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Neottianthe cucullata (L.) Schltr. (Orchidaceae Juss.), an endangered orchid in Central Russia

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Summary: Neottianthe cucullata (L.) Schltr. is an endangered orchid species. It grows in forest communities from Central Europe to Eastern Asia. Despite the wide range, this species is considered as rare and / or endangered plant everywhere. In Central Russia, the four *Neottianthe cucullata* populations are located in the Republic of Mordovia. The aim of our study was to assess the modern state and the endangerment of *Neottianthe cucullata* populations in Central Russia. We compared the age structure, abundance dynamics in populations, some morphological parameters of generative individuals, composition of the accompanying flora and environmental conditions of localities for all investigated populations. Our results show that *Neottianthe cucullata* inhabits plant communities, where permanent components are *Pinus sylvestris*, which forms the forest stand and *Pleurozium schreberi* (rarely *Climacium dendroides*), which forms the moss layer. Most important environmental factors determining *Neottianthe cucullata* presence in plant communities are moisture and light conditions, while soil fertility and pH are not so significant for this species. All investigated *Neottianthe cucullata* populations are vegetatively orientated. The percentage of generative individuals in the age spectrum varies from 4.9% to 46.1%. There is a threat to population decrease in the National Park 'Smolny' due to a very low percentage of young (juvenile and immature) individuals that varies from 8.0% to 9.2% despite to the relatively high level of flowers formed per one inflorescence. B2c (iv) status of the IUCN assessment should be attributed to this species due to the annual significant fluctuations in the number of individuals (about 10-times). A good strategy for conservation of *Neottianthe cucullata* in Central Russia might be further monitoring of studied populations as to their status in each habitat, and maintaining the special protection regime in the Mordovia State Nature Reserve and in National Park 'Smolny'.

Keywords: Neottianthe cucullata, endangered species, endangerment, population, age spectrum, morphometrics, environment indicator values, IUCN

The orchid family (Orchidaceae Juss.) is one of the most species-rich families of seed plants. It includes about 880 genera and more than 25 000 species in the world (Cribb et al. 2003). Due to mycorrhizal specificity (McCormick & Jacquemyn 2014), pollinator specialization (Cozzolino & Widmer 2005) and limited germination rates (Dearnaley et al. 2012), most orchids are extremely susceptible to habitat disturbance compared to other plants (Cozzolino & Widmer 2005; Jacquemyn et al. 2007). Frequently, orchids are distributed in specific habitats (Wotavova et al. 2004).

Currently, orchids are particularly vulnerable to climate and land-cover changes due to their narrow ecological preferences. Orchidaceae have a high share in species that have declined in abundance and are considered to be rare, threatened or endangered, primarily as a result of habitat loss (Kull & Hutchings 2006; Pillon & Chase 2007). Considering orchids' great value, endangered situation and its key role in ecosystem, Orchidaceae are frequently used as flagship group in biological conservation or as indicators of the degree of ecosystem disturbance

(Vakhrameeva 1992; Baillie et al. 2004). Patterns of orchid richness are regulated by habitat size and elevation range at large scales (Acharya et al. 2011), while by light availability, soil moisture, canopy height and area (especially for the epiphytic orchids) at fine scales (McCormick & Jacquemyn 2014).

About 130 orchid species belonging to 42 genera are known in Russia (Varlygina 2011). Many orchids are endangered, 65 species were included in the 'Red Data Book of the Russian Federation' (Bardunov & Novikov 2008). This can be explained by the increae of anthropogenic influences and biological and ecological features of orchids. 39 orchid species belonging to 20 genera are known in Central Russia (Averyanov 2014). Out of these, 19 orchids are included in the 'Red Data Book of Russian Federation' (Bardunov & Novikov 2008). The most vulnerable orchid species are often small plants with narrow ecological preferences like *Hammarbya paludosa* (L.) Kuntze, *Neottianthe cucullata* (L.) Schltr., *Calypso bulbosa* (L.) Oakes, etc.

We investigated population structure, biology and ecology of *Neottianthe cucullata* (L.) Schltr. (hooded neottianthe) in Central Russia. This species is distributed almost all over Eurasia in coniferous or mixed green-moss forests, but everywhere it is a rare and endangered plant (Bardunov & Novikov 2008; Varlygina 2011). There is a lack of data on the biology and ecology of this orchid as well as on its ecological preferences. So, *Neottianthe cucullata* is absent among species estimated according to the third edition of Ellenberg's indicator values (Ellenberg et al. 2001), while it is present in a species list of the phytoindication scale of Tsyganov (1983).

