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Development of the Hydrophytic Structure of the Bioplateau Type for the Purification of Water Bodies From ^{137}Cs

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Abstract. A floating structure of a bioplateau has been developed for the purification of water bodies from the toxic substances, the biotic component of which is terrestrial plants. Chemically inert floating materials were used as a substrate for the construction of the bioplateau: perlite, expanded clay, granular foam, vermiculite, cork. Substrate testing has shown that granular expanded polystyrene is the most optimal for the usage. The study of different options for seed germination in the design of the bioplateau has shown that its placement on the top of the substrate is the best option. The usage of perlite in combination with granular foam had created an additional capillary effect, due to which the seeds germinated at a faster rate. To optimize the hydrophytic structure a grid was used, which made it possible to increase the overall density of the bioplateau. An algorithm for creating a «rolled» plant that is suitable for transportation and placement in the surface water bodies that require purification from toxic substances has been developed. There was done a comparative study of the effectiveness of purification of the aquatic environment from ions of radiocesium bioplate with wheat plants of different varieties. It is confirmed that the efficiency of sorption of ^{137}Cs ions by terrestrial plants is influenced by the nature and concentration of macrocations in the aqueous medium.

Key words: water purification, phytoremediation, bio plateau, terrestrial plants, radionuclides, ^{137}Cs.

Introduction

Anthropogenic activities and emergencies have led to significant pollution of the environment, in particular water bodies, which is gaining global scale and causing undesirable consequences for humans and ecosystems (Isaienko, et al., 2018; Pshynko & Honcharuk, 2019). According to the forecasts of the Ukrainian experts, further intensive industrialization will lead to irreversible environmental changes and a catastrophic reduction of relatively clean freshwater resources in Ukraine as a source of drinking water (Iatsyk et al., 2014; Tomiltseva, 2017).

Taking into account the results of monitoring studies in recent years, surface waters of Ukraine are classified as water quality class 3-5, and the most environmentally hazardous toxicants are radionuclides (^{137}Cs) (Demchuk et al., 2010;

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Nigmatullina & Fazlyieva, 2018; Seregin & Ivanov, 2001).

Traditionally, chemical and physicochemical methods are used for wastewater treatment, which have a limited scope, certain advantages and disadvantages and mostly do not allow achieving the normative values of residual concentrations of pollutants entering and accumulating in surface aquatic ecosystems (Filatova, 2015; Pshinko et al., 2018; Vukcevic et al., 2014). Therefore, to improve the ecological status of water bodies, it is necessary to create effective, environmentally safe and cost-effective, compared to existing methods, systems for restoring the quality of large volumes of aquatic environments, which is developing new or improving existing methods of extracting these ecotoxicants from water bodies.

Nowadays much attention is paid to the use of phytoremediation technologies to improve the condition of water bodies. In world practice, various phytoremediation systems are used, in particular hydrophytic structures using higher aquatic plants and aquatic biota (Boog et al., 2019; Nivala et al., 2018; Romanenko et al., 2012; Sharma et al, 2015; Volkova et al., 2018; Xie et al., 2013).

Traditionally, the functioning of the bioplateau is based on the transiting of contaminated water through a system of biofilters located on the soil surface. This treatment scheme makes existing types of plateaus limited in terms of mobility and capacity, and the use of higher aquatic plants as biological components complicates the operation of the plateau. However, it is known that not only higher aquatic plants are characterized by high levels of accumulation of ecotoxicants, but also higher terrestrial plants in aquatic (hydroponic) culture have a high sorption capacity for radionuclides and toxic metals (Lapan et.al., 2019; Lapan et. al., 2020; Mikheev et. al., 2017). Therefore, despite the achievements in the usage of bioplateau, the need for scientific justification for the development of phytoremediation technology, which consists of studying the absorption capacity of terrestrial plants and creating an effective hydrophytic structure such as bioplateau, is an extremely important task.

The process of accumulation of radiocaesium bioplateau with plants can be influenced by a number of factors, in particular by the varietal characteristics of plants, the presence and concentration of macrocations typical of natural reservoirs.

The optimal content of macrocations, typical for natural waters - Na +, K +, Ca2 +, Mg2 +, in the environment is necessary to ensure the processes of metabolism, growth, gene expression, photosynthesis, synthesis of proteins, carbohydrates and other important vital processes in the plant body. It is known (Cornell, 1993) that under natural conditions, even with a relatively high level of radionuclide contamination of the environment (soils or water bodies), the concentration of ^{137}Cs is in many times lower than macrocations, so the efficiency of ^{137}Cs sorption largely depends on the presence and concentration of these elements that can act as specific and non-specific competitors (isotopic or non-isotopic carriers).

Physiologically complete mineral composition of drinking water must meet the following indicators by cations: K^+ – 2–20 mg/l, Ca^{2+} – 25–75 mg/l, Mg – 10–50 mg/l, Na – 2–20 mg/l (DSanPin 2.2.4-171–2010, 2010).

The study was conducted with the purpose of the experimental development of method of water purification of ^{137}Cs ions using a new design of a bio plateau, which is based on the use of terrestrial plants.

During the development of the floating bioplateau with the use of terrestrial plants that have the maximum ability to accumulate radionuclides, the following tasks were set and solved:

- searching for species of terrestrial plants that are able to grow in conditions of high humidity;
- testing of different types of substrates that provide high buoyancy and close connection with the root system of plants;

- testing of substrates and plants for the formation of the floating bioplateaus;
- searching of the optimal means of seed germination (upper location according to the substrate, mixing with the substrate);
- optimization of the hydrophytic structure;
- study of sorption properties of bioplateau with wheat plants of different varieties in relation to ^{137}Cs;
- study of the effect of macrocations on sorption ^{+}Cs.

Material and Methods

As biosorption material the usage of intact higher terrestrial plants and their isolated parts (mainly leaf-stem) is evaluabled. Plant material is an element of the biofilter, which is a system that uses the sorption properties of the root system of intact plants.

The second stage in the construction of a floating bioplateau was the searching for a substrate for the development and growth of the plants. The chemically inert floating materials, such as perlite, expanded clay, granular foam, vermiculite, cork are used. These substrates must meet the following requirements: non-toxicity to plants; minimum porosity – to minimize the ingrowth of roots into the granules of the substrate and ensure the buoyancy of the bioplateau structure.

The third stage in the construction of a floating bioplateau was the combination of different options of seed and substrate. To obtain the required hydrophytic system, a combination of seed variants of promising higher terrestrial plants and substrate was studied. The method of placing seeds on the top of the substrate, below and the method of mixing the substrate with plant seeds was used.

To construct a bioplateau by the method of placing seeds below the substrate, the bottom of the cuvette measuring 21 × 12.5 × 2.5 was covered with seeds, cm^3: peas (40), corn (40), barley (25), oats (25); poured granular foam (1.5 cm); added 100 ml of settled water from the water supply; placed in a thermostat at $t = 24$ ℃.

Next, a bioplateau was created by mixing the seeds with the substrate: pre-soaked for 8 hours corn (40 cm^3) and peas (40 cm^3) were used. Foam was poured into 21×12.5×2.5 cuvettes and mixed with seeds. 100 ml of settled water from the water supply was added and placed in a thermostat at $t = 24$ ℃.

The construction of the bioplateau by the method of placing the seeds on top of the substrate was performed in the following order: a cuvette of size 21×12.5×2.5 was used; the bottom was covered with a layer of granular foam (500 cm^3); the surface of the granular foam was moistened; 100 ml of settled water from the water supply was added; with a layer of perlite (70 cm^3) was covered; perlite was moisturized; on the surface was placed the seeds (cm^3): hemp (25), mustard (25), rye (25), oats (25), amaranth (3), flax (15), millet (15), barley (25), rape (10), corn (40), thyme (5), oatmeal (5); the seeds were covered with a layer of perlite (50 cm^3); then it was placed in a thermostat at $t = 24$ ℃.

To optimize the hydrophytic structure of the bioplateau, a supporting mesh and perlite was used, poured on top of granular foam (70 cm^3).

The design of the bioplateau for the using in the field.

A cuvette measuring 30 × 40 cm was used; a grid was placed on the bottom of a cuvette; the bottom was covered with a layer of granular foam 1.5 cm thick; a layer of perlite was poured on top of the foam; 200 ml of water was poured into the cuvette; during further germination another 600 ml of water was added; a spray to moisten the surface of the substrate was used; a mixture of seeds: corn (200 cm^3) - barley (100 cm^3) - meadow thyme (10 cm^3) was used; a thin layer of perlite was sprinkled on the seeds; the cuvette was placed in a plastic bag to create a wet chamber.

The cultivation of plants in a solution of cesium-137 chloride was performed in glass containers, which were pre-treated for 3 days with 0.1 mg solution of stable cesium chloride to prevent sorption of cesium radioisotope ions by the inner surface of the glass. Settled tap water was used. Once a day the solution was poured into a Marinelli vessel to determine the specific activity of the radionuclide on a gamma spectrometer SEG-001 "APK-C" -63. The initial specific activity of radiocaesium was 2.0-2.2 kBq / l, which according to previous experiments did not cause a significant effect on plant growth and development. Measurements of the specific activity of Cs-137 were performed with the measurement error of 3.8%.

The degree of purification from ^{137}Cs (*DP*, %) was calculated as follows:

$$DP = \frac{(A_0 - A_p)}{A_0} \cdot 100,$$

where A_0, A_p – respectively, the activity of ^{137}Cs ions in the initial solution and in the solution after sorption, kBq/l.

The concentration of Cs^+ ions was determined in the selected samples with a volume of 20 ml on the atomic absorption spectrophotometer C-115-M1 at a wavelength of λ = 852.1 nm. The concentration of Cs ions was determined using a mixture of acetylene with air. The optimal range for determining the concentrations of Cs^+ ions was 5–50 mg/l.

Results and Discussion

At the first stage of the study, pea, corn and barley seeds were placed at the bottom of the substrate - foam. After 3 days of seed germination – peas almost did not germinate, corn and barley showed good results - the seeds began to germinate. After 7 days of incubation, the barley germinated well - the length of the shoots reached about 10 cm; in the version of the bioplate with corn – about 3-5 cm. Peas have sprouted up to 3 cm, there is a bacterial infection of plants. Thus, the construction of a bioplate by seed germination from below does not meet the requirements for hydrophytic structure: the substrate of the bioplate is not necessarily bound by the root system of plants, the buoyancy of the structure is not ensured.

The pea and corn seeds were used to study the variant of seed germination mixed with the substrate. The polyfoam acted as a substrate. It was found that corn mixed with the substrate for 7 days sprouted about 3 cm, peas are not a promising plant, because it has a high level of bacterial infection. Thus, the construction of the bioplateau by mixing seeds with the substrate also does not meet the requirements for the bioplateau: the substrate of the structure is not necessarily bound by the root system of plants, the buoyancy of the bioplateau is not provided.

Another option for designing of the bioplateau was the germination of seeds on top of the substrate. The studies have shown that in all variants of the combination of foam with plants, there was an effect of binding the substrate to the root system and there was a high level of buoyancy of the bioplateau. A weak effect of substrate binding to the root system was observed by combining vermiculite with plants. The variants of the combination of the cork with plants showed a weak effect of binding of the substrate to the root system, as a result of which the buoyancy of the bioplateau is not ensured. Combining expanded clay with plants also gave a weak effect of binding of the substrate and the root system, due to the low buoyancy of the bioplateau.

In the course of experimental studies, it was found that the placement of seeds for germination on top of the substrate is the best option for its germination, which in its turn allowed to obtain a dense structure of the bioplateau for the further research.

The next task was to optimize the hydrophytic structure. To minimize the edge effect (looseness at the edges of the bioplateau), it was decided to use a fine-grained mesh, which allowed to increase the overall density of the bioplateau. For this purpose, when constructing the bioplateau, firstly, a grid was placed on the bottom of the cuvette, then polyfoam and plant seeds (Fig. 1).

Fig. 1. Bioplateau with the using of fine-grained mesh.

To ensure more complete contact of germinating seeds with the substrate, perlite was used, because it in the combination with the foam creates an additional capillary effect, which provides faster seed germination. The components of the bioplateau were placed in the following sequence: mesh - foam - perlite - seeds.

The next stage of the work was the study of the buoyancy of the constructed bioplateau in the laboratory (Fig. 2).

Fig.2. Checking of the floatation of the bioplateau.

Thus, the constructed hydrophytic structure differed from the previous versions by the maximum density and homogeneity of the system, which ensured a high level of buoyancy and ease of transporting the bioplateau to water bodies.

The usage of the bioplateau in the field.

Since it is planned to use a bioplateau to clean xenobiotic-contaminated water bodies, one of the tasks was to test the possibility of transporting the proposed type of bioplateau for placement on the mirror of the reservoir. First of all, an important task was to minimize mechanical damage to bioplateau plants during transportation, so it was decided to focus on the practice of transporting lawn grass in the form of a roll. It was planned to find out the possibility of twisting the plateau into a roll for the purpose of transporting the hydrophytic structure to the required water bodies. In fig. 3 a bioplateau of the rolled type is presented.

Fig. 3. The bioplate that was prepared for the transportation.

The germination of plants to create a rolled bioplateau using a mesh gave a very good result: the bioplateau is quite dense, the root system binds the substrate well, which allows them to be easily twisted into rolls, makes them transportable – in other words, it is possible to deliver and place the bioplateau on the surface of reservoirs (Fig. 4).

Fig. 4. The location of the bioplate on the river.

The laboratory-built floating bioplateau, designed to purify water bodies, has been successfully transformed and tested in an open surface body of water.

To study the effect of plant varietal affiliation on the accumulation of ^{137}Cs wheat plants, bioplates with ten-day-old seedlings were cultured in ^{137}Cs solution. Measurements of the specific activity of 137Cs were measured after 5 and 24 h of incubation of plants in ^{137}Cs solution (Fig. 5).

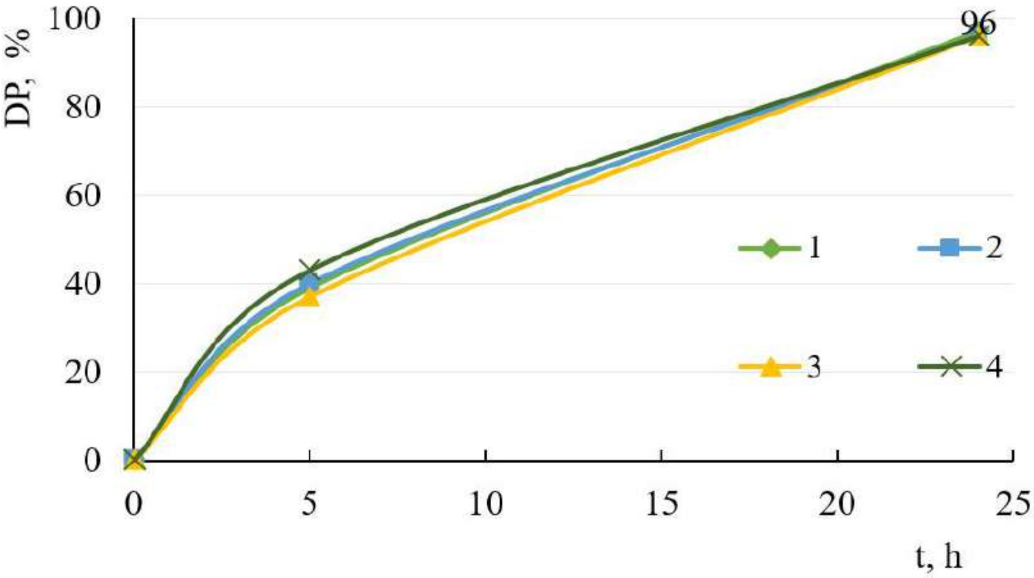

Fig. 5. Dynamics of ^{137}Cs uptake by wheat plants of different varieties; 1 – Polis’ka 90, 2 – Myronivs’ka 808, 3 – Favorytka, 4 – Dons’ka napivkarlyk, A (^{137}Cs)=2,0 кBq/l, Δ= 3,8 %.

It was found that the varietal affiliation of wheat plants at the end had almost no effect on the accumulation of ^{137}Cs. Thus, the degree of purification of water from ^{137}Cs ions will mostly be affected by the species characteristics of terrestrial plants, but taken the results above into consideration, even the species specificity is manifested only in the first stages of observation, and then it is leveled.

The effect of potassium, calcium, magnesium and sodium ions, characteristic of macrocations for natural aquatic environments, on the sorption of ^{137}Cs by terrestrial plants was studied. Fig. 6 presents the results of a study of the effect of the concentration of macronutrient ions on the sorption of Cs^+ by plants of common corn hybrid Dostatok 300 MW.

It is established that the influence of competing ions increases in the series $Mg^{2+} < Ca^{2+} < Na^+ < K^+$. Magnesium ions at the used concentrations had almost no effect on the sorption of ^{137}Cs, in contrast to calcium and sodium, the presence of which ions reduced the sorption of ^{137}Cs by only 10 % at a concentration of 100 mg/l. Potassium (a chemical analogue of cesium) inhibited the sorption of the latter to such a level at significantly lower concentrations - 10 mg/l. The reason for this may be the competition of these elements for K-transport proteins. It should be noted that the concentration of potassium in natural waters is quite low and is 3-10 mg/l.

The obtained results of the influence of Na^+, K^+, Ca^{2+}, Mg^{2+} on the sorption of ^{137}Cs ions by plants suggest a high level of purification of the water body provided that these competing ions are present in the aquatic environment even in high concentrations, which can be caused, for example, by potassium fertilizers or liming.

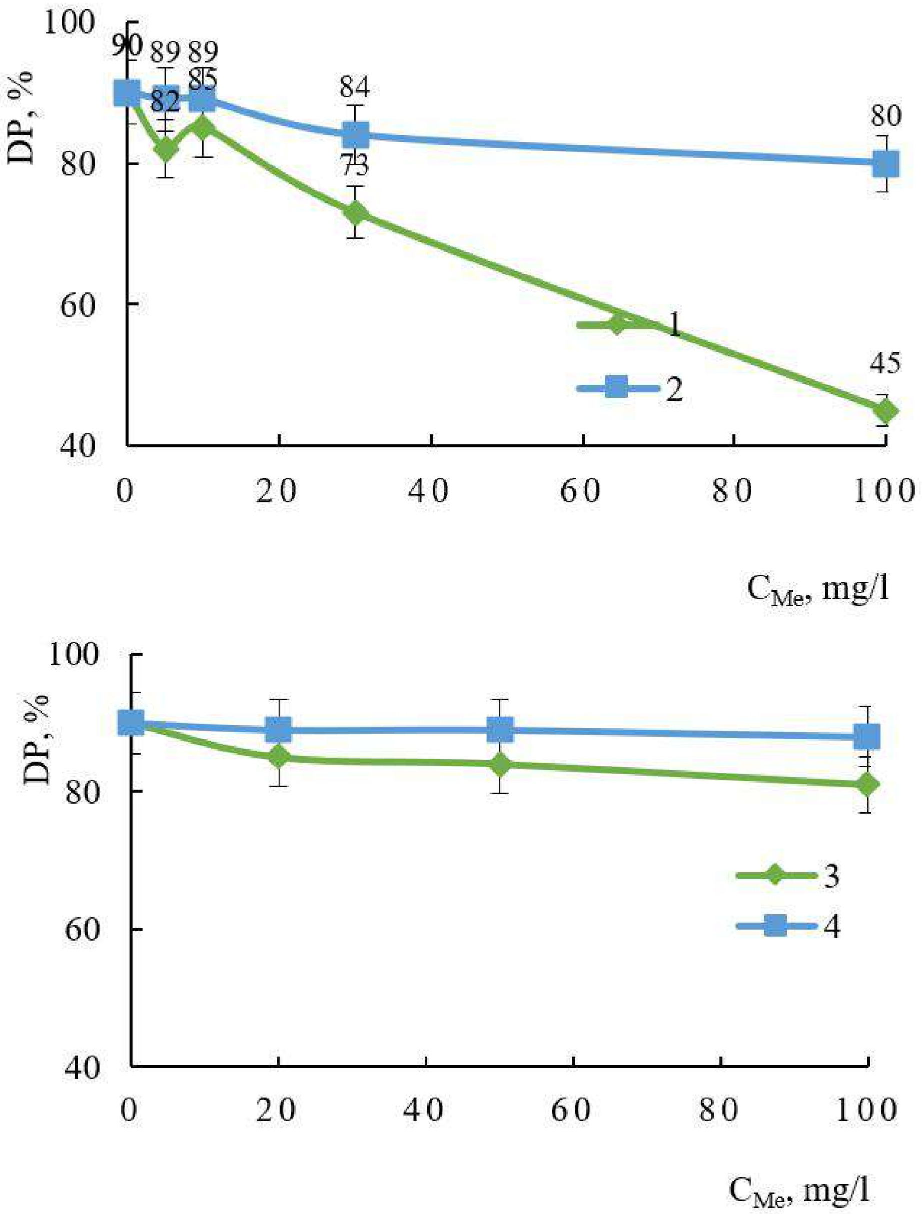

Fig. 6. The effect of the concentration of macrocations (C_{Me}) on the sorption of Cs^+, C_0 (Cs^+) = 10 mg/dm^3, $V_{solution}$ = 500 cm^3, $t_{sorption}$7 days, 1 – K^+, 2 – Na^+, 3 – Ca^{2+}, 4 – Mg^{2+}.

Conclusions

Thus, a new method of constructing a floating structure of a bioplateau for purification of reservoirs from toxic substances, the biotic component of which is terrestrial plants, has been developed.

Tests of several types of floating substrates have shown that granular expanded polystyrene is the most optimal for use. It is established that the placement of seeds on the top of the substrate is the best option for its germination. The use of perlite in combination with foam created an additional capillary effect, so that the seeds germinated faster. The required density of the bioplateau, in particular the sealing of the edges of the bioplateau, was provided by a mesh with a small mesh.

The parameters of the bioplateates obtained in the experiments make it possible to transport them in the form of a roll for placement on the surface of reservoirs that require purification from toxic substances.

The sorption properties of bioplateau with wheat plants of different varieties in relation to ^{137}Cs were studied. In the research it was possible to achieve a high (more than 90%) level of water purification from this radionuclide.

It is confirmed that the efficiency of sorption of ^{137}Cs ions by terrestrial plants is influenced by the nature and the concentration of macrocations in the aqueous medium, their influence decreases in a number $K^{+} > Na^{+} > Ca^{2+} > Mg^{2+}$.

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Demographic Parameters of the Grey Partridge (Perdix perdix L., 1758) in Upper Thracian Plain (Bulgaria)

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Abstract. The Grey Partridge (*Perdix perdix*) is a common gamebird which population decreased dramatically across Europe, including Bulgaria as well. While recent studies report continuing declines in numbers, sufficient data on basic demographic parameters of the species in Bulgaria are scarce or lacking. Main objective of the present study is to provide up-to-date information on Grey Partridge demography in the arable lands of the Upper Thracian Plain (south Bulgaria). Breeding density was 6.27 ± 4.73 SD (min – max 0 - 20) pairs/km^2 and density in August averaged 57.32 ± 41.82 SD (min – max 0 – 148.06) individuals/km^2 during the monitored period (2018 - 2020). The Generalized Linear Model showed significant decrease of both breeding (Wald stat. = 65.47, $p < 0.0001$) and August (Wald stat. = 533.860, $p < 0.0001$) densities from west to east. However, direct reproduction parameters showed no geographic gradients and had relatively high mean levels as mean brood size was 9.0 ± 5.3 SD (min – max 1 - 26) young/successful pair (n = 163), chick survival rate up to six weeks of age was 0.56 ± 0.22 SD (min – max 0.19 – 0.94), brood production rate was 84.6 ± 20.9 SD (min – max 0 – 100%) and reproductive success was 3.96 ± 1.85 SD (min – max 0 - 8.5) young/adult bird. The present study shows highly unequal Grey Partridge breeding densities within the study area and suggests that diverse approach for management and restoration of the Grey Partridge will be needed across different parts of the Upper Thracian Plain.

Key words: breeding density, brood size, chick survival rate, brood production, spatial trend.

Introduction

In the lowland and hilly areas of Bulgaria, as well as through most of its European range, Grey Partridge (*Perdix perdix*) was a common gamebird with a high population density. However, since the middle of 20th century it is declining probably in all 31 countries within its range (McGowan & Kirwan, 2015). The most rapid decline in continental Europe occurred in the period 1960 - 1980 and continued in lower rate afterwards (Kuijper et al., 2009). Nowadays Grey Partridge is considered non-threatened species but with a decreasing population trend (Birdlife International, 2016). It is well documented that the dramatic decline is caused mainly by intensification of agriculture (Báldi & Faragó, 2007; Potts, 1986). Reduction of chick survival and suitable nesting cover and as a consequence increased predation are the factors believed to reduce Grey Partridge numbers by the late 20th century to less than 10% of those in 1950s (Aebischer &

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Kavanagh, 1997). Over the last two decades Grey Partridge densities remain under 5 pairs/km^2 over the most lowland territories in many European countries (Bro et al., 2005; Newson et al., 2005; Panek, 2006; Ronnenberg et al., 2016; Šálek et al., 2002) while breeding densities > 20 pairs/km^2 are scarce and derived from local habitat factors more likely established by chance (Bro et al., 2005; Šálek et al., 2004). Recently reported results from long-term studies show continuing decrease in some reproduction parameters of the species (Panek, 2019). Breeding density, reproductive success, brood production and chick survival rate are amongst the most important demographic parameters indicating the adverse effects of modern agriculture on Grey Partridge populations. Therefore, they have been studied across Europe either to estimate population status and determine the causes of decline (Bro et al., 2005; Panek, 2019; Potts & Aebischer, 1995) or to plan and assess effectiveness of applied habitat improvement measures (Buckley et al., 2021; Ewald et al., 2012).

Decline of Grey Partridge population has been observed in Bulgaria as well. In the middle of the 20th century the species was described as common in plains and low mountains of the country (Patev, 1950) and during the first half of the 1980s breeding population reached about 600 000 ind. (Simeonov et al., 1990). Decrease in Grey Partridge numbers took place afterwards and two independent studies reported estimates of 20000 – 30000 pairs in 2004 (Nankinov et al., 2004) and 10000 - 25000 pairs in 2007 (Gerasimov & Mitev, 2007). Detailed data on breeding density and reproductive parameters of the Grey Partridge in Bulgaria are currently missing apart from a study in Sakar Mountain in which low breeding densities (1.23 ± 0.19 pairs/km^2) and a reproductive success of 2.97 young/adult individual are reported (Gruychev & Angelov, 2019). In Bulgaria, the Grey Partridge is subject to hunting (Hunting and Game Preservation Act, 2000) and it is one of the most preferred gamebirds by Bulgarian hunters, especially in lowland hunting districts of the country. In attempt to maintain sustainable harvest of the species, each year the hunting clubs set hunting bag limits based on the estimated spring density, theoretical reproductive success, expected winter losses (after closing the season at the end of November) and the target density for the next spring.

Nowadays, the western parts of the Upper Thracian Plain are believed to sustain one of highest Grey Partridge densities in Bulgaria but over the last 30 years there are no published data on basic demographic parameters of the species in this part of the country. Over this period major changes in agricultural practices took place, especially after Bulgaria joined the EU in 2007, but their influence on the population dynamics of the species remains unclear. The aim of this paper is to present up-to-date information on Grey Partridge density and reproduction parameters of the population in the Upper Thracian Plain as a basis for current population status assessment and adequate research and management planning.

Materials and Methods

Study area

The study area is located in the Upper Thracian Plain (South Bulgaria) (Fig. 1) and covers a total of 4869 km^2. It is enclosed between the foothills of Sredna Gora Mountain to the north and the Rhodope Mountain to the south. The eastern limit of the study area is defined by Sazliyka River. The surveyed territory falls into Pazardzhik, Plovdiv, Stara Zagora and Haskovo provinces. The elevation varies between 130 and 350 m a.s.l. Woodlands occur sporadically and the landscape is comprised mostly of arable land dominated by cereal (mainly wheat and barley) and oilseed (sunflower being most common) crops.

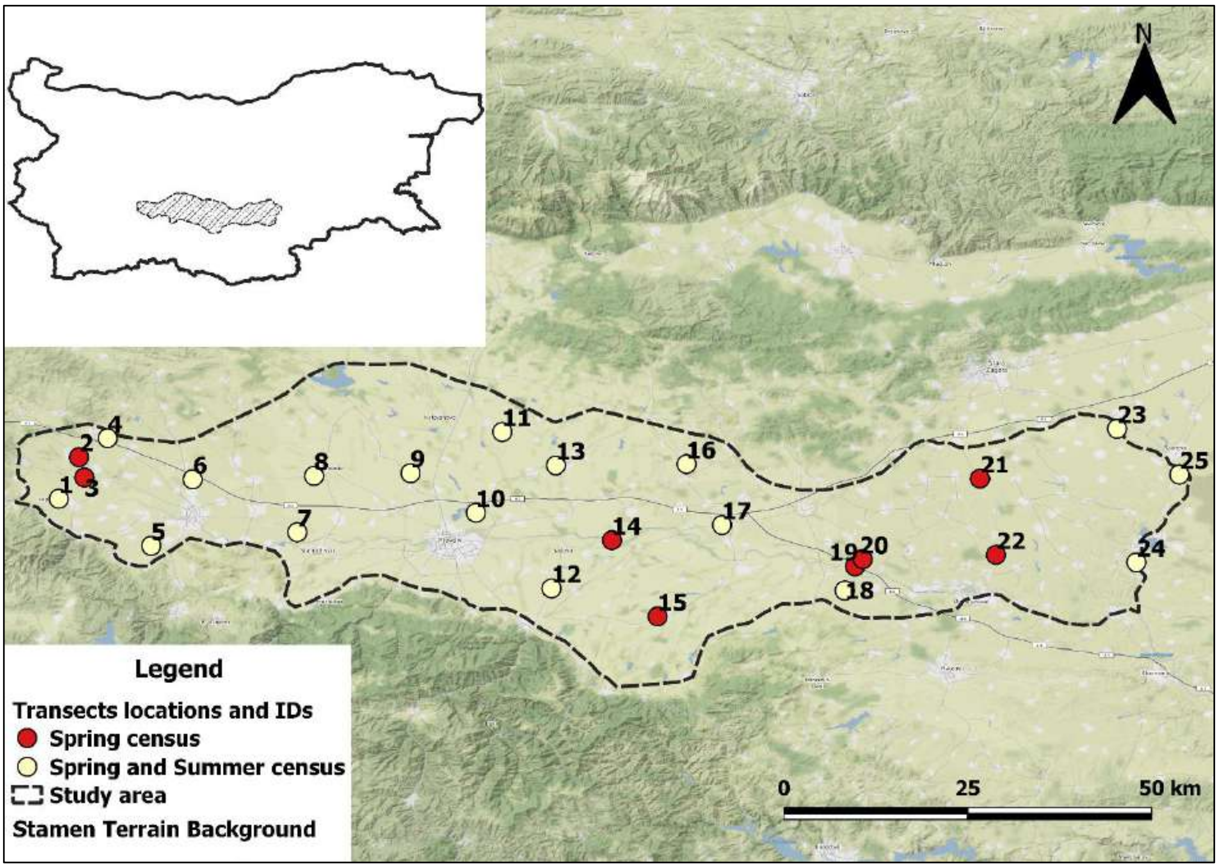

Fig. 1. Study area map. The transects are numbered according to their longitudinal distance from westernmost point of the study area. (Country borders on locator map are adapted from UNICEF (2019), under CC BY-IGO; Map tiles by Stamen Design (2021), under CC BY 3.0. Data by OpenStreetMap, under ODbL).

Breeding density

The breeding density of the Grey Partridge in the study area was estimated by a total of 25 line transects with average length of 5.13 ± 1.94 (min – max 2.10 – 8.89) km and width of 200 m (100 m on each side of the route). Playback calls were used alongside the transects in order to increase the detectability of Grey Partridges (Gruychev & Angelov, 2019; Rosin et al., 2010). The volume of the speaker was adjusted so that the sound was audible at about 100 m distance and the male partridge song was played every 200 m, thus covering the whole area of the transect. Each transect was visited at least twice from beginning of March to 10th of May between 2018 and 2020 in order to collect sufficient data. When playback is used during census even one visit on the transect could be enough as the vocal activity of male partridges remain relatively constant in March and April (Panek, 1998). The Grey Partridge density was determined by transforming the maximum number of counted pairs relative to 1 km^2 area (Bibby et al., 1992).

Summer census

Surveys during the period mid-July to mid-August were carried out on part of the transects used to estimate Grey Partridge density in the spring (Fig. 1). Fifteen sites were monitored between 2018-2020 and two transects (7 and 13) were visited in 2019 and 2020, thus a total of seventeen sites were censused in summer over the study period. The selection of transects to be surveyed in the summer and the number of visits on each of them were chosen in such a way that at least 30 – 40% of females counted in the spring had been observed in the summer (Bro et al., 2003). Trained hunting dog was used to flush the birds and thus increasing their detectability (Bibby et al., 1992). For every flock observed the number of young and adult birds were recorded. In order to assess the breeding success of the Grey Partridge during the monitored period four basic reproduction parameters were calculated. Brood production rate (BPR) was determined by the percent of pairs that have successfully reproduced, i.e. reared at least one chick during the survey. Brood size was calculated as arithmetic mean of number of young in each observed flock. Reproductive success was determined by the young-to-old ratio (including unsuccessful pairs) on surveyed sites (Rosin et al., 2010). The chick survival rate (CSR) up to six weeks of age was calculated according formulae proposed by Potts (1986), where CSR = 0.03665(geometric mean brood size)^{1,293} when geometric brood size < 10 and CSR = (geometric mean brood size)/13,84 when geometric brood size > 10.

The data collected during the summer censuses was used to estimate the August density of the Grey Partridge in the study area. The transect width was set on a total of 100 m (50 m on each side of the routes) as this was the mean distancing of the dog from the route. Given the census area and the number of recorded individuals, the August density was calculated as individuals/km^2. Obtained results on this demography parameter of the Grey Partridge were compared with the threshold of 20 birds/km^2 that should be maintained in order to conduct any shooting (Tapper, 2001). As losses between August and 1st October (start of hunting season) are possible and augmentation in abundance is very unlikely to happen, thus a minimum number of sites not capable of sustaining any harvest was derived.

Statistical analysis

In order to investigate geographical variation of estimated demographic parameters of the Grey Partridge a direct gradient analysis approach was adopted (Ter Braak & Prentice, 1988). As the distribution of the data was apart from the normal, a Generalized Linear Model (GLM) (Poisson distribution, Log link) was used. The aim of the analysis was not to predict the Grey Partridge density or values of the reproduction parameters at a given pair of geographical coordinates. As this would hardly be appropriate for a sedentary species inhabiting arable lands, the GLM was actually used to investigate the significance of spatial variation of the estimated demographic parameter within the study area. Using the points of mean coordinates of the transects, the longitudinal and latitudinal deviations of each study site (in km) from the westernmost and southernmost points, respectively, were calculated. The routes of the transects were recorded on each visit via Garmin Oregon 600 GPS and later mean coordinates (in Coordinate Reference System: WGS84/UTM zone 35N) were derived with QGIS 3.14 standard tools (QGIS.org, 2021). A separate model was constructed for the spring and August densities, as well as for every estimated reproduction parameter. Each demographic parameter was included in the particular model as response variable and longitudinal/latitudinal deviations as explanatory variables. Together with the geographical variable, the year of census was included in all models as categorical explanatory variable. At first, the effect of each covariate on the response variable of interest was tested using the Wald stat. (in test of all effects). For the significant predictors (at $p < 0.05$), the direction of relationship with the dependent variable was determined according to parameter estimates of the model (McCullagh & Nelder, 1989). All tests were performed with TIBCO Statistica 14.0.0.15 (TIBCO Software Inc., 2021).

Results

Breeding density

The mean breeding density of the Grey Partridge over the whole study period was 6.27 ± 4.73 SD (min - max 0 - 20) pairs/km^2. The test of all effects highlighted the year of census as an explanatory variable with significant effect (Wald stat. = 17.832, p = 0.0001) on the breeding density. According to parameter estimates (Table 1), the breeding density in 2018 (Appendix 1) was significantly lower than in the two successive years. Both, the longitudinal (Wald stat. = 65.466, $p < 0.0001$) and latitudinal (Wald stat. = 9.085, p = 0.0026) deviations, were determined by the test of all effects as significant predictors of the Grey Partridge breeding density within the study area. The breeding density decreased as the longitude increased (negative parameter estimate) and as the latitude decreased (positive parameter estimate), with the former showing higher significance (Table 1).

Reproduction parameters

The average values of Grey Partridge reproduction parameters in 2018, 2019 and 2020 are presented in Table 2. When performed for each of the estimated reproduction parameters of the Grey Partridge, the test of all effects showed statistically significant effect of years on brood size (Wald stat. = 12.3712, p = 0.0021) and BPR (Wald stat. = 12.064, p = 0.0024), but not on reproductive success (Wald stat. = 3.9036, p = 0.1421) and CSR (Wald ^{2} = 1.4512, p = 0.4840). According to parameter estimates, only the year 2018 (of all three categorical variables) had significant positive effect on the brood size (Parameter estimate = 0.2018, Wald stat. = 8.1532, p = 0.0043) and negative on the BPR (Parameter estimate = -0.0862, Wald stat. = 11.971, p = 0.0005). Hence, in 2018, the brood size was significantly higher and the BPR significantly lower than in the following years.

