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Relocation and formation of new local population of *Viola pumila* Chaix – an endangered species in Europe and identification of measures for improvement of its habitat

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Abstract

The meadow violet (*Viola pumila* Chaix) is an endangered species which is regionally extinct in many European countries. This is a stenobiontic species with a very limited distribution in Bulgaria. Only a few populations have been detected, and they consist of several tens to several hundreds of specimens. The meadow violet has limited reproductive abilities and weak competitiveness. The species is exposed to various anthropogenic threats, as the most significant of them are related to ploughing, conversion of the mesophilic meadows into arable lands, changes in the water regime of the habitats, the expansion of highly competitive species from the group of tufted cereal grasses and infrastructure construction. To date, information concerning the relocation of rare and endangered plant species in Bulgaria is rather scarce. In the present study we provide a protocol on our activities during a successful establishment of a new locality as part of our effort to successfully relocate one particular population of the meadow violet. The observations made after the relocation of the species over a two-year period (2020 and 2021) provided data on the successful relocation, and the creation of a new locality for the species. We discuss the conservation measures needed for this rare and endangered species, and efforts to increase its population, as well as the measures needed for effective management of the habitats of *V. pumila* (mainly 6510 "Lowland hay meadows").

Keywords

Biological restoration, conservation measures, eco-management, grassland habitat, plant ecology, *Viola pumila* Chaix

Introduction

The extinction of plant species is a well-recognized global problem. In the last 250 years about 571 plant species have become extinct (Humphreys et al. 2019) and that means that two or three plant species have been lost every year. The extinction of one particular plant species may directly impact other related organisms and even entire ecosystems (GBIF 2021). In recent decades, some plant species registered a dramatic decline in their population numbers. Several taxonomically closely related species of the genus *Viola* L. fall into this category: *V. elatior* Fr., *V. pumila* Chaix and *V. persicifolia* Roth (= *V. stagnina* Kit.). These species are among the most endangered plants in Central Europe (Eckstein et al. 2006; Holzel 2003). Due to the loss of suitable habitats or changes in their hydrological regime, these species have become extremely rare (Korneck et al. 1996) and are the subject of restoration programs in several European countries (Pullin and Woodell 1987). The meadow violet is suffering a severe decline in Europe. Reports attest to a diminishing number of this species (Eckstein et al. 2006) and this is the reason why the species is included in the red lists of several European countries: Switzerland (Info flora); Czech Republic (Grulich 2012); Italy (Buldrini and Dallai 2011) and also Bulgaria (Petrova and Vladimirov 2009).

V. pumila is a hemicryptophyt, in which the regenerative buds are located below the soil surface. The species is a representative of the spring ephemeroids and begins its growth in the early spring. It develops in a short time, but only by favorable soil moisture and scant competition for light by the syntopic species. According to Eckstein et al. (2006), *V. pumila* has a special type of life cycle and reproduction. The mechanisms of distribution of the species were not well studied. Pollination takes place in the flowers, but that mechanism has not been understood to date. Most likely, the species is a self-pollination (cleistogamic) plant, which is characteristic of members of the Violaceae family. This violet is a myrmecochor plant – ants are partially responsible for dispersal of the seeds, which is indicated by the presence of elaismosom in the seeds. During cracking, the seed boxes shoot the seeds at a distance of up to 1.19 m. In the soil, the scattered seeds are located in the 5 cm soil layer, where they undergo a period of stratification before beginning their active life cycle. The end of the growing season of *V. pumila* is in autumn, when the above ground parts of the plant dry out and the plant prepares for overwintering.

V. pumila has a Eurasian-continental distribution in the temperate climate zone (Buldrini et al. 2013). Populations of the species have been registered in Western Europe (Aeschiman and Burdet 1994), Central Europe (Danihelka et al. 2009) as well as in Eastern Europe (Gejdeman 1986). In Bulgaria, the species was considered extinct for a prolonged period, however, in the 1990s, the species was rediscovered by Andreev (1993). There were six known localities of the species, however two of them have disappeared in recent years (Apostolova and Meshinev 2015). In Bulgaria, the species can be found at altitudes between 500 and 1000 a.s.l. Currently, the largest populations are located around the villages of Kokalyane and Tsraklevtsi, as well as in the vicinity of Aldomirovsko and the Dragoman marshes (Apostolova and Meshinev 2015).

In the case of our study, a population of the meadow violet was threatened with direct annihilation during the construction of a motorway. The situation demanded fast and precise actions for localization of a possible new habitat, selection of relocation tactics and procedures for transporting and planting the violets, as well as developing a plan for sustaining the new local populations. Our activities resulted in a successful relocation of one particular population of *V. pumila*. To our knowledge this is the first successful establishment of a new locality following the relocation of an endangered plant species on the territory of Bulgaria. We discuss the necessary conservation measures for the habitat of *V. pumila* (mainly 6510 "Lowland hay meadows").

