Preslia 94: 255–273, 2022
doi: 10.23855/preslia.2022.255

Preslia

Conservation status and ecology of the highly threatened endemic *Gentianella bohemica*

Agnes Preinfalk, Dietmar Moser & Franz Essl*

BioInvasions, Global Change, Macroecology Group, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, AT-1030 Vienna, Austria
*corresponding author: franz.essl@univie.ac.at

Abstract: The Bohemian gentian *(Gentianella bohemica*) is a highly threatened endemic species of nutrient poor grasslands on the Bohemian Massif in Austria, Bavaria and the Czech Republic. Here, we analyse its microhabitat preferences and the role of historic and current land management and seasonal climatic variability in the changes in population sizes. A total of 114 populations of *G. bohemica* were recorded from 2003 onwards, of which only 50 were still extant in 2017. The highest number of flowering plants (29,200) was recorded in 2009 and the smallest in 2016 (4,084). Inter-annual population sizes fluctuated strongly and were partly synchronized with differences in the weather in preceding years, notably average temperature and precipitation in the growing season (May to August), i.e. wet and warm conditions in preceding years had a positive influence on population size. For analysing microhabitat preferences (using Ellenberg indicator values) and species of plants associated with *G. bohemica*, 122 plots (0.25 m^2) were established at 22 sites with *G. bohemica*, of which 45 were placed around a plant of this species, while 77 were randomly located where this species was absent. *Gentianella bohemica* preferred microhabitats characterized by nutrient poor vegetation and this preference was also indicated by several species that were highly over-represented in plots with *G. bohemica*. A high proportion of open soil in the plots also favoured the occurrence of *G. bohemica*, possibly because it facilitated seedling establishment. Data on land management from 2003 onwards revealed that dedicated conservation measures somewhat positively, but not significantly so, are associated with changes in the sizes of the populations of this species. The results of this study should improve conservation management and ensure the long-term survival of this highly threatened grassland specialist.

Keywords: community indices, conservation status, Ellenberg indicator values, grasslands, habitat preferences, Natura 2000, niche, population dynamics

Introduction

Many plant species are declining in Europe in response to anthropogenic pressures (e.g. Eichenberg et al. 2021) and about a quarter of the vascular species of plants in the European Red List are threatened (Bilz et al. 2011). As a corollary, many range-restricted (i.e. endemic) species are also declining. While most endemic plants in Europe are confined to natural habitats in mountain ranges, such as rocks, screes or alpine grasslands (Essl et al. 2009), a subset of them predominantly occur in semi-natural habitats such as extensively used grasslands (Hobohm & Bruchmann 2009). However, species-rich grasslands are

Received: 14 Sep 2021, Revised: 28 Jan 2022, Accepted: 17 Feb 2022, Published online: 13 Jun 2022

highly threatened by changes in land-use such as intensification, abandonment and afforestation (Janssen et al. 2016). Consequently, biodiversity of grasslands in Europe is declining (e.g. Habel et al. 2013) and the conservation status of many of the endemic species associated with semi-natural grassland ecosystems is poor (Hobohm & Bruchmann 2009).

In recent decades, there have been substantial efforts to reverse the decline of threatened species in grasslands. For instance, the agricultural policies of the European Union and adjacent countries provide subsidies for land owners in exchange for extensively using grasslands (Knop et al. 2006), the European Natura 2000-network strongly accounts for species and habitats of extensively used grasslands (European Union 1992), and complementary species-specific conservation plans targeted on species of particular concern have been implemented (European Commision 2008). While such targeted measures have been successful in improving the conservation status in some cases, other species of high conservation concern continue to decline (e.g. Bühler & Roth 2011, Eichenberg et al. 2021).

Here, we focus on a charismatic and highly threatened endemic flagship species of extensively used grasslands, *Gentianella bohemica* (Lennartsson 2000, Königer et al. 2012). This species is restricted to the Bohemian Massif in the region bordering on the Czech Republic, Germany and Austria. Land-use change caused a severe decline in recent decades, leaving remaining extant populations highly isolated (Brabec 2005, Engleder 2006, Königer et al. 2012). Population sizes of this short-lived species are known to fluctuate greatly between years (Bucharová et al. 2012, Königer et al. 2012, Plenk et al. 2016), which might further increase the risk of extinction. In addition, incipient climate change has potentially emerged as another novel threat for *G. bohemica*, as it is argued that extreme droughts or heavy precipitation events during germination may result in low germination success and poor survival (Bucharová et al. 2012, Plenk et al. 2016).

One key requirement for successful conservation of threatened species is to gain an in-depth understanding of their specific ecological preferences. This is a crucial prerequisite for developing conservation actions tailored to species preferences. In this context, analysing microhabitat preferences and evaluating the effects of different conservation measures on population size allow one to assess and improve conservation measures for the target species.

Given its poor conservation status, *G. bohemica* is included in Annex II of the Habitats Directive of the European Union (European Union 1992). Further, species conservation plans were implemented in each of the three countries with extant populations (e.g. Engleder 2006, Brabec 2010). These plans consist of population monitoring, optimizing land use, collecting information on species biology and ecology (Brabec 2010) and seed collection for establishing ex-situ populations (Zillig et al. 2010, Zehm et al. 2017). Despite these dedicated conservation efforts, the long-term survival of *G. bohemica* is not secure (Königer et al. 2012). Similarly, there is insufficient knowledge on its microhabitat preferences and the efficiency of different conservation measures (Zehm et al. 2017).

Thus, here we address the following research questions: (i) Have the populations of *G. bohemica* at the different sites changed recently? (ii) Which environmental (e.g. climate) and anthropogenic (e.g. land use, conservation measures) factors are associated with the differences in population trends at different sites? (iii) What are the microhabitat preferences of *G. bohemica*? (iv) Which species regularly co-occur with *G. bohemica*? Finally, we provide recommendations for improving the conservation prospects of this species.

Materials and methods

Study species

The Bohemian gentian (*Gentianella bohemica* Skalický) [= *G. praecox* A. et J. Kerner subsp. *bohemica* (Skalický) Holub] belongs to the *Gentianaceae*. Species delineation in the genus *Gentianella* is difficult due to poor genetic differentiation among taxa caused by introgression and hybridization (Jang et al. 2005) and is further complicated by high morphological variability, seasonal dimorphism and habitat-dependent polymorphism (Jang et al. 2005). In the most recent taxonomic assessment of the genus based on genetic and morphological data, Greimler et al. (2004) and Jang et al. (2005) recognize 21 species in Europe, of which five are considered to be currently insufficiently taxonomically supported and are therefore assigned to three species groups: *G. amarella* agg., *G. campestris* agg. and *G. germanica* agg. Due to the paucity of differentiating morphological and genetic features, the exact assignment of *G. bohemica* to one of these aforementioned species groups is uncertain (Greimler et al. 2004, Jang et al. 2005). The Bohemian gentian occurs in two genetically distinct ecotypes, an early flowering (= aestivale) one that flowers in June, and a late flowering (= autumnal) one that flowers in August and September (Skalický 1969, Plenk et al. 2016).

In spring (late April to early May), seeds of *G. bohemica* germinate mostly in small gaps in the vegetation (Dolek et al. 2010, Brabec 2012). It is a small (5–50 cm), biannual hemicryptophyte, which forms a rosette in the first year and the inflorescence develops in the second year (Dolek et al. 2010). This species has no mechanisms for long-distance seed dispersal and its seed is dispersed only over short distances by wind (Plenk et al. 2016). Seed either can stay dormant for several years, with the probability of germination after four years ~20% (Bucharová et al. 2012). Thus, *G. bohemica* forms a persistent seed bank (Bucharová et al. 2012). *Gentianella bohemica* is mostly associated with nutrient-poor mesic acidophilic grasslands dominated by *Nardus stricta* and less frequently occurs in moderately nutrient-rich or temporarily wet grasslands, or in nutrient-poor neutral grasslands (Engleder 2006, Dolek et al. 2010, Brabec 2012, Königer et al. 2012, Křenová et al. 2019).

