Ecological factors influencing the diversity and distribution of Orchidaceae members in the Intercontinental Biosphere Reserve of the Mediterranean of Northern Morocco

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Abstract

The complex interplay of ecological gradients, habitat heterogeneity, and species-specific traits usually shape orchid diversity and distribution in Mediterranean ecosystems. In this study, we surveyed terrestrial orchids across 65 diversified sites in northern Morocco's Intercontinental Biosphere Reserve of the Mediterranean (IBRM). Overall, 26 orchid taxa were recorded across different substrates and environments with calcareous substrates supporting 1,775 individuals compared to 128 on siliceous ones. The Outlying Mean Index analysis revealed clear patterns of ecological specialization across environmental gradients. Five orchid species (*Epipactis tremolsii, Limodorum trabutianum, Orchis anthropophora, Ophrys battandieri*, and *Orchis mascula* subsp. *laxifloriformis*) exhibited strong marginality indicating high specialization associated with narrow niches and specific environmental preferences (particularly in cooler, mid- to high-elevation habitats with distinct substrates), while others were moderately specialized or generalists displaying broader tolerance (more common at higher elevations and warmer sites). The main drivers influencing orchid distribution were temperature, elevation, precipitation, latitude, slope, moisture, forest and substrate type. Hierarchical clustering further distinguished four ecological groups linked to specific habitat types and environmental conditions. *Serapias* spp. mainly occurred in high-moisture habitats, whereas most *Ophrys* and *Orchis* taxa preferred forested calcareous sites. Our findings underscore the importance of habitat heterogeneity and substrate type in maintaining orchid diversity, thus providing a valuable framework for conservation strategies in Mediterranean North Africa and beyond.

Introduction

The Rif region is the most forested Moroccan region, with more than 40% of its territory covered by forests (ANEF 2024), thus being considered as the most important orchid diversity centre in Morocco (Valdès et al. 2002; El Karmoudi et al. 2025b). However, this region of Morocco is also the most threatened by climate change and human activities (Ajbilou et al. 2006; Cheddadi et al. 2017; El Karmoudi et al. 2025a, b). To date, water scarcity and human-induced pressures have led to a significant decrease to the range of several forest species in Rif region, such as *Cedrus atlantica*. The distribution of Manetti ex Carrière (Atlas cedar) decreased by about 75% over the last 50 years (Cheddadi et al. 2017). The conservation of forests and their orchid species requires an in-depth understanding of the ecological factors affecting the diversity and geographical distribution of these vulnerable plants.

Species distributions, abundance, and composition are shaped by both abiotic and biotic factors, whose effects vary across space and time (Rojas-Sandoval and Meléndez-Ackerman 2013). At the core of their range, species typically show high abundance and occupy multiple areas across diverse habitats, reflecting optimal conditions (Sagarin and Gaines 2002). Towards the range boundaries, however, population densities and the number of occupied areas often decline, indicating increasingly suboptimal environments (Pfeifer et al. 2010). Previous studies on the ecology and biogeography of Orchidacaeae taxa (species and subspecies), have focused primarily on the species interactions and the effects of environmental conditions on orchids diversity and distribution (Tsiftsis et al. 2008, 2018; Djordjević et al.

2014, 2016, 2020). At broad spatial scales, the geographic distribution of plant species is primarily shaped by macroclimatic conditions, along with their evolutionary background and migration history (Djordjević et al. 2014). At the regional level, however, species distribution and abundance are further influenced by a range of environmental and ecological factors, including soil characteristics, light availability, meso- and microclimatic conditions, altitude, habitat type, disturbance regimes, and biotic interactions (Landi et al. 2009; Tsiftsis et al. 2012).

Plant species can be classified along a specialist–generalist continuum, which reflects the breadth of their niche. Specialists occupy narrow environmental ranges and are typically restricted to specific, often stressful habitats, such as wetlands, alpine zones, and dry heathlands. In contrast, generalists tolerate a wide range of conditions and tend to be more broadly distributed. Despite their limited geographic range, specialists often thrive within their preferred habitats (Boulangeat et al. 2012). The relationship between orchid species and their surrounding plant communities is influenced abiotic and biotic factors, as well as the age and evolutionary development of the ecosystem (Djordjević and Tsiftsis 2020). For instance, the connection between orchids and nearby forest trees is often reinforced by their mutual association with fungi that form ectomycorrhizal relationships with tree roots while also facilitating orchid growth (Girlanda et al. 2006).

The Orchidaceae is among the largest and most diverse families of flowering plants, encompassing over 29,524 accepted species across 736 genera (Chase et al. 2015; Djordjević and Tsiftsis 2020; Pérez-Escobar et al. 2024). While the majority of orchid species are concentrated in tropical regions, the family exhibits a near-global distribution, inhabiting environments that range from tropical rainforests to Arctic tundra. They exhibit remarkable ecological adaptability and can colonize a wide range of habitats, including terrestrial, epiphytic, and lithophytic environments. In North Africa and Europe, orchid species are strictly terrestrial, occurring across diverse habitats such as forests, shrublands, grasslands, moorlands, peatlands, and wetlands (Dressler 1981; Delforge 2006; Djordjević et al. 2020), occurring in both herbaceous and forest communities, although may be restricted to one of these habitat groups (Delforge 2006). The distribution and abundance of orchids are strongly affected by vegetation types, which serve as a primary factor driving the differentiation of their ecological niches (Djordjević et al. 2016; Tsiftsis et al. 2008).

Globally, at least 331 studies have investigated orchids within protected areas. The majority of these studies primarily focus on the diversity and distribution of orchid species (Khapugin 2020). However, the combined effects of abiotic and biotic factors on the distribution and abundance of forest-dwelling terrestrial orchids have been relatively understudied (Tsiftsis et al. 2008, 2019; Djordjević et al. 2020). At finer spatial scales, numerous factors may influence the richness, distribution, growth, and reproductive success of terrestrial orchid species. These include pollinator availability, mycorrhizal associations, vegetation composition, geological substrates, soil characteristics, climatic conditions, latitude, longitude, elevation, and exposure (Jacquemyn et al. 2005; Delforge 2006; Bulafu et al. 2007; Rasmussen and Rasmussen 2018; Tsiftsis et al. 2018; Djordjević et al. 2020; Luo et al. 2020; Xi et al. 2020; El Karmoudi et al. 2025a, b).

In Morocco, research on orchid niche remains limited, with scarce knowledge regarding the key factors driving the orchids' distribution, abundance, and community structure across different ecosystems. The present study sought to identify the principal environmental determinants shaping the spatial orchids' distribution and abundance patterns, and community composition across distinct ecosystem types within the Moroccan part of the Intercontinental Biosphere Reserve of the Mediterranean of Northern Morocco (hereafter referred to as IBRM). The specific objectives were as follows: (a) to identify the predominant environmental variables influencing niche differentiation among orchid species within the studied habitats; (b) to determine species that characterize particular habitat types; and (c) to delineate coherent orchid ecological groups.

Materials and methods

Study area

The study area encompassed the Moroccan part of IBRM in northern Morocco. The IBRM spanning regions of northern Morocco and southern Spain boasts high biodiversity, with a rich variety of ecosystems and species (Molina and Villa 2008; ANEF 2024). The Moroccan part, covering mountains of the western Rif, known as Jbala region, contains numerous natural ecosystems of significant bio-ecological value, some of which have been designated as Sites of Biological and Ecological Interest (SIBE) (see El Karmoudi et al. 2025a). The present study area included the Talassemtane National Park, Bouhachem Natural Park, Jbel Lehbib, Jbel Mossa, Jbel Ghorghize, Jbel Bouzaitoun, and Ain Lahsan (Fig. 1).