Materials and methods

The most complete morphological description of the species *V. pumila* was provided by Eckstein et al. (2006) and from Buldrini and Dallai (2011). The species is a perennial herbaceous plant, with a rhizome up to 30 cm long and a root thickness of 0.5 to 1 cm. The stem is erected, with a height of between 5 and 20 cm. The leaves have 0.3 to 0.6 mm long hairs; the leaf length is 2–6 cm and their width is 1–2 cm; the edges are shallowly serrated. The flower stalks are 5–10 cm long, the petals are purple at the beginning of flowering and later they become pale purple to whitish, with cilia at the base and 3–5 dark longitudinal lines. The fruit box is elongated, with yellow to brown coloration with a 10 mm length and width of 6–7 mm. One box may contain 24 seeds which are brownish and about 1 mm in length. Flowering occurs in the period April–May, and fruit formation and fruiting is in the period May–June (Table 1).

In the late spring of 2019 we provided the first on spot field survey for identification of the exact position and ranges of the population of meadow violet, as well as the number of the plants that had to be relocated. We inspected the terrains along the road Sofia-Kalotina between the km. 21 and km. 22. The field study was performed by the use of the transect method. The transects were repeated three times in three days by three qualified experts with the necessary experience. The species identification was performed according to Delipavlov 1979, Delipavlov et al. (2011).

Table 1. Phenological peculiarities in *V. pumila.*

	Period of the year	Developmental phase
1.	Beginning of April	Appearance of the first aboveground stalks
2.	Middle of April to the end of the month	Buttonization phase – forming of the flower buds
3.	End of April – beginning of May	Beginning of blooming
4.	First half of May	Mass blooming
5.	Second half of May	End of the blooming phase
6.	End of May to the middle of June	Gradual ripening and cracking of the seed boxes
7.	Middle of June	End of mass dissemination
8.	October	The aboveground parts dry out and the plant prepares to overwinter

* Depending on the specific meteorological conditions during the year, the phenophases during which *V. pumila* passes, may occur at different times

In the processing of the digital data for the identification of the most suitable sites for relocation, the layer with the distribution of the natural habitat within the Natura 2000 site SCI BG0000322 Dragoman (in "shape" format) was intersected with the layer containing the boundaries of the national protected area "Aldomirovsko Blato" and the layer of the municipal property in the protected area. The data processing and statistical analyzes were performed with the standard tools of ArcGIS 10 and the corresponding extensions of the used software.

Two satellite images from the Sentinel-2A L2A satellite (https://apps.sentinel-hub.com/eo-browser/) were used to determine the boundaries of the fire that occurred in 2019. In accordance with the period of the fire, the images used were selected respectively before the fire (September 21, 2019) and immediately after its end (October 26, 2019). Standard Arc GIS 10 tools were used for drawing the boundaries of the burned area, as well as for the map visualization.

The violets were manipulated in the morning or late afternoon, when the intensity of sunshine is not very high and the temperatures are lower. With the use of suitable tools the plants were dug up along with the adjacent soil around their rhizomes (in a radius of 25 cm. from its center). The plants were then immediately placed in vegetation containers with a depth of 30 cm and some water was added (Fig. 1). The specimens were covered with a transparent foil to preserve the soil moisture and the freshness of the plants. In this form, the violets were transported to their new locality. The specimens were planted in pre-prepared and moistened pits – 3 holes of the size of the

Figure 1. Technology for the relocation **a** identification of the plant **b** extraction of a rhizome **c** replacement of the rhizomes in container **d** preparation of the rhizomes for transportation.

Figure 2. Implementation of the Action plan for permanent relocation of the species to a suitable habitat: **a** preparation of the relocation pits **b** planting of the rhizoms **c** stuffing of the soil around the newly planted rhizome.

rhizomes in a raw in 10 m distance, to ensure enough space for the root system. The rhizomes were carefully positioned in the pits and the soil was compacted afterwards for reduction of the soil air (Fig. 2). We then watered the plants with 60 cl to prevent further water stress, as well as for faster adaptation.

After planting the violets in the new location, special care was taken in situ until their full adaptation (3–6 months) and subsequent two-years monitoring was carried out. During this period, the following activities were performed: watering (monthly); mowing around the localities in periods of three months; monitoring of the seed dispersal; observation of the flowering of the specimens during the next vegetation cycle; monitoring for pressures (fire, pollution, ploughing). All of our activities were coordinated with the competent authorities.