The aim of our work was to evaluate the modern status and the endangerment of *Neottianthe cucullata* populations in Central Russia. We addressed following questions: (i) What main environmental factors influenced the vulnerability of *Neottianthe cucullata* in Central Russia? (ii) What is the status of *Neottianthe cucullata* populations in natural forest ecosystems of Central Russia?

Materials and methods

Neottianthe cucullata is a plant with erect or ascending stems, 6.5–28(35) cm long. Tubers ellipsoid to globose, 0.5–12 mm in length and 0.7–1.8 mm in width. 1–2(3) basal leaves elliptical to lanceolate: the bottom leaf 2.2–8.1 × 0.9–4.8 cm, the upper leaf 2.0–7.5 × 0.7–3.4 cm. Sometimes, 1–3 small, narrow-lanceolate cauline leaves can be observed. Inflorescence is a sparse one-sided raceme. Each raceme is up to 8 cm long and often has 6–24 irregular flowers. Perianth lobes 6–8 × 1–1.5 mm, acuminate. Labellum up to 12 mm, deflexed, deeply tripartite. Lateral lobes linear-lanceolate, 2–6 × 0.3–0.5 mm, middle lobe longer, linear-oblong, 4–8 × 1–2 mm. Spur up to 5 mm in length and 3 mm in width, globose-dilated. Capsule 6–14 mm in length and up to 3 mm in diam., contains abundant dust-like seeds (Vakhrameeva & Zhirnova 2003; Webb 1980; Vakhrameeva et al. 2014).

Neottianthe cucullata is distributed from Poland and Hungary to the Sakhalin Island and the South Kuriles and from 56–58°N to Belorussia and southern regions of Russia: Voronezh Region, Saratov Region, Orenburg Region, Republic of Bashkortostan, Chelyabinsk Region (Wisniewski 1976; Vakhrameeva & Zhirnova 2003; Webb 1980). However, there is a lack in distribution data for this species. So, recently, new locations of *Neottianthe cucullata* were found (e.g. in Tyumen region (Kuzmin & Drachev 2007)) and some locations disappeared (e.g. in 4 of 22 regions of Central Russia (Varlygina 2011)).

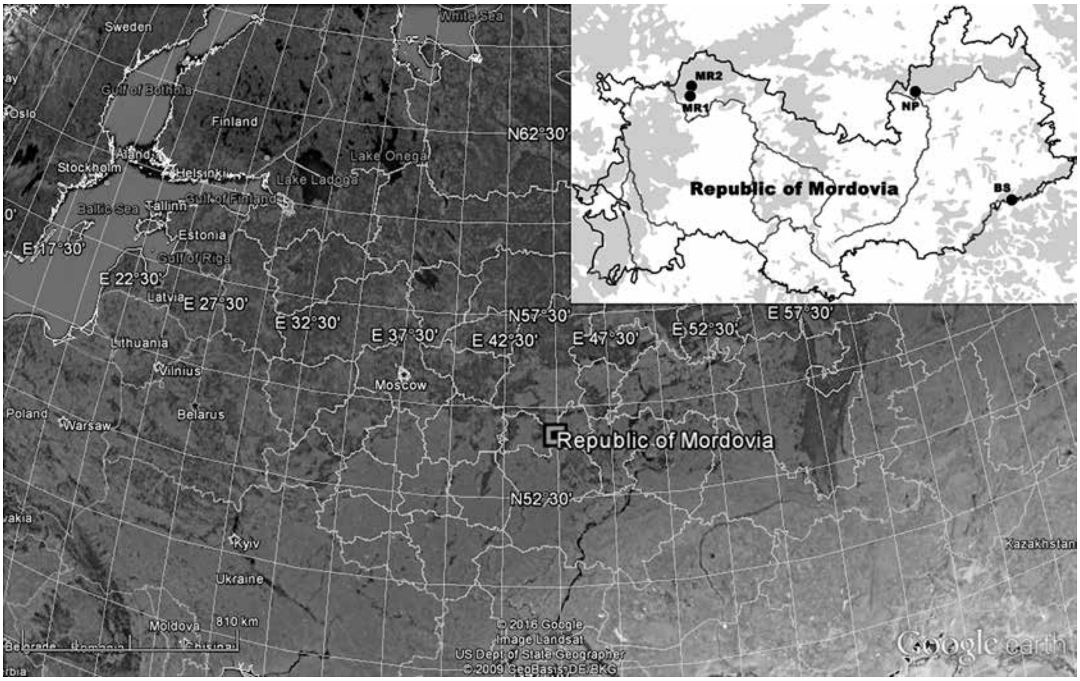

Figure 1. Geographical position of the Republic of Mordovia in Eastern Europe. Symbols on the Mordovia map: BS – population nearby the biological station of the Mordovia State University, NP – population in National Park 'Smolny', MR1 and MR2 – populations in the Mordovia State Nature Reserve (447 and 361 forest compartments respectively).

