Botanica
ISSN 2538-8657
https://doi.org/10.35513/Botlit.2022.2.2
2022, 28(2): 91–101

Original research

Current state of critically endangered *Neotinea ustulata* (Orchidaceae) in Lithuania and report on a new record of the species

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Abstract

Kazlauskas M., Taura L., Gudžinskas Z., 2022: Current state of critically endangered *Neotinea ustulata* (Orchidaceae) in Lithuania and report on a new record of the species. – Botanica, 28(2): 91–101. https://doi.org/10.35513/Botlit.2022.2.2

Neotinea ustulata (Orchidaceae) is a rapidly declining species throughout much of its range and is classified as critically endangered in Lithuania. Most of the historical occurrences of the species were concentrated in north-western Lithuania, while the largest extant population was in the southern part of the country. In July 2022, a new population of *Neotinea ustulata* was discovered in an urbanised habitat in Šiauliai in northern Lithuania. A total of 84 individuals of this species were recorded in the population. In the southern part of Lithuania (Varėna distr.), the studied population comprised 86 individuals in 2002 and had decreased to 64 individuals by 2022. The total currently known population of the species in the country consists of about 150 individuals and remains critically endangered. The individuals of *Neotinea ustulata* studied in Šiauliai were significantly lower and had shorter inflorescences than the plants studied in Varėna district in 2002 and 2022. Since the population of *Neotinea ustulata* in Šiauliai is in an urbanised area, its conservation poses a significant challenge, making it difficult to make long-term predictions on the development of the population. Therefore, it is essential to continue searching for populations of *Neotinea ustulata* in the country, perform detailed studies on the plants' reproductive potential, and properly organise the management and protection of extant populations and their habitats.

Keywords: conservation, distribution, fluctuation, grasslands, population size, red list, threats, urban habitat.

INTRODUCTION

Species loss caused by anthropogenic impacts and climate change is among the most concerning contemporary issues for nature conservation (Mantyka-Pringle et al., 2015; Attorre et al., 2018). In order to halt the decline of species, it is crucial to have accurate knowledge of population size, distribution and occupied habitats, as well as to assess the causes of population decline at the global and local scales (Pedersen et al., 2013; Mantyka-Pringle et al., 2015; Asamoah et al., 2022).

Following the most recent assessment of the species according to the IUCN (2012) criteria, the Red List of Lithuania has been compiled, which now includes 167 species of vascular plants. Among them, 23 species are critically endangered (CR), 66 are endangered (EN), 41 are vulnerable (VU), 23 are near threatened (NT), five are of least concern (LC) and nine species are data deficient (DD) (Rašomavičius,

2021). The latest list of legally protected plant species in Lithuania is considerably shorter than in 2007, when it included 234 species of vascular plants (Rašomavičius, 2007). The reduction in the number of red-listed species is not solely due to the improved status of populations (e.g., *Campanula cervicaria* L., *Dactylorhiza fuchsii* (Druce) Soó, *Dactylorhiza incarnata* (L.) Soó), but also because of the significant lack of data on the status of populations of certain species (e.g., *Isolepis setacea* (L.) R. Br., *Koeleria delavignei* Czern. ex Domin, *Taraxacum suecicum* G.E. Haglund.). This clearly illustrates the importance of information on the distribution of each species, the condition of its habitat, and the processes taking place in the populations. We expect the Red List will expand in the future with the availability of more data on the status of species that have not yet been assessed. In addition, new plant species native to Lithuania are being discovered, the assessment of which justifies the need for their legal protection (Gudžinskas & Taura, 2021, 2022; Ryla et al., 2021; Taura et al., 2022).

It has been noted that the populations of several species of the Orchidaceae family in Lithuania and other European countries have drastically declined over the last few decades, and this process is continuing. Among the most rapidly declining species are *Anacamptis morio* (L.) R.M. Bateman, Pridgeon et M.W. Chase, *Gymnadenia odoratissima* (L.) Rich., *Herminium monorchis* (L.) R. Br., *Neotinea ustulata* (L.) R.M. Bateman, Pridgeon et M.W. Chase., *Neottianthe cucullata* (L.) Schltr., etc. (Gudžinskas & Ryla, 2006; Tali et al., 2004, 2006; Roze et al., 2014; Gudžinskas, 2015; Uogintas, 2015; Jakubska-Busse et al., 2021; Štípková & Kindlmann, 2021). A new and relatively large locality of the rapidly declining *Neotinea ustulata* was discovered in Šiauliai (northern Lithuania) in 2022.

The genus *Neotinea* comprises eight species (among them, two are of hybrid origin) distributed in Europe, North Africa and Western Asia (Kretzschmar et al., 2007; Kühn et al., 2019). Most species of the genus occur in the Mediterranean region (Kühn et al., 2019). The range of *Neotinea ustulata* covers most of Europe, except for the Arctic regions and the eastern part of the continent. A few islands separated from the main range are found in central Siberia, the Caucasus and southwest Asia (Tali et al., 2004; Kretzschmar et al., 2007; Kühn et al., 2019). *Neotinea ustulata* is rare or very rare throughout most of its range and is considered one of the most rapidly declining species of the Orchidaceae in Europe (Tali et al., 2004, 2006).

