

## Spatial analysis of orchids diversity unveils hot-spots: The case of Zante Island, Greece

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### ABSTRACT

The current study aims to analyze the ecological niche and mapping the distribution of species belonging to Orchidaceae family, in Zante Island and to determine hotspots at sites of high species richness. 967 observations were recorded into 110 tracks in 2015 and 120 tracks in 2016, where 47 orchid species were identified belonging to 9 genera. Using Maxent, the ecological niche of each species was analyzed and habitat suitability map was created using 12 environmental parameters. The suitability maps were transformed into a binary format according to the threshold “10th percentile training presence” and the transformed maps used to compute hot-spot regions by applying the SMD toolbox using the software ArcGIS. Zante Island is characterized by remarkably high orchid species richness since the synthetic model indicates presence of at least 10 species for the 53.2% of the island area. Topography, geology and landscape openness are determining factors for orchids’ diversity conservation. Altitude is the determining factor of differentiation in species distribution. Hydro-lithology was the following most significant interpreting parameter. The mapping analysis of species abundance units revealed that 12.5% of the island surface could be characterized as hotspots of high value for the conservation of orchid diversity.

## 1. Introduction

Interactions between biotic organisms and environmental variables, such as the relation of heliophyte orchids with light intensity (Naveh 1982), are of crucial importance for ecosystem evolution especially in the Mediterranean landscape. Selecting the proper environmental parameters and collecting field work data can result in the mapping of species distribution and their habitats suitability; such maps depict the predicted spatial distribution of species at wider areas. The combination of environmental parameters and taxa recordings can generate more robust modeling outcomes of species distribution (Elith & Leathwick 2009) principally because proper quantitative methods and tools are valuable for deriving credible conclusions (Tremlová & Münzbergová 2007). Noteworthy is the fact that species occurrence modeling can produce multiple maps that are considered suited for floristic analyses because they reveal the existing reliance of species’ presence on biogeochemical requirements (Van der Maaten et al. 2012). The aforementioned are basic tools for

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protecting species and preserving their diversity in regions with hot spots of high value (Rodríguez et al. 2007).

Orchidaceae is one of the prime and most diverse plant families (Tsiftsis et al. 2008) numbering more than 25.000 species of 800 genera worldwide (Popova et al. 2016). Despite its high species number, this family exhibits the highest percentage of extinction rate in the world. In Europe orchid species are reported to have been severely declined the last decades (Kull & Hutchings 2006). In most studies orchids are mainly investigated for their anatomy (Aybeke et al. 2010), medicinal substances and ethnobotanical uses (De Vos 2010), innovations in their breeding (Popova et al. 2016) or their presence is recorded in species lists (Melendo et al. 2003), but less studied are their conservation priorities (Pfeifer et al. 2010). Hence the determination of the ecological niche is considered elementary for the conservation of Orchidaceae species (Tsiftsis et al. 2008). Related issues such as the reduction in the distribution range of orchid species have been investigated for instance in Northern Europe (Kull & Hutchings 2006).

It is widely known that the majority of orchids interact with compatible mycorrhizal fungi throughout their lives, due to lack of endosperm in their seeds. Since orchid seed lack an endosperm and consist only of an embryo and a seed coat, this association is obligate during seed germination with the mycorrhizal fungus supplying the critical nutrients for germination and continue to rely on fungi for water and nutrient to some extent at adulthood (Rasmussen 2002; Warcup 1990; Jacquemyn 2016). Mariangela Girlanda and Silvia Perotto (2011), in their research on the of mycorrhizal fungal diversity in four photosynthetic Mediterranean meadow orchids, indicates that meadow orchids may prefer specific fungal partners in natural conditions.

In parallel, it is recognized that our knowledge of orchid ecology needs to be enhanced especially in terms of their geological and altitudinal preferences (Hágsater & Dumont 1996), while usually the studies investigating orchid ecological preferences are too few (Tsiftsis et al. 2008, Phillips et al. 2011) and most often such studies are conducted after a decline in the orchid distribution ranges has taken place (e.g. Kull & Hutchings 2006). A major factor resulting in such declines is habitat loss (Fischer & Stöcklin 1997). Consequently, studying the distribution of species' abundance and how it is affected by geographical and environmental factors, as well as determining their ecological niche, is essential for enhancing the protection planning of the species of Orchidaceae family (Crain et al. 2014).

Nowadays the number of threatened species exceeds well our available resources for the protection and conservation of these species, indicating a firm need for prioritizing biodiversity protection with a focus on regions where are detected the earnest protection requirements. A promising approach is to determine 'hotspots' in areas of high endemism threatened by habitat loss and in areas of high species richness or presence of rare taxa (Myers et al. 2000). This may be particularly the case of areas undergoing fragmentation and such areas can be traditionally species-rich areas, entailing habitats that are hotspots, but due to fragmentation (or other threats) their occurring species run the risk of extinction (Piqueray et al. 2011). Species respond differently to threats like fragmentation (Lindborg 2007) and this is a main reason that species preferences are worth-investigating as they can be pivotal in conservation management (Piqueray et al. 2011).

The main aims of the study were 1) to analyze the ecological niche and mapping the distribution of species belonging to Orchidaceae family in Zante Island and 2) to determine hotspots at sites of high species richness belonging to Orchidaceae. The determination of ecological factors and the distribution of species as well as the spotting of areas of high values will lead to protection and conservation of orchid. Likewise, this study sets the basis to plan the scientific monitoring of Orchidaceae in the Ionian region.