In Mordovia, *Neottianthe cucullata* is known in the Mordovia State Nature Reserve (MR), National Park 'Smolny' (NP) and in the neighborhood of the biological station of the Mordovia State University (BS). One location has not been found since 1890 in Temnikov district nearby the Mordovia State Nature Reserve (Kosmovskiy 1890). *Neottianthe cucullata* is known in MR since 1966 (Tsinger 1966). Populations of endangered plants cover small areas in green-moss pine forests predominantly in the western part of Mordovia Reserve (Chugunov et al. 2011). Until 2011, no regular population-based studies of *Neottianthe cucullata* had been carried out, excluding insignificant data on the presence / absence of plants in some stationary plots. In the southern part of National Park 'Smolny', *Neottianthe cucullata* is known since 1999 (Silaeva et al. 2010). Population-based studies of *Neottianthe cucullata* were not carried out here until 2014. In 2003, this species was included in the 'Red Book of the Republic of Mordovia' (Silaeva 2003). After that, a third locality of *Neottianthe cucullata* was found in the neighborhood of the biological station of Mordovia State University (Silaeva et al. 2004). Since that time, studies of this population had been carried out until 2015. In 2008, this endangered orchid was included in the 'Red Data Book of the Russian Federation' (Bardunov & Novikov 2008) as a rare and sporadically distributed species.

After publication of the 'Red Book of the Republic of Mordovia', we investigated *Neottianthe cucullata* in Mordovia State Nature Reserve (two populations), in National Park 'Smolny' (one population) and in the neighborhood of the biological station of the Mordovia State University (one population) (Fig. 1).

We compared results of population-based studies in the Mordovia State Nature Reserve (MR) in 2011–2015, in National Park 'Smolny' (NP) in 2014–2015, using results of abundance investigations in the population nearby the biological station of Mordovia State University (BS) in 2004–2015. The field investigations were carried out in 2011–2015 for both populations in the Mordovia State Nature Reserve (MR1 – 54.722602 N, 43.237436 E; MR2 – 54.774908 N, 43.249433 E), in 2014–2015 for population in National Park 'Smolny' (NP – 54.745357 N, 45.280732 E) and in 2004–2015 for population in the neighborhood of the biological station of Mordovia State University (BS – 54.179159 N, 46.156401 E). For field investigations of MR1, MR2, NP, transect with 5 (MR2, NP) and 6 (MR1) square plots (1 × 1 m) were established in each location.

We carried out the assessment of the *Neottianthe cucullata* populations status on the basis of the individual parameters of plants (height of plants, number and size of leaves per each individual, number of flowers per one generative individual), composition of accompanying flora and abundance dynamics in each population. The density (number of individuals per 1 m^2) of *Neottianthe cucullata* per plot was revealed. We determined population type of *Neottianthe cucullata* in Central Russia according to Gorchakovskii & Igosheva (2003).

Based on revealed morphometrical data, individuals of *Neottianthe cucullata* were divided into four age-state classes: juvenile (j), immature (im), mature vegetative (v) (= non-flowering adults) and generative (g) (= flowering adults) according to Vakhrameeva & Zhirnova (2003) and personal observations. Comparison of generative individual's parameters of *Neottianthe cucullata* was carried out for populations MR1, MR2, NP.

Composition of accompanying flora was recorded within each plot. Comparison of accompanying floras from studied localities was carried out. We calculated a Jaccard's similarity index, $JS = 100 \times C/(A+B-C)$, where A = number of species in locality A; B = number of species in locality B; C = number of species shared between two (A and B) localities (Jaccard 1901).

