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Research Article

Evaluation of phenotypic diversity in *Anacamptis coriophora* (Orchidaceae) populations from South Romania

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Abstract

Romanian flora includes a high diversity of orchids, any with a high conservationist interest, but many 0f themnot yet sufficiently investigated from an ecological, phytosociological or genetic point of view. Phenotypically analysis is also scarce and only a few data have been published in relation to the effects of site factors on the morphological and anatomical characteristics of the family Orchidaceae. This study analyses the variability of two populations of *Anacamptis coriophora*, based on their coenological affiliations, morphological observations and biometrical measurements. The data were collected from two grassland areas in Romania Stoenesti, Vâlcea County and Cobia, Dâmbovița County. The results showed that both populations are influenced by temperature, light and humidity and develop normally under optimal conditions. In the most favorable conditions encountered in Vâlcea county, orchids offer the splendor of flowers for a long time. Orchids in our country usually bloom in March, sometimes lasting until August, they have a delicate appearance, varied colors and the most diverse perfumes. The information obtained regarding the morphological diversity of the two populations could be suitable for designing strategies for their conservation in both locations.

Keywords: *Anacamptis coriophora*; population variation; phenotypic diversity; South Romania

Introduction

A good knowledge of orchids is especially important, as many species of this family are not very abundant and ecological, phytosociological, or morpho-anatomical studies are very limited. Orchids play an essential role for the conservation of natural heritage and for the development of sustainable landscape management strategies. The diversity of relief, soil types and ecological factors in Romania have an overwhelming influence on the diversity of orchid species, but also on the habitats and landscapes in which they are found. In addition, the effects of the current climate changes on orchid diversity must be taken into account. Many species have a high ornamental value and inspired a series of artists, poets, designers, photographers or popular creators of legends or myths (Tomescu, 2018).

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According to Chase *et al*. (2015), Orchidaceae family comprises almost 28.000 species, divided into five subfamilies and 736 genera. Most species are found in tropical forests, but there are also a considerable number of taxa in the temperate region, growing in grasslands and deciduous or coniferous forests.

Orchid species are of particular importance from the point of view of biodiversity conservation, once they have been identified in a particular site. Orchids are generally shallow-rooting plants and can survive on well-drained soils with low fertility. They maintain symbiotic relationships with soil fungi which allows them to obtain sufficient nutrients and be able to compete successfully with other plants. In order to ensure their preservation, great attention is paid to the development and protection of their genetic diversity outside natural habitats (*ex situ*). Orchids are an important part of plant biodiversity in certain habitats. In Romania, the Orchidaceae family is represented by 3 subfamilies and 26 genera, comprising approximately 71 species and 14 subspecies (De Angelli and Anghelescu, 2020).

Orchids are present in a large number of plant communities in Romania, including here the vast majority of natural and semi-natural habitats of meadows, marshy areas, shrub communities or forests habitats. Most of the orchid species in Romania are rare and require protection, considering that the state of conservation of the natural habitats in which they are found is not always favorable. The main factors that lead to the degradation of the habitats of these species are: changes of land use, invasive species expanding in their habitats, storage of garbage and waste, intensive grazing, among others. In Romania, *A. coriophora* (L.) R.M.Bateman, Pridgeon & M.W.Chase, or the (bug orchid), is found in meadows, on poor soils, with an alternating or swampy moisture regime, in sunny locations, from plains to the mountain region, mostly on hills. In Europe the species can be found in the central and southern part of the continent. The conservation status of the species is generally favorable, with particularities for each region or protected area where it grows.

Orchids from Romania have not been sufficiently investigated from an ecological, phytosociological, genetic point of view, but especially phenotypically, and the specialized literature highlights only a few data on this group of plants. The scanty information on these species may be linked to several factors, such as their scarcity, the small and scattered populations as well as to the degradation and even extinction of their natural habitats (Savić, 2001). Diversity and morphological analysis are useful for resource management, conservation or other activities for genetic improvement (Prayoga *et al*., 2022).

There are only few studies related to the effects of site factors on the morphological and anatomical characters of the family *Orchidaceae*. Therefore, the present study was carried out to bring new data on the morphological characters of the species *A. coriophora* identified in South Romania.

Materials and Methods

Biological material was analysed *in situ* in the natural populations from two types of sites:

Population *A. coriophora* 1 - hilly region, beech forest, altitude 400 m, acid brown soils, flat or slightly sloping terrain, meadow of *Festuca rubra*, swampy, sunny – the Govora Stream basin, Stoenești, Vâlcea County, location: 45.132116 latitude N- 24.174707 longitude E.

Population *A. coriophora* 2 - plain region, oak forests, altitude – 254 m, pre-podzolized soils (very good, feri-illuviale, brown-podzolized), flat or slightly sloping terrain, mesophyll meadow of *Arhenatherrum elatius*, partially sunny Cobia, Dâmbovița County. location: 44.789602 latitude N- 25.342674 longitude E.

The identification of taxa was based on Romanian Flora (Săvulescu, 1972) and *Flora Europaea* (Tutin *et al*., 1964-1980). For the analysis of the plant community in the study area was used the method of phyto-sociologic research of the Central European Phyto-Sociologic School, which is based on the principles and methods elaborated by Braun-Blanquet (Braun-Blanquet and Jenny, 1930). The plant communities were identified according to the characteristic, edifying, dominant and differential species. For the classification and phytosociology study were used synthesis papers on the Romanian (Coldea, 1991; Coldea *et al.*, 1997) and European vegetation (Sanda *et al.*, 2001; Rodwell *et al.*, 2002; Mucina *et al.*, 2016).

