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Research Article

The age structure of a *Cypripedium guttatum* (Orchidaceae) population in the Tyumen Region, Western Siberia

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Abstract

Despite active botanical studies around the world, there are still regions that lack data about the distribution, ecology, and biology of some threatened plants. During the field surveys in 2021, the new location of a threatened plant, *C. guttatum* (Orchidaceae), has been found in the Tyumen Region. The age structure of the found population has been studied by measuring morphometric traits (plant height, number of leaves, size (length, width), and number of veins of the second leaf, and, for generative individuals, the number of flowers and (if any) fruits, and fruit set) and classifying *C. guttatum* individuals to several age classes (juvenile, immature, virginile (mature vegetative), generative). The obtained results demonstrated that the found orchid population is assigned to *C. guttatum* f. *albostriatum* nom. prov. Age structure of *C. guttatum* population (j:im:v:g: 1%:5%:64%:30%) is characterized by dominance of vegetative individuals. The obtained data on *C. guttatum* population serve as a starting point for further population studies in both Tyumen Region and the entire Western Siberia. The establishment and extension of a plan for long-term population studies can allow us to make an unbiased evaluation of the conservation status of *C. guttatum* using methods of the IUCN Red List assessment.

Keywords: Age population structure, new record, orchid, Red Data Book, threatened species

Introduction

At present, biodiversity loss remains a global issue challenging the attention of various stakeholders (Heydari et al., 2020; Lees et al., 2021). Naturally rare and threatened species are at

special risk of extinction (e.g., Melnyk et al., 2021; Anselmo & Rizzioli, 2022). Among plants, Orchidaceae is one of the largest and most threatened families in the world. That is why many aspects of orchid biology (e.g., Tsiftsis & Djordjevic, 2018), ecology (e.g., Djordjevic et al., 2016), taxonomy (e.g., Łobas et al., 2021; Botes et al., 2020), and phylogeny (e.g., Calevo et al., 2022) are being studied in various regions worldwide. Orchid distribution is especially important being relevant for conservation-based issues, such as IUCN Red List assessment, the development of conservation plans, establishment of new Protected Areas serving orchid preservation (e.g., Efimov et al., 2022). The studying of orchid distribution, like other plants and animals, is especially relevant in hard-to-reach areas. There, each new record can change an understanding of the species range and its conservation status in a region or worldwide.

In Russia, the family Orchidaceae includes 135 species and 13 subspecies belonging to 38 genera (Efimov, 2020), while the highest species richness occurs in the Russian Far East and South Russia (e.g., Popovich et al., 2020). According to Glazunov et al. (2017), 30 orchid species from 19 genera are noted in the Tyumen Region. Among them, 25 orchids are included in the Red Data Book of the Tyumen Region (Petrova, 2020), including *Cypripedium guttatum* Sw. with protection category 3 (rare species). This plant is one of the species, most frequently included in Red Data Books of Russian regions (Khapugin et al., 2020). Throughout its range, *C. guttatum* is characterized by the infraspecific polymorphism of the flower coloration (Shirokov et al., 2020), which requires extended population studies to solve this issue. Despite the wide distribution of *C. guttatum* both in Russia (Averyanov, 1999; Khapugin et al., 2020) and Tyumen Region (Petrova, 2020), no population-based studies have been conducted previously in this region of Russia. This makes it difficult to assess the unbiased conservation status (e.g., based on the IUCN Red List assessment) of *C. guttatum* in the Tyumen Region.

This short communication aimed to study the age structure of the *Cypripedium guttatum* population in a newly found location in the Tyumen urban district, Tyumen Region, Western Siberia. For this purpose, we have established the following research tasks: measure some morphological parameters of *C. guttatum* individuals; determine the age structure of *C. guttatum* population.

Material and methods

The study area is situated in the Tyumen Region, Western Siberia. Tyumen Region covers about 160 122 km^2. The climate is temperate continental (Degefie et al., 2014); about 2/3 of the

precipitation amount occurs in May – September (Gvozdetskiy, 1973). The study area is located in the ecoregion of Kazakh forest-steppe in the biome of temperate grasslands, savannas, and shrublands, according to the classification of Dinerstein et al. (2017, 2019), where macro-mosaic light birch (*Betula pendula* Roth) forests and grasslands are occurred (Gvozdetskiy, 1973).