Based on the compositions of accompanying floras, environment factors of habitats with *Neottianthe cucullata* were calculated for conditions of Central Russia (on the example of Mordovia). Calculations were carried out according to Ellenberg's (Ellenberg et al. 2001) and Tsyganov's (1983) scales. In the first case, mean environmental indicator values (EIVs) were weighted by projective cover (Käfer & Witte 2004, with modifications according to Bulokhov 2004). In the second case, ecological indicator values are arranged as interval scales. It means that for each plant species we can define the range of its existence in relation to a concrete factor, for instance, soil nitrogen, moisture, etc. It could be evaluated in conventional units covering the total factor longitude from minimum up to maximum in relation to the concrete species. Mean EIVs were calculated using algorithm suggested by Buzuk & Sozinov (2009). In both cases we used six environmental factors: light (L), temperature (T), continentality (C), moisture (M), pH (R) and soil nitrogen (N).

Statistical analyses were carried out using PAST (Hammer et al. 2001) and Microsoft Excel.

Results

Abundance dynamics of *Neottianthe cucullata* populations in Central Russia was presented by wave amplitudes which were similar for all studied localities (Fig. 2). The *Neottianthe cucullata* population from MR1 consisted of 41 (in 2011) to 171 (in 2013) individuals; the population from MR2 consisted of 20 (in 2011) to 214 (in 2013) individuals; the population from NP consisted of 76 to 100 individuals; and the population from BS consisted of 2 (in 2010) to 35

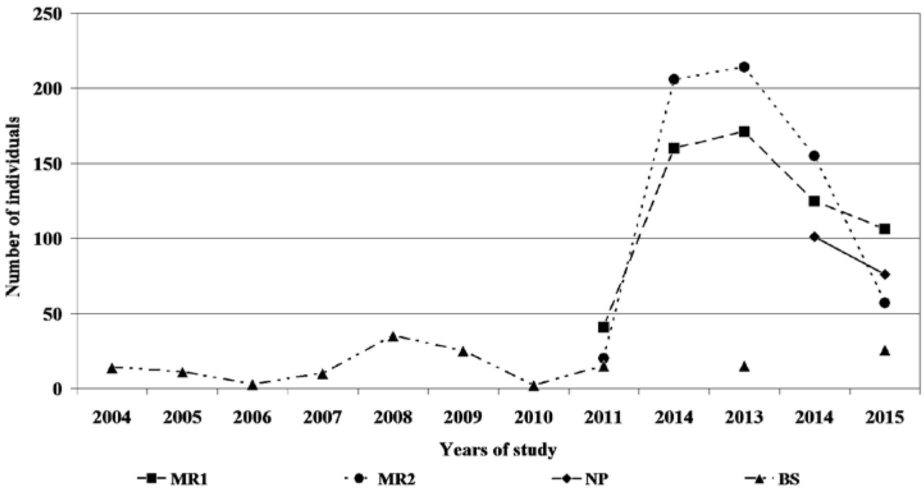

Figure 2. Abundance dynamics in *Neottianthe cucullata* populations in Central Russia.

(in 2008) individuals. Density of plants varied highly among different populations: from 1 (BS in 2006 and 2010) to 73 individuals per 1 m^2 (MR2 in 2012).

Age spectrum in *Neottianthe cucullata* populations includes four age-state classes. Independently of origin, non-flowering individuals (juvenile, immature and mature vegetative age-state classes) significantly predominate in the age structure in MR1, MR2, NP populations (Fig. 3). The highest percentage of generative individuals was marked for NP populations (43.6% in 2014 and 46.1% in 2015), while values of this parameter for MR1 and MR2 populations were significantly lower. In these localities, they varied from 3.8% (in 2012) to 34.1% (in 2011) within MR1 and from 4.9% (in 2012) to 18.7% (in 2014) within MR2. It should be noted that *Neottianthe cucullata* population in NP had very few young (juvenile and immature) individuals.

There were no significant differences between studied *Neottianthe cucullata* populations relative to the height of generative individuals and number of flowers per one inflorescence (Table 1). However, values of these parameters were slightly higher in MR1 compared to the lower in BS.

The accompanying floras were revealed for all four localities with *Neottianthe cucullata* participation. It included 63 species of vascular plants and mosses from 53 genera and 36 families. Of these, the accompanying floras included 23 species from 19 genera and 15 families in MR1, 19 species from 19 genera and 17 families in MR2, 20 species from 15 genera and 12 families in NP and 51 species from 43 genera and 30 families in BS. Jaccard's similarity index amongst accompanying floras varied from 20.0% (amongst MR1 and MR2) to 44.8% (amongst MR1 and NP) (Table 2).