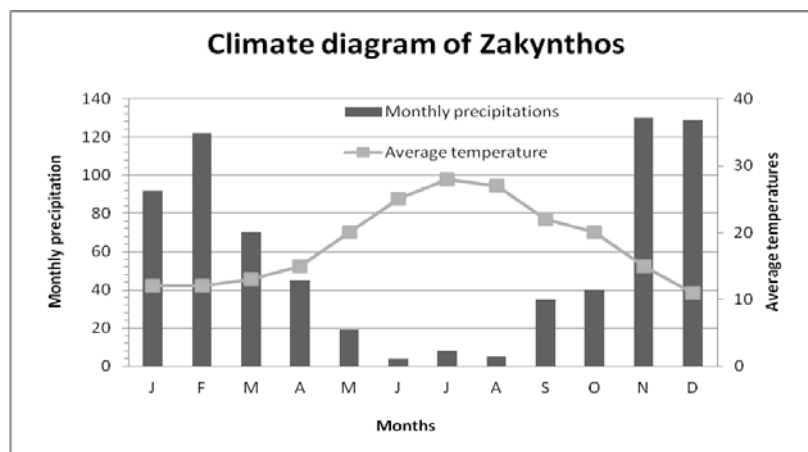
## 2. Methodology

### 2.1. Study area and floristic recordings

The Zante is located in the southwestern of Greece, in the Ionian Sea, between Greece and Italy. It is about 150 km south of Corfu (Kerkira) island and forms with Lefkada, Kefalonia, Ithaki, Paxi, Kythira and many others small islands, the Ionian archipelago (Eptanisa). Zante covers an area of 406 km<sup>2</sup> with a population of about 42,000 people (Figure 1). The study area covers all the surface of this small island, in which the climate is of Mediterranean type, with intense annual precipitation ranging from 800 to 900 mm (average of approximately 850 mm), showing a decreasing trend over time. The wet season starts in September and ends by May (Figure 2). The area is characterized by low and middle altitudes, where the largest altitude is located at the top of the mount Vrachionas and reaches 792 m. As consequence, there is an overlap of vegetation zones and the distribution of species. Thus, a high diversity of habitats is exhibited with a diverse mosaic of varied vegetation types (*Pinus halepensis* woods, shrubs, meadows, agricultural areas, olive groves, open burnt areas, sand dunes etc.).

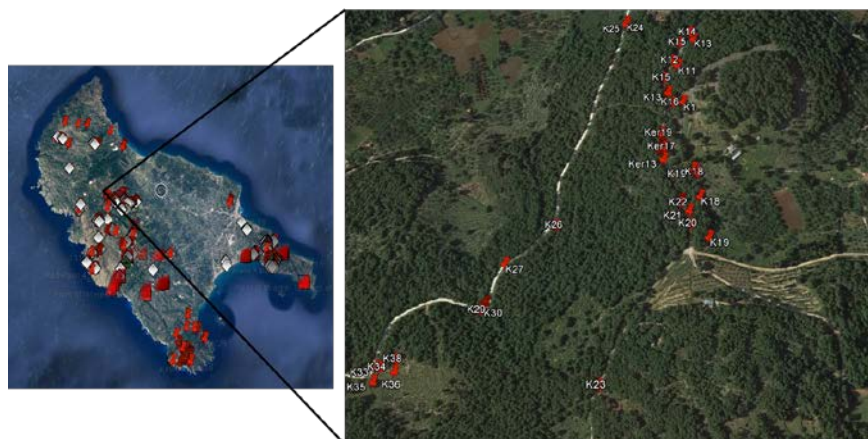


**Figure 1.** Location of Zante Island in the Ionian Sea



**Figure 2.** Average monthly precipitation and temperature in Zante Island. Temperature in Celsius Degrees (°C) and precipitation in mm.

The first recordings of orchids' distribution in Zante Island took place early in the 19th century (Delforge 1993, Gözl & Reinhard 1995). In our case, a total of 967 recordings were completed between 2015 and 2016, following different tracks with a 2-4 km length and 2-meter belts of either side of each track. Specifically, 110 tracks were trailed in 2015 and 120 tracks were trailed in 2016. All these routes were homogeneously distributed across the habitats of Zante Island (Figure 3). In totally two years of recording, were trailed 230 tracks with an overall length of approximately 650 km.



**Figure 3.** On the left, recordings in study area (white points: recordings in 2015 and red points: recordings in 2016). The right image shows some indicative recordings of orchids in the mountainous region of Loucha - Gyri (Zante Island - Greece)

Each year (2015 and 2016), orchid recording took place between mid-February and end of May. In sites of high orchid abundance, the recording was repeated (in the beginning and at the end of spring). For each sampling site, a designed protocol was used for the recording of coordinates, observed species, analytical photographical images for species documentation, specific data for vegetation and geo-topographical parameters as well as data of their conservation status and threats. In particular, the orchid species recording was conducted only using photographs and no orchid individual were sampled for conservation reasons. Orchid species were recorded at all representative biotopes of the island, open and shadowed forest, macquis, phrygana, previously burnt, and wetland ecosystems, with the aim to cover a wide spectrum of environmental parameters so that the suitability models approximate the real species distribution. The orchids were identified based on standard floras (Tutin et al. 1980) and named in line with the binomial concept of Linnaeus (Jarvis, Cribb 2009) following Euro+Med (2006-) and the Flora Ionica Inventory (Flora Ionica Working Group (2016 onwards)) cross-checked with Dimopoulos et al. (2013).