Study region

The range of this species, and thus the region studied, is the Bohemian Massif of the southern Czech Republic, north-eastern Bavaria and northern Austria (Fig. 1). In addition, there are three populations of this species in the Sudeten Mountains in southern Poland (Smoczyk 2014); however, as data on the population sizes of these populations are not available, they were not included in this study. The Bohemian Massif is an ancient and heavily eroded mountain range formed during the Variscian mountain building event in the Paleozoic. Acidic bedrocks such as granites, diorites and gneisses are characteristic, resulting in nutrient poor and acidic soils. The climate on the Bohemian Massif is suboceanic temperate and is mostly shaped by altitudinal gradients from the lowest valleys (300 m a.s.l) to the highest mountain tops (Grosser Arber, 1,455 m a.s.l). Further, from west, the climate becomes more continental with lower precipitation, colder winters and warmer summers. Mean annual temperatures range from 6.5 °C in valleys to 3.5 °C at high altitudes (Fink 1993).

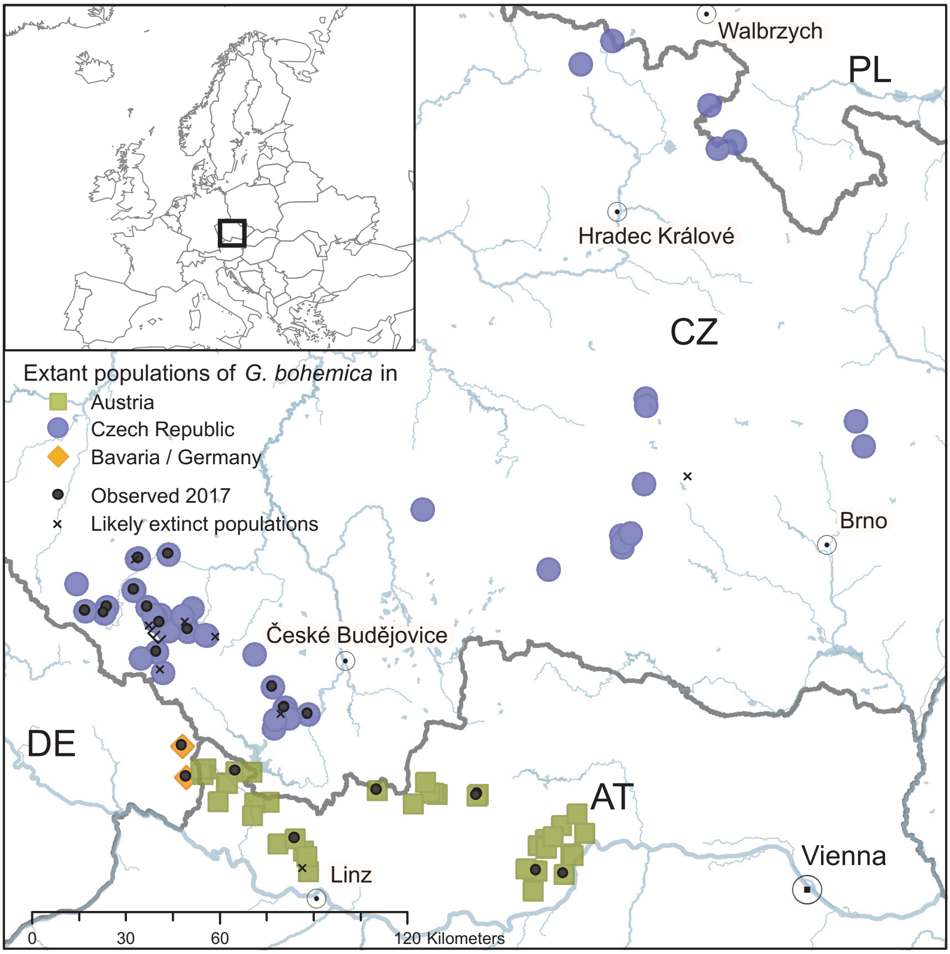


Fig. 1. The distribution of all known extant populations of *Gentianella bohemica* on the Bohemian Massif. Populations where plots for studying microhabitat preferences were established are denoted by a black dot.

Study design

A total of 114 populations of *G. bohemica* were recorded from 2003 onwards (with data for four sites extending back to 1993), of which 50 populations were still extant in 2017 (Table S1). Of these, 22 sites were selected for assessing the microhabitat preferences of the Bohemian gentian, of which 14 were located in the Czech Republic, six in Austria and two in Bavaria (Fig. 1). Field data were collected from July to September 2017, thus only the late-flowering ecotype, which is the more widespread, was sampled. At most sites, six plots giving a total of 122 plots of 0.25 m^2 were established (Table S2): 45 were placed around a plant of *G. bohemica* and 77 randomly located where this species was absent; at some sites, the number of plots differed somewhat (e.g. because fewer flowering individuals were found, or sites were partially mown). At each plot, the following data were collected:

occurrence and abundance of vascular plants using the semi-quantitative Braun-Blanquet scale (Braun-Blanquet 1964), total cover of herbaceous vegetation (in %), total cover of bryophyte vegetation (in %), total area of open soil (in %), exposure and inclination, and land management (mowing, grazing, abandoned). The nomenclature and taxonomy of vascular plants follows Fischer et al. (2008). For analyses, we converted the semi-quantitative cover classes of Braun-Blanquet to %-values according to van der Maarel (1979). For all vascular species of plants, we retrieved Ellenberg indicator values, which characterize the ecological preferences of species for light (L), water availability (W), temperature (T), nitrogen/nutrient supply (N) and soil reaction (R), from https://statedv.boku.ac.at/zeigerwerte (see Ellenberg et al. 2001).

Further, we collected data on historic land management for all sites for which this was documented (n = 76). This data was compiled by the conservation managers of *G. bohemica* in the three countries within its range. Specifically, we received management data and data on annual population sizes from T. Engleder for Austria, T. Zipp for Bavaria and J. Brabec for the Czech Republic. This allowed the recording for each year from 2004 onwards of the land management at each site (i.e., mowing, grazing, abandoned and implementation of targeted conservation measures such as removing moss and creating small gaps in the vegetation). Similarly, population size of *G. bohemica* (number of flowering individuals) was counted annually at these sites over the same period (Table S3). In addition, the size of each site (in m^2) was calculated in QGis.

For analysing climatic dependencies, we obtained data on seasonal weather for all sites of *G. bohemica* from 2002 to 2013 (time series data from CHELSA, Karger et al. 2017). Specifically, we obtained monthly precipitation and mean temperature values for every site and year.

Analyses

All statistical analyses were performed in R 3.5.2 (R Core Team 2018).

Population trajectories

For analysing the trajectories of the sizes of the populations of *G. bohemica*, we restricted the dataset to the period 2004 to 2015 in order to obtain the most complete time series for all the sites (n = 61). Since the response variable (individual numbers per year) are count data, a Poisson family generalized linear mixed model (function glmmPQL (penalized quasi-likelihood) from the R package MASS, Venables & Ripley 2002) was used. The models included sites as a random effect to account for non-independent multiple measurements over time; in addition, we used a correlation structure parameter (corCAR1) to account for temporal autocorrelation within the groups. As the original Poisson model indicated overdispersion, i.e. the residual deviance was larger than the degree of freedom (tested using the dispersiontest function in the AER library (Kleiber & Zeileis 2008) and the dispersion glmer function in the blmeco library (Korner-Nievergelt et al. 2015) we used a Pseudopoisson family assumption for the models. To explore whether spatial autocorrelation was an issue, we calculated spline (cross-)correlograms of the raw data as well as of the regression residuals using the spline.correlog function in the ncf R package (Bjornstad 2020). Since neither raw data nor residuals showed signs of autocorrelation we did not include additional random effects to further structure the data. Marginal pseudo-R-squares used to explore the variance explained by the fixed effects was calculated using the r.squaredGLMM function in the MuMIn R library (Bartoń 2020).

As population size is likely to depend on the spatial extent of the site, the size of a site (log area) was taken as a fixed effect predictor. To test for the temporal development of populations, the year of observation was used in the model as a second fixed effect predictor variable. Since *G. bohemica* is a biannual hemicryptophyte, its population size depends, inter alia, on seed abundance. The counts in our data are for flowering plants, thus we used the population size of two years prior to the counts (which was considered, apart from seed bank germination, as the most relevant parental generation) as a third fixed effect predictor. These three variables (area, year of observation and population size two years previously) were included in the basic model.