The model showed no statistically significant effect of the geographical location on the brood size (Long: Wald stat. = 1.0395, p = 0.308; Lat: Wald stat. = 1.7879, p = 0.1812), the BPR (Long: Wald stat. = 0.247, p = 0.6194; Lat: Wald stat. = 2.753, p = 0.0971), the reproductive success (Long: Wald stat. = 0.5321, p = 0.4657; Lat: Wald stat. = 2.2251, p = 0.1358) and the CSR (Long: Wald stat. = 0.0622, p = 0.8030; Lat: Wald stat. = 0.4320, p = 0.511) for the test of all effects.

August density

Mean August density of the Grey Partridge over the 3-year period was 57.32 ± 41.82 SD (min – max 0 – 148.06) individuals/km^2. It averaged 59.06 ± 59.01 SD (min – max 0 - 210.67), 60.53 ± 64.66 SD (min – max 0 - 250) and 44.40 ± 28.93 SD (min – max 0 - 89.56) individuals/km^2 in 2018, 2019 and 2020, respectively. According to the test of all effect, the categorical variables (the years of censuses) were significantly related to the August density (Wald stat. = 52.336, $p < 0.0001$). The parameter estimates of years 2018 and 2019 (Table 3) suggest significantly higher August densities of the Grey Partridge in these years compared to 2020. The test of all effects determined the longitude as the only geographical variable related significantly (Wald stat. = 533.860, $p < 0.0001$) to the density of the Grey Partridge in August, while the latitude had no significant effect (Wald stat. = 2.392, p = 0.1219) on this demographic indicator. Parameters estimates showed inverse relationship between longitude and the August density (Table 3).

At least five (IDs: 21 - 25, Appendix 2) of all surveyed sites are not expected to yield numbers over 20 individuals/km^2 which should sustain shooting. In sites 21 and 22 no birds were observed during spring census, so the species is considered to be absent from these areas.

Table 1. Parameter estimates of the effect of each predictor on breeding density (Poisson distribution, LOG link, the year 2020 was set as a reference categorical variable, bolded values denote significant effects).

Effect	**Level of Effect**	**Estimate**	**Standard Error**	**Wald Stat.**	**Lower CL 95,0%**	**Upper CL 95,0%**	**p**
Intercept		**1.8281**	**0.2430**	**56.590**	**1.3519**	**2.3045**	**0.0000**
Longitude		**-0.0094**	**0.0011**	**65.466**	**-0.0118**	**-0.0072**	**0.0000**
Latitude		**0.0174**	**0.0058**	**9.085**	**0.0061**	**0.0289**	**0.0026**
Year	**2018**	**-0.2851**	**0.0714**	**15.909**	**-0.4252**	**-0.1451**	**0.0001**
Year	2019	0.0758	0.0650	1.358	-0.0517	0.2034	0.2440
Scale		1.0000	0.0000		1.0000	1.0000	

Table 2. Grey Partridge reproduction parameters per year and mean values over the study period (2018 - 2020).

	2018	**2019**	**2020**	**Mean**
Mean brood size ± SD	12.2 ± 4.9	8.7 ± 4.7	7.8 ± 5.4	9.0 ± 5.3
Min – Max	1 – 21	1 – 22	1 - 26	1 – 26
Chick survival rate ± SD	0.80 ± 0.31	0.49 ± 0.20	0.42 ± 0.16	0.56 ± 0.22
Min – Max	0.22 – 1.15	0.22 – 0.84	0.15 – 0.83	0.15 – 1.15
Brood production rate ± SD	77.1 ± 29.4	87.5 ± 14.9	88.4 ± 15.9	84.6 ± 20.9
Min – Max	0 - 100	0 - 100	0 - 100	0 – 100
Reproductive success ± SD	4.83 ± 2.58	3.61 ± 1.39	3.55 ± 1.23	3.96 ± 1.85
Min – Max	0 – 8.5	1.15 – 5.8	2 – 5.75	0-8.5

Table 3. Parameter estimates of the effect of each predictor on August density (Poisson distribution, LOG link, the year 2020 was set as a reference categorical variable, bolded values denote significant effects).

Effect	**Level of Effect**	**Estimate**	**Standard Error**	**Wald Stat.**	**Lower CL 95,0%**	**Upper CL 95,0%**	**p**
Intercept		**4.9016**	**0.0963**	**2591.855**	**4.7129**	**5.0904**	**0.000**
Longitude		**-0.0126**	**0.0005**	**602.632**	**-0.0136**	**-0.0116**	**0.000**
Latitude		-0.0047	0.0024	3.722	-0.0094	0.0001	0.0536
Year	**2018**	**0.0875**	**0.0276**	**10.042**	**0.0334**	**0.1416**	**0.0015**
Year	**2019**	**0.1112**	**0.0266**	**17.514**	**0.0591**	**0.1633**	**0.0001**
Scale		1.0000	0.0000		1.0000	1.0000	

Discussion

Breeding density

The spring density in the studied part of the Upper Thracian Plain has increased between 2018 and 2020 but as Grey Partridge populations often tend to be highly fluctuating (Bro et al., 2005) longer monitoring period is needed in order to clearly determine trends of demographic parameters. Grey Partridge average breeding density is higher than recently reported in Sakar Mountain (Gruychev & Angelov, 2019) which is likely to be a result of different habitat quality provided by both regions. Precise comparison between breeding density in the study area and on the remaining territory of Bulgaria could hardly be reliable due to lack of sufficient data. However, the mean breeding density of the species estimated in the present study is higher than the commonly reported across Europe and North America (Carroll et al., 2021; Montagna & Meriggi, 1991; Newson et al., 2005; Ronnenberg et al., 2016) although far from levels observed in some areas in France and Czech Republic (Bro et al., 2005; Šálek et al., 2004). The current estimated densities in the Upper Thracian Plain are comparable to the levels observed in Poland (Panek, 2005) and over most of France (Bro et al., 2015). Found average density of the Grey Partridge in spring is above the expected level of 4 pairs/km^2 in modern farmlands where no particular measures for species' habitats improvement are carried out (Aebischer & Ewald, 2004). As the breeding density significantly decreases from west to east this statement is mostly true for the western half of the study area. Considering only these parts of the Upper Thracian Plain, estimated densities (Appendix 1) are comparable to those achieved after Grey Partridge habitat improvements in United Kingdom and Hungary (Ewald et al., 2020; Faragó et al., 2012). On the other hand, the sites in eastern half of the study area sustain density levels similar or lower than reported for areas with modern agriculture and no habitat enhancements (Ewald et al., 2020). However, most sites maintain breeding densities of more than 2 pairs/km^2 (Appendix 1), thus enabling restoration of Grey Partridge population levels without releases of farm birds (Buner et al., 2011). The releases of commercially reared birds produced under intensive methods are proven ineffective for conservation purposes (Rymešová et al., 2012), yet they are still widespread among hunting clubs in Bulgaria.

As emphasized by Bro et al. (2005) Grey Partridge density levels vary considerably across space due to local factors, thus obscuring trend detection. It can be hypothesized that the significant spatial trends observed in the present study may be a result of global factors either related or not to human activity. Further investigations are needed in order to determine the specific factors causing decrease of density levels towards the southern and eastern parts of the study area.

Reproduction parameters

Mean brood size of the Grey Partridge in the Upper Thracian Plain is lower than reported in Sakar Mountain (Gruychev & Angelov, 2019) but similar to pre-decline levels in Poland (Panek, 2019) and those observed in Italy (Rosin et al., 2010). Brood size decreased over the monitored period but, unlike reported in some papers (Bro et al., 2015), it did not cause decrease in breeding density. The BPR and reproductive success are higher than those in Sakar Mountain (Gruychev & Angelov, 2019). The average estimates of these parameters in the present study, as well as estimates of the CSR, are similar to those observed after applying special habitat management in some areas of United Kingdom and Ireland (Buckley et al., 2021; Draycott, 2012; Ewald et al., 2012), although the chick survival tend to be higher in continental Europe than in Britain, due to the different climatic characteristics of these regions (Potts, 1988). Observed reproductive success is higher than the one reported in 1980s in Poland (Panek, 2006).

August density

Mean August density of the Grey Partridge is higher than the one estimated in Hungary (Faragó et al., 2012) and such as the breeding density decreases towards eastern limit of the study area. Results of the present paper show that at least 24% of surveyed sites in summer will produce autumn densities lower than the minimum required, according to Tapper (2001), for conducting shooting. The same author emphasizes that Grey Partridge population with such density in the autumn, after 55% winter losses, is capable to sustain density of 4.5 pairs/km^2 in the next spring, on farmland where no species' habitat improvements are applied. However, in sites 16 and 17 (Appendix 1), mean spring densities fail to reach these expected levels, albeit the mean estimated August densities were higher than 20 individuals/km^2 (Appendix 2). This could be caused by multiple environmental or management factors but, more importantly, these results suggest setting even higher autumn density threshold for conducting Grey Partridge shooting in some parts of the Upper Thracian Plain.

Conclusions

Despite the fact that the reproduction parameters of the Grey Partridge show no spatial trends across the study area and sustain relatively high levels, both spring and August densities remained low in eastern parts over the monitored period. This could be a result of multiple factors such as high winter losses, overshooting or emigration due to the lack of suitable nesting habitats. In general, further research on factors determining high spatial variation is necessary as the results of the present study suggest that diverse management approach will be needed across different parts of the Upper Thracian Plain. It is likely that restoration and management of the populations trough habitat enhancement will be most appropriate.

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Appendix 1. Grey Partridge breeding densities on monitored sites.

Transect ID	**Breeding density (pairs/km^2)**			
	2018	**2019**	**2020**	**Mean**
1	7.76	13.44	17	12.73
2	4.87	7.67	12	8.18
3	11.27	8.97	8.2	9.48
4	8.64	16.86	9.6	11.70
5	4.71	7.06	7.4	6.39
6	8.69	12.77	11	10.82
7	3.61	11.11	13	9.24
8	8.49	8.57	13	10.02
9	4.16	6.56	6.4	5.71
10	7.98	8.37	13	9.78
11	8.07	10.04	12	10.04
12	2.8	5.42	9.5	5.91
13	8.22	14.69	20	14.30
14	1.65	4.1	4.3	3.35
15	0.91	1.01	5.1	2.34
16	2.96	0.77	3.5	2.41
17	2.75	5.72	4.4	4.29
18	10.97	7.65	3.1	7.24
19	0.83	1.02	3.2	1.68
20	0	3.3	4.8	2.70
21	0	0	0	0.00
22	0	0	0	0.00
23	2.62	2.99	5.3	3.64
24	0	1.49	0	0.50
25	3.44	5.98	3.4	4.27
Mean ± SD	4.62 ± 3.63	6.62 ± 4.77	7.57 ± 5.31	
Min - Max	0 – 11.27	0 – 16.86	0 – 20.00	

Appendix 2. Grey Partridge reproduction parameters on monitored sites.

Transect ID	**Brood size**	**BPR**	**Reproductive success**	**CSR**	**August density**
1	10.6	100.0	5.75	0.61	95.8
4	9.9	83.6	4.06	0.62	110.0
5	8.4	95.2	3.97	0.50	44.6
6	8.8	70.8	3.29	0.53	90.0
7	10.6	81.3	4.25	0.61	105.5
8	10.5	91.7	4.37	0.54	61.8
9	7.8	66.7	3.16	0.40	24.7
10	10.9	70.0	3.19	0.60	60.2
11	7.7	90.5	3.20	0.38	52.8
12	10.5	84.7	4.45	0.70	60.4
13	8.5	90.0	3.89	0.49	148.1
16	5.8	50.0	2.88	0.83	20.6
17	5.7	83.3	2.50	0.34	24.8
18	12.1	100.0	6.37	0.84	55.9
23	8.5	100.0	4.25	0.58	8.6
24	3.0	100.0	3.00	0.37	7.9
25					0.0

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New Records of Peniophora Species (Basidiomycota) for the Bulgarian Mycota

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Abstract. This study provides new data for two corticioid species of genus *Peniophora* Cooke for the Bulgarian mycota, *P. piceae* and *P. pini* as wood-decaying saprobes, causing a white rot in coniferous wood. These fungal species have restricted range that follow the spread of the coniferous hosts. *P. piceae* is known only as saprotroph on dead spruce wood while *P. pini* prefers dead wood of pine. The work includes information on the morphology and ecological features as well as the distribution pattern of the species in Bulgaria.

Key words: Bulgarian mycota, *Peniophora*, lignicolous fungi, white rot.

Introduction

The genus *Peniophora* Cooke is a member of the Corticiaceae sensu lato (Basidiomycota). Cooke (1879) described *Peniophora* as the first genus of corticioid fungi based on microscopic features (incrusted cystidia). *Peniophora* is a widespread genus of corticioid fungi with more than 60 species (Kirk et al., 2008).

The genus *Peniophora* includes species with a resupinate basidiome, margin adnate or loosening from the substrata, hymenial surface smooth to tuberculate, grey, violaceous, orange, red or brown, hyphal system monomitic, hyphae hyaline to brown, thin-to-thick walled with clamps in most species, few with simple-septate hyphae. Dendrohyphidia, gloeocystidia and lamprocystidia present or lacking. Basidia subclavate to cylindrical, with 4-sterigmata, a basal clamp present in most species. Basidiospores usually large, ellipsoid, cylindrical to allantoid, smooth, thin-walled, IKI$^-$, spore print pinkish to reddish (Gorjón, 2020). The species within the genus *Peniophora* cause decaying of a white rot type.

In Bulgaria, up to date, ten species of *Peniophora* have been published, as follows: *Peniophora cinerea* (Pers.) Cooke (Pilát, 1937; Hinkova, 1955; Denchev & Assyov, 2010), *P. incarnata* (Pers.) P. Karst. (Pilát, 1937; Kuthan & Kotlaba, 1989; Hinkova, 1955; Bencheva, 2006; Denchev et al., 2006; Denchev & Assyov, 2010; Gospodinov et al., 2018), *P. laeta* (Fr.) Donk (Kuthan & Kotlaba, 1989; Denchev & Assyov, 2010), *P. lycii* (Pers.) Höhn. & Litsch. (Kuthan & Kotlaba, 1981, 1989; Gyosheva, 1997; Bencheva, 2006; Denchev & Assyov, 2010; Gyosheva et al., 2016; Gospodinov et al., 2018); *P. nuda* (Fr.) Bres. (Kuthan & Kotlaba, 1989; Denchev &

Assyov, 2010; Gospodinov et al., 2018), *P. pilatiana* Pouzar & Svrček (Kuthan & Kotlaba, 1981, 1989; Denchev & Assyov, 2010); *P. pini* (Schleich.) Boidin (Gyosheva & Stoykov, 2019); *P. quercina* (Pers.) Cooke (Kuthan & Kotlaba, 1981, 1989; Pencheva et al., 2009; Denchev & Assyov, 2010; Stoyneva & Uzunov, 2015; Gyosheva et al., 2016; Gospodinov et al., 2018; Lacheva, 2018); *P. rufa* (Fr.) Boidin (Denchev et al., 2007; Denchev & Assyov, 2010) and *P. violaceolivida* (Sommerf.) Massee (Kuthan & Kotlaba, 1989; Denchev & Assyov, 2010).

In addition, two other new species, *P. meridionalis* Boidin and *P. junipericola* J. Erikss. have been found recently (Lambevska-Hristova et al., 2020), whereby the number of the *Peniophora* species for the Bulgarian Mycota becomes 12. During field investigations related to the study of coniferous communities in the ecological network NATURA 2000, two more species of the genus have been identified. *P. piceae* (Pers.) J. Erikss. has been recorded for the first time for the Bulgarian mycota while *P. pini* (Schleich.) Boidin has been earlier reported from Bulgaria only once (Gyosheva & Stoykov, 2019).

This article aims to add new data to the knowledge about the diversity of the genus *Peniophora* in Bulgaria. The purpose of this study is to report the first descriptions of two species, *P. piceae* and *P. pini* for the Bulgarian mycota and to provide information about their morphological and ecological characteristics and distribution in the country (Fig. 1).

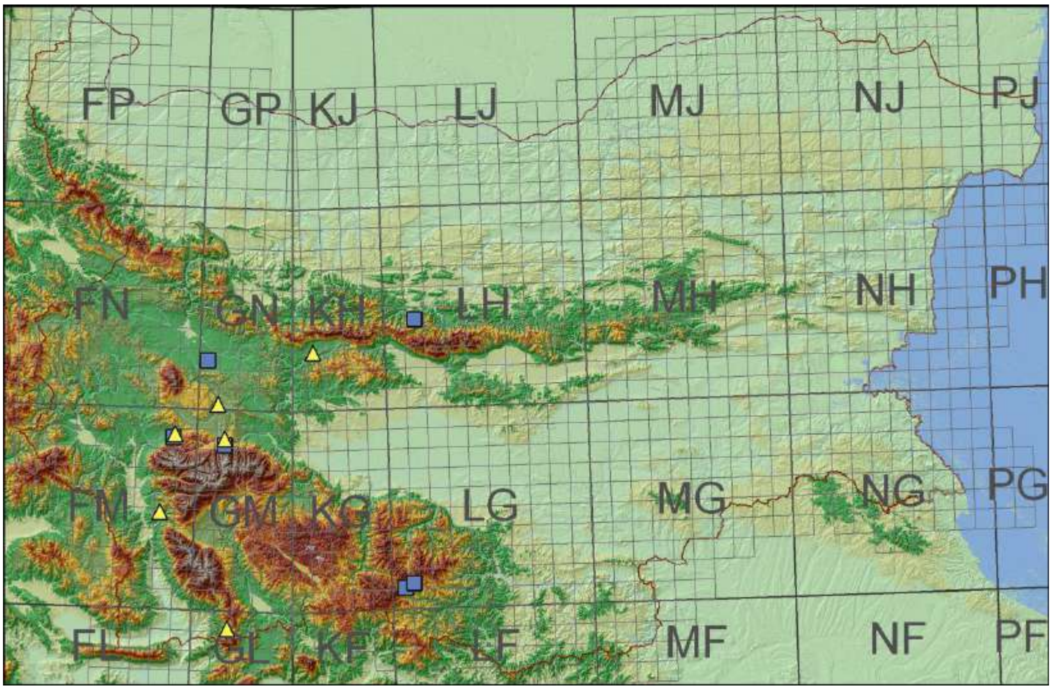

Fig. 1. A distribution map of *P. piceae* (blue square) and *P. pini* (yellow triangle) in Bulgaria.

Material and Methods

The field studies were conducted during the period 2015-2021 in different parts of the country. For identification of the lignicolous fungi, standard methods were applied, implying microscopy and application of reagents (Melzer's reagent and 5% KOH). Measurements and photographs were examined at magnification up to 1000×, with a LW scientific microscope and MiniVID camera. The specimens were identified by the following sources: Eriksson et al. (1978); Jülich (1984); Breitenbach & Kränzlin (1986);

Hansen & Knudsen (1997); Bernicchia & Gorjón (2010); Yurchenko (2010). The abbreviations of the authors of fungal names follow Kirk & Ansell (2004). The studied specimens were deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, within the Bulgarian Academy of Sciences (SOMF).

Results and Discussion

1. *Peniophora piceae* (Pers.) J. Erikss., Symb. Bot. upsal. 10(5): 49 (1950).

Bas.: *Thelephora piceae* Pers., Mycol. Eur. (Erlanga) 1: 123 (1822).

Morphological description. Basidiome resupinate, effused, adnate, but margin loosening from the substratum, hymenophore smooth or tuberculate, reddish grey to grey to dark violaceous grey, margin whitish, hyphal system monomitic, hyphae with clamps, basal hyphae brown, thick-walled, 3-4 µm wide, subhymenial hyphae hyaline to brown, thin-to-thick walled, 2.5-4 µm wide, lamprocystidia hyaline to brown, encrusted, 40-80 x 6-18 µm, basidia subcylindrical, 30-50 x 5-7 µm, with 4-sterigmata and with a basal clamp, basidiospores allantoid, 6.5-9 x 2-2.5 µm, smooth, thin-walled, hyaline (Fig. 2, A-B).

The species is very similar to *P. pithya* (Pers.) J. Erikss., which occurs on conifers, but differs in the lack of sylfocystidia and the loosening from the substratum, and with the closely related species *P. limitata* (Chaillet ex Fr.) Cooke, it differs in the size and shape of basidiospores (Eriksson et al., 1978).

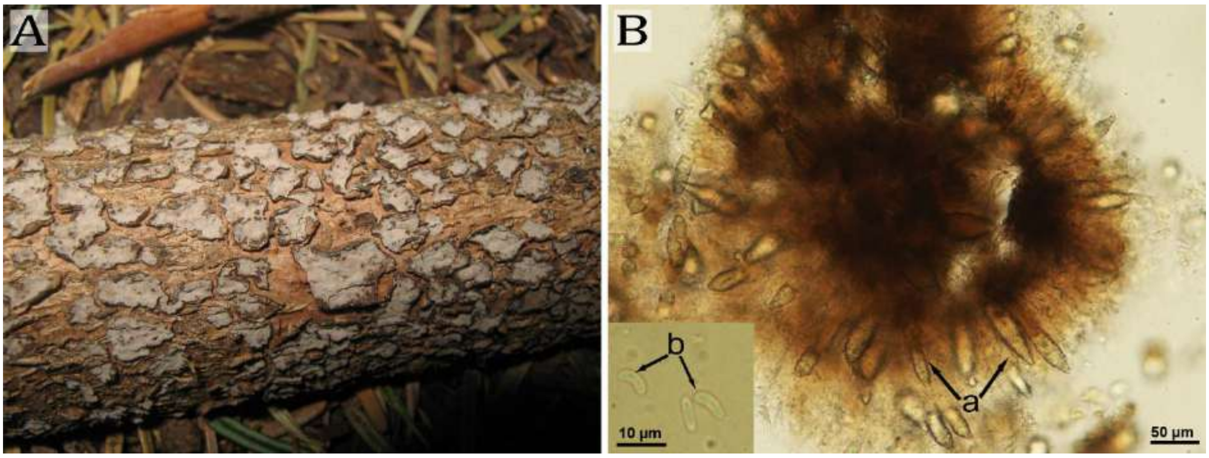

Fig. 2. A) A macroscopic view of basidiome of *P. piceae*; B) Microscopical features of *P. piceae*: section of basidiome, a) presence of numerous incrusted cystidia (lamprocystidia), b) basidiospores.

Distribution in Bulgaria. The species has been found in 4 regions of the country: Sofia region, Stara Planina Mts., Rila Mts. and Rhodopes Mt.

General distribution. The species is generally distributed in Europe, Caucasus, Asia, North America, South America (Liberta & Navas, 1978; Klán & Kotilová-Kubičková, 1982; Mukhin & Stepanova, 1983; Mukhamedshin, 1992; Boidin, 1994, Sesli & Denchev, 2005). It has not been previously reported for Bulgaria.

Ecological remarks and host. The species causes decay of a white rot type on dead wood of conifers (Lambevska et al., 2013). According to the available literature, the taxa prefers dead wood of spruce species, but it is also known from *Abies*, *Juniperus*, *Pinus*, *Thuja*, *Tsuga* and *Pseudotsuga* (Mukhin & Stepanova, 1983; Tortić, 1985; Grosse-Brauckmann, 1987; Ginns & Lefebvre, 1993; Boidin, 1994; Dämon, 2001;

Polemis et al., 2002; Bernicchia & Gorjón, 2010).

Remarks. In northern Europe, the species has been described as destructor of processed wood materials (Bondartseva et al., 1999). At the same time, it is included in the Red List of Threatened Macrofungi of Poland (Wojewoda & Ławrynowicz, 1992).

Material examined. Sofia region, Park-Museum "Vrana", in mixed forest of white pine (*P. sylvestris*) and *Abies* sp., on fallen branches of coniferous tree, N 42.63808° E 23.43195°, 574 m, 04.04.2018, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 282); Stara Planina Mts., 2 km S of Stoynovskoto village, hydroelectric power station (VEC) Cherni Osam, in coniferous forest (*P. nigra*, *Abies* sp. and *Picea* sp.), on fallen branches of a coniferous tree, N 42.80382° E 24.77489°, 623 m, 24.02.2018, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 283); Rila Mts., 5.5 km S of Beli Iskar village, coniferous forest, on a fallen cut trunk of *P. abies*, N 42.22783° E 23.54761°, 1 305 m, 13.07.2019, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 281); Panichishte village, near Panorama Hotel, in mixed forest with white pine (*P. sylvestris*) and *Abies* sp., on fallen branches of a coniferous tree, N 42.26864° E 23.29274°, 1 456 m, 09.09.2017, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 280); Rhodopes Mt., Pamporovo ski resort, *P. abies* forest, on fallen branches of *P. abies*, N 41.65156° E 24.69824°, 1 566 m, 25.07.2019, leg. A. Lambevska-Hristova, I. Todorov & P. Boyadzhiev; det. A. Lambevska-Hristova (SOMF 30 279); the road to Rozhen village, in *Picea abies* forest, on fallen branches of *P. abies*, N 41.67365° E 24.73205°, 1 420 m, 25.07.2019, leg. A. Lambevska-Hristova, I. Todorov & P. Boyadzhiev; det. A. Lambevska-Hristova (SOMF 30 278).

2. *Peniophora pini* (Schleich.) Boidin, Revue Mycol., Paris 21: 123 (1956).

Bas.: *Thelephora pini* Schleich., in de Candolle & Lamarck, Fl. franç., Edn 3 (Paris) 5/6: 31 (1815).

Morphological description. Basidiome resupinate, confluent and effused, adnate, margin loosening from the substratum, hymenophore smooth to tuberculate, violaceous grey to dark bluish violaceous, hyphal system monomitic, hyphae with clamps, thin-walled, 2.5-7 µm wide, hyaline to pale brownish, more or less gelatinized, denser and vertically arranged in the subhymenium, thick-walled and swollen in the subiculum, lamprocystidia, present in the subhymenium, 25-40 x 5-8 µm, at first thin-walled, pointed or obtuse, finally strongly encrusted in the apical part, both externally and internally, encrusted part 12-20 x 5-8 µm, gloeocystidia, thin- or with distinct walls, very variable in shape and size, cesicular to cylindrical, 20-50 x 10-25 µm, with granular contents, basidia subclavate, 20-40 x 4.5-6 µm, with 4-sterigmata, and a basal clamp, basidiospores allantoid, 6-9 x 2.5-3 µm, smooth, thin-walled, hyaline (Fig. 3, A-B).

Distribution in Bulgaria. The species has been found in 4 regions of the country: Vitosha Mt. (Plana Mt.), Slavyanka Mt., Rila Mts. and Sredna Gora Mt. The species has previously been reported only once, from Rila Mts., Ibur Reserve.

General distribution. It occurs in Europe, North America and Asia (Davydkina, 1980; Jülich & Stalpers, 1980; Ginns & Lefebvre, 1993; Boidin, 1994; Hansen & Knudsen, 1997; Bernicchia & Gorjón, 2010). According to the data provided by Eriksson (1958) and Eriksson et al. (1978), it occurs in the northern taiga above the Arctic Circle and follows the distribution of pine.

Ecological remarks and host. Davydkina (1980) mentioned this species as a boreal geographical element, distributed only in the northern hemisphere, with a panboreal type of distribution. *P. pini* is considered a white rot saprobe that is apparently restricted only to dead wood of pine species.

To date, it has been known from *P. halepensis* Mill., *P. hamata* (Steven) Sosn. [non Roezl], *P. montezumae* Lamb., *P. mugo* Turra, *P. nigra* J. F. Arnold, *P. nigra* subsp. *pallasiana* (D. Don) Holmboe, *P. peuce* Griseb., *P. pityusa* Steven, *P. strobus* L., *P. sylvestris* L. and *P. wallichiana* A. B. Jacks. (Eriksson et al., 1978; Tortić, 1985; Breitenbach & Kränzlin, 1986; Renvall, 1995; Dämon et al,. 2009; Yurchenko, 2010; Lambevska-Hristova et al., 2013).

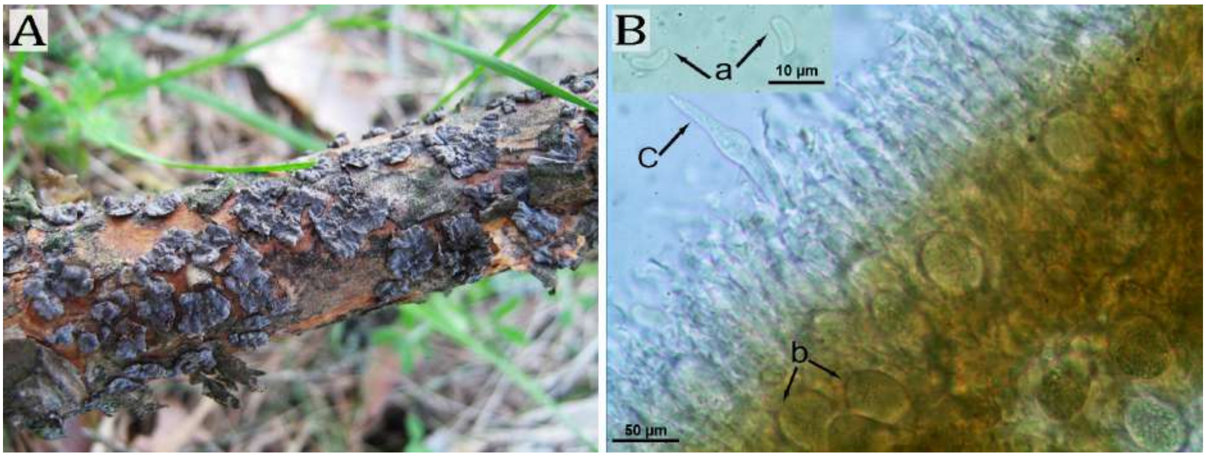

Fig. 3. A) A macroscopic view of basidiome of *P. pini*; B) Microscopical features of *P. pini*: section of basidiome, a) basidiospores, b) presence of numerous gloeocystidia with granular content, c) sulfocystidium.

Remarks. According to the data published by Domański (1976), *P. pini* occurs most commonly on fallen twigs of *Pinus sylvestris* or in a complex with pathogenic fungi *Cenangium ferruginosum* Fr. and *Valsa pini* (Alb. & Schwein.) Fr. Sometimes it manifests as necrotrophic species, and it causes necrosis of coniferous trees (Davydkina, 1980). Domański et al. (1976) consider *P. pini* as a possible agent of crown necrosis in forest communities of *Pinus sylvestris*.

Material examined. Plana Mt., 1 km NW of Mechkata villa area, in *P. sylvestris* forest, on fallen branches of *P. sylvestris*, N 42.44214° E 23.52360°, 918 m, 29.04.2018, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 284); Slavyanka Mt., 3 km SE of Goleshovo village, near Livade place, in mixed forest, on fallen branches of *P. nigra*, N 41.40811° E 23.60829°, 1 513 m, 19.06.2020, leg. A. Lambevska-Hristova, S. Lukanov; det. A. Lambevska-Hristova (SOMF 30 285); Rila Mts., 3.6 km S of Beli Iskar village, in mixed coniferous forest, on fallen branches of *P. sylvestris*, N 42.241916° E 23.542250°, 1 232 m, 13.07.2019, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 288); above Gorno Harsovo village, in mixed forest, on fallen branches of *P. sylvestris*, N 42.02510° E 23.16348°, 732 m, 10.09.2021, leg. G. Hristov, det. A. Lambevska-Hristova (SOMF 30 277); Panichishte village, near Panorama Hotel, in mixed forest of white pine (*P. sylvestris*) and *Abies* sp., on fallen branches of *P. sylvestris*, N 42.26864° E 23.29274°, 1 456 m, 09.09.2017, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 287); Sredna Gora Mt., 2 km SW of Koprivshtitsa town, in coniferous forest, on fallen branches of coniferous trees, N 42.62812° E 24.33837°, 1 316 m, 25.04.2015, leg. A. Lambevska-Hristova, G. Hristov, det. A. Lambevska-Hristova (SOMF 30 286).

In conclusion, it can be noted that the present work contributes to the knowledge of the diversity of the genus *Peniophora* in Bulgaria. However, given that the diversity in this group in Bulgaria has not yet been fully explored, the finding of new taxa is not surprising.

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Current State of Iris L., Subgenus Iris (Iridaceae) in Bulgaria

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Abstract. The distribution of genus *Iris* subgenus *Iris* was presented and notes of its taxonomy were given in the paper. Based on the critical processing of the collections in the national herbaria (SOM, SOA and SO), the literature sources, and our field observation we have produced distribution maps. New chorological data are reported for *Iris reichenbachii*, *I. suaveolens,* and *I.* ×*germanica*. *Iris mellita,* whose taxonomical status is under investigation, was reviewed as a synonym of *I. suaveolens*. The distribution of *I. aphylla* in Bulgaria was not confirmed until now, with the current collection for the Bulgarian flora.

Key words: chorology, herbarium collections, subgenus *Iris*, taxonomy.

Introduction

Genus *Iris* L. comprises approximately 300 species, native to Europe and Asia (Rodionenko, 1987). The interest in the genus has existed from a long time ago, because of the diversity of forms and colors, the wide ornamental cultivation, and the presence of medicinal plants. The most accepted classification systems divide the rhizomatous European species into two subgenera. The typical subgenus *Iris* unites the species with bearded falls (external perygon leaves). Subgenus *Limniris* (Tausch) Spach contains the species with glabrous falls (Mathew, 1981; Rodionenko, 1987). Despite the existence of intensive literature taxonomy investigations, the genus *Iris* is still considered to be problematic from the taxonomical viewpoint. The *Iris* species, especially the members of subgenus *Iris,* are difficult to be distinguished due to the lack of clearly definitive morphological characters and the presence of great variability.

Following the Bulgarian authors, the genus is represented by 9 or 10 species in wild, and 2 cultivated species (Stojanov & Stefanov, 1924; 1933; 1948; Stojanov et al., 1966; Delipavlov & Cheshmedzhiev, 2003; Assyov & Petrova, 2012). The genus is taxonomically developed in volume II of Flora Reipublicae Popularis Bulgaricae (Radenkova, 1964). No summary floristic examinations of the subgenus have been conducted over the last 70 years. The information about the taxonomy, chorology, and conservation status of the members of genus *Iris* in Bulgaria is outdated and indisputably needs revision and actualization. The review of the taxonomical literature shows that the representatives of genus *Iris* have not been an object of purposive studies in Bulgaria for decades past. During the last decades, the taxonomy of the genus *Iris* has changed. The molecular approaches proved that *Iris* s.l. contains 23 separate genera (Mavrodiev et al., 2014).

The present study is based on literature data, reviewed herbarium specimens, and field research. The goal is to update and generalize the existing information about the Bulgarian representatives of genus *Iris* subgenus *Iris*.

Materials and Methods

The field studies on the status of the localities, accenting on the critically targeted species from the investigated subgenus, were taken during 2010-2021. The reviewed herbarium samples of genus *Iris* subgenus *Iris* L., deposited in Bulgarian herbaria, were generally 494 in number, respectively 301 in number till now (incl. the deposited in the present study) from the Herbarium of Agricultural University – Plovdiv (SOA), 76 in number from the Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Science (SOM), and 117 in number from the Biological Faculty – Sofia University (SO). Additionally, the GBIF entries from the herbaria MNHN (Chagnoux, 2021), W (Natural History Museum, Vienna, 2019), BRNU (Masaryk University, Department of Botany and Zoology, 2019), G (Conservatoire et Jardin botaniques de la Ville de Genève - G, 2016), as well as from recent photo observations (Affouard et al., 2020; de Vries & Lemmens, 2018-2019; Ueda, 2021) were reviewed. The collections of our field studies were deposited in SOA. Separated by species, the collections from Bulgaria are represented as follows: *Iris reichenbachii* (289 samples), *I. suaveolens* (125 samples), *I. pumila* (44 samples), *I. variegata* (92 samples), and *I.* × *germanica* (14 samples). All reviewed data from the herbarium specimens and literature were imported to the electronic documentation system in the Herbarium of Agricultural University – Plovdiv (SOA) (Stoyanov, 2009) and were exported to chorological maps. The floristic regions were described and numbered according to Jordanov (1966) as follow: Black Sea Coast (1), Northeast Bulgaria (2), Danubian Plain (3), Forebalkan (4), Balkan Range (5), Sofia region (6), Znepole region (7), Vitosha region (8), West Frontier Mts (9), the Valley of Strouma River (10), Mt Belasitsa (11), Mt Slavyanka (12), the Valley of River Mesta (13), Pirin Mts (14), Rila Mts (15), Mt Sredna Gora (16), Rhodopi Mts (17), Thracian Lowland (18), Tundzha Hilly Country (19) and Mt Strandzha (20). The subregions were marked with letters: **w** – western; **s** – southern; **e** – eastern; **n** – northern and **c** – central. The same codes were shown on the maps. The oldest herbarium materials have been labeled without exact geographic coordinates, but they contain pretty enough geographical information. The coordinates of them were taken from topographic maps. All coordinates are recalculated to UTM 10×10 km squares following the accepted standard (Kozhuharov et al., 1983) and represented in the text as MGRS codes.

