

Ophrys insectifera L.—Update of the Status of a Critically Endangered Orchid in Bulgaria

Andrey Popatanasov

Faculty of Biology, Plovdiv University “Paisij Hilendarski”, Plovdiv 4000, Bulgaria

Abstract: Aim: *Ophrys insectifera* L. due to its peculiar biology and environmental requirements is among the most threatened and endangered vascular plants in Bulgaria. On the territory of Bulgaria the species fulfills the IUCN criteria for critically endangered [CR C2a(i); D] and is included also in the Red Data Book of Republic Bulgaria and Biodiversity Act. The present study aimed to explore and evaluate the status of its populations and their threats. Materials and methods: Exploration and monitoring of the populations of the critically endangered orchid *O. insectifera* in Bulgaria was done from 2012 till 2017. The shoot count and some morphometric parameters were recorded for evaluation of their distribution and population dynamics. Results and discussion: At one location *Ophrys insectifera* can be considered extinct. However two new locations were found so the total number of known locations is increased. Approximately over 50% of the occupied territories fall outside protected sites and many of the habitats are endangered by anthropogenic or other factors. At all of the known locations there are less than 50 shoots per place. These facts put a high stress on the perspectives of their populations’ survival in Bulgaria.

Key words: *Ophrys insectifera*, fly orchid, bioconservation, conservation, orchids.

1. Introduction

Ophrys insectifera (fly orchid) is one of the rarest and most threatened plants in Bulgaria. It is a perennial plant with two egg-shaped tubers. The stems are 15-40 (60) cm tall, with 2-5 rosette leaves and 1-3 stem sheathing-leaves. Inflorescences have 2-10 (15) flowers. Sepals are yellowish green and spread, 6-9 mm long and 3-4 mm wide. Both petals are linear-filiform, purple-brownish, velvety 4-7 mm long. The labellum is brown with middle whitish area, velvety, three lobed, 9-12 mm long, 6-10 mm wide. The flower resembles the body of a wasp, which perform the pollination. It flowers in May-June and fruits in June-July. Propagation is by seeds [1, 2]. It can be found in fens, grassland, wet meadows, shrubs, open pine woods and wooded meadows, moss and sedge swamps, rarely on peat-bogs. It grows in dry to wet, calcareous to neutral soil, in full sunlight to moderate shade [1-4].

The largest biodiversity of *Ophrys* genera is in the Mediterranean region but the areal of *O. insectifera* starts at its north border. The species has the northernmost distribution in the genera—from North Greece till Karelia in Russia and from Ireland to Mt. Ural [1, 3-6]. Although its areal is in well studied region and its rather early records (1597 by Gerard [7]) there are still big countries like Belarus where it was found just in 21st century [3]. This is probably due to its extreme rarity, low numbered populations and relic disjunctive areal. On the Balkans the species was reported for: Romania in 1915 [8], Greece in 1940 [9], but for Serbia in 1989 [10] and for Bulgaria in 1978 at Mt. Golo Bardo and “re-discovered” there in 2000 by Petrova and Venkova [11]. Shortly afterwards in 2004 new locations were found in south-central Bulgaria—at Buynovsko gorge by Vladimirov and Trigrad gorge by Tsvetanov [11]. In 2014 Popatanasov reported the last two locations—in West and Central Mt. Rhodopes [12].

In the past the orchid was common in middle and north Europe but with the dramatic rise of the

Corresponding author: Andrey Popatanasov, Ph.D.
candidate, research field: biology. E-mail:
and_atanasov@abv.bg.

human-used lands most of its habitats were lost and nowadays it is very rare [1, 4, 7]. Currently IUCN assigns LC globally however in many countries it has CR status [4]. In Bulgaria the species fulfills the IUCN criteria for CR-D status with tendency for CR-B [13] and is protected by the Biodiversity Act, also it is included in the Red Book of Bulgaria and Red List of the Bulgarian vascular plants [2, 13].

2. Materials and Methods

The study sites are located in the Znepole floristic region (Mt. Golo Burdo), Mt. Rhodopes West (Buynovsko gorge, Derinkouk gorge and Trigrad gorge) and Mt Rhodopes Central floristic regions (Chairite gorge) (Fig. 1). The central point coordinates and altitude of the locations are: Mt. Golo Bardo—N 42°32'42.72"; E 23°02'30.33", 990 m a.s.l.; Buynovsko gorge (subdivided in two parts)—1/. N 41°37'58.86"; E 24°20'29.77", 1,100 m a.s.l.; 2/. N 41°37'58.28"; E 24°20'11.34", 1,050 m a.s.l.; Trigrad gorge—N 41°37'09.51"; E 24°22'48.06" and 1,100 m a.s.l.; Derinkouk gorge—N 41°40'39.27"; E 24°20'37.49", 980 m a.s.l.; Chairite gorge—N 41°35'40.85"; E 24°26'13.81", 960 m a.s.l. The location at Mt. Golo Bardo is in the temperate continental and the ones in Mt. Rhodopes are in the mountain climate region [14].