## 2.2. Data analyses and Individual models of habitat suitability

All recordings were introduced into a geographical database and individual models of habitat suitability were developed with the program Maxent (of maximum entropy) (Phillips et al. 2006), using 13 environmental parameters that affect the presence and distribution of Orchidaceae species, in three parameter groups of topography, vegetation and geology (Remm et al. 2008):

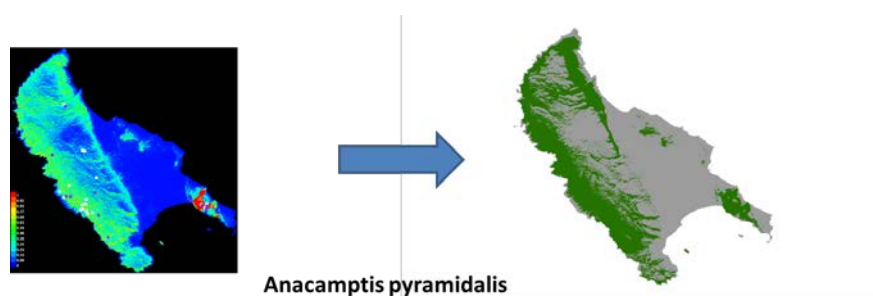
- The first group includes six parameters of topography: altitude, terrain gradient, effect of the relief exposure (quantitative northbound effect variable and eastbound quantitative variable) and relative topographic location (as a quantitative and qualitative variable).
- The second, includes four vegetation indices to characterize the density of the forest area, namely Normalized Difference Vegetation Index (NDVI), Normalized Difference Moisture Index (NDMI), Modified Soil-adjusted Vegetation Index (MSAVI), Enhanced Vegetation Index (EVI) and soil temperature as a surrogate of the openness, recorded by the thermal band of satellite images.

- The third, includes two qualitative geological parameters: lithological and hydro-lithological rock formations, with 10 and 6 classes for the formations correspondingly (Appendix 1).

### 2.3. Habitat suitability models – Hot spot analysis

Habitat suitability models were estimated for each species, using Maxent (Phillips et al. 2006). These models were based on a mean value of 10 replicates, with the method of crossvalidated. The resulted habitat suitability maps, that its scale was firstly continuous between 0 and 1, was transformed into a binary file (presence – absence) using the threshold “10th percentile training presence” to consider the environmental complexity of the region and thus depict the species presence more conservatively, where 90% of the training samples are correctly classified (Figure 4). Considering this threshold, the 10% of excluded presences are potentially represent recording errors, ephemeral populations, or occurrence of unusual microclimatic conditions within an area.

The current models for the suitability prediction for species do not require data of verified species absence but they do compare the sites of the species presence with the range of the total change of the niche in the studied area, on the basis of determined factors. However, the statistical models do not recognise the most significant factors that define ecologically the presence of a species. On the other hand, if proper factors are used in the models, it is highly probable that this will result in a proper outcome (for instance a robust distribution of the species presence), though the ecological and management importance of this outcome may only have a trivial meaning. For the above reasons, the selection of the environmental parameters was based on two criteria: a) percentage over a 5% participation in the interpretation of the species distribution, and b) degree of correlation between the selected variables less than 0.8. The best regularization number (at a scale from 0.5 to 3 in increments of 0.5) for each model generality was calculated based on the criterion AICc, and when AICc was the same in 2+ models, then the most proper one was selected, namely the model with the lowest AUC.Diff using statistics in R. Note that the highest generality value corresponding to a spread distribution is the default value of 1 (Phillips and Dudik 2008).



**Figure 4.** Transformation of the individual suitability map (left) of *Anacamptis pyramidalis* to a binary file (right).

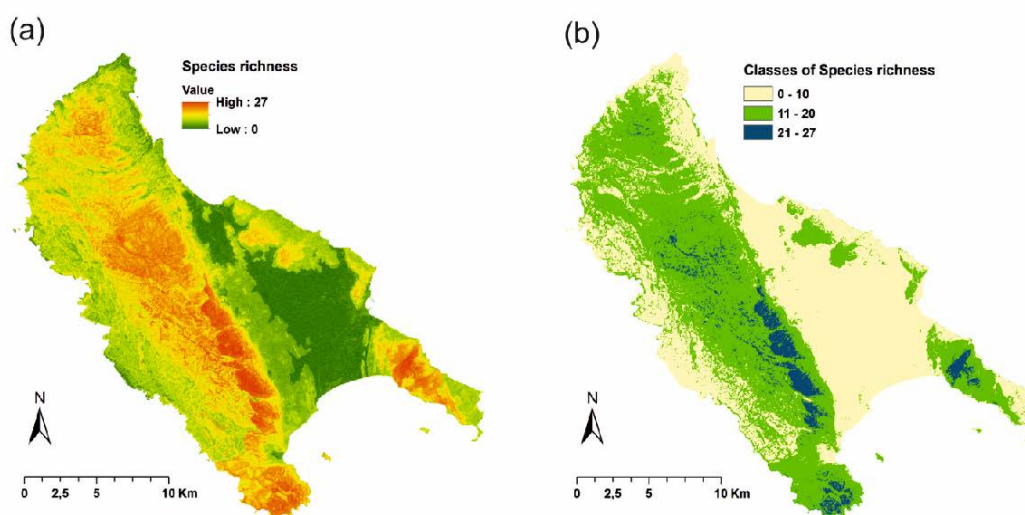
Based on the individual binary models, hot spot analysis was formed by applying the SMD toolbox (Brown et al. 2017) using the software ArcGIS (analysis at scale of 5000-meter squares). Then six spatial units based on species richness were computed via the tool Hot Spot Analysis (Getis-Ord  $G_i^*$ ) and the Euclidean distance.