Results and Discussion

The chorological information, according to the latest field investigation and relevant literature dates, was compared with the existing herbarium collections from the territory of Bulgaria (Appendix 1). The representativeness of the collections is summarized in Table 1.

Iris reichenbachii Heuff. (Fig. 1A). syn.: *Iris balkana* Janka; *I. bosniaca* auct. non Beck.

The species is a Balkan endemic. The native distribution on the Balkan Peninsula is on the territory of Albania, Bulgaria, Greece, Eastern Aegean islands, Serbia, N. Macedonia, Bosnia and Herzegovina, Croatia, Slovenia (Webb & Chater, 1980; Dimopolous et al., 2018-2019).

The data from Serbia (Adamović, 1901; Randolph & Rechinger 1954; Stepanović-Veseličić, 1976; Urumov, 1905a), Republic of N. Macedonia, the northern part of Greece (Dimopoulos et al., 2018-2019; Randolph & Rechinger, 1954), were confirmed with samples from Bulgarian herbaria (SOA, SOM).

Table 1. Representativeness of the *Iris* collections in the Bulgarian herbaria – number of specimens vs. represented UTM squares.

Species name	**SOA**		**SOM**		**SO**	
	Number of specimens	**UTM squares**	**Number of specimens**	**UTM squares**	**Number of specimens**	**UTM squares**
I. reichenbachii	159	36	78	33	41	29
I. suaveolens	44	20	32	16	30	26
I. aphylla	9	2	-	-	-	-
I. pumila	28	18	7	7	8	7
I. variegata	62	18	3	3	22	18
I. ×germanica	4	3	1	1	-	-

The distribution of this species is wider than known from the literature. The floristic records from the literature are from the following regions: **1s** (Tashev et al., 2016), **3** (Assyov & Petrova, 2012), **4w** (Assyov & Petrova, 2012; Randolph & Rechinger, 1954), **4e** (Urumov, 1900, 1901a, 1898), **5w** (Assyov & Petrova, 2012; Ueda, 2021; Urumov, 1905b), **5c** (Assyov & Petrova, 2012; Popova & Cheschmedjiev, 1975; Randolph & Rechinger, 1954; Urumov, 1901b), **5e** (Assyov & Petrova, 2012, Randolph & Rechinger, 1954), **6** (Randolph & Rechinger, 1954; Toshev, 1903); **7** (Apostolova-Stoyanova & Stoyanov, 2009; Assyov & Petrova, 2012; Ueda, 2021; Urumov, 1905b), **8** (Assyov & Petrova, 2012; Ueda, 2021). **10s** (Dimitrov & Vutov, 2013), **9**, **12**, (Assyov & Petrova, 2012), **14** (Assyov & Petrova, 2012; Ueda, 2021) , **15** (Assyov & Petrova, 2012), **17** (Randolph & Rechinger, 1954) – **17w** (Assyov & Petrova, 2012), **17c** (Assyov & Petrova, 2012; Popova & Cheschmedzhiev, 1975; Randolph & Rechinger, 1954), **17e** (Assyov & Petrova, 2012), **18** (Randolph & Rechinger, 1954). The species has not been reported till now for **10n**, **11**, **19**. There is no chorological data about the floristic regions 2, 13, 16 and 20. The vertical distribution of the species is up to 2900 m above sea level.

In the herbarium collections were found samples signed as hybrids *Iris reichenbachii* × *I. aphylla* (SOA 30430, 25904, 30431, 30436, 30905, 25907, 30252). These samples have been collected around the city of Kardzhali (17e) and the town of Peshtera (17w), then grown in the former botanical garden. Our revision assigned them to *Iris reichenbachii*. In SOA have been deposited 3 herbarium specimens signed as *Iris reichenbachii* var. *intermedia* Česchm. – "Rhodopi centralis, in saxosis supra urbeum Asenovgrad", SOA holotypus: 029276, isotypus: 025909, 047202.

Iris suaveolens Boiss. & Reut. (Fig. 1B). Syn.: *I. mellita* Janka.

This species is very mutable, according to the scape, and the size and color of the flower. As a result of this variation, many taxonomical interpretations can be found.

Comparatively a little count of species has been deposited under the name *I. suaveolens* (e.g. SOA 14562; SOM 1401, 1402, 1410, 1901, 1911; SO 101663).

The largest amount of materials under the name *I. mellita* are deposited in SO. Single herbarium specimen is deposited in SOA as a new variety – *I. mellita* var. *macrantha* Stoj. (SOA 02286, the region of Petrich). We accept these data as part of the circumscription of *I. suaveolens.*

Iris suaveolens is reported for the following floristic regions: **1** (Assyov & Petrova, 2012; Davidov, 1905b; Randolph & Rechinger, 1954; Ueda, 2021), **2** (Davidov, 1905b, Urumov, 1901), **5e**, **10** (Assyov & Petrova, 2012), **11** (Assyov & Petrova, 2012; Topalova, 2006), **17** (Randolph & Rechinger, 1954) – **17w** (Chagnoux, 2021), **17e** (Assyov

& Petrova, 2012); **18** (Assyov & Petrova, 2012; Dimitrov, 2014; Randolph & Rechinger, 1954), **19** (Assyov & Petrova, 2012; Toshev, 1903), **20** (Assyov & Petrova, 2012; Gussev et al., 1998).

The species has been noticed for regions **3** (Popova & Cheschmedjiev, 1975), **13** (Assyov & Petrova, 2012; Goranova et al., 2013) and **14** (Assyov & Petrova, 2012). The data of the herbarium materials added the regions **5w**, **5c**, **7**, and **17c**. The species has not been reported or noticed for regions 4, 6, 8, 9, 12, and 15.

The type specimen of *I. suaveolens* (G-G-173989/1 - !), signed as "Bulgarie – Kustendje", has been collected near the city with the recent name Konstantsa, North Dobroudja, today in the territory of Romania. The species has been reported with exact data for Serbia (Adamović, 1901; Randolph & Rechinger, 1954; Stepanović-Veseličić, 1976), Greece (Dimopolous et al. 2018-2019; Randolph & Rechinger, 1954), Turkey (Dane et al. 2009; Randolph & Rechinger, 1954), Republic of N. Macedonia (Randolph & Rechinger, 1954), Romania (Randolph & Rechinger, 1954; Prodan & Nyarady, 1966). Vertically the species is distributed up to 1362 m above sea level.

Iris aphylla L. (Fig. 1C).

Iris aphylla is a steppe species. It is classified as exclusively rare and endangered in the red lists of many European countries (Allen et al. 2014; Holub & Procházka, 2000; Kaźmierczakowa & Zarzycki, 2001; Ludwig & Schnittler, 1996; Maglocky & Feráková, 1993). During the last years, the species is a subject of monitoring programs, with conclusions for the decreasing strength of the populations (Wróblewska et al., 2003).

This species is known as continental and subcontinental element (Borhidi, 1995). The recent distribution of *I. aphylla* covers Ukraine, Central and Southern Russia, Caucasus, and Minor Asia. The insulated populations of this species in Poland, Belarus, Germany, Czech Republic, Slovakia, Hungary, and Romania appear as a terminal border of the geographic distribution (Kaźmierczakowa & Zarzycki, 2001; Webb & Chater, 1980). For the Balkans, it is noticed only in isolated localities in Croatia, Romania, and Serbia (Obradović et al., 1986; Prodan & Nyarady, 1966; Purger et al., 2008). The species is indicated as critically endangered according to the Red lists and Red Data Book (Ivanova, 2009; 2011).

Iris aphylla has been reported for a first time for Bulgaria (Cheschmedzhiev, 1967) for the region of Zhelezni-Vrata and Kardzhali, and following these data is included in the Red Data Book (Ivanova 2011). The collections of SOA consist of 12 materials, determined as *I. aphylla*.

The locality "Zhelezni-Vrata train stop" is highly damaged by anthropogenous factors. After the last collection of the species from the only known locality for the country, more than 50 years ago, *I. aphylla* has not been found (Ivanova, 2011). The slope reported as a locality of *I. aphylla* has been used as a stone carrier. The same authors determine some specimens as *I.* ×*germanica* (SOA, 45790) on the locality of *I. aphylla*. Our detailed field research, as well as investigation of the Herbaria, showed that there are no recent data to confirm the species, and the status of the species could be suggested as "Regionally extinct". Samples of this species are not deposited in SO and SOM. On the grounds of the represented above, the occurrence of *I. aphylla* is considered as doubtful in the recent Bulgarian flora and the current botanical survey did not confirm its distribution.

Iris pumila L. (Fig. 1D).

This species has Pontic-Pannonian distribution: Austria, Bulgaria, Croatia, Serbia, Romania, Czech Republic, and Slovakia (Koca, 2003; Purger et al., 2008).

The species is noticed as distributed in the whole country (Assyov & Petrova, 2012), up to 1000 m above the sea level. The exact records in the literature were found for the following floristic regions: **1n** (Urumov, 1901a; Davidov, 1905b; Ueda, 2021); **2** (Urumov, 1901a, 1901b, 1904; Davidov, 1904, 1905b); **3** (Popova & Cheschmedzhiev 1975), **4e** (Neichev, 1903; Urumov, 1897, 1898, 1904; Randolph & Rechinger, 1954); **5c** (Urumov, 1904), and noticed without herbarium materials for **4w** (Urumov, 1897) , **7** (Apostolova-Stoyanova & Stoyanov, 2009), **9** (Urumov, 1904), **16w** (Glogov & Pavlova, 2018).

Despite the information for the whole country in the floristic summaries (Delipavlov & Cheschmedzhiev, 2003; Assyov & Petrova, 2012), in the herbaria were not found samples from the regions **4w, 5w**, **5e**, **6**, **8**, **9**, **11**, **12**, **13**, **14**, **15**, **16**, **17w**, **17e** and **20**. The vertical distribution of the species is up to 760 m above sea level.

Iris variegata L. – (Fig. 1E).

The natural distribution area covers Central and South-Eastern Europe – Austria, Czech Republic, Germany, Hungary, Bulgaria, Romania, Serbia, and Slovakia, east to Ukraine, and naturalized in Italy and Switzerland (Dimopolous et al., 2018-2019; Webb & Chater, 1980). This species is widely cultivated as ornamental.

The species is noticed for the whole territory of Bulgaria (Assyov & Petrova, 2012). Exact localities are reported in the literature for floristic regions: **1n** (Davidov, 1905b), **2** (Davidov, 1904, 1905b; Urumov, 1901a, 1904, 1905a), **3** (Davidov, 1904; Urumov, 1901a), **4w** (Urumov, 1905a), **4e** (Randolph & Rechinger 1954; Urumov, 1897, 1898), **5w** (Popova & Cheschmedzhiev, 1975; Urumov, 1905b), **6** (de Vries & Lemmens, 2019; Randolph & Rechinger, 1954), **7** (Affouard et al., 2020; Apostolova-Stoyanova et al., 2009; Urumov, 1905b; Ueda, 2021), **8** (Urumov, 1905b), **17c** (Popova & Cheschmedjiev, 1975), **18** (de Vries & Lemmens, 2019; Randolph & Rechinger, 1954; Toshev, 1903).

The herbarium data add exact localities for 6 regions. The herbarium data can not confirm the distribution in the regions: **9**, **10**, **11**, **12**, **13**, **14**, **17w**, and **20**.

In the known literature the vertical distribution has been noticed up to 2900 m above sea level (Assyov & Petrova, 2012). The confirmed vertical distribution of this species is between 10 and 1217 m above sea level.

Iris ×germanica L. (Fig. 1F). syn. *Iris florentina* L.

Iris ×germanica, including *I. florentina*, is distributed in SW Europe, Iberia, France, EC Europe, Turkey, Palestine, NW Africa (Dimopolous et al., 2018-2019; Seidemann, 2005). It is native in Europe, also cultivated ornamental species.

Following the data of Urumov (1898a, b) and Davidov (1915), in the country are known natural populations of this species in **4e** – the region of Lovech, Veliko Turnovo, and Gorna Oryahovitsa.

Wild populations, probably garden escapes, could be found in many regions of the country. In the national herbaria are deposited samples from 3 floristic regions. A recently published observation, probably of a garden escape, has been found from 15. 34TFM96. Rila Monastery, 29.05.2005. Despite the wide distribution, the specimens under the name *I. ×germanica* in the herbarium collections are found only 5 exsiccates. Only one sample is determined as *I. florentina* (SOA 44729, cultivated).

Conclusion

The observed 564 samples of genus *Iris*, subgenus *Iris* display vertical distribution up to 2900 m above sea level. The highest altitude for *I. reichenbachii* is about 2900 m. *Iris suaveolens* and *I. variegata* reach up to 1500 m. *Iris pumila* can be found only in the low parts of the country, up to 800 m. The distribution of *I. ×germanica* is up to 1000 m, as this is related to its secondary distribution.

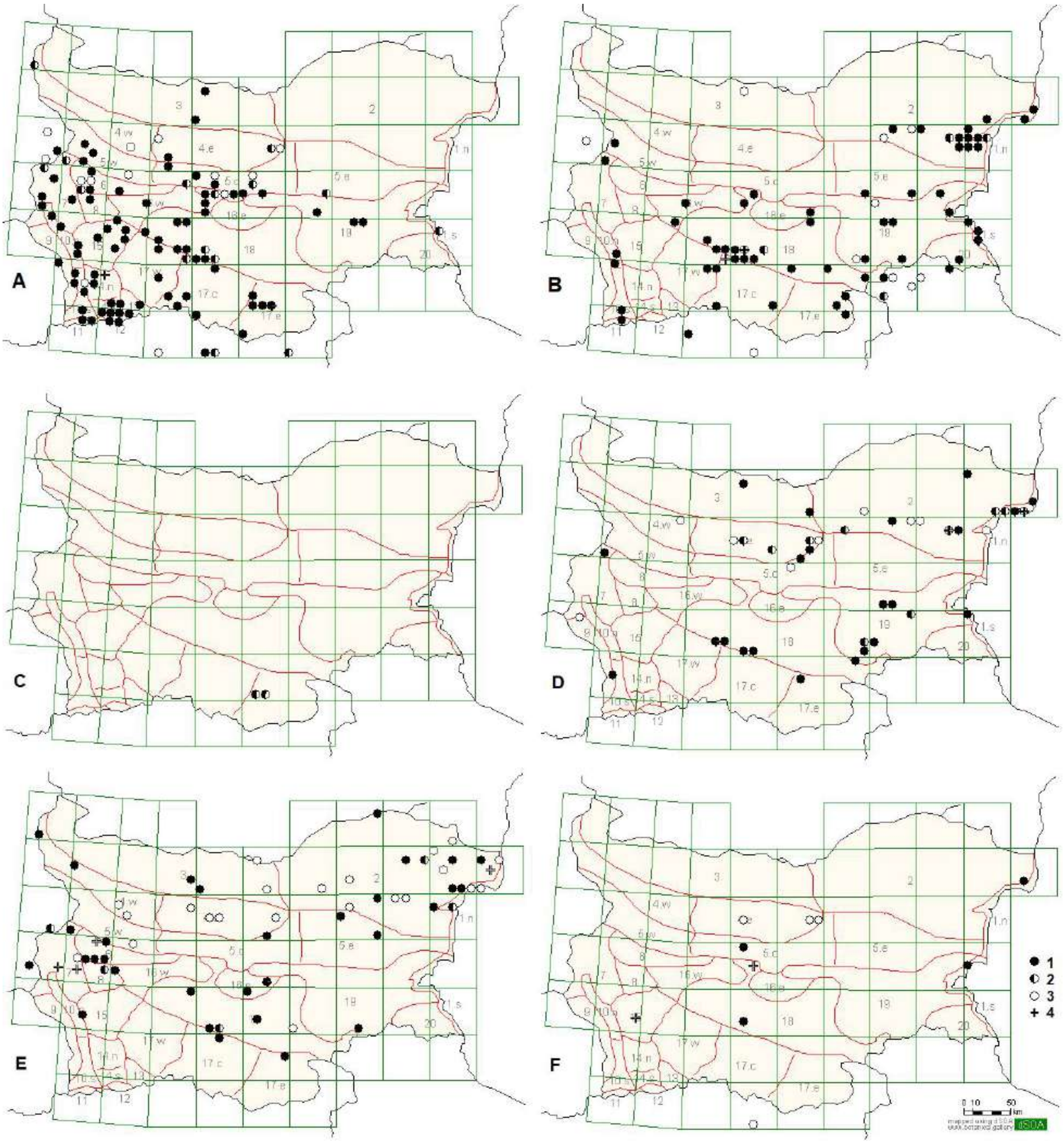

Fig. 1. Distribution maps: A – *Iris reichenbachii*; B – *I. suaveolens*; C – *I. aphylla*; D – *I. pumila*; E – *I. variegata*; F – *I. ×germanica*; 1 – localities of herbarium specimens; 2 - confirmed localities with herbarium specimens; 3 – reported localities from the literature; 4 – additional confirming records of observations without revised herbarium specimens.

According to the UTM grid (10x10 km), the most distributed species is *Iris reichenbachii*, represented by 60 squares, *I. suaveolens* by 48 squares, *I. variegata* by 35 squares, *I. pumila* by 23 squares, and *I. ×germanica* by 7 squares. The richest regions are Black Sea Coast, Balkan Range, and Thracian lowland – 5 species. Regions with 4 species are Danubian Plain, Znepole region, Rhodope Mts, and Toundja Hilly Country. Regions with 1 species are Slavyanka, the Valley of Mesta River, Pirin, and Mt Strandzha. Regions without representatives of West Frontier Mts and Mt Sredna Gora (east).

Iris aphylla is currently known only from a single locality in Bulgaria (65 years ago). It is not confirmed with recent literature data, terrain study, and herbarium samples, and to be still considered unverified.

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Appendix 1. Studied herbarium specimens of genus *Iris*, subgenus *Iris* with Bulgarian origin. Abbreviations: Collector's names (in brackets): Aht. – B. Akhtarov; Ch. – I. Cheschmedhiev; Dav. – B. Davidov; Delip. – D. Delipavlov; D.Dim. – D. Dimitrov; D.S. – D. Stoyanov; I.K. – I. Kovachev; I.P. – I. Penev; Jord. – D. Jordanov; Kit. - Kitanov; KS – K. Stoyanov; Mar. – Y. Marinov; Mrkv. – I. Mrkvička; Neic. – I. Neichev; Pop. – M. Popova; Stoj. – N. Stojanov; Stef. – B. Stefanov; Rad. – I. Radenkova; S.G. – S. Georgiev; S.Dim. – S. Dimitrov; Strib. – V. Stribrny; T.G. – T. Georgiev; TR – T. Raycheva; Urum. – I.Urumov; V.V. – V. Velchev; Vih. – N. Vihodzevsky; Z.B. – Zh. Barzov. The herbarium specimens from L, MA, MW, P, and PRK are studied on photo scans via GBIF.org. New regions marked with *.

Iris reichenbachii **Heuff.** (sub *I. bosniaca* Beck signed with index 1).

1s: **35TNG68**: Primorsko, 6.05.2014 (*A. Tashev*) SOM 171895; ***3***: **35TLJ00**: Pleven, 5.1996 (*B. Assyov*) SO 98549; **35TLJ13**: Shiyakovo, 04.1963 (*Ch.*), SOA 45737; ***4w***: **34TFP15**: Vrashka-Chouka peak, 16.04.1966 (*Ch.*), SOA 045721, 045732; 02.05.1975 (*Y.Koeva*) SO 91200, 95635; ***4e***: **35TLH87**. Veliko-Turnovo, 1896 (*Urum.*) SOM 14189; ***5w***: **34TFN75**: Dedina-Glava peak, 1.05.1920 (*Stoj. & Stef.*) SOA 2281; Belidie-Han, 05.1980 (*E. Bozhilova*) SO 91824; **34TFN77**: Petrohan, 5.05.1965 (Ch.) SOA 045731, 045736, 045738; 35TKH76: Glozhene (*Urum.* det. *Dav.*) SOM 14274; **34TFN86**: Chepurna peak, 05.1994 (*D.Dim.*) SO 98609; ***5c***: 1897 (Strib. det. Velenovsky sub *I. r.* var. *tubifera*, <u>holotypus</u>) PRC 451886; **35TKH93**: Kozyata-Stena hut, 1562 m, 4.04.1958, SOA 04386, 04387, 04388; 5.06.1962 (*Ch.*) SOA 45722; 6.05.1963 (*Ch.*) 045739; 19.05.1965 (*Ch.*) SOA 45734; 30.04.1965 (*Ch.*) SOA 045573, 034367; 30.05.1968 (*Ch.*) SOA 30191, 30192; **35TLH04**: Kozyata-Stena peak, 1600 m, 24.06.1930 (*Stoj.*) SO 13446; 14.06.1995 (*T.Meshinev*) SOM 152979; **35TLH12**: Sopot, 04.05.1976 (*S.Dim., Delip. & Ch.*) SOA 033589; 16.05.1976 (*Pop.*) SOA 032561; **35TLH22**: Karlovo, 1902 (*Urum.* det *Dav.*) SOM 14186, 14187; **35TLH23**: Kaloferski-Koupen peak, 6.08.1923 (Jord.) SO 13563; **35TLH42**: Kuru-Dere river, 28.06.1965 (*Delip.*) SOA 045664, 045667; **35TLH52**: Ak-Dere, 9.06.1965 (*Ch.*) SOA 045730; **35TLH63**: Malousha peak, 14.05.1898 (*Neic.*) SOM 14269; **35TKH75**: Teteven Balkan Mt, 1904 (*Urum.* det. *Dav.*) SOM 14276; **35TLH72**: Stara-Reka locality, 29.04.1939 (*A.Yurkovskiy*) SOM 14194; ***5e***: **35TMH42**: Tyulbeto locality, N42.71964 E26.36308, 1038 m, 19.05.2020 (*I. Kostadinov*) SOA 062750; Sinite-Kamani locality, 2.05.1962 (*V.V.*) SOM 105141; Sliven (*J. Wagner*) P 02163558; ***6***: **34TFN72**: Lyulin Mt, 2.05.1901 (*A. Toshev*) SOM 14239; 22.05.1938, SOM 14231 (sub *I. virescens* var. *bosniaca* Beck); **34TFN75**: Tsrucklevtsi, N42.947369 E23.131372, 794 m, 23.05.2020 (*TR & KS*) SOA 062768; **34TFN84**: Dobroslavtsi, 1912 (*J. Kelleler* det *Rad.*) SOM 14076; **34TGN12**: Aprilovo, 19.05.1982 (*D. Stoyanov*) SO 90972; ***7***: **34TFN30**: Silni peak, 18.06.1933 (Aht,) SOM 14183; 1000 m, 23.05.2009 (*A.Assenov*) SO 105829; **34TFN43**: Paramoun Mt, 29.05.1958 (*I.P. & Vih.*) SO 32104^{1}; **34TFM49**: Risha Mt, 26.05.1957 (*A. Yanev*) SO 102986; **34TFM58**: Gorna-Koznitsa, 8.05.1979 (*Ch.*) SOA 35865; **34TFN31**: Treklyano, 13.05.1939 (*K. Stoikov*) SOM 14229; **34TFN72**: Raylovo-Gradishte locality, 16.09.1947 (*I. Ganchev*) SO 83707; **34TFN34**: Zdreloto locality, 1904 (*Urum.* det. *Dav.*) SOM 14245^{1}; **34TFN43**: Lyubash Mt, 1200 m, 24.05.1987 (*V. Nikolov*) SOM 146599; **34TFN55**: Chepan, 28.05.1932 (det. *Aht.*) SOM 14247; **34TFN61**: Ostritsa peak, 1100 m, 10.05.1936 (*Aht.*) SOM 14227; 950 m, 20.05.1951 (*B. Zhelezova* det. *Aht.*) SOM 14230^{1}; 850 m, 05.1940 (Stoj.) SO 13500; 15.05.1941 (*Aht.*) SOM 14184; 18.05.1941 (*Aht.*) SOM 14232^{1}; 1000 m, 24.05.1955 (Vih.) SO 83708; 1100 m, 26.05.1957 (*Stoj. & Kit.*) SO 13447; 2.05.2005, SO 103401; ***8***: Vitosha, subalp., 1912 (Urum.) SOM 14236^{1}; 05.1920 (Stoj.) SOA 2250; (Jord.) SO 13451; 05.1922 (Strib.) SOA s.n.; **34TFN82**: Boyanski waterfall, 1895 (*S.G.*) SO 13456; 3.06.1923 (*Jord.*) SO 13449; Dol Rakovets locality, 05.06.1957 (*G. Dimitrov*) SO 13564; Dragalevtsi (*A. Drenovski*) SOM 14233; 05.1902 (Mrkv.) SOM 14228; 05.1905 (*Strib.*)

SOM 14235; 15.05.1920 (*Aht.*) SOM 14238, 14240[1]; 24.05.1936 (Jord.) SO 13452; Knyazhevo, 05.1890 (*S.G.*) SO 13450; ***9*: 34TGM20**: Delchevo border post, 14.05.1968 (*V.V.*) SOM 157568; ***10s***: **34TFM72**: Kresnensky-Prolom, 30.03.1920 (*Tsar Boris III*) SOM 14234, 14249[1]; Tisata reserve, 5.05.2007 (*D.Dim.*) SOM 164000; **34TFM73**: Stara-Kresna station, 5.05.1930 (*N.Fenenko* det. *Stoj.*) SOM 14250[1]; **34TFM81**: Kresna Station, 19.04.1964 (*Ch.*) SOA 045686; 34TFL89: Malak-Kozhukh hill, 14.05.1992 (*D.S.*) SO 96019-96020; ***10n****: **34TFM75**: Blagoevgrad, 600 m (*N. Fenenko* det. *Stoj.*) SOM 14185 (sub *I. chamaeiris* Bert. var. *balcana* Beck); ***11****: **34TFL88**: Ruzhdack, 16.07.1964 (*Ch.*) SOA 045682; N41.403606, E23.265164, 210 m, 15.04.2021 (*Mar.*) SOA 063105; **34TFL98**: Ruzhdack, N41.396207 E23.274501, 311 m, 15.04.2021 (*Mar.*) SOA 063104; ***12***: 28.05.1933 (*A. Drenovski* det. *Rad.*) SOM 14075; **34TGL09**. Sveti-Iliya locality, 26.04.1902 (*I. Bondev* det. *Rad.*) SOM 124533; **34TGL18**: Petrovo, 6.05.1992 (*I. Pashaliev*) SOM 151590; Suhoto-Ezero locality, 1800 m (*D.S.*) SO 92425; **34TGL19**: Gorno-Spanchevo, N41.5145 E23.5412778, 764 m, 1.04.2012 (*TR*) SOA 061728; **34TGL28**: Koynareto – Hambar Dere, 1700 m, 20.07.1983 (*Bozhlova, Tonkov & D.S.*) SO 91935; Hambar Dere locality, 04.05.1964 (*Ch.*) SOA 045710, 045719; Parilski-Dol locality, 8.08.1974 (*Ch.*) SOA 45760; 5.06.1983 (*R. Krusheva*) SO 91857; 19.05.1988 (*N. Valyovska*, det. *D. Peev*) SOM 147435; Nova-Lovcha, 17.06.2006 (*I. Pashaliev* det. *D.Dim.*) SOA 151589 (sub *I. r.* var. *balcanica* f. *bosniaca* (Beck) Acht.); **34TGL29**: between Lovcha and Paril, 6.05.1980 (*Ch.*) SOA 039619; 13.05.1981 (*Ch.*) SOA 045050; **34TGL39**: Pazlaka locality, 15.05.1970 (*Delip.*) SOA 030623; 25.05.1984 (*Ch.*) SOA 043958; ***14n***: **34TFM92**: Vihren peak, 1921 (*Kelleler* det. *Dav.*) SOM 14272, 14275[1]; **34TFM93**: Yavorov hut, 25.04.1976 (*I.K.* det. *Ch.*) SOA 047761; ***14s***: **34TGM10**: Baba peak, 1900 m, 1.06.1941 (*Kit.* & *Stoj.*) SO 93579; **34TGM20**: Pilentseto peak, 1522 m, 20.08.1988 (*D.S.*) SO 93912; ***15***: 06.1894 (*Strib.*) P 01840847; 30.05.1955, 1340 m (*I. Bondev*) SOM 106174, L 1472722; MW 0769512, MA 01-00192473; **34TFM76**: Rila, 1.05.1984 (*D.S.*) SO 92436; **34TFM97**: Droushlyavitsa river, 24.06.1974 (*Delip. & Ch.*) SOA 45748, 45749; **34TGM08**: Dospey, 1000 m, 20.05.1909 (*Dav.*) SOM 14253, 14259-14260, 14261 (rev. sub var. *davidofii* Akht.); **34TGM19**: Dragoushnovo, 950 m, 13.05.1912 (*Dav.*) SOM 14252; **34TGM27**: Ajran-Dere, 2150 m, 18.08.1911 (*Dav.*) SOM 13241, 14242 (sub *I.r.* var. *bosniaca* Beck); 18.08.1911 (Dav.); **34TGM28**: Dolna-Banja, 650 m, 24.04.1912 (Dav.) SOM 14254, 14256-14258, 14262-14264; ***16w***: **34TGN41**: Poibrene, 1904 (*Urum.* det. *Rad.*) SOM 14123; **35TLH10**: Voden-Kamak locality, 4.05.1974 (*Pop.*) SOA 032583, 32602; **35TLH11**: Mihiltsi, 22.04.1976 (*Pop.*) SOA 034177; ***17w***: **34TGM48**: Sestrimo, 9.05.1931 (*Trifonov*) SOM 14248; **35TKG63**: Beglika, 06.1956 (*S. Kozhukharov*) SO 13566; **35TKG66**: Tsepina station, 28.04.1978 (*Delip. & Ch.*) SOA 035278, 035279, 035280; **35TKG67**: Eli-Dere (Vetren-Dol), 14.04.1914 (*Mrkv.*) SOA 19050; ***17c***: **35TKG71**: Тешел, 4.06.1974 (*J.Koeva*) SO 86798; **35TKG80**: Trigrad, 1600 m, 31.07.1940 (*Jord.*) SO 13567; 26.06.1960 (*Ch.*) SOA 045695, 045725-045728; **35TKG91**: Bedenski-Bani, 15.05.1972 (*Petkova*) SO 35712; **35TKG95**: Oustina, 28.05.1967 (*I.K.* det. *Ch.*) SOA s.n.; Manastira locality, 10.08.1963 (Ch) SOA 04391; 13.05.1966 (*I.K.* det. *Ch.* sub *I. aphylla* x *I. reichenbachii*) SOA 030430, 030435-030436, 030905, 034671-034675; 30.05.1966 (*Ch.*) SOA 030252, 030423-030426, 030428-030429, 030432, 034663-034665, 034667-034669; 034677-034680, 034682, 034685; 17.04.1967 (*I.K.* det. *Ch.*) 025906-025907, 030252; 05.05.1968 (*Ch.*) SOA 033603; N42.02534 E24.54629, 812 m, 24.03.2019 (*TR*) SOA 062594; 30.04.2019 (*TR & KS*) SOA 063120; **35TLG15**: Kaloyanov peak, N42.04393 E24.70802, 637 m, 28.09.2018 (*TR & KS*) SOA 062434; Kouklen, 16.05.1966 (*Ch.*) SOA 045699; **35TLF09**: Ouhlovitsa cave, 19.05.1985 (*Pop.*) SOA 045025, 045030-045032; **35TLG05**: Hrabrino, 23.04.1891 (*S.G.*) SO 13565; **35TLG24**: Bachkovo, 28.04.1888 (Strib.) SO 13455; Bachkovo Monastery, 5.04.1915 (Mrkv. det Dav.) SOM 14266[1]; 7.05.1986 (*Pop.*) SOA 044835; Dobrostan, 1220, 1905 (*Urum.*) SOM 14273 (sub *I. r.* var. *tenuifolia* Vel.); Lale Bair locality 6.05.1975 (*Ch.*) SOA 033759-033760, 045740; Koru-Dere locality 05.1910 (*Strib.*) SOM 14179 (sub *I. r.* var. *tenuifolia*), 14268; 11.04.1915 (*Mrkv.* det. *Dav.*) SOM 14265; 30.04.1968 (Ch.) SOA 034349; Sveta-Petka Monastery, 560 m, 05.1915 (*Strib.*) SOM 14271 (sub *I. r.* var. *tenuifolia* Vel.); **35TLG25**: Asenovgrad (Stanimaka), 1891 (*Strib.*) PRC 451887 (sub *I. r.* var. *tenuifolia* Vel.); 05.1893, SOA 14567, 14569; 05.1894 (*Strib.*) P 02159332, 02159329, L 1472312^{1}; 6.06.1896 (Strib.) SO 13448; 05.1899 (*Strib.*) SO 13562, P 01840848; 04.1903 (*Strib.*) MA 01-00023208 (sub *I. r.* var *tenuifolia* Vel.); 05.1906 (*Strib.*) P01793712 (sub *I. r.* var. *tenuifolia* Vel.); 04.1914 (*Strib.*) SOA 14564, 19049; (*Mrkv.*) SOA 19051, 27.04.1914 (*Mrkv.*) SOM 14180 (sub *I.r.* var. *tenuifolia* Vel. locus classicus), 14192^{1}; (*Aht.* det. *Dav.*) SOM 14190, 14267^{1}; 11.04.1915 (*Strib.*) SOM 14270^{1}; 21.04.1919 (*Tzar Boris III* det *Dav.*) SOM 14178^{1}, 14193^{1}; 2.04.1966 (*Ch.*) SOA 045696; 17.04.1968 (*Ch.*) SOA 030190; 30.04.1968 (*Ch.*) SOA 025908, 029276 (sub *I. r.* var. *intermedia* Česchm., holotypus), 025909 (sub *I. r.* var. *intermedia* Česchm., isotypus), 047202 (sub *I. r.* var. *intermedia* Česchm., isotypus); 04.05.1975 (*Ch.*) 034351; 26.04.1989 (Delip.) 045309^{1}; Anatema locality, 8.04.1967 (*Ch.*) SOA 04389-04390, 045672, 045743, 045745-045746; 17.04.1968 (*Ch.*) SOA 030190, 030433-030434 (sub *I. r.* f. *balcana*), 034348 (sub *I. r.* f. *balcana*); N41.99099 E24.87531, 315 m, 28.04.2020 (*TR & KS*) SOA 062754; N41.9907 E24.88458, 640 m, 28.04.2019 (*KS*) SOA 063005, 063006; N41.9891667 E24.8772222, 500 m, 1.05.2020 (*TR & KS*) SOA 062755; ***17e*:** **35TLF57**: Chakalarovo, 25.05.2000 (*A.S.Petrova*) SOM 155586; **35TLG60**: Kardjali, 04.05.1937 (Stoj.) SO 13561; 25.06.1968 (*Ch.*) SOA 030431 (sub *I. reichenbachii* x *I. aphylla*), 45785-45786, 47762; Momchilgrad – Kardjali, 8.05.1964 (*Ch.*) 045741-945742; **35TLG80**: Golemiya-Sipey locality, N41.6292639 E25.6134851, 428 m 28.04.2021 (*V. Trifonov* det. *TR & KS*) SOA 063122, 063126-063127, 063167; ***18*:** **35TKG86**: Novo-Selo, N42.1030556 E24.4586111, 401 m, 3.06.2010 (*KS*) SOA 062495; **35TKG89**: Smilets, 20.04.1977 (*Pop. & Ch.*) SOA 031256-031257, 031258; **35TKG96**: Kourtovo-Konare, 20.06.1995 (*D. Georgiev*) SO 199158; 35TKG99: Dragomir, 08.1898 (*S.G.*) SO 13560; **35TLG16**: Plovdiv (cult.), 5.05.1973 (*Pop.*) SOA 029646-029647, 5.03.2018 (*Ch.*) SOA 062408; ***19****: **35TMG79**: Yambolski-Bakadjik ridge, 5.05.1965 (*Ch.*) SOA 045229, 45687; **35TMG89**: Voynishki-Bakadjik ridge, 7.05.1965 (*Ch.*) SOA 045665, 045667, 45681; **35TMH30**: Konjovo, 1891 (*J. Velenovsky*) PRC 451885.; Bulgaria, without data: 06.2021, SOA 14566; (A. Toshev) SOA 23587.