The Syn-Tax 2000 program was used for the statistical analysis of the floristic richness of plant communities in which the studied species were found. The calculation of the correlation quantitative index was performed by the Group-Average method (UPGMA) and dendrograms according to Podani (2001).

For the study of the phenotypic diversity of the two populations of *A. coriophora,* three factors were taken into consideration: humidity level, sun brightness and location. The humidity (factor A) had two levels: swampy and low humidity. The B factor, sun brightness, had also two levels: in the sun and in the shade. The C factor, location, included also two levels: Vâlcea and Dâmbovița County, both situated in southern part of Romania. To analyze the variability of the two populations, observations and determinations of 20 individuals were made in the field for a series of morphological characters such as: average plant of height, leaf no./plant, leaf length, leaf width, inflorescence length, flowers no./inflorescence, flower length and diameter.

Data were statistically analyzed using IBM SPSS software. All determinations were performed in five repetitions in a randomized block design model and mean values were processed by analysis of variance (ANOVA). The significance of the differences was estimated with the LSD multiple comparison test at the $p \leq 0.05$ level. The significance of differences between means was presented on letter basis, with significant differences between means marked with different letters (a, b, c - for genotype × year comparisons; A, B, C - for year comparisons). The correlation coefficients between characters were also calculated, their significance being determined by Pearson values. The principal component analysis method explained by Harman (1976) was followed in the extraction of the components. The percentage variability explained by each component were determined (Harman, 1976; Sharma, 1996; Tadesse and Bekele, 2001). Correlation and principal component analysis as well as bi-plot graphical display were performed.

Results

Plant coenology

Considering the variability of the plant communities in which the species was identified, it is important to present some aspects in this regard. The studied species is found in the floristic composition of an important number of plant communities belonging to Festuco-Brometea and Molinio-Arrhenatheretea classes (Sanda, 2001; Oroian *et al.*, 2007), as well as the alliances Molinion caeruleae*,* Calthion, Arrhenatherion, Nardo-Agrostion (Sanda, 2001; Tashev *et al.*, 2010).

In the analytical section dedicated to the two populations of orchid species found in the research area, we addressed various aspects related to the conservation and sustainable protection of the orchid species populations analyzed from the two locations in southern Romania, Stoenești and Cobia, which are the subject of this study (Figure 1). The analyzed populations of *A. coriophora* belong to two communities of meadow plants: *Festucetum rubrae-Agrostietum capillaris* Horv. 1951 (Horvat, 1951) and *Arrhenatheretum elatioris* Br.-Bl. et Scherrer 1931 (Braun-Blanquet and Scherrer, 1931). These two plant communities are of particular importance from the point of view of biodiversity conservation.

Festucetum rubrae-Agrostietum capillaris Horv. 1951 (Horvat, 1951) is a widespread plant community in the Govora basin, being found in the hilly and mountainous belts. It grows on flat or slight slopes, preferring eutricambosols. In the studied territory, such phytocoenoses were analyzed in the middle basin of the Govora stream, in the Stoenești village. The analysis of the phytocoenoses according to the main ecological indices highlights the mesophilic, micro-mesothermic and euriionic character. The floristic composition of this plant community is one of the most complex, with a fairly large number of cormophytes in the six relevés analyzed. Numerous forage species participate in the composition of the vegetation floor: Festuca pratensis, *Arrhenatherum elatius*, *Anthoxanthum odoratum*, *Cynosurus cristatus*. The phytocoenoses analyzed show a high cohesion, achieving a 90-100% vegetation coverage. Such phytocoenoses were identified in the hilly floor, in the Stoenești village at an altitude around 400 m.s.l., growing on sunny and slightly inclined lands. Thus, it was found that the phytocoenoses analyzed within this plant community are very rich in terms of floristic composition, comprising a number of 62 species, including *A. coriophora*. Within the analyzed phytocoenoses, this species shows an abundance-dominance that varies depending on the ecological conditions, especially taking into account soil moisture and exposure. In some phytocoenoses analyzed on the hills around Stoenești village, only allow abundance-dominance of the species *A. coriophora* (Figure 2) was found. This can be explained by the aridizationtendency of these meadows, due to the southern and south-east geographic exposure and the soils on which these communities develop. In order to observe the floristic richness of the phytocoenoses in which the studied species is also found, a dendrogram was performed using the Syn-Tax 2000 statistical program and correlation quantitative indexes were calculated by the Group-Average method (UPGMA) (Podani, 2001). Analyzing the dendrogram (Figure 2), it is noticeable the grouping of two clusters, with well-individualized branches, in which the relevés 1, 2, 3, 4 (REL_01_A, REL_02_A, REL_03_A, REL_04_A) are highlighted, the abundance-dominance of *A. coriophora* being higher as compared to relevé 5 (REL_05_A) where the abundance-dominance of the species is lower. In the relevé 6 (REL_06) from the second cluster the species is missing.

Figure 1. *Anacamptis coriophora* in the meadows from Stoenesti

Following intensive grazing, these meadows can degrade and evolve into Festuca rupicola or *Dichanthium ischaemum* meadows. These meadows, built of red grass and wind grass, are of particular importance from the point of view of biodiversity conservation, including in their floristic composition a series of botanically interesting taxa, including the here analyzed. These meadows also represent pastures and hayfields with a special role in providing green and dry fodder. The superior quality is given by Festuca rubra, *Agrostis capillaris*, *Trifolium pratense*, *Medicago lupulina*, *Trifolium pannonicum*. As hay they are cut twice a year. For the protection of species with high value for biodiversity and especially for the protection of orchid species, their exploitation from an economic point of view must be conducted in compliance with the current protection norms.

