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Habitat preferences of orchids in the Republic of Mordovia (European Russia): insights from (un)published floristic data

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Summary: Orchidaceae are considered one of the most diverse and threatened plant families in the world. Because of the high conservation value of orchids around the world, it is highly relevant to estimate their habitat preferences and environmental factors that affect their distribution range and abundance. This paper presents the bibliographic survey (2002–2021) of published and unpublished data on the floras aassociated with orchid species in the Republic of Mordovia (European Russia). The study was aimed to estimate habitat preferences of orchids in this region based on associated flora composition. We have conducted the bibliographic survey to find any information on the composition of floras associated with orchids in the Republic of Mordovia. In addition, we have included our personal still unpublished data. Based on the floristic lists, we have evaluated the environmental conditions of orchid habitats using phytoindication methods (Tsyganov scale). We have distinguished four habitat groups of orchids, namely bog, meadow-mire, coniferous forest and mixed forest. The environmental conditions of sites inhabited by orchids of all these groups differed significantly ($p < 0.05$). This result highlights that various orchid species with close environmental condition preferences can be found and have to be protected at the same sites (e.g. so-called 'orchid glades'). In addition, we have found that for many orchids, even the most common ones, there are no data on the associated floras. This makes it difficult to understand their environmental preferences and, consequently, the search of their localities in conditions of the highly pronounced floristic mosaics in the Republic of Mordovia. We propose the wide researching and publishing of data on plants (incl. orchids) associated with floras in the Republic of Mordovia and other regions for a better understanding of their habitat preferences.

Keywords: flora, bibliographic survey, Orchidaceae, plant distribution, threatened plants, Tsyganov scale

Orchidaceae are recognized as one of the richest plant families in the world, comprising more than 28 000 accepted species and 763 genera (Christenhusz & Byng 2016). Orchids inhabit a variety of biotopes, although they are most common in forests (e.g. Khapugin 2020). Many species are endemics or rare and threatened taxa (e.g. Djordjević et al. 2017; Besi et al. 2020; Fateryga et al. 2020). More than half (56.5%) of the 948 orchid species assessed according to the IUCN Red List guidelines are considered threatened (e.g. Gale et al. 2018; Kottawa-Arachchi & Gunasekara 2020). Besides the global IUCN assessments (e.g. Wagensommer et al. 2020), there is a number of regional IUCN Red List assessments of orchids (e.g. Eliáš et al. 2015; Djordjević et al. 2017; Juiling et al. 2020). Ecological studies of orchids thematically vary. Specifically, there are studies on orchid species' state depending on weather conditions (e.g. Pfeifer et al. 2006; Kirillova & Kirillov 2020; Mursal et al. 2020; Romano et al. 2020), habitat degradation (e.g. Khapugin et al. 2017a; Tatarenko et al. 2020; Tsiftsis & Djordjević 2020), altitude (e.g. Tsiftsis et al. 2008; Djordjević et al. 2016a, 2020), light availability (e.g. Djordjević et al. 2016b; Perazza & Decarli 2020), geological patterns (e.g. Djordjević & Tsiftsis 2019), height and area of the forest canopy (e.g. Djordjević et al. 2016b). Sometimes, molecular phylogenetic methods (e.g. Szlachetko et al. 2019; Terentieva et al. 2020) or studies

of morphological variation (e.g. Paušič et al. 2018; Shirokov et al. 2020) are strongly necessary to understand the ecology and apply the conservation efforts of orchids. Thus, studies on a variety of ecological and biological aspects of orchids are being carried out in various worldwide regions.

In Russia, Orchidaceae are represented by 135 species and 13 subspecies from 38 genera (Efimov 2020), with the highest diversity occurring in the Russian Far East and South Russia (e.g. Popovich et al. 2020). In the Republic of Mordovia (European Russia), 24 orchid species are known (Khapugin & Chugunov 2021). During the last two decades, many studies were specifically devoted to Orchidaceae, like their population status (e.g. Khapugin et al. 2016a,b, 2017a; Khapugin & Korochkina 2017), morphometric traits of certain species (Khapugin et al. 2015, 2016b, 2017a), conservation issues (Shigaeva & Silaeva 2010; Khapugin et al. 2017a,c). Despite the high threat status and conservation importance of orchids, there is a certain lack in the published data on floras associated with orchids in the Republic of Mordovia. The composition of associated flora is of high relevance to reveal the habitat's environmental conditions using phytoindication methods (e.g. Khapugin 2021). This paper aims to estimate habitat preferences of Mordovian orchids based on data on associated floras. We established the following research tasks: I) to test the applicability of the phytoindication methods for characterizing the habitat preferences of orchids in the Republic of Mordovia; II) to check the availability of the published data on floras associated with orchid species in the Republic of Mordovia.