Next, we tested for the dependence of population sizes on climate. Since it was a priori unclear for which part of the life cycle of *G. bohemica* weather conditions are most relevant, we screened the climate data (taken from CHELSA, Karger et al. 2017) for significance. We first calculated a time series of summer (May to August) and winter (December to February) precipitation sums and average temperatures for every site and every year. To cover the full life cycle, we updated the basic model (see above) by adding the climatic predictors for the year of observation, then the previous year (rosette stage), then two years previously (seedling stage and flowering of parental plants) and finally three years previously (rosette stage of parents). We did this for summer and winter climate variables separately. Moreover, since models with both, temperature and precipitation as two additional additive predictors did not converge, we calculated separate models with either precipitation or temperature as additional predictor to the area of the site, the year of observation and the population size two years previously.

Microhabitat preferences

For analysing microhabitat preferences of *G. bohemica*, we used data recorded for the plots. We tested the dependency of the probability of occurrence of *G. bohemica* in plots in terms of moss cover, cover of bare soil and nutrient levels. For nutrients, we obtained a community-weighted index of Ellenberg indicator value for each plot by calculating an abundance-weighted mean Ellenberg N-value based on abundance information of all species recorded in a plot (Käfer & Witte 2004). Since plots (n = 122) were nested in sites (n = 22), we again calculated GLMMs (function glmer of the R package lme4, Bates et al. 2015) with site as random effect predictor. Presence and absence of *G. bohemica* was taken as a binary (binomial) response and covers of moss, bare soil and the community weighted Ellenberg N-value as fixed effect predictors.

Co-occurrence patterns

To identify the association of *G. bohemica* with other plants, we identified species that are significantly over- or under-represented in plots with *G. bohemica* by performing a Dufrene-Legendre Indicator Species Analysis using the R-package labdv (Roberts 2016).

Success of targeted conservation measures

To analyse the success of specific conservation measures, we used information from all sites for which there was information for the period 2004 to 2017. First, we calculated the average population size in the first three years (as initial population size) and the average population size in the last three years (as current population size). In addition, we restricted the data to sites with an initial population of at least 10 individuals (46 sites). Next, we calculated the number of years in which particular conservation measures (i.e. shrub removal, moss layer removal, mechanical scarification, harrowing, seed dispersal) for *G. bohemica* were undertaken. Finally, we classified populations as either increasing or decreasing, and in which conservation measures were taken for more or less than 2/3 of the period of study. The success of specific conservation measures was tested by means of a proportional test (prop.test function from the R stats library). Specifically, we tested whether the proportion of populations that were stable or increased was greater at sites that were managed compared to those not managed.

Results

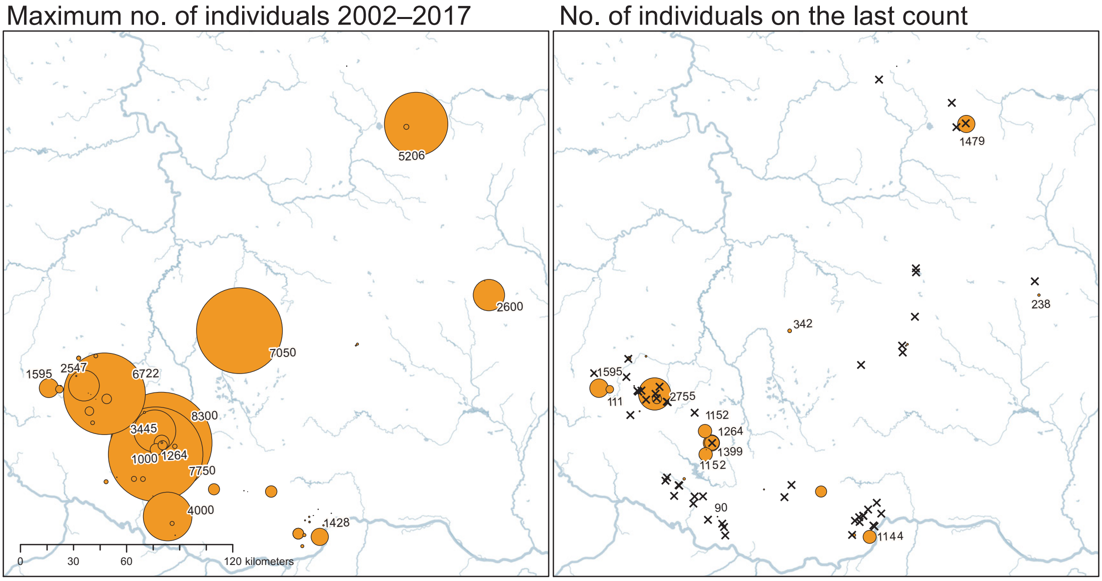
Population sizes and trends

The number of flowering individuals of *G. bohemica* per population differs greatly among sites and years. Inter-annual fluctuations in numbers of flowering individuals were very pronounced, with changes in mean population sizes by up to a factor of five between two subsequent years (Fig. 2). The highest total number of 29,200 flowering plants was documented in 2009, of which 22,427 plants were recorded in the Czech Republic, 6,297 in Austria and 476 in Bavaria (Fig. 3A). Smallest numbers of flowering plants were recorded in 2016 in all three countries with a total of only 4,084 specimens. In 2017, the total numbers of flowering plants increased again to 16,064 individuals. Interestingly, temporal fluctuations were partly synchronized between the countries, i.e. between the Austrian and Czech populations [rs (128) = 0.77, $P < 0.01$]. However, no significant correlation was found between the annual population sizes at the Bavarian and Czech and Bavarian and Austrian sites, respectively.

In addition, population sizes of *G. bohemica* declined during the period studied. Across all sites (n = 114), the number of flowering individuals per site was 141±641 (mean±SD) (2003–2005) and decreased to 81±286 (2015–2017). For the Czech Republic, the average number of *G. bohemica* individuals recorded per site was 142±744 in 2003–2005 and 72±286 individuals in 2015–2017. The number recorded in Austria was slightly lower, 140±400 individuals in 2003–2005, which decreased to 98±278 in 2015–2017. Substantially lower numbers of *G. bohemica* of only 52±112 individuals per site in 2006–2008 and 5±6 flowering individuals per site in the years 2015–2017 were recorded in Bavaria.

The influence of weather on the population size of Gentianella bohemica

Our analyses showed that *G. bohemica*-population size is significantly influenced by mean precipitation and temperature during the growing season (Table 1). Interestingly, climate is most closely related to population size of *G. bohemica* (lowest P-value) with


Maximum no. of individuals 2002–2017

No. of individuals on the last count

Fig. 2. Fluctuations in population sizes of *Gentianella bohemica*. (A) Maximum number of flowering individuals per year over the period 2002–2017, (B) population size in the last year for which data were available. Numbers in the map denote populations with more than 1,000 individuals at any time in the study period. Symbol size is proportional to population size. Most probably extinct populations are denoted by an “x”.

Table 1. Result of GLMMs of individual numbers of *Gentianella bohemica* populations. The following predictors were used: size of the site, precipitation and temperature in the vegetation period (May to August), the number of individuals in preceding years and year of observation. Time lag for precipitation was taken as three years, for temperature and parent individual numbers as two years preceding the observation of individual numbers at a site. The analysis comprises all available individual number counts across the whole time series from 2004 to 2015. Site identity was used as random factor to account for temporal replication of sites. The tables show the results of two separate models (A, B) since the model failed to converge when both precipitation and temperature were included in one model. Significance values (Sign.): * P > 0.01–0.05; ** P > 0.001–0.01; *** P < 0.001.