Neottianthe cucullata grows in pine forest plant communities (*Pinus sylvestris* L.) with green mosses (*Pleurozium schreberi* Mitt., *Climacium dendroides* (Hedw.) F. Weber & D. Mohr and rarely *Dicranum polysetum* Sw.) in the ground layer.

Due to absent environmental indicator values in Ellenberg's scale (Ellenberg et al. 2001), we defined these on the basis of synphytoindication methods according to Didukh (2011) for conditions of Central Russia as mean EIVs amongst the four studied localities (Table 3). These data show that *Neottianthe cucullata* in studied localities grows in semi-shade habitats (generally

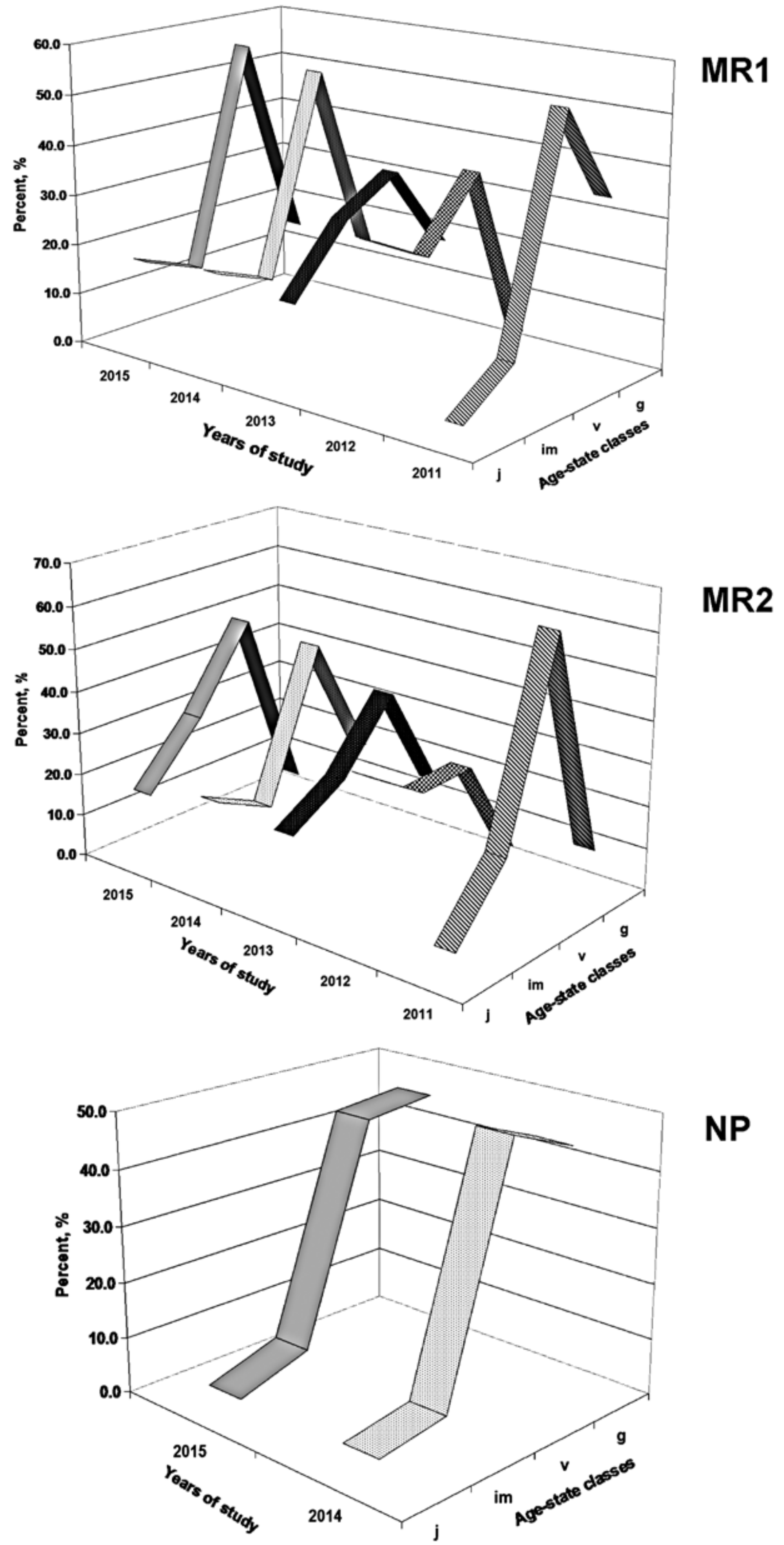

Figure 3. Age structure in *Neottianthe cucullata* populations. Age-state classes of individuals: juvenile (j), immature (im), mature vegetative (v), generative (g).