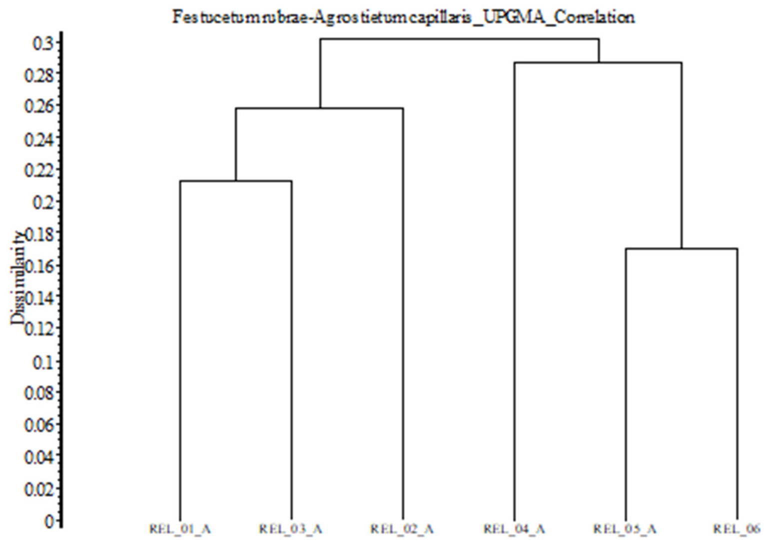


Figure 2. Dendrogram of the *Festucetum rubrae-Agrostietum capillaris* plant community

Arrhenatheretum elatioris Br.-Bl. et Scherrer 1931 (Braun-Blanquet and Scherrer, 1931) phytocoenoses were identified in the vicinity of Cobia, alt. 254 m. s.l., on a flat land surrounded by forest, where it grows on soils with moderate moisture. This type of meadow is dominated everywhere by oat, a tall species that covers a high percentage of the ground. The floristic structure of the phytocoenoses is formed by species characteristic of mesophilic grasses, some of which belong to the order Arrhenatheretalia. Among them, *Bromus commutatus*, *Anthoxanthum odoratum*, *Leucanthemum vulgare* and *Campanula patula* appear with high constancy. In the floristic composition of this plant community are found 53 species, and *A. coriophora* in some phytocoenoses has a rather high abundance-dominance. As with the previous plant community, in order to highlight the floristic richness, and the abundance-dominance of the analyzed species, we performed a dendrogram and we gave a special attention to the calculation of the correlation quantitative index (Podani, 2001). Analyzing the dendrogram (Figure 3), one can notice the grouping of two clusters, with well-individualized branches. Relevés 3, 4 and 5 (REL_03_A, REL_04_A, REL_05_A) are highlighted, in which the abundance-dominance of *A. coriophora* is greater compared to the relevés 1, 2 and 6 (REL_01_A, REL_02_A, REL_06) where the species abundance-dominance is very low. Relevé 3 of the first cluster is distinguished by the highest abundance-dominance of the species. The habitat of the species is sometimes disturbed by some invasive species, such as *Ambrosia artemisiifoloia*, *Erigeron annuus*. It is observed that the dominant abundance of the analyzed orchid is lower in these phytocenoses. It should be noted that in the analyzed communities, apart from the species belonging to the Molinio-Arrhenatheretea class, the characteristic elements of the other syntaxonomic categories are poorly represented. Due to the seasonal variation of the edaphic humidity of the populated stations, in addition to mesophilic and meso-hygrophilic species, they reach quite high values. Regarding the need for light, micro-mesotherms are dominant. *Arrhenatherum elatius* hay has a superior pratological value. It is worth noting that, in addition to the oat, which reaches heights of over 1 m, many of the accompanying forage species are also tall. In addition to fodder plants, honeydew, medicinal and toxic species also appear here. Among the limiting factors that act at the level of this habitat can be mentioned grazing, garbage and solid waste, invasive species, oil extraction.