	Estimate ± SE	t value	Pr(>|z|)	Sig.
A				
Intercept	3.86±0.26	14.828	< 0.001	***
Site size (log)	0.85±0.27	3.182	0.002	**
Seasonal precipitation (three years before)	0.27±0.05	5.372	< 0.001	***
Parental generation individual numbers (two years before)	0.06±0.02	2.874	0.004	**
Year of observation	–0.15±0.05	–2.945	0.003	**
B				
Site size (log)	3.85±0.27	14.16	< 0,001	***
Log(area)	0.74±0.28	2.64	0.011	**
Seasonal temperature (two years before)	0.35±0.06	5.63	< 0.001	***
Parental generation individual numbers (two years before)	0.06±0,02	2.87	0.004	**
Year of observation	–0.11±0.05	–2.04	0.042	*

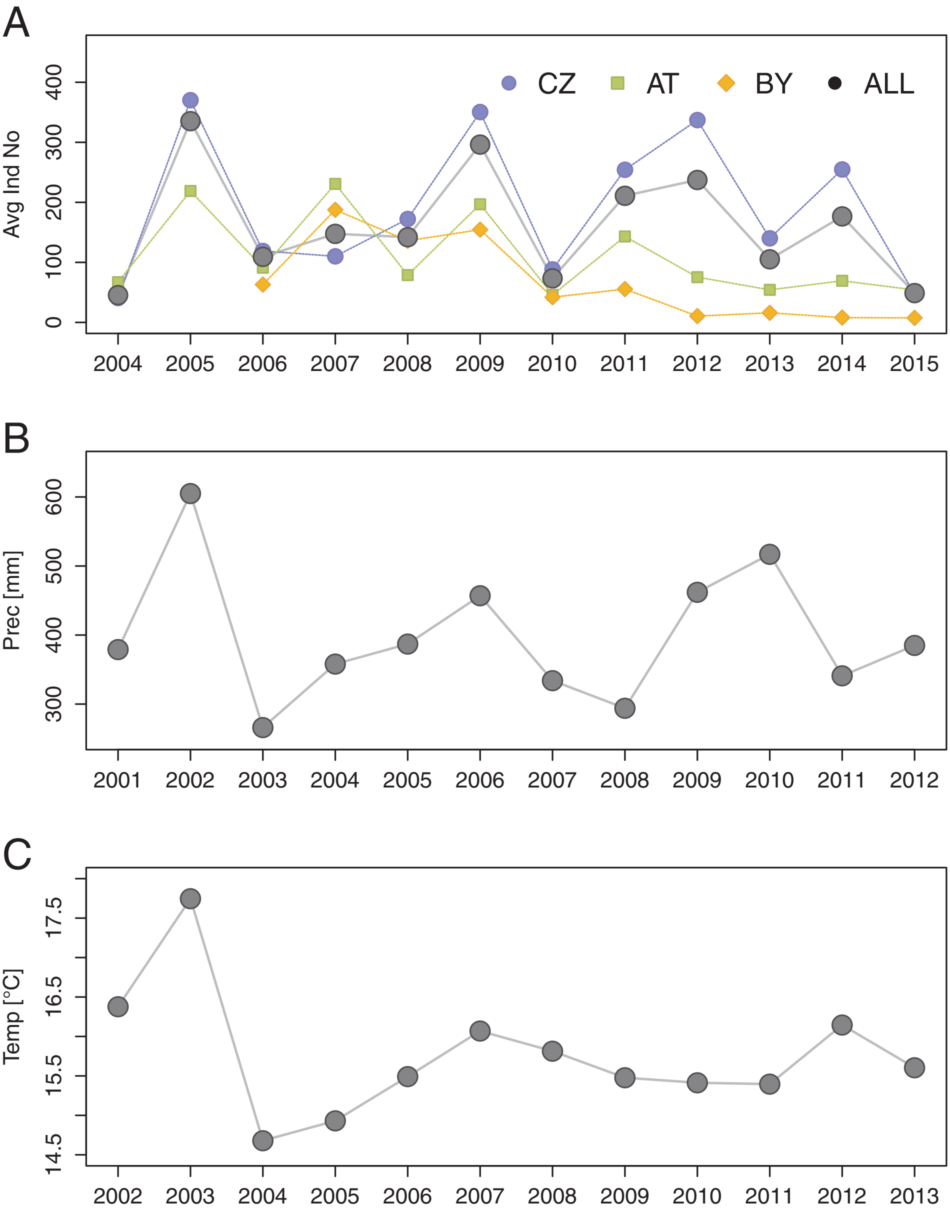


Fig. 3. Average numbers of flowering individuals of *Gentianella bohemica* per site and seasonal weather conditions (in preceding years) for 114 sites on the Bohemian Massif. Shown are (A) average numbers of flowering individuals, (B) average precipitation (mm) in the growing season (May to August) three years before, and (C) average mean annual temperature (°C) in the growing season (May to August) two years before. Note that the x-axis displays the explanatory variables with a time lag.

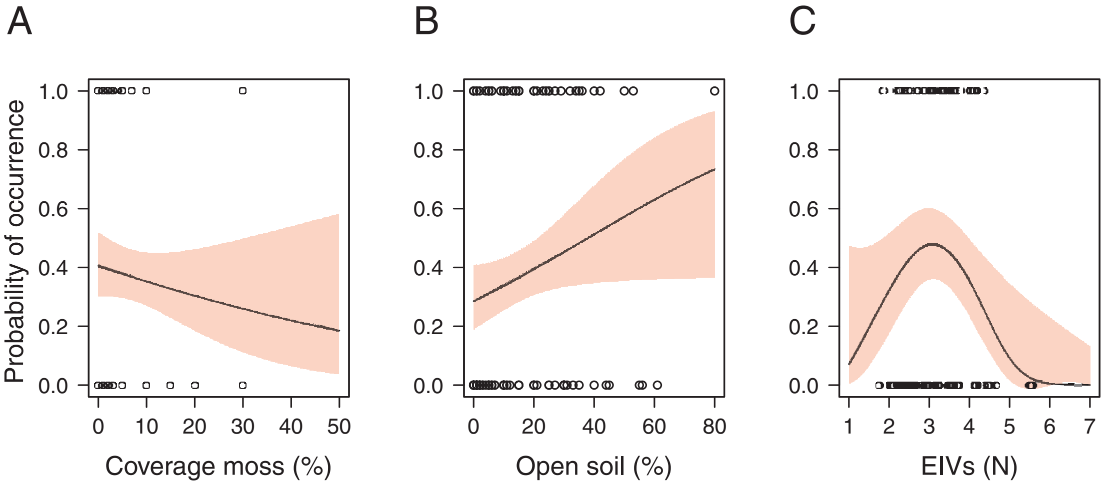


Fig. 4. Probability of occurrence of *Gentianella bohemica* depending on (A) the cover of the moss layer, (B) open soil and (C) weighted Ellenberg community index for nitrogen in 122 plots (0.25 m^2) located at 22 sites. The shaded area shows the 95% confidence interval; presence and absence of *G. bohemica* is plotted as dots of 1 and 0.

a lag time of three (precipitation) and two (temperature) years respectively between their effect (seasonal weather) and the response (population size). In addition, population size is positively correlated with the size of the sites and the population size two years previously. Regarding the effect of size, site area has the strongest effect, followed by precipitation, temperature and parental population size. Overall, the negative estimate for the year of observation indicates a decrease in the sizes of the *G. bohemica* populations.

Microhabitat preferences

We found that *G. bohemica* had distinct preferences for specific microhabitats at the 122 plots established at 22 sites. First, the probability of occurrence increased significantly with the proportion of open soil (Fig. 4, Table 2) and showed a unimodal response to nitrogen values, with the highest occurrence probabilities at low, but not very low, weighted community index values for nitrogen. Occurrence of *G. bohemica* was limited to plots with a mean nitrogen value ranging from 1.5 to 5.0. High proportions of open soil significantly favoured the occurrence of *G. bohemica*. While low moss cover favoured the occurrence of *G. bohemica*, this relationship was not significant.

Co-occurrence analysis of species

The co-occurrence analysis shows that nine vascular plant species are closely affiliated with the presence of *G. bohemica* in the 122 plots (Table 3); the majority of these are characteristic species of nutrient poor grasslands. Five species (*Pimpinella saxifraga*, *Lotus corniculatus*, *Festuca ovina* agg., *Leontodon hispidus*, *Nardus stricta*) occurred in > 50% of the plots where *G. bohemica* was present (Fig. 5). On the other hand, two species that are typical of nutrient rich grasslands are significantly under-represented in plots with *G. bohemica* (*Dactylis glomerata*, *Taraxacum officinalis*).