Table 1. Some morphometrical parameters of generative individuals of *Neottianthe cucullata* in Central Russia.

	Height of plants, cm				Number of flowers per one inflorescence			
Parameter	MR1	MR2	NP	BS	MR1	MR2	NP	BS
2013*								
M	16.1	11.1	–	9.0	9.4	6.3	–	6.6
m	1.0	0.9	–	0.6	1.3	1.1	–	0.5
min	5	6	–	6.8	2	2	–	5
max	27	18	–	12.0	29	17	–	9
2014**								
M	17.1	8.1	12.5	–	12.7	11.6	16.7	–
m	1.1	0.6	0.8	–	0.9	0.5	0.7	–
min	11	3	1	–	8	6	4	–
max	27	17	26	–	21	17	26	–
2015***								
M	21.0	15	16.8	14.7	12.8	8.7	11.5	8.3
m	1.2	2.1	0.7	1.1	1.2	0.7	0.7	1.2
min	13	12	9	7.0	6	8	6	3
max	30	19	25	19.0	23	10	20	17

M – mean value, m – error of the mean, min – minimum value, max – maximum value; * – nMR1 = 25, nMR2 = 17, nBS = 10, ** – nMR1 = 18, nMR2 = 27, nNP = 44, *** – nMR1 = 16, nMR2 = 3, nNP = 30, nBS = 14.

with more than 10% relative illumination) on fresh, moderately acidic soils with fertility level from poor (MR2) to intermediate (MR1).

The scale of Tsyganov (1983) contains indicator values for *Neottianthe cucullata* in the subzone of coniferous-broadleaved forests. So, we tested whether these values equal to our experimental data obtained on the basis of phytoindication methods (Table 4). These data show that *Neottianthe cucullata* is a mesophyte to permesophyte, and in studied localities it inhabits plant communities intermediate between light-coniferous forest and forest edges, cut lines etc. on acidic (pH ≈ 4.7), nutrient-poor soils.

Spatial arrangement of studied localities with *Neottianthe cucullata* using principal correspondence analysis (PCA) was significantly similar for both scales of environmental indicator values (Tsyganov 1983; Ellenberg et al. 2001) (Fig. 4). Thus, in both cases, MR2 is considerably separated from the three other localities, while MR1 shows a certain affinity to NP (Fig. 4A) or to BS (Fig. 4B).

Table 2. Compositional similarity (Jaccard's index, 100 × J) of the accompanying flora in four localities with *Neottianthe cucullata* in Central Russia.

	MR2	BS	NP
MR1	20.0	23.3	44.8
MR2		29.6	22.6
BS			29.6

Table 3. Mean environmental indicator values for *Neottianthe cucullata* in Central Russia based on Ellenberg et al. (2001).

	Light	Temperature	Continentality	Moisture	Reaction	Nitrogen
MR1	5.4	4.1	4.9	5.0	4.4	4.9
MR2	5.4	4.0	5.0	4.7	3.4	2.4
NP	5.9	3.5	5.2	4.3	3.8	4.3
BS	5.6	5.1	4.8	4.9	4.7	3.7
Mean	5.6	4.2	5.0	4.7	4.1	3.9

Discussion

Annual changes in the individuals' abundance in almost all studied *Neottianthe cucullata* populations can be considered as typical of this orchid species. Decreasing in the individuals' abundance in populations had a positive correlation with the dry summer seasons in 2006 and especially in 2010. After that, an increase in the individuals' abundance in population was observed. Thus, abundances of individuals in MR2 and BS changed 10.7-times and 17.5-times respectively. Most likely, this is typical of the other studied populations, but it can not be shown here due to a lack of data for a longer period. Such annual 10-times fluctuations in the number of individuals in *Neottianthe cucullata* populations must be considered as 'extreme fluctuations' according to IUCN criteria and categories (2012a, 2012b, 2014). Taking this into account, the B2c (iv) status (= extreme fluctuations in number of mature individuals) according to the IUCN assessment is required for *Neottianthe cucullata* in Central Russia as well as in other areas, where this species shows similar features.