Exploration and monitoring of the populations of *O. insectifera* in Bulgaria was done from spring 2012 till autumn 2017. The shoot count and flowers per shoot were recorded for evaluation of their distribution and population dynamics.

The marking of the populations of the species was done using GPS receiver Garmin Colorado 400 and/or Solmeta Pro Geotagger. The mapping and the measurement of the distances were done with the software Garmin BaseCamp ver 4.6 and SASPlanet ver. 15.

The data were processed with Originlab Origin Pro ver. 7 and Microsoft Office ver. 2007.

The species in the habitats were determined using the Identification guide of the plants in Bulgaria [15], Flora of Republic Bulgaria [16], Guide to the mosses of Bulgaria [17]. The names are given according Conspectus of the Bulgarian Vascular Flora [18]. The soil determination was according to Soils of Bulgaria [19]. The vegetation structure was characterized according to Edwards' structural classification [20] and the other characteristics of the vegetation cover were estimated according to the Braun-Blanquet method [21, 22]. Presented are only the most abundant and/or characteristic species for the habitat descriptions and analysis which had constant occurrence at the observed spots during the monitored period.

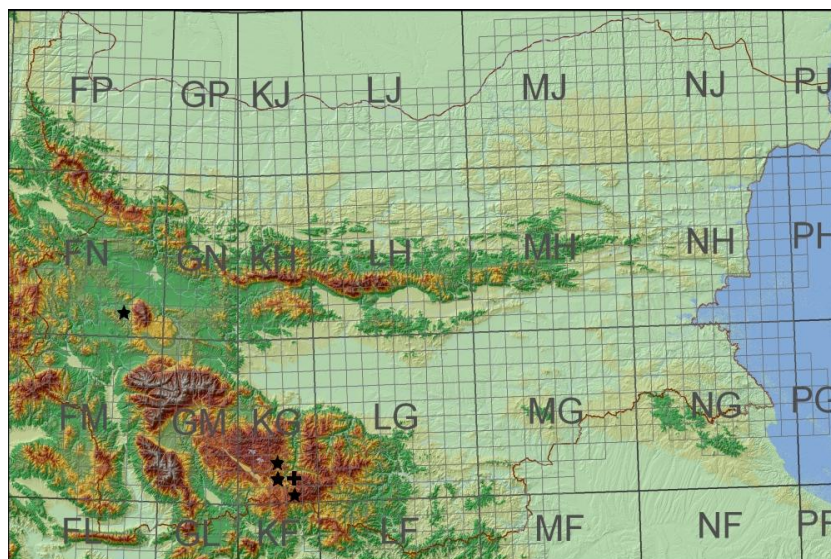


Fig. 1 Distribution of *O. insectifera* in Bulgaria (black star—confirmed locations; black cross—extinct).

3. Results and Discussion

The location at Trigrad gorge was visited several times since 2012 during the flowering season however no plants were found—neither germinative nor vegetative, so the species can be considered extinct from this site.

The location at Buynovsko gorge comprises two sublocations separated by few hundred meters in two close but different habitats. The first is on north facing slope at the transitory zone of the inclination where there is big inclination change ranging from 10 to 30 degrees. It is an old shady mixed coniferous forest dominated by *Pinus nigra* and fewer *Abies alba*. The forest has unusually well developed and spread rather dense and thick moss ground-cover layer with nearly full coverage. It comprises from such large and tall moss species as *Rhytidiadelphus triquetrus*, *Hylocomium splendens* etc., which is relatively typical element for the north temperate and boreal coniferous forests [23, 24]. All of the orchids at this sublocation grow among the mosses. The herbaceous layer is poorly developed and comprises of such taxa as *Sesleria latifolia*, *Euphorbia amygdaloides*, *Geranium sanguineum*, *Hieracium murorum*, *Monotropa hypopitys*, etc., and the rare orchid—*Goodyera repens* [12].