The species currently known as *Neotinea ustulata* has traditionally been regarded as a representative of the genus *Orchis* L. However, genetic studies have shown significant differences between the members of *Orchis* s.l., and on this basis, the taxonomy of this group of plants has been significantly restructured (Pridgeon et al., 1997; Bateman et al., 2003; Tyteca & Klein, 2008). This has led to a significant broadening of the monophyletic genera *Anacamptis* Rich. and *Neotinea* Rchb.f., which included the well-known *Orchis* s.l. species, *Orchis morio* L. and *Orchis ustulata* L., which became *Anacamptis morio* (L.) R.M. Bateman, Pridgeon et M.W. Chase, and *Neotinea ustulata* (L.) R.M. Bateman, Pridgeon et M.W. Chase., respectively (Bateman et al., 2003; Tyteca & Klein, 2008). The genus *Orchis* s.str. retained two species occurring in Lithuania, *Orchis mascula* (L.) L. and *Orchis militaris* L.

After discovering a new population of *Neotinea ustulata* in Šiauliai, we also decided to examine other species' populations in previously recorded localities in Lithuania. The objectives of this study were to assess the size of the species populations and their long-term changes in Lithuania, as well as the conservation potential of the newly recorded.

MATERIALS AND METHODS

The new population of *Neotinea ustulata* in Šiauliai (northern Lithuania) was discovered on 4 July 2022 and studied between 7 and 9 July. In the village of Kapiniškės (Varėna distr., southern Lithuania), a species population was investigated on 20 July 2022, whereas, in 2002, investigations were carried out on 18 July. During the field studies, the species composition of the plant communities occupied by *Neotinea ustulata* was registered, and the area covered by the population was estimated. All detected individuals of *Neotinea ustulata* were divided into two maturity groups: mature (flowering) and immature (non-flowering). The height of each mature individual from the soil surface to the apex (cm) and inflorescence length from the lower flower to the inflorescence apex were measured using a measuring tape.

The population status and distribution of the species in Lithuania were assessed based on the information stored in the Protected Species Information System (SRIS), literature sources and data presented on the labels of herbarium specimens. The herbarium specimens stored in the Herbarium of Vilnius University (WI) and the Herbarium of the Institute of Botany of the Nature Research Centre (BILAS) were analysed. A map of the distribution of *Neotinea ustulata* was compiled by applying a grid system arranged according to the geographical coordinates. All records made in the same grid cell were marked with a single symbol. Previously published information on the distribution of the species in Lithuania was also used to create the map (Gudžinskas, 2001; Gudžinskas & Ryla, 2006; Uogintas, 2015, 2021).

Statistical analysis of the research data was performed with PAST 4.10 software (Hammer et al., 2001). The results of the descriptive statistics represent the mean and standard deviation (mean ± SD). As the sample sizes varied between years and locations, non-parametric methods of analysis were applied. Data from different survey years and locations were compared by using the Mann-Whitney U test.

RESULTS

New locality in Šiauliai

At the beginning of July 2022, a new locality of *Neotinea ustulata* was discovered in the northern part of Lithuania, in the city of Šiauliai, in Dainai Park. Individuals of this species were spread over an area of approximately 120 m^2 (40 m long and 30 m wide). The plants grew in mesophyte grassland and along its margins near trees and shrubs. A total of 43 mature (flowering) individuals and 41 immature (non-flowering) individuals were counted at the site. Thus, the total population of *Neotinea ustulata* in 2022 comprised 84 individuals. Individuals of *Neotinea ustulata* were unevenly distributed throughout the area. Often, 2–3 individuals formed compact groups, with distances of 5–10 m between groups.

Most individuals of *Neotinea ustulata* grew in mesophyte grassland with *Briza media* L., *Dactylis glomerata* L., *Daucus carota* L., *Hypericum perforatum* L., *Leucanthemum vulgare* Lam., *Phleum pratense* L., *Plantago media* L., *Taraxacum officinale* F.H. Wigg., *Trifolium pratense* L., *Veronica chamaedrys* L. and *Vicia cracca* L., being the most abundant (Fig. 1). A small number of individuals grew in slightly moister grassland, which, in addition to the above-mentioned species, also included *Filipendula ulmaria* (L.) Maxim., *Molinia caerulea* (L.) Moench, *Poa palustris* L. and *Potentilla erecta* (L.) Raueusch. The grassland was somewhat influenced by trees such as *Acer platanoides* L., *Betula pubescens* Ehrh., *Picea abies* (L.) H. Karst. and *Sorbus aucuparia* L., growing along its edges. Almost half of all individuals of *Neotinea ustulata* grew in the part of the grassland exposed to partial tree shade.