Table 2. Probability of occurrence of *Gentianella bohemica* depending on (A) the cover of the moss layer, (B) open soil, and (C) weighted Ellenberg community index for nitrogen (EIVn) in 122 plots (0.25 m^2) at 22 sites. The table shows the results of binary mixed effects models (GLMMs) with binomial distributed responses (presence/absence of *G. bohemica* on 0.25 m^2 plots). Site identity was used as random effect term to account for multiple plots within sites. Significance values (Sign.): . P > 0.05–0.10; * P > 0.01–0.05; ** P > 0.001–0.005; *** P < 0.001.

	Estimate ± SE	z value	Pr(>|z|)	Sign.
A				
Intercept	–0.38±0.23	–1.64	0.101	
Moss cover (%)	–0.02±0.02	–1.06	0.291	
B				
Intercept	–0.92±0.27	–3.37	0.001	***
Open soil (%)	0.02±0.01	1.99	0.046	*
C				
Intercept	–0.68±0.22	–3.11	0.002	**
EIVn	–6.66±3.42	–1.95	0.051	.
EIVn (quadratic)	–8.97±4.01	–2.24	0.025	*

Table 3. Dufrene-Legendre Indicator Species Analysis of species association with *Gentianella bohemica*. Shown are species that are associated in 122 plots (0.25 m^2) with the occurrence of *G. bohemica*, and species that are significantly under-presented in plots with *G. bohemica*.

	Indicator value	P-value
Co-occurring species:		
Alchemilla vulgaris agg.	0.1794	<0.05
Festuca ovina agg.	0.3873	<0.05
Hieracium pilosella	0.1815	<0.05
Leontodon hispidus	0.3829	<0.01
Linum catharticum	0.2026	<0.05
Lotus corniculatus	0.4273	<0.01
Nardus stricta	0.3714	<0.001
Pimpinella saxifraga	0.4712	<0.01
Trisetum flavescens	0.1699	<0.05
Under-represented species:		
Dactylis glomerata	0.3274	<0.05
Taraxacum officinalis agg.	0.1667	<0.001

The role of land management and land-management history

For almost a third of the *G. bohemica* sites, there is no data on land management for the first years of the millenium, but information on land management increased subsequently (Fig. 6). In the year 2000, about 30% of the sites were mown. In all three countries, this type of land management was the most common form of land use. From the years 2003 to 2017, mowing increased to 41%. Abandoned (fallow) sites had a share of 18% in 2003, which declined to 11% in 2017. Grazing was used at 16% of the sites in 2003, and this value remained constant until 2017. In the early 2000s, only about 7% of the *G. bohemica* sites were managed, but since 2006 increased substantially to almost 30% of the sites in 2017. This type of land management includes a variety of conservation measures that aim

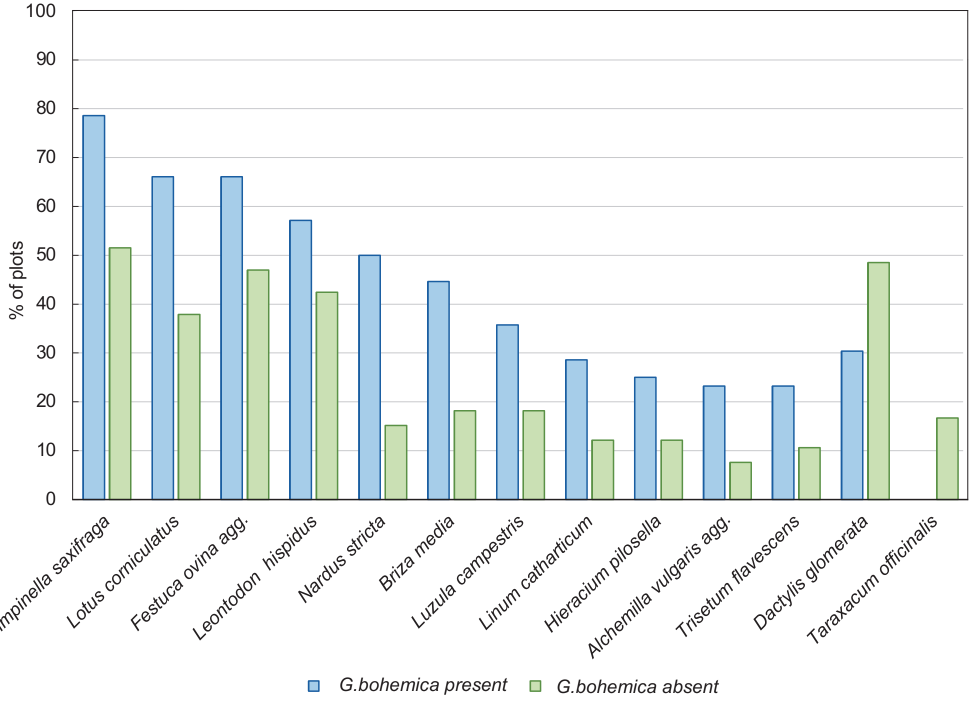


Fig. 5. The most frequently occurring species in plots (n = 122) shown as percentage of plots with (dark grey) and without (light grey) *Gentianella bohemica*.

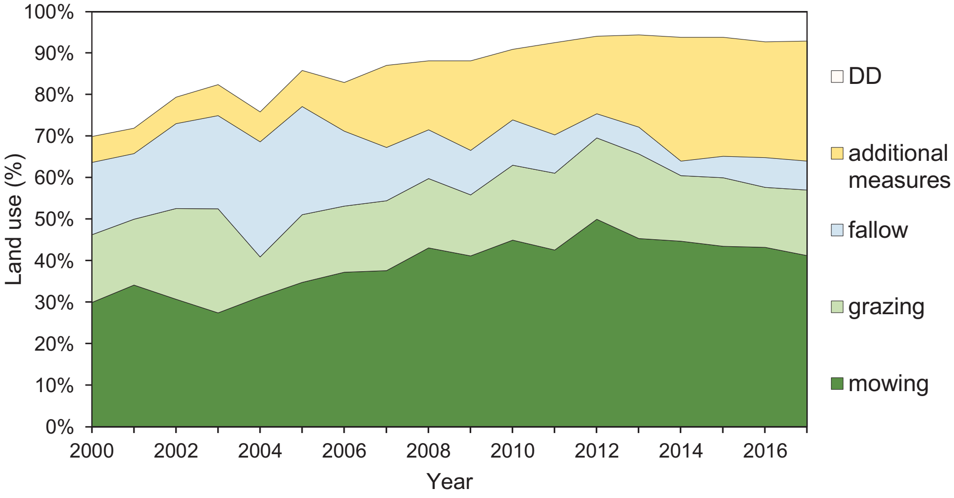
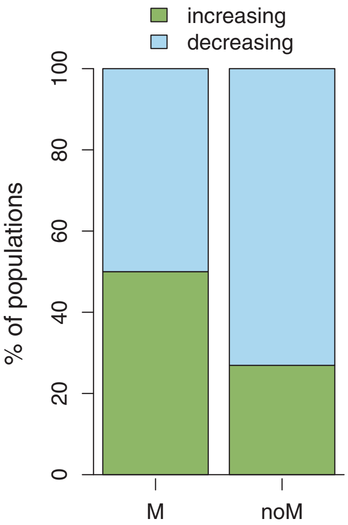


Fig. 6. Percentage of three different land management types (fallow, mowing, grazing) and conservation measures at 81 sites for the years 2000 to 2017. DD = Data deficient.

Fig. 7. Percentage of increasing and decreasing populations of *Gentianella bohemica* at sites with (M, n = 20) and without (noM, n = 26) targeted conservation measures. The dataset comprises populations with a maximum of more than 10 individuals in 2004 to 2015. Despite the difference in the percentages (50% for managed vs. 27% for not-managed populations), a proportional test did not show a significant difference (P = 0.09).



to improve the conservation status of *G. bohemica*. Sites with increasing populations of *G. bohemica* were subjected to a higher proportion of targeted conservation measures than sites with declining populations (Fig. 7). However, the proportional test revealed no significant difference.