Materials and methods

Study area

The present research has been carried out in the Republic of Mordovia (26 200 km^2, 54.42°–54.56° N, 43.04°–43.36° E), central part of European Russia. This region is situated on the border of the forest and forest-steppe zones. This results in high habitat diversity. Coniferous and mixed forests (i.e. coniferous and deciduous) are distributed predominantly in the west and northwest of the Republic of Mordovia. Broadleaf forests are located in the center and east of the region. Forest-steppe landscapes dominate eastern and southeastern parts of the Republic of Mordovia (Yamashkin 2012).

Research design

For the study, we searched and analyzed two groups of data on floras associated with orchid species in the Republic of Mordovia. The first group includes our personal unpublished relevés obtained in the field. The second group consists of publications found through an expert search in bibliographic databases including elibrary (http://elibrary.ru), Google Scholar, Scopus, Web of Science Core Collection. For this purpose, we have checked all indexed publications authored by botanists who have ever worked in the Republic of Mordovia. As a result, we have found data on floras associated with 19 species collected in 2002–2021 (Table 1).

In Table 1, we present brief information on data obtained, including Latin names of orchids, their abbreviations used in the paper, the numbers of locations, where the data were extracted, and sources containing such information. Based on Silaeva et al. (2010), we added preliminary characteristics of the orchid habitat preferences by distinguishing bog group, meadow-mire group, coniferous forest group and mixed forest group. In this paper, we also tested the concordance of data in Silaeva et al. (2010) and the results of the conducted analysis based on associated floras.

Table 1. Characteristics of background reference data obtained for orchids in the Republic of Mordovia (European Russia).

Species	Abbreviation	Habitat*	Number of locations	Source(s)
Cephalanthera rubra (L.) Rich.	CeR	CF	2	Khapugin et al. (2015); Senchugova et al. (2017)
Corallorhiza trifida Châtel.	CoT	CF	1	Khapugin et al. (2015)
Cypripedium calceolus L.	CyC	CF	6	Khapugin et al. (2017a,b)
Cypripedium guttatum Sw.	CyG	CF	1	Kiryukhin (2004)
Dactylorhiza fuchsii (Druce) Soó	DaF	CF	7	Kuznetsov & Silaeva (2008); Khapugin et al. (2015)
Dactylorhiza incarnata (L.) Soó	DaI	MM	1	Kuznetsov & Silaeva (2008)
Dactylorhiza maculata (L.) Soó	DaM	CF	1	Khapugin et al. (2015)
Epipactis helleborine (L.) Crantz	EpH	MF	18	Kuznetsov & Silaeva (2008); Ageeva (2011); Khapugin & Senchugova (2018); Khapugin (2017, 2021); Khapugin et al. (2017b); Khapugin (2017, 2021: personal unpublished data)
Epipactis palustris (L.) Crantz	EpP	MM	2	Khapugin et al. (2016b)
Goodyera repens (L.) R.Br.	GoR	CF	4	Kuznetsov & Silaeva (2008); Khapugin (2016, 2021: personal unpublished data)
Hammarbya paludosa (L.) Kuntze	HaP	B	1	Chugunov (2002: personal unpublished data)
Hemipilia cucullata (L.) Y.Tang, H.Peng & T.Yukawa	HeC	CF	4	Khapugin et al. (2016a)
Herminium monorchis (L.) R.Br.	HeM	MM	1	Khapugin et al. (2016b)
Malaxis monophyllos (L.) Sw.	MaM	MM	2	Kuznetsov & Silaeva (2008); Khapugin et al. (2016b)
Neottia nidus-avis (L.) Rich.	NeNA	MF	7	Ageeva (2011); Khapugin & Senchugova (2018); Khapugin (2017, 2020, 2021: personal unpublished data)
Neottia ovata (L.) Bluff & Fingerh.	NeO	CF	3	Kuznetsov & Silaeva (2008); Khapugin et al. (2017a); Senchugova et al. (2017)
Orchis militaris L.	OrM	MM	2	Kiryukhin (2004); Khapugin et al. (2016b)
Platanthera bifolia (L.) Rich.	PlB	MF	32	Kiryukhin (2004); Kuznetsov & Silaeva (2008); Ageeva (2011); Utorova et al. (2014); Khapugin & Senchugova (2018); Khapugin & Korochkina (2017); Khapugin (2017, 2021); Khapugin et al. (2015, 2017b); Senchugova et al. (2017); Khapugin (2017, 2021: personal unpublished data)
Platanthera chlorantha (Custer) Rchb.	PlC	MF	2	Ageeva (2011)

Note: *MM – meadow-mire group, B – bog group, CF – coniferous forest group, MF – mixed forest group.

