orchid belonging to the sexually deceptive orchid genus *Ophrys* (Orchidaceae) was discovered and characterized.

The genus *Ophrys* consists of a large number of distinct Orchids. Most notably these plants are recognized by their flowers resembling the bodies of hymenoptera and other insects which they rely on for their pollination. *Ophrys* orchids are very common throughout the Mediterranean region extending up to the southern regions of Scandinavia. These plants rely on sexual deception of their pollinators as a reproductive strategy. Flowers of the genus resemble morphologically and chemically a virgin, receptive female of their corres-

ponding pollinator insect. A male of the receptive population is stimulated into pseudo-copulation. This effect is achieved both through the use of volatile chemicals (allomones) produced in mimicry of the female pheromones released by the pollinator species and through visual stimuli provided by the structure and texture of the flowers highly modified dorsal petal, the labellum to imitate various prototypical female signals (Streinzer et al., 2010; Sciestl et al., 1999; Devey et al., 2008). During this act, the male insect attaches itself to the flower and its body is exposed to the pollinia of the plants column. The insect, which upon termination of its association transfers the material to the stigmatic cavity of another flower when enticed again, acts as the pollinator of the species. The pollination of the majority of *Ophrys* species

relies heavily on individual species of pollinator and in most cases with high specificity (Kullenberg, 1961).

Taxonomically, the genus belonging to the family Orchidaceae, subfamily orchidoideae, tribe Orchidae, subtribe Orchidinae, consists of an indeterminate number of different species with 142 already recognized (Delforge, 2005) and new species being continually described, making its taxonomy confusing in the absence of full molecular phylogenetic data (Soliva et al., 2001). The new *Ophrys* presented here morphologically belongs to the *heldreichii* group. This group includes 13 species and two varieties, of which the following are distributed in Greece (in alphabetical order): *Ophrys calypsus*, *Ophrys chiosica*, *Ophrys colossaea*, *Ophrys heldreichii*, *Ophrys homeri*, *Ophrys polyxo* and *Ophrys schlechteriana*, with only the latter representing the group in mainland Greece (Delforge, 2005), yet being different in morphology and distribution to the new *Ophrys* of Mt. Hymettus.

Hymettus is a mountain of the Attica prefecture of Greece. It forms the eastern physical border of the Athens plateau extending from Agia Paraskevi to Voula municipalities. The geological formations of the mountain belong to the Attica unit of the Attic-Cycladic zone. Composed mainly of lime stone, schist and marble, the mountain has shallow soils (less than 30 cm) demonstrating intense erosion and are characterized by a pH greater than 7 (Gouvas, 2001). The flora of the mountain is extensively diverse demonstrating well over 700 different taxa (Zerlentis, 1965; Gouvas and Theodoropoulos, 2007). Furthermore, over 41 different species of Orchidaceae members of the *Anacamptis*, *Ophrys*, *Orchis* and *Serapias* genus have been identified.

The ecosystem of the ACG, comprising of approximately 260,000 m² of the western slopes of Mt Hymettus, is restricted and partially isolated from mammalian herbivores through a series of fences, walls and building works. Furthermore, extensive cultivation of various non-native plantations has altered the biodiversity of this microsystem. Extending north to northwest of the Hymettus Mountain, its more than 47 years of existence and protection has so far been uncharacterized with respect to the micro-system that has developed. An early indication of the importance of the project to quantify and characterize this territory is the discovery of the novel species of *Ophrys dicipulus*, identified in three locations on the campus and up to this point appear limited to this specific area.

