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Some morphological features and distribution of *Limodorum abortivum* (L.) Sw. (Orchidaceae) in Azerbaijan

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Abstract: *Limodorum abortivum* is a threatened rhizomatous orchid species in Azerbaijan. In this study, we investigate the distribution areas of *L. abortivum* in the Great Caucasus mountains and the Talysh mountains within Azerbaijan, based on field surveys in 2018-2021 years. The potential distribution areas of the species have been clarified using species distribution modeling (SDM). As a result of the model, it was found that the species is expected to occur in areas outside the current areas of occurrence. Seed morphological characteristics of *L. abortivum* were investigated using binocular stereomicroscopy and light microscope for classification and identification of the species by its seed. Morphological characters included the size and shape of the seeds. Based on the analysis, it was determined that the seed length ranged from 665.4 μm to 1339.6 μm, while the width ranged from 352.5 μm to 399.8 μm. The results of the morphological characteristics of the seeds will be helpful for the identification of *L. abortivum* species in the future.

Keywords: *characters, conservation, morphology, population, SDM, seed, vulnerable species*

INTRODUCTION

Plants are the most vulnerable part of biological diversity in the world and the extinction of any species can cause an indispensable loss for the whole ecosystem [Ibadullayeva, Huseynova, 2021]. The rapid decrease in the distribution of many wild species due to human activity and climate change sharply raises the issue of biodiversity conservation.

Limodorum abortivum (L.) Sw. is a plant belonging to the *Orchidaceae* Juss. family, *Neottieae tribe* and the species was recorded in the flora of Azerbaijan in the mid of the last century [Flora..., 1952]. Based on the literature and herbarium data, *L. abortivum* is mainly distributed in the mountainous areas of Azerbaijan [Askerov, 2011; Alizade, et al., 2019]. Although the species is distributed in several districts of the country, there are few populations in the wild, and the populations tend to be small [Ibrahimova, 2019, 2020].

The species is most often found in deciduous forest habitats, less often in mixed forests in the lower and middle mountain zones [Flora..., 1952, Perebora, 2008, Alizade et al., 2019]. It is native to Europe, western Asia and the Mediterranean area [Magrini, Vitis, 2016]. *L. abortivum* is a threatened species included in the Red List of Azerbaijan [Red Book of the Republic of Azerbaijan, 2023], as well as those of neighboring countries (Russia, Ukraine, and etc.) [Red Book of Ukraine, 2009; Red Book of the Russian Federation, 2008].

Considering the rarity of *L. abortivum* in the wild, it is very important to determine the distribution areas of the species in the present and its potential distribution in the future. The distribution of this species has been investigated by a number of scientists in Azerbaijan [Heydarova, 2016; Alizade, et al., 2019], but more remains to be done. Species distribution modeling (SDM) is widely used to study the geographical ranges of plant species [Phillips, 2006]. In this study we used the MaxEnt algorithm in SDM. This method is appropriate for small occurrence datasets, or for threatened species for which distribution areas are not well known.

The species of the family *Orchidaceae* usually have extremely small seeds, which can be examined only through a microscope. According to R.L. Dressler's classification the seeds of *L. abortivum* belong to the *Limodorum*-type of seeds of the *Orchidaceae* family [Dressler, 1993]. The seeds of species belonging to this family are described in a number of publications [Rasmussen, 1995; Arditti, Ghani, 2000; Chase, Pippen, 1990; Kurzweil, 1993; Şeker, 2022].

The present research has two aims: 1) to clarify the potential distribution patterns of *L. abortivum* in

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the Great Caucasus mountains (GCM) and the Talysh mountains (TM) within Azerbaijan, and 2) to conduct studies on the morphological characteristization of *L. abortivum* seeds (shape, size, colour, etc.).