Once associated floras were extracted from background references (Table 1), we used the Tsyganov (1983) ecological scale (Tsyganov scale) to obtain the environmental status of the studied orchid locations through the average values (measured in scale scores) of the used environmental factors. In the Tsyganov scale, each factor is represented by gradation values reflecting the survival intervals of plants (for more explanation, see Khapugin 2021). In the present study, we used environmental parameters based on the Tsyganov scale, namely thermoclimatic scale (TM), climate continentality scale (KN), climate humidity scale (OM), cryoclimatic scale (CR), habitat shading (LC), moisture of soils (HD), nitrogen availability in soils (NT), soil pH (acidity) (RC), soil trophicity (TR), soil moisture variability (FH). For each location, the average values of environmental factors were calculated as a mean of the total set of species found there. Calculations were performed using the following formula (see Khapugin 2021):

$$mEFV = \frac{\left(x_1^{\min} + x_2^{\min} + \ldots + x_n^{\min}\right) + \left(x_1^{\max} + x_2^{\max} + \ldots + x_n^{\max}\right)}{2n}$$

where

$mEFV$ = average environmental factor value;

$x_n^{\min}$ = the minimal score value of a certain factor for n plant species;

$x_n^{\max}$ = the maximal score value of a certain factor for n plant species;

n = the number of certain plant species in the floristic list obtained in the study plot.

Since we had data on orchids from several locations, we have used environmental factor values averaged on the basis of values from all found sites. The obtained average values were implemented in the linear discriminant analysis (LDA). The set of environmental factor values was subjected to multivariate analysis of variance in one-way PERMANOVA (permutation-based MANOVA) with 9999 permutations to test, if there is a statistically significant difference ($p \leq 0.05$) in the environmental status of habitats (group centroids) among the distinguished habitat groups of orchids. All calculations were performed using PAST v. 4.09 (Hammer et al. 2001).

Results and discussion

Availability of data on the flora associated with orchids

The bibliographic survey demonstrated that among 24 orchid species of the Republic of Mordovia, data on associated flora are available for 19 species only (Table 1). There were no available data on the flora associated with *Dactylorhiza viridis* (L.) R.M. Bateman, Pridgeon & M.W. Chase, *Epipogium aphyllum* Sw., *Neotinea ustulata* (L.) R.M. Bateman, Pridgeon & M.W. Chase, *Neottia cordata* (L.) Rich., *Gymnadenia conopsea* (L.) R.Br. Almost all of them are extremely rare and threatened in the Republic of Mordovia (Khapugin et al. 2017c; Khapugin & Chugunov 2021) except *G. conopsea*. Despite the last orchid being known from seven locations, no data on its associated flora are available.

Noteworthy, other orchid species were represented by one to 32 locations, where floristic data are available. There was no correlation ($p > 0.05$) between the total number of locations in the Republic of Mordovia and the number of locations, where data on associated flora were found in published or unpublished sources. For instance, for some orchids (e.g. *Cypripedium guttatum*, *Hammarbya paludosa*), we had data from 100% of known locations in the region. In contrast, for rather common *Dactylorhiza incarnata*, we have found data from one location only, while for

also common *Platanthera bifolia*, our analysis was based on 32 locations. Previously, Khapugin & Senchugova (2018) suggested a need for publication of floristic lists and separate relevés to make them available. Accordingly, for orchids of the Republic of Mordovia, we showed a considerable lack of data on floras associated with both threatened (Critically Endangered *Neottia cordata*) and common (*Dactylorhiza fuchsii*) species. In the context of the present study, it means that we are not able to estimate the environmental status of habitats occupied by many orchid species.

Habitat confinement of orchids in the Republic of Mordovia

Results of the linear discriminant analysis of 19 orchids based on the average values of environmental factors are presented in Fig. 1. The most separated group has only one orchid species, *Hammarbya paludosa*. This is caused by the highly specific environmental conditions of the raised bog inhabited by the orchid in the National Park 'Smolny'.