The second sublocation is on a steep north-west facing slope at the transitory high open shrubland located between an upper mixed closed coniferous forest and a lower steeper closed beach forest. The sparse formations of low height trees and shrubs are formed by *Ostrya carpinifolia*, *Fagus sylvatica*, *Juniperus communis*, *Genista carinalis*, *Abies alba*, etc. The grassy openings and herbaceous layer comprise of *Sesleria latifolia*, *Poa pratensis* as dominants and fewer *Centaurea triumfettii*, *Globularia aphyllanthes*, *Dorycnium herbaceum*, *Astragalus monspessulanum*, *Knautia drismaeja*, *Hieracium murorum*, *Leontodon crispus* etc.

Soils are brown forest type (eutric and dystric

cambisols) over calcareous rocks.

The location at Derinkouk gorge near Kastraklii Reserve is tall open shrubland at the transitory zone between closed coniferous forest with dominant *Pinus nigra* and calcareous rocky slopes with chasmophyte vegetation. At the site there is pronounced slope inclination change ranging from 10 to 20 degrees. There are well developed patches of moss layer of *Hylocomium splendens* etc. at the appropriate sites of the rocks providing water accumulation and reducing the evaporation and soil erosion. The shrubland comprise from low height *Pinus nigra*, *Fagus sylvatica*, etc. The herbaceous layer has unusual dominant elements as *Gymnadenia conopsea* and *Dactylorhiza saccifera* and fewer *Hieracium vulgatum*, *Campanula rapunculoides*, *Geranium sanguineum*, *Euphorbia amygdaloides* etc.

The Derinkouk gorge habitat and adjacent areas are notoriously rich of forest terrestrial orchids some of which are quite numerous in quantity. In this area can be found rare and protected species as *Cypripedium calceolus* [25] along with more common ones as *Cephalanthera rubra*, *Corallorhiza trifida*, *Epipactis helleborine*, *Epipactis atrorubens*, *Platanthera chlorantha*. Also at some areas the orchids population density is relatively high for this family in the country easily outscoring 20 shoots/m² (or much more at some spots) which resembles the presence of rather rich and moist soil with well developed mycoflora that can support such number of partly myco-heterotrophic plants as a recent study reveals [26].

Soils are litosols, rendzic leptosols and brown forest type (dystric cambisols) over calcareous rocks.

The location at Chairite gorge is situated in old open mixed coniferous forest of *Pinus nigra* and fewer *Picea abies*, *Abies alba* etc. The herbaceous and shrub layer is poorly developed and is presented by *Alopecurus pratensis*, *Sesleria latifolia* and fewer *Polygala vulgaris*, *Euphorbia cyparissias*, *Euphorbia amygdaloides*, *Juniperus communis* etc.

Soils are brown forest type (dystric cambisols) over calcareous rocks.

The location at Mt. Golo Bardo is tall open shrubland comprising of *Crataegus monogyna*, *Carpinus orientalis*, *Genista januensis* etc. The most common herbaceous species are *Carex humilis*, *Artemisia alba*, *Filipendula vulgaris* and fewer *Globularia aphyllanthes*, *Dorycnium herbaceum*, *Centaurea orientalis*, *Euphorbia cyparissias*, *Hippocrepis comosa*, *Hyacinthella leucophaea*, etc.

Soils are litosols and rendzic leptosols over calcareous rocks.

During the observed period this polycarp species showed typical for many European orchids fluctuations in the shoots number.

The population at the location at Derinkouk gorge showed little overall fluctuations in the number of shoots with slight prevail of the germinative over the vegetative shoots (Fig. 2).

The population at the location at the Chairite gorge showed more pronounced fluctuations in the overall number of shoots with slight prevail of the vegetative over the germinative shoots (Fig. 3).