Iris suaveolens Boiss. & Reut. (sub "*I. rubromarginata mellita*" signed with index 1; sub "*I. mellita* Janka" signed with index 2):

1s: **35TNG59**: Kavatsite locality, 19.04.1975 (*Gerginov, Markova & Cherneva*) SOM 134213^{2}, L 1472899, MA 01-00210537, U 1343276; Sozopol, 8.05.1929 (*Jord.*) SO 13510^{2}; **35TNG67**: Primorsko, 30.04.1965 (Ch.) SOA 045709^{2}; **35TNG68**: Arkoutino locality, 19.04.1975 (Markova, Cherneva & Gerginov) 19.04.1975; Ropotamo Estuary, 22.07.1938 (*L. Tzvetkov & Vih.*) SO 13524^{2}; **35TNH30**: Bourgas, 27.04.1921 (*Aht. det Dav.*) SOM 14018;.***1n*:** **35TNH78**: Varna, 13.04.1903 (*Dav.*) SOM 14022^{1}, 14015^{1}, 14125^{1}; 15.05.1904 (*Dav.*) SOM 14012^{1}, 14094^{1}, 14111^{1}; **35TPJ10**. Kaliakra, 29.03.1901 (*Dav.*) SOM 14101; 26.03.1998 (*Delip.*) SOA 047882; 01.04.2002 (*Ch.*) SOA 049452; 24.04.2003 (*Ch.*) SOA 059898; Kalekayryak, N43.3891111 E28.4382778, 76 m, 14.04.2012 (*TR & KS*) SOA 061743-061746; **35TPJ21**: Yailata, N43.4333333 E28.5333333, 50 m, 16.05.2010. (*TR*) SOA 062264; N43.4355556 E28.5445, 11 m, 14.04.2012 (*TR & KS*) SOA 061742, 061747; ***2:*** Deli-Orman area, 14.02.1902 (*Dav.*) SOM 14109; **35TMH79**: Manastirtsi, 4.1918 (*Strib. det. Rad.*) SOM 14105^{2}; **35TNH09**: Madara, 6.04.1966 (*A.Yanev*) SO 86633^{2}; **35TNH38**: Provadia, 12.04.1902 (*Dav.*) SOM 14108^{1}, 14011^{1}, 14014^{1}; 35TNH48: Devnya, 4.05.1926 (*Jord.*) SO 13509; **35TNH47**: Sindel, 04.1940 (*Stoj.*) SO 13551^{2}; **35TNH57**: Avrenska Mt, 15.04.1901 (*Dav.*) SOM 14015, 23.04.1903 (*Dav.*) 14028; **35TNH58**: Beloslav, 12.04.1957 (*Jord.*) SO 13525; **35TNH59**: Kalimantsi 4.04.1902 (*Dav.*) SOM 14023; **35TNH67**: Priseltsi, 20.04.1903 (*Dav.*) SOM 14103; **35TNH68**: Aksakovo, 11.04.1901 (*Dav.*) SOM 14107^{1}; Vladislavovo, 30.03.1903 (*Dav.* rev. *Rad.*) SOM 14104^{2}; **35TNJ70**: Batovo, 14.04.1905 (*Dav.*)

SOM 14110; ***5w*:*** **34TFN67**: Komshtitsa, 17.06.2006 (*coll. G. Stoyanov* det. *D. Dimitrov*) SOM 162787; ***5c*:*** **35TLH22**: Karlovo, 1.05.1967 (Vih.) SO 13526^{2}; ***5e***: **35TMH42**: Sliven, 28.04.1924 (*Jord.*) SO 13523-13514^{2}; 30.05.1927 (Stoj. & Stef.) SOA 02285; **7*: 34TFN55**: Chepan, 23.05.192 (*Jord.*) SO 13523^{2}; ***10s***: **34TFL89**: Malak-Kozhukh hill, 7.05.1964 (*Delip.*) SOA 045666^{2}; ***10n***: **34TFM75**: Blagoevgrad, 5.05.1930 (*Stoj.*) SOA 02287^{2}; ***11***: **34TFL88**: Petrich, 1929 (*Zhekov* det. *Stoj.*) SOA 02286; ***16w:*** **34TGM39**: Zaichy peak, 19.04.1891 (*S.G.*) SO13518^{2}; **34TGN41**: Poibrene, 1904 (*Urum.* det. *Dav.*) SOM 14188; **35TLH11**: Mihiltsi, 359 m, 22.04.1976 (*Delip.*) SOA 38356^{2}; ***17.*** Rhodopi Mts 1929 (*Stransky*) SOA 02284^{2}; ***17w***: **35TKG74**: Nova-Mahala, 27.05.1895 (*Strib.*) P02163464; **35TKG84**: Ravnogor, 05.1952 SOA 04385^{2}; ***17c*:*** **35TKG80**: Trigrad, 22.07.1938 (*Jord.*) SO 13553^{2}; **35TLG05**: Hrabrino, 13.04.1998 (*Ch.*) SOA 060059^{2}; **35TLG15**: Brestnik, 25.04.1971 (*Pop.*) SOA 25899^{2}; 20.04.1976 (*G. Stoychev & Ch.*) SOA 34048-30349^{2}; Kouklen, 1.05.1984 (Ch.) SOA 040585^{2}; 35TLG40. Dyavolsky bridge, 2.05.1961 (*Vih.*) SO 46740^{2}; 35TLG25: Asenovgrad, 6.06.1896 (*Strib.*) SO 13515; ***17e***: **35TMF29**: Ivaylovgrad, 03.05.1932 (*Stoj.*) SOA 14573; **35TMG10**: Dabovets – Kamilski-Dol, 11.05.1996 (*I.Gerasimova, D.Venkova & A.S.Petrova*) SOM 53449; ***18***: Bessapara ridges, 16.04.1966 (*Ch.*) SOA 045705; **35TKG86**: Elenski peak, 25.04.1937 (*S.G.*) SO 13547^{2}; Glavinishki ridge, N42.13971 E24.34422, 260 m, 28.04.2021 (*KS)* SOA 063106; **35TKG96**: Trivoditsi, 13.04.1889 (*S.G.*) SO 13517*i*; **35TLG06**: Purvenets, 05.05.1980 (*Ch.*) SOA 38619^{2}; **35TLG16**: Plovdiv, 05.1894 (*Strib.*) SOM 14112^{1}, P02163466; 05.1902 (*Mrkv.*) SOM 14116^{1}; (*Strib.*) SOA 14571^{2}; 04.1903 (*T.Pichler*) MA 01-00023207^{2}; Dzhendemtepe, Plovdiv, 06.1891 (*V.Janka*) JE 00022413^{2}; (*E.Halacsy*) 02163468; (T. Pchler) 01793703^{2}, 02163467^{2}; (P.Sintenis) P02163465; 05.1893 SOA 14572^{2}; 06.05.1893 (*Strib.*) SOM 14114^{1}, P 02163469^{2}; US 1312870^{2}; 13.05.1893 (Strib.) SO 13516^{2}, L 1472900^{2}; 23.05.1896 (*Strib.*) SO 13520-13521^{2}; 04.1910 (*Strib.*) SOM 14106^{1}; 29.03.1914 (*Mrkv.* rev. *Rad.*) SOM 14024^{2}; 7.04.1965 (*Ch.*) SOA 45702^{2}; Bounardjik (*Strib.*) P 02163470^{2}; **35TLG36**: Popovitsa, 05.1895 (*Strib.*) P01840773^{2}; **35TLG64**: Mineralni-Bani, 22.04.1987 (*Delip.*) SOA 045351; **35TLG89**: Stara-Zagora, 12.04.1902 (*Aht.* det. *Dav.*) SOM 14126; 28.03.1911 (*Aht.* det. *Dav.*) SOM 14020-14021^{2}; **35TLG94**: Bryagovo, 25.04.1962 (*Vih.*) SO 13522^{2}; **35TLH80**: Ayazmoto park, 7.05.2005 (*S. Radanova*) SOM 163477; **35TMG21**: Karatepe, 22.04.1940 (*Kit.*) SO 32686^{2}; **35TMG63**: Matochina, 26.05.1962 (*Jord. & A. Yanev*) SO 26800^{2}; ***19:*** **35TMG43**: Shtit – Dervisha-Mogila, 25.04.1937 (*Jord.*) SO 13549^{2}; **35TMG45**; Topolovgrad, 25.04.1971 (*I.Panova* det. *Ch*) SOA 045658; **35TMG69**: Kalchevo, 28.04.1943 (*Jord.*) SO 13546^{2}; **35TMG79**: Yambolski-Bakadjik ridge, 20.04.1891 (*S.G.*) SO 13511; 07.05.1964 (*Ch.*) SOA 045657^{2}, 045700^{2}, 045703^{2}, 04705^{2}, 07.05.1965 (*Ch.*) SOA 045708^{2}, 045795-045797^{2}; 7.05.1976 (*Ch.*) SOA 042581; **35TMH92**: Karnobat, 23.04.1930 (*Jord.*) SO 13512^{2}; **35TNH22**: Aitos, 05.1905 (*Str.*) SOA 14562; 04.05.1929 (*Jord.*) SO 13510^{2}; 12.05.1965 SOA 04400-04401^{2}; ***20***: **35TNG34**: Vitanovo reserve, 19.04.1995 (*D.S.*) SO 98982; **35TNG45**: Stoilovo, 15.08.1934 (*Jord.*) SO 13545^{2}, Sredoka reserve, 04.2000 (*B. Georgiev & D.S.*) SO 101663.

Iris aphylla **L.**

17e. **35TLG60**, The Bridge south of Kardzhali, 275 m. 8.05.1964 (*Ch.*) SOA 045642, 045643, **35TLG70**. Zhelezni-Vrata train stop, 240 m, 1964-05-08 (*Delip. & Ch.*) SOA 45642-45645, 45647-45649; 1965-05-08, 1975-06-13 (*Pop.*) SOA 31118.

Iris pumila **L.**

1s: **35TNG59**: Sozopol, 1932 (*Stoj.*) SOA 02283; ***1n***: **35TNJ90**: Balchik, 24.04.1988 (*Ch.*) SOA 45612; 35TPJ00: Touzlata, 06.04.1957 (*Vih.*) SO 13554; **35TPJ10**: Bulgarevo, N43.408545 E28.398388, 90 m, 9.04.2020 (*Z.B.*) SOA 062785; Tyulenovo, N43.485668 E28.575025, 11 m, 9.04.2020 (*Z.B.*) SOA 062786; Bolata, N43.38991 E28.47254, 48 m, 9.04.2020 (*Z.B.*) SOA 062787; 35TPJ21: Yailata, 10.04.2007 (A.Asenov) SO 104378; ***2:*** **35TMH79**: Manastiritsa, 04.1918 (*Strib.*) SOA 04636-04637; **35TNH38**: Kairyaka locality, N43.18959 E27.47539, 268 m 14.04.2020 (*Z.B.*)

SOA 062780; Provadiya, N43.1766667 E27.4425, 35 m (*S.G.*) SO 1354?; **35TNH48**: Devnya, 1902-04-11 (det. Dav.) SOM 14016; Zederlikaylack hill, N43.18907 E27.54436, 186 m, 14.04.2020 (Z.B.) SOA 062782; **35TNJ54**: Lyaxovo, 7.05.1987 (*V.V.*) SOM 15172-158173; ***3:*** **35TLJ13**: Shiyakovo, 08.04.1966 (Ch.) SOA 45660-45662, 45667, 45668; 35TLJ80: Polsko-Kosovo, 04.1931 (K.Popov) SO 13544; ***4w***: **35TLH17**: Lovech, 1901 (Urum.) SOA 14565; ***4e***: **35TLH46**: Sevlievo, 03.1904 (*Neic.*) SOM 14065; 35TLH86: Veliko-Turnovo station, 07.06.1966 (Vih.) SO 13552; 35TLH87: Veliko Turnov, 1897 (*I. Stamboliev*) SOM 14067; ***5c***: 35TLH75: Dryanovo monastery, 04.1901 (*Neic.*) 14063; **7**. **34FN55**, Chepan, 05.1914 (Stoj. & Stef) SOA 02278; **10s**: **34TFM72**, Kresna gorge, 17.04.1970, (*Delip.*) SOA 30687; **17c**: **35TLG15**: Brestnik, 24.04.2011 (*TR*) SOA s/n; 35TLG25: Asenovgrad, 05.1893 SOA 14563; **35TLG02**: Zornitsa village, 20.04.1970 (*Jord.*) SO 13555; ***18***: **35TKG86**: Elenski peak, 19.06.1965 (*Ch.*) SOA 45674-45676; 35TKG96: Trivoditsi, 04.04.1892 (*S.G.*) SO 13550; **35TMG34**: Kostur, N41.9796811 E26.2701371, 580 m (*V.Trifonov* det. *TR & KS*) SOA 063125; ***19***: **35TMG46**: Topolovgrad, 04.07.1974 (*Ch*) SOA 45688; **35TMG56**: Golemiy-Kamuk locality, N42.09524 E26.50081, 150 m, 13.04.2021 (*TR & KS*) SOA 063103; **35TMH60**, Yambolski Bakadzhik, 06.05.1963 (*Ch.*) SOA 45659, 45692; 24.04.1968 (*Pop.*) SOA 25903.

Iris variegata L

1n: **35TNJ73**: Kranevo, 27.05.1997 (*Delip.*) SOA 047633; **35TNH78**: Varna, 6.06.1904 (*Dav.*) SOM 14214; 35TNJ80: Batova, 2.06.1968 (*Kit.*) SO 91612; ***2:*** **35TMH99**: Shoumen, 05.1951 (*S.Dim. & Delip.*) SOA 04402-04404; **35TMJ98:** Popina, 25.06.1946 (*Jord.*) SO 13630; **35TNH58**: Beloslav, 81 m, N43.210928 E27.685363 (*Z.B.*) SOA 062858; **35TNJ23**: Zurnevo (*Kit. & I.P.*) SO 72124; **35TNJ43**: Karapelit, N43.649 E27.5831, 195 m (*Z.B.*) SOA 062859; 35TNJ70: Prilep, 2.06.1975 (*I. P. & Kit.*) SO 32543; **35TPJ03**: Surnino, 26.05.1958 SOA 04405-04407; ***3***: **35TKJ91**: Ostrata-Mogila locality, 23.05.1998 (*R.Tsonev*) SO 99629; **35TLJ00**: Pleven (*B. Ivanov*) SOA 14559; ***4w***: **34TFP15**: Vrushka-Chuka peak, 30.05.1967 (Ch.) SOA 045779-045781; 34TFP52: Белотинци, 12.06.1949 (*Jord. & S.Valev*) SO 13629; ***5w***: **34TFN94**: Kutinski-Piramidi locality, 18.06.1974 (*J. Koeva*) SO 69477; ***5c****: **35TLH75**:Momini-Skali locality, N42.95473 E25.444563, 518 m, 1.07.2020 (*Mar.*) SOA 062983; ***5e****: 35TMH57, Omourtag, 23.06.1923 (Jord.) SO 13662; 35TMH95: Malka-Orlitsa peak, 650 m, 08.07.2011 (*D.S.*) SO 101664; ***6:*** 03.06.1921 (Jord.) SOA 14560; **34TFN72**: Divotino Monastery,14.06.1931 (Stoj & *T.G.*) SOA 02276; 16.06.1931 (Stoj. & *T.G.*) SOM 14211; **34TFN72**: Souhodolska river, 30.06.1967 (Vih.) SO 13631; 34TFN82: Gorna-Banya, 20.06.1948 (I.Ganchev) SO 83703; Suhodol, 02.05.1929 (Vitanov, det. T.G.) SOA 02274; **34TFN92**: Lozenets, 04.06.1888 (*S.G.*) SO 13626; ***7:*** 34TFN55: Chepan, 7.07.1930 (B.Stefanov & T.G.) SOA 02273; ***8***: 4.06.18889 (*S.G.*) SO 13623; 34TFN82: Boyana, 06.1932 (K.Popow) SO 13627; ***15****: **34TFM76**: Rila, 850 m, 02.05.1989 (*D.S. & M.Langov*) SO 94208; ***16w***: **34TGN01**: Pasarel, 18.07.1975 (*L. Drazheva*) SO 41972; ***16e****: 35TLH70: Starozagorski-Bani, 7.06.1961 (Vih.) SO 30624; ***17c****: **35TLG15**: Kouklen, 20.05.1964 (Ch.) SOA 45772; N42.0271389 E24.7682778, 500 m, 17.06.2012 (KS) 061734; **35TLG24**: Bezovo hut, 26.05.1963 (Ch.) SOA 045459-045460, 045462, 045464-045467; **35TLG25**: Asenovgrad, 11.05.1892 (*S.G.*) SO 13628; 21.05.1893 (*Strib.*) SO 13625; 06.1894 (*Strib.*) SOA 14561; 10.06.1963 (*Ch.*) SOA 045458; 1.07.1964 (*Ch.*) SOA 045463; 19.05.1974 (*Ch.*) SOA 045783-045784; Asenova-Krepost fortress, 1.06.1967 (*Ch*) 033318-033320; ***17e***: **35TLG92**: Maluk-Izvor, 1.06.1964 (*I.K.*) SOA 047694; ***18****: **35TLG25**: Dolni-Voden, 20.05.1965 (*Ch.*) SOA 045461; 15.05.1967 (*Ch.*) SOA 045712 (sub *I. v.* var. *pigmea* Českhm.), 037370; 30.05.1967 (*Ch.*) 045450-045457, 045750, 045787-045791, 045793-045794; **5TLG59**: Malko-Dryanovo, 06.04.1972 (*S.Dim.*) SOA 025900-025902, 25948; 35TLG66: 06.1915 (det. *Stoj. & Stef.*) 02275; ***19***: **35TMG75**: Razdel, 27.06.1941 (*Jord.*) SO 14624.

Iris* ×*germanica L.

1s. **35TNH52**: Kaloyanova-Bakhcha locality, N42.714, E27.7233333, 22 m, 17.04.2011 (*TR & KS*) SOA s/n; **1n**. 35TPJ11: Sovata Bay, N43.5206389 E28.4349167, 1 m, 17.05.2010 (*TR & KS*) SOA 062265; **5c**. **35TLH14**, Knezhki Lag, 1893 (*Urum.*) SOM 100643; 18: **35TLG16**: Plovdiv, 13.05.1980 (*Pop.*) SOA 044729, 04762.

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Non-native Brook Trout Salvelinus fontinalis in Bulgaria: an Established Population in the Palakariya River (Balkan Peninsula, Iskar River Basin)

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Abstract. In the present study, we provide data on the first established, self-sustaining population of non-native brook trout (*Salvelinus fontinalis* Mitchill, 1814), family Salmonidae, in Bulgaria. The brook trout was detected in upland section of the Palakariya River (Iskar basin) at an altitude between 1350 and 1500 m a.s.l. Distribution, abundance and size structure of *S. fontinalis* were studied in the period 2019-2021. The coexistence of individuals of different sizes (from 4.1 cm to 24.6 cm); no restocking activities in the last 10 years and the suitable environmental habitat features support the contention of a self-reproducing population of *S. fontinalis* in the Palakariya River.

Key words: non-indigenous species, salmonid fish, self-reproducing, Bulgaria.

Introduction

The brook trout *Salvelinus fontinalis* (Mitchill, 1814) is a native salmonid species for North America (Page & Burr, 1991; Karas, 1997; Scott & Crossman, 1973). This fish inhabits oligotrophic streams, rivers and lakes and prefer cool, clear, well-oxygenated waters, pools with large woody debris and temperatures up to 20 °C (FishBase, 2021; Page & Burr, 1991; Karas, 1997).

Brook trout is a highly desirable fish for both sport fishing and aquaculture (Jansson, 2013). Since brook trout is relatively tolerant of acidic waters withstanding a pH as low as 5.0 (Dunson & Martin, 1973), it has been used as a replacement when populations of native salmonid species become locally extinct due to acidification of some Scandinavian lakes and rivers (Hesthagen et al., 1999). *S. fontinalis* were introduced in more than 50 countries all over the world (FishBase, 2021; Jansson, 2013; Welcomme, 1988). Reports for established population of *S. fontinalis* outside the species native range exist for 20 countries (Jansson, 2013). In Norway alone, 202 established populations of the species have been identified (Hesthagen et al., 2018).

In many countries *S. fontinalis* is considered as a medium-risk invasive species among non-native freshwater fish (Britton et al., 2010; Copp et al., 2009; Simonović et al., 2013). Many introductions of *S. fontinalis* were to previously fishless lakes which resulted in a dramatic reduction of amphibian populations, zooplankton and other invertebrates (Benjamin et al., 2013; Dunham, 2004; FishBase, 2021; Fuller &

Neilson, 2014; Jansson, 2013; Levin et al., 2002; Nakano et al., 1998; Spens et al., 2007). Brook trout have been found to compete with, displace, or replace many fish species throughout the world including golden trout (*Oncorhynchus aguabonita*), brown trout (*Salmo trutta*), cutthroat trout (*O. clarki*), bull trout (*Salvelinus confluentus*), and chinook trout (*O. tshawytscha*) (Levin et al., 2002; Korsu & Huusko, 2009; Korsu et al., 2009; Öhlund et al., 2008; Reiman et al., 2006). A study of 193 boreal lakes in Sweden found that introduced *S. fontinalis* pose a detrimental impact to native brown trout. Twenty percent of brown trout populations exposed to brook trout went extinct versus an only 2% extinction rate in unexposed lakes (Spens et al., 2007). Natural hybridization and other reproductive interactions between brook trout and brown trout that might detrimentally affect on native species have been recorded (Cucherousset et al., 2008). Hesthagen et al. (2018) supposed that invasiveness of the brook trout depends on site and conditions.

In Bulgaria, *S. fontinalis* has been introduced in the 1930 from Czechoslovakia (Wellcomme, 1988). To middle of 2010s, *S. fontinalis* was produced mainly on a state hatchery in the town of Samokov. Annual production varies between 5 and 12 tones. Since the middle of the 20th century, brook trout has been introduced into more than 20 alpine lakes and rivers throughout the country (T. Trichkova, G. Raikova, unpublished data). Some of these water bodies, such as glacial lakes were naturally fishless. There are observations that in the areas where stocking with rainbow trout and brook trout is carried out, the native brown trout is not found or has a low density. However, the reasons for this have not been studied. Despite suspicions of a negative impact of these nonnative salmonid species on local fauna, no research has been conducted in this regard (Uzunova, 2006). At the beginning of this century, the official policy in Bulgaria regarding the distribution of alien species was changed and brook trout stocking ceased. However, probably due to the lack of effective control over stocking conducted by individual and private organizations, brook trout continue to be released in wild.

River Palakariya is a part of Iskar River basin. The valley of the Palakariya River is included in the European ecological network Natura 2000 (BG0000617) (38.1 rkm). The uppermost part of the river (6.6 rkm) is on the territory of Vitosha Nature Park. At the beginning and middle of the last century the ichthyofauna of the Iskar River and its tributaries was studied by Drenski (1921) and Paspalev & Peshev (1955). More recent studies on ichthyofauna of the Palakariya River show totally 15 fish species and subspecies from three families (Dikov et al., 1988; Kenderov et al., 2017; Pavlova & Pehlivanov, 2009). The following fish species listed in Annex 2 of the Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora are found in the river: *Sabanejewia aurata*, *Rhodeus amarus*, *Barbus petenyi*, *Cobitis taenia*, *Gobio uranoscopus* and *Gobio kessleri*. In 2015, the reintroduction of bullhead (*Cottus gobio*) was carried out with the aim to restore its population in the river (Uzunova et al., 2017). Faunistic research has not documented the presence of brook trout so far, but the surveys did not cover the uppermost sections of the river. However, a few angler records of this species posted in different internet platforms suggested that a small population of brook trout exist. The first confirmed record of brook trout in the Palakariya River was in 2016 when ten adult individuals were caught near to the village of Yarlovo (lat. 42°27' 55, long. 23°16' 55, 1120 m a.s.l.) (EU pers. obs.). The following year, five sexually mature brook trout were caught at the same place (EU pers. obs.). Despite the long period of acclimatization of *S. fontinalis* in Bulgaria, the existence of a self-sustaining population has not been established so far.

The aim of the present study is to find evidences that the non-native brook trout has formed a self-sustaining population in the Palakaria River.

Material and methods

Study area. The study was conducted on the Palakariya River. With its 44, 7 km of length it is the one of largest left tributaries of the Upper Iskar River (Danube River Basin). The watershed area is 400 km^2 (Hristova, 2012). The Palakaryia River rises at the foot of the Kupena peak in the south part of Vitosha Mountain at the altitude of about 2113 m a.s.l. The study covered the area from weir located above the village of Yarlovo to the spring zone of the river or a total of 6.1 km. The entire study area is natural without any significant modifications except two fragmentations caused by weirs higher than two meters (Fig. 1). Regarding habitat characteristics, fast flowing, shallow riffles with some interspersed pools (0.9 m max depth) were dominated. Bottom substrate was coarse, composed by gravel, boulders and rocks. River wetted width was between 1 and 3.5 m. Above site 4 the river passes into its spring zone, characterized by significant steepness and width ~1 m (in low water period). Water level varied from 0.1 m to 0.5 m depending on the hydrological conditions. Water temperature during winter months varied between 1.5 and 4 °C, in summer max temperature reached 12 °C. Dissolved oxygen range from 9.2 to 11.5 mg/l; conductivity was ranged from 20 to 40 µS cm^{-2}; pH - from 7.7 to 8.4. Just above weir 2 (site 1) a pool ~ 15 m^2 and 1.2 m max depth was formed.

Data collection. Samples were collected between 2019 and 2021 in five expeditions (May, July, October, November, and December). The fish sampling was performed during the day time (9.00:17.00). Two different collection types were conducted. Point Abundance Sampling by Electrofishing approach (PASE) SAMUS-725G device (12 cm diameter ring anode, average voltage of 200 - 350 V, operating at an average of 3 - 8 A) was used in area from weir 1 located upstream of Yarlovo village to weir 2 (site 1, where the presence of brook trout has been found). 261 sampling points or 1 point every 20 m were randomly selected in an upstream direction. Anode was activated for a 10-s period at each point sample (Copp, 1989).

Abundance of *S. fontinalis* in river section between site 1 and site 4 was assessed by using CEN standard (CEN, 2003) with the same equipment except ring anode, which was replaced with a 30 cm diameter. A single pass electrofishing without block nets was applied. Sampling transects were between 30 and 165 m long, depending on river width. The total fishing area per site varied between 380 and 420 m^2. The electrofishing was conducted from one bank to the other over the entire river stretch. The electrofishing crew was consisted of one operator and one netter. Electrofishing was performed in zigzag pattern, upstream covering all available habitats.

All the collected brook trout specimens were fixed in formaldehyde solution and stored in the collection of the Department of General and Applied Hydrobiology (GAH). All other fish species caught have returned to places of their capture after recovering from electronarcosis. Each brook trout was photographed with a Canon EOS Digital 350D camera and the photos were used for morphometric measurements (Digimizer Version 4.6.1 MedCalc Software). The collected fish were counted and measured: total length (TL), standard length (SL) in 0.1 cm accuracy and total weight (W) in 0.1 g accuracy. The measurement of the plastic and meristic characteristics was performing according to the scheme proposed by Pravdin (1966). The identification keys of Bacon (1954), Karas (1997), Kottelat & Freyhof (2007), Martinez (1984) and Stauffer & King (2014) were used.

At each site, the physical and chemical parameters, i.e. conductivity (µS/cm), pH, dissolved oxygen content (D.O., mg/l), and water temperature (°C) were measured, using Portable Hanna Instr.

Data analysis. All sampled specimens were split in size groups (every 10 mm TL) and the number of individuals in each group was plotted as a histogram. The total length frequency formula is as follows:

$$TLi = Ni/ N \times 100\%$$
$$(i = 4{,}1 – 5{,}0 \text{ cm}, 5{,}1 – 6{,}0 \text{ cm}...24{,}1 – 25{,}0 \text{ cm}),$$

where TLi is the frequency for a certain interval, Ni is the number of specimens in one total length interval, and N is the total number of specimens.

The fish density was expressed as number of fishes caught per 100 m^2 and the biomass in kg per 100 m^2. Abundance and biomass of *S. fontinalis* were calculated separately for each sampling site.

Results

The following fish species were collected in the river section between weir 1 and weir 2 (Fig.1): *Salmo trutta* (n=34), *Phoxinus phoxinus* (n=14) and *Barbus petenyi* (n=7). The survey detected *S. fontinalis* (n=51) in the highest parts of the Palakariya River at altitude of 1352 m (Site 1 - N 42° 30' 44,43"; E 23°16' 2.56") to about 1500 m a.s.l. (Site 4 - N 42° 30' 48.78"; E 23°17' 4.515") (Fig. 1). The length of the river section inhabited by brook trout was about 900 m. No other fish species than *S. fontinalis* were found in the river section upstream weir 2. The zone above site 4 was fishless.

Collected fish specimens in sites 1 to 4 had a spindle-shaped body with an adipose fin close to the tail. The adult specimens had dark green to brown back, with a distinctive marbled pattern of lighter color, described as vermiculations. These vermiculations break up into light spots and relatively small red spots surrounded by blue halos along their sides. Pectoral, pelvic, and anal fins have white edges followed by a contrasting black stripe (Fig. 2). Juvenile fish have 8-10 parr marks.

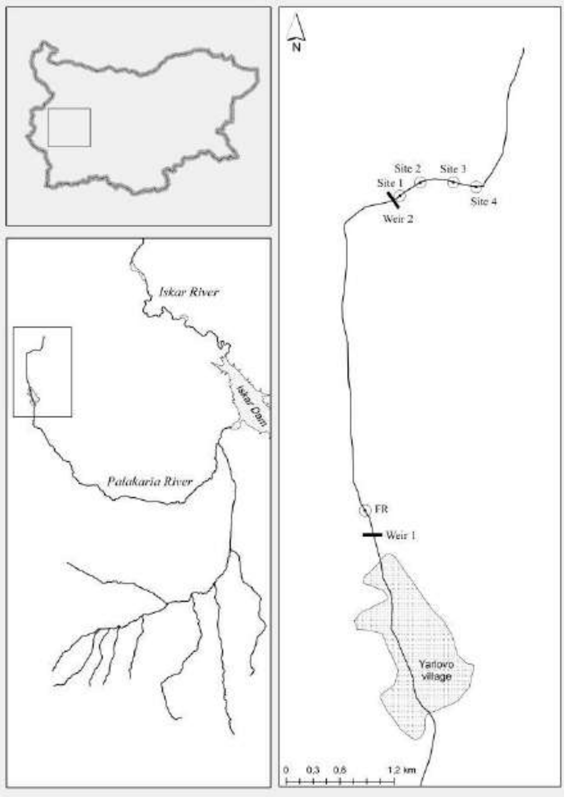

Fig. 1. Confirmed records of *S. fontinalis* in the Palakariya River: Site 1,2,3,4 - sites of established brook trout in period 2019-2021; FR – site of established brook trout in period 2016-2017; (▬) – unpassable fragmentation (weir).

The total body mass of the collected *S. fontinalis* individuals ranged from 2.0 g to 155 g. The longest specimen was a female of 246 mm (TL), and the smallest was 4.1 cm (TL). The most common were fish with size from 4 to 10 cm (Fig. 3). No external malformations were found among collected individuals. The adult brook trout caught during the autumn months were sexually mature and released eggs and milt when their abdomens were gently pressed.

The density of *S. fontinalis* in sites 1-4 varied from 6 to 28 ind. 100 m^{-2}. Mean density (mean number of fish per 100 m^{-2} ± SE) was 13 ± 5.07. The highest density was observed at the site 1, while the lowest at site 4 (Fig. 4). Site 1 was dominated by fish of the smallest size classes, so the biomass measured here is the lowest, while in site 2 was found few, but relatively large fish (Fig.4).

Fig. 2. Brook trout specimens caught in the Palakariya River. A) Specimen Cat. N GAH 123194, 13.5 cm TL; B) specimen Cat. N GAH 12531912, 4.1 cm TL, (on the right). Photographs by Eliza Uzunova.

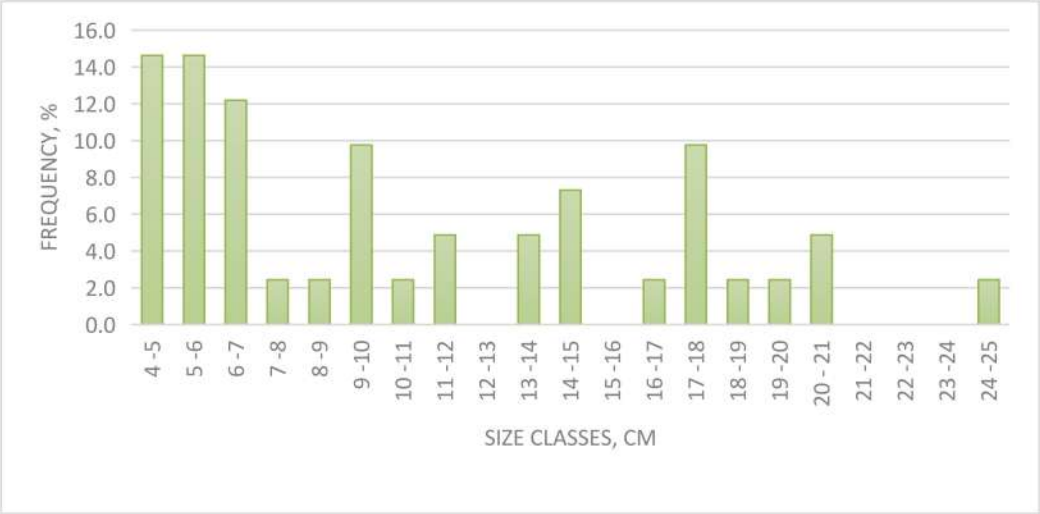

Fig. 3. Frequency distribution of the body size (TL, cm) of brook trout specimens caught in the Palakariya River in the period 2019-2021. (n = 51).

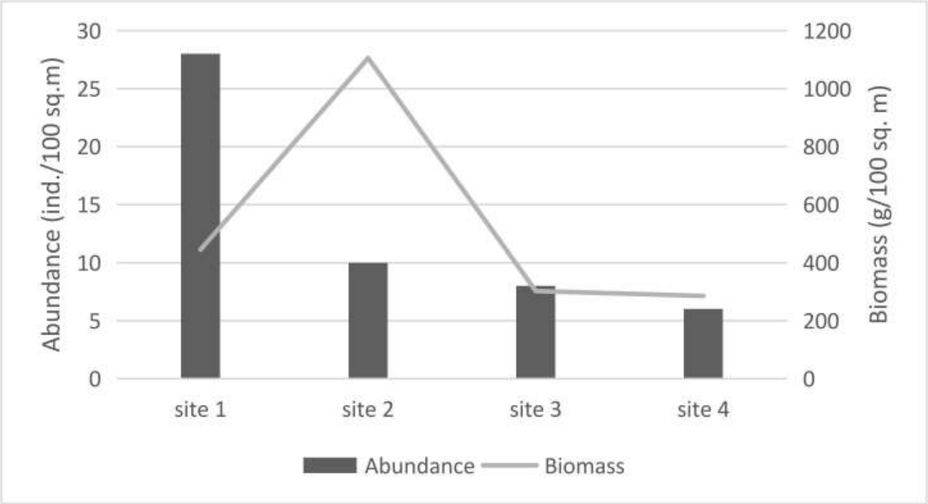

Fig. 4. Abundance (ind.100 m^{-2}) and biomass (g100 m^{-2}) of brook trout in the Palakariya River (2019-2021).

Fish density calculated from 261 sampling points in area between weir 1 and weir 2 were as follow: *S. trutta* - 141.1 ind. 100 m^{-2}; *P. phoxinus* - 84.8 ind. 100 m^{-2}; *B. petenyi* – 42.4 ind.100m^{-2}.

Discussion

According to internet posts, *S. fontinalis* in the Palakariya River originated from a single introduction of brook trout juveniles in the early-2010s. Anglers' posts are proving to be a very important source of information related to the distribution of invasive fish species (e.g. Banha et al., 2015; Kalous et al., 2018). That is how we found the person who carried out the single illegal stocking of brook trout in Palakaryia River in the 2010s. There is no evidence that this introduction has been repeated since then. We discovered that in the 10 years following that introduction, the brook trout has spread 1 km upstream from its point of initial release. This is probably due to the fact that the brook trout is a highly mobile invader that has spread extensively in stream ecosystems, occupying steep stretches of streams, even those with gradients up to 31% (Korsu et al., 2007). It was found that brook trout have a high capacity to disperse and a single introductory population may spread throughout an entire river system in search of territory and food resources (Hesthagen et al. 2018; Karas, 1997). The spread of the brook trout to the lower reaches of the Palakariya River is prevented by weir 2 (site 1, 1350 m a.s.l.), serving as a fish passage barrier. However, in periods with high water levels it could be possible for a few individuals to overcome this fragmentation in the downstream direction. This explains the discovery of single specimens in the lower reaches of the river.

The self-sustaining status of *S. fontinalis* in the Palakariya River is supported by information obtained from the body size structure of the collected specimens. The brook trout population had an overall balanced body size distribution consisting of individuals of all size classes between 4 and 25 cm. The dominating size for juvenile fish was between 4 and 10 cm.