In the IBRM Moroccan part, the climate is predominantly temperate and dry, with hot summers across most of the territory. The mountains of Talassemtane National Park (TNP) and Bouhachem Natural Park (BNP) experience a temperate, dry climate with warm summers (Köppen-Geiger Climate Classification System 2024). The mean annual precipitation is approximately 892 mm, and the annual mean temperature is 16.46°C (https://www.worldclim.org/). Geologically, most of the IBRM is underlain by a limestone-dolomite bedrock (Michard 1976), except for a large portion of BNP, which primarily consists of acidic layers of flyschs and sandstones.

Data collection

To explore the diversity and distribution patterns of Orchidaceae in the IBRM, various habitats were surveyed during the growth and/or flowering seasons of orchid plants (from 2019-05-01 to 2024-05-18), across altitudes ranging from 77 to 1969 m above sea level. To ensure representative sampling, multiple visits were made to different ecosystems (forest, matorral, grassland, wetlands, etc.) (see El Karmoudi et al. 2025a, b). At each site, we collected data on Orchidaceae species richness and abundance as well on plant community type and vascular plant cover. Taxonomic identification was based on regional floras (Valdés et al. 2002; Fennane et al. 2014) and the specialized work of Delforge (2006), while species

nomenclature was standardized according to *Plants of the World Online* (PoWo 2024), as referenced in El Karmoudi et al. (2025a, b).

Plant communities associated with orchids were analyzed, resulting in the identification of 14 distinct habitat types within the study area, detailed as follows: (1) *Abies marocana* forest; (2) Degraded forest with *Pteridium aquilinum* understory; (3) *Quercus canariensis* forest; (4) *Quercus ilex* forest; (5) Mixed *Quercus ilex* and *Quercus pubescens* subsp. *pubescens* (= *Q. faginea*) forest; (6) *Quercus pyrenaica* forest; (7) *Quercus suber* forest; (8) Siliceous substrate *Quercus suber* forest; (9) *Pistacia atlantica* matorral; (10) *Pistacia lentiscus* matorral and/or mixed *Pistacia lentiscus* - *Myrtus communis* matorral; (11) *Quercus coccifera* matorral; (12) *Quercus ilex* matorral; (13) Chamaephytic scrub dominated by *Chamaerops humilis* and *Stachys fontqueri*; (14) Pelouses dominated by herbaceous Asteraceae, Boraginaceae, and Poaceae.

At each sampling site, geographic coordinates were recorded using a GPS, and elevation was measured using a smartphone-based global positioning system. Slope was visually estimated as the percentage of ground inclination (i.e., the angle of the terrain relative to the vertical axis). Aspect (exposure) was used to calculate the Heat Load Index (HLI) for each site, following the formula proposed by McCune and Keon (2002): Heat Load Index = 1-cos (θ-45)/2, where θ represents the azimuth in degrees east of north. HLI values range from 0 (coolest slopes; northeast-facing, 45° E of N) to 1 (warmest slopes; southwest-facing, 225° E of N). Field assessments of soil moisture were based on estimated water content, with sites grouped into three distinct moisture classes: dry, moderate, and moist. Substrate type was determined through field observations and reference to the geological map of the Rif region (Michard 1976), allowing classification of sites as either calcareous or siliceous. Mean annual temperature and precipitation data were extracted from the WorldClim climate database (Hijmans et al. 2005). All categorical variables, including habitat type, soil moisture, and substrate, were converted into binary variables for inclusion in subsequent analyses.

Statistical analysis

We explored the relationship between orchid species richness and abundance with elevation using Generalized Additive Models (GAM, Hastie 2017), to capture unimodal relationship without assuming a specific functional form of the relationship. We assumed Poisson error distribution and log link function. The analysis was implemented with the *mgcv* R package (Wood 2001).

The effects of environmental and topographic variables on orchid community structure and composition and orchid species niches were evaluated by the Outlying Mean Index analysis (OMI, Doledec et al. 2000). Prior to analysis, we implemented Principal Component Analysis to the environmental data (latitude, longitude, elevation, slope, moisture, substrate, mean annual temperature, mean annual precipitation and habitat type) with the R function *dudi.pca* of the *ade4* package (Dray et al. 2007). The first three PCA axes were used in the OMI analysis and their correlation with the environmental variables was evaluated by Pearson's correlation coefficients. The OMI analysis is a two-table ordination

technique, i.e. species table and explanatory data table that assigns equal weights to sites regardless of their species richness, without making any assumption about the type of the relationship between species response and explanatory variables. This analysis allows the identification of key environmental drivers and the visualization of complex interrelationships among variables, thereby providing an integrative understanding of the ecological requirements of species. The orchid abundance table was used as species-table and the explanatory data table included the first three PCA axes. It should be noted that orchid species present in only one site, i.e. *Neotinea conica*, *Orchis spitzelii* subsp. *cazorlensis*, and *Serapias vomeracea*, were excluded from the analysis. This multivariate analysis provides three essential indices: (a) species marginality, the Euclidean distance between species' mean habitat conditions, and the mean habitat conditions of the study area; it reflects species niche position relative to the mean environmental conditions, (b) the tolerance, the range of habitat conditions that a species occupies along an environmental gradient, i.e. reflecting its niche breadth, and (c) and residual tolerance, the proportion of species niche variability that is not explained by the environmental variables included in the analysis. Species with high values of marginality tend to occur in marginal habitats, i.e. habitats not common in the study area, while species with low values of marginality tend to occur in common habitats. High values of tolerance indicate generalist species, i.e. species that occur in wide range of environmental conditions, whereas specialist species exhibit low values of tolerance. Regarding tolerance, residual quantifies how effectively the considered environmental variables describe species' niche, with lower values signifying that environmental variables capture niches' variability. The significance of the marginality was tested by performing random permutation tests (1000 permutations) to assess whether observed marginality differs from random expectations. The OMI analysis was performed with the *niche* function of the R package *ade4* (Dray et al. 2007).

In the next step, we estimated the Species Specialization Index (SSI) as SSI = 1-%Tolerance/$\%Tolerance_{max}$, where $Tolerance_{max}$ is the maximum value of tolerance. The index ranges between 0 and 1, with values close to 0 denoting generalist species and values close to 1 denoting specialist species. We calculated the mean specialization index per site and tested whether the index varies with latitude, longitude and elevation using Generalized Additive Models (GAM, Hastie 2017), assuming Gaussian error distribution and identity function in all cases.

Indicator Species analysis was implemented to identify whether orchid species or specific species were strongly associated with specific habitat and substrate types. The analysis was performed separately for habitat and substrate types. We used the function of *multipatt* of the R package *indicspecies* (De Cáceres et al. 2016). Finally, we applied a hierarchical clustering analysis to detect ecological groups of orchid species and subspecies. To do so, we estimated the Euclidean distance based on the species position in the niche space using the first three OMI axes. The Euclidean distance was estimated with the function *dist* of the R package *vegan* (Oksanen et al. 2007). Clustering was performed with the Unweighted Pair Group with Arithmetic Mean (UPGAM) with the R base function *hclust*. Then, SIMPROF (Similarity Profile, Clarke et al. 2008) test was implemented to assess statistical different clusters of orchid species, setting threshold value equal to $p < 0.05$. The SIMPROF test was applied with the function *simprof* of the

R package *clustsig* (Whitaker et al. 2014). The clusters were visualized with a dendrogram, with each cluster of orchid species highlighted in different color.

All data preparation, analysis and visualization were performed in R version 4.4.1 (R Development Core Team 2024).

Results

Richness and abundance of orchid species

A total of 65 sites were surveyed across the IBRM (Fig. 1), encompassing a broad altitudinal gradient ranging from 77 to 1969 m above sea level (mean 798.2 ± 533.09 m) reflecting considerable topographic and ecological heterogeneity. This gradient spanned diverse ecological zones and habitat types that supported a wide variety of Orchidaceae species. Among sites, 29 sites were of low altitude (below 500 m), primarily characterized by open woodlands, and scrublands, often under strong anthropogenic pressure, eight sites were of mid-altitude (500–1000 m), including mixed deciduous forests and matorrals, while 28 sites were at high altitude (above 1000 m), dominated by montane habitats such as coniferous and *Quercus* forests (Fig. S1). The majority of the sites were situated on steep slopes (63% sites in steep slope, 31% in moderate slope, and less than 10% of sites in very steep slopes or gentle slopes). Very steep slopes (class 4) and gentle slopes (class 1) were comparatively uncommon, characterizing less than 10% of the sites. In total, 87.7% of the studied sites had a limestone substrate. Soil moisture conditions across the sites were predominantly intermediate (moisture class 2 in 83% of sites). Wet habitats (moisture class 3) were less frequent, found in only 9 sites, while dry conditions (class 1) were restricted to three sites.