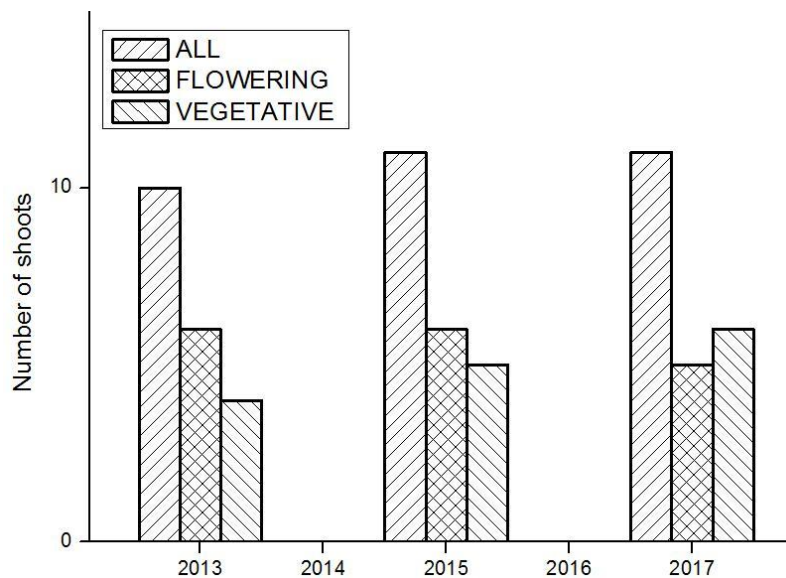


Fig. 2 Population dynamics at Derinkouk gorge location.

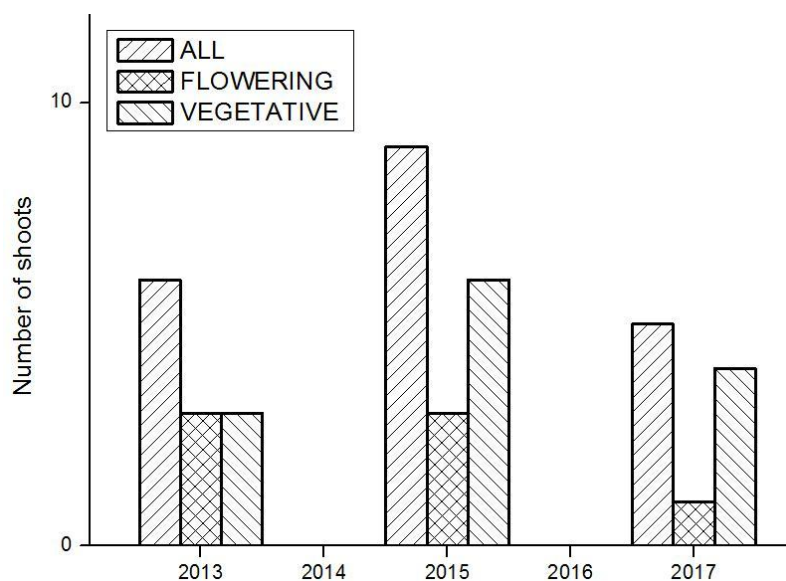


Fig. 3 Population dynamics at Chairite gorge location.

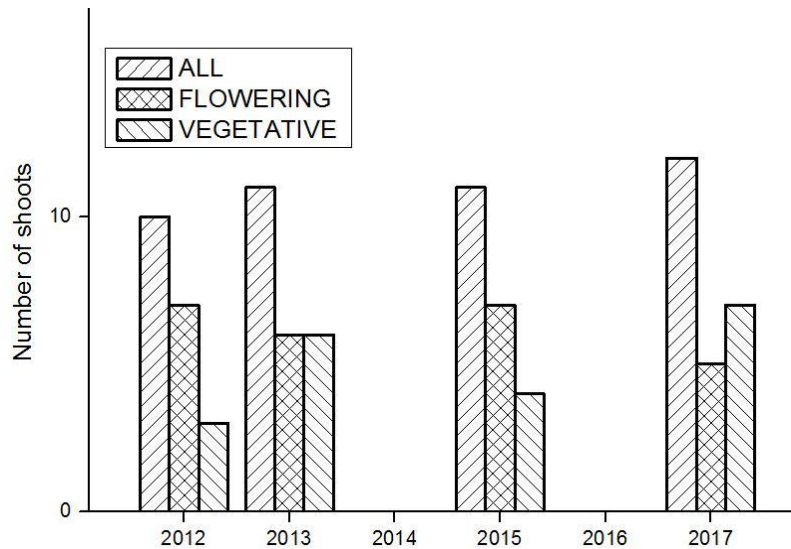


Fig. 4 Population dynamics at Buynovsko gorge sublocation 1.