In the urban forests on the periphery of Tyumen city, two subpopulations of *C. guttatum* of one population have been explored during the route surveys in June – July 2021. Once a subpopulation is found, stationary 1 × 1-m study plots have been established. On each 1 × 1-m study plot, we counted the number of *C. guttatum* individuals, and morphometric parameters of each orchid plant, namely the plant height, number of leaves, size (length, width), and number of veins of the second leaf, and, for generative individuals, the number of flowers and (if any) fruits, and fruit set. An above-ground shoot was considered a counting unit (hereinafter – individual). Based on morphometric traits, all individuals were classified into a number of age classes, namely juvenile (j), immature (im), virginile (mature vegetative) (v), and generative (g) individuals. In accordance with Bychenko (2009), non-flowering generative individuals were considered virginile plants contrary to some other authors (e.g., Tatarenko, 1996; Kirillova et al., 2017). We determined the population type of *C. guttatum* according to Gorchakovskii & Igosheva (2003). Both *C. guttatum* subpopulations had a relatively small area, namely 8 m^2 in site 1, and 20 m^2 in site 2. Therefore, we were able to establish only two 1 × 1-m study plots in site 1, and three ones in site 2. Because these sites are closely located, we considered them a single population investigated using five 1 × 1-m study plots. Based on photographs of *C. guttatum* flowers (Fig. 1), we have identified its coloration form in the studied population according to Shirokov et al. (2020).



Figure 1. The coloration of *Cypripedium guttatum* flowers in the Tyumen urban district, Tyumen Region, Western Siberia.

For each age class, the mean values of morphometric parameters were calculated. To test the correctness of the attribution of non-flowering generative individuals to the virginile group, the linear discriminant analysis of all studied individuals was performed. All calculations and statistical treatments have been performed in PAST 4.11 (Hammer et al., 2001).

Results

The new location of *Cypripedium guttatum* in the Tyumen Region

During field surveys of 2021 in the Tyumen urban district, two closely located *C. guttatum* subpopulations have been found. They have not been mentioned in the recent edition of the Red Data Book of the Tyumen Region (Petrova, 2020). Both sites are situated 100 m from each other and can be considered one location in addition to the Red Data Book of the Tyumen Region (Petrova, 2020). Site 1 is situated in the light forb birch (*Betula pendula*) forest, while site 2 is located in the moist birch-pine (90% *Betula pendula*, 10% *Pinus sylvestris* L.) forest. These floristic records supplement the information in the Red Data Book of the Tyumen Region (Petrova, 2020) about *C. guttatum* distribution.

Characteristics of the *Cypripedium guttatum* population

In all five study plots, 102 *C. guttatum* individuals have been registered, with a density of 20.4±5.1 individuals per 1 m^2. Morphometric traits were measured for each individual (Table 1).

Table 1. Summary statistics of morphometric traits (Mean±SD) of *Cypripedium guttatum* individuals in the Tyumen urban district, Tyumen Region, Western Siberia.

Stage	LN	IH, cm	LL, cm	LW, cm	NV	FloN	Fruit set
j (n=1)	2	2.5	3.5	1.5	2	–	–
im (n=5)	2.0±0.0	4.6±2.4	5.1±1.1	2.3±0.4	5.0±0.0	–	–
v (n=65)	2.4±0.5	14.4±2.9	10.2±1.6	5.8±1.2	7.2±0.6	–	–
g (n=31)	3.0±0.2	29.6±2.5	11.5±1.1	7.3±0.9	8.5±1.0	1.0±0.0	6.5%

Note: LN – number of leaves, IH – the height of the individual (cm), LL – leaf length (cm), LW – leaf width (cm), NV – number of veins, FloN – number of flowers, Fruit set – total number of fruits / total number of fruits × 100%.