In total, 1903 orchid individuals were recoded belonging in 26 species and nine genera of Orchidaceae, which represent 46.4% of the total known orchid diversity of Morocco. The genus *Ophrys* L. members were the richest (nine taxa), followed by *Orchis* Tourn. Ex L. spp. and *Serapias* L. spp. with four taxa each. *Serapias parviflora* emerged as the most widespread and abundant species, recorded at 16 sites with a total of 270 individuals. Other highly represented taxa included *Ophrys apifera* (196 individuals at 8 sites), *Ophrys tenthredinifera* (169 individuals at 11 sites), *Himantoglossum robertianum* (166 individuals at 4 sites), and *Orchis mascula* subsp. *laxifloriformis* (156 individuals at 9 sites). In contrast, some taxa exhibited restricted distributions and low abundances such as *Neotinea conica*, *Orchis spitzelii* subsp. *cazorlensis*, and *Serapias vomeracea* which were present in only one site with eight, 13 and 12 individuals, respectively, and *Himantoglossum hircinum* which was recorded with only five individuals across two sites.

In the study area, habitat types are major determinants of Orchidaceae species' distribution and abundance. A total of 1903 individuals were observed across 14 habitat types (Fig. 2; Table S1). The data revealed clear patterns in orchid abundance relative to habitat structure and vegetation composition. The result outlined the dominance of matorral and forest habitats. The matorral habitats,

particularly those dominated by *Pistacia lentiscus-Myrtus communis*, support the highest number of orchid individuals (699), accounting for approximately 36.7% of the total. However, forests dominated by *Quercus suber* rank second with 348 individuals (18.3%), followed by *Quercus ilex* forests with 234 individuals (12.3%) and *Abies marocana* forest with 183 individuals (9.61%). The marginal habitats such as *Quercus pyrenaica* forest, *Pteridium aquilinum*-dominated degraded forest, and *Chamaerops humilis-Stachys fontqueri* vegetation hosted low-density orchid communities. While the chamaephytic vegetation hosting the species-poorer communities. As expected, given that calcareous substrate was dominant, it hosted the majority of orchid species in terms of both richness and abundance.

OMI analysis

The OMI analysis revealed that five out of the 23 analysed orchid species and subspecies (*Epipactis tremolsii, Limodorum trabutianum, Orchis anthropophora, Ophrys battandieri*, and *Orchis mascula* subsp. *laxifloriformis*) had statistically significant ($p < 0.05$) marginality (Table 1), thus indicating that these species are strongly specialized to certain environmental conditions. Additionally, eight species displayed significant OMI marginality value, suggesting a weaker specialization to specific environmental conditions. The percentage (%) OMI value ranged between approximately 36% (*Ophrys lutea*) to 77% (*Ophrys apifera*). The highest values of percentage of inertia attributed to tolerance, i.e. generalist species, were observed for *Himantoglossum robertianum*, *Serapias lingua* and *Serapias strictiflora* (over 40%). In general, species with high OMI values tended to exhibit narrow niche breadth (tolerance values) and higher values of specialization (SSI). However, there was two notable exceptions namely *Serapias parviflora* and *Ophrys lutea* which exhibited moderate marginality, but narrow niche breadth and high specialization index (Table 1). Yet, these two species had the higher residual tolerance (%), meaning that other environmental conditions not considered herein are probably more relevant to their niches. Yet, the remaining orchid species showed low residual variance, thus the included environmental variables explained effectively their distribution and abundance across the IBRM area.

Table 1

Niche parameters of Moroccan orchid species of the study area as estimated by the Outlying Mean Index analysis. Occ: occurrences; Inertia: total variability; OMI outlying mean index percentage (%): the statistical significance of species marginality; Tol: species tolerance percentage (%), ResTol: residual tolerance; SSI-species: specialization index. Asterisks in the brackets in the OMI value indicate whether the observed values differ from random expectations. Significance levels: (.): 0.05 < $p \leq 0.1$; * $p \leq 0.05$; * * $p \leq 0.01$; * ** p ≤ 0.001; absence of asterisks indicates non-significant result).

Species (abbreviation)	**Occ**	**Abundance**	**Inertia**	**OMI**	**Tol**	**ResTol**	**SSI**
Serapias parviflora (Serpar)	16	270	5.93	49.75	15.35	34.91	0.67
Ophrys lutea (Ophlut)	13	130	5.93	36.26 (.)	16.19	47.55	0.65
Ophrys apifera (Ophapi)	8	196	10.24	77.44	22.56	0.00	0.51
Ophrys scolopax (Ophsco)	8	45	8.46	76.83 (.)	23.17	0.00	0.50
Ophrys lutea subsp. *phryganae* (Ophphr)	2	28	8.74	75.86	24.14	0.00	0.48
Ophrys speculum (Ophspe)	4	61	13.08	73.85	26.15	0.00	0.43
Gennaria diphylla (Gendip)	2	16	6	72.67	27.33	0.00	0.41
Himantoglossum hircinum (Himhir)	2	5	12.23	72.61 (.)	27.47	0.00	0.41
Neotinea maculata (Neomac)	7	75	5.93	59.53 (.)	29.17	11.47	0.37
Orchis mascula subsp. *laxifloriformis* (OramasLx)	9	156	10.73	68.87 (**)	31.13	0.00	0.33
Ophrys bombyliflora (Ophbom)	4	54	9.67	67.84 (.)	32.16	0.00	0.30
Limodorum trabitianum (Limtra)	3	8	13.43	67.76 (*)	32.24	0.00	0.30
Neotinea tridentata (Neotri)	4	52	10.31	65.57	34.43	0.00	0.25
Orchis anthropophora (Oraant)	5	82	23.03	64.26 (*)	35.78	0.00	0.23
Ophrys battandieri (Ophbat)	7	76	19.35	62.74 (**)	37.26	0.00	0.19
Orchis mascula (Oramas)	4	27	10.77	61.56 (.)	38.44	0.00	0.17
Ophrys fusca (Ophfus)	5	26	5.93	53.79	38.45	7.76	0.17

Species (abbreviation)	Occ	Abundance	Inertia	OMI	Tol	ResTol	SSI
Epipactis tremolsii (Epitre)	3	36	21.12	60.89 (*)	39.11	0.00	0.15
Ophrys tenthredinifera (Ophten)	11	169	7.31	60.74 (.)	39.26	0.00	0.15
Cephalanthera longifolia (Ceplo)	7	79	5.93	49.75	39.80	10.46	0.14
Himantoglossum robertianum (Himrob)	4	166	9.27	56.74	43.15	0.00	0.06
Serapias lingua (Serlin)	7	88	5.93	51.10 (.)	44.01	4.89	0.05
Serapias strictiflora (Serstr)	3	25	7.83	53.90	46.23	0.00	0.00

In the OMI analysis, we used the first PCA axes which accounted for 60.66% of the total variability (first PCA axis: 35.43%, second PCA axis: 13.85% and third PCA axis: 11.39%). The first OMI axis was significantly and positively correlated to temperature and latitude and negatively correlated to elevation, precipitation, and longitude, while it exhibited significant correlations with the substrate, slope (northern and south-eastern) and specific habitat types, primarily forests (Table 2 and Fig. 3). The second axis was strongly related to the substrate, moisture (dry and moist), and some forest types (Table 2 and Fig. 3), while the third axis was strongly related to moisture (mainly dry conditions), the slope, and substrate (Table 2).