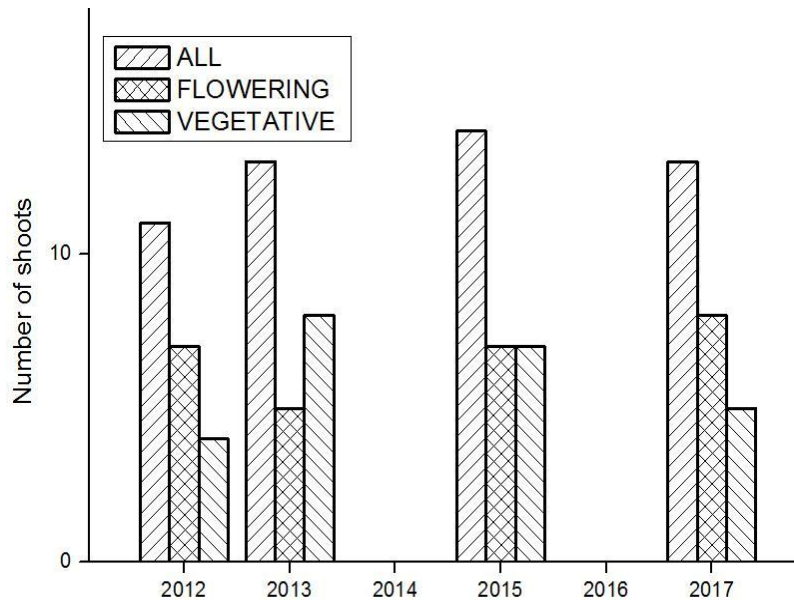


Fig. 5 Population dynamics at Buynovsko gorge sublocation 2.

The population at Buynovsko gorge sublocation 1 showed minimal fluctuations in the overall number of shoots with prevail of the germinative over the vegetative shoots in most of the years during the monitoring period (Fig. 4).

The population at Buynovsko gorge sublocation 2 showed more pronounced fluctuations in the overall number of shoots than the neighbor forest population with prevail of the germinative over the vegetative shoots in most of the years during the monitoring period (Fig. 5).

The population at Mt. Golo Bardo location at showed most pronounced fluctuations in the overall number of shoots than the rest of the locations with prevail of the vegetative over the germinative shoots in most of the years during the monitoring period (Fig. 6).

All of the locations at Mt. Rhodopes are located in gorges. Gorges are unique landforms for many karst areas [27] as are these parts of west and central Mt. Rhodopes. Being narrow, steep and relatively deep clefts they have reduced length time of exposure to the direct solar radiation, specific hydrologic regime etc.

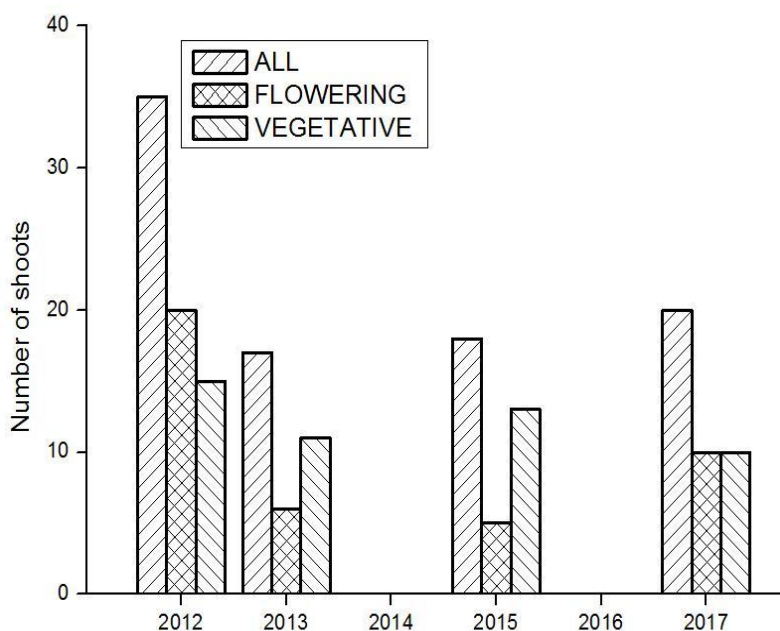


Fig. 6 Population dynamics at Mt. Golo Bardo location.

which provide conditions for formation of cold-air-pools and specific microclimate with increased moisture, reduced temperature fluctuations and protection from winds which mitigate partly the regional climate hardships and fluctuations [28-30]. Additionally the sites at Buynovsko gorge (sublocation 1) and Derinkouk gorge are on north-facing steep slopes which means they further receive reduced solar radiation compared to the other orientations [31]. This along with the canopy effects results in greatly reduced surface energy budget [32]. These microclimate and environmental specifics can explain the presence of mesic temperate elements and the development of rather thick moss layer ranging from 5 cm to over 30 cm among which a great part of the populations grow that further protects them from the droughts during the vegetation season. Additionally the relatively large inclination change accompanied with local convex micro-landforms at the sites provides conditions for increased water/moist exfiltration and accumulation [33, 34] facilitating the population's wellbeing and survival. This is in accordance with the observed population dynamics at both sites which showed minimal overall shoot

number fluctuations and prevalence of the germinative individuals over the vegetative in most of the observed years.