As the grassland with *Neotinea ustulata* locality is in a park, the grass is mown at least twice during the warm season. The grassland where this species grows was mown on 21 July 2022, before the plants had finished flowering. No information is available on the mowing regime of the grassland in the previous years.

State of the population in Kapiniškės

Eighty-six flowering individuals were recorded in Kapiniškės in 2002, while the number of immature individuals in the population was not assessed. In 2022, 62 flowering individuals and two immature individuals were recorded in the same population. In Kapiniškės, the number of individuals of *Neotinea ustulata* declined by 25.6% between 2002 and 2022. The area occupied by the population has also decreased by approximately 30%. In 2002, 23 individuals were found in the grassland on the top of the hill, while in 2022, only one individual was recorded in that part of the grassland. Almost the entire grassland area on the hilltop, where *Neotinea ustulata* was present in 2002, was already covered by dense swards of *Calamagrostis epigejos* (L.) Roth in 2022.

The number of individuals in the grassland on the hillside remained unchanged during the two decades (63 individuals in 2002 and 64 in 2022). However, the distribution pattern of individuals in Kapiniškės has changed substantially over that period. In 2002, most individuals of *Neotinea ustulata* grew solitarily, and five small groups of two or three individuals were recorded. In contrast, eight groups of two to eight compactly growing individuals were recorded in the locality in 2022 (Fig. 2).

Fig. 1. Two individuals of *Neotinea ustulata* growing in a mesic grassland (Šiauliai, northern Lithuania) in 2022. Photo by M. Kazlauskas

The dry hillside grassland, which is formed in carbonate-rich soils, has a high diversity of plant species (Fig. 3). The most abundant species were *Anthericum ramosum* L., *Briza media* L., *Centaurea scabiosa* L., *Centaurea jacea* L., *Festuca rubra* L., *Galium verum* L., *Gentiana cruciata* L., *Helianthemum nummularium* (L.) Mill., *Medicago falcata* L., *Poa angustifolia* L., *Thymus serpyllum* L. In addition to *Neotinea ustulata*, the grassland supports many protected species such as *Cephalanthera rubra* (L.) Rich., *Epipactis atrorubens* (Hoffm.) Besser, *Prunella grandiflora* (L.) Scholler and *Thesium ebracteatum* Hayne.

Plant size within populations

In Kapiniškės, the lowest generative individual in 2022 was 16 cm, and the tallest was 49 cm, whereas in 2002, the lowest generative individual in this population was 19 cm, and the tallest was 48 cm. In Šiauliai, the lowest generative individual was 7 cm, and the tallest was 46 cm. The mean height of generative individuals of *Neotinea ustulata* in 2022 in Kapiniškės ($n = 62$) was 37.9 ± 7.4 cm, whereas in the same population in 2002, the mean height of individuals ($n = 86$) was 32.6 ± 6.9 cm (Fig. 4). The generative individuals in this population in 2022 were significantly taller ($U = 1583.5$; $p < 0.001$) than in 2002. In Šiauliai, in the population studied in 2022, the mean height of generative individuals ($n = 43$) was 26.6 ± 7.1 cm. Plants in Šiauliai were significantly lower than those in Kapiniškės in 2002 ($U = 984.5$; $p < 0.001$) and 2022 ($U = 354.5$; $p < 0.001$).

The shortest recorded inflorescence in 2022 was 3 cm, and the longest was 17 cm long in Kapiniškės,

Fig. 2. A group of six compactly growing mature individuals of *Neotinea ustulata* in Kapiniškės (Varėna distr., southern Lithuania) in 2022. Photo by Z. Gudžinskas

whereas in the same population in 2002, the shortest inflorescence was 3 cm, and the longest was 13 cm long. In Šiauliai, the shortest inflorescence was 1 cm, and the longest was 12 cm long. In the population of *Neotinea ustulata* in Kapiniškės, the mean length of the inflorescence (n = 62) in 2022 was 9.7 ± 3.5 cm, whereas, in the same population in 2002, the mean length of the inflorescence (n = 86) was 7.9 ± 2.4 cm (Fig. 4). In 2022, the inflorescences in this population were significantly longer (U = 1834.5; $p = 0.001$) than in 2002. In the population studied in Šiauliai, the mean length of the inflorescence (n = 43) was 5.8 ± 2.1 cm. In Šiauliai, the inflorescences of *Neotinea ustulata* were significantly shorter than in Kapiniškės both in 2002 (U = 924.0; $p < 0.001$) and in 2022 (U = 503.0; $p < 0.001$).