Table 2

Pearson's correlation coefficients showing the relationship between environmental variables and the first three axes used in the Outlying Mean Index analysis. Inside the brackets, the statistical significance of the relationship is provided (significance levels: (.) $0.05 < p \leq 0.1$; * $p \leq 0.05$; * * $p \leq 0.01$; * ** $p \leq 0.001$; absence of asterisks indicates non-significant result).

Environmental variable	**OMI axis 1**		**OMI axis 2**		**OMI axis 3**	
Latitude	0.75	***	0.38		0.08	
Longitude	-0.55	***	-0.65		0.19	***
Elevation	-0.84	***	-0.41		-0.05	
Slope	0.36	*	-0.27		-0.29	***
Temperature	0.87	***	0.38		0.05	
Precipitation	-0.72	***	-0.02	.	-0.11	*
Substrate						
Calcareous	0.52	*	-0.75	***	0.24	***
Siliceous	-0.52	*	0.75	***	-0.24	***
Exposure						
East	-0.19		-0.26		-0.14	***
N	0.34	***	0.16		-0.18	.
S	0.14		0.00		0.04	
W	0.01		-0.16		-0.14	
NE	0.11		-0.08		-0.15	
NW	-0.28	.	0.22	*	-0.08	
SE	-0.21	*	-0.11		0.05	
SW	0.05		0.07	***	0.62	
Moisture						
Dry	-0.14		-0.11	***	0.60	
Moderate	0.46	*	-0.54		-0.47	***
Moist	-0.43	.	0.69	***	0.15	***
Habitat type						
Abies marocana forest	-0.36	***	-0.30	***	0.54	
Mixed *Quercus ilex* and *Quercus faginea* forest	-0.10		-0.10		-0.06	*

Environmental variable	OMI axis 1		OMI axis 2		OMI axis 3	
Quercus ilex forest	-0.28	**	-0.31	*	-0.14	
Quercus canariensis forest	-0.22		0.15	.	-0.36	*
Quercus pyrenaica forest	-0.11		0.14		-0.28	
Quercus suber forest	0.27	**	0.01		-0.24	
Siliceous Substrate *Quercus suber* forest	-0.34		0.62	**	-0.02	***
Pistacia atlantica matorral	0.70	***	0.14	**	0.34	
Quercus coccifera matorral	0.18		0.02		-0.13	
Quercus ilex matorral	-0.23	*	-0.40		-0.21	*
Endorsed *Pistacia lentiscus* matorral and Mixed *Pistacia lentiscus - Myrtus communis* matorral	0.13		0.02		-0.12	
Degraded Forest with *Pteridium aquilinum* understory	-0.27		0.42		0.14	**
Chamaephytic scrub dominated by *Chamaerops humilis* and *Stachys fontqueri*	-0.08		-0.11		-0.05	
Pelouses	-0.13		-0.01		0.04	

We detected a significant longitudinal and latitudinal gradient in the mean Species Specialization Index (SSI) per site (longitude: $GAM_{Deviance\ Explained}$=26.1%, $p < 0.05$; latitude: $GAM_{Deviance\ Explained}$=21.1%, $p < 0.05$). Specifically, the mean SSI per site increased with increasing latitude and then reached a plateau, while decreased linearly with increasing longitude, with the observed relationships suggesting that more specialized species are found in the northern area, while more generalist species are found toward the eastern areas. The relationship between mean SSI and elevation was significant, linear and negative ($GAM_{Deviance\ Explained}$= 9.4%, $p = 0.01$), suggesting that more generalist species occur at higher elevation.

The hierarchical clustering combined with SIMPROF test revealed four distinct ecological groups of orchid species and subspecies (Fig. 4). The first group included *Limodorum trabutianum*, *Orchis mascula, Orchis mascula* subsp. *laxifloriformis, Serapias lingua*, and *Serapias strictiflora*. Inspecting the environmental conditions where the species of cluster 1 occurred in the wild, we observed that these species tended to occupy cooler to moderately warm habitats of mid- to high elevations and of moderate slope. Although, the calcareous substrate was dominant, *Limodorum trabutianum* and *Orchis mascula* subsp. *laxifloriformis* were indicator species of the siliceous substrate. *Limodorum trabutianum* was a specialist species according to its OMI score and SSI, which was narrowly distributed to very specific environmental conditions, and indicator species of *Quercus canariensis* forests and mixed forests of *Q. ilex* and *Q. faginea* forests. Similarly, *Orchis mascula* subsp. *laxifloriformis* was a specialist that tended to occupy cool habitats of higher elevation.

The second group included nine species, most of which were abundant, with two of them, *Serapias parviflora* and *Ophrys lutea*, being also the most widespread in the study area. Most of these species inhabited warmer sites in lowlands, but *Ophrys lutea* which occurs in a wide elevational range can persist in various ones. This finding contradicts the high value of species specialization index, but the species showed the highest value of residual tolerance, suggesting that the included environmental variables do not adequately describe its niche. Additionally, two species; namely *Ophrys bombyliflora* and *Ophrys speculum* were identified as indicator species of the endorsed *Pistacia lentiscus* matorral and the mixed *Pistacia lentiscus–Myrtus communis* matorral.

Ophrys battandieri, *Orchis anthropophora*, *Neotinea maculata*, and *Ophrys fusca* formed the third distinct ecological group which was the poorer species one. Most of these species occurred in a broad elevational range. These species exhibited broader environmental preferences, as confirmed by their moderate to rather high tolerance values. The species of the cluster showed high marginality but differed in their specialization index.

The last cluster included five orchid species, most of which had a high number of individuals, namely *Himantoglossum hircinum*, *Himantoglossum robertianum*, *Epipactis tremolsii*, *Cephalanthera longifolia*, and *Ophrys tenthredinifera*. The species of this cluster covered a broader range of environmental conditions, i.e. from low to higher elevations, cooler to warmer habitats, a span of light conditions and slopes, but occurred only to moderate to moist levels of moisture and exclusively to calcareous substrate, i.e. the dominant one in the study area. Their wider environmental preferences were reflected in their tolerance values, i.e. species with moderate to wide niche breadth, but with wide to moderate marginality. *Himantoglossum hircinum* constituted an exception, however; this specialist orchid species occured at upland cooler zones (found in *Abies* and *Quercus* forests), and had narrow niche breadth with a strong association to the specific environmental conditions.

Exposure

To cope with soil drought and reduce the impact of evapotranspiration driven by solar radiation, Orchidaceae in the study area predominantly occupy habitats with limited sun exposure. Specifically, 75.38% of the surveyed sites were oriented toward the North, Northeast, Northwest, West, or Southwest directions that receive less direct sunlight, helping to preserve soil moisture and maintain cooler microclimatic conditions favourable for orchid growth and survival.

However, despite this preference for shaded environments, many orchids also exhibit a tendency to colonize more open microhabitats to avoid interspecific competition for light, nutrients, and space. This is particularly evident in 21 sites where orchids were found thriving in canopy gaps, vegetation clearings and the edges of adjacent roads and pathways. These semi-open environments likely provide an optimal balance between reduced competition and sufficient light availability, supporting both photosynthesis and reproductive success. This dual strategy highlights the ecological adaptability of many orchid species to microhabitat variability within the broader landscape.

Role of basic/acid substrate in the differentiation of orchid groups

Substrate-specific distribution patterns of orchid species revealed a marked preference for calcareous soils within the IBRM region. Out of 26 recorded taxa, 24 were found exclusively or predominantly on calcareous substrates, accounting for over 93% of the total 1903 individuals surveyed. In contrast, only six taxa occurred on siliceous soils, and just one species (*Serapias vomeracea*) was restricted solely to this substrate type. A limited number of generalist species for substrate, such as *Orchis mascula*, *O. mascula* subsp. *laxifloriformis*, *Limodorum trabutianum*, *Serapias lingua*, *S. parviflora*, and *S. strictiflora*, were able to establish on both substrate types, though they were significantly more abundant on calcareous soils. These findings highlight the ecological importance of calcareous habitats in supporting orchid richness and abundance, likely due to favorable soil chemistry and compatible mycorrhizal associations. Consequently, conservation efforts should prioritize these alkaline environments to safeguard the region's orchid diversity.