Results

The population of *V. pumila*, which was the focus of the present study, was endangered by a large linear infrastructure project. The project envisages the enlargement of an existing road section in the Sofia-Kalotina direction and its restructuring in "Europe Motorway". To protect the specimens, it was necessary to relocate the plants away from the route of the future motorway (Fig. 3), to create a new locality and to identify appropriate measures for its new habitats.

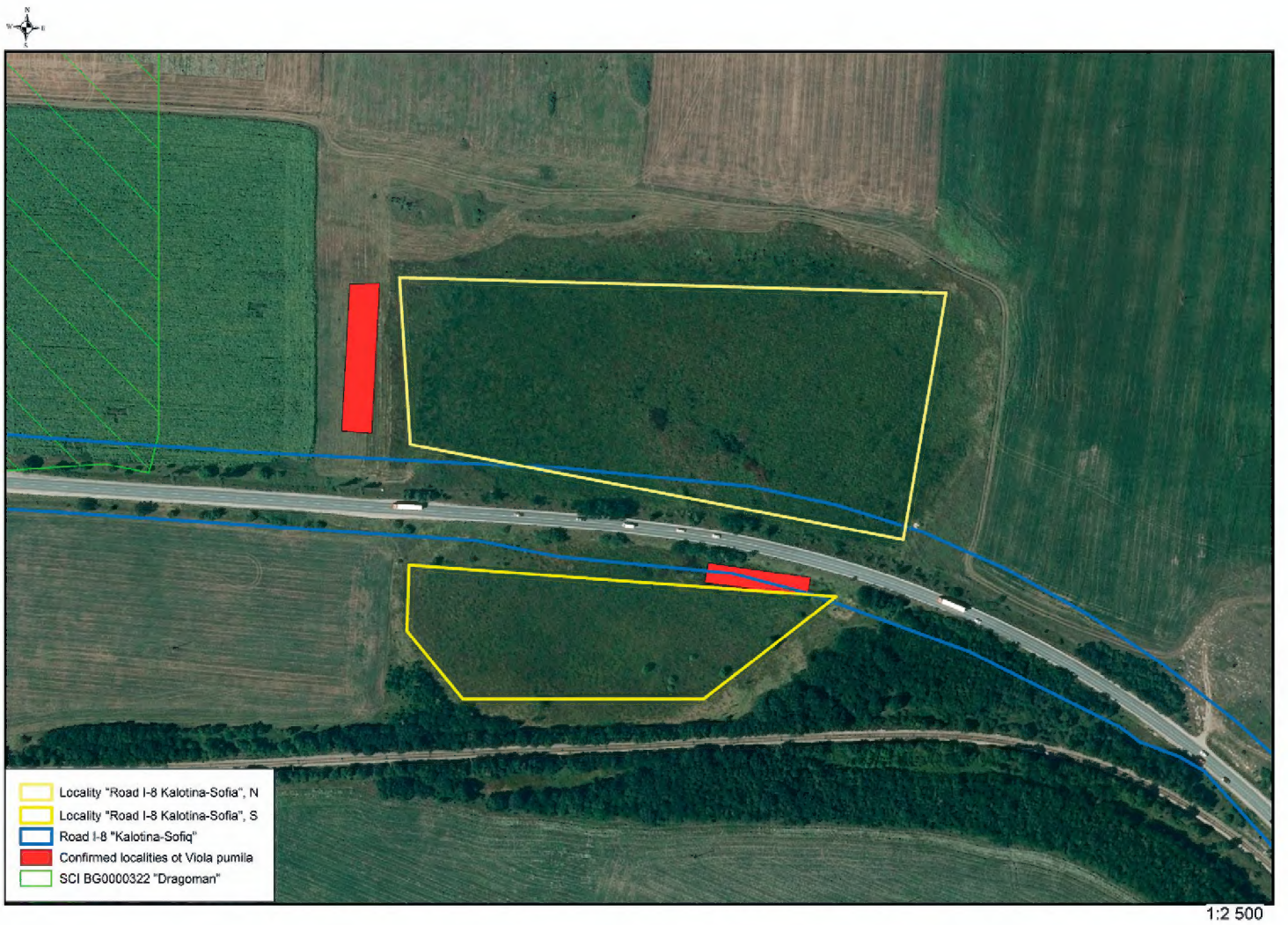

Figure 3. Plan for reconstruction of the existing road as part of Europe Motorway and position of two localities of *V. pumila*.

During our field surveys we identified a total of 50 individuals of *V. pumila* formed in 8 tufts. The other species detected around the violets were: *Alopecurus pratensis*, *Deschampsia cespitosa*, *Lysimachia vulgaris*, *Molinia caerulea* and *Potentilla reptans*.