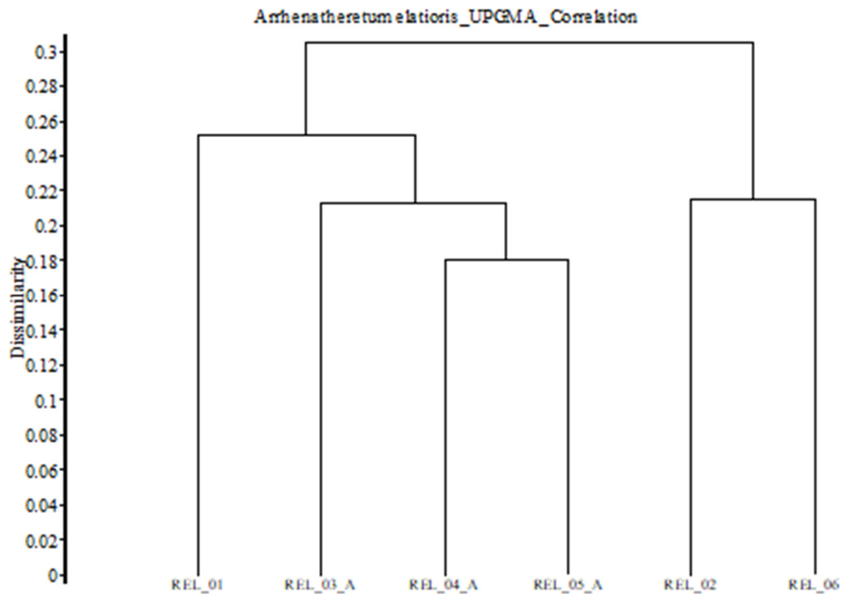


Figure 3. Dendrogram of the *Arrhenatheretum elatioris* plant community

Statistical analysis of morphological characters

Generally, morphological variability is due to ontogenetic programming of each plant, but abiotic variables can exert a significant effect on characters. We considered three factors in relation to the morphological variability of the analyzed plants. Factor A, humidity: the average values calculated for graduation A1, swampy registered significant positive differences compared to the values from graduation A2, dryer environment, on most of the analyzed characters. The exception is the inflorescence length character, between the two average values calculated at A2 graduation, where no statistically significant difference was recorded (Table 1).

Table 1. The influence of factor A, humidity, on the studied characters

Character / A level	Mean±S.D.							
	Plants height (cm)	Leaf no./plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./ inflorescence	Flower length (cm)	Flower diameter (cm)
A1' (swampy)	31.375±4.54 a	9.45±2.48 a	9.91±1.53 a	1.09±0.15 a	6.255±2.25 ns	20.1±7.34 a	1.18±0.10 a	0.51±0.09 a
'A2' (low humidity)	22.625±3.67 b	7.1±1.41 b	8.195±1.65 b	0.86±0.12 b	5.635±0.76 ns	14.65±1.95 b	0.92±0.14 b	0.41±0.08 b
LSD 5%	0.416	0.840	0.997	0.080	1.219	3.111	0.107	0.038

Different letters between populations denote significant differences (LSD test, $p < 0.05$).

Factor B, light: for the character plant height, flower length and respectively flower width, the average values calculated for the B1 graduation, respectively sunny environment, register significant positive differences compared to the average values calculated for the B2 graduation, darker environment. In the case of the analysis of the leaf length character, the value for grading B2, i.e., darker environment, registers a significant positive difference compared to the value calculated for graduation B1, sunny environment. In the case of character analysis leaf width, inflorescence length and no. of flowers/pl., no statistically significant differences were recorded between the two gradations of factor B (Table 2).

Table 2. The influence of factor B, light, on the studied characters

Character / B level	Mean±S.D.							
	Plants height (cm)	Leaf no. /plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./ inflorescence	Flower length (cm)	Flower diameter (cm)
'B1' (sunny)	30.26±5.30 a	8.6±2.64 ns	8.625±2.04 b	0.985±0.16 ns	5.795±2.00 ns	18.45±6.97 ns	1.075±0.17 a	0.49±0.10 a
'B2' (shade)	23.74±4.89 b	7.95±1.96 ns	9.485±1.45 a	0.965±0.20 ns	6.095±1.34 ns	16.3±4.73 ns	1.025±0.18 b	0.43±0.09 b
LSD 5%	0.826	1.526	0.826	0.145	1.246	3.145	0.044	0.036

Different letters between populations denote significant differences (LSD test, $p < 0.05$).

Related to the influence of the C factor, the location, on the analyzed characters, for the character plant height, leaf width, and respectively, flower width, the average values calculated for the C1 graduation, respectively the Vâlcea location, register significant positive differences compared to the values averages calculated at C2 graduation, location Dâmbovița, Cobia, between the other characters no statistical differences were recorded (Table 3).

Table 3. The influence of factor C, location, on the studied characters

Character / C level	Mean±S.D.							
	Plants height (cm)	Leaf no./plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./ inflorescence	Flower length (cm)	Flower diameter (cm)
Vâlcea	28.85±6.07 a	8.4±2.44 ns	9.245±1.87 ns	1.015±0.16 a	5.73±1.48 ns	16.1±4.99 ns	1.06±0.18 ns	0.495±0.11 a
Dâmbovița	25.15±5.50b	8.15±2.25 ns	8.865±1.75 ns	0.935±0.19 b	6.16±1.88 ns	18.65±6.71 ns	1.04±0.18 ns	0.425±0.07 b
LSD 5%	0.690	1.341	0.900	0.059	1.196	2.783	0.076	0.046

Regarding the influence of the interaction of factors A and B on the studied characters, for the character of plant height, significant differences were calculated between all average values, the highest value being recorded at the A1B1 graduation. In the case of character no. leaf average/pl., the value calculated at the A1B1 graduation registered significant differences compared to all other values, less compared to the second classified value from the A1B2 graduation. In the case of the leaf length character, the first three calculated values, from the A1B1, A1B2 and A2B2 graduations, show significant differences compared to the last recorded value, a situation that we also find in the analysis of the flower length character. For the leaf width character, the first calculated average value, the one from the A1B1 graduation, shows significant differences compared to all other calculated values, less compared to the second calculated value. An almost similar situation is also found in the case of characters no. average flower/inflorescence, respectively the average width of the flower, the first calculated average value, the one from the A1B1 graduation registering significant differences compared to all other calculated values (Table 4).