DESCRIPTION OF SPECIES

Description

Terrestrial, perennial, herb, 290 to 470mm tall *RHIZOME* tuberous subterranean with spherical tuberoid on short stolon. Stem is erect, single, 4 mm, glabrous, enveloped by tubular sheath at base. Leaf basal rosette at base (32

to 87 mm) with upper leaves (17 to 31 mm), erect, enveloping flowering stalks, lanceolate, entire with parallel venation. Inflorescence 4 to 9 flowers, unbranched, elongated. Flowers are orchidaceous, hanging, bracts leaf-like elliptical 11-14 x 3-5 mm, larger than the inferior to the inflorescence ovary. Sepals 3, identical ovate-lanceolate to triangular, 12-19 x 4-8mm, slightly keeled towards inflorescence or forming a hood, pink to light violet dissected through single thick green vein, identical, dorsal sepal erect in right angle to the lateral. Petals are 3, erect, arranged at 120° angles; lateral petals are inconspicuous, triangular 4-6 x 2 mm pink to light violet. Labellum is composed of one central and two lateral lobes. Central lobe is pubescent outside, glabrous inside, oval, heavily curved behind the median region 17-22 x 12-16 mm at widest point, lateral edges of the lobe are 2-4 mm apart, intricate sixicolor including brown, red-brown, orange to orange-brown and light yellow green with blue-gray speculum, orange pigmentation marking the center of the labellum on a pale yellow floor, extended apical appendage, leaning forward, 3-4 x 3-4 mm with 3 points. Lateral lobes are very prominent, elongated extending proximately to 6-10 mm with fine, curved tip at the apical side. Base is tomentose, brown on the outside, slightly brown pigmented green inwards, width of 5-6 mm, apical region pointed, hispid, light green. Column is elongated, 10-15 x 3 mm extending forward, pronounced, light green covering the red brown stigmatic cavity; pollinia are two, with dark-green to brown pigmentation equilateral to viscidium. Scent: grassy, extremely faint to none, unattractive for *Homo sapiens* pseudo-copulation. Phenology: Flowering takes place from April to mid-May and fruiting from May to early June.

Type

Greece: Attica Prefecture, Aghia Paraskevi, NNW root of Mt Hymettus on shallow soil 24 cm, pH 5.8, on top of rocky limestone in openings of pine-tree forest. Longitude: 38.0040 N, 23.8335 E Altitude 281 m, 5 April 2011, Valahas. Holotype (ATH) isotypes (ACG collection) (Figure 1).

Etymology

O. dicipulus is named after the locality of the species namely the three pockets inside the grounds of an academic institution on a height overlooking classrooms.

Additional specimens examined

Greece: Attica Prefecture, Aghia Paraskevi, NNW Mt. Hymettus altitude 278 m 38.0040 N, 23.8335 E, Valahas (ACG collection) (Figure 2).

Habitat and ecology

The new *Ophrys* was found in three locations (Figure 3a,



Figure 1. Holotype of *O. dicipulus* from Mt. Hymettus, flowering (photo 11.05.2011 M. Valahas).

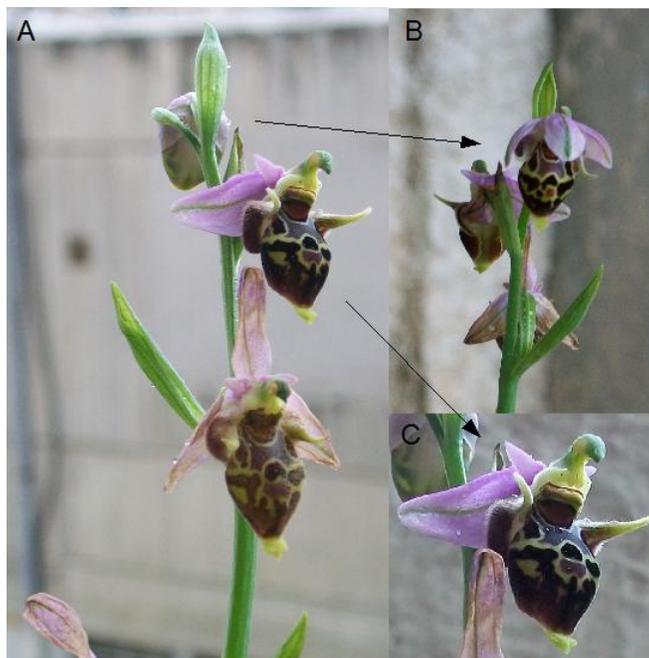


Figure 2. Inflorescence of *O. dicipulus* isotype brought into cultivation at the American College of Greece laboratories (photo 11.05.2011 M. Valahas) (A). Top flower of the same plant displaying the pigmentation characterizing the inflorescence and the distance that separates the edges of the folded medial lobe of the flower (photo 13.05.2011 M. Valahas) (B). Magnification of (A) the penultimate flower of the plant displaying the lack of pseudo-eyes that characterizes the species as well as the hirsutiness of the lateral lobes of the labellum (C).