State of the species in Lithuania

Most of the historical localities of *Neotinea ustulata* were concentrated in the northwestern part of Lithuania. In contrast, in other parts of the country, only sporadic

Fig. 3. Habitat of *Neotinea ustulata* on a hill slope in Kapiniškės (Varėna distr., southern Lithuania) in 2022. Photo by Z. Gudžinskas

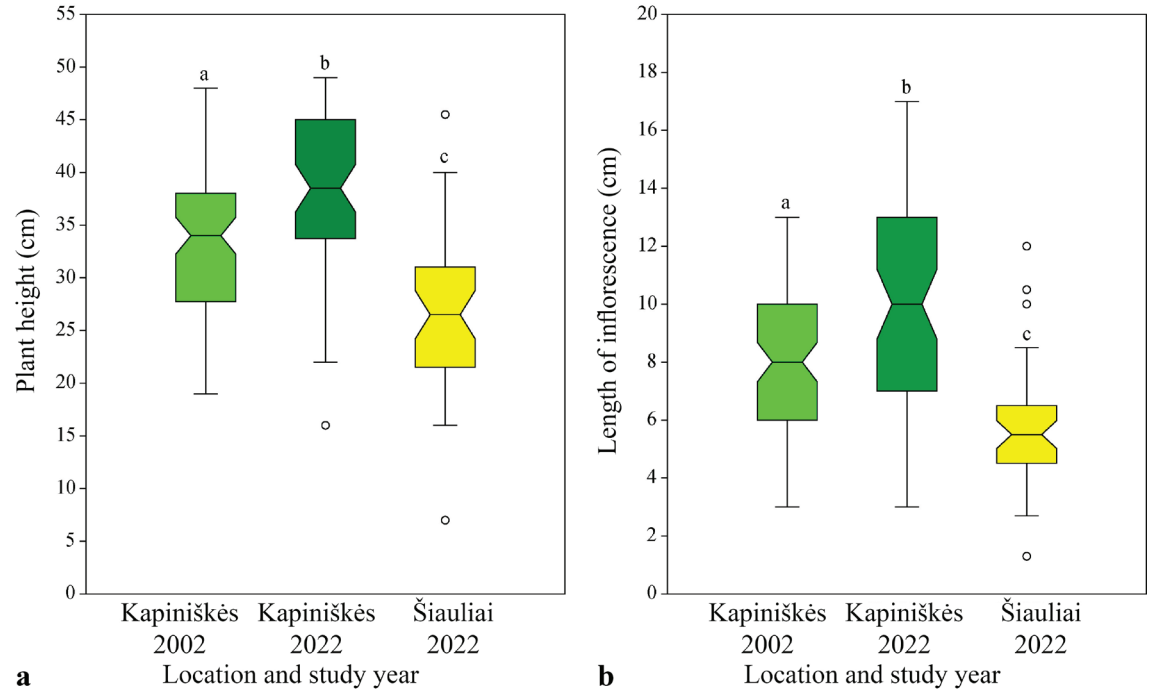

Fig. 4. *Neotinea ustulata* plant height (a) and inflorescence length (b) in Kapiniškės and Šiauliai populations. The notch indicates the mean, and the whiskers refer to the standard deviation. Empty circles represent outliers. Different letters above the whiskers indicate significant differences according to the Mann-Whitney test

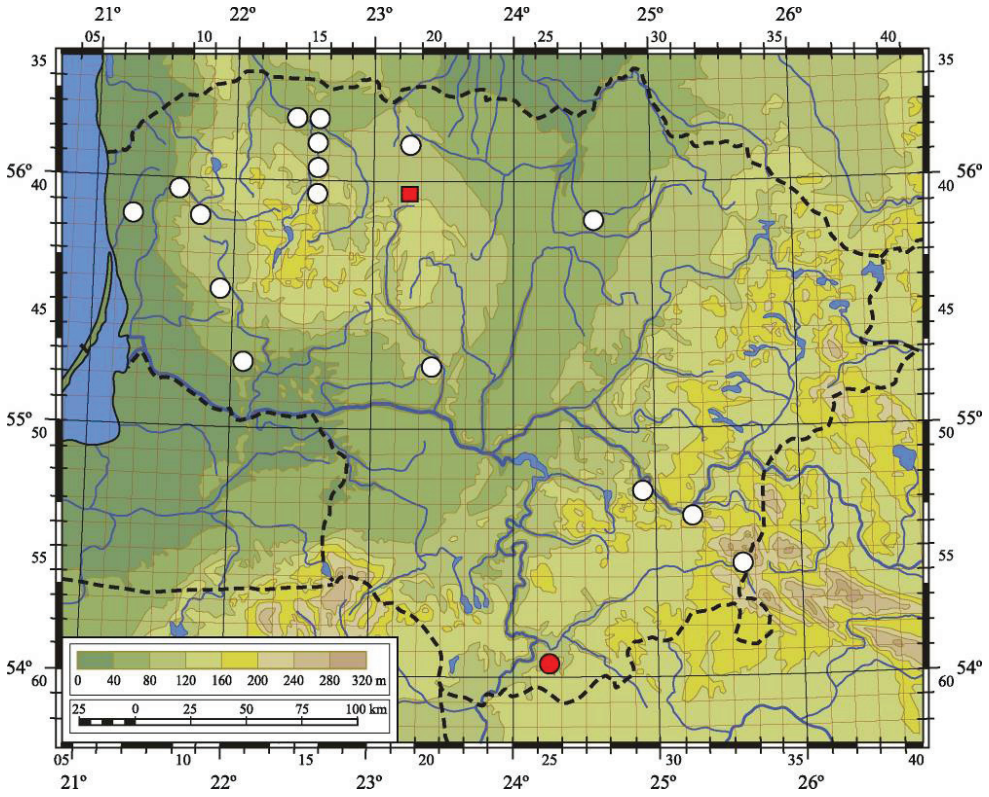

Fig. 5. Distribution of *Neotinea ustulata* in Lithuania. A rectangle indicates a newly discovered locality, a red circle indicates the studied Kapiniškės population, and white circles indicate historical records and localities where no individuals have been found in the last ten years