Moisture

The combination of steep terrain and moderate moisture levels suggests that the majority of orchid habitats are situated in mesic montane environments, with a subset of taxa potentially adapted to either xeric or hydric microhabitats. These environmental gradients provide a valuable context for interpreting species distribution patterns and ecological preferences within the orchid assemblage. Although terrestrial orchids typically favour moist and/or shaded environments, they are capable of thriving across a range of habitat types, including coniferous and oak forests, matorrals, meadows, and wetlands. In the study area (sites 8, 25, 29, and 30), *Serapias* species are generally associated with habitats characterized by elevated soil moisture levels.

Discussion

A total of 26 orchid taxa, representing nine genera of the Orchidaceae family, were recorded across 65 sites within the IBRM. This diversity represents approximately 46.4% of Morocco's total orchid flora. The genus *Ophrys* was the most diverse, comprising nine taxa. However, this number remains lower than that reported in temperate forest of western Serbia, where 42 taxa were identified (Djordjević et al. 2020), or the temperate Balkan Mountains (Greece), where 55 taxa were identified (Tsiftsis et al. 2008).

In the IBRM, most species (23 out of 26) were found at fewer than 10 sites, and only three taxa were present in more than 10 sites. This pattern indicates a lower overall species abundance compared to western Serbia, where the majority of orchid species occurred at more than 10 sites (Djordjević et al. 2020).

Previous studies (e.g., Djordjević et al. 2014) have shown that orchid richness is typically higher in habitats with alkaline substrates. While species adapted to acidic soils can sometimes occur in both substrate types, alkaline habitats generally support greater orchid diversity. Consistent with these

observations, *Serapias vomeracea* in the IBRM was found exclusively on acidic bedrock (site 29), although it is known to tolerate alkaline substrates as well (Delforge 2006). This suggests that the seven species recorded on siliceous bedrock in the IBRM may be considered facultative with respect to substrate acidity. However, contrary to what is reported by Djordjević et al. (2014), *Neotinea tridentata* which is associated with acidic soils, was found growing on alkaline substrates in the IBRM, indicating some ecological flexibility.

Although siliceous substrates are typically low in nutrients, they may offer favorable conditions for some orchids due to open vegetation structure and reduced interspecific competition. Furthermore, high moisture availability may mitigate the effects of substrate acidity. The presence of *Serapias vomeracea* and *Serapias parviflora* in the Bouhachem Natural Park supports this hypothesis, as both species, which generally prefer slightly acidic substrates (Delforge 2006), were observed in water-saturated acidic soils, a phenomenon also noted by Djordjević et al. (2014). Rare species such as *Orchis spitzelii* subsp. *cazorlensis* and *Serapias vomeracea* were each recorded at a single site, while forest specialists like *Cephalanthera longifolia* and *Limodorum* spp. were more consistently associated with woodland habitats, echoing findings from Djordjević et al. (2016).

While terrestrial orchids are typically associated with moist and shaded environments, many display a broad ecological amplitude, occupying habitats such as coniferous and oak forests, matorrals, meadows, and wetlands. In the study area (sites 8, 25, 29, 30), *Serapias* species showed a strong preference for moist soil conditions. These findings align with previous studies highlighting soil moisture as a key factor influencing orchid distribution at fine spatial scales (Zhang et al. 2015; Diez and Pulliam 2007). Likewise, Djordjević et al. (2016) reported that orchid species richness was highest in habitats with semi-dry to moderately moist soils, while fewer species were observed at both ends of the moisture gradient. These moisture-dependent orchids were further classified into ecological groups according to vegetation type and underlying bedrock composition (Djordjević et al. 2016).

The OMI analysis results identified eight key environmental variables influencing orchid distribution within the IBRM; namely temperature, elevation, precipitation, latitude, slope, moisture, forest type, and substrate type. These findings align partially with previous studies highlighting geological substrate (Tsiftsis et al. 2008; Landi et al. 2009; Djordjević et al. 2014, 2016), moisture availability (Diez and Pulliam 2007; Djordjevic et al. 2016), and altitude (Tsiftsis et al. 2008; Djordjevic et al. 2016) as primary drivers of orchid distribution. At finer geographic scales, however, altitude tends to exert less influence, whereas geological substrates and soil properties (Djordjevic et al. 2014), soil moisture and light availability (Zhang et al. 2015) become more significant factors. Contrary to what was reported by Jacquemyn et al. (2005), in the IBRM area, species richness increased with elevation, suggesting a unique altitudinal pattern in this region.

Our study showed that 22% of species were strongly specialized; 35% were moderately specialized and 43% were generalists or weak specialists. The percent of the specialized species is low, compared to the orchids of Balkan Mountains (Greece) with 75% of species with significant marginality (Tsiftsis et al.

2008) or in the temperate forests of Serbia, with 72.5% (Djordjević et al. 2020). In a fine local scale, our study found a higher mean Species Specialization Index (SSI) in north sites and lower SSI eastward, and that the northern sites host more specialist species compared to the eastern areas, which are habitat of more generalist species. The difference between the northern orchids of Serbia and Greece and the southern ones of Morocco could be explained by the climate type (Temperate in Balkan Mountains and temperate forests in Serbia vs. Mediterranean in the IBRM), and the adaptation of the Moroccan orchids to habitat changes (presence of impacted non-protected sites in the study area). In general, our study showed that the species specialization drivers are climate, slope, and habitat heterogeneity. However, in the temperate forests of Serbia, bedrock type, forest type, and soil properties were the factors that shaped orchid assemblages (Djordjević et al. 2020).

Our study reported that generalist species occur at higher elevations [significant, linear and negative relationship between mean SSI and elevation ($GAM_{Deviance\ Explained}$= 9.4%, p = 0.01)]. However, the specialists dominate mid-altitudes. This pattern was not observed in the temperate forests in Serbia. Where orchids are segregated by moisture, slope, soil pH and forest type (Djordjević et al. 2020). In addition, our study showed that the high mean Species Specialization Index was linked to northern latitudes and lower elevations. However, the higher specialization was weakly linked to altitude and latitude in the temperate forests in Serbia (Djordjević et al. 2020).

Residual tolerance was emphasized in our study (e.g. *Serapias parviflora* and *Ophrys lutea*, up to 47%) and the study of Tsiftsis et al. (2008) suggest that additional environmental or biotic factors, not considered herein, may influence the orchid niches. Yet, the remaining orchid species of the IBRM showed low residual variance. Thus, the environmental variables included in the analysis effectively explained the distribution and abundance of orchids across the study area, suggesting that these selected factors exert a significant influence; consistent with findings reported by Djordjević et al. (2020).

In addition, habitat types highly influenced the distribution and abundance of orchids in the IBRM. Thus, the woody formations such as matorral and forest habitats, host 99.2% of the total orchid individuals. The latter is consistent with the findings of Bulafu et al. (2007), Tsiftsis et al. (2008), and Djordjevic et al. (2016), suggesting a significant influence of vegetation type on the distribution and abundance of orchid species.

In the IBRM area, four ecological groups of orchids were distinguished according to the hierarchical clustering combined with SIMPROF test. This study suggested that the specialist species was associated with cooler to moderately warm habitats of mid- to high elevations and of moderate slope, namely *Limodorum trabutianum*, *Orchis mascula, Orchis mascula* subsp. *laxifloriformis, Serapias lingua* and *Serapias strictiflora*. However, in western Serbia, these specialist orchid species occur in habitats characterized by extreme conditions along gradients of light, temperature, soil pH, moisture, and elevation (Djordjević et al. 2020).