Figure 3. Map and localities (image cropped from Google Maps(c) bar represents 50 m) of the three identified *O. dicipulus* populations. Holotype (ATH) in location (a) and isotype (ACG collection) in location (b) (photo of locations 11.05.2011 M. Valahas).

b and c) consisting of a: 16, b:7 and c:13 flowering stems with a close proximity to each other dispersed in an area of less than 500 m² all within a restricted 259000 m² region of the mountain slopes of Mt. Hymettus. No major project for the characterization of these grounds has been previously conducted.

Abiotic factors

Abiotic consistency for the area is maintained throughout and similar to that of the western slope of the mountain for sunlight and rainfall. The areas flanking the western parts of the *O. dicipulus* populations have been subject to extensive urbanization and building development in the past 20 years adding material to the limestone and schist formations of the mountain. Soil pH in the proximity of the holotype (radially 10 cm three measurements in 120°) ranging from 5.7 to 6.0 (measured on site).

Biotic factors

Taxa occurring in proximity to the populations of *O. dicipulus* represent typical limestone flora of Mt. Hymettus. Some have been introduced after extensive forestation, these species are indicated as including (in alphabetical order): *Anthyllis hermaniae*, *Arbutus unedo*, *Asphodelus aestivus*, *Calicotome villosa*, *Cercis siliquastrum*, *Cistus parviflorus*, *Erica manipuliflora*, *Myrtus communis*, *Nerium oleander*, *Olea europea (i)*, *Pinus Brutia (i)*, *Pinus Halepensis*, *Pinus pinea*, *Quercus coccifera*, *Robinia pseudoacacia*, as well as a staggering variety of wildflowers including orchids with the notable

absence of any other scolopaxoid orchid within measurable range.

The locality of the populations as that of the entire ACG area is guarded by large herbivores and human activity through an extensive network of fences. Moreover, the presence of a large number of *Canis lupus* and feral *Felis catus*, as well as the occasional sighting of *Vulpes vulpes* has led to the exclusion of the vast numbers of *Leporidae* that dominate other parts of the mountain.

DISCUSSION

In terms of gross morphology, *O. dicipulus* has distinct features as well as a completely isolated geographical position from other long horned scolopaxoid *Ophrys* species and members of the heldreichii group. *O. schlechteriana* (Devillers et al., 2004; Delforge, 2005; Antonopoulos, 2004), is the only member of the group that shares a Greek mainland distribution and appears to be related to the larger *O. dicipulus* (Figure 1). *O. dicipulus* is substantially greater in height than *O. schlechteriana* (100 to 320 mm only rarely extending to 420 mm) in cases more than 100 mm and flower size (*O. schlechteriana* 15-18 mm, *O. dicipulus* 17-22 mm). Table 1 displays a comparison between the two species. Sepals size appear similar in size, yet different in structure as the extending oblique angle from the base is more pronounced resulting in a triangular-lanceolate shape (Figure 2A and B). Lateral petals of *O. dicipulus* display substantial difference in morphology (lanceolate-triangular fashion of the sepals is reversed) and the size (one of the main distinguishing characters of *O. schlechteriana*) being smaller not only proportional to the

Table 1. Important morphological and qualitative differences between *O. dicipulus* and *O. schlechteriana*.