Discussion

Population size and conservation status

Our results show that despite substantial conservation efforts, there has been an ongoing decline in *G. bohemica* from 2003 to 2017. Similar declines are also evident in other European short-lived *Gentianella* species (e.g. Greimler & Dobeš 2000, Stadler et al. 2010). Further, we found that populations of *G. bohemica* exhibit pronounced inter-annual fluctuations in size previously reported by Dolek et al. 2010, Bucharová et al. 2012, Königer et al. 2012, Plenk et al. 2016, Engleder 2017 and Křenová et al. 2019). Here, we revealed that these fluctuations are largely synchronized among populations and countries due to the important role of the prevailing seasonal climatic conditions (temperature, precipitation) in the vegetation period, with interesting time lags of three (precipitation) and two (temperature) years in their effects on population sizes (Fig. 3B, C).

In short-lived plants, strong inter-annual fluctuations in population size are common (Runge 1963, Dierschke 1986), as generative regeneration depends on favourable conditions for germination and establishment of seedlings, which is often the most sensitive life-cycle phase (Dolek et al. 2003). Křenová et al. (2019) recently argued that the yearly fluctuations in size of *G. bohemica* populations can be attributed to changes in land management and seasonal weather conditions. We confirmed the latter hypothesis, as seasonal climatic conditions in preceding years had a strong effect on population sizes (Fig. 3, Table 1). Similar relationships between seasonal weather conditions and population development of congeneric species are reported by Dierschke (1986). For *G. bohemica*, seed germination seems to be the life-cycle phase that is the most susceptible to seasonal climatic fluctuations (Brabec 2012). Bucharová et al. (2012) show that drought in summer ultimately leads to an increase in the sizes of the populations of this species. The underlying mechanism seems to be the creation of more open gaps in the vegetation, which promote the germination of *G. bohemica* in subsequent years (Křenová et al. 2019). Increase in seedling establishment due to reduced competition caused by drought in preceding years has been repeatedly reported for short-lived species of plants (e.g. Gross et al. 1998, Smith et al. 2005, Toräng et al. 2010). Thus, there seems to be a trade-off between increase in germination and establishment success (caused by wet springs) and increase in creation of suitable sites for germination in the following year (caused by drought periods in summer). Overall, our analyses show that wet and warm conditions during the vegetation period in preceding years favour *G. bohemica*. This finding has important implications for the conservation of this species as it reveals that there are substantial carry-over effects of seasonal climatic conditions in preceding years, which may mask the effects of management.

Microhabitat preferences

We found clear evidence of the specific microhabitat preferences of G. *bohemica*. There was a positive correlation between *G. bohemica* presence in plots and the proportion of open soil, as well as avoidance of dense layers of moss. As a light-demanding, small species, *G. bohemica* is dependent on disturbance, particularly for seedling establishment (Rösler 2001, Engleder 2006, Dolek et al. 2010, Plenk et al. 2016). Open gaps around the mother plant facilitate germination of *G. bohemica* (Dolek et al. 2003). Similar microhabitat preferences are reported in several studies on closely related species, e.g. for *G. germanica* (Verkaar & Schenkeveld 1984, Fischer & Matthies 1998), *Gentiana pneumonanthe* and *Gentiana punctata* (Kobiv 2018).

Similarly, a dense moss layer may prevent the development of seedlings of *G. bohemica* (Bucharová et al. 2012). There is evidence of the negative effects of dense layers of moss on seedlings of vascular plants (e.g. Keizer et al. 1985, van Tooren 1988, Ingerpuu et al. 2005). The germination success, especially of plants with small seeds, such as *G. bohemica*, is often greatly reduced (van Tooren 1988). Further, germination of seeds is often stimulated by light of a specific wavelength (photosynthetically active radiation, PAR), which is reduced under a dense layer of moss (e.g. Keizer et al. 1985, Fenner & Thompson 2005, Jeschke & Kiehl 2008).

The analysis of microhabitat preferences based on Ellenberg indicator values (Fig. 6) revealed that *G. bohemica* prefers nutrient-poor microhabitats, but avoids the most nutrient-poor sites. As a poor competitor dependent on gaps for seedling recruitment, it is rapidly outcompeted in more nutrient-rich conditions. This finding supports empirical knowledge of experts on this species (Brabec 2012, Plenk et al. 2016). The avoidance of fertilized sites has also been shown for congeners like *G. campestris*, which mainly grows in nutrient-poor calcareous grasslands (Oostermeijer et al. 2002).

Regarding other environmental factors such as availability of water, light and soil reaction, Ellenberg indicator values revealed no distinct preferences for *G. bohemica* (results not shown). One likely reason for this is that only microhabitats within sites of *G. bohemica* were sampled, thus the above-mentioned environmental parameters allow in principle for the occurrence of this species, which may mask microhabitat preferences, because unsuitable sites were not sampled. For instance, all sites are characterized by Ellenberg indicator values that indicate high light requirement, which is in line with previous studies (Rösler 2001, Engleder 2006, Dolek et al. 2010). Further, Ellenberg indicator values indicate a moderate preference for moderately acidic sites, while highly acidic and neutral sites are less suitable; this finding supports the results of Dolek et al. (2003). The analysis of species co-occurrence showed that species that are over-represented in plots with *G. bohemica* are characteristic species of nutrient-poor grasslands, while species that are over-represented in plots where the study species is absent, are species with high nutrient demands (Table 2, Fig. 7).

The role of current and historic land use

On the Bohemian Massif, wide-spread land-use intensification after World War II started somewhat later than in agriculturally more favourable regions in central Europe (Bičík & Kupková 2007). Former small-holder farms were collectivized in the Czech Republic in the early 1950s during the communist era, while in Germany and Austria there were still mainly private family-owned farms. Land-use on large collectivized farms in the Czech Republic was less intensive than on private farms in Austria and Bavaria (Bičík & Kupková 2007) and this may be one factor for the considerably higher number of extant populations o*f G. bohemica* in the former country.

The data on the land management of *G. bohemica* sites from 2003 and 2017 showed that it changed over time: the proportion of abandoned grasslands has declined, while mowing and the implementation of targeted conservation measures has increased (Brabec 2012, Křenová et al. 2019). Conservation measures focus on removing litter, creating open gaps and adjusting the timing of mowing or grazing to the development of *G. bohemica* (Brabec 2012, Zehm et al. 2017). Such management measures may increase seedling mortality, but this is compensated for by reduced competition and improved development of surviving plants of *G. bohemica* (Brabec 2012, Bucharová et al. 2012). Křenová et al. (2019) report the effects of long-term conservation efforts and indicate that intensive conservation measures positively affect populations under current climatic condition (Křenová et al. 2019). Our study confirmed the importance of dedicated management measures for population survival (Fig. 7).

Conclusions

Despite several decades of dedicated conservation measures aimed at improving the conservation status of *G. bohemica*, we found that they are likely to have reduced population decline, but are insufficient to ensure long-term survival. In addition, we show that *G. bohemica* is highly responsive to seasonal climatic fluctuations in preceding years and that seedling emergence and establishment seems to be the most critical phase in their life cycle. This conclusion is corroborated by the microhabitat preferences of this species for small gaps in the vegetation. As extant populations are highly isolated, recolonization currently is only possible with human assistance once the seed-bank of the species is exhausted at a site. Microhabitat preferences of this species have shown that the presence of open soil and absence of a dense moss layer are essential for the establishment of its seedlings. Small populations are often prone to genetic erosion, which may result in reduced genetic diversity and fitness (Ellstrand & Elram 1993, Fischer & Matthies 1998, Jacquemyn et al. 2009) and it has already been shown that the genetic diversity of many of the extant *G. bohemica* populations is low (Königer et al. 2012).

Thus, it is obvious that the long-term survival of *G. bohemica* critically depends on dedicated and ambitious conservation measures. As current conservation measures were not able to stop the decline of this species, additional conservation efforts should be established, including sharing of best practice information among conservation managers, rigorous monitoring of the success of conservation measures and the development and use of novel methods. Given that our results have shown that the seedling stage is the most critical, conservation measures should be targeted at improving germination and establishment. Protecting this species also protects many associated biotas of nutrient-poor acidic grasslands, a habitat that has declined dramatically in the last few decades.