The combination of morphological characteristics and specific body coloration allow for the definitive conclusion that the adult fish found in the uppermost section of the Palakaryia River are in fact brook trout and not any other synoptic species (Karas, 1997; Kottelat & Freyhof, 2007; Page & Burr, 1991; Scott & Crossman, 1973; Stauffer & King, 2014). The identification of the smallest brook trout (< 6-7 cm) was made on the basis of the length of the pectoral fins and the length and pigmentation of the adipose fin (Bacon, 1954; Martinez, 1984). Lastly, the distinction between wild and stocked salmonid fish, although remaining quite difficult, was done on the basis that among farm *S. fontinalis*, malformations are quite common. The absence of such malformations in the specimens caught in the Palakariya River suggests that the hypothesis for the presence of successful natural reproduction of the brook trout in the river still stands.

In the upper reaches of the Palakaryia River, the main native fish is the brown trout. The consequences of coexistence of brown trout and brook trout are contradictory. According to Spens et al. (2007) brook trout actively displace brown trout through competition, predation, disease spreading, or a combination of these. A long-term detrimental impact of brook trout on brown trout was documented in higher-altitude lakes (Eby et al., 2006; Spens et al., 2007). Cucherousset et al. (2008) observed mixed spawning groups composed of native brown trout and non-native brook trout, interspecific subordinate males, and presence of natural hybrids („tiger trout") and suggested that these reproductive interactions might detrimental effects on native species. In the majority of cases where the two species coexist, however, it has been documented that brown trout dominate and gradually displace brook trout from river ecosystems (Fausch, 1988; Öhlund et al., 2008). Due to the lack of previous ichthyological studies in the headwater parts of the Palakariya River, we cannot conclude with certainty whether the absence of brown trout in this section of the river is a result of displacement by the brook trout, or the area was originally fishless. It seems possible that the brook trout have found an empty niche in the upper headwater reaches of the Palakaryia river system and that is why no brown trout were found. Moreover, environmental conditions in the upper section of the Palakaryia River are more favourable for brook trout than brown trout. *S. fontinalis* prefer colder waters and generally inhabit areas with higher altitudes than *S. trutta* (Rahel & Nibbelink, 1999). Water temperature is considered the single most important factor that limits its geographic range (MacCrimmon & Campbell, 1969). It has been suggested that brook trout have a competitive advantage against brown trout in such cold habitats because of their higher juvenile growth rates (Öhlund et al., 2008). Brook trout also favour smaller streams, as they mature at a smaller body size and can reproduce in shallow systems with limited spawning habitats (Rahel & Nibbelink, 1999). Hesthagen et al. (2018) conclude that in small streams, brook trout will sustain viable populations and are unlikely to be wiped out by brown trout through competition. Korsu et al. (2007) and Korsu & Huusko (2009) found that in Finland, brook trout are mostly established in tributary streams, where they may form dense populations that can be harmful to the local brown trout, while in larger streams brown trout are usually not affected. Therefore, the brown trout population in the lower parts of the Palakariya River (600-1350 m a.s.l.) is vulnerable to a potential increase in the number of brook trout. The influence of the non-native brook trout on native species such as *Barbus peteyi, Sabanejewia aurata, Gobio kessleri, G. uranoscopus* has not been studied at this stage and we can only assume that the presence of a predator such as *S. fontinalis* would have negative consequences for the listed species.

Despite the fact that studies consider the brook trout as a non-native species with moderate invasiveness in countries of the Balkans region, its potential harmful effects to native ecosystems should not be underestimated (Simonović et al., 2013). The control over the resettlement of brook trout must be increased and the release of fish in the oligotrophic lakes, as well as in the high-altitude mountain streams inhabited by brown trout, must be prevented.

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Assessment of Endemic Lycian Salamanders Habitats Impacted by 2021 Mega Forest Fires in Turkey

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Abstract. The Lycian salamanders consist of seven allopatric endangered and endemic species restricted to Mediterranean Turkey and some adjacent Aegean islands. Mega forest fires occurred consecutively over a prolonged period of time in the distribution areas of six of the species in 2021, leading to habitat fragmentation and habitat loss. In this study, we find that a total of 751.9 hectares of Lycian salamander habitats were lost due to the mega-fires that occurred in summer 2021. Our results suggest that *L. atifi* is the most vulnerable with a loss of 285.84 hectares of habitat area, followed by *L. flavimembris* with 242.54 hectares, and *L. antalyana* with 124.16 hectares. *L. fazilae* is the species which suffered the least habitat loss, at 25.83 hectares. *L. billae* and *L. luschani* suffered habitat losses of 30.83 and 43.40 hectares respectively. When the transformation of morphological classes was examined, a significant decrease of all species was observed in the core areas which ensure spatial connectedness, and the edge magnitude, which was taken as an indicator of fragmentation, increased. Bridges providing connectedness were observed to have increased for some species. This indicates that while existing connections in habitats were fragmented due to the forest fires, potential connections may be formed after the forest fires. When the fragmentation values were examined according to the results of pattern analysis, the most notable marginal increase in fragmentation before and after the fires was found to have occurred in the habitat of *L. atifi*. In addition, we discuss recommendations for the sustainability of species populations in habitat restoration and forest management.

Key words: *Lyciasalamandra*, wildfire, habitat loss, spatial pattern, southwestern Anatolia.

Introduction

Amphibians are among the most vulnerable organisms on Earth and changes in land use and global warming constitute the most critical threats to their existence (Hof et al., 2011; Nelson et al., 2021). Forest fires, one of the causes of habitat loss, may affect caring capacity and population density by damaging habitats. After habitats have been fragmented, the inability of species to

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change their home ranges may cause the local density to drop below caring capacity and reduce their survival rates (Lappan et al., 2021). The disturbance of ecological processes due to forest fires also poses a threat to biological diversity (Driscoll et al., 2021). Only a few studies have been undertaken on the effects of forest fires on salamander populations, particularly following forest fires (Renken, 2006; Greenberg & Waldrop, 2008; Ford et al., 2010; O'Donnell & Semlitsch, 2015; Margolis & Malevich, 2016). The direct and indirect impacts of forest fires on terrestrial salamanders are assumed to be affected by a variety of factors including seasonality, the frequency of the forest fires, the intensity of the forest fires and the historical fire regime (Greenberg & Waldrop, 2008). While (Stebbins & Cohen, 1995) argue that amphibians' humid and permeable skin and eggs make them more vulnerable to heat and the drying up of microhabitats, (Komarek, 1969) claims that amphibians do not seem to be uncomfortable near fire and respond with adaptive behaviour that reduces their death rate.

Forest fires are historically the primary form of disruption in the scrub and coniferous forests along the Mediterranean coast. Due to the region's various physiographical and climatic characteristics and the accumulation of combustible materials, fire regimes also vary. The Mediterranean has witnessed its highest temperatures and lowest humidity in the last ten years likely as a result of climate change, and has experienced drought in the last two years. There was a tendency in the region towards an increase in the frequency and severity of forest fires, and – again within the last two years – there have been predictions of mega forest fires (Jiménez-Ruano et al., 2017; Moreira et al., 2020). There are grave concerns that climatic factors such as extreme temperatures and prolonged periods of drought along the European Mediterranean coast will extend the forest fire season in the near future (Cardil et al., 2014). In order to understand the effects of forest fires on endemic species and to monitor and assess ecological processes, spatial relationships need to be measured in relation to short- or long-term changes over time (de Vries et al., 2003; Corona, 2016). Remote sensing, geographic information systems (GIS) and the integration of landscape metrics combine to provide the detailed, spatially consistent information required for ecosystem services, sustainable resource management and land use planning (Kayiranga et al., 2016; Dutta et al., 2020). Monitoring the fragmentation of forest habitats due to fires in the context of land use planning and sustainable environment management is important for preventing irremediable negative consequences (Hansen et al., 2013).

The Lycian salamanders (*Lyciasalamandra* (Veith & Steinfartz, 2004); Amphibia: Salamandridae) consist of the following seven allopatric species: *L. antalyana* (Başoğlu & Baran, 1976), *L. atifi* (Başoğlu, 1967), *L. billae* (Franzen & Klewen, 1987), *L. fazilae* (Başoğlu & Atatür, 1974), *L. flavimembris* (Mutz & Steinfartz, 1995), *L. helverseni* (Pieper, 1963), and *L. luschani* (Steindachner, 1891) (see (Sparreboom, 2014; Veith et al., 2016). These amphibian species represent an exceptional case of micro-endemism (Veith et al., 2020). They are all slender, terrestrial, and viviparous. The Lycian salamanders are to be found in limited areas and in relatively small patches. Endemic along the 385-kilometer stretch of the Mediterranean coast from the west of Mt. Menteşe to the southeastern Asar mountains and the southern Taurus mountains in Turkey, and on some of the nearby Greek islands in the Aegean (Veith et al., 2001; Öz et al., 2004; Eleftherakos et al., 2007; Franzen, 2008; Sparreboom, 2014; Göçmen & Karış, 2017), these salamanders live at altitudes of 40-1,150 metres on north-facing slopes (Franzen, 2008; Göçmen et al., 2013) and pine forest and maquis shrubland habitats (Veith et al., 2001; Rödder et al., 2011). The species have adapted to live deep within humid crevices in boulder fields at the foot of karstic limestone slopes (Sparreboom, 2014), since their habitats are hot and dry in summer, and warm and damp in winter. Within this naturally restricted range, the genus faces the risks of habitat loss as a result of forest fires and over-collection for scientific purposes. On the International Union for the Conservastion of Nature (IUCN) red list, two of the Lycian salamanders (*L. luschani* – (Steindachner, 1891)– and *L. helverseni* – (Pieper, 1963) are listed as vulnerable (VU). Four (*L. atifi* – Başoğlu, 1967, *L. antalyana* – Başoğlu & Baran, 1976, *L. fazilae* – Başoğlu & Atatür, 1974, and *L. flavimembris* – Mutz & Steinfartz, 1995) are listed as endangered (EN), and one (*L. billae* – Franzen & Klewen, 1987) is listed as critically endangered (CR). The populations of *L. flavimembris* in its northernmost limits and *L. antalyana* in its westernmost limits are on the decline (Kaska et al., 2009; Arslan et al., 2020).

Turkey's first mega fires occurred along the Mediterranean shoreline, the habitat of the Lycian salamander, in 2021. From now on, the Mediterranean basin will most likely experience mega fires more frequently due to global warming. This study, which focuses on the impact of the forest fires on the habitats accommodating the six species of Lycian salamanders with endemically limited distribution abilities, discusses (i) the extent to which salamanders were affected by the forest fires, (ii) the burn ratios of their habitats, and (iii) how ecological connectedness has changed before and after the forest fires. The results of this study reveal the effects of forest fires on the habitats of salamanders, which are endemically restricted at the local level. Finally, in addition to its findings, the study also discusses which areas attention should be paid to with respect to these species during the course of habitat restoration efforts in the wake of the forest fires.

Materials and Methods

Our study focuses on six Lycian salamanders (*L. antalyana, L. atifi, L. billae, L. flavimembris, L. fazilae, L. luschani*) that are restricted to the southwestern coast of Turkey (Fig. 1). The study area has a Mediterranean climate (Köppen: Csa) characterised by hot, dry summers and warm, wet winters. In addition, the temperature and precipitation may have effects on the local distribution ranges of the species. The study area is covered with scrubland and *Pinus brutia* forests, which primarily include *Olea europea, Juniperus oxycedrus, Phillyrea latifolia, Myrtus communis, Pistacia terebinthus, Quercus coccifera,* and *Arbutus andrachne*.

Field studies were carried out at intervals in November–February between 2010–2020 and all records of the species were confirmed from previously published literature (Başoğlu & Atatür, 1974; Mutz & Steinfartz, 1995; Veith et al., 2001; Öz et al., 2004; Johannesen et al., 2006; Beukema et al., 2009; Akman et al., 2011; Göçmen & Akman 2012; Göçmen et al., 2013; Akman & Godmann, 2014; Üzüm et al., 2015; Göçmen & Karış, 2017; Arslan et al., 2018; Oğuz et al., 2020; Veith et al., 2020). A total of 249 presence records were gathered for the six species. In all, 62 records were obtained for *L. antalyana*; 35 for *L. atifi*; 35 for *L. billae*, 24 for *L. fazilae*, 42 for *L. flavimembris* and 51 for *L. luschani*. Where locality information lacked coordinates, it was referenced to the closest location provided in earlier studies using Google Earth Pro v. 7.1.5 (Google Inc.). All records were georeferenced using a WGS84 coordinate system and their accuracy was checked with ArcGIS (v10, ESRI, California, USA). The extent of occurrence (EOO – (Joppa et al., 2016) of each species was calculated, to establish its distribution, with the help of the IUCN Red List Toolbox for ArcMap. The EOO measures the spatial spread of the areas currently occupied by the taxon. It is ascertained by applying a Minimum Convex Polygon (as in the IUCN

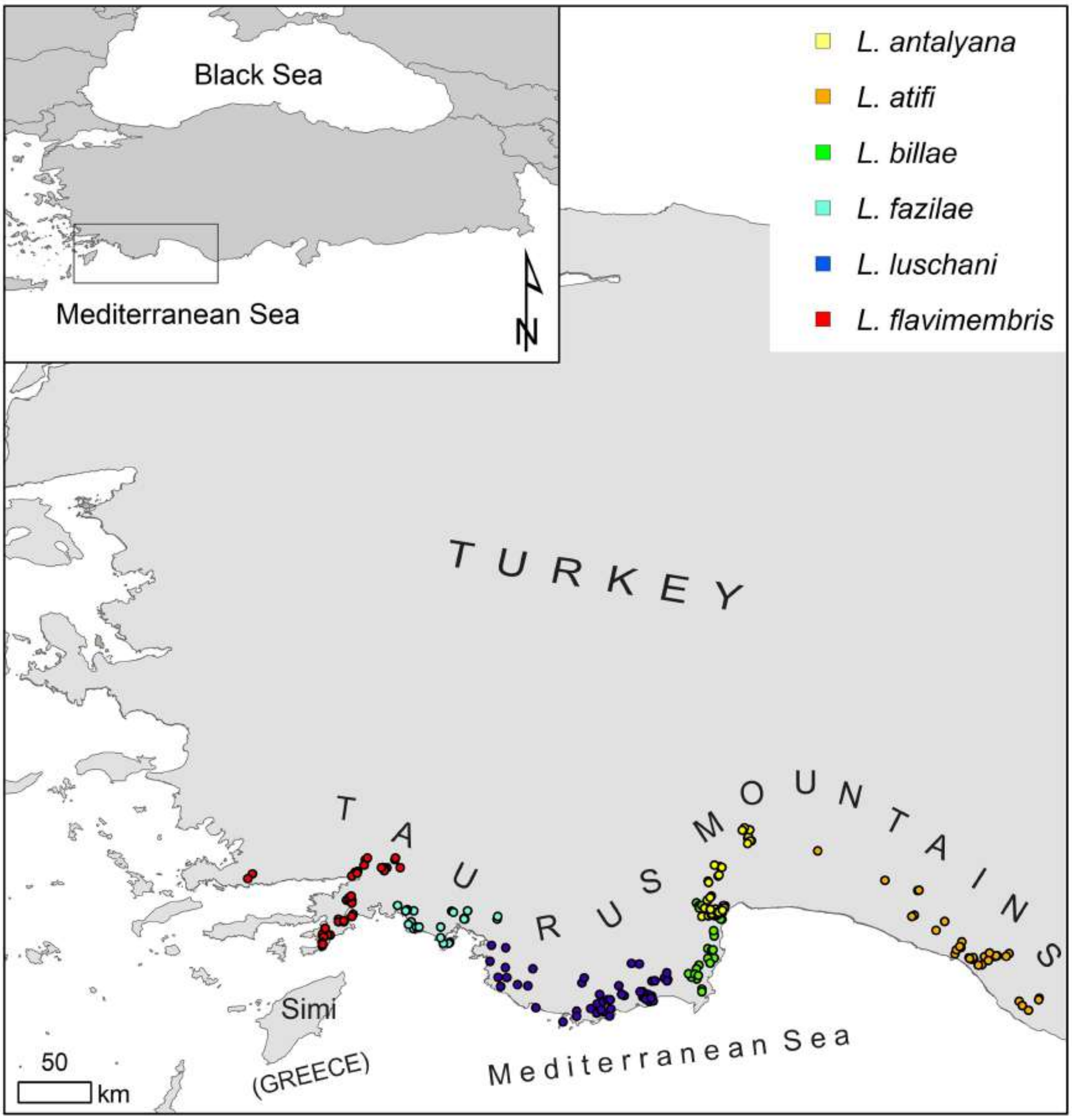

Fig. 1. The distribution of Lycian salamanders in south-western Anatolia.

Red List Criteria) to measure how far risks from threats are spread spatially across the geographical range of the taxon (Bland et al., 2017; IUCN Standards and Petitions Committee 2019).

The methodology for the study consists of three parts – namely, identifying habitat losses in accordance with burn rates based on the calculation of spectral indices, examining transformations in morphological classes using Sankey diagrams, and measuring the degree of fragmentation. Spectral indices (NDVI and NBR) were measured in the R 4.0.3 environment. The degree of fragmentation was determined using the GuidosToolbox (Graphical User Interface for the Description of image Objects and their Shapes) software. Here, we used Level-1 C products of Landsat-8 OLI data with 30 m spatial resolution that was downloaded from the U.S. Geological Survey (USGS) website. The dates when the vegetation was still active were taken into consideration while downloading satellite images before the fire, and a total of 12 pre-fire and post-fire images with a cloud cover less than 2 per cent were downloaded. Since cloudy areas did not overlap with forest areas, masking was not necessary. The Landsat 8 observation satellite system has two different sensors: the Operational Land Imager (OLI) and the Thermal Infrared Sensor (TIRS). With the help of these sensors, 11 different wavelength images were captured. The satellite system also has calibrated and scaled Digital Numbers (DN). These numbers are represented in 16-bit unsigned integers and using the radiometric re-scale factors from Landsat metadata files (MTL), can be re-scaled according to Top of Atmosphere (ToA) reflectance values.

During the initial stage, surface reflectance correction was carried out using ToA and dark object subtraction (DOS) for the radiometric calibration of satellite images. ToA corrections often result in negative reflectance values. Landsat atmospheric correction and surface recovery algorithms are not ideal for bodies of water due to the naturally low level of water brightness and the resulting low signal-to-noise ratio.

After radiometric calibration, the bands were merged. Bands 4, 5 and 7 were merged and the obtained raster map was framed according to boundaries of the study area. Then the Normalized Difference Vegetation Index (NDVI) was calculated. NDVI uses the red (R) and near infrared (NIR) values, since energy reflected in those wavelengths is correlated with the amount of surface vegetation. NDVI values are standardised and vary between -1 and +1. NDVI values which are greater than 0.3 are assumed to represent vegetation.

Pixels that do not represent vegetation (< 0.3) can be masked out (Bhandari et al., 2012; Gandhi et al., 2015). In R, the "*calc*" function is used for arithmetic calculations using raster data (in addition to other applications). In that case, a function is written to select all values smaller than 0.3 and to mark these values as n/a (not applicable). This way, the value of the image acquired will be bigger than 0.3. This operation is called "thresholding".

The Normalised Burn Ratio (NBR) is used to define the burned areas. This index uses near the infrared (NIR) band where the vegetation has high absorption levels and the short-wave infrared (SWIR) band for damaged woody vegetation with higher reflectance values. The NBR is calculated as follows:

$$\mathrm{NBR} = \frac{\mathrm{NIR}-\mathrm{SWIR}}{\mathrm{NIR}+\mathrm{SWIR}}.$$

The NBR index was originally developed for use with the Landsat 4-5 Thematic Mapper (TM) and the Enhanced Thematic Mapper Plus (Landsat 7 ETM +). However, it works with any multispectral sensor with an NIR of band between 760-900 nm and a SWIR band between 2080-2350 nm (Cocke et al., 2005). As a result, the SWIR2 band is used for Landsat 8.

The Normalised Burn Ratio (dNBR) is better suited to understanding the scope and severity of forest fires when used after calculating the differences in pre-fire and post-fire conditions. This difference is best measured by collecting data just before and after the fire (Miller & Thode, 2007):

$$\mathrm{dNBR} = \mathrm{prefire\ NBR} - \mathrm{postfire\ NBR}.$$

The burn severity map is categorised according to the table below (Table 1). It is important to underline that these values, which quantitatively display burn severity, may represent various outcomes and be interpreted differently.

After forming the dNBR map, the areal distribution of pixel values was calculated by burn severity levels, and the change in the number of habitat areas following the forest fires was examined.

In the second stage, presence/absence data was formed (vegetation 1, no vegetation 0) using a pre-fire NDVI image. The maps formed using ArcMap 10.7 in raster format were transferred to GuidosToolbox software. Landscape analysis for habitat connectivity was carried out using GuidosToolbox (v. 3.0), an integrated platform for the analysis of habitat composition, connectivity and fragmentation, independent from the unique reactions of various species (Vogt & Riitters, 2017).

Table 1. Burn severity levels obtained calculating dNBR, proposed by USGS.

Severity level	**dNBR range (scaled by**	**dNBR range (not scaled)**
Enhance	-500 to - 251	-0.500 to -0.251
Enhance	-250 to -101	-0.250 to -0.101
Unburn	-100 to -99	-0.100 to -0.99
Low	100 to 269	0.100 to 0.269
Moderat	270 to 439	0.270 to 0.439
Moderat	440 to 659	0.440 to 0.659
High	660 to 1300	0.660 to 0.1300

Morphological Spatial Pattern Analysis (MSPA) is a series of specialised mathematical morphologic operators that intend to describe the geometry and connection of image components (Soille & Vogt, 2009; Saura et al., 2011). This method, which is solely based on geometrical concepts, can be implemented on any digital image at any scale and implementation area (Vogt, 2016). MSPA operates with presence/absence data. The morphological structure is categorised into seven classes by the MSPA assessment (Saura et al., 2011). These are: (1) cores (area inside excluding the outside environment); (2) islets (small and distinct areas too small to include cores); (3) loops (areas connected to the same core); (4) bridges (areas connecting different cores); (5) perforations (areas on the periphery of an internal object); (6) edges (areas on the periphery of an external object), and (7) branches (areas with one side connected to an edge, perforation, corridor or loop). Morphological spatial pattern classes are among the significant indicators of habitat fragmentation and loss in forests. For instance, core areas (forests) indicate unfragmented habitat potential for the species living in the forest while edges show the boundaries formed in forests after perforation. Compared to core areas, the edges accommodate invasive species or the species dependent on border areas. Corridors providing connectivity represent the potential mobility of species, the fragmentation of which would cause ecological harm. Branches that can provide connections between core areas are recognised as potential corridors. Certain classes in the interpretation of the analysis can be paired in various combinations. For instance, classes indicating corridors can stand for fragmented or broken connections of three kinds (branches with corridors, branches with an inner corridor and branches formed from the boundary) (Vogt et al., 2007). In total, MSPA segmentation results in 25 classes of characteristics which correspond to the initial foreground area when combined. Since most of the microclimatic and biophysical parameters of forests (temperature, humidity, shade, and radiation) are affected by edge impacts located between 0 and 5 metres from the forest edge, the edge width in the MSPA was adjusted to 3 metres (or 2 pixels) (Ossola et al., 2019). The transformation of morphological classes during the pre-fire and post-fire periods was visualised using Sankey diagrams. The height of each component in the columns representing units in a Sankey diagram is proportional to the relative abundance of morphological classes represented in the study area and the categories were arranged taking into consideration the spatial dimensions (vertical) of all categories, from the smallest to the largest. The thickness of the lines in between columns indicating the units between which change has occurred are important for showing the degree of transformation. A thicker line denotes a higher degree of transformation.

In the final stage of the method, hypsometric values were used to calculate the fragmentation value in percentages. The hypsometric value is scaled to the maximum distance between the foreground (the forest area) and the background (the non-forest area).

The fragmentation of the foreground and background are calculated using the following formulae:

$$\mathrm{frag}_{FG} = \int_{0}^{1} \mathrm{NLCH}_{FG} - \int_{0}^{1} \mathrm{NLCH}_{FGMIN}$$
$$\mathrm{frag}_{BG} = \int_{-1}^{0} \mathrm{NLCH}_{BG} - \int_{-1}^{1} \mathrm{NLCH}_{BGMIN},$$

where $Frag_{FG}$ in the formula represents foreground fragmentation and $frag_{BG}$ stands for background fragmentation. $NLHC_{FG}$ / $NLHC_{BG}$ is the normalised HE value for the foreground/background class of a specific landscape and $NLHC_{FGMIN}$ / $NLHC_{BGMIN}$ indicates the maximum foreground grouping (minimum fragmentation) for the same foreground ratio.

The degree of fragmentation of a specific image is defined as the area between minimum fragmentation and maximum fragmentation (Kozak et al., 2018). The landscape fragmentation index used in the formation of the normalised HE is represented with values of between 0% and 100% and is used in the calculation of the direction and degree of fragmentation:

$$\mathrm{frag} = f(x) = \left(\frac{A_{FG}}{100} \times \mathrm{frag\_FG}\right) + \left(\frac{A_{BG}}{100} \times \mathrm{frag\_BG}\right),$$

where *frag* indicates fragmentation, A_{FG} indicates foreground, A_{BG} indicates background, *frag_FG* indicates foreground fragmentation and *frag_BG* indicates background fragmentation (Vogt & Riitters, 2017; Kozak et al., 2018).

Results

We find that the NDVI and NBR values in the extent of occurrence (EOO) areas of the species at the study area have significantly changed with the fire. When all the EOO areas were selected from maps belonging to the two spectral indices and compared, we found that the threshold values had dropped. The changes in the EOO areas by burn severity are visualised in Fig. 2. When the burn ratios in different EOO areas are compared, the severity of the fire is observed to have varied. In this study, we find that a total of 751.9 hectares of Lycian salamander habitats were lost due to the mega-fires occurring in summer 2021. Our results suggest that *L. atifi* was the most vulnerable with a loss of 285.84 hectares of habitat area, followed by *L. flavimembris* with 242.54 hectares, and *L. antalyana* with 124.16 hectares. The habitat loss of *L. atifi* within the boundaries of the EOO is concentrated in the northeast and the northwest. The habitat loss of *L. flavimembris* within the boundaries of the EOO is concentrated in the southeast and the northwest. *L. antalyana* suffered habitat loss mainly of low severity within the boundaries of the EOO. *L. fazilae* is the species which suffered the least habitat loss, at 25.83 hectares. The *L. fazilae* habitat suffered low severity fires within the boundaries of the EOO. This is also true of *L. billae* and *L. luschani*. *L. billae* and *L. luschani* suffered habitat losses of 30.83 and 43.40 hectares respectively. The habitats of *L. fazilae*, *L. billae*, and *L. luschani* were thus less affected by the forest fires than those of the other species. The number of EOO areas outside the habitats of *L. flavimembris* and *L. atifi* which suffered high and moderate-high severity fires is low. Table 2 shows that the amount of burn severity in the EOO area for *L. flavimembris* was the most homogenously distributed. *L. flavimembris* and *L. atifi* are the species with the most habitat loss at moderate-high severity. Fig. 3 visualises losses according to burn severity. It shows that *L. antalyana* suffered a 17.40% habitat loss in the EOO area, *L. atifi* a loss of 11.90%, *L. flavimembris* a loss of 9.49%, *L. billae* a loss of 4.44%, *L. fazilae* a loss of 3.10% and *L. luschani* a loss of 1.29%.

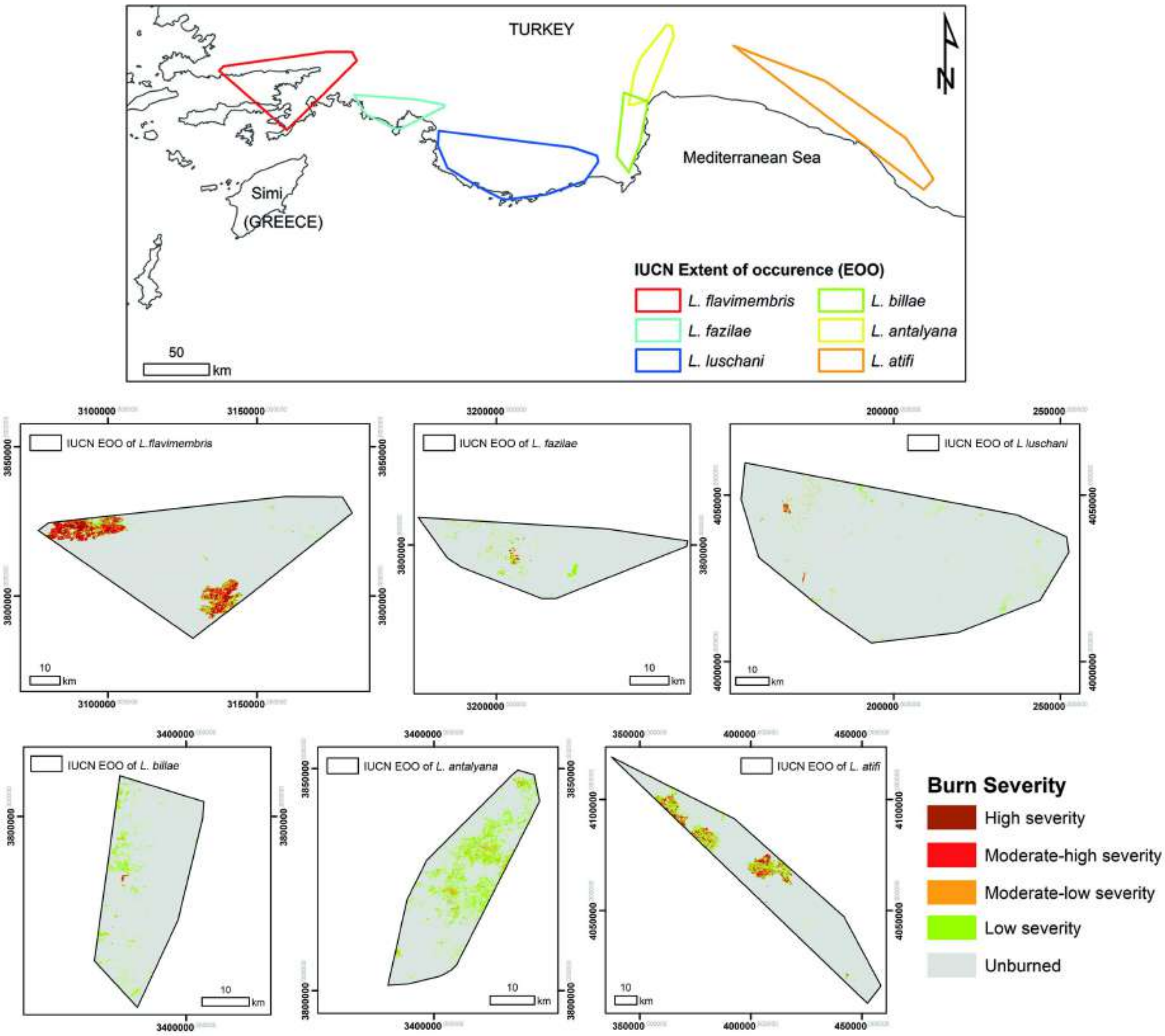

Fig. 2. Burn severity maps for Lycian salamander habitats.

Table 2. Changes in habitats of Lycian salamanders according to burn severity (ha).

Burn severity	***L. antalyana***	***L. atifi***	***L. billae***	***L. fazilae***	***L. flavimembris***	***L. luschani***
Low severity	107.36	145.67	27.61	20.04	56.13	35.74
Moderate-low severity	16.30	79.18	2.55	3.51	54.56	4.27
Moderate-high severity	0.49	40.29	0.60	1.53	67.34	2.82
High severity	0.01	20.69	0.07	0.05	64.52	0.58
Total (ha)	124.16	285.84	30.83	25.13	242.54	43.40

An examination of the MSPA maps displaying morphological classes revealed significant changes in habitat units (Fig. 4). The decrease in the number of core areas demonstrates that all habitats were fragmented after the fire. The loss in core areas overlaps with the EOO areas that suffered habitat losses. For instance, since the habitat loss of *L. atifi* was concentrated in the northeast and the northwest, the loss of core areas was greatest in those regions. Fig. 4 demonstrates the similar spatial results obtained for other species. In addition, the amount of islets, edges, loops, and branches in areas with habitat loss increased and the amount of perforations decreased. Interestingly, in the habitat of *L. atifi*, all morphological classes except for cores increased. However, although the number of bridges in the range of *L. atifi* increased, the increases in the marginal distribution of all classes except cores indicate a very high degree of fragmentation.

When the transformation of morphological classes was examined, a significant decrease was observed in the core areas, which provide spatial connectedness, for all the species, along with an increase in the magnitude of edges, which was taken as the fragmentation indicator (Table 3). In the habitat of *L. atifi*, which suffered a significant loss of habitat, the core areas were seen to have decreased. This was also the case for *L. flavimembris* and *L. luschani*. Bridges providing connectedness were observed to have increased for all species other than *L. fazilae*. This indicates that while existing connections in habitats were fragmented due to the forest fires, potential connections may be formed after the fires.

When we examined the Sankey diagrams, we found that all morphological classes had interchanged after the fire (Fig. 5). Among the morphological classes of *L. flavimembris*, core areas transformed into edges after the fire, whereas for *L. fazilae* core areas transformed into loops. By contrast, a significant proportion of the core areas for *L. luschani* transformed into edges and bridges and some of the branches transformed into islets. Among the morphological classes of *L. billae*, core areas transformed into edges, loops, perforations and bridges. The amount of transformation is similar. While the transformation of the morphological classes for *L. antalyana* resembles that of those for *L. billae*, more transformations from cores to edges occurred in the case of *L. billae*. The greatest marginal transformation in the Sankey diagram was observed for *L. atifi*. While the great majority of the core areas of *L. atifi* transformed into edges, a significant proportion transformed into bridges, islets and branches.

According to the examination of hypsometric values, the fragmentation value of *L. antalyana*, which was 54% before the fire, increased to 58.56% after the fire (Fig. 6). Fragmentation increased from 48.26% to 55.91% in the case of *L. atifi*. There were no significant changes in *L. billae*. The fragmentation value of *L. fazilae* increased slightly from 59.35% to 59.97%. The fragmentation value of *L. flavimembris* increased from 54.76% to 57.74%. In contrast, the fragmentation value of *L. luschani* decreased from 79.47% to 79.04%. In conclusion, the marginal increase in fragmentation before and after the fire was most notable in the habitat of *L. atifi*.

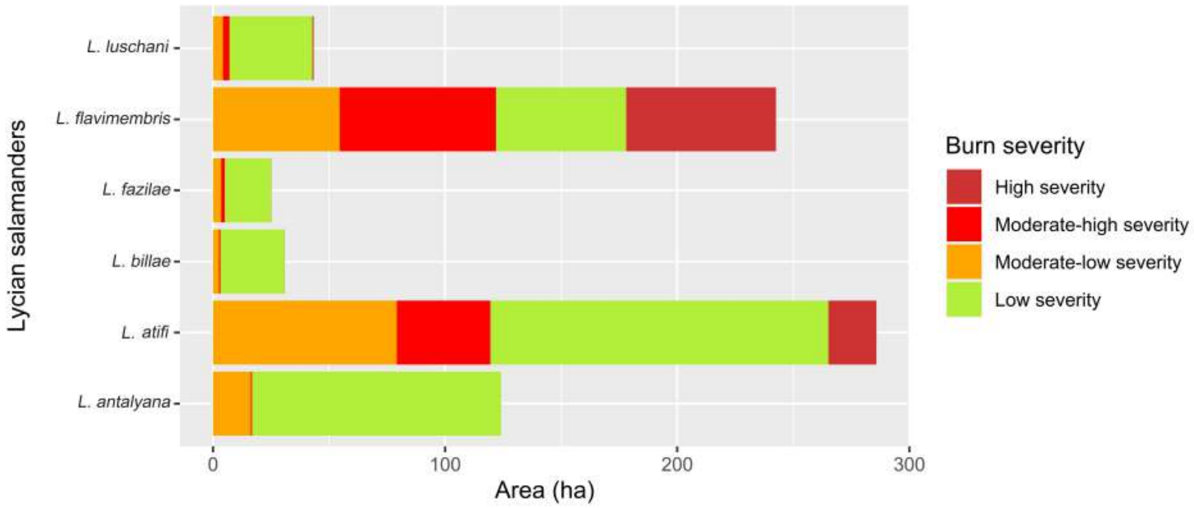

Fig. 3. Comparison of habitats of Lycian salamanders according to burn severity (ha).