## 3. Results and Discussion

### 3.1. Floristic results and Synthetic maps

Totally 47 different orchid species of 9 genera (*Ophrys*, *Orchis*, *Anacamptis*, *Serapias*, *Dactylorhiza*, *Limodorum*, *Neotinea*, *Spiranthes*, *Himantoglossum*) were identified (Appendix 2). Among them, the endemic *Serapias neglecta* subsp. *ionica*, *Dactylorhiza romana*, *Ophrys speculum*, and the rare *Spiranthes spiralis*, are indicative of the excessively high species richness of orchids in Zante. Delforge (1993) distinguished 41 species distributed across Zante corresponding to 37 species according to current nomenclature. From these 37 species, 33 were recorded in our study comprising

90% of Delforge database. From the 47 species of our study, 29 of 7 genera were found to have adequate enough data to provide reliable models. Most of the models found to perform well with AUC values more than 0.8 (mean AUC value of all models equal to 0.849, with a standard deviation of 0.07). Combining independent models, a synthetic map of species richness was created (Figure 5a). This map is of particular value for designing and planning the conservation and protection of the Orchidaceae species, demonstrating that at a 53.2% of the island area, the presence of at least 10 orchid species is estimated (Figure 5b). At particular sites (comprising the 4.7% of the island area) it is estimated that at least 20 species up to 27 are present, mainly at the southwest of the Island (Figures 5b).

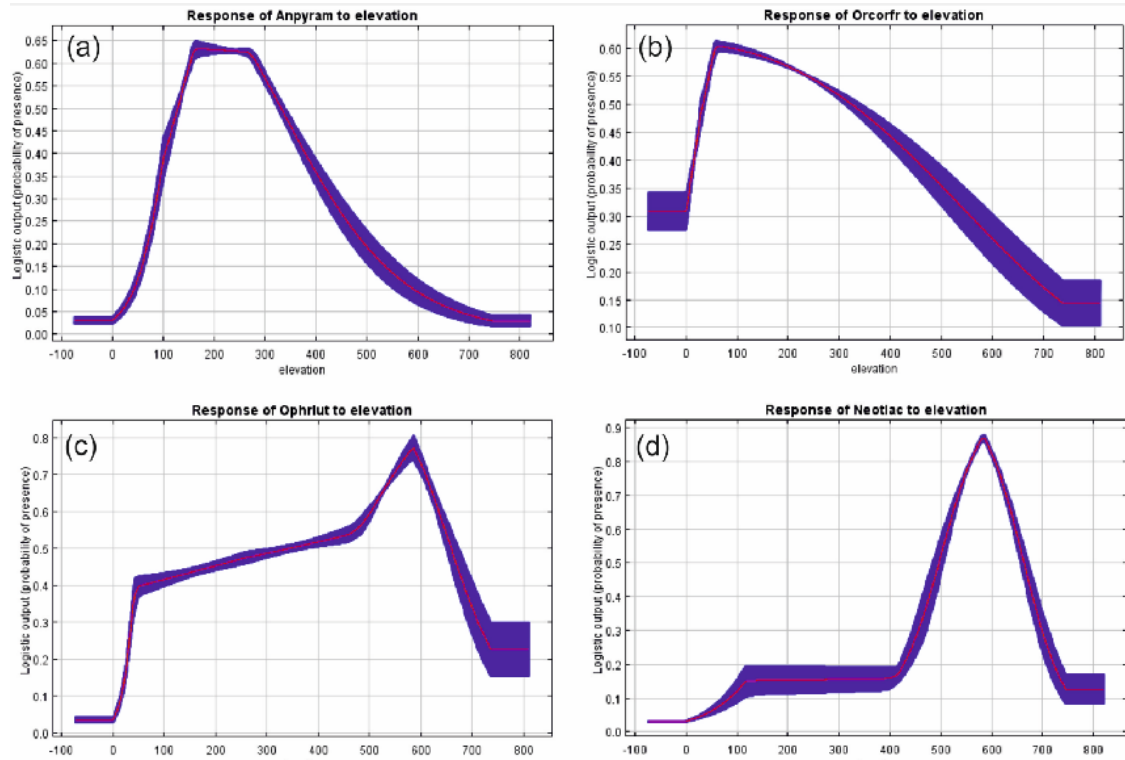


**Figure 5.** Synthetic map of species richness of Orchidaceae family (a) and three classes of species richness of Orchidaceae (b) in Zante Island.