MATERIAL AND METHODS

Study area and field survey. Azerbaijan is one of several countries of the Caucasus region, which lies on the Caspian Sea. The high mountains play an important role in intercepting cold air masses coming from the north, making the air conditions colder on northern slopes and relatively mild on southern slopes [Museyibov, 1998; Alizade et al., 2019]. Field surveys were conducted in the north GCM and south parts TM of Azerbaijan in 2018-2021, data on the observed distribution of *L. abortivum* were recorded. Data were also extracted from the Herbarium Fund (BAK) of the Institute of Botany of the MSE and the Global Biodiversity Information Facility (GBIF 2020).

To study the potential distribution of *L. abortivum*, we used MaxEnt algorithm in an SDM approach [Phillips et al., 2006; Guisanet et al., 2005] in R version 3.6.2 [R Core Team, 2019]. To evaluate the model, we used the receiver operator characteristic (ROC), summarized by the area under the curve (AUC) statistical test [Lobo et al., 2008]. The combinations of bioclimate variables used in the final model were BIO1 (the annual mean temperature), BIO4 (the temperature seasonality), BIO7 (the temperature annual range), BIO11 (the mean temperature of coldest quarter), BIO15 (the precipitation seasonality) and BIO17 (the precipitation of driest quarter). The suitability of different areas was represented by different colours: white (0.00-0.10) – not suitable, pink (0.11-0.40) – low suitability, yellow (0.41-0.70) – moderate suitability, and green (0.71-1.00) – highly suitable. The protected areas of the country were indicated with blue lines on the maps.

Seed material. Seeds were collected from Ismayilli and Siyazan districts located in the north part of country and Lerik district in the south part. Five to ten samples were collected from each population. In each sample100 mature seeds taken from fruits were analyzed. Seeds were examined by light microscope (Axio Imager Vert. A1 Carl Zeiss), measurements and photographs were taken.

The seed materials were collected within the joint project "Threatened biodiversity hotspots programme", carried out by the Millennium Seed Bank Partnership, Royal Botanic Gardens, Kew and the Institute of Botany of the MSE.

RESULTS AND DISCUSSION

Limodorum abortivum is common in the lower and middle mountain belts of the forests, mainly occurring between 500 and 1400 m a.s.l. in the GCM and TM regions (Fig. 1, 2). During our field surveys in 2018-2022, *L. abortivum* was recorded in Guba, Siyazan, Gakh, Ismayilli (GCM), and Lerik districts (TM).The species is mainly distributed in a few populations, which

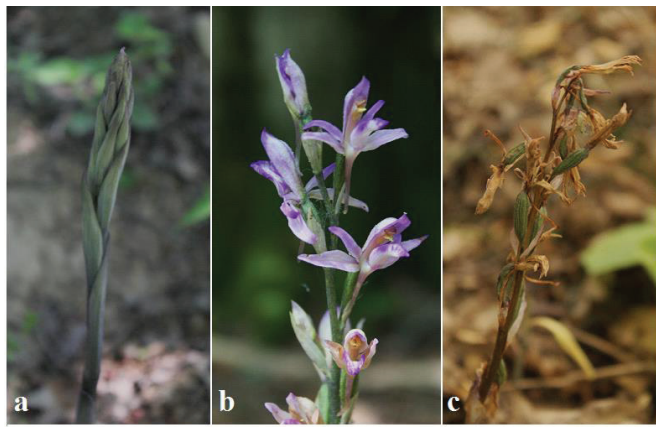

Figure 1. Inflorescences of *Limodorum abortivum* before flowering (a), in flowering (b) and in fruiting (c) periods.