Similarly to what was reported by Delforge (2006), Tsiftsis et al. (2008), and Djordjević et al. (2020), our study reported that *Cephalanthera longifolia* is a generalist species. This species exhibited broad ecological tolerance, being present in several forest communities, across multiple substrates, and throughout a wide elevation spectrum (Djordjević et al. 2020). In the IBRM area, *Cephalanthera longifolia* could show also a wide ecological scope, from 415 to 1366m of elevation, in different habitat types, such as matorrals of *Quercus coccifera, Quercus ilex*, and *Pistacia lentiscus*, or forest of *Quercus ilex_Q. faginea*. However, it was recorded only on calcareous substrate.

Although *Ophrys apifera* has a high value of marginality (77.44%), and high SSI (0.51), this species has considered as generalist species. This finding could be explained by its high value of occurrence and abundance (8 sites and 196 individuals), and occurring in a wide range of habitat types, such as *Quercus suber* forest, and matorral of *Pistacia atlantica, Pistacia lentiscus*, and *Quercus coccifera*, but in small range of elevation (77 to 587m), on calcareous substrate. However, this Mediterranean and sub-Mediterranean orchid species has considered as a specialist species, occupying few forest types and geological formations, it is predominantly found within a restricted elevation range by Djordjević et al. (2020).

Toward the periphery of a species' geographic range, both mean population densities and the number of occupied sites tend to decline, reflecting increasingly marginal or suboptimal ecological conditions (Pfeifer et al. 2010). The Talassemtane National Park could be considered as the centre of orchids diversity in the IBRM. The most orchids occur in this area, with a great number of individuals. Towards the edge of the IBRM, a decreasing diversity and abundance of orchids may be detected. For example, *Cephalanthera longifolia* occur in six sites in the Talassemtane National Park, however, we found only one site of this species in the remaining areas of the IBRM, while 16 out of 26 taxa of the study area occur in Talassemtane National Park. This suggests the multiplication of in situ conservation efforts of this protected area.

European orchid populations located at the southern edge of their range have been adversely affected by intensified land use and reduced rainfall (Pfeifer et al. 2010). The same situation could be raised for the orchid populations of North Africa, and Morocco, deserving more priority for conservation because they occur in the extremely southern range of species distribution and contain some rare and endemic species, such as *Dactylorhiza maurusia*, last reported over 15 years ago by Mateos and Valdes (2010).

Compared to the centre of distribution of *Himantoglossum hircinum* in France, the southern Spanish populations have low number of plants (up to 200 plants) and flowering plants (up to 100 plants) (Pfeifer et al. 2010). However, in our study area, the two sites of occurrence of *Himantoglossum hircinum* have only five individuals, at elevation ranging from 1174 m to 1608 m a.s.l. The latter confirms that the Moroccan population of this species occur in the extreme edge of the species distribution. In addition, this protected park probably constitute the only site of occurrence of *Orchis spitzelii* subsp. *cazorlensis*.

Overall, woody and forested habitats tend to support a greater diversity and abundance of orchids compared to open or degraded areas. Thus, the matorral *Pistacia lentiscus*, forests of *Quercus suber* and

Quercus ilex communities supports the highest orchid diversity, with 18, 10 and 9 species respectively. The same trend occurred in western Serbia, where *Fagus* forests supported the richest orchid assemblage, with 24 recorded species and subspecies (Djordjević et al. 2020).

Conclusion

The Intercontinental Biosphere Reserve of the Mediterranean (IBRM) harbors a significant portion of Moroccan orchid diversity, with 26 taxa of nine genera recorded at 65 sites, representing 46.4% of the national total. Despite this notable richness, species abundance is low, with most taxa occurring at fewer than ten sites.

The OMI analysis of terrestrial orchids in The IBRM (northern Morocco) revealed clear patterns of ecological specialization across environmental gradients. Five orchid species exhibited strong marginality, indicating high specialization, while others showed moderate specialization or generalist behavior. High specialization was typically associated with narrow niche breadth and specific environmental preferences, particularly in cooler, mid- to high-elevation habitats with distinct substrate types. Conversely, generalist species displayed broader ecological tolerance and were more common at higher elevations and in warmer conditions.

The main environmental drivers influencing orchid distribution were temperature, elevation, precipitation, latitude, slope, moisture, forest type, and substrate type. The analysis highlighted a significant latitudinal and longitudinal gradient in species specialization, with more specialized orchids found in northern areas and more generalists in eastern regions. Hierarchical clustering further distinguished four ecological groups, each linked to specific habitat types and environmental preferences. Overall, the study demonstrates that orchid specialization in this Mediterranean region is closely tied to both abiotic conditions and microhabitat characteristics, underlining the importance of habitat heterogeneity in orchid conservation strategies.

Environmental variables including elevation, latitude, longitude, soil moisture, slope, substrate type, and annual mean temperature significantly shape the spatial distribution of orchid taxa, as revealed by principal component analysis. Soil substrate and moisture emerged as particularly influential factors. Although alkaline substrates are traditionally associated with greater orchid richness, the presence of species like *Serapias vomeracea* and *Serapias parviflora* on acidic, water-saturated soils suggests facultative adaptation and niche flexibility. This observation reinforces the role of soil moisture as a key determinant of orchid distribution, especially in compensating for otherwise limiting substrate conditions.

Moreover, orchids in the IBRM display broad habitat plasticity, occupying coniferous and oak forests, meadows, matorrals, and wetlands. However, the rarity of some taxa, coupled with their dependence on specific ecological conditions such as moisture and low interspecific competition, underscores their vulnerability. These findings emphasize the ecological value and conservation importance of the IBRM,

which serves as a critical refuge for both generalist and specialist orchid species within the Mediterranean biodiversity hotspot.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

The study conception and design were performed by Y.E. (Yahya El Karmoudi), M.L. (Mohamed Libiad), and N.K. (Nikos Krigas). Material preparation and data collection were performed by Y.E., M.L., and A.K. (Abdelmajid Khabbach), and analysis was performed by M.La. (Maria Lazarina). The first draft of the manuscript was written by Y.E., M.L., I.S. (Ioulietta Samartza) and M.La. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Figures