The identification of a suitable new location was performed with the idea that it had to be identical to the current habitat of the plants. The new habitat had to possess similar physical characteristics, species composition, soil-climatic conditions and the water regime had to be similar to those of the original habitat. Several possible variants of suitable terrains were considered for creating the new habitat for the violets.

As a result of the analysis of all collected data, we calculated that possible sites for relocation of the species were the following four areas from the territory of the village of Aldomirovtsi: land properties with the following numbers 00223.257.32, 00223.257.62, 00223.257.68 and 00223.257.186. These terrains were municipal property and were managed by the municipality of Slivnitsa. All four land properties fall within the boundaries of the Aldomirovsko Blato protected area. The area was declared for the purpose of preserving the natural habitats of protected and rare species of waterfowl and 40 species of higher plants. The land properties also fall within the boundaries of a Natura 2000 site (SCI BG0000322 Dragoman). Another land property was identified as a possible variant – 00223.257.294 from the territory of the village of Aldomirovtsi. In this sector is located one of the most numerous populations of *V. pumila* in the country. However, the crucial disadvantage of this land was that it is private property and falls outside the boundaries of the Aldomirovsko Blato protected area.

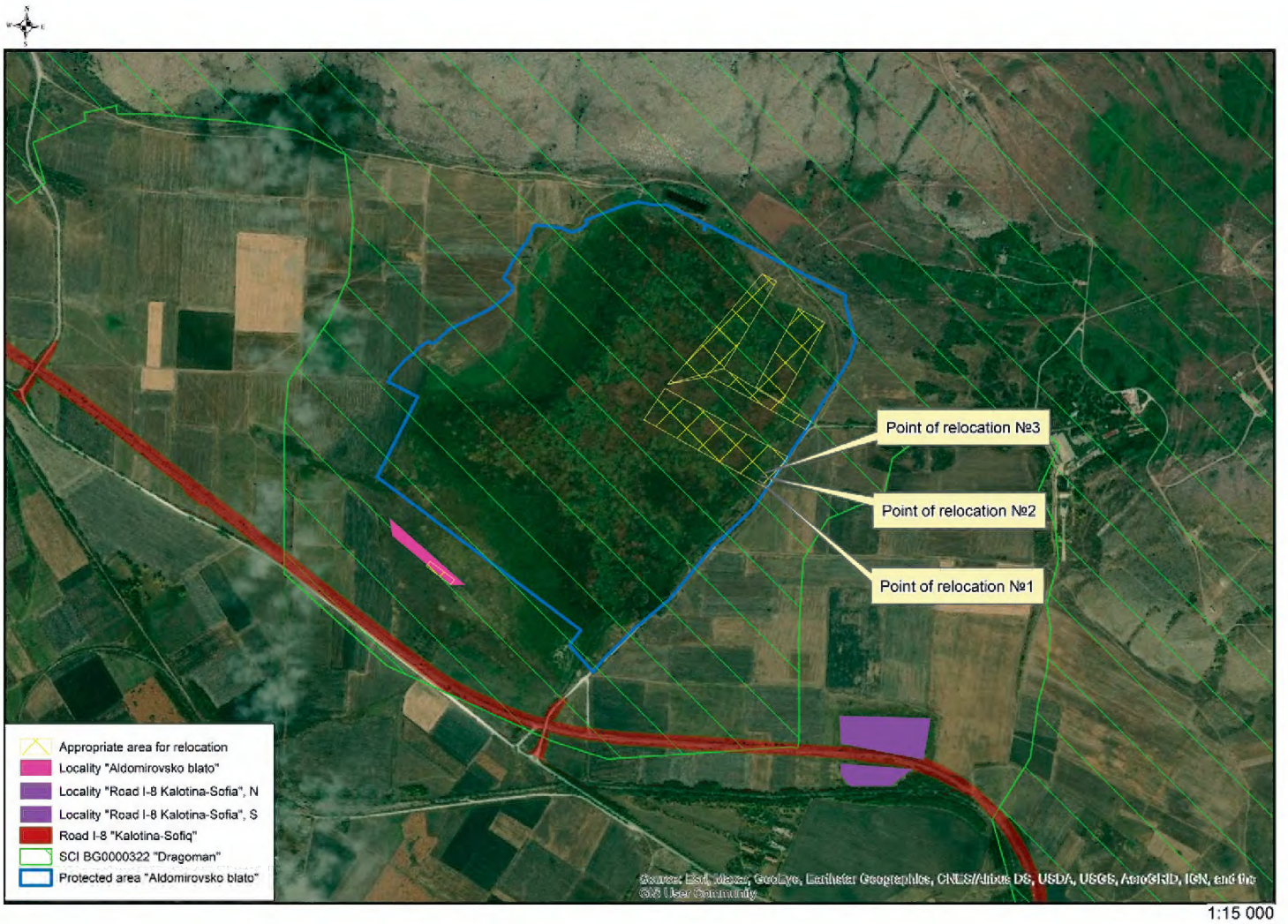

Figure 4. Sites of relocation of *V. pumila*.