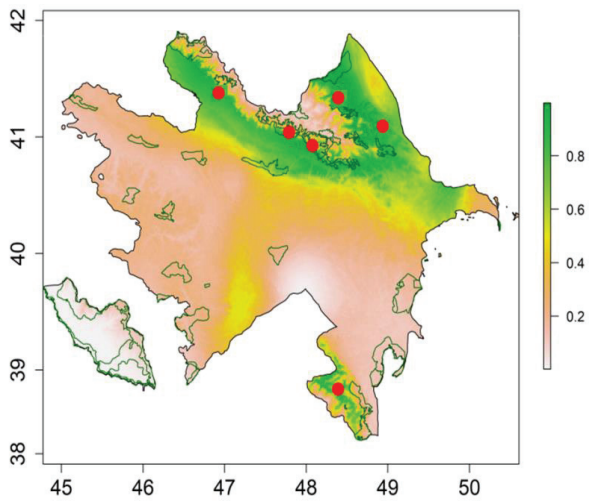

Figure 2. Distribution of *Limodorum abortivum* according to field surveys in 2018-2022 (●) and the potential distribution of the species in Azerbaijan based of the SDM model.

are characterized by small numbers (2-5) of individuals. It was also found singly, and most populations consisted only of generative individuals.

In the TM region, *L. abortivum* was found in Durgan village of the Lerik district, in the middle mountain belt at an altitude of 923 m. Based on records from

the mountain forest vegetation dominated by *Quercus* L., the species has a population consisting of max. 11 individuals per 1 m^2. The population of the species was quite small in 10 m^2. The area was also rich with other representatives of orchids such as *Platanthera chlorantha* (Custer) Rchb., *Dactylorhiza romana* subsp. *georgica* (Klinge) Soó ex Renz & Taubenheim, *Neottia ovata* Bluff & Fingerh. It seems that the ecological conditions of this area are quite suitable for the spread of orchid species. However, strong anthropogenic factors including intensive grazing, trampling, etc. were observed which effect the growth conditions of the species.

In the GCM region, *L. abortivum* was found in the forest areas of Guba, Siyazan, Gakh and Ismayilli districts. Two populations of *L. abortivum* were recorded in Ismayilli district. The first population was recorded in Ismayilli part of Shahdag National Park, which is a protected area.The population is distributed in the mountain forest dominated by *Fagus orientalis* L. in the middle mountain belt at an altitude of 1029 m. In contrast to Lerik district, in Ismayilli district, *L. abortivum* is distributed in an area of 500 m^2, but here the number of individuals is small and they grow at different distances from each other, there were one or two individuals per 1m^2, and rarely five individuals. Nevertheless, since this distribution area is protected from the influence of many environmental factors, the natural recovery of the species is secured here. The second populations were recorded in an area with a predominance of *Fagus orientalis* and *Carpinus betulus* L. A total of seven individuals of the species were recorded in the area, with a maximum of two individuals per 1m^2 area.

In Siyazan district two populations of *L. abortivum* were recorded. The first populations was found around Qalaalti village in a mixed forest area of the middle mountain belt at an altitude of 709 m. The area was also rich in species belonging to the *Orchidaceae* family such as *Cephalanthera longifolia* (L.) Fritsch, *Ophrys caucasica* Woronow, *Dactylorhiza romana* subsp. *georgica* etc. species.

In Gakh district, *L. abortivum* was found mainly in the forest areas of the lower and middle mountain belt, between an altitude of 600 m to 1250 m. The species is distributed in shady and humid forest areas dominated by *F. orientalis* and *Carpinus betulus* species, as well as in mixed forests. In all populations of the species, there were 1-4 individuals per 1 m^2 area.

Considering the rarity of *L. abortivum* it is very important to discover new distribution areas of the species within the territory of Azerbaijan. In this regard, we have evaluated the potential geographical distribution areas of the species in Azerbaijan using SDM. The model showed performance with AUC scores above 0.99, indicating a high reliability for the results of the model.

Based on the model, the actual range of *L. abortivum* is only half of its potential range, as modeled from climate data. Thus, the most suitable areas are identified mainly in the lower and middle mountain belt of the southern foothills of the GCM and in the Lerik and Yardimli districts of the TM (Fig. 2). According to the model, it can be said that there are additional areas suitable for the potential distribution of *L. abortivum* in Azerbaijan. Considering this, additional fieldwork in the potential new distribution areas for this species will be needed to check that the species does occur in these areas.