localities were registered (Fig. 5). In most of the historical localities, where *Neotinea ustulata* was recorded in the 1940s–1980s, it is now absent, and there are no more recent data on the status of these populations (Gudžinskas, 2001; Gudžinskas & Ryla, 2006). The species has probably become extinct because of habitat destruction or land-use changes. The populations recorded in the last decades of the 20th century were small and consisted of solitary (usually 3–5) individuals. Only occasionally, more individuals were found. A relatively large population of the species was discovered in 1997 in the Pasvalys district, consisting of 18 individuals (Stukonis, 1998). Still, the population has severely declined or even become completely extinct (Uogintas, 2021). A small population of this species was found in the environs of Baltamiškis (Elektrėnai distr.), but it has not been detected in the last decade, although potentially suitable habitats persist.

The largest and most viable population of *Neotinea ustulata* in Kapiniškės village (Varėna distr.) has been, and remains, particularly important for the conservation of this critically endangered species. With the discovery of a new population in Šiauliai, the total number of individuals recorded in Lithuania has doubled, but remains critically low. According to available data, about 150 individuals of this species are now in the country, and about 98% are concentrated in two populations. Even though the discovery of a new population of *Neotinea ustulata* in Šiauliai has significantly increased the number of recorded individuals in the country, its status according to the IUCN (2012) criteria remains unchanged since the latest assessment (Uogintas, 2021).

Two varieties of *Neotinea ustulata*, var. *ustulata* and var. *aestivalis* (Kümpel) Tali, M.F. Fay et R.M. Bateman, are distinguished, which differ significantly by the time of flowering. The type variety flowers in late spring, usually at the end of May, while var. *aestivalis* usually in mid-summer, from mid-June to the end of July. The labels of the flowering specimens of *Neotinea ustulata* in the herbarium indicate that they were collected between mid-June (at the very beginning of flowering) and the end of July. The same flowering time for this species in Lithuania has

also been reported in the literature (Stukonis, 1998; Gudžinskas & Ryla, 2006; Uogintas, 2015, 2021). The long-term observations of the population of *Neotinea ustulata* in Kapiniškės show that the flowering time of individuals is relatively constant, with the first plants starting to flower at the end of June. This leads to the conclusion that only var. *aestivalis* grows in all extant populations in Lithuania. In contrast, information on the presence of plants of the type variety (var. *ustulata*) is absent.

DISCUSSION

New locality in Šiauliai

The discovery of a new population of *Neotinea ustulata* in the northern part of Lithuania was not unexpected, judging by the overall species distribution in the country. Several small populations of the species have previously been recorded in this region (Gudžinskas & Ryla, 2006; Uogintas, 2015, 2021). It is now difficult to determine whether the population of *Neotinea ustulata* in Šiauliai, in Dainai Park, is extant from earlier times, when the area was non-urbanised, or whether the plants have settled in later periods from seeds introduced by wind or other means. It has been found that the small seeds of the Orchidaceae are efficiently dispersed by wind over relatively long distances (Vanden Broeck et al., 2014; Kotilínek et al., 2020).

There are no data on the growth of *Neotinea ustulata* in heavily disturbed habitats. The species has been found to be very sensitive to fertilisers, pesticides and herbicides, as well as to intensive grazing or frequent mowing (Tali et al., 2004, 2006). Other endangered Orchidaceae species often colonise anthropogenic habitats (roadsides, ditches, quarries, abandoned lands, etc.). Such populations may even be very abundant for a specific time (Pedersen et al., 2013; Norkevičienė & Gudžinskas, 2016). When a large proportion of the natural habitats of rare plant species have been destroyed, anthropogenic or heavily human-impacted habitats often become their only refuges. However, habitats in urbanised environments are frequently only temporary refuges for endangered species, as conserving such habitats and species poses many challenges (Norkevičienė & Gudžinskas, 2016; Soanes & Lentini, 2019; Soanes et al., 2020).