After geodetic and ecological field surveys were performed, we selected as the most suitable property for the relocation of *V. pumila* – 00223.257.68 from the territory of the Aldomirovtsi village (Fig. 4). That terrain met the requirements of the meadow violet concerning the conditions of the soil, the vegetation and the water regime. We selected floodplain meadows, which were moist as water was retained above the soil surface in the local depressions. The soil in the habitat was of "meadow-swamp" type. It was covered by hydrophilic, mesophilic and xeromesophilic grass communities.

Part of the selected terrain was occupied by natural habitat 3150 "Natural eutrophic lakes with Magnopotamion or Hydrocharition", and another part by natural habitat 6510 "Lowland hay meadows" (Fig. 5). Because we worked with a very rare plant species with few remaining localities in the country, as well as because of the small population subject for relocation (only 50 specimens), we decided not to divide the individuals and provide experiments to grow them in different conditions. We identified the most optimal suited location and planted all specimens together.

The other three potential land properties from the territory of the Aldomirovtsi village were occupied only by a natural habitat "3150 Natural eutrophic lakes with Magnopotamion or Hydrocharition" and were flooded almost all year round. On that basis, we assessed them less suitable for the relocation of the violets.

A key step in the initial phase of our efforts was the coordination of our actions with the local municipality. The new habitat was in a municipal property, which allowed for regular grooming of the terrain and monitoring of the plants. With the next