The morphological study of the seeds of *L. abortivum* revealed that the shape of the seed coat varies significantly within one fruit, depending on the place where the seed was located (Fig. 3). In addition, differences in the size of the seeds were observed according to the area where the species was collected. For this study, the morphological characteristics of fully mature seeds were studied. In compressed, curved seeds, the clarity of the shape is lost, so it can be difficult to determine the main morphological features. It has been observed that the deformed seeds in each fruit are no less than well-shaped ones.

Several seed shapes were found in each fruit: fusiform, filiform, ellipsoid, and clavate (Fig. 4).The embryo of the species was generally ellipsoid and located at the center of the seed. Based on the color of

Figure 3. Seed diversity of *Limodorum abortivum* species taken in a light microscope.

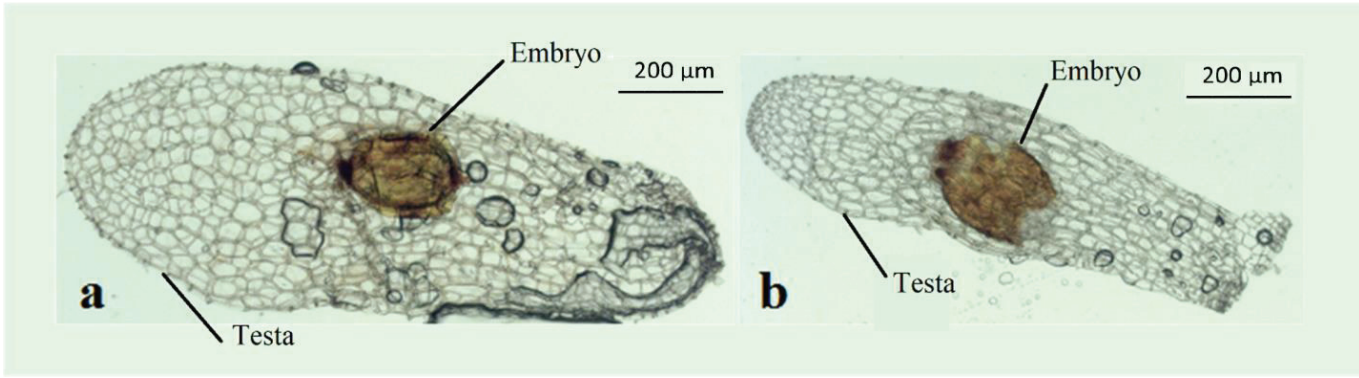

Figure 4. Seeds of *Limodorum abortivum* observed in scanning electron microscopy: a) fusiform, b) fusoid.

the embryo, the seeds of *L. abortovum* are brown or dark brown in color.

The seeds showed significant difference in their length and width size and seed length ranged from 665.4 µm to 1339.6 µm, while the width ranged from 352.5 µm to 399.8 µm (Table 1). The larger-sized seeds were observed among seeds collected from Siyazan district, and smaller-sized seeds were observed among seed collected from Lerik district (Tab. 1).

As a result of our observations during the field surveys in Azerbaijan, it was found that most species belonging to the *Orchidaceae* family, including *L. abortivum*, are under threat due to the influence of environmental factors, especiallyin Lerik district. The distribution of the species located in other areas besides the protected ones may be in danger in the future. The annual mean temperature and the precipitation of the driest quarter are the most important climatic factors determining the present distribution of the species. It is interesting that its actual geographical range is only half of the potential range in Azerbaijan, as predicted by the SDM.

Based on data from the literature, *L. abortivum* is very difficult to introduce to new areas and its seeds are hard to germinate [Magrini & Vitis, 2016]. From this point of view, it is important to study this species within its natural distribution area.