Based on the flower coloration (Fig. 1), we tend to attribute plants of the found population to *Cypripedium guttatum* f. *albostriatum* nom. prov. (see Shirokov et al., 2020). Although, the lip coloration is something intermediate between this form and *C. guttatum* f. *rubrosaccos* nom. prov., the lip is not almost evenly purple-colored (see Fig. 1), like it is proposed in the identification key in Shirokov et al. (2020).

Based on them, all plants have been classified into four age classes, including one juvenile plant,

five immatures, 65 virginile plants, and 31 generative individuals. According to the age structure (j:im:v:g: 1%:5%:64%:30%), the studied C. guttatum population is classified as vegetatively oriented. Table 1 shows that values of morphometric parameters are well applicable to classify plant individuals to age classes. The studied C. guttatum population is dominated by vegetative plants, i.e., virginile, immature, and juvenile individuals. Although generative individuals are relatively numerous, the fruit set is quite low (6.5%) which indicates difficulties in seed reproduction.In the framework of this study, we have classified non-flowering generative *C. guttatum* plants as virginile individuals. To test the correctness of this attribution, we have performed the linear discriminant analysis of all studied individuals where morphometric traits were considered environmental variables (Fig. 2).

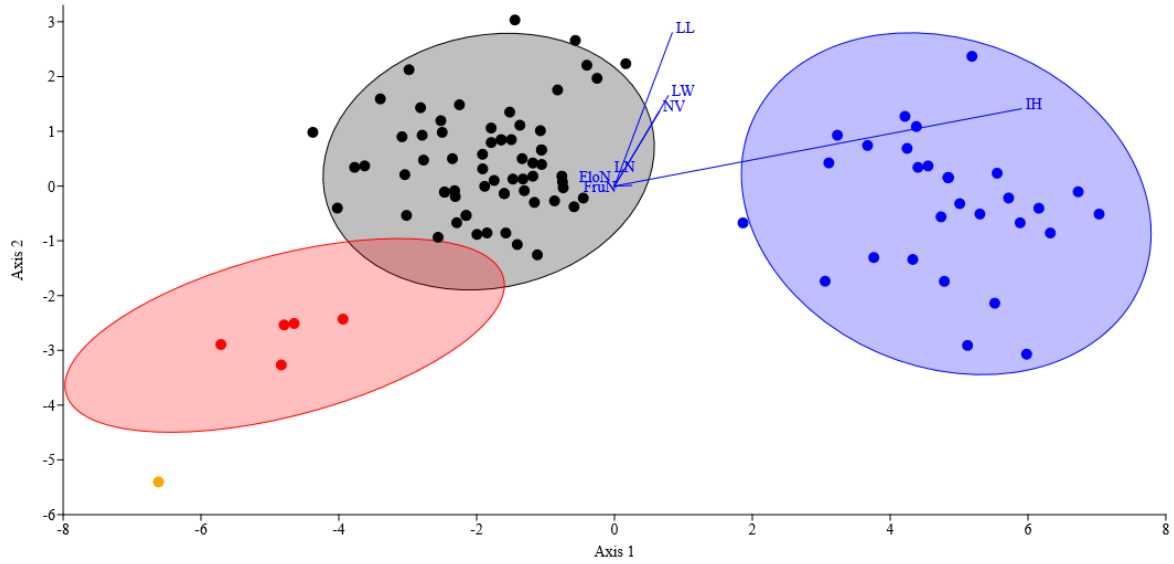


Figure 2. Discriminant analysis of *Cypripedium guttatum* individuals based on their morphometric traits. Designations: black dots – virginile individuals, blue dots – generative individuals, red dots – immature individuals, orange dots – juvenile individuals; colored ovals designate areas containing 95% of analyzed points, where they are expected to fall (see Hammer et al., 2001); variables on the biplot: LN – number of leaves, IH – the height of the individual, LL – leaf length, LW – leaf width, NV – number of veins, FloN – number of flowers, FruN – number of fruits.

Figure 2 shows a quite clear distinction of individuals of all age classes based on morphometric traits. Noteworthy, the number of flowers and fruits have not been considered as key traits for

distinguishing generative individuals from vegetative (virginile, immature, juvenile) ones, while traits of vegetative organs (leaf size, number of veins, and plant height) were considered the most important parameters.