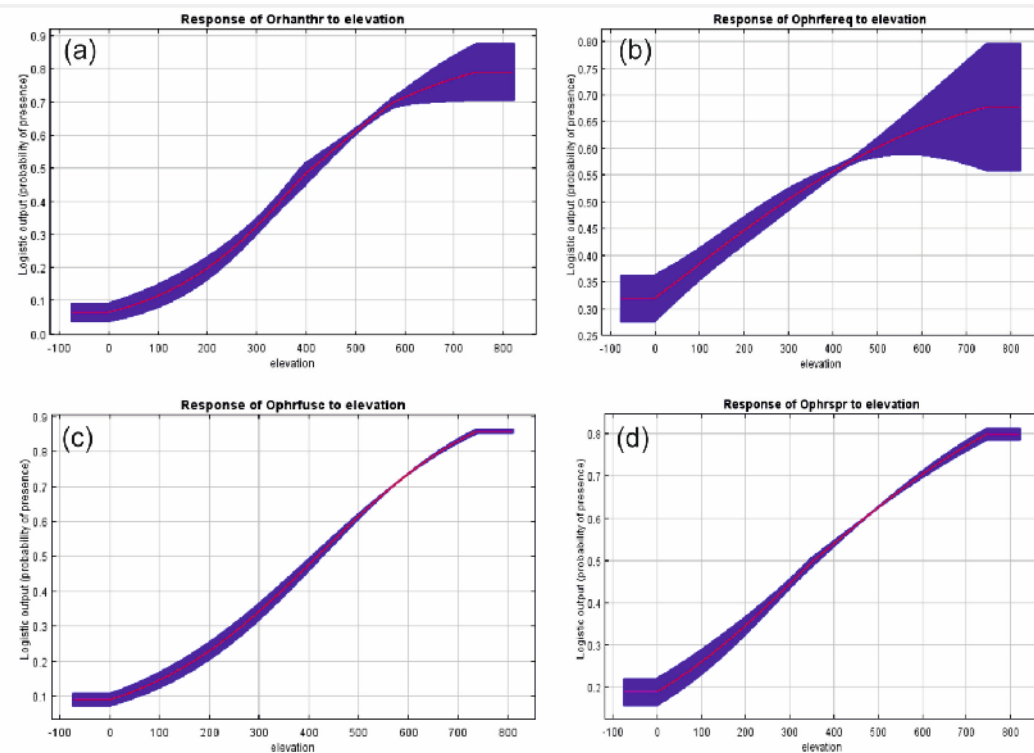
### 3.2. Environmental parameters in orchid distribution analyses (niche analyses)

The relation between species abundance and altitude is considered a key factor in floristic studies, as in relatively small geographical distances there are often significant climatic variations that largely determine the biodiversity of a region (Grytnes et al. 2006, Acharya et al. 2011), despite the fact that in many cases and for various taxa, altitude is not a defining factor of species abundance (Tsiftsis et al. 2008, Trigas et al. 2013). Apart from altitude, the range of species dispersion is also associated with other ecological and environmental parameters such as soil pH, aspect, slope, soil temperature, and climatic conditions, mainly precipitation and temperature. Taxa exhibit some degree of physiological tolerance to environmental pressures, while wide variations in species richness are strongly correlated with the climate (Lomolino 2001). Some researchers argue that species' morphology and abundance are mainly due to an environmental factor, which may be different for each species (McCain 2007, Currie & Kerr 2008). In any case, apart from the altitude, the most important factors seem to be water and energy for many species (O'Brien 1998, Hawkins et al. 2003, Fu et al. 2006). In the particular case of orchids, the species distribution is critically correlated to edaphic and mycorrhizal specialization (Phillips et al. 2011). For rare orchids' conservation, this implies the importance of microhabitats where present are the suitable endophytes, and fungi are properly distributed within the microhabitats. Hence changes in orchid species composition are not yet known to be driven by changes in endophytes across environmental gradients (Phillips et al. 2011).

In the case of Zante Island, despite the small altitudinal differences, altitude is apparently the most determining factor of variation in species distribution, since it was selected as one of the most important variables in 27 individual suitability models out of a total of 29 models. In eleven species the altitude exhibited a bell-shaped adaptation (at 200 meters or at 600 meters as peaks, Figure 6), and in nine species showed an incremental linear adjustment (Figure 7).



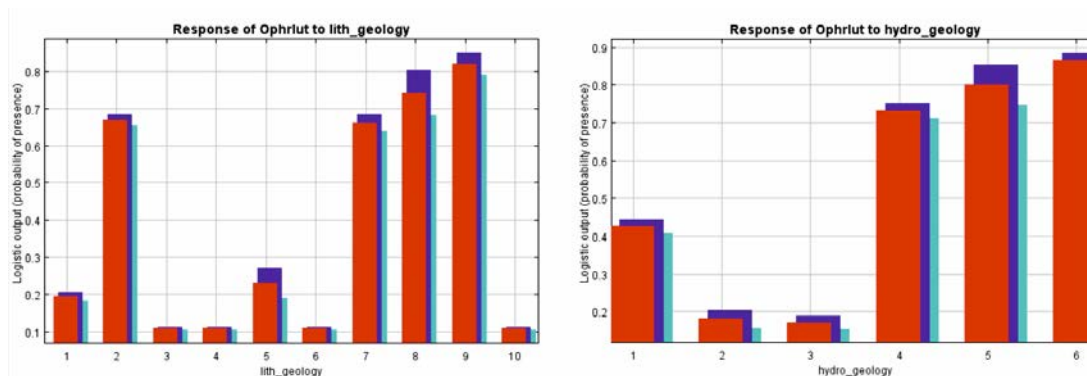
**Figure 6.** The impact of altitude, where the peak appears at an altitude of approximately 200 m on *Anacamptis pyramidalis* (a) and *Anacamptis coriophora* (b), and the impact of altitude where the peak appears at an altitude of approximately 600 m on *Ophrys lutea* (c) and *Neotinea lactea* (d)



**Figure 7.** The impact of altitude where an incremental linear adjustment is shown on *Orchis anthropophora* (a), *Ophrys ferrum-equinum* (b), *Ophrys fusca* (c), and *Ophrys spruneri* (d)

Topography, geology and landscape openness are determining factors for orchids' diversity conservation. This is congruent with the findings of Tsiftis et al. (2011) that highlighted the significance of the geological substrate as a surrogate of the soil conditions that inflict on orchid distribution. Hydro-lithology was the following most significant interpreting parameter (17 out of 29 models), with granular deposits dominating as suitable substrate, at south and east aspects of moderate inclination covered by less dense to sparse vegetation.