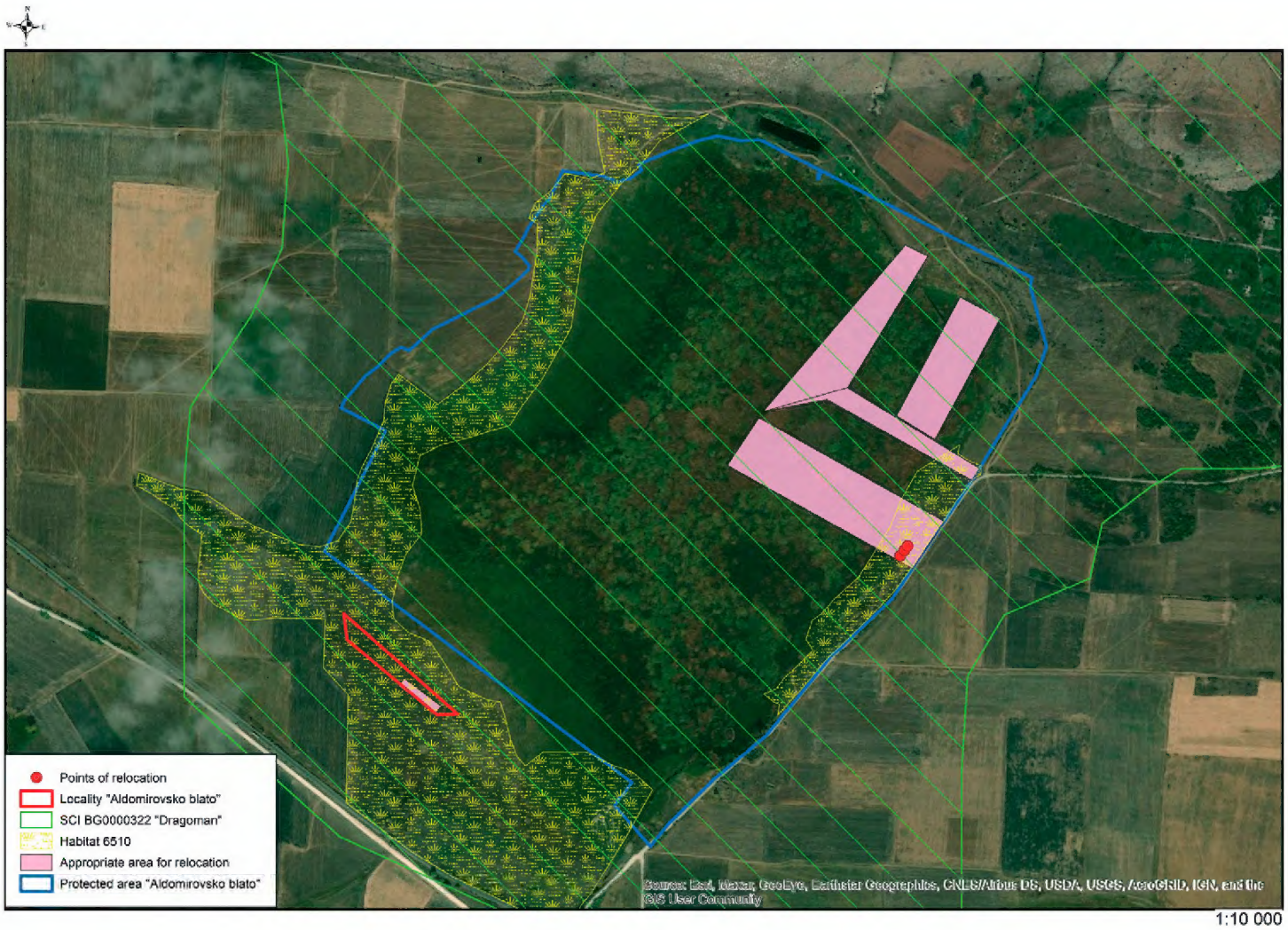

Figure 5. Location of the new habitats of *V. pumila* in natural habitat 6510 Lowland hay meadows.

step we prepared an Action plan for all activities. The plan and the technology for the permanent relocation of the species were reconciled by the relevant competent authorities and implemented in full scale.

The relocation of the *V. pumila* specimens was accomplished before the end of the vegetation period – in our case before the full rupture of the seed boxes (Table 1). We aimed the rupture of the seed boxes and the dissemination to occur on the spot of the new location in order to improve the chance of successful seed generation and development of the next vegetation (see Volis 2019). According to Eckstein et al. (2006), the vegetation period of the species ends in the October – November period when the upper sections of the plant die out and the violets prepare for overwintering.

After the establishment of the new locality, we performed in situ care until the complete adaptation of the plants. This included watering and mowing around all of the three new micro- localities. In the first month after the relocation (June), regular watering was carried out (every 3 days), and during the next two months (July and August) – every 7 days. Mowing was carried out on an area of 4 square meters around each of the micro-localities in the middle of July. The two-year follow-up monitoring of the new sites and adjacent areas of habitat 6510 "Lowland hay meadows" showed success in preserving the relocated specimens and also their successful reproduction (Fig. 6)

In October 2019, a large-area fire was detected within the Natura 2000 site SCI BG0000322 Dragoman and it reached the territory of the new locality of *V. pumila* (Fig. 7 and Fig. 8). None of the three plots with relocated plants was affected by that fire.

Figure 6. Condition of the relocated plants: **a** successful replanting **b** rupture of the seed boxes **c** dissemination.

Volis (2019) described two main successive stages that plant species go through after reintroduction to a new location. The first stage begins with the flowering of a mature (relocated) plant. The second involves the establishment of an entirely new reproductive individual at the site of transmission (reintroduction). The second stage consists of smaller phases associated with the appearance of reproductive organs, feeding flowers and seeds, scattering the seeds in places, with conditions for germination and survival of the young plants. In *V. pumila* we detected flowering in a mature adult in the year after reintroduction – the first stage indicated by Volis was successfully completed. The implementation of the second stage began in the year of relocation (2019) with the scattering of seeds from the seed boxes of the transferred adults. The full completion of the second stage required more time due to the specific features of the species *V. pumila*. The fact that there are flowers from adults a year after reintroduction, (Fig. 9), as well as cracked seed boxes in the year of reintroduction (Fig. 6b), gives us hope for the successful completion of the second stage.

In the early summer of 2021 (early July) we started the monitoring of the new locality of the violets in the second year after relocation. We identified new juvenile specimens with different heights from 2 to 7 cm, which were in the vegetation phase between the first and third pair of true leaves (Fig. 10). They all sprouted after the plants were relocated. All of the young plants were found at relocation points 2 and 3 (Fig. 4) – in point 2 there were found seven young specimens, and in point 3 there were two young specimens. The young plants were located outside the main tuft.