According to Soanes & Lentini (2019), successful conservation of species in cities can only be expected if its policies are legislated, and biodiversity professionals are involved in implementing the policies. The General Plan of Šiauliai City identifies the areas of the natural framework, which includes the habitat of *Neotinea ustulata*. This document also specifies the need for detailed urban biodiversity assessments to identify areas of nature conservation value and provide recommendations for their protection. Awakening a sense of ownership among residents is essential for successfully conserving rare species and biodiversity in urban areas (Andersson et al., 2014; Soanes & Lentini, 2019). Habitat fencing is recommended in areas with a risk of unintentional destruction of rare species. Fencing also protects the most sensitive populations from trampling, plant gathering and the impact of domestic and wild animals (Rankou, 2011). The staff of the Department of Urban Economy and Environment of Municipality of Šiauliai City Administration and specialists from the Botanical Garden of VU Šiauliai Academy could contribute to the protection of the *Neotinea ustulata* population in Šiauliai. The Botanical Garden can undertake population monitoring, raise public awareness about the conservation of endangered species, and organise habitat management actions.

State of the population in Kapiniškės

The population of *Neotinea ustulata* in Kapiniškės village (Varėna distr.) has been monitored periodically since 2000. That year, 180 flowering individuals were recorded in this locality (Gudžinskas & Ryla, 2006), but in 2002 the number decreased to 63. Three years later (in 2005), 50 flowering individuals were found in this locality. After that, no counts were conducted, and in 2022, 62 flowering and two immature individuals were recorded in the population. Thus, the most significant shift in the number of individuals occurred between 2000 and 2002. In contrast, subsequent changes may be explained by fluctuation, a break in the flowering of mature plants, or a temporary dormancy of individuals (Shefferson & Tali, 2007).

Relatively stable or low fluctuation in the number of individuals in a population over the last 20 years is, on the one hand, a good indication of habitat stability. On the other hand, the very slow recruitment of

the population with very few immature individuals is a matter of concern. The reasons for the slow recruitment are not yet understood, as no assessment has been made of the fruit set. The low percentage of fruit set and low seed viability could be caused by the inbreeding of the individuals that make up the population or by a lack of pollinating insects in the habitat (Vöth, 1984; Mrkvicka, 1991; Tali, 2004). Thus, future studies on assessing the fruit set and seed quality of *Neotinea ustulata* should be performed to understand the real reasons for the slow population recruitment.

Plant size within populations

The analysis of the height and inflorescence length of *Neotinea ustulata* individuals showed that these parameters differed significantly between years within the same population and between different populations in the same year. As the studies in Kapiniškės were performed in different years at the same time (two days apart), the differences in plant height and inflorescence length cannot be explained by variation in the flowering phase. It is possible that variations in the meteorological conditions could cause differences during the years of the study, such as the amount of precipitation.

The differences between the plant height and inflorescence length in Šiauliai and Kapiniškės in 2022 could be explained by the different timing of the study (10 days difference). It is known that the flowers in the inflorescence of *Neotinea ustulata* open from the bottom upwards and that the inflorescence grows longer, and the height of the plant increases simultaneously. Nevertheless, differences in plant height may have been influenced by different habitat conditions. In Šiauliai, the plants grew in a mesophyte grassland surrounded by trees and with little shade, whereas in Kapiniškės, they grew in a completely open area on the slope of the southwest exposure. *Neotinea ustulata* is known to favour sunny, open habitats (Tali, 2004), so the relatively dense mesophyte grassland and partial shade from trees may have led to the lower height of the individuals and the shorter inflorescences.

We tried to minimise the impact on *Neotinea ustulata* individuals during the study and therefore did not count flowers in the inflorescences. However, we noted that inflorescences of the same length were laxer in Šiauliai than in Kapiniškės. Habitat conditions and competition from surrounding plants likely affect the height and reproductive traits of *Neotinea ustulata* (Tali, 2004).

State of the species in Lithuania

The decline of *Neotinea ustulata* populations in Lithuania, as in other European countries, over the last few decades, has mainly been determined by the direct destruction of suitable habitats in the past and the current loss of suitable habitats by changes in farming practices (Gudžinskas, 2001; Tali, 2004; Tali et al., 2004, 2006; Gudžinskas & Ryla, 2006; Rankou, 2011; Štípková & Kindlmann, 2021). In addition, small areas of grasslands have been abandoned, overgrown with scrub and trees or converted into forest plantations. However, these reasons alone cannot explain the population decline and the number of individuals. We believe that some of the populations in Lithuania have become extinct due to critically low numbers of individuals (Gudžinskas & Ryla, 2006; Uogintas, 2015; 2021). When the number of individuals is low, the potential for generative reproduction is reduced, and the population collapses once the biological lifespan of the plant is over (Tali, 2004).

The conservation of critically endangered *Neotinea ustulata* should be organised to protect the extant populations and their habitats from further decline. A continuous, preferably annual inspection of the sites of the former populations is essential, and immediate conservation measures should be taken in the event of discovering individuals of this species. Implementing conservation measures is preferable from the perspective of nature conservation and is more economically feasible than restoring populations of already extinct species (Dobson et al., 1997). Targeted searches for *Neotinea ustulata* should also be undertaken, particularly in the western and northern parts of Lithuania, where most of the localities now considered extinct were located. We assume that *in situ* propagation trials of *Neotinea ustulata* could be undertaken using seeds originating from the same population. This critically endangered species can only be protected from further extinction by implementing all possible conservation measures.