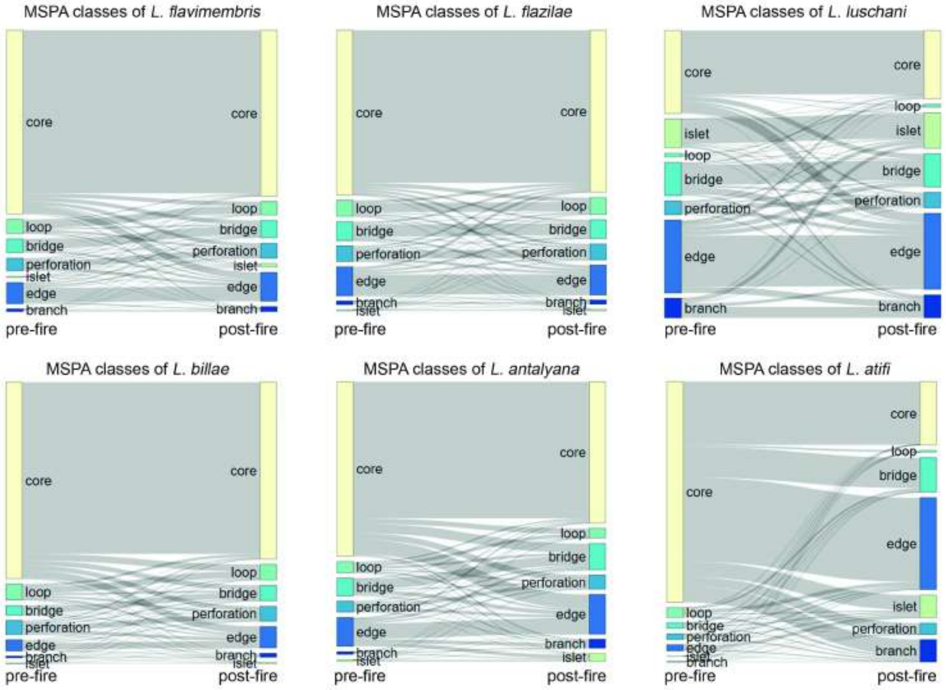

Fig. 4. Change between morphological classes of Lycian salamanders' habitats.

Table 3. Changes in MSPA classes (morphological classes) by species based on pre- and post-fire binary images (km^2).

Species	Core	Islet	Perforation	Edge	Loop	Bridge	Branch
L. flavimembris	-131.37	25.81	-15.85	97.89	9.20	40.13	21.98
L. fazilae	-3.24	0.81	2.43	0.08	0.00	-0.40	0.57
L. luschani	-128.48	64.07	-15.10	22.48	15.43	16.44	24.82
L. billae	-38.68	1.60	-9.10	29.03	0.14	12.08	4.93
L. antalyana	-47.59	16.41	-13.63	31.46	2.07	1.07	10.27
L. atifi	-1215.93	215.51	250.59	589.83	24.75	434.62	195.09

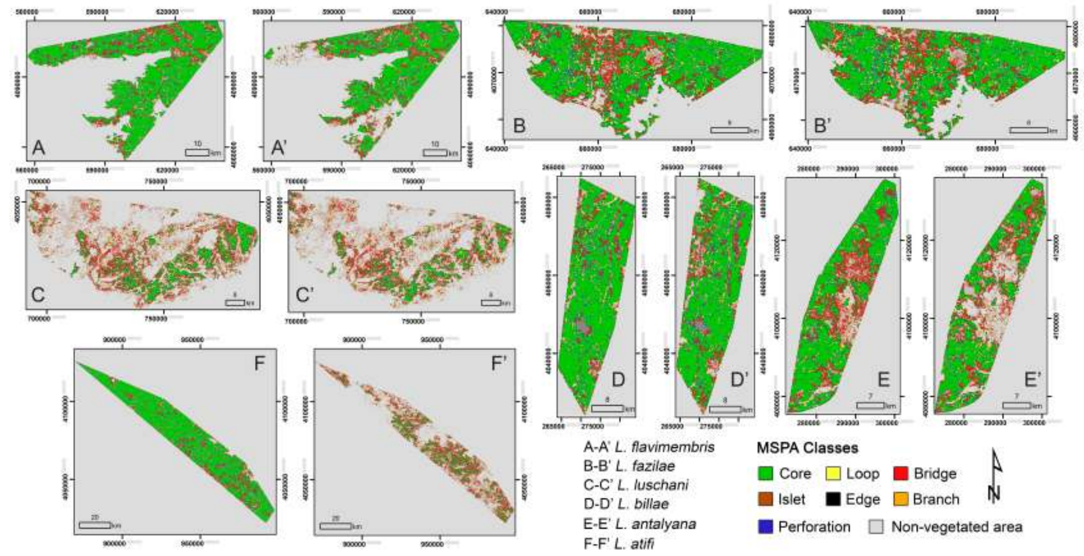

Fig. 5. Sankey diagrams illustrating the transformations of morphological classes of Lycian salamander habitats.

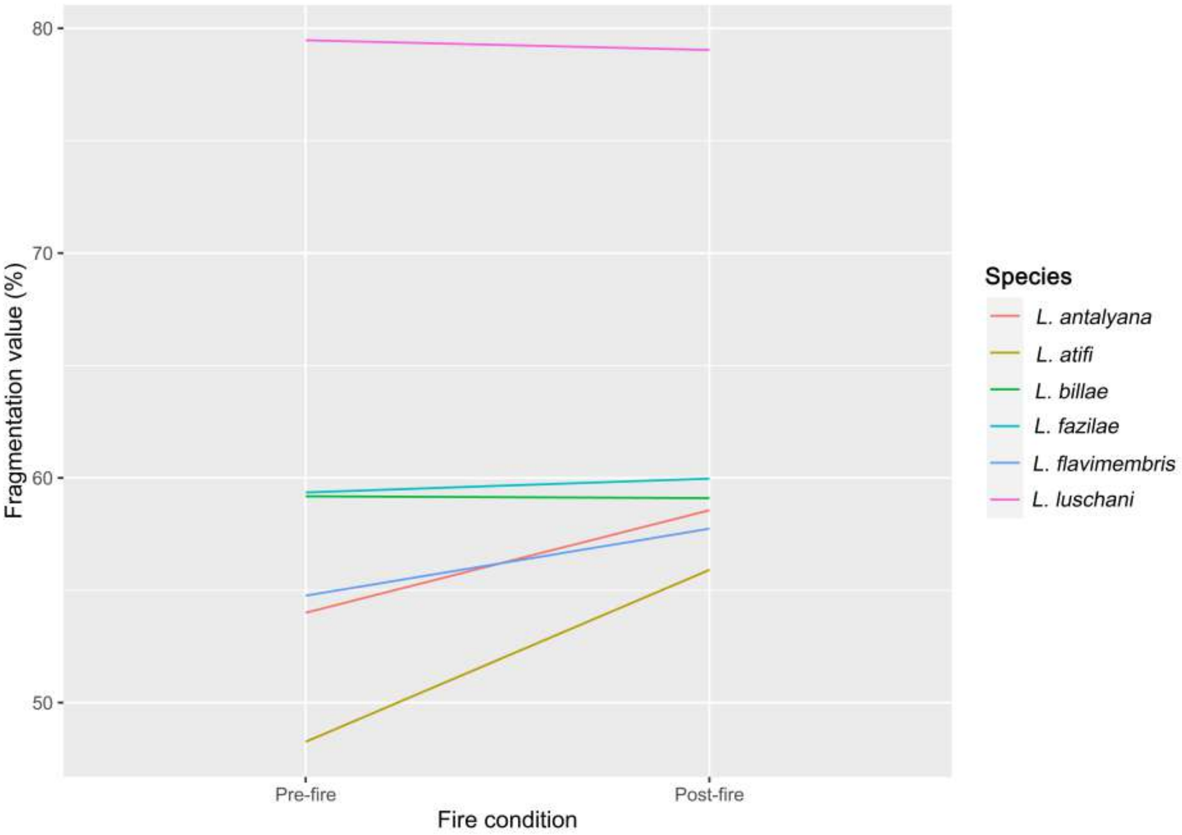

Fig. 6. Change in fragmentation values before and after the fire based on hypsometry values.

Discussion

Seven species of Lycian salamanders, some of which also have various subspecies, are found in the Western Taurus Mountains along the Turkish Mediterranean coastline and the Karpathos Islands of Greece. Lycian salamanders are restricted to karstic limestone areas in arid Mediterranean environments. The species' typical habitat is pine forests and maquis shrublands on north-exposure slopes (Veith et al., 2001). Lycian salamanders live in specific isolated environments and do not display signs of mixing even when living in close proximity (Johannesen et al., 2006). The major threat towards amphibian populations worldwide is habitat loss and habitat fragmentation (Chanson et al., 2008; Sodhi et al., 2008; Hof et al., 2011). The biggest potential threats to Lycian salamanders, which live in naturally isolated environments, are forest fires, urbanisation, overcollection for scientific purposes and habitat loss (Kaska et al., 2009; Başkale et al., 2018; 2019; Arslan et al., 2020).

One of the key natural disruptions to Mediterranean ecosystems, on both small and large scales, is wildfires (Izhaki, 2012). The vertebrate communities of the region have been shaped mostly by fire (Prodon, 1987; 2000). Aside from damaging vegetation, forest fires cause severe habitat and landscape transformations, which affect the dynamics and structures of vertebrates including their population and community levels (Izhaki, 2012). Forest fires reduce the availability of habitats for the species, but the general impact of fires does not have to be negative from the viewpoint of the preservation of biodiversity. This is because they create a heterogeneous landscape through forest patches, and provide open habitats which are significant for the species. Fires affect wildlife directly by causing injury or death and indirectly through their impacts on the quality of habitats and food resources (Smith & Lyon, 2000; Harper et al., 2016). The short-term ecological effects which fires have on animal populations and communities as a result of habitat modification can be even greater than their direct effects (Izhaki, 2012). However, fires occurring in the Mediterranean basin have rather varied effects on biodiversity. These effects depend on the extent, severity and frequency of the fire, the initial condition of the ecosystem and the spatial arrangement and isolation of burned and unburned patches after the fire, as well as various abiotic conditions. In terms of forest management in the Mediterranean, it is of great importance to conserve the landscape mosaic of habitats with different fire histories in order to preserve the high biodiversity of the vertebrate population. The impacts of wildfires on birds and mammals have been documented more extensively than their effects on reptiles; in the case of amphibians, there is only limited data (Izhaki, 2012).

Terrestrial salamanders generally live in shelters, which they leave for foraging if conditions are favourable. Lycian salamanders have been observed to be active from early November to mid-April depending on environmental conditions (Olgun et al., 2001; Gautier et al., 2006; Sparreboom 2014). During this period of activity, the salamanders live in shelters (under stones, in wood or in crevices) and move on the surface when foraging at night (Olgun et al., 2001; Sparreboom, 2014). During the dry season, they avoid the surface and live a secluded life underground, many individuals coming together to share a single shelter (Gautier et al., 2006). Terrestrial salamanders have been sheltered from the direct effects of wildfires due to these underground habits. Plethodontid salamanders retreat underground in wildfires, which most frequently occur during the summer drought (Hossack & Pilliod, 2011). The severity of forest fires consists of two components: intensity and duration. Fire intensity is the speed with which a fire produces thermal energy. Although heat is transferred more rapidly and penetrates deeper in humid soil, the latent heat of vaporisation prevents the soil temperature from exceeding 95°C until the water has evaporated completely (Campbell et al., 1994). The temperature then typically rises to 200-300°C (Franklin et al., 1997). If heavy fuels area present, the temperature on the soil surface may reach 500-700°C (DeBano et al., 1998) and momentary values up to 850°C have been recorded on occasion (DeBano, 2000). The combination of burn and heat transfer produces vertical thermal gradients in the soil. Temperatures at 5 centimetres in mineral soil rarely exceed 150°C and soil 20-30 centimetres from the surface does not generally heat up (DeBano, 2000). The possibility of individuals dying directly from the heat is therefore low. Studies of various species have indicated that direct damage is negligible. The real threats that may impose constraints on the populations of species are increased vulnerability to predators, food scarcity and the microclimatic fluctuations that occur in parallel with the decrease in the vegetation cover ratio. Moreover, the nature and timing of the forestry activities conducted for the recovery of the area after a fire are of great importance.

The Mediterranean flora and fauna have developed considerable defences against forest fires. These species started radiating at least 12.3 and 10.2 mya around the final emergence of the mid-Aegean trench (Veith et al., 2016; 2020). Phylogenetic studies have postulated their intraspecific evolution by the Messinian Salinity Crisis (5.3–5.6 mya) as well as climatic alterations since the Late Pliocene and throughout the Pleistocene (Veith et al., 2016; 2020; Ehl et al., 2019). The demographic and growth model analyses conducted by (Sinsch et al., 2017) show that the deep crevice systems in the calcareous limestone on the shores of the Mediterranean have enabled the Lycian salamanders to survive both the cold winters and the hot dry summers However, forestry activities after the fire may be of greater concern. With the recent wildfires occurring in Turkey, there has been public pressure for the burned areas to be restored. In fact, some civil society organisations have raised donations for the afforestation of burned areas. The forest law of Turkey makes it obligatory to reafforest burned areas within a year. At present, the forestation of these areas is seen as unavoidable in Turkey. Forestation is carried out mechanically by means of deep soil cultivation. In some areas, fragments of rock are cleaned up. Soil cultivation can generally be carried out at all times except the winter months. Unless the salamanders move deeper under the surface for the summer months in the wake of the fire, they will clearly be affected by these cultivation processes.

Logging takes place continuously in the Calabrian pine and scrub forests of Mediterranean Turkey. After the fire, all the trees in some areas are likely to be removed. Coastal giant salamanders (*Dicamptodon tenebrosus*) responded to clear-cut loggings by staying near streams, spending less time in their shelters and decreasing their home range (Johnston & Frid, 2002). Riparian zones are therefore of particular importance in burned forest areas. The higher humidity of these areas enables the vegetation to recover more quickly. These areas are already suitable as salamander habitats. For this reason, they constitute priority zones in the habitat restoration of forest areas.

Logs in burned forest areas that are economically valuable are collected and sold. Considering the magnitude of the area affected by this mega fire, removing the logs will require heavy-duty machinery, which brings with it the risk of greater soil compaction during the removal of logs (Grialou et al., 2000). The use of heavy-duty machinery should be prohibited in areas with high potential for habitat suitability as identified by scientific studies. Operations of this kind would not only affect salamanders but also the endemic fossorial *Ophiomorus kardesi* (Kornilios et al., 2018), as well as other amphibians and reptiles living in these areas.

Clearing away the plant residue covering the soil surface could cause problems. (Morneault et al., 2004) argue that terrestrial salamanders will be more affected by the disappearance of the humid microclimate provided by woody residues than by timber logging. Moreover, surface cover could permit salamanders to move more freely without being attacked by predators. For instance, *Ambystoma opacum* moves for relatively short distances until it finds shelter (Graeter et al., 2008). Of course, the surface cover does not consist only of vegetation. The distribution areas of the Lycian salamanders consist of deep crevices and calcareous rocks with rough surfaces. This constitutes an advantage for the salamanders.

Another factor to be considered during the habitat restoration of burned areas is timing of operations. The only period when Lycian salamanders are active on the soil surface is winter (Franzen, 2008). This indicates that this period is important for the survival of the population (Sinsch et al., 2017). Because of this, the area should be screened extensively before each and every operation to be carried out in the winter and precautions should be developed and applied instantly depending on the populations identified.

Populations with restricted distribution, including Lycian salamanders, are more prone to extinction unless their distribution is secured by ecological connections (Bani et al., 2015). The degree of isolation between the populations can be determined by ecological and geographical distances. Karstic limestone bedrock, rivers, and rock formations have been seen as the greatest obstacles to the gene flow of Lycian salamander populations (Klewen, 1991). On the contrary, Veith et al. (2020) claim that karstic limestone could even connect adjacent populations, making the distribution through river systems easier. Nevertheless, due to their environmental tolerance limits and limited dispersal capacities, the species were unable to spread during the climate changes in the history of the Mediterranean, where southern Anatolian environments are even more suitable for the survival of Lycian salamanders today (Veith et al., 2016; 2020). All the Lycian salamander species are similar in terms of their ecological niches and selection of habitats, and there are no significant differences between them (Franzen, 2008).

Spatial planners need easy-to-use and effective alternative planning tools to minimise habitat loss, prevent fragmentation and strengthen ecological connectedness (Babí Almenar et al., 2019). To preserve ecological assets and ensure their balanced use, preservation methods should be researched, analysed and planned. The implementation of planning decisions should be inspected, and interventions should be made if necessary. Throughout this process, there is a need to produce base maps to direct planning decisions. In this context, it is important to monitor the transformations in ecological connectedness units and to assess the changes in the importance of connectedness as part of conservation planning.

There is a strong correlation between measurements of habitat configuration and habitat volumes (Fahrig, 2003). Landscape ecologists argue that forests, which cover large spaces in natural ecosystems, may contain a particularly large variety of habitat types. A greater diversity of species may emerge in large and continuous forests, since larger areas generally possess a regional pool of species. For this reason, most studies argue that forest fragmentation leads to a reduction in biodiversity (Jackson & Fahrig, 2013). Although this study does not focus on the impacts of direct fragmentation on biodiversity, fragmentation occurring over time is thought to damage habitat areas. Significant fluctuations may occur in the presence of individual species and/or their transformation as a result of the decrease in forest areas under the impact of fragmentation. Additionally, a significant edge impact may occur, and the survival rate of trees in forest areas that are opened up in mountain ecosystems may decrease due to increased exposure to wind (Laurance, 2008; Kettle & Koh, 2014). For this reason, species may be lost in forest areas affected by fragmentation or the suitability of these areas as habitats for species may change. Identifying positive and negative transformations may lead to practical solutions for conservation management in landscape planning.

Identifying the species most affected by fragmentation over time could be useful for understanding the condition and dynamics of forest ecosystems, habitat conditions and ecosystem functions (Hermosilla et al., 2019). This study has focused on the short-term transformations over time on Lycian salamander habitats as a result of forest fires. Due to the lack of data on habitats after the fire, it has not been possible to make an assessment of functional connectedness. Subsequent studies may develop both structural and functional connectedness models to assess the connectedness of green spaces more comprehensively. To this end, we recommend adopting data collection methods that are based on field observations and that make it possible to understand the mobility of the species in the landscape.

The available research suggests that data on land cover or pixel sizes on different spatial scales can lead to different results during fragmentation analysis. For instance, Riitters et al. (2002) emphasise that smaller pixel sizes or fragmentation analysis conducted with a higher resolution database will not show greater fragmentation than larger pixels or lower resolution data. However, other studies indicate that higher resolution data can display more detailed anthropogenic fragmentation (Shen et al., 2020).

The capacities of forests and other natural habitats to sustain biodiversity and maintain ecosystem services will depend on the total amount and quality of the fragmented habitats, on their connectivity levels and on how they are affected by climate change. In order to explain and estimate the long-term environmental impacts of transformations in habitats in forest areas, more research needs to be done using a variety of scenarios. Subsequent studies should address various ecosystems and spatial connectedness as a whole, and present experimental efforts within a holistic approach (Hansen et al., 2013).

For the sustainable planning and management of areas with strong natural landscape characteristics, it is crucial to analyse the spatial transformations of habitat units over time using the latest data and methods so as to obtain accurate and useful results. Taking into account the interaction between the species and the landscape, it has been argued that the continuity of local populations must be ensured in core areas consisting of forests. Analysing maps of the ecological connectedness provided by green spaces may prove beneficial in identifying priorities for the conservation of areas with high rates of fragmentation.

This study underlines that the areas of burned forests and the burn severity can be analysed using remote sensing methods. In many studies, NBR and dNBR have performed well in identifying burned areas and analysing burn severity (French et al., 2008; Sabuncu & Özener 2019; Roy et al., 2019). However, since we could not acquire information on ignition points, we were unable to validate the classification of the Landsat-8 images. This may be considered a limitation of the study.

Conclusions

The present study has assessed the fragmentation in the distribution areas of six species due to forest fires using Landsat 8 satellite images and remote sensing and pattern analysis methods. Habitat loss increased substantially as a result of the fire, depending on burn severity. The changes in morphological classes showed that while the core areas in habitats decreased the formation of new bridges hints at the possibility of a recovery of spatial connectedness. However, since the formation of new bridges was not able to prevent an increase in other morphological classes, it is included that the fragmented areas need to be restored urgently. For these reasons, the survival of the salamander populations in the fragmented landscapes depends on the preservation or restoration of the functional (ecological) connectedness between the remaining habitat areas, which needs to be assured by the presence of ecological corridors.

Large-scale forest fires are one of the environmental disruptions that negatively affect habitats, particularly in Mediterranean Turkey. This study therefore emphasises that landscape administrators and environmental policy-makers need to come up with appropriate strategies that will prevent forest fires from occurring. Monitoring the spatial structure of forest areas will be decisive in whether these strategies achieve their aims. This process will succeed if effective, well-planned and well-coordinated measures are taken that also contribute to forest ecosystem management. For the purposes of the planning activities to be carried out in this context, it is proposed that fire risk maps should be developed using satellite image and pattern analysis techniques, and that these maps should be incorporated into environmental monitoring activities.

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An Ecological Perspective on Cities: the Benefit of Urban Vegetation and Parks in Prishtina City, Kosovo

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Abstract. Urban vegetation and green spaces are considered as indicators of both quality of life and urban sustainability. The purpose of this paper is to present how quality of public spaces and urban vegetation contribute positively to people's quality of life. Green space quantity is measured by the ratio of green space, while quality consists of plant community structure (deciduous, evergreen, annual plants, flowering shrubs and perennials plants). The study was conducted in four urban parks (Germia Park, City Park, Tauk bashçe Park and Dardania Park) in Prishtina City, Republic of Kosovo. Urban vegetation plays an important role when it comes to cope with the challenges that are related to the urban infrastructure of XXI century. From the historical time period, human being has appreciated greenery to find the flavor of life, form some physical and psychological dependency on nature. Cities have been the centers of economic and social developments, as well as sources of many major environmental problems.Issues regarding urban vegetation, public green spaces per inhabitant, public parks and recreation areas are frequently highlighted as important factors to make the more sustainable, pleasant and attractive for its citizens.

Key words: plant community structure, vegetation, quality of life, ecology, greenery, parks.

Introduction

Urban green spaces include a wide variety of vegetated public spaces, from large parks on the urban periphery to small green spaces located in densely populated urban neighborhoods (Maruani & Amit-Cohen, 2007). Urban green spaces are often viewed in different lights because ecologists and other stakeholders have contrasting opinions on their role in biodiversity conservation and their value to society.

Air quality is a major problem in many urban areas in the Republic of Kosovo and therefore has an impact on human health. In the Republic of Kosovo there is an increased the tendency for environmental regulation with different ornamental plants, in parallel with the expansion of urban centers, large cities, new houses, residential areas.

Urban green spaces comprise a range of habitat types that cross a continuum

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from intact remnant patches of native vegetation, brownfields, gardens, and yards, to essentially terraformed patches of vegetation that may or may not be representative of native community associations (Cilliers et al., 2013). Urban vegetation provides a wide variety of ecosystem services, including air quality improvement, climate regulation, and other elements that enhance urban environmental quality (Bolund & Hunhammar, 1999).

A variety of land use practices and environmental factors affect urban park biodiversity and vegetation structure, composition, and ecological function, but few studies have compared plant taxonomic composition, structural complexity, and species traits across different types of urban green spaces.

Green and open areas can be public or private properties and are differentiated by their function, their ecological value and their belonging to other land use types. Such green space is diverse, varying in size, vegetation cover, species richness, environmental quality, and proximity to public transport, facilities, and services.All forms of vegetation contribute to visual improvement and in this context they are of aesthetic value and contribute to urban architecture (Smardeon, 1988).

Urban park planted with various species provides a wide range of ecosystem benefits including regulating, supporting, cultural, and provisioning services (Arnberger & Eder, 2012). Urban green spaces provide critical ecosystem services to urban area residents. While urban green spaces provide a range of ecosystem services, cultural ecosystem services may be the most prominent to residents. Cultural ecosystem services provide benefits through educational, recreational, social, and spiritual opportunities (Almas, 2016).

As a result of rapid population growth and a lack of urban planning, cities in developing countries tend to have higher population densities and lower environmental quality compared to cities in developed countries (Peschardt & Stigsdotte, 2013). In this context, the creation of green spaces has been a strategy for the improvement of environmental quality, due to the positive effects that such spaces have on social and environmental dimensions connected to quality of life.

This study investigates the relationship between neighborhood green spaces and residential satisfaction considering both the quantity and quality of green space. Green spaces play an important role in supporting urban communities both ecologically and social.

Material and Methods

Study area

Location of this study was in Pristina City in Republic of Kosovo. During the study we have analyzed the vegetation in different locations in the central and northern parts of the Pristina City (Fig. 1). The study was conducted in four urban parks (Germia Park, City Park, Tauk bashqe Park and Dardania Park) in Pristina City, between May 2020 and October 2021. The city covers 572 km^2 and has a population of over 600.000 inhabitants. Kosovo is located in the central part of the Balkans. It lies between 41°50′58″and 43°51′42″ of northern geographic latitude and between 20°01′3“and 21°48′02″ of eastern geographic length.

Data of type of parks categories were compiled from urban green maintenance maps, Pristina Municipality, cadastral maps, and land maps. Plant identification at the site was done using the botanical key. The structure and function of urban park vegetation were analyzed using Summed Dominance Ratio (SDR), indices of diversity, species richness, evenness, and similarity. SDR index was used to analyze the plant species dominance and frequency of the four urban parks in Pristina City. SDR was calculated using the formula below (Muhlisin et al., 2021):

$$\mathrm{SDR} = \frac{\mathrm{FR}+\mathrm{DR}}{2},$$

where: FR is Relative Frequency and DR is Relative Dominance. Relative Frequency (FR) was calculated by dividing the frequency of species-i by the sum frequency of all species then multiplying by 100 percent. Dominance Relative (DR) was calculated by dividing the individual number of species-i by the dominance of all species multiplied by 100 percent. Dominance of species-i (Di) was obtained by dividing the individual number of species-i by the individual number of all species.

The species diversity index was calculated using the Shannon-Wiener formula (Spellerberg & Fedor, 2003).

Results and Discussion

In total, across four urban parks in Prishtina City observed in this study, there were 13.500 individuals of plants recorded, consisting of 76 species belong to 16 families.

Table 1 shows species and cultivars with the highest Summed Dominance Ratio (SDR)in four urban parks in (*Tilia cordata, Platanus orientalis, Catalpa bigninoides, Betula alba, Aesculus hippocastanum, Magnolia grandiflora, Prunus cerisfera, Thuja occidetalis, Tagetes patula, Salvia splendens, Petunia x hybrida, Salix babylonica, Fagus sylvatica, Robinia pseudoacacia, Photinia fraseri, Buxus sempervirens*).

Fig. 2 shows species and cultivars with the highest Summed Dominance Ratio(SDR) in city parks in Prishtina City (*Tagetespatula "Carmen", Salvia splendens "Scarlet Sage", Petunia "Million Bells", Thujaocidentalis "Smaragd", Pynuscaricifera "Kanzan", Tiliatametosa "Brabant"*).

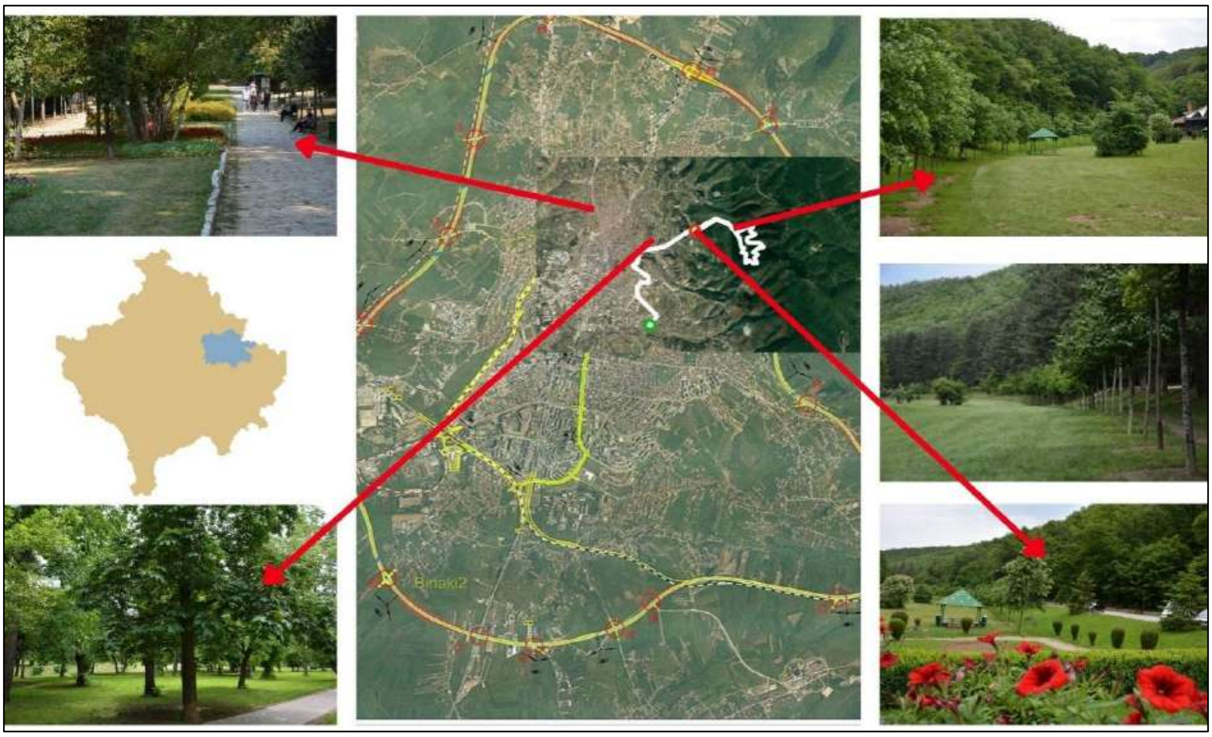

Fig. 1. Photographs of the analyzed green spaces. Geographical location of selected neighborhoods and green spaces in area of Pristina City.

Table 1. Twenty plant species with the highest Summed Dominance Ratio (SDR) in for urban parks in Pristina.

Botanical name	Common name	Plant category	Color leaf
Tilia cordata	Lime	Deciduous	Green
Platanus orientalis	Platanus	Deciduous	Green
Catalpa bigninoides	Catalpa	Deciduous	Green
Betula alba	Brich	Deciduous	Green
Aesculus hippocastanum	Aesculus	Deciduous	Green
Magnolia grandiflora	Magnolia	Evergreen	Green
Prunus cerisfera	Japonese Cherry	Deciduous	Red
Thuja occidetalis	Americanarborvitae	Evergrenn	Green
Tagetes patula	French marigold	Annual plant	Orange
Salvia splendens	Scarlet sage	Annual plant	Red
Zinnia elegans	Zinnia	Annual plant	Green
Petunia hybrida	Petunia	Annual plant	Green
Salix babylonica	Weeping willow	Deciduous	Green
Fagus sylvatica	European Beech	Deciduous	Purple
Robinia pseudoacacia	Black locust	Deciduous	Green
Platanus acerifolia	London plane	Deciduous	Green
Berberis thunbergii	Japanese barberry,	Deciduous	Purple
Forsythia x intermedia	Forsytia	Deciduous	Yellow
Photinia fraseri	Photina	Evergreen	Green
Buxus sempervirens	Boxwood	Evergreen	Green

Plant species diversity index in urban parks in Prishtina City is ranged from 1.21 to 1.67 point in the medium category, except in Dardania Park which was in the low category (0.68). The high diversity of plant species in urban parks provides an important value in biodiversity conservation (Goddard et al., 2010) and puts urban parks as an important habitat of biodiversity (Lepczyk et al., 2017).

The CityPark in Pristina City is one of the oldest parks in the city with an area of 7.6 ha. This park is located near the city center, which is frequented by a large number of visitors.

Fig. 3 shows species and cultivars with the highest SDR in Dardania urban park in (*Viola wittrockiana "Majastic Gaint", Viola wittrockiana "Dynamite", Thuja smaragd, Legustrum ovalifolium, Abies normandiana, Viola x hybrida*).

Bedding plants can be annuals, biennials or perennials. Annuals are plants which are grown from seed, produce flowers and die in one growing season. Biennial plants grow leaves, stems and roots the first year, and then go dormant for the winter. Perennials are plants which are live longer than two years and are typically cold-hardy plants that will return each year in the spring. Perennials play an important role every garden and green public spaces.

Table 2 shows species and cultivars with the highest SDRin Germia park (*Corylus avellana, Tilia platyphyllos, Catalpa bigninoides, Viburnum lantana, Rosa sp., Carpinus betulus, Picea abies, Betula alba, Fagus sylvatica, Quercus pubescens, Acer campestre*).

Table 3 shows species and cultivars with the highest SDR in Taouk bashçe Park in (*Aesculus hippocastanum, Tilia platyphyllos, Potentilla micrantha, Viburnum lantana, Ulmus campestris, Carpinus betulus, Picea pungens, Betula alba, Fagus sylvatica, Hedera helix,Catalpa bigninoides*).

Fig. 2. Plant species diversity and SDR in Pristina City Park.

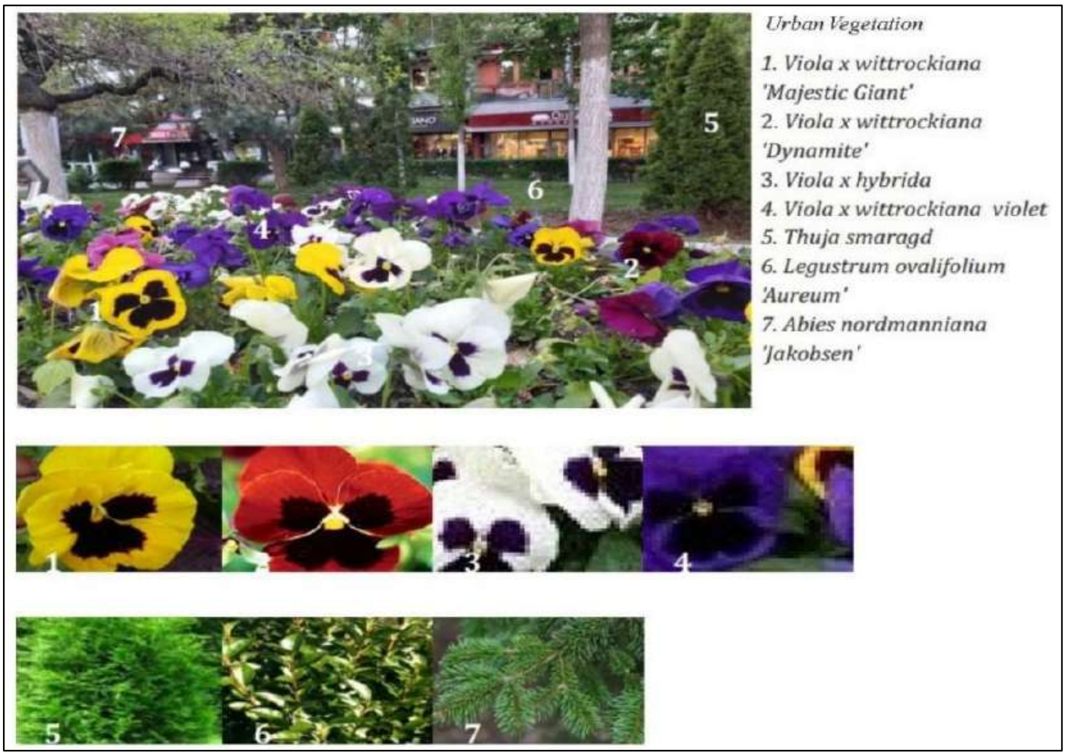

Fig. 3. Plant species diversity and SDR in Prishtina Dardania Park.

Table 2. Ten plant species with the highest SDR in Germia Park in Pristina City.

Botanical name	**Common name**	**Plant category**	**Color leaf**
Corylus avellana	Hazel	Deciduous	Green
Tilia platyphyllos	Lime	Deciduous	Green
Catalpa bigninoides	Catalpa	Deciduous	Green
Viburnum lantana	Brich	Deciduous	Green
Rosa species	Rosa	Deciduous	Green
Carpinus betulus	Hornbeam	Deciduous	Green
Picea abies	European spruce	Evergreen	Green
Betula alba	Brich	Deciduous	Green
Fagus sylvatica	European Beech	Deciduous	Green
Quercus pubescens	Pubescent oak	Deciduous	Green

Table 3. Ten plant species with the highest SDR in Taoukbashçe Park in Pristina City.

Botanical name	**Common name**	**Plant category**	**Color leaf**
Aesculus hippocastanum	Aesculus	Deciduous	Green
Tilia platyphyllos	Lime	Deciduous	Green
Potentilla micrantha	Pink barren strawberry	Deciduous	Green
Viburnum lantana	Brich	Deciduous	Green
Ulmus campestris	Olmo	Deciduous	Green
Carpinus betulus	Hornbeam	Evergreen	Green
Picea pungens	Blue spuce	Evergreen	Red
Betula alba	Brich	Deciduous	Green
Fagus sylvatica	European Beech	Deciduous	Orange
Hedera helix	Ivy	Deciduous	Red

Conclusions

The manifestation of the concept of a sustainable city of the XXI century, raised the themes at analyzing the role of vegetation and green spaces in urban and suburban areas in the Republic of Kosovo. Urban vegetation of Pristina City is comprised of species of landscaping shrubs, urban trees, flowers, woodland, tall shrub, conifers, herbaceous and other vegetation.