Environmental conditions differed significantly among the distinguished habitat groups of orchids (PERMANOVA, $F = 7.54$, $p = 0.0001$). Specifically, meadow-mire group significantly differed from both mixed forest group (PERMANOVA, $F = 8.498$, $p = 0.0082$) and coniferous forest group (PERMANOVA, $F = 5.962$, $p = 0.0012$). In its turn, we found a statistically significant difference in environmental conditions between the mixed forest group and the coniferous forest group (PERMANOVA, $F = 4.771$, $p = 0.0133$).

Habitat groups distinguished based on environmental conditions underline the possible joint findings of different orchids in the same locations. Already now, we may highlight several sites, where populations of various orchids occur. For instance, these are so-called 'orchid glades' in surroundings of the Simkino village, where 11 orchids (e.g. such species of the coniferous forest group as *Cephalanthera rubra*, *Cypripedium calceolus*, *C. guttatum*, *Malaxis monophyllos* and others) occur together as well as surroundings of the Pushta settlement (Mordovia State Nature

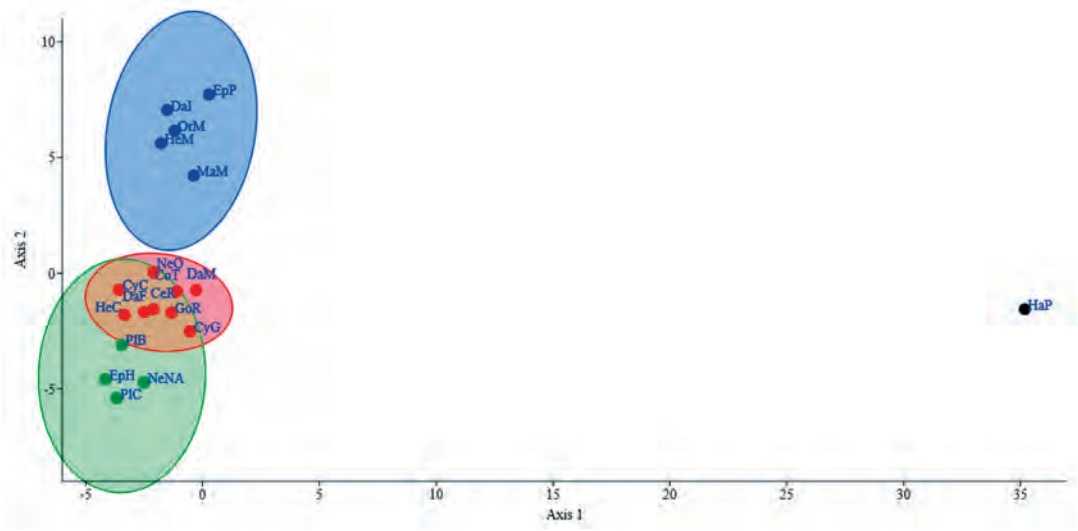

Figure 1. Linear discriminant analysis (LDA) ordination of 19 orchid species. Points represent orchid species displayed according to the environmental factor values based on the Tsyganov (1983) scale. Each plot was colored according to the preliminary habitat classification from Silaeva et al. (2010): blue – meadow-mire group, black – bog group, red – coniferous forest group, green – mixed forest group. 90% concentration ellipses are shown. Orchid species abbreviations refer to Table 1.

Reserve), where *Cypripedium calceolus*, *Goodyera repens*, *Dactylorhiza fuchsii* and *Platanthera bifolia* are known in the same site. In other Russian regions, such 'orchid glades' are well known (e.g. Golubeva et al. 2007; Dubynin et al. 2021). Such sites rich in threatened species diversity are of great conservation importance as locations proposed for protected area establishment (Cañadas et al. 2014).

Conclusion and research implications

We have found a lack of floristic information on floras associated with orchids of the Republic of Mordovia. It requires filling this gap in understanding the environmental status of the occupied habitats. As a valuable source for obtaining the environmental status of plant habitats using phytoindication methods, floristic lists were proposed previously (Khapugin & Senchugova 2018; Khapugin 2021). Thus, we strongly recommend studying and publishing data on floras associated with various common and threatened plants.

A statistically significant difference among habitat groups of orchids cannot be considered a final result, because no data on associated floras were revealed from all locations of orchids in the Republic of Mordovia (Table 1). The inclusion of floristic data from other locations and for other (lacking) species will provide a complete understanding of the habitat preferences of orchids including the environmental status of sites inhabited by the plants in the Republic of Mordovia. Finally, the knowledge on habitat preferences of orchids allows us to direct research efforts to habitats, where various orchids are expected to be found in the region.

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