All investigated *Neottianthe cucullata* populations could be called 'vegetatively-orientated' according to Gorchakovskii & Igosheva (2003). Data on the age-state structures in MR1 and MR2 are similar due to a large share in young (juvenile and immature) individuals. In contrast, the percentage of young plants in NP is much lower. That can imply a threat of population extinction due to the fact that young individuals could become generative individuals later. Large percentage of juvenile and immature plants in *Neottianthe cucullata* populations is a result of the successful seed germination (Vakhrameeva & Zhirnova 2003).

Table 4. Mean environmental indicator values for *Neottianthe cucullata* in Central Russia based on Tsyganov (1983).

	Light	Temperature	Continentality	Moisture	Reaction	Nitrogen
MR1	4.8	7.7	8.4	12.9	5.6	5.2
MR2	4.3	7.6	8.6	12.6	4.8	3.9
NP	4.4	7.9	8.6	12.2	5.7	5.0
BS	4.7	7.9	8.5	12.7	5.6	4.6
Mean	4.5	7.8	8.5	12.6	5.4	4.7
Amplitude of values according to Tsyganov (1983)	3–7	6–10	7–15	9–13	not determined	not determined

Original designations of environmental factors in Tsyganov (1983): light – LC, temperature – TM, continentality – KN, moisture – HD, reaction – RC, nitrogen – NT

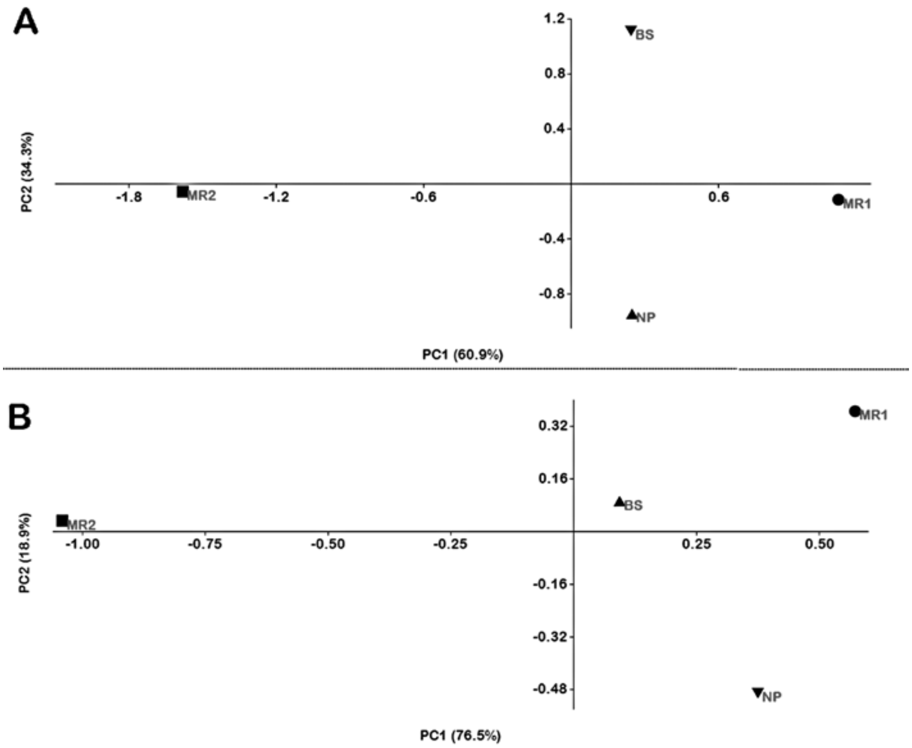

Figure 4. Principal correspondence analysis (PCA) ordination diagram for the four localities with *Neottianthe cucullata* participation, based on mean environmental indicator values according to Ellenberg et al. (2001) (A) and Tsyganov (1983) (B).

Studied morphological parameters of generative individuals (the height and the number of flowers per inflorescence) are similar in all investigated *Neottianthe cucullata* populations. It should be noted that a low percentage of juvenile and immature individuals was observed in NP despite the relatively high number of flowers per one inflorescence in this location (see Table 1). Perhaps, there are additional, unknown reasons for inhibition of *Neottianthe cucullata* seed germination in National Park 'Smolny'.