Plant species diversity index in urban parks in Prishtina City is ranged from 1.21 to 1.67 point in the medium category, except in Dardania Park which was in the low category (0.68). The CityPark in Pristina is one of the oldest parks in the city with an area of 7.6 ha. This park is located near the city center, which is frequented by a large number of visitors.Issues regarding urban vegetation, public green spaces per inhabitant, public parks and recreation areas are frequently highlighted as important factors to make the more sustainable, pleasant and attractive for its citizens.

As the city of Pristina is facing some of the same challenges,is an initiative to optimize the provision of urban green environments by establishing 6 new "Parks projects" to 2024 has been implemented.

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Anatomical Investigation of Marrubium friwaldskyanum Boiss. and Marrubium peregrinum L. (Lamiaceae) from Bulgaria

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Abstract. The present study presents data on the anatomical characteristics of the leaf and stem in *Marrubium friwaldskyanum* and *Marrubium peregrinum*. The leaves in both species are amphistomatic with diacytic and anomocytic stomata. The leaf lamina shows differentiation of palisade and spongy tissue, and collateral vascular bundles. Non-glandular and glandular trichomes have been found on the epidermis of the leaves and stem. The non-glandular trichomes are unicellular linear and multicellular branched. Glandular trichomes are peltate, with a single-celled or two-celled structure. There is a difference between the two species in terms of the width of the epidermal cells of the stem, the thickness of the cortex, the thickness of the xylem and the phloem in the stem. The established differences in the anatomical features can be useful in future taxonomic studies within the genus *Marrubium*.

Key words: Plant anatomy, Marrubium, Lamiaceae.

Introduction

The genus *Marrubium* L. belongs to the family Lamiaceae and is represented worldwide by about 40 species (Akgül et al., 2008). Representatives of the genus are herbaceous, annual and perennial plants, distributed mainly in the Iranian-Turanian and Mediterranean phytogeographical region (Hedge, 1992). Some of them have medicinal properties and are used as spices, and others are grown as ornamental plants (Meyre-Silva & Cechinel-Filho, 2010; Estilai & Hatemi, 1990; Büyükkartal et al., 2016). The genus *Marrubium* is represented with the greatest diversity in Turkey (Akgül & Ketenoğlu, 2014), where 21 taxa are found, 12 of which are endemic (Cullen, 1982; Ekim et al., 2000; Aytaç et al., 2012). In the flora of Europe, the genus is represented by 12 species (Tutin et al., 1972). There are 4 species in the Bulgarian flora – *M. friwaldskyanum* Boiss., *M. vulgare* L., *M.*

peregrinum L., *M. parviflorum* Fisch et Mey (Andreev et al., 1989).

M. friwaldskyanum Boiss. is a rare species and Bulgarian endemic (Kozhuharov, 1992; Delipavlov & Cheshmedzhiev, 2011), included in the Red Book of Bulgaria (Meshinev, 2015), as well as in the Red list of Bulgarian vascular plants (Petrova & Vladimirov, 2009) with category "vulnerable". The plant is perennial, herbaceous with yellow to creamy yellow flowers, gathered in short verticillaster and elliptical to oval leaves.

M. peregrinum is also a perennial herbaceous plant with lanceolate to elliptical leaves, narrowed at the base and to the top. The species is included in the list of medicinal plants of the Medicinal Plants Act (2000).

So far, cytotoxic activity of methanolic extraction from *M. friwaldskyanum* herbs (Kozyra et al., 2020), antioxidant (Kaurinović et al., 2010, 2011; Stanković, 2011) and antimicrobial activity of *M. peregrinum* extracts (Radojević et al., 2013), composition of diterpenoids and phenolic compounds in *M. friwaldskyanum* and *M. peregrinum* (Piozzi et al., 2006; Hennebelle et al., 2007; Kozyra et al., 2020; Zheljazkov et al., 2022), have been studied. Studies on the reproductive biology and reproductive capacity, leaves, stem, calyx, corolla, seeds and pollen surfaces analysis by Scanning Electron Microscopy of *M. friwaldskyanum* have been held by Zheljazkov et al. (2022). Data concerning anatomical structure of vegetative organs of these species are missing in the literature.

Since the main characteristics of taxonomic value in members of the Lamiaceae family are the type of trichomes (Marin et al., 1994; Navorro & El-Qualidi, 2000), the type of epidermal structure is covered in the Cantino studies (1990), which described two main types - non-glandular and glandular trichomes, as non-glandular are more common. In species of the genus *Marrubium*, stellate non-glandular and branched non-glandular trichomes are found on all parts of plants (Bosabilidis, 1990; Kaya et al., 2003; Upson & Andrew, 2004; Ahvazi et al., 2016).

The structure of glandular trichomes of *M. cuneatum* and *M. vulgare* has been studied by Baher et al. (2004), Belhattab & Larous (2006), Dmitruk & Haratym (2014).

Haratym & Weryszko-Chmielewska (2017) conduct a detailed study of the structure of glandular trichomes in *M. vulgare*. The authors describe peltate trichomes with a single-celled stalk and an 8-cell head and capitate trichomes of 3 types: with long stalk and unicellular head, with a short stalk and a two-celled head, and with a short stalk and a four-celled head. Two types of non-glandular trichomes are also mentioned: multicellular linear (found on the stem and the lower surface of the leaves and calyx) and multicellular branched (located mostly on the upper leaf surface).

There are descriptive anatomical studies of *M. vulgare* concerning bioaccumulative properties (Moreno–Jimenez et al., 2006) and M. anisodon (Talebi et al., 2019a) concerning leaf structure. In another study, Talebi et al. (2019b) examine the anatomical structure of the stem of 6 species of the genus *Marrubium* found in Iran. Ahvazi et al. (2017) describe the taxonomic, morphological and pharmacological features of *M. vulgare* from Iran.

It is obvious that at present research on the genus mainly concerns the features of pollen, anatomomorphological and chemical characteristics (Akgül et al., 2008; Ahvazi et al., 2016; Kharazian & Hashemi, 2017).

Cross-sectional studies of the leaf lamina of *M. trachyticum* show a bifacial structure with collateral vascular bundles (Akçin & Camili, 2018). The stomata are of the anomocytic type, located on both leaf surfaces, which defines the structure as amphistomatic.

The analysis of the literature data and the identified lack of information on comprehensive anatomical studies on *M. friwaldskyanum* and *M. peregrinum* determined the purpose of the present study, namely the anatomical study of *M. friwaldskyanum* and *M. peregrinum*, in order to enrich the information on the genus and Bulgarian endemic plants.

Material and Methods

Plant material

Plant material (leaves and stems) from *M. friwaldskyanum* and *M. peregrinum* (Fig. 1) was collected from natural habitats during the 2020 growing season (Table 1). In order to prepare histological preparations, the vegetative organs were initially fixed in 70% ethanol.

Fig. 1. General view of *M. friwaldskyanum* (A) and *M. peregrinum* (B).

Table 1. List of studied taxa and localities of their collection.

Species	Locality(latitude/longitude and altitude)	Floristic region and year of sampling
M. friwaldskyanum Boiss.	42°09' N; 24°12'E/1136 m	Fortress Cepina, Rhodopes Mts. (western), 2020
M. peregrinum L.	42°04' N; 24°47'E/389 m	Krichim, Rhodopes Mts. (western), 2020

Anatomical methods

The analysis of leaves and stems was done following the classical methods of Metcalfe & Chalk (1950). For easier description of the epidermal structures, as well as the underlying tissues, a large amount of trichomes on the leaf and stem surface has been removed. The following qualitative and quantitative features were observed: type of trichomes on the leaf and stem surface; length and width of leaf and stem epidermal cells (µm); type, number (mm^2), length and width (µm) of the stomata on the adaxial and abaxial epidermis; mesophyll thickness (palisade and spongy) in the leaves (µm); stem cortex thickness (µm); thickness of xylem and phloem in the stem (µm). In order to ensure the reliability of the results for each quantitative trait, 30 prominent fields were examined and 30 measurements were made, respectively. The light microscope images were taken with a Magnum T Trinocular microscope, equipped with a Si5000 photo documentation system in the Department of Botany and Biological Education at the Faculty of Biology, Plovdiv University "Paisii Hilendarski".

Statistical methods

Statistical analyzes were performed with statistical program SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The main descriptive statistical values of the variation variables: mean value (mean), median (median), standard deviation (SD) and standard error (SE) were calculated according to standard methods. Quantitative (variational) variables were tested for normality of distribution with the Kolmogorov-Smirnov D-Test. The difference between the mean values of the normally distributed quantitative variables of two independent groups was estimated using the non-pair t-test for independent groups (Independent-samples t-test).

The possibilities of graphic analysis are used to illustrate the processes and phenomena.

In all analyzes for a level of statistical significance at which the null hypothesis is rejected (i.e. there is a difference between the compared values) p <0.05, respectively confidence probability greater than 0.95 (95%).

Results and Discussion

Leaf anatomy

Analyzing the leaf surface of *M. friwaldskyanum* and *M. peregrinum*, the epidermis was found, including ordinary epidermal cells, stomata and trichomes of different structures. The basic cells in both species have undulated cell walls and different sizes, which is in accordance with the classification of Aneli (1975) and the studies of Mladenova et al. (2019) in other members of the Lamiaceae family. In both taxa, the predominant stomata are of the diacytic type, and in some places an anomocytic type of stomata is found (Fig. 2).

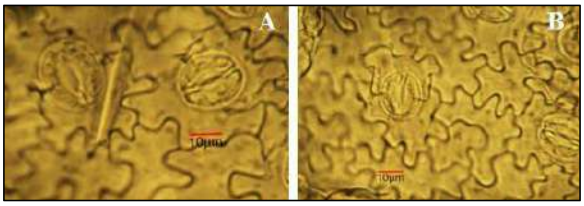

Fig. 2. Stomatal apparatus in *M. friwaldskyanum* (A) and *M. peregrinum* (B).

Their location on the two leaf surfaces defines the leaves as amphistomatic, which is in line with the statement of Büyükkartal et al. (2016) and Tüylü et al. (2017), for other species of the genus *Marrubium*. The indumentum in *M. friwaldskyanum* is represented by two types of trichomes - non-glandular and glandular. Non-glandular trichomes are unicellular linear and multicellular strongly branched (Fig. 3A, B). Glandular trichomes are peltate, stacked (Fig. 3C), with a short unicellular stalk (Fig. 3D, E) or bicellular structure (Fig. 3F).

These results are similar to those reported by Zheljazkov et al. (2022) for the same species collected from another locality. In *M. peregrinum*, only a large number of multicellular branched non-glandular trichomes (Fig. 4A) were found, while in structure glandular trichomes are peltate, stacked (Fig. 4B), with a unicellular stalk and a secretory cell (Fig. 4C) and with a unicellular stalk and a bicellular secretory cell (Fig. 4D).

The cross-section of the leaf lamina in the two studied species showed epidermal cells who are arranged in one row, oval in shape. In *M. friwaldskyanum*, the adaxial and abaxial epidermises are distinguished with a mesophyll consisting of one row of palisade tissue to the adaxial surface and spongy cells to the abaxial surface (Fig. 5A). In *M. peregrinum*, palisade tissue is found on both surfaces (Fig. 5B). The central vein of the leaves in the two studied species is represented by closed collateral vascular bundle (Fig. 5C, D).

The data from the statistical processing of the quantitative indicators reflecting the leaf structure of *M. friwaldskyanum* are presented in Table 2.

Based on the performed statistical correlation analyzes between leaf traits within the species, no statistical reliability was established ($p > 0.05$). Analyzing the mark length of the stomata on both epidermis ($p = 0.250$), the obtained average values on the abaxial epidermis are close to those on the adaxial epidermis. As for the width of the stomata on the two leaf surfaces, similar mean values are again established ($p = 0.648$). Regarding the number of stomata (per mm^2), despite the significant difference in the average values of the scar on the two epidermis, no statistically significant difference was found ($p = 0.890$). This fact determines the amphistomatic nature of the leaves, as noted by Büyükkartal et al. (2016) and Akcin & Camili (2018) for other species of the genus *Marrubium*. The values of p when comparing the length and width of epidermal cells are 0.322 and 0.147, respectively. Palisade and spongy tissue occupy almost the same space in the leaf structure, which is evident from the close values of the two scars ($p = 0.147$).

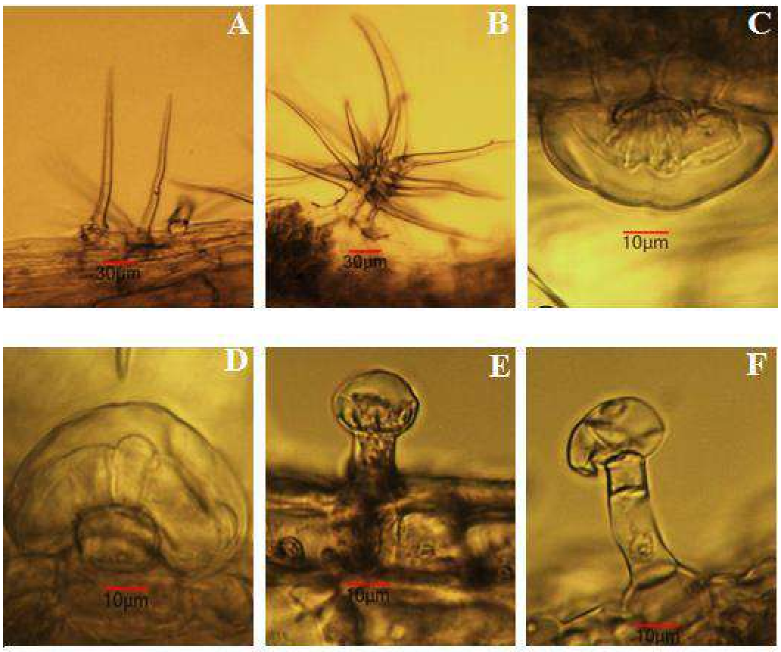

Fig. 3. Non-glandular and glandular trichomes in *M. friwaldskyanum*. A - unicellular linear non-glandular trichomes; B - multicellular branched non-glandular trichome; C – glandular peltate trichome; D, E - glandular trichomes with unicellular stalk; F - glandular trichomes with bicellular stalk.

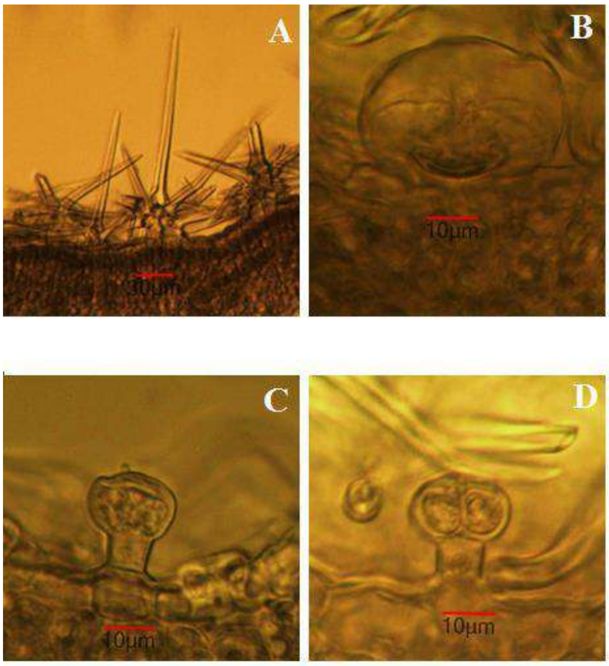

Fig. 4. Non-glandular and glandular trichomes in *M. peregrinum*. A - multicellular branched non-glandular trichomes; B - glandular peltate trichome; C – glandular trichome with a unicellular stalk and secretory cell; D – glandular trichome with a unicellular stalk and a bicellular secretory cell.

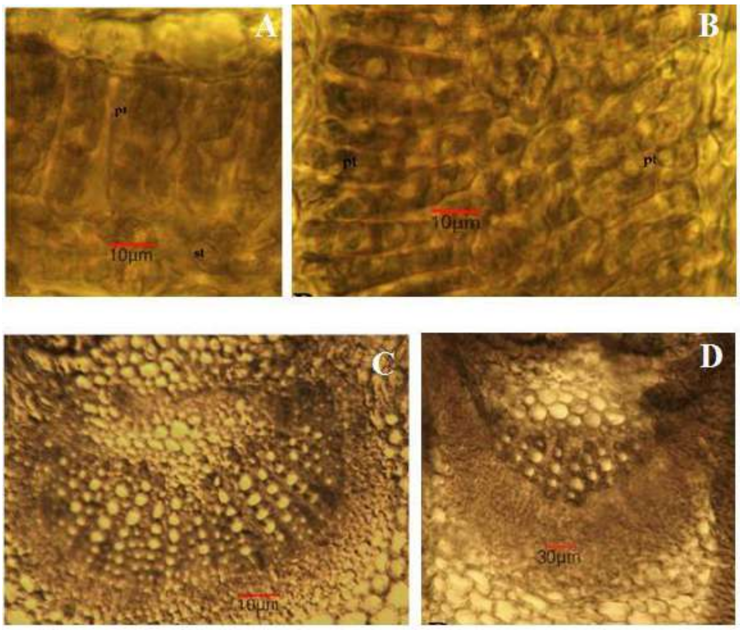

Fig. 5. Cross section of the leaf lamina. A – *M. friwaldskyanum*; B – *M. peregrinum*; C – Vascular bundle by *M. friwaldskyanum*; Vascular bundle by *M. peregrinum*; pt – palisade tissue; st – spongy tissue.

Table 2. Descriptive statistics *of M. friwaldskyanum*. Legend: SD – Standard deviation; SEM – Standard Error of Mean.

Feature	Mean±SD	95% Confidence Interval	Median	SEM	Minimum	Maximum	One-Sample Kolmogorov-Smirnov Test
Abaxial epidermis stomata (Length, µm)	5.12±0.28	5.02-5.22	5.06	0.05	4.14	5.52	p=0.054
Abaxial epidermis stomata (Width, µm)	3.95±0.34	3.82-4.07	3.91	0.06	3.22	4.37	p=0.470
Abaxial epidermis stomata (Number, per mm^2)	140.57±18.85	133.53-147.61	146.00	3.44	98	171	p=0.052
Adaxial epidermis stomata (Length, µm)	4.81±0.31	4.70-4.93	4.83	0.55	4.37	5.29	p=0.247
Adaxial	3.90±0.17	3.84-3.97	3.91	0.03	3.68	4.14	p=0.117

epidermis stomata (Width, μm)							
Adaxial epidermis stomata (Number, per mm^2)	45.47±20.15	37.94-52.99	49.00	3.68	24.00	98.00	p=0.083
Epidermal cells abaxial epidermis (Length, μm)	3.57±0.24	3.48-3.66	3.68	0.04	3.22	3.91	p=0.156
Epidermal cells abaxial epidermis (Width, μm)	2.51±0.33	2.38-2.63	2.30	0.06	2.07	3.22	p=0.008
Epidermal cells adaxial epidermis (Length, μm)	4.38±0.86	4.06-4.70	4.37	0.16	2.53	5.75	p=0.035
Epidermal cells adaxial epidermis (Width, μm)	2.97±0.47	2.79-3.14	3.10	0.08	1.84	3.45	p=0.043
Palisade tissue (Thickness, μm)	9.03±0.60	8.81-9.25	9.20	0.11	7.59	10.12	p=0.280
Spongy tissue (Thickness, μm)	9.12±1.39	8.60-9.64	8.28	0.25	7.82	11.96	p=0.034

The results obtained from the analysis of leaf characteristics in *M. peregrinum* are presented in Table 3.

The same structure in the internal structure of the two epidermis of *M. peregrinum* determines the smaller number of features described in the table. The stomata frequency on both surfaces is close in average to that of the lower epidermis in *M. friwaldskyanum*.

Stem anatomy

The cross-section of the stem of the two studied species shows the typical for the representatives of the family Lamiaceae four-edged shape. Cortex and pith are clearly distinguishable. Parenchymal cells are mainly present in the cortex, with several layers of collenchyma being observed in the corners. The vascular tissues have a non-bundled structure (Fig. 6).

The epidermis consists of round or rectangular cells covered with a thick cuticle. These features of the covering tissue are also mentioned by Akçin & Camili (2018) in another species of the genus *Marrubium*. On the stem surface of *M. friwaldskyanum* and *M. peregrinum* a large number of trichomes are observed, which in structure and type highly correspond to the trichomes covering the leaves. In *M. friwaldskyanum*, both unicellular linear and multicellular branched non-glandular trichomes are found (Fig. 7A, B). Glandular trichomes in this species are of two types: with a unicellular stalk and a secretory cell, and with a bicellular stalk and a secretory cell (Fig. 7C, D).

In *M. peregrinum*, the non-glandular trichomes are multicellular branched (Fig. 8A), and the glandular trichomes are composed of a bicellular stalk and a bicellular secretory cell (Fig. 8B).

The analyzed values of the quantitative indicators affecting the stem of the studied taxa are presented in Table 4.

The comparative analysis (Independent Samples T-test) between the stem indicators of the two taxa showed that there was no statistically significant difference in epidermal cell length alone. For the other studied traits, the values for the two species show significant differences (p <0.0001). The identified significant differences are presented graphically in Fig. 9.

Table 3. Descriptive statistics *of M. peregrinum*. Legend: SD – Standard deviation; SEM – Standard Error of Mean.

Feature	**Mean±SD**	**95% Confidence Interval**	**Median**	**SEM**	**Minimum**	**Maximum**	**One-Sample Kolmogorov-Smirnov Test**
Stomata (Length, µm)	4.41±0.24	4.31-4.50	4.37	0.04	3.91	4.83	p=0.089
Stomata (Width, µm)	3.15±0.37	3.01-3.29	3.22	0.07	2.53	3.68	p=0.111
(Number, per mm^2)	120.47±24.63	111.27-129.66	122.00	4.50	73.00	171.00	p=0.060
Leaf epidermal cells (Length, µm)	4.73±0.54	4.53-4.93	4.83	0.10	3.45	5.98	p=0.713
Leaf epidermal cells (Width, µm)	2.94±0.68	2.68-3.19	2.99	0.12	1.84	4.60	p=0.679
Palisade tissue (Thickness, µm)	5.95±0.91	5.61-5.29	5.75	0.16	4.60	7.59	p=0.479

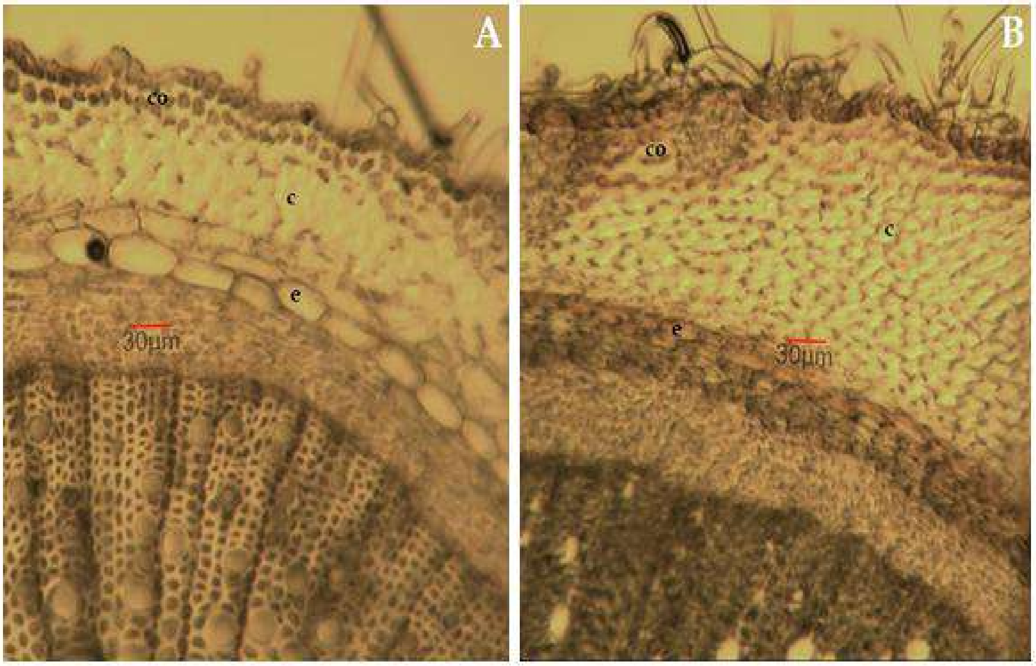

Fig. 6. Cross section of the stem. A - *M. friwaldskyanum*; B – *M. peregrinum*; co – collenchyma; c – cortex; e – endodermis.

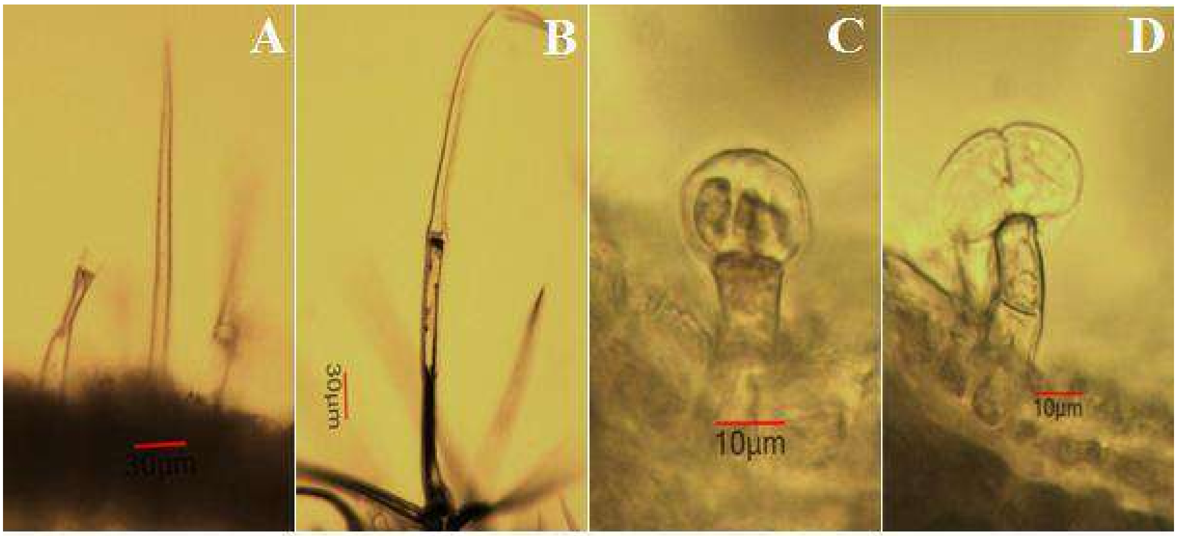

Fig. 7. Type of trichomes on the stem surface of *M. friwaldskyanum*. A - unicellular linear non-glandular trichome; B - multicellular branched non-glandular trichome; C – glandular trichome with a unicellular stalk and a secretory cell; D – glandular trichome with a bicellular stalk and a secretory cell.

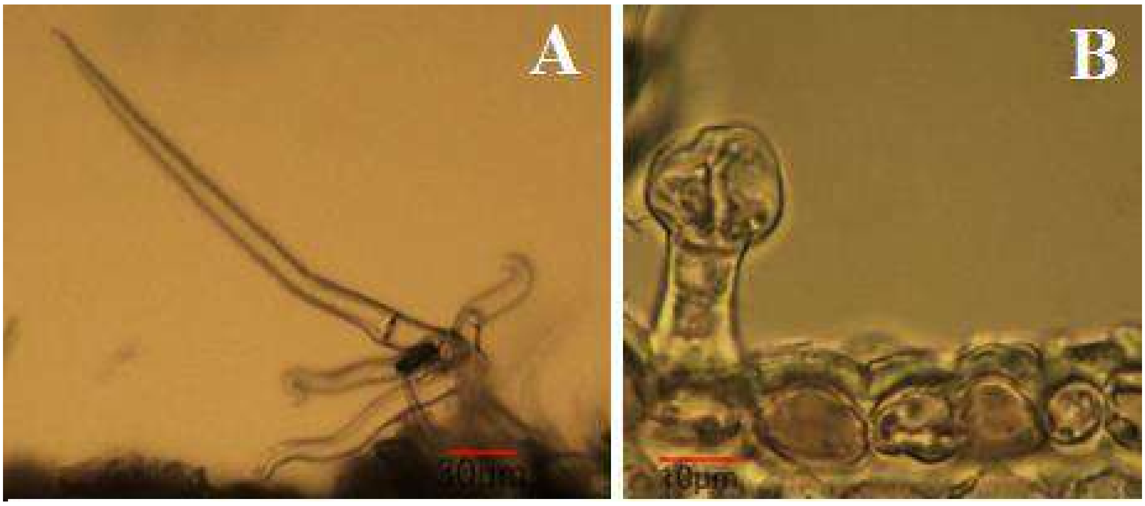

Fig. 8. Type of trichomes on the stem surface of *M. peregrinum*. A - multicellular branched non-glandular trichome; B – glandular trichome with bicellular stalk and a secretory cell.

Table 4. Descriptive statistics of the studied stem features of *M. friwaldskyanum* and *M. peregrinum*. Legend: SD – Standard deviation; SEM – Standard Error of Mean.

Feature	Mean±SD	95% Confidence Interval	Median	SEM	Minimum	Maximum	One-Sample Kolmogorov-Smirnov Test
			M. friwaldskyanum				
Stem epidermal cells (Length, μm)	4.00±0.58	3.78-4.22	4.02	0.11	2.99	4.83	p=0.305
Stem epidermal cells (Width, μm)	2.81±0.24	2.72-2.90	2.76	0.04	2.53	3.22	p=0.070
Stem cortex (Thickness, μm)	36.57±5.88	34.38-38.77	36.57	1.07	23.92	45.08	p=0.142
Xylem (Thickness, μm)	81.16±2.53	80.21-82.10	81.65	0.46	74.98	84.18	p=0.316
Phloem (Thickness, μm)	14.60±1.56	14.02-15.19	15.64	0.28	11.96	16.10	p=0.006

M. peregrinum							
Stem epidermal cells (Length, µm)	4.05±0.44	3.89-4.22	4.14	0.08	3.22	4.60	p=0.059
Stem epidermal cells (Width, µm)	1.93±0.23	1.84-2.02	1.95	0.04	1.61	2.30	p=0.096
Stem cortex (Thickness, µm)	26.56±3.15	25.38-27.73	24.95	0.57	23.00	34.04	p=0.080
Xylem (Thickness, µm)	97.14±7.13	94.47-99.80	95.45	1.30	86.94	113.16	p=0.349
Phloem (Thickness, µm)	13.62±1.87	12.92-14.31	13.80	0.34	9.66	16.33	p=0.606

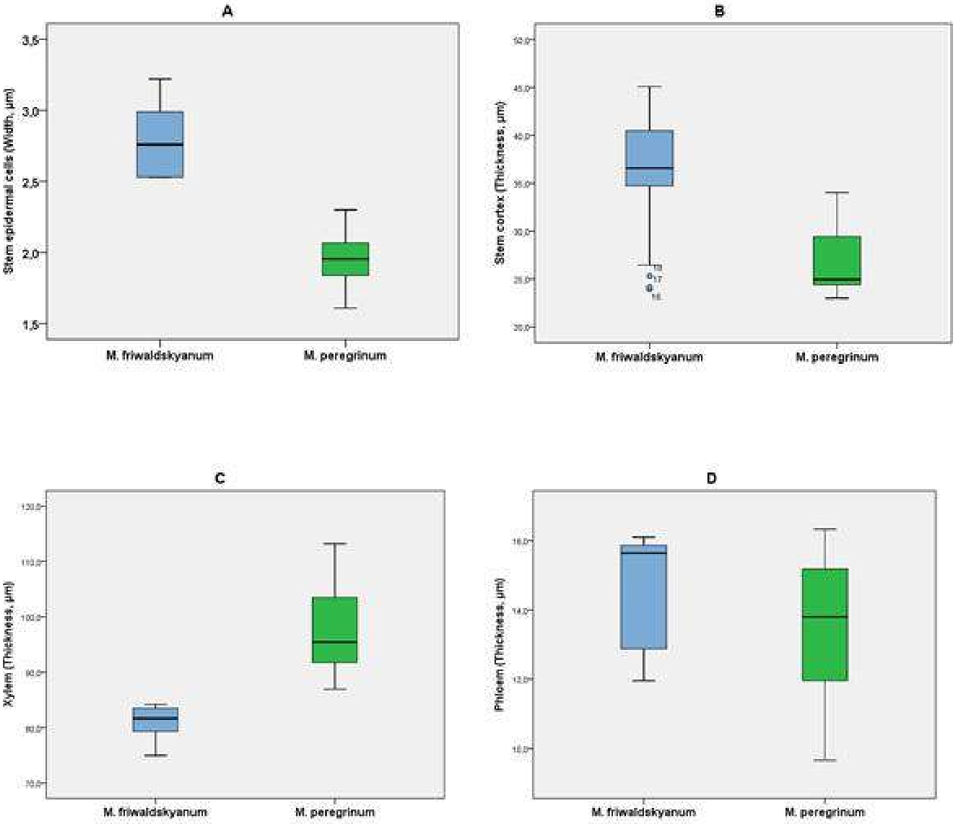

Fig. 9. Significant differences between the mean values of the stem indicators of *M. friwaldskyanum* and *M. peregrinum*.

M. friwaldskyanum is characterized by wider epidermal cells than *M. peregrinum* (Fig. 9A). The cortex of the stem in *M. friwaldskyanum* is statistically significantly thicker than that of *M. peregrinum* (Fig. 9B). Comparing the thickness of the two vascular tissues in both species, it was found that *M. friwaldskyanum* had a thinner layer of xylem (Fig. 9C) and a thicker layer of phloem (Fig. 9D).

Conclusions

The anatomical study of the species *M. friwaldskyanum* and *M. peregrinum* enriches the information about the genus and the endemic plants of Bulgaria. Described are: amphistomatic leaf structure with diacytic and anomocytic stomata, palisade and spongy chlorenchyma, unicellular and multicellular non-glandular trichomes with linear and branched structure, peltate, stacked, unicellular and bicellular glandular trichomes covering the leaf and the stem surface. The structure of the stem in both species is typical of the Lamiaceae family. Differences resulting from statistical processing of trait data: stem epidermal cell width, cortex thickness, and xylem and phloem thickness can be useful in future taxonomic studies within the genus *Marrubium*.

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Soil Magnetic Susceptibility Properties as Indicators of Heavy Metals Pollution in "Bobov Dol" TPP Area (Bulgaria)

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Abstract. All the coal used in the power plants in Bulgaria are mainly high ash coals, which consequently leads to the release of big quantities of ash during their combustion. This cause a significant pressure on ecological status of the soil cover in the plants areas. Recently it has been proved that the magnetic susceptibility of polluted soils increases due to their enrichment with different metal oxides. The main goal of this research is to be analyzed the magnetic susceptibility values in the soils around "Bobov dol" thermal power plant (TPP) as an indicators of heavy metals pollution. More than 60 field measurements of the soil magnetic susceptibility were conducted around "Bobov dol" TPP. This measurements were evenly distributed around the power plan in accordance with the established grid network. The measured field value of the magnetic susceptibility range between 1.9358x10^{-6} SI and 475.233x10^{-6} SI. The higher values of the magnetic susceptibility are observed around the "Bobov Dol" TPP and along the transport line that transport the waste from the burning process to the nearby dump site. In addition frequency-dependent susceptibility - χFD% of the same samples have been measured and very good positive correlation between higher magnetic susceptibility values and lower frequency-dependent susceptibility have been found which proved that the high MS values are due to the presence of coarse spherical particles with anthropogenic origin.

Key words: magnetic susceptibility, heavy metals, contaminated soils.

Introduction

In recent years, soil has been under increasing environmental pressure, driven or exacerbated by human activity. One of the most common soil pollutants that are extremely harmful for the human health are the trace elements/heavy metals. Some of these elements are essential for plants and animals in small amounts but in the recent years there has been an increasing ecological and global public health concern associated with environmental contamination by these metals (Tchounwou, 2012).

Solid fuel (coal) thermal power plants are one of the major industrial pollutants and emitters of heavy metals. Modern thermal power plants are usually equipped with sophisticated filters, however, some of the ash inevitably passes through the filters and is discharged into the atmosphere and accumulated in the surrounding soils. These are predominantly fine ash particles which are the most dangerous, since they usually contain elevated concentrations of some heavy metals and toxic elements (Helble & Sarofim, 1993; Helble, 1994; Ratafia-Brown, 1994, Mokreva et al., 2017; El Bagdadi et al., 2011).

The traditional detection methods of heavy metals in soil have strong specificity and high sensitivity, but they also are expensive and unsuitable for rapid or filed determination. Because of that during recent years, the measurements of magnetic susceptibility have become a broadly applied method to research the spatial distribution of pollution and to identify polluted sources. Measurements of magnetic susceptibility (MS) provide a fast and cheap alternative to conventional chemical analysis and it was proved to be extremely useful in disclosing industrial pollutants, traffic emission and other atmospheric pollutants (Morris et al., 1995; Dearing et al., 2006; Bityukova et al., 1999; Durza, 1999; Hoffmann et al., 1999; Kapicka et al., 1999; Matzka & Maher, 1999; Shu et al., 2000; Lecoanet et al., 2001; Hanesch et al., 2003; Goddu et al., 2004, Mokreva et al., 2017, El Bagdadi et al., 2011).