*Ophrys lutea* exhibits an incremental linear adaptation and the species presence was recorded throughout the altitudinal range of the island. According to recordings, the individuals of *O. lutea* are more robust and in complete flowering at medium altitudes, while at lower and higher altitudes their flowers are less and smaller. The species exhibits wide distribution also in relation to the geological substrate. Apart from the alluvial deposits, the species is recorded in all other types of geological substrates of the island, while in relation to water permeability, it prefers deposits with moderate, small and very low water permeability (Figure 8).

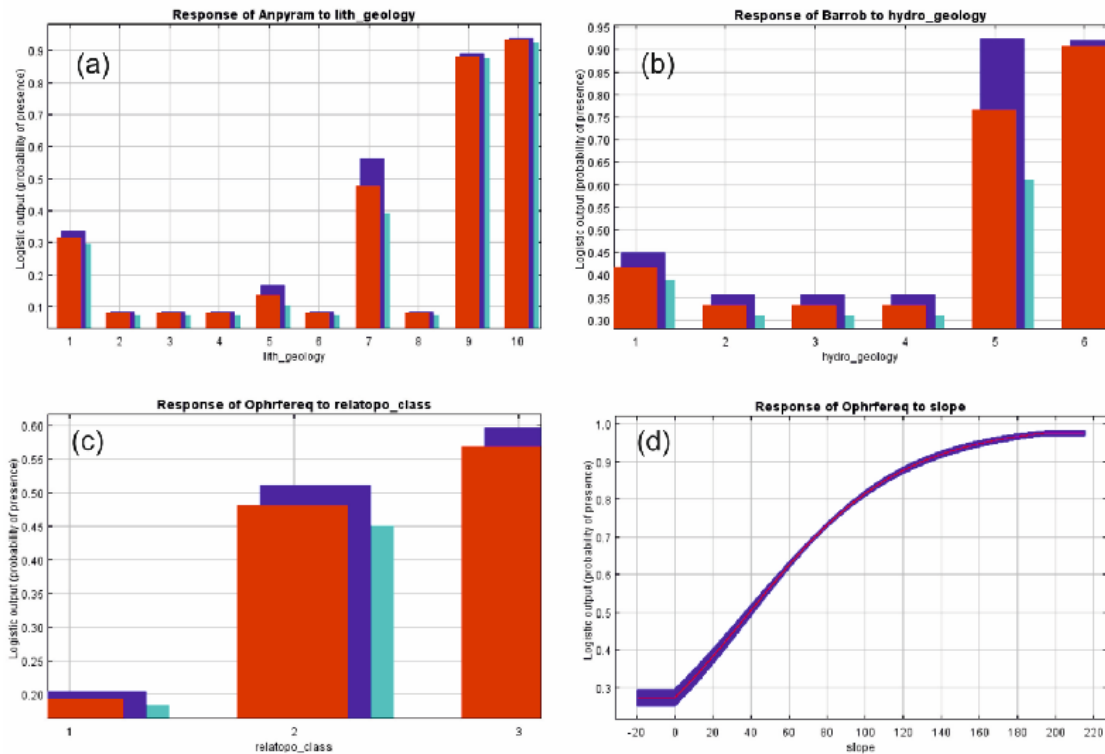


**Figure 8.** Geological and hydrological preferences of *Ophrys lutea*.

*Ophrys tenthredinifera* was recorded on limestone rocks and marble limestones, while in relation to the altitude it is present along the whole range of the altitude in the island (10-790 m), with robust individuals in full flowering. *Anacamptis papilionacea* also shows the same tolerance to altitude, while it prefers granular deposits with fluctuating permeability. On the contrary, the distribution of *Orchis italica* appears to be strongly influenced by the altitude. *Orchis italica* has robust populations up to altitude of 580 m and beyond a decline is observed in the population and in the individuals' robustness. *Serapias lingua* is recorded at large populations in the island, at altitudes of 10-200 m, and besides alluvial deposits, it shows particular tolerance to all other types of rocks and water-permeability. A preference to low-middle altitudes in open areas of the above species has been also reported by Tsiftsis et al. (2008).

The erythrocyte deposits, mainly of carst cavities, as well as the limestones and dolomites, form the appropriate geological substrate for *Anacamptis pyramidalis* (Figure 9a); the limestone, and also marl's limestone, and gypsum substrates are appropriate for *Ophrys ferrum-equinum* (Figure 9c,d), while *Himantoglossum robertianum* shows preference to alluvial deposits of low water permeability and to granular deposits of fluctuating water permeability (Figure 9b). While the slope increases, the suitability of the site increases (Figure 9d). Calcareous soils and limestones have been associated with orchid diversity by others (e.g. Tsiftsis et al. 2011) while bedrock reactivity was identified as an important factor for species of *Dactylorhiza* (Štípková et al. 2017).





**Figure 9.** Individual models of the effect of abiotic parameters on *Anacamptis pyramidalis* (a), *Himantoglossum robertianum* (b) and *Ophrys ferrum-equinum* (c, d).

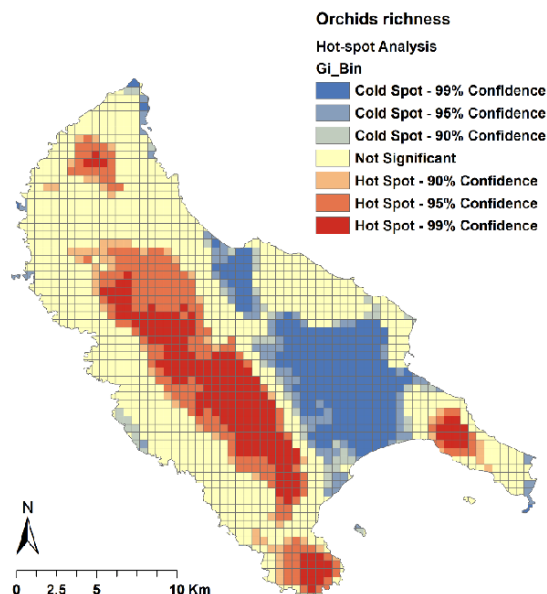
In relation to the rest of parameters, the majority of species were recorded at southern and eastern expositions, mainly on medium - and upper - slopes of moderate inclinations with more or less sparse vegetation.