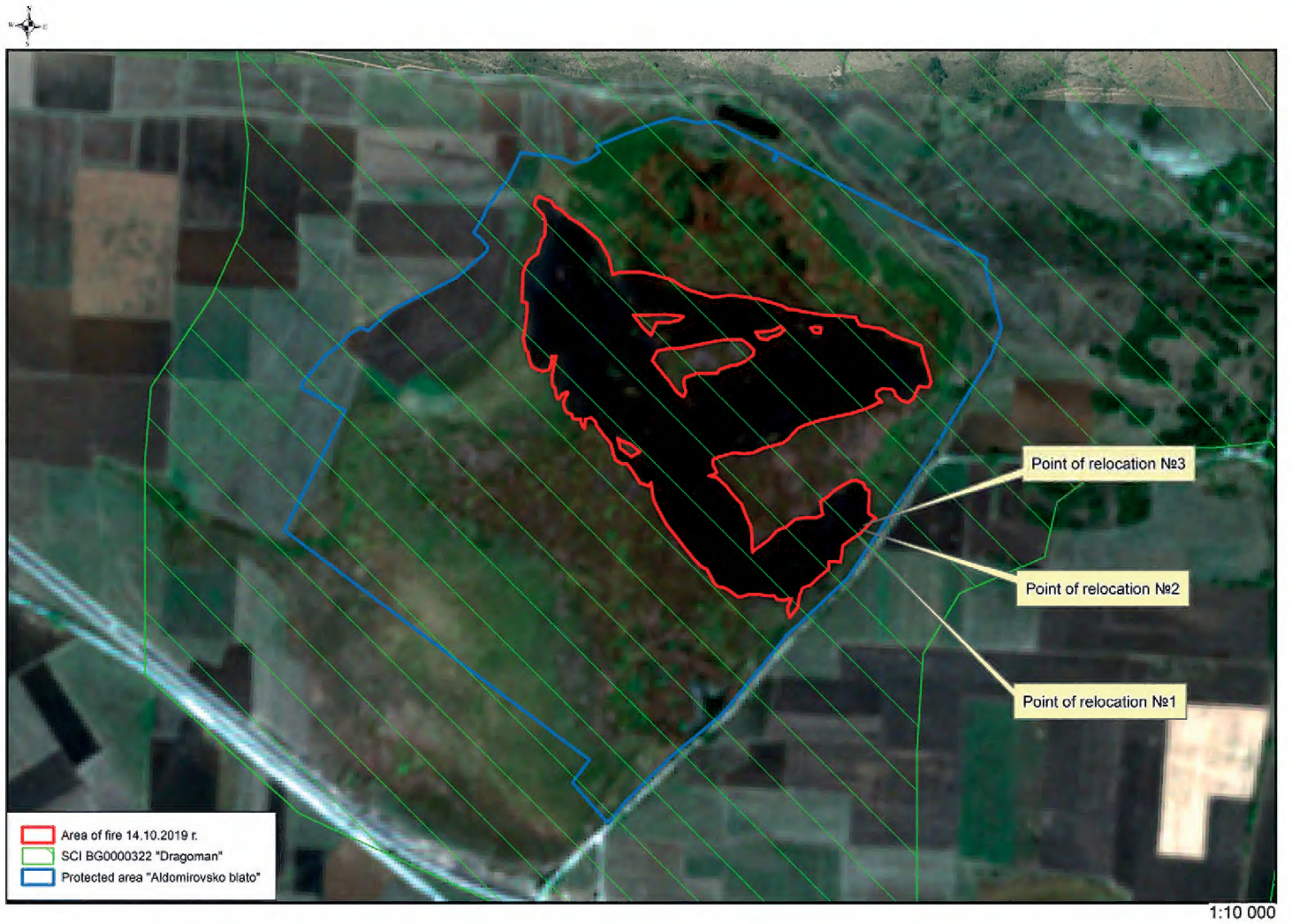

Figure 7. Map of the fire in the area of the new locality of *V. pumila*.

Figure 8. Pictures of the three working polygons of the new locality which are not affected by the fire: **a** picture of polygon number 1 represented on Figure 07 **b** picture of polygon number 2 represented on Figure 07 **c** picture of polygon number 3 represented on Figure 7.

Figure 9. Photograph of blooming (late phase) in *V. pumila* detected in the spring after the fire.

Figure 10. New young plants obtained from seeds of different heights and vegetation phases.

Discussion

The cycle of regeneration of the species at the new location (including transfer from the original location to the new one) included several successive stages. The process started with the planting of the mature adult specimens in a new location and ended with the establishment of a new reproductive local population. Intermediate phases in the establishment of that new locality included: production of living pollen, building of knots, development of seeds, dissemination and growing of a viable young plant (Volis 2019). With the discovery of juvenile (young) specimens of *V. pumila* at the site of relocation, it can be considered that the second (final, concluding) phase described by Volis (2019) has been successfully completed.

The newly established localities of *V. pumila* were saved from the fire in 2019 (see results) due to the special algorithm of the cultivation of the relocated plants and, especially, measures related to mowing the habitat.

The fires are often related to the functioning of the entire ecosystems. They can start spontaneously or may have anthropogenic origins. The fires impact the biodiversity and may change the structure of the ecosystems and the landscape (Whelan 2009, Argañaraz et al. 2015). In 2000, a bulletin was published for Australia, which determined the impact of fires on wetland flora (similar to that of the habitat of *V. pumila*). According to Allen (2000), the plants can survive the effects of fires by regenerating dormant buds from stems or roots, while fire can cause the germination of seeds of some plants stored in the soil. If a fire occurs during an inappropriate growing season, it would result in the loss of stocks of seeds or mature plants. The strength and intensity of the fire depends on the season of occurrence, which affects the plant communities inhabiting the wetlands. Fires in this type of habitat may destroy a significant part of the existing dry biomass, which is above the water level, as well as nearby terrains with mesophilic vegetation. It should be noted that due to the cares taken during the first year of reintroduction (cleaning of the area and mowing around the nests with the reintroduced plants) the fire's boundaries (demonstrated on Fig. 7) did not affect the violets, but all of the vegetation in the vicinity was burned out (Fig. 8 a,b,c). We can conclude that taking proper measures for the plants in the first year of their relocation is key to both their successful adaptation and the prevention of damage.