Table 4. The influence of the interaction of factors A and B on the studied characters

Character / Variant	Mean±S.D.							
	Plants height (cm)	Leaf no./plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./ inflorescence	Flower length (cm)	Flower diameter (cm)
A1B1	34.91±10.76 a	10.4±3.88 a	10.23±3.36 a	1.1±0.35 a	6.15±3.22 ns	23.1±9.75 a	1.2±0.37 a	0.54±0.19 a
A1B2	27.84±8.92 b	8.5±3.35 ab	9.6±3.29 a	1.08^{ab}±0.37	6.36±2.52 ns	17.1±8.03 b	1.16±0.37 a	0.48±0.16 b
A2B1	25.61±8.04 c	6.8±2.40 b	7.02±2.33 b	0.87±0.29 b	5.44±1.76 ns	13.8±4.46 b	0.95±0.32 b	0.44±0.15 b
A2B2	19.64±6.14 d	7.4±2.65 b	9.37±3.08 a	0.85±0.28 b	5.83±1.92 ns	15.5±5.01 b	0.89±0.29 b	0.38^{c}±0.14
LSD 5%	1.169	2.158	1.169	0.205	1.762	4.448	0.062	0.050

Different letters between populations denote significant differences (LSD test, $p < 0.05$).

Regarding the influence of the interaction of factors A and C, for the character of plant height, significant differences were calculated between all calculated average values, the highest value being recorded at the A1C1 graduation. In the case of characters no. of leaves/pl., leaf length and flower length, respectively, the values calculated at the A1C1 and A1C2 graduations show significant differences compared to all other values. For the leaf width character, the first calculated value, the one from the A1C1 graduation, registers significant differences compared to all the others, less compared to the second calculated value. We find an almost similar situation in the case of the character width and no. of flowers/inflorescence, the first calculated value, the one from the A1C2 graduation registering significant differences compared to all other calculated values (Table 5).

Table 5. The influence of the interaction of factors A and C on the studied characters

Character / Variant	Mean±S.D.							
	Plants height (cm)	Leaf no./plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./ inflorescence	Flower length (cm)	Flower diameter (cm)
A1C1	33.52±3.92 a	9.7±2.41 a	10.1±1.53a	1.11±0.15 a	5.66±1.96 ns	17.5±6.47 b	1.2±0.08 a	0.56±0.08 a
A1C2	29.23±4.22b	9.2±2.66 a	9.73±1.58 a	1.07±0.15 ab	6.85±2.46 ns	22.7±7.54 a	1.16±0.11 a	0.46±0.05 b
A2C1	24.18±3.74 c	7.1±1.73 b	8.39±1.84b	0.92±0.10 b	5.8±0.88 ns	14.7±2.54 b	0.92±0.12 b	0.43±0.08 b
A2C2	21.07±3.00 d	7.1±1.10 b	8.0±1.52 b	0.8±0.12 c	5.47±0.61 ns	14.6±1.26 b	0.92±0.16 b	0.39±0.07 b
LSD 5%	0.976	1.896	1.273	0.083	1.692	3.936	0.108	0.064

Different letters between populations denote significant differences (LSD test, $p < 0.05$).

Regarding the influence of the interaction of factors B and C: for the character plant height, significant differences were calculated between all calculated values, the highest value being recorded at the B1C1 graduation. In the case of character no. of leaves/pl., the values calculated for the first two classifications, respectively B1C2 and B2C1, show significant differences compared to the last calculated value, the one from the B2C2 classification. For the character width of the leaf, and respectively the length of the flower, the first calculated value registers significant differences compared to all other calculated values, less compared to the second calculated value. An almost similar situation is also found in the case of the character width of the flower, and respectively no. flower/inflorescence first average value calculated, registering significant differences compared to all other calculated values (Table 6).

Table 6. The influence of the interaction of factors B and C on the studied characters

Character / Variant	Mean±S.D.							
	Plants height (cm)	Leaf no./plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./infl.	Flower length (cm)	Flower diameter (cm)
B1C1	32.22±5.34 a	7.9±2.64 abc	8.8±2.31 ns	1.02±0.16 a	5.13±0.94 ns	15.1±2.81b	1.04±0.19 ab	0.54±0.10a
B1C2	28.3±4.72 b	9.3±2.58 a	8.45±1.83 ns	0.95±0.16 ab	6.46±2.56 ns	21.8±8.35a	1.11±0.15 a	0.44±0.07b
B2C1	25.48±4.91 c	8.9±2.23 ab	9.69±1.26 ns	1.01±0.17 a	6.33±1.72 ns	17.1±6.52b	1.08±0.17 a	0.45±0.10b
B2C2	22.00±4.42d	7.0±1.05 c	9.28±1.65 ns	0.92±0.22 b	5.86±0.87 ns	15.5±1.78b	0.97±0.19 b	0.41±0.07b
LSD 5%	0.976	1.896	1.273	0.083	1.692	3.936	0.108	0.064

Different letters between populations denote significant differences (LSD test, $p < 0.05$).

Regarding the influence of the interaction of factors A, B and C upon the studied characters, it can be said that for the character of plant height, significant differences were calculated between all the average values calculated, the highest value being recorded for the variant A1B1C1. For characters no. of leaves/pl., leaf length, leaf width and flower length, respectively, the highest values were calculated for the variants in both locations in high humidity, the lowest values being recorded for the variants in dry and shaded environments. For character no. of flowers/inflorescence, the average value calculated for the A1B1C2 variant obtains significant differences compared to all other calculated values, a sign that the increased humidity in high light conditions in the Vâlcea location leads to the potentiation of this character. A similar situation is recorded in the case of the average flower width character, where the variant A1B1C1 obtains significant differences compared to all other calculated values (Table 7).