The significance of different ecological preferences of species which may be related to the high species richness, has already been identified for Zante Island (Fourth author et al. 2015), suggesting that the landscape heterogeneity is a critical factor in preserving biodiversity. The present study confirmed the previous survey, since the research results of both studies are consistent, and also highlighted the importance of modeling and spatial-analysis that result in useful implications for conservation planners regarding protection of the rarer recorded species.

### 3.3. Hot Spot analysis

*Hot Spot* analysis revealed that four areas of Zante, which account for a total of 12.5% of the island area, are of great importance for protecting species biodiversity and could be classified as hotspots of high value for the conservation of orchid diversity (Figure 10). It is worth of noticing that in Zante Island have been recorded the rare *Ophrys speculum* (for the first time at only one site) as well as the rare *Dactylorhiza romana*, *Limodorum abortivum* and *Spiranthes spiralis* the only one species with autumn flowering. Alike, Štípková et al. (2017) also concluded on the usefulness of Maxent tool in determining hotspots of rare orchids in Czech Republic.

Emphasis on species extinction, fluctuations and ecological preconditions needs to be enabled (Godefroid 2001). Changes in distributions of orchid species depend largely on local conditions and identification of areas -such as hot spots- on which protection management should focus, is important (Kull & Hutchings 2006) as important is the selection of which management or conservation practices need to be implemented. In our study, environmental parameters were associated with the orchid distribution (niche analyses) for both abundant and rarer orchid species indicating that rare species of limited distribution should not be underestimated in conservation and protection goals (Tsiftsis et al. 2008).



**Figure 10.** Hot Spot analysis for orchid diversity in Zante Island.

### Perspectives and conclusion

The results of the present study, that it is the first time it is conducted in Zante Island, confirmed the richness of orchid species in the area, included rare species and recorded the most significant environmental parameters influencing the species distribution; whereas the hot spots of the highest significance for orchid species conservation were detected and mapped. For these species, it is indicated that apart from climatic conditions, the altitude is a determining factor for the majority of orchid species as well as the geological substrate, the slope and the aspect. It was also confirmed that the maps of species distribution which are derived from combining field work with geographical and spatial analysis have the potential to provide safe tools for decision making at spatial scales aiming at biodiversity conservation, through proper prioritization and development of strategies for conservation and protection of sensitive orchid species especially under climate change.

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**Appendix 1: Lithological and Hydro-lithological formations in the study area**

| <b>Geological Parameters</b>     | <b>Code</b>      | <b>Description</b>   |
|----------------------------------|------------------|--|
| <b><i>Lithological</i></b>       |                  |  |
| 1                                | <b>E-O.k</b>     | Eocene to Oligocene marly limestones with thin marly intercalations. At the contact of the marly series with the underlying limestones there is a thin intercalation of coarse - conglomerate with pebbles of Cretaceous to Eocene age limestones with no unconformity |
| 2                                | <b>Ks-Pc.k</b>   | Thin to thick bedded limestones intercalated with microbrecciated limestones   |
| 3                                | <b>Mi</b>        | Conglomerates, phsammite, brecciated limestone and marles  |
| 4                                | <b>Pl</b>        | Pliocene deposits of light-grey to white sandstones with clayey-marly intercalations. The lower horizons consist of blue marls and marly sandstones  |
| 5                                | <b>Pt</b>        | Pleistocene coastal deposits of loose or cohesive conglomerates with marly cement, blue marls and calcarenites   |
| 6                                | <b>T.br</b>      | Triassic breccia. Gypsum and anhydrite highly disturbed due to diapirism   |
| 7                                | <b>Ts-Ji,k.d</b> | Triassic to Jurassic shallow-water limestones and dolomites  |
| 8                                | <b>al</b>        | Holocene alluvial deposits deriving from the transportation of various material into the lower parts of the island   |
| 9                                | <b>g</b>         | Gypsum coarse crystalline with well developed crystals and in beds of 5-20 m thickness   |
| 10                               | <b>tr</b>        | Holocene terra rossa consisted of loam and sandy loam of intense red colour  |
| <b><i>Hydro-lithological</i></b> |                  |  |
| 1                                | <b>A3</b>        | Practically impermeable or with selective water circulation formations, with small or very small permeability  |
| 2                                | <b>K1</b>        | Limestones, dolomites, crystalized limestones, marbles with high to moderate permeability  |
| 3                                | <b>P2</b>        | Miocene, Pliocene and Pleistocene deposits with moderate to small permeability   |
| 4                                | <b>P3</b>        | Granular non sedimentary formations with small to very small permeability  |
| 5                                | <b>P4</b>        | Granular sedimentary deposits with variable permeability   |
| 6                                | <b>g</b>         | Gypsum   |

**Appendix 2:** The presence of orchid species in Zante Island and the number of recordings in 2015-2016 per species are displayed. Species nomenclature in line with Euro+Med (2006-) cross-validated with Dimopoulos et al. 2013 & Flora Ionica Working Group (2016 onwards): (\*) asterisk in parenthesis indicates the species is listed as heterotypic synonym of the taxon. # indicates the taxon name as included in Dimopoulos et al. 2013.