We selected to relocate the saved plants in one particular habitat of type 6510 "Lowland hay meadows", which has good representativeness and degree of conservation in SCI BG0000322 Dragoman.

However, at biogeographical level, the habitat 6510 is not in good condition due to anthropogenic threats (Eionet CDR 2020). In the RUHD (2015) for Bulgaria, no habitats were reported to be in "Unfavorable – Bad" condition. According to the second report for Republic of Bulgaria concerning the period 2013–2018 (RUHD 2020a), for the Continental Biogeographic Region (CON) the assessment of the conservation status of three grassland habitats was "Unfavourable – Bad" (U2) and one of these habitat was habitat 6510 "Lowland hay meadows". Because of the poor assessment of the condition of the habitat 6510 "Lowland hay meadows" in RUHD

(2020b), concerning the parameters "Future perspectives", the conservation measures have to be focused on the reduction of the pressures and the threats.

According to Apostolova and Meshinev (2015) the most important threats for the habitats of *V. pumila* are related to the conversion of mesophilic meadows into arable land through habitat drainage. As necessary measures, the authors pointed out the maintenance of the haymakingregime in the sites and maintaining a moderate degree of disturbance within populations. Tzonev and Gusev (2020) identified the following main threats to habitat 6510 "Lowland hay meadows": the abandonment of haymaking, overgrazing, the ploughing for arable land, and changes in the water regimes. The measures they offer for the conservation of the habitat are related to the restoration of the haymaking use, change of the mowing terms and the reduction of the grazing intensity. Brzank et al. (2019) reported that the mowing delay or sporadic mowing lead rapidly to significant changes in species composition in the habitat. The drying of marshy soils leads to fast mineralization, carbon dioxide and nitrogen release, encroachment of nitrophilous plants, and the disappearance of peat earth layer. According to Martin et al (2018), pressures such as agricultural intensification, abandonment, lack of mowing and the application of natural fertilizers (such as slurry) are the largest threats to the conservation of the habitat 6510

"Lowland hay meadows". As appropriate conservation measures, the authors propose implementation of management plans and targeted agri-environment schemes, and engagement with landowners and other stakeholders.

Our results indicate that the most important conservation measures which are essential for the management and protection of habitat 6510 "Lowland hay meadows" in the CON Biogeographical Region (incl. the Natura 2000 site SCI BG0000322 Dragoman) are as follows: i) regulation of the haymaking regime of the meadows; ii) regulation of grazing of farm animals; iii) conversion of abandoned arable land into meadows; iv) restoration of the water regime of the meadows where it was disturbed; v) creation and implementation of incentives and compensatory mechanisms for the owners of private lands in which the habitat is distributed; vi) fire prevention; vii) surveillance of the status of the meadows and data collection on the structure and function of the habitat in the particular Natura 2000 sites; viii) updating of the information in the Standard Data Forms (SDFs) of Natura 2000 sites; ix) raising the awareness of the stakeholders by conducting regular information campaigns aimed at the local public, as well as by providing publicly available up-to-date information by the competent authorities, and scientific and environmental non-governmental organizations.

Conclusions

The successful relocation and the creation of new habitats for rare and endangered plant species can be achieved by adhering to a certain algorithm of actions. By setting the site specific conservation objectives (Commission note, 2012) and the necessary conservation measures for habitat 6510 "Lowland hay meadows" in SCI BG0000322

"Dragoman", the original and the new localities of *V. pumila* should be taken into consideration. The determination of the site specific conservation measures in SCI BG0000322 "Dragoman" and the territories where they will be applied (the specific landfills / properties) should be implemented with the active participation of the relevant stakeholders (Commission note 2013). The management plans for Natura 2000 sites could represent an appropriate tool for this (NEC 2019). The availability of reliable up-to-date information on the distribution and condition of *V. pumila* and its habitats is crucial in order to implement proper management and conservation policy.

Author's contribution

MK and NN provided the field surveys, all of the relocation activities and the monitoring of the condition of the plants. MK issued the time tables, NN organized the coordination with the competent authorities and the government institutions. Both authors wrote the manuscript, prepared the maps and designed the figures.

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