The locations at Mt. Golo Bardo and the sublocation 2 at Buynovsko gorge resemble more xerothermic, heliophile type phytocenosis having some common Mediterranean-Central European geoelements as *Globularia aphyllanthes*, *Genista* sp., *Dorycnium herbaceum*, *Centaurea* sp. [16, 18] which may be attributed to their similarities in exposure and light regime, slope inclination, soil thickness, altitude etc. This seems to put higher stress upon this species which prefers cooler and moister habitats as is reflected in the global distribution areal and the studies on its biology [1, 3]. This is especially true for the largest known location in Bulgaria—at Mt. Golo Bardo which in “good” years can comprise nearly 150 shoots during the flowering season [D. Venkova—personal communication], while in the observed period it did not pass 40 shoots. So it seems that the population is in suppressed state and most of the individuals reside either in vegetative or in secondary dormancy state. However these effects seem to be mitigated at the location at Buynovsko

gorge probably by its specific microclimate.

The location at Chairite gorge also presents more xeric conditions as is reflected by the presence of geoelements as *Euphorbia cyparissias*, *Juniperus communis* and the population seems to be in suppressed state.

Another important factor for this species is its specific pollinators—the male *Argogorytes* sp. wasps and the complex ecological network needed for their development and living. At several of the locations were observed some of the plants which are preferred by the female wasps as broad-leaf *Euphorbia* sp. (and in particular *E. amygdaloides*) and some umbellifers on which to feed, and appropriate sites for egg-laying [1]. This may explain the unusually high percentage of fruit set reaching over 80% at Buynovsko, Derinkouk and Chairite gorge, while at Mt. Golo Bardo the percentage is less than 30% probably because some of the elements of this complex network are missing or too far from the location of *O. insectifera*.

Perspectives: In Bulgaria the perspectives for the species survival are neutral to negative. Bulgaria is at the southernmost border of *O. insectifera* areal which means that the species here leaves at its tolerance limits toward some of the environmental variables and factors. All populations occupy less than 1 ha areas and no or minimal increase of their area was observed during the monitoring, new juvenile plants (10-20%) were observed only at Derinkouk and Chairite gorge (however juvenile plants may remain unnoticed at the other locations due to their small size and the thick/dense diverse plant and organic cover there).

The locations at Mt. Rhodopes have better perspectives for long-term survival in regard to the advance of the global climate changes and warming. They are located at deep and steep gorges which can partly decouple the local climatic conditions from the regional circulation patterns and can serve as climate changes refugia as a recent study reveals [35]. Additionally at Buynovsko and Derinkouk gorge the habitats resemble the ones from the northern

temperate and boreal zones of Europe where this orchid has the largest and most numerous distribution and therefore are most favorable for the species survival regarding its biology and environmental requirements. The populations there have low fluctuations and overall prevail of germinative shoots, high reproductive success but rather low seedling recruitment.

The location at Mt. Golo Bardo is facing rather large valley where there are minimal conditions for local accumulation of cold moist air to moisture the ground as in Mt. Rhodopes. This along with the rather shallow soil layer over a steep solid rock base makes the population wellbeing very dependent on the precipitation and its fluctuations.

Limiting factors and threats for this species are its relatively narrow ecological amplitude, overgrowth of the biotopes with shrubs, low seedling recruitment, critical myco- symbiont dependence, grazing and various anthropogenic factors. For example by anthropogenic factors (flower collection) in 2017 was damaged almost 50% of the population at Buynovsko gorge and grazing by farm herds at the adjacent territories of Mt. Golo Bardo location was observed in 2015 and 2017.

Conservation measures and recommendations: The locations at Mt. Golo Bardo and Buynovsko gorge received protected status unlike the ones at Derinkouk and Chairite gorge. Since the latter ones have stable fly orchid population and beneficiary microclimate conditions it is reasonable at least the site at Derinkouk gorge to receive protected status either by enlargement of the nearby Katraklii reserve or by creating dedicated to the species microreserve as it was the case with the site at Mt. Golo Burdo. Other actions: (1) active site management; (2) development of *ex situ* cultivation and strengthening of the populations; (3) placement of informing boards at their entrance points about the restricted activities and other informatory initiatives for the residents and tourists.

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