| Nr | Orchid species   | Nr of recordings |
|----|--|------------------|
| 1  | <i>Anacamptis coriophora</i> subsp. <i>fragrans</i> (Pollini) R. M. Bateman, Pridgeon & M. W. Chase #                          | 257              |
| 2  | <i>Anacamptis laxiflora</i> (Lam.) R. M. Bateman, Pridgeon & M. W. Chase   | 5                |
| 3  | <i>Anacamptis papilionacea</i> (L.) R. M. Bateman, Pridgeon & M. W. Chase  | 71               |
| 4  | <i>Anacamptis pyramidalis</i> (L.) Rich.   | 186              |
| 5  | <i>Dactylorhiza romana</i> (Sebast.) Soó subsp. <i>romana</i> #  | 3                |
| 6  | <i>Himantoglossum robertianum</i> (Loisel.) P. Delforge  | 130              |
| 7  | <i>Limodorum abortivum</i> (L.) Sw.  | 16               |
| 8  | <i>Neotinea lactea</i> (Poir.) R. M. Bateman, Pridgeon & M. W. Chase   | 63               |
| 9  | <i>Ophrys apifera</i> Huds.  | 4                |
| 10 | <i>Ophrys umbilicata</i> Desf. subsp. <i>umbilicata</i>  | 8                |
| 11 | <i>Ophrys holoserica</i> (Burm. f.) Greuter<br>(* <i>Ophrys bombyliflora</i> Spreng.)  | 137              |
| 12 | <i>Ophrys holoserica</i> (Burm. f.) Greuter subsp. <i>holoserica</i> #<br>(* <i>Ophrys episcopalis</i> Poir.)                  | 16               |
| 13 | <i>Ophrys ferrum-equinum</i> Desf. subsp. <i>ferrum-equinum</i>  | 118              |
| 14 | <i>Ophrys ferrum-equinum</i> subsp. <i>gottfriediana</i> (Renz) E. Nelson  | 15               |
| 15 | <i>Ophrys fusca</i> subsp. <i>cinereophila</i> (Paulus & Gack) Faurh.  | 29               |
| 16 | <i>Ophrys scolopax</i> subsp. <i>heldreichii</i> (Schltr.) E. Nelson   | 2                |
| 17 | <i>Ophrys holoserica</i> subsp. <i>candica</i> (Greuter & al.) H. A. Pedersen & Faurh.   | 1                |
| 18 | <i>Ophrys sphegodes</i> Mill. subsp. <i>sphgodes</i> #<br>(* <i>Ophrys sphegodes</i> subsp. <i>atrata</i> (Rchb. f.) A. Bolòs) | 17               |
| 19 | <i>Ophrys fusca</i> subsp. <i>iricolor</i> (Desf.) K. Richt.   | 45               |
| 20 | <i>Ophrys lutea</i> subsp. <i>melena</i> Renz  | 7                |
| 21 | <i>Ophrys lutea</i> subsp. <i>galilaea</i> (H. Fleischm. & Bornm.) Soó   | 21               |
| 22 | <i>Ophrys lutea</i> Cav. subsp. <i>lutea</i>   | 232              |
| 23 | <i>Ophrys sphegodes</i> subsp. <i>mammosa</i> (Desf.) Soó ex E. Nelson   | 197              |
| 24 | <i>Ophrys scolopax</i> Cav. subsp. <i>scolopax</i>   | 16               |
| 25 | <i>Ophrys scolopax</i> Cav.  | 7                |
| 26 | <i>Ophrys speculum</i> Link  | 3                |
| 27 | <i>Ophrys sphegodes</i> Mill.  | 13               |
| 28 | <i>Ophrys sphegodes</i> subsp. <i>spruneri</i> (Nyman) E. Nelson   | 55               |
| 29 | <i>Ophrys tenthredinifera</i> Willd.   | 262              |
| 30 | <i>Ophrys umbilicata</i> Desf.   | 7                |
| 31 | <i>Orchis anthropophora</i> (L.) All.  | 31               |
| 32 | <i>Orchis italica</i> Poir.  | 122              |
| 33 | <i>Orchis simia</i> Lam.   | 5                |
| 34 | <i>Orchis mascula</i> (L.) L.  | 3                |
| 35 | <i>Orchis pauciflora</i> Ten.  | 16               |
| 36 | <i>Anacamptis palustris</i> (Jacq.) R. M. Bateman, Pridgeon & M. W. Chase  | 3                |
| 37 | <i>Orchis provincialis</i> Balb. ex Lam. & DC.   | 178              |

| <b>Nr</b> | <b>Orchid species</b>                                     | <b>Nr of recordings</b> |
|-----------|---|-------------------------|
| 38        | <i>Orchis quadripunctata</i> Cirillo ex Ten.              | 260                     |
| 39        | <i>Serapias bergonii</i> E. G. Camus                      | 29                      |
| 40        | <i>Serapias cordigera</i> L.                              | 126                     |
| 41        | <i>Serapias lingua</i> L.                                 | 77                      |
| 42        | <i>Serapias neglecta</i> De Not.                          | 49                      |
| 43        | <i>Serapias neglecta</i> subsp. <i>ionica</i> #           | 237                     |
| 44        | <i>Serapias orientalis</i> (Greuter) H. Baumann & Künkele | 80                      |
| 45        | <i>Serapias parviflora</i> Parl.                          | 31                      |
| 46        | <i>Serapias vomeracea</i> (Burm. f.) Briq.                | 20                      |
| 47        | <i>Spiranthes spiralis</i> (L.) Chevall.                  | 3                       |