Supplementary materials

Table S1. – Geographic coordinates and IDs of the sites of *G. bohemica* included in this study.
Table S2. – Data sampled in the 122 co-occurrence plots.
Table S3. – Data on population sizes at the sites of *G. bohemica* studied.

Supplementary materials are available at www.preslia.cz

Acknowledgements

We are deeply indebted to the conservation managers of *G. bohemica* in Austria (T. Engleder), Bavaria (T. Zipp) and the Czech Republic (J. Brabec), who provided data on annual population sizes and management measures for their respective countries. We further thank M. Strauch, A. Zehm, J. Königer and M. Dolek for valuable information. FE appreciates being funded by the Austrian Science Foundation FWF (grant I 3757-B29). We appreciate the constructive feedback of two anonymous reviewers. We thank T. Dixon for correcting the English.

References

Bartoń K. (2020) MuMIn: Multi-Model Inference. R package version 1.43.17. – URL: https://CRAN.R-project.org/package=MuMIn.

Bates D., Maechler M., Bolker B. & Walker S. (2015) Fitting linear mixed-effects models using lme4. – Journal of Statistical Software 67: 1–48.

Bičík I. & Kupková L. (2007) Land use development in the Czech Republic and possibilities of generalization and modelling. – In: Dostál P. & Langhammer J. (eds), Modelling natural environment and society, p. 179–202, P3K, Praha.

Bilz M., Kell S. P., Maxted M. & Lansdown R. V. (2011) European Red List of vascular plants. – Publication Office of the European Union, Luxembourg.

Bjornstad O. N. (2020) ncf: Spatial covariance functions. R package version 1.2-9. – URL: https://CRAN.R-project.org/package=ncf.

Brabec J. (2005) Současný stav rozšíření hořečku mnohotvarého českého (*Gentianella praecox* subsp. *bohemica*) v ČR (Recent distribution of *Gentianella praecox* subsp. *bohemica* in the Czech Republic). – Zprávy České botanické společnosti 40: 1–44.

Brabec J. (2010) Záchranný program hořečku mnohotvarého českého (*Gentianella praecox* subsp. *bohemica*) v České republice [Programme to protect *Gentianella praecox* subsp. *Bohemica* in the Czech Republic]. – Ms., Depon in: AOPK ČR, Praha.

Brabec J. (2012) Optimising management at *Gentianella praecox* subsp. *bohemica* sites. – In: Jongepierová I., Pešout P., Jongepier J. W. & Prach K. (eds), Ecological restoration in the Czech Republic, p. 56–58, AOPK ČR, Praha.

Braun-Blanquet J. (1964) Pflanzensoziologie. Ed. 3. – Springer, Vienna.

Bucharová A., Brabec J. & Münzbergová Z. (2012) Effect of land use and climate change on the future fate of populations of an endemic species in central Europe. – Biological Conservation 145: 39–47.

Bühler C. & Roth T. (2011) Spread of common species results in local-scale floristic homogenization in grassland of Switzerland. – Diversity and Distributions 17: 1089–1098.

Dierschke H. (1986) Untersuchungen zur Populationsdynamik der *Gentianella*-Arten in einem Enzian-Zwenken-Kalkmagerrasen. – Natur und Heimat 46: 73–81.

Dolek M., Königer J. & Zipp T. (2010) Böhmischer Fransenenzian *Gentianella bohemica* Skal. – In: Bayerisches Landesamt für Umwelt, Augsburg (eds), Merkblatt Artenschutz, Vol. 6, URL: https://www.lfu.bayern.de/natur/artenhilfsprogramm_botanik/merkblaetter/doc/06lfumerkblatt_gentianella_bohemica.pdf.

Dolek M., Sukopp U., Geyer A., Dötterl M. & Freese A. (2003) Artenhilfsprogramm Böhmischer Enzian (*Gentianella bohemica* Skalický). – Unpublished report, Bayerisches Landesamt für Umweltschutz (LfU), Augsburg.

European Commision (2008) LIFE and Europe's grasslands. Restoring a forgotten habitat. – Publications Office, Luxembourg, URL: https://data.europa.eu/doi/10.2779/23028.

Eichenberg D., Bowler D. E., Bonn A., Bruelheide H., Grescho V., Harter D., Jandt U., May R., Winter M. & Jansen F. (2021) Widespread decline in Central European plant diversity across six decades. – Global Change Biology 27: 1097–1110.

Ellenberg H., Düll R., Weber H. E., Werner W. & Paulißen D. (2001) Zeigerwerte von Pflanzen in Mitteleuropa. – Scripta Geobotanica 18: 1–248.

Ellstrand N. C. & Elram D. R. (1993) Population genetic consequences of small population size: implications for plant conservation. – Annual Review of Ecology, Evolution and Systematics 24: 217–243.

Engleder T. (2006) Der Böhmische Kranzenzian / *Gentianella bohemica* (*Gentianaceae*) im österreichischen Teil der Böhmischen Masse (Böhmerwald, Mühl- und Waldviertel). – Neilreichia 4: 215–220.

Engleder T. (2017) Artenschutzprojekt Böhmischer Enzian (*Gentianella praecox bohemica*) & Holunderknabenkraut (*Dactyloriza sambucina*). – Unpublished report, Naturschutzabteilung des Landes Oberösterreich, Linz.

Essl F., Staudinger M., Stöhr O., Schratt-Ehrendorfer L., Rabitsch W. & Niklfeld H. (2009) Distribution patterns, range size and niche breadth of Austrian endemic plants. – Biological Conservation 142: 2547–2558.

European Union (1992) Council Directive 94/43/EEC on the conservation of natural habitats and of wild fauna and flora. –
URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31992L0043&from=EN.

Fenner M. & Thompson K. (2005) The ecology of seeds. – Cambridge University Press, Cambridge.

Fink M. H. (1993) Geographische Gliederung und Landschaften Österreichs. – In: Mucina L., Grabherr G. & Ellmauer T. (eds), Die Pflanzengesellschaften Österreichs. Teil I. Anthropogene Vegetation, p. 29–42, Gustav Fischer Verlag, Jena.

Fischer M. & Matthies D. (1998) RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (*Gentianaceae*). – American Journal of Botany 85: 811–819.

Fischer M., Oswald K. & Adler W. (2008) Exkursionsflora für Österreich, Liechtenstein, Südtirol. – Biologiezentrum der Oberösterreichischen Landesmuseen, Linz.

Greimler J. & Dobeš C. (2000) High genetic diversity and differentiation in relict lowland populations of *Gentianella austriaca* (A. and J. Kern.) Holub (*Gentianaceae*). – Plant Biology 2: 628–637.

Greimler J., Hermanowski B. & Jang C. G. (2004) A re-evaluation of morphological characters in European *Gentianella* section *Gentianella* (*Gentianaceae*). – Plant Systematics and Evolution 248: 143–169.

Gross K., Lockwood J. R., Frost C. C. & Morris M. F. (1998) Modelling controlled burning and trampling reduction for conservation of *Hudsonia montana*. – Conservation Biology 12: 1291–1301.

Habel J. C., Dengler J., Janišová M., Török P., Wellstein C. & Wiezik M. (2013) European grassland ecosystems: threatened hotspots of biodiversity. – Biodiversity and Conservation 22: 2131–2138.

Hobohm C. & Bruchmann I. (2009) Endemische Gefäßpflanzen und ihre Habitate in Europa: Plädoyer für den Schutz der Grasland-Ökosysteme. – Tüxenia 21: 142–161.

Ingerpuu L., Liira J. & Pärtel M. (2005) Vascular plants facilitated bryophytes in a grassland experiment. – Plant Ecology 180: 69–75.

Jacquemyn H., Brys R., Adriaens D., Honnay O. & Roldan-Ruiz I. (2009) Effects of population size and forest management on genetic diversity and structure of the tuberous orchid *Orchis mascula*. – Conservation Genetics 10: 161–168.