Characters	<i>Ophrys dicipulus</i>	<i>Ophrys schlechteriana</i> (Soó) Devillers-Tersch. & Devillers
Height	280-470 mm	100- 320 mm
Inflorescence	(4-9)	(4-10)
Sepals	3,ovate-lanceolate to triangular (12-19 x 4-8) mm	3 Ovate-lanceolate (13-16 x 6-10 mm)
Petals	3, ovate-lanceolate (3.5- 5 x 2-4 mm)	3, Ovate-lanceolate to triangular (4-8 x 2.5-4 mm)
Labellum	<i>hanging</i>	Sub-horizontal to hanging
Size	17-22 x 12-16 mm (<i>Lateral edges typically 3-4 mm apart</i>)	15-18 x 13-16mm (<i>Lateral edges in contact</i>)
Pigmentation	<i>Highly intricate and irregular formation of yellow to pale green inside, brown, red-brown, orange to orange-brown, orange and light yellow green with blue-gray speculum</i>	<i>Regular formations of yellow to pale green inside, brown, red-brown, orange-brown and light yellow green with blue to violet speculum</i>
Stigmatic cavity	Small, <50% to the width of the lip	Large, >60% to the width of the lip
pseudo-eyes	None	Yes, obvious black brown shiny
Cornuta (pronounced lateral lobes)	2	0 or 2
Morphology	<i>Always pronounced base is tomentose apical region pointed, hispid to glabrous</i>	<i>Occasionally pronounced, tomentose throughout</i>
Size	6-10 mm	3-8 mm when existent

labellum, but also actual dimensions (3.5- 5 x 2-4 vs. 4-8 x 2.5-4 mm). In addition, all *O. dicipulus* flowers are substantially more intricate in pigmentation and pattern including extensive orange-brown pigmentation below its dark navel uncharacteristic of *O. schlechteriana*. *O. dicipulus* lacks the formation of pseudo-eyes encountered in other members of *O. heldreichii* group instead it displays a dark pigmentation equilateral to the viscidium, appearing darker as the flower matures. Furthermore, the most pronounced difference is that *O. dicipulus* displays a modest, in comparison to its labellum (50%), stigmatic cavity than *O. schlechteriana* (typically more than 60%) and smaller petals in comparison with its sepals and labellum (Figure 2C).

The species has up to this point been only identified in the locations mentioned above. While extensive investigation of the northern slopes of Hymettus have revealed *Ophrys* species including *O. lutea*, *O. scolopax*, yet none of them in the protected area, members of *O. dicipulus* have been yet isolated anywhere. The area contained within the campus of ACG is relatively virgin as it is inaccessible from the periphery due to an intricate fencing project and access from the campus is restricted to staff and students. Furthermore it has been guarded from the major herbivores and other large non burrowing animals for over 45 years. Forestation projects and works conducted in the area have resulted into a unique hybrid

of naturally occurring trees and introduced species. *O. dicipulus* was retrieved from soil that was remarkably acidic for Mt. Hymettus with an average pH value of 5.8. Furthermore, extensive building works on the periphery have introduced substantial amounts of marble dust to augment the limestone profile of the mountain (Figure 3). All the above elements and observations have contributed to providing novel conditions and selective pressures for native species thus creating a distinct microsystem within the greater Hymettus ecosystem.

Following these findings, extensive molecular studies are already underway to further characterize *O. dicipulus*, to fully address its taxonomical position by contrasting it with the molecular markers of other seemingly related species (*O. heldreichii*, *O. schlechteriana* and *O. colossaea*) and to locate the genetic factors responsible for the unusual pigmentation of its flowers and its general divergence. Additionally, studies during next spring will be initiated in order to identify the *O. dicipulus* pollinator. Of potential importance was the surprising finding that upon removal of specimens from two distinct populations with a separation of 48 m in association with the rhizome, two egg chambers of ant colonies were revealed in direct association with the root of the plant. The ants that inhabited the rhizome were small, approximately 2 mm and numerous. While the first finding was considered coincidental and left uncovered, the second colony was

quickly covered with soil to prevent further exposure. This observation will be investigated further during the next growing season.

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