Table 7. The influence of the interaction of factors A, B and C on the studied characters

Character / Variant	Mean±S.D.							
	Plants height (cm)	Leaf no./plant	Leaf length (cm)	Leaf width (cm)	Inflorescence length (cm)	Flowers no./ inflorescence	Flower length (cm)	Flower diameter (cm)
A1B1C1	37.06±1.00 a	9.6±2.70 ab	10.8±1.10 a	1.14±0.05 a	4.76±0.98 b	17.6±0.55 b	1.2±0.07 a	0.62±0.04 a
A1B1C2	32.76±0.11 b	11.2±2.05 a	9.66±1.55 ab	1.06±0.13 a	7.54±3.39 a	28.6±6.35 a	1.2±0.07 a	0.46±0.05 bc
A1B2C1	29.98±1.51 c	9.8±2.39 ab	9.4±1.69 ab	1.08±0.22 a	6.56±2.38 ab	17.4±9.69 b	1.2±0.10 a	0.50±0.07 b
A1B2C2	25.70±2.99 e	7.2±1.30 bc	9.8±1.79 ab	1.08±0.18 a	6.16±0.97 ab	16.8±0.84 b	1.12±0.13 ab	0.46±0.05 bc
A2B1C1	27.38±2.12 d	6.2±1.10 c	6.8±0.87 d	0.90±0.14 b	5.50±0.83 ab	12.6±1.34 b	0.88±0.11 cd	0.46±0.05 bc
A2B1C2	23.84±0.55 f	7.4±1.34 bc	7.24±1.22 cd	0.84±0.11 bc	5.38±0.56 ab	15.0±1.00 b	1.02±0.16 bc	0.42±0.08 bcd
A2B2C1	20.98±1.20 g	8.0±1.87 bc	9.98±0.72 ab	0.94±0.05 b	6.10±0.90 ab	16.8±1.30 b	0.96±0.13bcd	0.40±0.10 cd
A2B2C2	18.30±0.84 h	6.8±0.84 c	8.76±1.51 bc	0.76±0.11 c	5.56±0.72 ab	14.2±1.48 b	0.82±0.08 d	0.36±0.05 d
LSD 5%	1.380	2.682	1.801	0.118	2.393	5.567	0.152	0.091

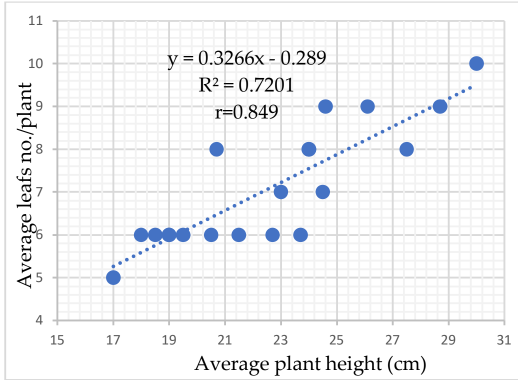
Different letters between populations denote significant differences (LSD test, $p < 0.05$).

Related to the analysis of the correlations between the analyzed characters, this was carried out according to the two locations, where the researches were carried out. Thus, between plant height and no. leaf/plant medium, leaf length, inflorescence length and respective no. of flowers/inflorescence from the Dâmbovița location records very significant or distinctly significant values, while in the Vâlcea location, the values of the same coefficient of correlation are insignificant. In this location, only in the case of the correlation between the size pl. and flower width a very significant value is obtained (Table 8).

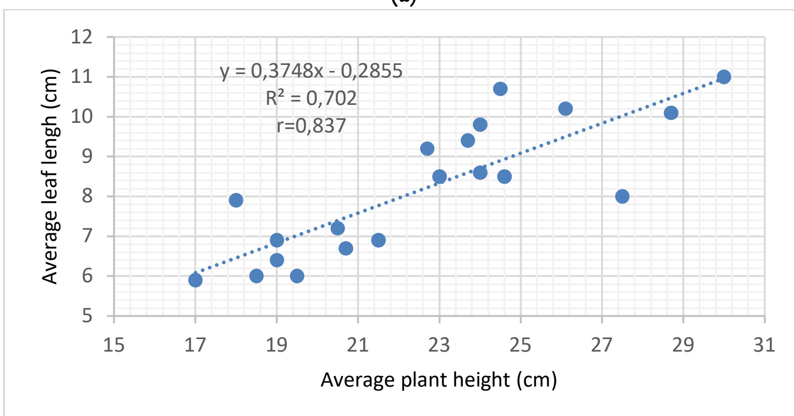
Table 8. Comparative analysis of the correlation coefficients between the characters studied according to the location

Trait	Mean±S.D.													
	Plants height (cm)		Leaf no./plant		Leaf length (cm)		Leaf width (cm)		Inflorescence length (cm)		Flowers no./inflorescence		Flower length (cm)	
	Vl	Db	Vl	Db	Vl	Db	Vl	Db	Vl	Db	Vl	Db	Vl	Db
Leaf no./plant	0.388 ns	0.849 ***												
Leaf length (cm)	0.379 ns	0.837 ***	0.173 ns	0.668 ***										
Leaf width (cm)	0.249 ns	0.224 ns	0.170 ns	0.176 ns	0.539 *	0.229 ns								
Leaf width (cm)	-0.060 ns	0.638 **	-0.383 ns	0.505 *	-0.145 ns	0.551 **	-0.030 ns	0.010 ns						
Flowers no./ inflorescence	0.264 ns	0.830 ***	0.125 ns	0.815 ***	-0.136 ns	0.792 **	-0.096 ns	0.267 ns	0.707 **	0.411 ns				
Flower length (cm)	0.409 ns	0.392 ns	0.174 ns	0.603 **	0.447 *	0.214 ns	0.507 *	0.263 ns	0.133 ns	0.028 ns	-0.080 ns	0.489 *		
Flower diameter (cm)	0.706 **	0.214 ns	0.127 ns	0.417 ns	0.294 ns	-0.084 ns	0.383 ns	0.423 ns	-0.179 ns	0.117 ns	-0.061 ns	0.126 ns	0.221 ns	0.506 *