REFERENCES

Andersson E., Barthel S., Borgström S., Colding J., Elmqvist T., Folke C., Gren A., 2014: Reconnecting cities to the biosphere: stewardship of green infrastructure and urban ecosystem services. – Ambio, 43: 445–453. https://doi.org/10.1007/s13280-014-0506-y

Asamoah E.F., Di Marco M., Watson J.E.M., Beaumont L.J., Venter O., Maina J.M., 2022: Land-use and climate risk assessment for Earth's remaining wilderness. – Current Biology, 32(22): 4890–4899. https://doi.org/10.1016/j.cub.2022.10.016

Attorre F., Abeli T., Bacchetta G., Farcomeni A., Fenu G., De Sanctis M., Gargano D., Peruzzi L., Montagnani C., Rossi G., Conti F., Orsenigo S., 2018: How to include the impact of climate change in the extinction risk assessment of policy plant species? – Journal for Nature Conservation, 44: 43–49. https://doi.org/10.1016/j.jnc.2018.06.004

Bateman R.M., Hollingsworth P.M., Preston J., Yi-Bo L., Pridgeon A.M., Chase M.W., 2003: Molecular phylogenetics and evolution of *Orchidinae* and selected *Habenariinae* (*Orchidaceae*). – Botanical Journal of the Linnean Society, 142: 1–40. https://doi.org/10.1046/j.1095-8339.2003.00157.x

Dobson A.P., Bradshaw A.D., Baker A.Á., 1997: Hopes for the future: restoration ecology and conservation biology. – Science, 277(5325): 515–522. https://doi.org/10.1126/science.277.5325.515

Gudžinskas Z., 2001: Diversity, state, and protection of Orchidaceae species in Lithuania. – Journal Europäischer Orchideen, 33(1): 415–441.

Gudžinskas Z., 2015: Miškinė plikaplaiskė. *Neottianthe cuculata*. – In: Vaitonis G. (ed.), Lietuvos griežtai saugomos rūšys: 88–89. Vilnius.

Gudžinskas Z., Ryla M., 2006: Lietuvos gegužraibiniai (Orchidaceae). Vilnius.

Gudžinskas Z., Taura L., 2021: *Scirpus radicans* (Cyperaceae), a newly-discovered native species in Lithuania: population, habitats and threats. – Biodiversity Data Journal, 9: e65674. https://doi.org/10.3897/BDJ.9.e65674

Gudžinskas Z., Taura L., 2022: Rediscovery of endangered species *Laphangium luteoalbum* (Asteraceae) in Lithuania. – Botanica, 28(1): 49–55. https://doi.org/10.35513/Botlit.2022.1.7

Hammer Ø., Harper D.A.T., Ryan P.D., 2001: PAST: Paleontological statistics software package for education and data analysis. – Palaeontologia Electronica, 4(1): 9.

IUCN, 2012: Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. Gland–Cambridge.

Jakubska-Busse A., Tsiftsis S., Śliwiński M., Křenová Z., Djordjević V., Steiu C., Kolanowska M., Efimov P., Hennigs S., Lustyk P., Kreutz K., 2021: How to protect natural habitats of rare terrestrial orchids effectively: A comparative case study of *Cypripedium calceolus* in different geographical regions of Europe. – Plants, 10(2): 404. https://doi.org/10.3390/plants10020404

Kotilínek M., Těšitelová T., Košnar J., Fibich P., Hemrová L., Koutecký P., Münzbergová Z., Jersáková J., 2020: Seed dispersal and realized gene flow of two forest orchids in a fragmented landscape. – Plant Biology, 22(3): 522–532. https://doi.org/10.1111/plb.13099

Kretzschmar H., Eccarius W., Dietrich H., 2007: The Orchid Genera *Anacamptis*, *Orchis* and *Neotinea*. Phylogeny, Taxonomy, Morphology, Biology, Distribution, Ecology and Hybridisation (ed. 2). Bürgel.

Kühn R., Pedersen H.A., Cribb V., 2019: Field Guide to the Orchids of Europe and the Mediterranean. Kew.

Mantyka-Pringle C.S., Visconti P., Di Marco M., Martin T.G., Rondinini C., Rhodes J.R., 2015: Climate change modifies risk of global biodiversity loss due to land-cover change. – Biological Conservation, 187: 103–111. https://doi.org/10.1016/j.biocon.2015.04.016

Mrkvicka A., 1991: Bestaeuber, Chromosomenzahl und weitere Beobachtungen zu *Orchis ustulata aestivalis* Mitteilungsblatt. – Arbeitskreis Heimische Orchideen Baden Würtemberg, 23: 331–338.

Norkevičienė E., Gudžinskas Z., 2016: Protected plant species and their state in anthropogenic habitats of south-western Lithuania. – In: Mildažienė V., Balsevičius A., Narijauskas R., Gudžinskas Z., Norkevičienė E., Obelevičius K., Žalneravičius E. (eds) Conservation of Botanical Diversity in South-Western Lithuania: 170–178. Kaunas.