The accompanying floras in investigated localities are slightly similar. Highest value of Jaccard's index was amongst MR1 and NP, while the lowest compositional similarity of the accompanying floras was amongst MR1 and MR2 despite their close geographical location relative to each other. Low similarity in the accompanying floras can be explained by high species diversity in plant communities with *Neottianthe cucullata* participation. However, there is a group of species common to all investigated localities. These are *Luzula pilosa*, *Rubus saxatilis*, *Pinus sylvestris* and *Pleurozium schreberi*. Thus, only these four species are constant components of plant communities with *Neottianthe cucullata* in conditions of Central Russia. Additionally, 10 species (*Calamagrostis epigejos* (L.) Roth, *Carex digitata* L., *Cytisus ruthenicus* Woł., *Climacium dendroides*, *Fragaria vesca* L., *Melica nutans* L., *Rubus idaeus* L., *Sorbus aucuparia* L., *Vaccinium vitis-idaea* L., *Veronica officinalis* L.) were found in 3 of 4 localities.

Environment indicator values for *Neottianthe cucullata* are new for Ellenberg's scale. Despite of the fact that results of studies outside Central Europe are sometimes questioned (Godefroid & Dana 2007) or considered as 'too good to be true' (Zelený & Schaffers 2012), Ellenberg's indicator values are the most popular indicators in whole Europe (Hill et al. 1999; Seregin 2014). That is why our results may qualify for inclusion into available tables of Ellenberg's indicator values for Eastern Europe, e.g. Borhidi (1995).

Comparison of obtained environmental indicator values according to the scales of Ellenberg and Tsyganov allow to show the high spatial similarity of studied localities. In both cases, MR2 was considerably separated from the others due to the lowest values of soil pH and fertility, while MR1, NP and BS show higher values of these environmental factors in both tested indicator scales. In case of soil nitrogen, mean EIVs changed 2 times according to Ellenberg's scale (from 2.4 in MR2 to 4.9 in MR1). Taking this fact into account, soil pH and soil fertility are not determining factors of *Neottianthe cucullata* presence in a plant community. In contrast, values of light and moisture of habitats were relatively stable with low variance coefficients. Thus, it can be suggested that exactly these environmental factors determine *Neottianthe cucullata* presence in forest communities. We propose that significant changes of these environmental factors can be the cause of a short-term transition from secondary dormancy to buffer stress without increase of mortality risk.

Conclusion

Our results show that all investigated *Neottianthe cucullata* populations in Central Russia are arranged in habitats which are slightly similar relative to the composition of accompanying floras. Its permanent components are *Pinus sylvestris* determining habitat conditions and green mosses (*Pleurozium schreberi*, rarely *Climacium dendroides*) determining conditions for germination and development of *Neottianthe cucullata* seeds. Most important environmental factors for *Neottianthe cucullata* are moisture and light conditions, while soil fertility and pH are not so significant for this species. These environmental factors are also important for the formation of the whole vegetation cover (Seregin 2014; Van Landuyt et al. 2008).

The study of the age-state structure of *Neottianthe cucullata* populations shows that all of these are vegetatively-orientated (percentage of generative individuals varied from 4.9% to 46.1%). There is a threat to NP by population decrease due to very a low percentage of young (juvenile and immature) individuals that varied from 8.0% to 9.2% despite of the relatively high level of flowers formed per one inflorescence.

Long-term observations of the abundance dynamics in *Neottianthe cucullata* populations show that annual fluctuations in the number of individuals (about 10-times) are typical for this orchid species in Central Russia and outside of this area. That is why the B2c (iv) status of IUCN assessment should be attributed to this species.

In our opinion, *Neottianthe cucullata* populations in the Mordovia State Nature Reserve and National Park 'Smolny' are relatively protected due to the special protection regime. This regime suitable for the protection of *Neottianthe cucullata*, but not for some other species (e.g. *Lunaria rediviva* L. (Khapugin & Chugunov 2015) or steppe perennial bunchgrasses (Khapugin & Silaeva 2013)), which may suffer from human or animal impacts (e.g. decrease in area). The smallest *Neottianthe cucullata* population is located within the planned protected area 'Simkino Nature Preserve', where populations of 28 rare plant species are known (Ageeva et al. 2011–2013). Its establishment would contribute to the conservation of populations of many species including *Neottianthe cucullata*.

Among all investigated *Neottianthe cucullata* populations, the populations in the Mordovia State Nature Reserve (MR1, MR2) are the most stable and well-developed in Mordovia due to high abundance of individuals, completeness in the populations age structure and special protection regime within this area. At present, a good strategy for *Neottianthe cucullata* conservation might be (i) to continue the monitoring of these populations relative their status in each habitat and (ii) to maintain the special protection regime in the Mordovia State Nature Reserve and in National Park 'Smolny'.

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