Based on the R^2 coefficient determination, two valid trend models were identified based on the simple linear regression equation, these being between plant height (cm) and leaf no./plant, respectively, between plant height and leaf length, both in Dâmbovița location. Thus, a regression coefficient of 0.3266 was identified between plant height and leaf no./plant, which means that when plant height increases by 1 cm, leaf no./plant increases by 0.3266 (Figure 4). Also, a regression coefficient of 0.3748 was identified between plant height and leaf length, which means that when plant height increases by 1 cm, average leaf length increases by 0.3748.



(a)



(b)

Figure 4. Trend model between the analyzed parameters: a) average plant height and average leaf no./plant based on simple linear equation; b) average plant height and average leaf length based on the simple linear equation

Principal components analysis

Regarding the Principal Components Analysis, the first two components explain 85.451% of total variance, the first component registering 63.367% and the second component 22.084% (Table 9).

Table 9. Total variance explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.069	63.367	63.367	5.069	63.367	63.367
2	1.767	22.084	85.451	1.767	22.084	85.451
3	0.658	8.229	93.680			
4	0.300	3.745	97.424			
5	0.131	1.639	99.063			
6	0.071	0.886	99.949			
7	0.004	0.051	100.000			
8	-4.087E-016	-5.108E-015	100.000			
Extraction Method: Principal Component Analysis.						

Related to the first component, the studied elements that obtain high positive values are plant height, leaf no per plant, leaf length, leaf width, inflorescence length, flower length and flower diameter. The first component can also be named the plant's ability to have height, well-developed leaves and flowers. For the second component, high positive values are obtained by inflorescence length and flowers no./inflorescence. The second component can be named the ability of the plant to develop inflorescences with a raised number of flowers (Table 10).

Table 10. Principal component analysis method, 2 components extracted

Character \ Comp. value	Component	
	1	2
Plant height	0.861	-0.357
Leaf no per plant	0.904	0.274
Leaf length	0.698	-0.007
Leaf width	0.919	-0.216
Inflorescence length	0.415	0.858
Flowers no per inflorescence	0.751	0.587
Flower length	0.946	-0.048
Flower diameter	0.740	-0.660

The variants with both positive components are A1B1C2, A1B2C1 and A1B2C2. Those ones have high results for flower, inflorescences and leaf characters;

-The variant with the first component positive and the second negative are A_1B_1C1. This variant has high values for leaf and flowers characters and medium values for the inflorescences characters;

-The variants with both negative components are A2B1C1, A2B1C2 and A2B2C2. Those ones have poor values for leafs and flowers characters and medium results for inflorescences characters;

-The variant with the first component negative and the second one positive is A2B2C1 which has poor values for leaf and flowers characters and high values for inflorescences characters.

The four groups based on the value of the two components were presented in Figure 6.

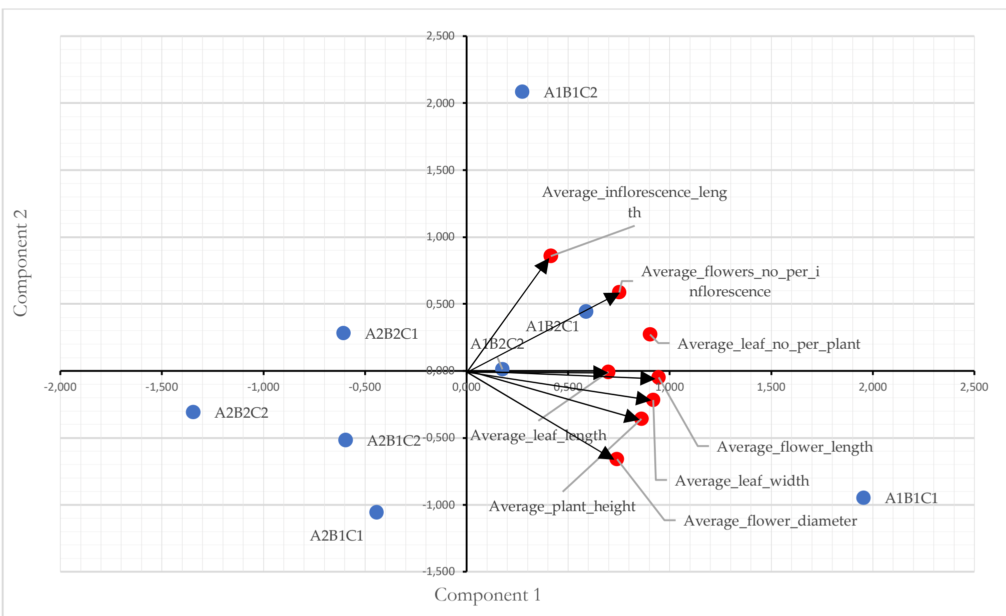


Figure 6. Plot of the two principal components (PCA1 and PCA2), the plant's ability to have height, well-developed leaves and flowers and the ability of the plant to develop inflorescences with a raised number of flowers.