Early literature reports that *Orchidaceae* family are characterized by tiny seeds, ranging in size from 0.25 to 1.22 mm long [Knudson, 1922; Rasmussen, 1985], which make them suitable for wind dispersal [Rasmussen, 1995]. However, as a result of our research, it can be said that the seeds collected from the territory of Azerbaijan differ in larger sizes.

Studies on the seeds of the *Orchidaceae* family are rare in Azerbaijan, and thus the development of research in this direction is considered very important. Apart from the physical characteristics of the seeds collected throughout the country, it will also be imporant and interesting to conduct research on the viability and germination of these seeds in the future.

Table 1. Seed size of *Limodorum abortivum*.

District	Seeds (n)	Seed dimensions (µm)						
		Length			Width			
		min.	max.	mean (±)	min.	max.	mean (±)	length/ width ratio
Siyazan	100	678.4	1339.6	1203.3 (±22.9)	203.6	480.1	399.8 (±8.8)	3.010
Ismayilli	100	675.4	1326.9	1219.0 (±15.0)	195.4	435.8	356.7 (±5.8)	3,417
Lerik	100	665.4	1305.4	1185.9 (±17.7)	201.4	427.8	352.5 (±6.1)	3.364

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Limodorum abortivum (L.) Sw. (Orchidaceae) növünün bəzi morfoloji xüsusiyyətləri və Azərbaycanda yayılması

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Limodorum abortivum (L.) Sw. Azərbaycanda itmə təhlükəsi altında olan kökümsovlu səhləb növüdür. Məqalədə 2018-2021-ci illərdə həyata keçirilən çöl tədqiqatları əsasında *L. abortivum* növünün Böyük Qafqaz sıra dağları və Talış dağları ərazilərində yayılması tədqiq edilmişdir. Növün potensial yayılma əraziləri Növlərin Yayılma Modelindən (NYM) istifadə olunaraq dəqiqləşdirilmişdir. Model nəticəsində müəyyən edilmişdir ki, bu növə hazırki yayılma ərazilərindən başqa ətraf ərazilərdə də rast gəlinməsi mümkündür. *L. abortivum* növünün toxum vasitəsilə təsnifatı və təyinatı üçün binokulyar stereomikroskop və işıq mikroskopdan istifadə edilərək toxum morfoloji xüsusiyyətləri araşdırılmışdır. Morfoloji əlamətlərə toxumların ölçüsü və forması daxildir. Təhlillər nəticəsində müəyyən olunmuşdur ki, toxumun uzunluğu 665.4 µm - 1339.6 µm, eni isə 352.5 µm - 399.8 µm aralığı ölçüsündədir. Toxumların morfoloji xüsusiyyətlərinin nəticələri *L. abortivum* növünün gələcəkdə təyinatı zamanı faydalı ola bilər.

Açar sözlər: *əlamət, qorunma, morfologiya, populyasiya, SDM, toxum, həssas növlər*

Распространение *Limodorum abortivum* (L.) Sw. (Orchidaceae) в Азербайджане и некоторые морфологические особенности

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Limodorum abortivum (L.) Sw. корневищная орхидея, находящаяся под угрозой исчезновения в Азербайджане. В данной статье на основе полевых исследований 2018-2021 гг. уточнены места распространения этого вида в горах Большого Кавказа и Талыша. Также потенциальные области распространения вида уточнены с использованием моделирования распространения видов (МРВ). В результате анализа установлено, что вероятно, вид встречается и в других ареалах помимо обнаруженных. Для классификации и идентификации вида по семенам исследовались их морфологические особенности с помощью бинокулярного стерео- и светового микроскопа. Морфологические признаки включали размер и форму семян. В результате анализа установлено, что длина семян находится в пределах от 665.4 мкм до 1339.6 мкм, а ширина – от 352.5 мкм до 399.8 мкм. Результаты морфологической характеристики семян в будущем могут быть использованы для идентификации видов *L. abortivum*.

Ключевые слова: *признаки, охрана, морфология, популяция, SDM, семена, уязвимые виды*