Discussion

Cypripedium guttatum individuals from the new location in the Tyumen Region have been assigned to the form of *C. guttatum* f. *albostriatum* nom. prov. based on Shirokov et al. (2020). Although *C. guttatum* is widely distributed being included in Red Data Books of more than half of Russian regions (Khapugin et al., 2020), its populations have been studied only in a few regions, namely the Republic of Komi (Kirillova et al., 2017; Kirillova & Kirillov, 2020b; Shirokov et al., 2020), Republic of Yakutia (Afanasieva et al., 2013; Nikolin et al., 2016), Primorsky Krai (Bondarchuk & Averkova, 2013; Nikiforova & Sivtsev, 2015; Shirokov et al., 2020), Vologda Region (Kuznetsova et al., 2015), Republic of Khakassia (Lebedeva, 2019), Krasnoyarsky Krai (Raiskaya, 2020); Shirokov et al., 2020), Sverdlovsk Region (Shirokov et al., 2020), Permsky Krai (Shibanova, 2016). Thus, data obtained in the Tyumen urban district can be considered novel for both Tyumen Region and Western Siberia.

The age spectrum of *C. guttatum* should be compared with care due to two different methodological viewpoints to distinguishing virginile and generative individuals. According to one author (e.g., Tatarenko, 1996; Kirillova et al., 2017), the generative group includes both flowering and non-flowering individuals, while according to other authors (e.g. Bychenko, 2009; Shibanova, 2016), all non-flowering individuals are considered in the virginile group. Therefore, we compared our results on the population age structure with data from the latter authors. Like in the studied location, *C. guttatum* populations in the Permsky Krai (Shibanova, 2016), and Krasnoyarsky Krai (Raiskaya, 2020) were found to be vegetatively oriented with a dominant of virginile plants, while in the Krasnoyarsky Krai (e.g., Raiskaya & Timoshok, 2019; Raiskaya, 2020), bimodal or generatively oriented population types were also observed. It must be noted that vegetatively oriented types of the populations are typical for many other orchid species, such as *Cypripedium calceolus* L. (Gorchakovskii & Igosheva, 2003; Khapugin et al., 2017; Stetsuk, 2013), *Hemipilia cucullata* (L.) Y.Tang, H.Peng & T.Yukawa (Khapugin et al., 2016), *Dactylorhiza majalis* subsp. *lapponica* (Laest. ex Hartm.) H.Sund. (Kirillova & Kirillov, 2020a). This strategy allows plants to survive even under unfavorable conditions by reproducing predominantly vegetatively.

The fruit set recorded in the studied population (6.5%) is of relatively low level compared to other parts of *C. guttatum* range. For instance, the average fruit set is 21.0% in the Republic of Komi (Kirillova et al., 2017), 5.0–8.5% in the Republic of Bashkortostan (Ishmuratova et al., 2005), 26% in the Primorsky Krai (Tatarenko, 1996), 16.7% (with variation between 0% and 44%) in Central Yakutia (Afanasieva et al., 2013), 7.1–23.8% in the Permsky Krai (Shibanova, 2016), 8.5% in the Vologda Region, North European Russia (Kuznetsova et al., 2015). Therefore, the main reproduction way of *C. guttatum* in the Tyumen urban district is vegetative by division of the rhizome. However, to make reliable conclusions on the reproductive biology of this species, long-term investigations are required.

Thus, in this paper, we supplement materials of the Red Data Book of the Tyumen Region (Petrova, 2020) with a new population represented by several small subpopulations of *C. guttatum*. The obtained population parameters serve as a starting point for further population studies of both *C. guttatum* and other orchids in both the Tyumen Region and the whole of Western Siberia. The establishment and extending a plan for the long-term population studies of orchids will allow us to reveal the unbiased conservation status of species using methods of the IUCN Red List assessment. Unfortunately, we have not found published results on the structure of *C. guttatum* populations outside Russia to compare our results with them. Therefore, the obtained results are highly important for all regions within the natural range of *C. guttatum*.

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