Discussion

Only two populations were chosen as the species is rare in Romania, being found only in a few locations and usually in a very small number of specimens (1-2). After an extensive search throughout several regions, we were able to identify two populations with a larger number of individuals that allowed us to perform this study. An analysis of the coenology was necessary as the species appeared in the two locations in different plant communities with different abundance-dominance. Regarding phytosociological aspects there is great variability, both at European level and in Romania. Thus, in some countries the species is very rare, endangered, appearing only in certain plant communities: *Scheuchzerio palustris - Caricetea nigrae* (Nordhagen, 1936) Tüxen 1937 (Podani, 2001; Nordhagen, 1936; Tüxen, 1937) em. Julve 1999 (Sanda, 2001; Julve, 1999), *Molinio caeruleae - Caricetalia davallianae* Julve 1988 (Sanda, 2001; Julve, 1988) em. de Foucault 1984 (Sanda, 2001; Foucault, 1984), *Molinio caeruleae* subsp. *caeruleae - Caricenea nigrae* subsp. *nigrae* (Julve, 1983) (Sanda, 2001; Julve, 1988), de Foucault, 1984 (Sanda, 2001; Foucault, 1984), *Molinio caeruleae - Caricetalia davallianae* Julve 1988 (Sanda, 2001; Julve, 1988) em. de Foucault 1984 (Sanda, 2001; Foucault, 1984) (*https://www.tela-botanica.org/bdtfx-nn-4309-synthese*). In Romania the species is frequently found in plant communities of *Festuco-Brometea* and *Molinio-Arrhenatheretea* classes. In the two locations here analyzed the species was present in two associations and had different abundance-dominance.

Although our results are, in general, in accordance with the description in "Flora Romania", some remarkable differences were also identified. Average plant height is a character influenced by humidity, light, but also location. Thus, in the presence of humidity and light in the Vâlcea location, this character obtains the highest average values. This trait was significantly different in the eight experimental variants analyzed. A large phenotypic variation was reported also by Tanveer *et al*. (2016) in all analyzed characters indicating also environmental influence. Average leaf no./plant is a character that is influenced only by humidity, not by light or location, as it is shown in Table 7. The variants with highest number of leaf no./plant are those found in swampy sites (A1B1C1; A1B1C2; A1B2C1 and A1B2C2) which are in the superior part of the rank. Moreover, humidity also influences the interaction with the other two factors, the variants at high humidity (regardless of the level of the other two factors) obtaining statistically superior values compared to the other variants. The character average leaf length is influenced by humidity and light, but not by location. Thus, in the presence of humidity and light, regardless of the location, this character obtains the highest values, these being recorded for the variants with high humidity located in the shade, and the lowest values being calculated for the variants located in the dry regardless of the light. Average leaf width is influenced by humidity and location, but not by light, but as in the previous case the higher average values calculated were obtained in locations with light. Similar values were reported by Erzurumlu *et al.* (2018) in a comparative morphological trait analysis on different wild populations of terrestrial orchid species. Average inflorescence length is the only analyzed character that is not influenced separately by the three experimental factors studied, and not even by the interaction of two of them. This character presents high values both at high humidity and at low humidity, among the variants analyzed, only the first classified variant differing significantly in value from the last classified one. Average flowers no./inflorescence is a character that is influenced only by humidity, not by light or location, as experienced factors analyzed individually. Moreover, humidity also influences the interaction with the other two factors, the variants at high humidity regardless of the level of the other 2 factors, obtaining statistically superior calculated average values compared to the other variants. Average flower length is influenced by humidity and light, but not by location. Thus, in the presence of humidity and light, regardless of the location, this character obtains the highest average values, average values being recorded for the variants with high humidity located in the shade, and the lowest values being calculated for the variants located in the dry regardless of the light. Average flower diameter was found to be influenced by humidity, light, but also location. Thus, in the presence of humidity, light and in the Vâlcea location, this trait revealed the highest average values.

Related to the eight variants analyzed according to the interaction of the three tested factors, the first variant A1B1C1 is distinguished by high values for all characters, except those related to the inflorescence. In other words, this variant, although it is well developed, does not produce many flowers, although it benefits from both light and especially humidity. The variants A1B1C2, A1B2C1 and A1B2C2 show high values for the stem and leaf and the highest values for the inflorescence. These varieties stand out for their well-developed flowers, which leads to adequate fruiting. Variants A2B1C1, A2B1C2 and A2B2C2 stand out for low values for leaf and waist characters, as well as for flowers, but medium values for inflorescences. In other words, this variety produces many small flowers on medium-sized inflorescences. The last variant according to the PCA analysis, variant A2B2C1, stands out for its modest values of the leaf characters, but high values of the flower and inflorescence characters, a fact that leads to good fruiting. Related to the characteristics of the leaf, this is not surprising, given the fact that this variant does not benefit from a consistent supply of moisture and light.

Conclusions

When temperature, light and humidity have optimal values and between them there is a harmonious relationship, orchids will develop normally. In the favorable conditions encountered in Vâlcea county, orchids offer the splendor of flowers for a long time, they could stay flowered from April to August. Although the flowers are small in size, they are particularly appreciated by nature lovers due to the symmetry of the floral formula, as well as the perfume they emit. The information obtained regarding the morphological diversity of the two populations could be useful suitable for designing a practical plan for their conservation in both locations.

Authors' Contributions

Conceptualization: MN; Data curation: PI; Formal analysis: OFP; Funding acquisition: ISN; Investigation: OFP; Methodology: MN and PI; Project administration: MN; Resources: ISN; Software: OFP; Supervision: MN; Validation: MN and PI; Visualization: MN, PI and ISN; Writing - original draft: PI; Writing - review and editing: PI.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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