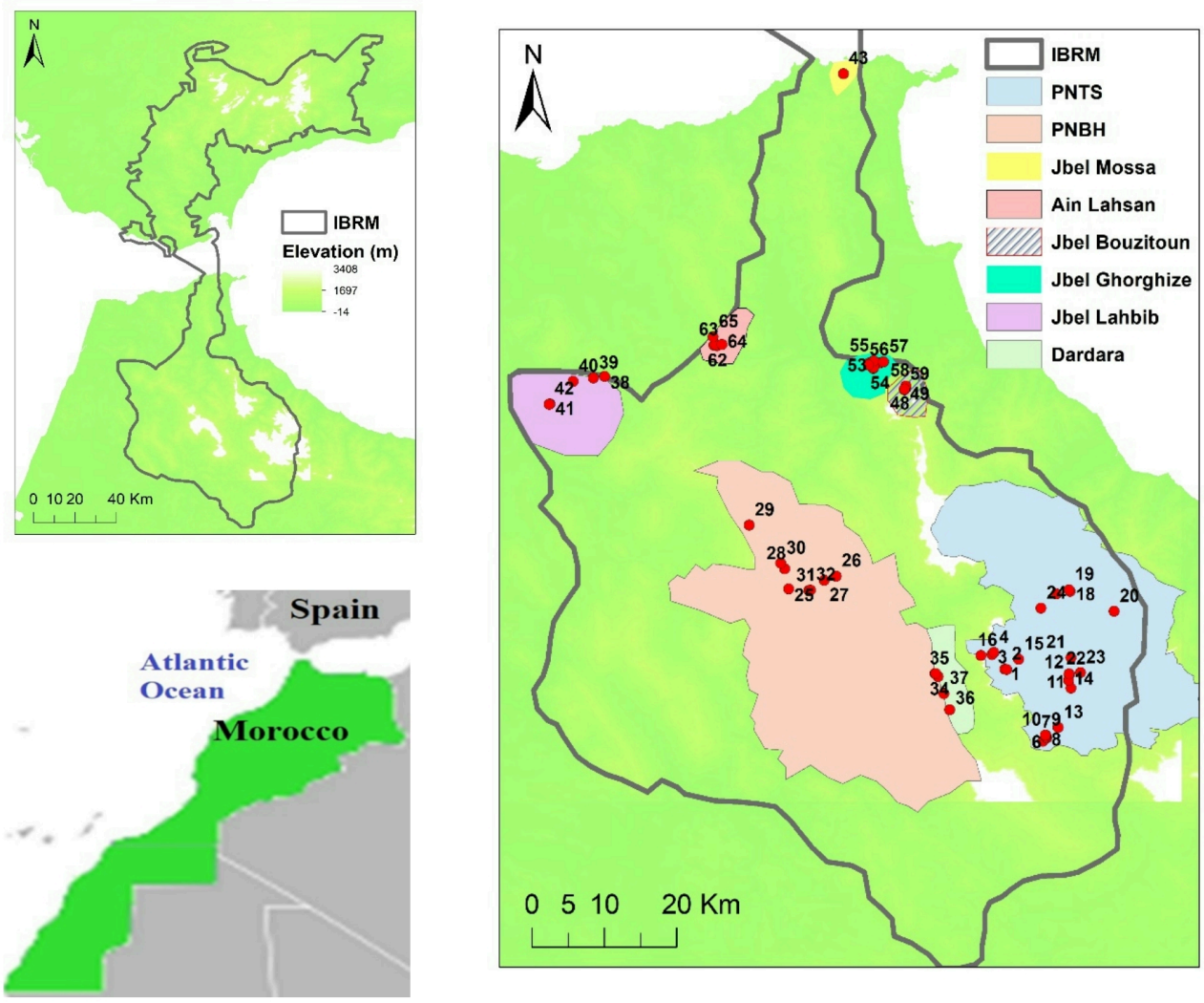

Figure 1

Map of the study area with explored protected areas (Sites 1-24: Talassemtane National Park; Sites 25-37: Bouhachem Natural Park; Sites 38-42: Jbel Lahbib Reserve; Site 43: Jbel Moussa Reserve), and

surveyed non-protected areas (Sites 44-46, 50-57: Jbel Ghorghize; Sites 47-49, 58-59: Jbel Bouzitoun; Sites 60-65: Ain Lahsan).

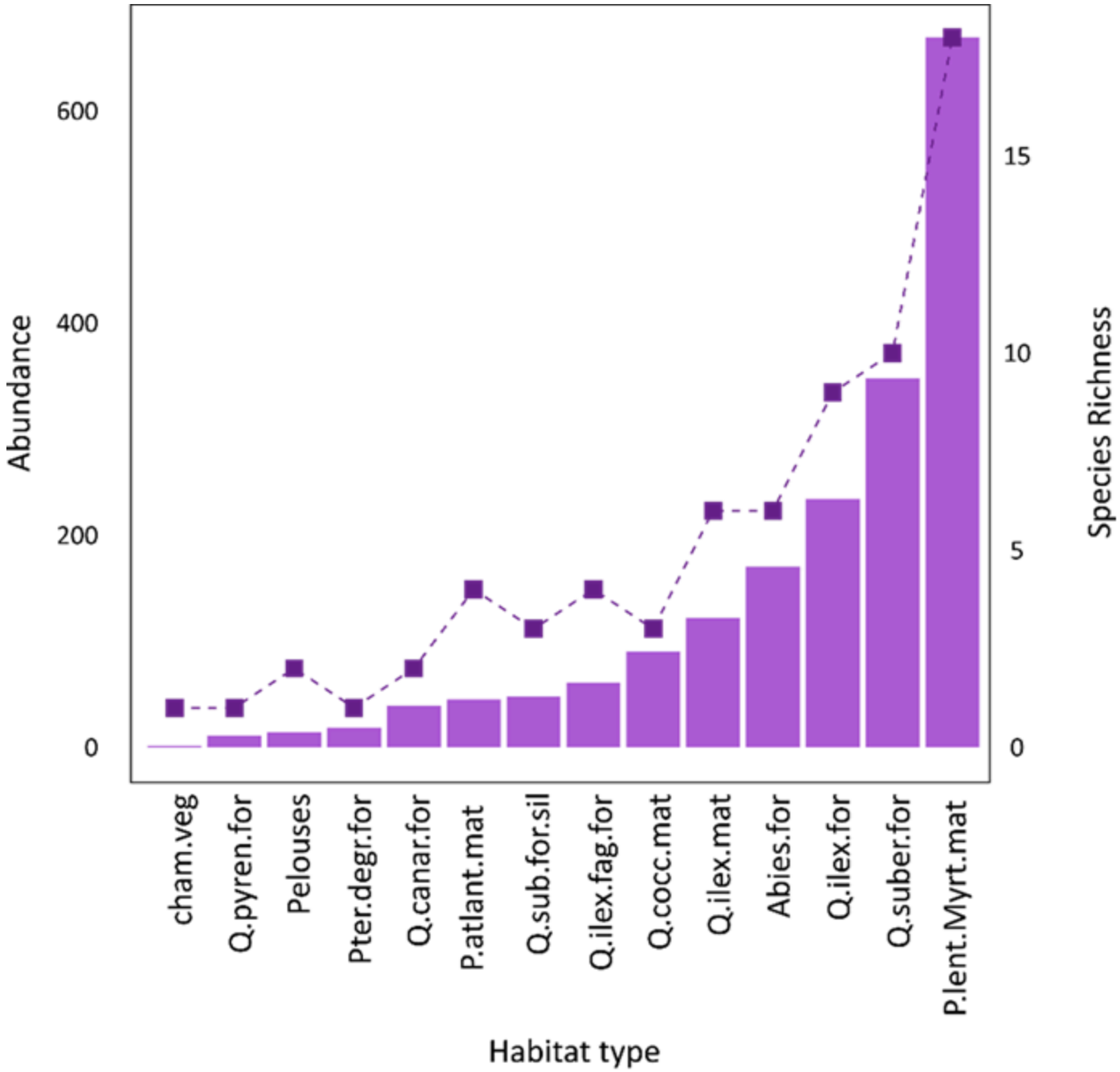

Figure 2

Orchid abundance and species richness across habitat types. Abbreviations for habitat types are as follows: **cham.veg:**Chamaephytic scrub dominated by *Chamaerops humilis* and *Stachys fontqueri*; **Q.pyren.for:** *Quercus pyrenaica* forest; **Pelouses:**Pelouses dominated by herbaceous Asteraceae, Boraginaceae, and Poaceae; **Pter.deg.for:**Degraded forest with *Pteridium aquilinum* understory; **Q.canar.for:** *Quercus canariensis* forest; **P.lent.Myrt.mat:** *Pistacia lentiscus* matorral and/or mixed *Pistacia lentiscus - Myrtus communis* matorral; **Q.sub.for.sil:**Siliceous substrate *Quercus suber* forest; **Q.ilex.fag.for:** Mixed *Quercus ilex* and *Quercus faginea* forest; **Q.coc.mat:** *Quercus coccifera* matorral;

Q.ilex.mat: *Quercus ilex* matorral; **Abies.for:** *Abies marocana* forest; **Q.ilex.for:** *Quercus ilex* forest; **Q.suber.for:** *Quercus suber* forest; **P.atlant.mat:** *Pistacia atlantica*matorral; **P.lent.mat:** *Pistacia lentiscus* matorral.