Pedersen H.A., Watthana S., Srimuang K., 2013:

Orchids in the torrent: on the circumscription, conservation and rheophytic habit of *Epipactis flava*. – Botanical Journal of the Linnean Society, 172: 358–370. https://doi.org/10.1111/boj.12023

Pridgeon A.M., Bateman R.M., Cox A.V., Hapeman J.R., Chase M.W., 1997: Phylogenetics of subtribe *Orchidinae* (*Orchidoideae*, *orchidaceae*) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis sensu lato*. – Lindleyana, 12(2): 89–109.

Rankou H., 2011: *Neotinea ustulata* (Europe assessment). The IUCN Red List of Threatened Species: e.T176036A7180745. – https://www.iucnredlist.org/species/176036/7180745 (accessed on 18 November 2022).

Rašomavičius V. (ed.), 2007: Red Data Book of Lithuania. Vilnius.

Rašomavičius V. (ed.), 2021: Red Data Book of Lithuania. Animals. Plants. Fungi. Vilnius.

Roze D., Jakobsone G., Megre D., Belogrudova I., Karlovska A., 2014: Survival of *Liparis loeselii* (L.) as an early successional species in Engure region described based on ecological peculiarities during the annual cycle. – Proceedings of the Latvian Academy of Sciences, 68(1–2): 93–100. https://doi.org/10.2478/prolas-2014-0008

Ryla M., Kinduris R., Nurczyński B., Žilinskienė A., Pranaitis A., 2022: *Epipactis albensis* (Orchidaceae) species new to the flora of Lithuania. Data from the northeastern limit of the species distribution area. – Botanica, 28(1): 46–59. https://doi.org/10.35513/Botlit.2022.1.6

Shefferson R.P., Tali K., 2007: Dormancy is associated with decreased adult survival in the burnt orchid, *Neotinea ustulata*. – Journal of Ecology, 95: 217–225. https://doi.org/10.1111/j.1365-2745.2006.01195.x

Soanes K., Lentini P., 2019: When cities are the last chance for saving species. – Frontiers in Ecology and the Environment, 17(4): 225–231. https://doi.org/10.1002/fee.2032

Soanes K., Threlfall C.G., Ramalho C.E., Bekessy S., Fuller R.A., Garrard G.E., Ikin K., Kendal D., Lee K.E., Mumaw L., Rowe R., Shanahan D.F., Valentine L.E., Williams N.S.G., Parris K.M., Lentini P.E., 2020: Conservation Opportunities for Threatened Species in Urban Environments. Wollongong.

Štípková Z., Kindlmann P., 2021: Orchid extinction over the last 150 years in the Czech Republic. – Diversity, 13(2): 78. https://doi.org/10.3390/d13020078

Stukonis V., 1998: Naujos retųjų augalų radvietės. – Raudoni lapai, 6: 33.

Tali K., 2004: Species Structure of *Neotinea ustulata*. A Dissertation of Philosophiae Doctor. Tartu.

Tali K., Foley M., Kull T., 2004: Biological flora of the British Isles No. 232. *Orchis ustulata* L. – Journal of Ecology, 92: 174–184.

Tali K., Fay M.F., Bateman R.M., 2006: Little genetic differentiation across Europe between early-flowering and late-flowering populations of the rapidly declining orchid *Neotinea ustulata*. – Biological Journal of the Linnean Society, 87: 13–25. https://doi.org/10.1111/j.1095-8312.2006.00550.x

Taura L., Kamaitytė-Bukelskienė L., Sinkevičienė Z., Gudžinskas Z., 2022: Study on the rare semiaquatic plant *Elatine hydropiper* (Elatinaceae) in Lithuania: Population density, seed bank and conservation challenges. – Frontiers in Bioscience. Landmark, 27(5): 162. https://doi.org/10.31083/j.fbl2705162

Tyteca D., Klein E., 2008: Genes, morphology and biology. The systematics of Orchidinae revisited. – Journal Europäischer Orchideen, 40(3): 501–544.

Uogintas D., 2015: Smulkiažiedė gegužraibė. *Orchis ustulata*. – In: Vaitonis G. (ed.), Lietuvos griežtai saugomos rūšys: 85–87. Vilnius.

Uogintas D., 2021: *Neotinea ustulata*. – In: Rašomavičius V. (ed.), Red Data Book of Lithuania. Animals. Plants. Fungi: 413. Vilnius.

Vanden Broeck A., Van Landuyt W., Cox K., De Bruyn L., Gyselings R., Oostermeijer G., Valentin B., Bozic G., Dolinar B., Illyés Z., Mergeay J., 2014: High levels of effective long-distance dispersal may blur ecotypic divergence in a rare terrestrial orchid. – BMC Ecology, 14(1): 1–15. https://doi.org/10.1186/1472-6785-14-20

Vöth W., 1984: *Echinomyia magnicornis* Zett. Bestäuber von *Orchis ustulata* L. – Orchidee, 35: 189–192.

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