Jang C. G., Müllner A. N. & Greimler J. (2005) Conflicting patterns of genetic and morphological variation in European *Gentianella* section *Gentianella*. – Botanical Journal of the Linnean Society 148: 175–187.

Janssen J. A. M., Rodwell J. S., García Criado M., Gubbay S., Haynes T., Nieto A. et al. (2016) European Red List of habitats. Part 2. Terrestrial and freshwater habitats. – Publications Office of the European Union, Luxembourg.

Jeschke M. & Kiehl K. (2008) Effects of a dense moss layer on germination and establishment of vascular plants in newly created calcareous grasslands. – Flora 203: 557–566.

Käfer J. & Witte J. P. (2004) Cover-weighted averaging of indicator values in vegetation analyses. – Journal of Vegetation Science 15: 647–652.

Karger D. N., Conrad O., Böhner J., Kawohl T., Kreft H., Soria-Auza R. W., Zimmermann N., Linder H. P. & Kessler M. (2017) Climatologies at high resolution for the Earth land surface areas. – Scientific Data: 4: 170122.

Keizer P. J., van Tooren B. F. & During H. J. (1985) Effects of bryophytes on seedling emergence and establishment of short-lived forbs in chalk grassland. – Journal of Ecology 73: 493–504.

Kleiber C. & Zeileis A. (2008) Applied econometrics with R. – New York, Springer.

Knop E., Kleijn D., Herzog F. & Schmid B. (2006) Effectiveness of the Swiss agri-environment scheme in promoting biodiversity. – Journal of Applied Ecology 43: 120–127.

Kobiv Y. (2018) Trends in population size of rare plant species in the alpine habitats of the Ukrainian Carpathians under climate change. – Diversity 10: 62.

Königer J., Rebernig C. A., Brabec J., Kiehl K. & Greimler J. (2012) Spatial and temporal determinants of genetic structure in *Gentianella bohemica*. – Ecology and Evolution 2: 636–648.

Korner-Nievergelt F., Roth T., Von Felten S., Guélat J., Almasi B. & Korner-Nievergelt P. (2015) Bayesian data analysis in ecology using linear models with R, BUGS and Stan. – Elsevier, Amsterdam.

Křenová Z., Brabec J., Rössler S. & Kindlmann P. (2019) Can we learn from the ecology of the Bohemian gentian and save another closely related species of *Gentianella*? – PLoS ONE 14: e0226487.

Lennartsson T. (2000) Management and population viability of the pasture plant *Gentianella campestris*: the role of interactions between habitat factors. – Ecological Bulletins 48: 111–121.

Oostermeijer J. G. B., Luijten S. H., Ellis-Adam A. C. & Den Nijs J. C. M. (2002) Future prospects for the rare, late-flowering *Gentianella germanica* and *Gentianopsis ciliata* in Dutch nutrient-poor calcareous grasslands. – Biological Conservation 104: 339–350.

Plenk K., Göld F., Kriechbaum M. & Kropf M. (2016) Genetic and reproductive characterization of seasonal flowering morphs of *Gentianella bohemica* revealed strong reproductive isolation and possible single origin. – Plant Biology 18: 111–123.

R Core Team (2018) R: A language and environment for statistical computing. Version 3.1.1. – URL: http://www.R-project.org.

Roberts D. W. (2016) labdsv: Ordination and multivariate analysis for ecology. R package version 1.8-0. – URL: https://CRAN.R-project.org/package=labdsv.

Rösler S. (2001) Das Artenhilfsprojekt Böhmischer Enzian (*Gentianella bohemica* Skalický): Ergebnisse der Dauerbeobachtung 1991–1998. – Schriftenreihe Bayerisches Landesamt für Umweltschutz 156: 89–104.

Runge F. (1963) Die Artmächtigkeitsschwankungen in einem nordwestdeutschen Enzian-Zwenkenrasen. – Vegetatio 11: 237–240.

Skalický V. (1969) Die Sammelart *Gentianella germanica* (Willd.) E.F. Warburg s.l. im Böhmischen Massiv. – Preslia 41: 140–147.

Smith M., Caswell H. & Mettler-Cherry P. (2005) Stochastic flood and precipitation regimes and the population dynamics of a threatened floodplain plant. – Ecological Applications 15: 1036–1052.

Smoczyk M. (2014) *Gentianella bohemica* Skalický. Goryczuszka czeska (goryczka czeska). – In: Zarzycki K., Kaźmierczakowa R. & Mirek Z. (eds), Polska Czerwona księga roślin [Polish red data book of plants], p. 397–399, Institute of Nature Conservation, Krakow.

Stadler K., Koch M., Bernhardt K. G. & Greimler J. (2010) Spatial arrangement and genetic structure in *Gentianella aspera* in a regional, local, and temporal context. – Plant Systematics and Evolution 286: 7–19.

Toräng P., Ehrlen J. & Agren J. (2010) Linking environmental and demographic data to predict future population viability of a perennial herb. – Oecologia 163: 99–109.

van der Maarel E. (1979) Transformation of cover-abundance values in phytosociology and its effects on community similarity. – Vegetatio 39: 97–114.

van Tooren B. F. (1988) The fate of seeds after dispersal in chalk grassland: the role of the bryophyte layer. – Oikos 53: 41–48.

Venables W. N. & Ripley B. D. (2002) Modern applied statistics with S. Ed. 4. – Springer, New York.

Verkaar H. J. & Schenkeveld A. J. (1984) On the ecology of short-lived forbs in chalk grasslands: life-history characteristics. – New Phytologist 98: 659–672.

Zehm A., Lauermann M., Königer J., Neumann S., Diball O. & Kerskes A. (2017) Situation der Gefäßpflanzen der Fauna-Flora-Habitat-Anhänge in Bayern. – Anliegen Natur 39: 8–10.

Zillig P., Lebermann K., Morba D., Arneth G. & Lauerer M. (2010) *Gentianella bohemica*: erfolgreiche Ex-situ-Erhaltungskultur im Botanischen Garten. – Naturschutz und Landschaftsplanung 42: 78–84.

Současný stav a ekologie vysoce ohroženého endemického druhu *Gentianella bohemica*

Gentianella bohemica je vysoce ohrožený endemický druh, rostoucí v travních porostech na živinami chudých substrátech Českého masivu v Rakousku, Bavorsku a České republice. V článku analyzujeme, jak mikrostanovištní preference tohoto druhu spolu s vlivem historického a současného hospodaření a sezónní klimatické variability ovlivňují velikost jeho populací. Od roku 2003 bylo evidováno celkem 114 populací *G. bohemica*, z nichž v roce 2017 existovalo pouze 50. Nejvíce kvetoucích rostlin (29200) bylo zaznamenáno v roce 2009, nejméně v roce 2016 (4084). Velikosti populací meziročně silně kolísaly a byly částečně synchronizovány s průběhem počasí v předchozích letech, zejména s průměrnými teplotami a srážkami ve vegetačním období (květen až srpen); vlhké a teplé podmínky v předchozích letech měly pozitivní vliv na velikost populace. Mikrostanovištní preference (vyjádřené pomocí pomocí Ellenbergových indikačních hodnot) a druhové složení porostů, v nichž se *G. bohemica* vyskytuje, jsme sledovali na 122 plochách o velikosti 0,25 m^2, rozmístěných na 22 lokalitách; 45 ploch bylo umístěno v místě výskytu *G. bohemica*, 77 tam, kde druh chyběl. *Gentianella bohemica* preferuje živinami chudé mikrobiotopy, čemuž odpovídal i výskyt několika dalších druhů s podobnými nároky. Výskyt *G. bohemica* dále stoupal s podílem obnažené půdy, který pravděpodobně usnadňuje uchycení semenáčů. Údaje o hospodaření na lokalitách s *G. bohemica* ukázaly, že ochranářská opatření pozitivně ovlivňují velikosti populací, efekt však není nikterak významný

How to cite: Preinfalk A., Moser D. & Essl F. (2022) Conservation status and ecology of the highly threatened endemic *Gentianella bohemica*. – Preslia 94: 255–273.

Preslia, a journal of the Czech Botanical Society

www.preslia.cz