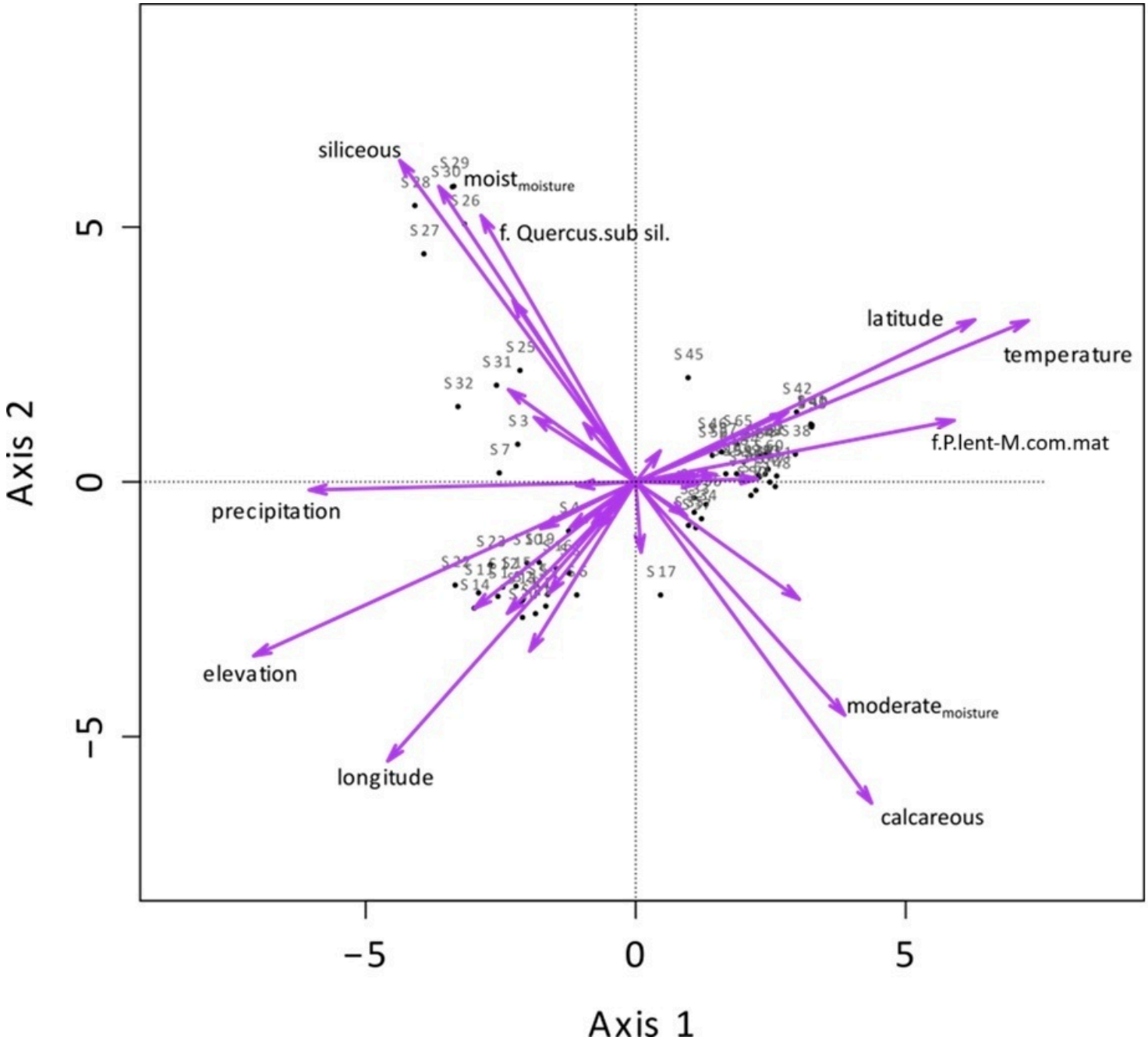

Figure 3

Principal Component Analysis biplot showing the scores of sampling sites on the first two PCA axes. The environmental variables are represented with arrows, while arrows' direction and length represent the correlation and the strength of the correlation with the PCA axes, respectively. Note that only variables with strong correlations are labeled.

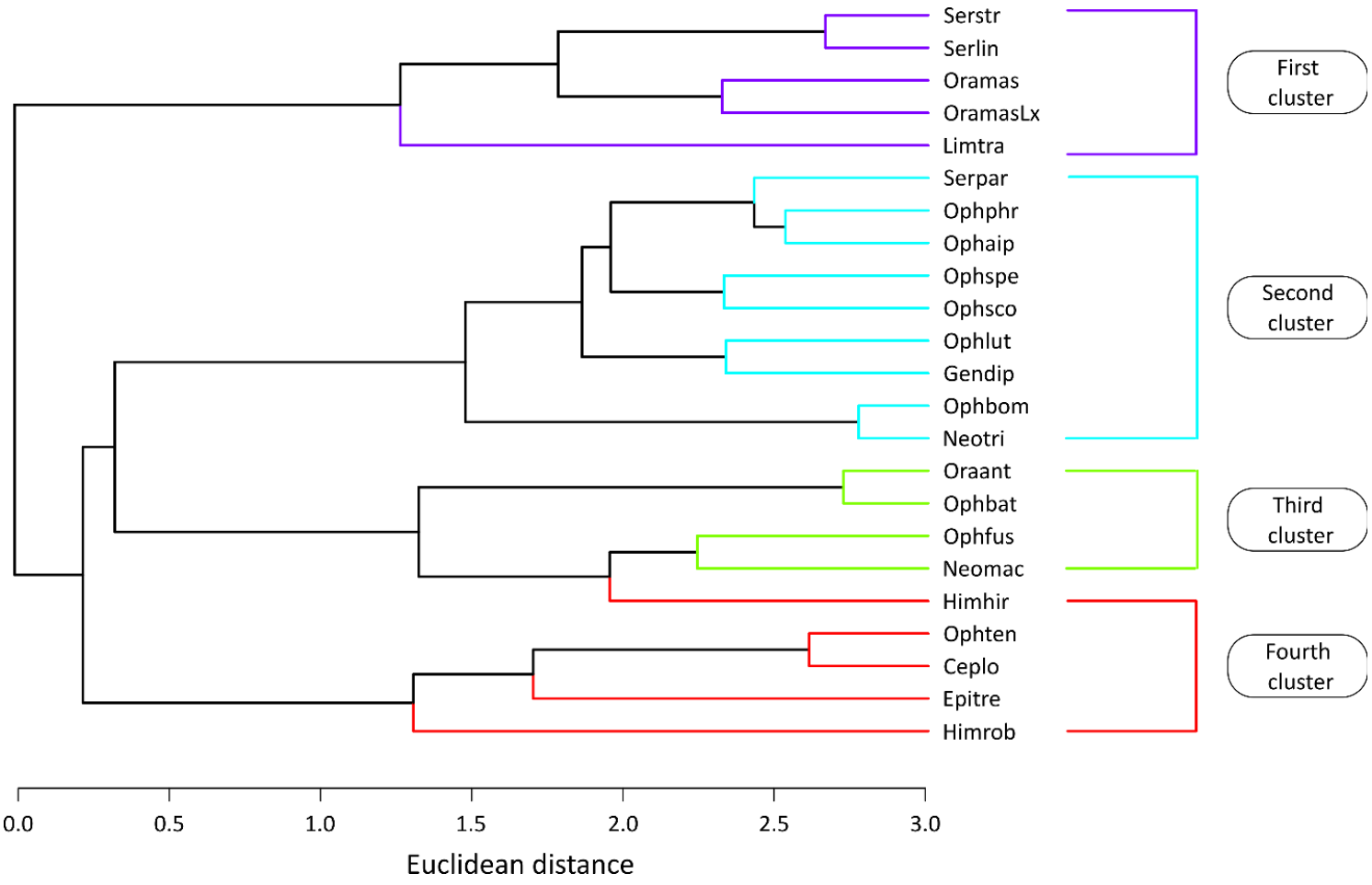

Figure 4

Dendrogram showing the relationships between clusters of orchid species and subspecies as were defined from the SIMPROF test. Different colours represent distinct ecological clusters. The abbreviations of the species are presented in the Table 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SMOrchidsIBRMPlantEcology.docx