The main goals of this research is to present the results of the field measurements of the soil magnetic susceptibility (MS) conducted in the proximity of the "Bobov dol" TPP and to compare these data with the data acquired by the laboratory measurements of Frequency-dependent susceptibility which can be used as indicator for the relative contribution of the anthropogenic and pedogenic ferromagnetic minerals to the total magnetic fraction in the soil.

Material and Methods

Test site

"Bobov dol" TPP is located in the southwestern part of Bulgaria, near the town with the same name. The area of interest in this research represent a square with a side of 6 km. and enclose an area of 36 sq. km.. The "Bobov dol" TPP is situated in the centre of the square. (Fig. 1). "Bobov dol" TPP was commissioned in 1973 - 1975 with three 210 MW units. Since then, mainly lignite coal from the Chukurov, Beloberezh and Stanyantzi basins is burned there, as well as a small amount of brown coal from the Bobov Dol Town and Pernik Town basins as well (Kortensky & Zdravkov, 2008).

The soil cover around "Bobov dol" TTP is diverse (according to WRBSR, 2006): Leached and Typical Cinemon Forest Soils, Endocalcic Luvisols and Haplic Luvisols, Vertic Luvisols, with some steep terrain poorly developed, shallow eroded soils (Cambisols and Leptosols) have been detected, Alluvial meadow soils (Mollic Fluvisols) are located in the lowest parts of the area near the river beds. The foothills of the valley slopes, the dry valley bottoms and the floodplains are occupied of deluvial materials. Alluvial deposits are deposited along the Razmetitsa River. The rest of the basin is composed of Paleogene soft sediments that are easily erodible (Nikova et al., 2013).

In the lands of Bobov Dol Town, close to Palatovo Village, a reclaimed tailing pond was built. All the coal used in the power plant are mainly high ash coals, which consequently leads to the release of significant quantities of ash during their combustion. According to Donchev et al. (2001) the plant generates about 900,000 tonnes of ash annually. Therefore, the degraded ecological status of the soil cover in the plant area could be due to the continued use of solid fuel for the production of electricity in the "Bobov dol" TPP. According to Kortensky & Zdravkov (2008), the ash of the coal used as a fuel in "Bobov dol" TPP is characterized by the relatively high concentration of SiO_2, Al_2O_3, TiO_2 and other metal oxides.

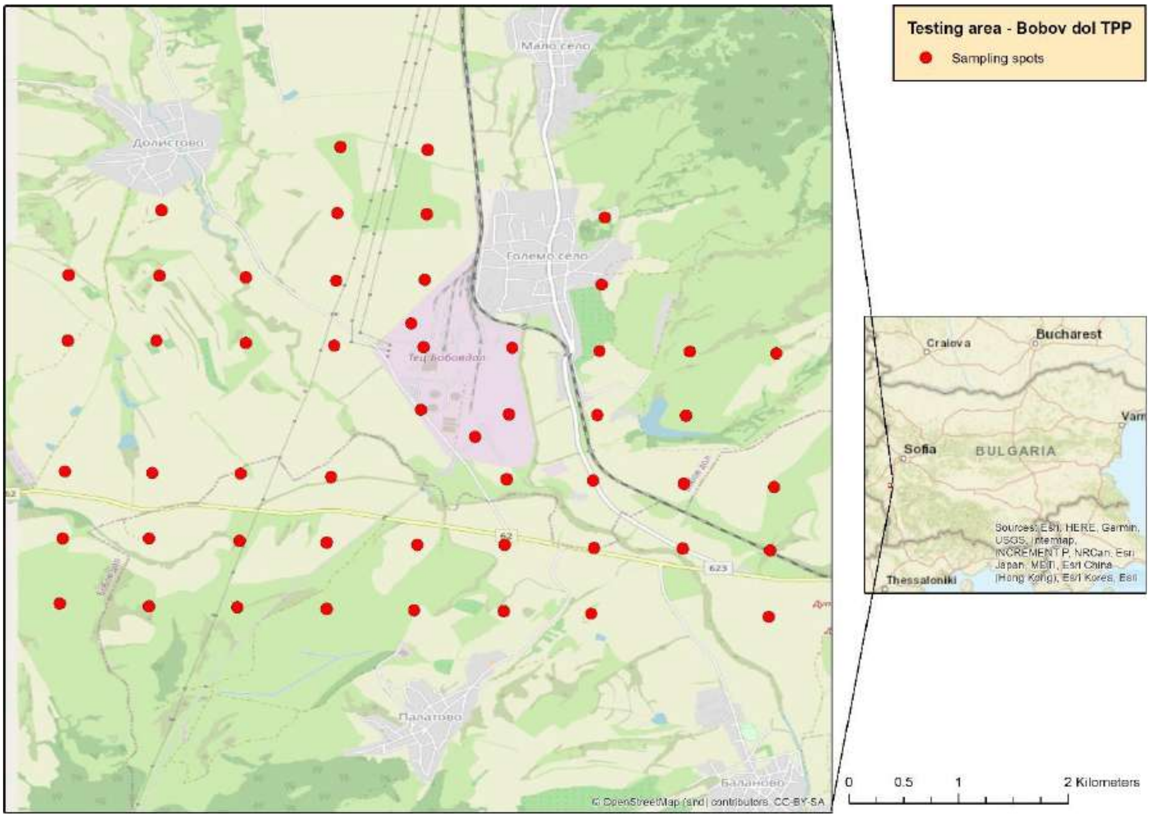

Fig. 1. Map of the "Bobov dol" TPP testing site with the sampling spots.

Field measurements for rapid assessment of the magnetic susceptibility

The assessment is implemented with Bartington MS3 susceptibility system which includes MS3 meter, MS2D surface scanning probe and MS2 probe handle. The MS2D loop is designed for rapid assessment of the magnetic susceptibility (Bartington Manual). In order to receive reliable scientific information the square shaped area of interest is divided by georeferenced grid that forms 700 x 700 m squares each grid cell was examined in a field campaign in order to select suitable spots (Fig.1) for assessment of the concentration of ferromagnetic materials in the top 60 to 80 mm of the land surface. At the suitable locations inside each grid cell, a visibly homogeneous, undisturbed squares with L=1 were selected. The geographical coordinates of each square is measured by portable GPS (WGS 84). Five independent soil magnetic susceptibility measurements were implemented at the vertices and in the center of a square. The mean value are assumed as the magnetic susceptibility of the testing square. For each measurement the grass was removed from the soil for better soil contact. The sensitivity of the Bartington MS3 susceptibility meter is 1x10^{-6} SI (Bartington Manual). The depth of penetration when is used with MS2D surface scanning loop is about 8–10 cm when the sensor is in contact with the surface; 50 % at 15 mm and 10 % at 60 mm from the surface (Bartington Manual). All measures were implemented when the sensor is in contact with the surface.

Sample collection for assessment of the frequency depended susceptibility

Sample extraction are implemented according the methodology of Yordanova et al. (2003; 2004; 2008) and Mokreva et al.

(2017). Material of about 50 g was taken from the top-soil at each corner and from the center of the square. It resulted in a pooled sample of roughly 250 g, considered as representative for the point. The material was kept and transported to the laboratory in closed plastic bags.

Measurements of Frequency-dependent susceptibility

Frequency-dependent susceptibility - χFD% was calculated and used as indicator for the relative contribution of the anthropogenic ferromagnetic minerals to the total magnetic fraction in the soil. The formula is $\chi FD\% = 100 \times (\chi LF - \chi HF) / \chi LF$ (Yordanova, 2008; Mokreva et al., 2017). Following the protocol developed by Yordanova et al. (2008) magnetic susceptibility was measured in the laboratory on homogenized material, sieved through 1 mm-mesh. The material was filled in 10 cm^3 plastic cylindrical containers and magnetic susceptibility is measured by Bartington MS3 meter equipped with dual-frequency sensor (MS2B) at two frequencies – 0.46 (KLF) and 4.6 kHz (KHF). The weight of the samples was measured and used for calculation of the mass-specific magnetic susceptibility (χLF). In order to achieve reliable results the signal and the weight of the empty containers was subtracted from the readings

Results and Discussion

Magnetic susceptibility values around "Bobov dol" TPP

During the field campaign a total of 60 measurements of the magnetic susceptibility were conducted around "Bobov dol" TPP. This measurements were evenly distributed around the power plan in accordance with the established grid network. The value of the magnetic susceptibility range between 1.9358×10^{-6} SI and 475.233×10^{-6} SI. The higher values of the magnetic susceptibility are observed around the "Bobov Dol" TPP and along the transport line that transport the waste from the burning process to the nearby dump site. Whereas background soils are dominated by thin superparamagnetic and single domain particles, large multi- domain particles prevail in urban soils (Veneva et al., 2004). Field measurements for assessment of the magnetic susceptibility are useful instrument for detecting the presents of ferromagnetic materials in the soils, however field sensors do not provide absolute values of mass-specific susceptibility, but a kind of relative volume-related data instead, because of that additional investigation is needed to obtain the frequency depended susceptibility and to distinguish if the high values of the magnetic susceptibility are due to presence of primary minerals that are connected with the process of pedo-genesis or because of the presents of industrial pollutant. The frequency dependent susceptibility in the research area varies between 1 and 17.78 percent. There is very good connection between the high values of the magnetic susceptibility and low values of the frequency dependent one (Fig.2). This fact confirm the conclusion that the majority of the magnetic minerals are with natural pedogenic origin and only in a small area around "Bobov dol" TPP the source of the magnetic minerals are the anthropogenic activities in the power plant.

Spatial distribution of the magnetic susceptibility values

The measurements of the magnetic susceptibility were evenly distributed around the "Bobov dol" TPP (Fig. 1). The value of the magnetic susceptibility range between 1.9358×10^{-6}SI and 475.233×10^{-6}SI. The higher values of the magnetic susceptibility are observed around the "Bobovdol" TPP and along the transport line that transport the waste from the burning process to the nearby dump site (Fig. 3). Because of the local wind patterns and the presents of the transport line the area with high magnetic susceptibility values has an elongated towards the South shape.

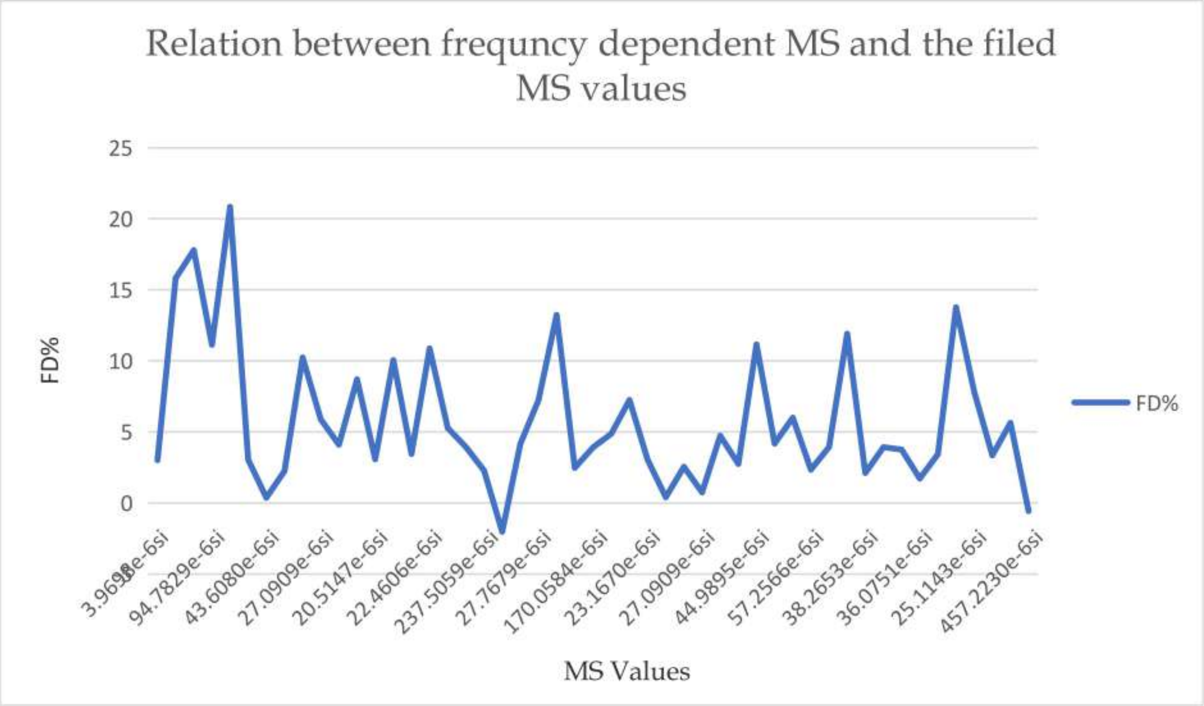

Fig. 2. Relation between frequency dependent MS and the field MS values around "Bobov dol" TPP.

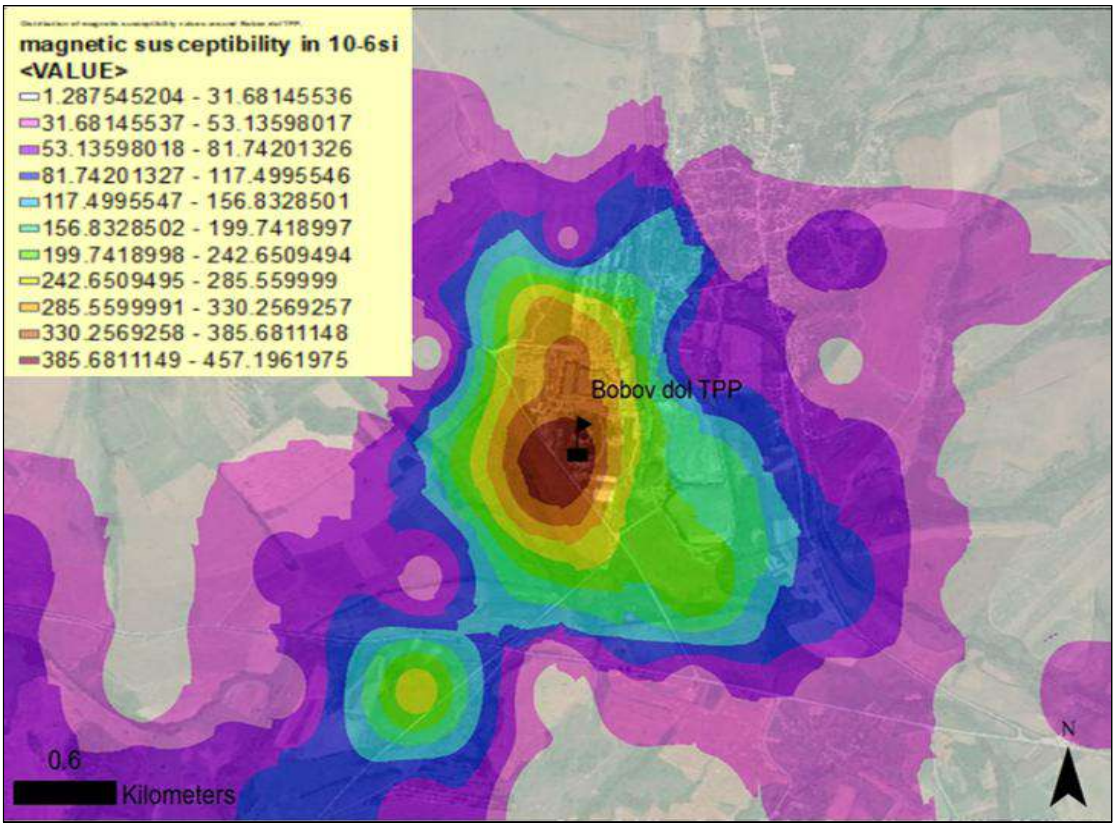

Fig. 3. Distribution of magnetic susceptibility values around "Bobov dol" TPP.

Heavy metal and magnetic susceptibility relation

The magnetic fractions of the urban topsoil are highly enriched in heavy metals. Many previous studies have reported the strong relationships between magnetic susceptibility and heavy metals in polluted soils (Hu et al., 2007; Chaparro et al., 2008; Bijaksana & Huliselan, 2010; El Bagdadi et al., 2011; Mokreva et al., 2017).

El Bagdadi et al. (2011) for example found a very good correlation between higher MS values and the presence of heavy metals in the urban soils close to roads and automobile repair depos in Beni Mellal City (Morocco). El Bagdadi et al. (2011) *in situ* magnetic susceptibility measurements closely to the road ranged from 67.9 to 577x 10^{-5} SI with median value 206 x 10^{-5} SI which is significantly higher than the magnetic susceptibility value of background soils ranging from 15 x 10^{-5} to 114 x 10^{-5} SI. El Bagdadi et al. (2011) also found that the content of Cd, Cu, Zn, Fe and Pb in the studied areas is significantly higher than those of background soils in Beni Mellal region with average concentrations of Cd, Cu, Zn, and Fe up to three times higher than the mean values of rural soils; but these of Pb is six time higher El Bagdadi et al. (2011).

One of the first regional magnetic maps was created in the Czech Republic for the area near the brown coal fired TPP (Kapicka et al., 1999). In the testing area, the *in situ* magnetic susceptibility ranged from 16 × 10^{-5} to 118 × 10^{-5} SI. Within the same area, the total content of cobalt varied from 1.5 to 12.6, that of arsenic from 0.5 to 6.8, and copper from 6 to 62 mg/kg (Kapicka et al., 1999). On the maps, contamination by these heavy elements is found to be consistent with magnetite contamination of soils (through magnetic susceptibility) and depends on the wind rose and distance from TPP (Kapicka et al., 1999).

The same conclusions were made by Mokreva et al. (2017) in a research about the degree of anthropogenic pollution of soils in the area of the Maritza-East coal mining and power plant complex located in central south Bulgaria. In this research Mokreva et al. (2017) used the magnetic susceptibility as proxy method to detect soil pollution with heavy metals and they reaffirm the conclusions made by Veneva et al., (2004) that a linear relationship has been obtained between the high magnetic susceptibility of fly ashes from different Bulgarian TPPs (including "Maritza iztok" TPP) and the content of Fe_2O_3. Furthermore Mokreva et al. (2017) also proved that the samples showing the highest MS values all have low frequency dependent susceptibility (Xfd%), generally below 4%. Which can be used as evidence that their magnetic mineralogy is dominated by magnetically strong, but coarse iron oxide particles (Mokreva et al., 2017). This reaffirm the fact that magnetic fly ash grains are generally coarse spherical particles (Veneva et al., 2004; Jordanova et al., 2006).

Similar results were obtained during the filed measurements of magnetic susceptibility (MS) values around "Bobov dol" TPP. The value of the magnetic susceptibility range between 1.9358x10^{-6} SI and 475.233x10^{-6} SI. The higher values of the magnetic susceptibility are observed around the "Bobov dol" TPP and along the transport line that transport the waste from the burning process to the nearby dump site (Fig. 3). Furthermore the higher MS values are very well correlated with the lower values of the frequency dependent susceptibility (Xfd%) of the same samples. The values of the χFD% of the samples with higher MS values are well below 4 % which according the Mokreva et al. (2017), Veneva et al. (2004) and Jordanova et al. (2006) can be used as evidence that higher MS values of the soil around "Bobov dol" TPP are due to the presence of fly ash grains which are generally coarse spherical particles with anthropogenic origin and are not related to the natural pedogenic processes. However the prevailing soil type in the proximity of the "Bobov dol" TPP and at the sampling spots with higher MS values is Vertic Luvisols. This soil type is in close relation with the Vertisols and according Jordanova (2016) this soil type is characterized by quite low magnetic susceptibility. This fact also confirms the conclusion that the higher MS values around "Bobov dol" TPP are due to the presents of magnetic particles with anthropogenic origin.

Conclusions

Although there are areas around "Bobov dol" TPP where the measured values of the magnetic susceptibility are high, generally the MS values show that the contamination of the soil around the power plant is limited to the very close perimeter of the power plant and one tight strip of land along the transport line that transport the waste products of burning process to the tail pond. As a whole except for the above mention territories the magnetic susceptibility values around the testing area are in line with the mean MS values for the presented in the test site soil types. These conclusions are reaffirm by the data obtain from the laboratory measurements of the frequency dependent susceptibility. Moreover these conclusions are also in line with the findings of the previous researches (Nikova & Tsolova, 2017) that regard the presence of trace elements in the soil cover around "Bobov dol" TPP area which are made by traditional methods. The magnetic susceptibility measurements are proved to be a reliable and cheap alternative of the traditional chemical methods for analyses of the anthropogenic pollution and the presents of heavy metals in the soils. However the reliability of the results and the effectiveness of the methodological approach need additional validation by the traditional chemical analyses for the presence of trace elements/heavy metals in the soils.

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Assessment of the Motivation for Participation in Citizen Science Initiatives for Invasive Alien Species in Bulgaria

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Abstract. The article presents an analysis of the motivation for participating in Citizen Science (CS) initiatives for invasive alien species (IAS) of professionals involved in the management and use of biological resources and general public. The motivation of the interviewees was assessed through a questionnaire containing questions about: Level of awareness of the existing opportunities for CS initiatives related to biodiversity in Bulgaria; Experience in initiatives for monitoring or registration of plants and animals; Reasons that would motivate or disappoint the respondents to participate in the CS; The most appropriate way for respondents to participate in CS initiatives, as well as the most appropriate form of raising their awareness of IAS. The results indicate that the main reasons for the failure to participate in CS initiatives was the lack of accessible information on the problems posed by the IAS and tools and initiatives for the IAS CS in Bulgaria. Possible measures and policies for involvement of various stakeholders in the IAS SC initiatives in Bulgaria are discussed.

Key words: data analysis, biodiversity, invasive alien species, citizen science.

Introduction

Good information on introduction routes and the negative impact of invasive alien species (IAS) is crucial for their early detection and rapid removal. IASs can significantly hamper efforts to conserve and restore biodiversity and cause significant damage to nature and the economy. The EU's 2030 Biodiversity Strategy aims to achieve the management of identified invasive alien species and reduce by 50% the number of endangered species on the Red List, which currently stands at 354 (EC, 2020). Citizen science (CS) can play an important role in involving a wide range of stakeholders in various initiatives that contribute to preventing the introduction and dissemination of IAS, but in Bulgaria it is underdeveloped. Insufficient volume of related information, as well as access in an understandable form through digital exchange channels is the reason for the poor knowledge of the state of CS for IAS. Improving this trend should be sought by providing and learning about activities aimed at increasing the experience of individual groups of participants in the monitoring of IAS. With the appropriate tools, a higher level of motivation can be achieved for their involvement in the process of registration of individuals from the flora and fauna. The level of awareness about the existing opportunities for CS initiatives for biodiversity in Bulgaria, the available experience in initiatives for registration/monitoring of plant and animal habitats, the main reasons that would motivate or disappoint the respondents to participate in CS. Together with the most appropriate way for respondents to participate in CS initiatives and the forms for raising their awareness about IAS, they are criteria whose assessment and analysis can be used to solve the problem of low interest and motivation of non-professionals in this field.

Approaches related to the assessment of motivation. It is essential here to study the importance of the relationship between assessment and motivation from the perspective of both biodiversity professionals and non-professionals. One way to do this is to combine a survey with separate data sets (Wu et al., 2014) to obtain the appropriate number of interrelated topics, with the subsequent analysis performed on the basis of an accepted standard thematic process of coding responses and inductive code generation. In order to increase motivation, it is necessary to provide activities related to the assignment of authentic and diverse evaluation tasks, providing effective feedback, as well as clarification of the set goals together with internal motivation. When the response rate is low, it is impossible to correctly interpret and use the responses of the respondents, which creates a number of problems (Hoel & Dahl, 2019). In such a case, it is necessary to assess where the motivation to participate breaks down, and where and how it makes sense to focus efforts on overcoming the problem. Regarding the behavior of those who rarely or never participate in online initiatives, in most cases the reasons can be found in their willingness to invest up to 5 minutes in the process. In terms of motivation, factors such as: willingness to participate at all; perception of autonomy and competence; significance; personal value, commitment to the participation of others; understanding the value of their own participation for the benefit of others. The approaches for assessing the motivation should also take into account the connections and in terms of knowledge of the subject related to the various activities and initiatives, which determines the effectiveness of the individual groups of participants in them. Dependence has been found according to which the internal motives for engagement with the problems of the surveyed professionals increase in a positive direction, while that of the others decreases over time (Tasgin & Tunc, 2018). Approaches such as the 'Effective Participation Scale' or the 'Internal External Motivation Scale' can be used to collect and process data from different surveys. The first is suitable for use at both the institutional and peripheral levels, allowing for an assessment of the impact of interventions to improve social participation - data allowing for comparison between participants, interventions and programs (Van Brakel et al., 2006). The scale allows data collection on participation and assessment of the impact of interventions to improve participation. In other approaches aimed at measuring motivation to participate, in particular in initiatives involving physical activity and leisure, in order to establish a comprehensive measure, a check on the internal coherence and validity of the criteria of the Motivation Scale for Physical Activity and Leisure (PALMS) is added, as well as testing individual models in confirmatory factor analysis (Roychowdhury, 2018). Despite the fact that the data from the surveys provide valuable information for both institutions and citizens, there is a worldwide trend of decreasing participants in them and, respectively, in the degree of answers given. Negative impact on future research may be caused by attempts to persuade or pressure for mandatory participation, which is counterproductive in the long run. An alternative approach to achieving higher levels of response may be to focus on the self-motivation of respondents in the surveys (Wenemark et al., 2011). Based on the use of the 'Self-Determination Theory', which provides a theoretical framework on how to stimulate inherent motivation, a redesign approach to relevant surveys can be used, measuring the effect in terms of response rate, participant satisfaction and data quality. Such an approach will also have an effect on the accumulation of positive experience by the respondents. Regardless of the approach used to assess motivation, it should be borne in mind that even a small dropout rate can affect bias and unrepresentative information (Ekholm et al., 2009).

CS for the IAS. CS plays an important role in engaging a wide range of stakeholders and the general public on the issue of IAS, whose biological invasions are an increasing challenge to individual countries in terms of measures needed to prevent environmental problems at local and national level. level. Any collection and analysis of environmental and/or biological data, including data quality control, undertaken by members of the general public, as individuals or as organized groups of citizens, with the guidance and/or assistance of scientists to solve environmental problems and/or community issues are treated as CS (Encarnação et al., 2021). In this process, the measures and initiatives taken need to be widely available and known to the general public in order to achieve a good level of its involvement. Good road awareness and the negative impact of IAS are crucial for early detection and prevention of their spread. In this sense, CS can be very successfully involved and greatly increase the effectiveness of the actions taken to prevent the introduction of IAS. When assessing the propensity to participate in such initiatives, surveys should take into account the extent to which they are applicable and possibly whether it is necessary to adapt methods and approaches to the studied ecosystems and species, along with the profile of respondents - motivation, level of engagement, social status, gender and age. In order to avoid obtaining fragmented data collected from various citizen initiatives related to the IAS, it is necessary to use an open and accessible platform for uploading data from CS sources (Cardoso et al., 2017), which reflects validated data. in an easy-to-use web service, in line with the EU's strategic open science priority. Once the data has been validated by the competent authorities of the Member States regarding the IAS Regulation, they will contribute to the early warning of the IAS by complementing the official monitoring systems, while raising citizens' awareness. The emergence of new, potentially invasive taxa, in addition to adverse effects on the environment, may directly affect some economic activities. Early detection and action is most cost-effective in terms of the resources needed to address the problem. For this reason, from a public point of view, CS's initiatives in this direction are extremely important. Volunteers providing information are a key part of such initiatives. In order for their participation to become more widespread, it is necessary to develop methods for quantifying their efforts and, accordingly, to assess their contribution to the detection of alien species (Pocock et al., 2017). In this way, there will be a tool for the objective assessment of the use of CS as a monitoring tool in the discovery of IAS deposits. Qualitative data collected by volunteers can greatly increase the range of the sample, fill gaps in the distribution of species, and improve habitat suitability patterns compared to professionally generated datasets used alone (Crall et al., 2015). The addition of voluntary data does not significantly change the performance of models generated by professional data sets, but changes the fitness surface generated by the models, making them more realistic. This helps CS to expand the scope of data collection and monitoring for IAS. The establishment of monitoring networks involving a wide range of professionals and non-professionals involved in the monitoring process, as well as the pooling of data, are key to the effectiveness and success of any initiatives related to improving knowledge about the dissemination of IAS.

In order to avoid obtaining fragmented data collected from various citizen initiatives related to the IAS, it is necessary to use an open and accessible platform for uploading data from CS sources (Cardoso et al., 2017), which reflects validated data. In an easy-to-use web service, in line with the EU's strategic open science priority. Once the data has been validated by the competent authorities of the Member States regarding the IAS Regulation, they will contribute to the early warning of the IAS by complementing the official monitoring systems, while raising citizens' awareness. The emergence of new, potentially invasive taxa, in addition to adverse effects on the environment, may directly affect some economic activities. Early detection and action is most cost-effective in terms of the resources needed to address the problem. For this reason, from a public point of view, CS's initiatives in this direction are extremely important. Volunteers providing information are a key part of such initiatives. In order for their participation to become more widespread, it is necessary to develop methods for quantifying their efforts and, accordingly, to assess their contribution to the detection of alien species (Pocock et al., 2017). In this way, there will be a tool for the objective assessment of the use of CS as a monitoring tool in the discovery of IAS deposits. Qualitative data collected by volunteers can greatly increase the range of the sample, fill gaps in the distribution of species, and improve habitat suitability patterns compared to professionally generated data sets used alone (Crall et al., 2015). The addition of voluntary data does not significantly change the performance of models generated by professional data sets, but changes the fitness surface generated by the models, making them more realistic. This helps CS to expand the scope of data collection and monitoring for IAS. The establishment of monitoring networks involving a wide range of professionals and non-professionals involved in the monitoring process, as well as the pooling of data, are key to the effectiveness and success of any initiatives related to improving knowledge about the dissemination of IAS.

Approach to assessing the level of awareness regarding CS for IAS in Bulgaria. In Bulgaria, in recent years, studies on the motivation for public involvement in various environmental initiatives have been conducted by Bancheva (2015), Bancheva-Preslavska & Bezlova (2018), Bancheva-Preslavska & Dallmer (2020) and others. Very little is known about the state of CS for IAS in Bulgaria. COST action CA17122 'Increasing the understanding of alien species through citizen science', addresses multidisciplinary research in connection with the development and implementation of CS. In implementation of this COST action, the project 'State and prospects of citizen science for invasive alien species in Bulgaria' is being implemented, funded by the Research Fund at the Ministry of Education and Science. The project has developed a specialized web-platform for registering information about the IAS and its sharing (https://invazivnividove.ltu.bg/) with a survey on public attitudes to IAS-related initiatives.

The main goal of this study is to assess the motivation of the general public in Bulgaria to participate in CS initiatives for IAS.

Material and Methods

Questionnaire surveys were conducted to assess the existing CS initiatives related to IAS, the level of IAS awareness of different groups of society and their motivation to participate in CS were assessed through a questionnaire survey asking respondents to make judgments about their own level of knowledge about: the level of awareness of about existing IAS CS initiatives in Bulgaria; the experience in monitoring or registration of plants and animals; the motivation and appropriate ways to participate in CS; the most appropriate approach for IAS awareness raising.

Two groups of respondents were questioned: professionals (with educational background in biology or with professional activities related to the bio resources) and non-professionals (not connected with bio resources).

Results and Discussion

The survey included 616 respondents, including 259 of them professionally dealing with biological objects, and the rest are representatives of the general public - non-professionals. The distribution by age and sex in the two groups is identical, except that among professionals the group of 15-20 year olds is missing (Fig. 1). The share (over 93%) of the respondents who are professionally engaged in biological sites and are in the active working age 21-60 years is significant, which makes the results of the survey representative in terms of the implementation of European policies related to biodiversity conservation in Bulgaria. Fulfilling one of the specific objectives of the survey, in terms of citizen society participation in citizen science initiatives, over 82% of non-professionals surveyed are under the age of 40, with more than 50% of them being young people under the age of 20. The knowledge, motivation and activity of young people is essential for solving modern environmental problems. The relevance of the study is confirmed by the result that respectively for about 58% of professionals and 42% of non-professionals, working with biological objects is related to their hobby. The main share is of tourists, naturalists, wildlife photographers and others. About 1/3 of the surveyed professionals have noted that they fall into the group of students, respectively master, doctor or bachelor 3, 4 course. The share of non-professionals who are students or bachelors from the initial courses of study is significant, these are almost 2/3 of the respondents.

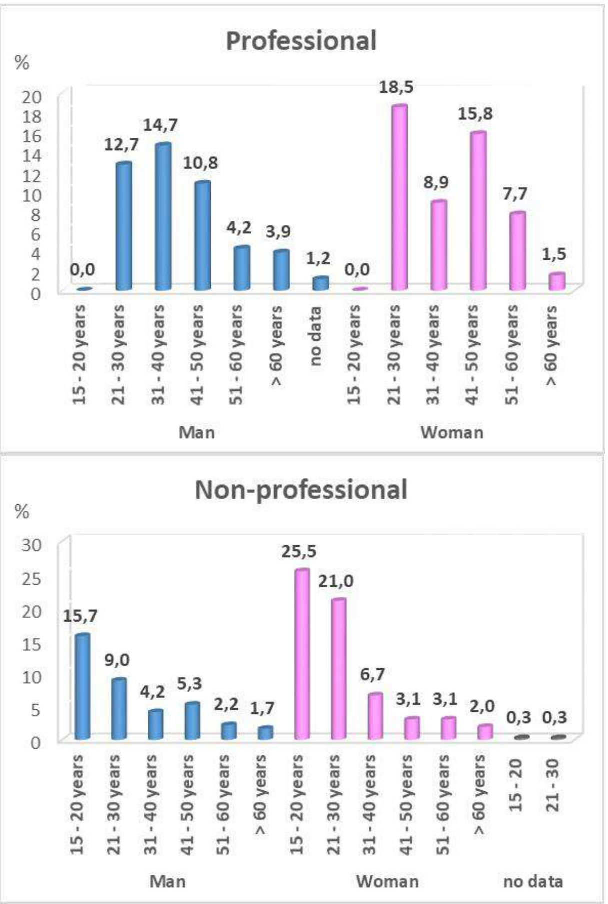

Fig. 1. Distribution of respondents by gender, age and professional status

For the majority of respondents (professionals and non-professionals) awareness of the importance of IAS is from medium to unsatisfactory level (Fig. 2). As expected, the share of positive responses among professionals is higher. The only exceptions are two issues concerning the distribution of IAS in their natural habitats and their socio-economic impact. There are no significant differences in the answers to these questions between professionals and non-professionals.

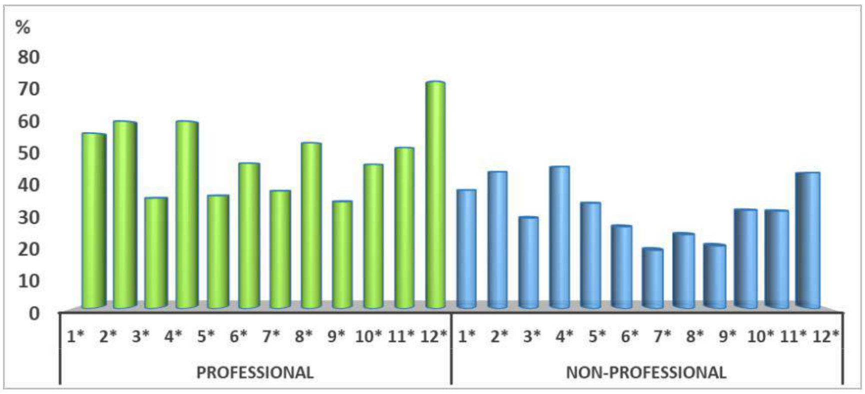

Fig. 2. Awareness of Invasive Alien Species (IAS).

Legend:
1*. Do you think that the term "invasive species" should refer to organisms that have a negative impact, regardless of whether they are expanding their range?
2*. Do you think that the term "invasive species" should refer to organisms that expand their range, regardless of their impact?
3*. Do you think that some species can be considered "invasive" in the places of their natural distribution?
4*. Do you consider invasive species to be one of the main threats to biodiversity?
5*. Do you think that invasive species can have a positive socioeconomic impact?
6*. Have you encountered information about invasive species in various information sources?
7*. Did you know that a Regulation on invasive alien species has been adopted in the European Union (Regulation No. 1143/2014)?
8*. Can you name one or more invasive alien species relevant to the European Union?
9*. Would you name a pet that is an invasive species of importance to the European Union?
10*. Can you give an example of the negative impact of invasive species?
11*. Can you indicate a way/way for introduction (transfer) of alien species in Bulgaria?
12*. Can you name a structure/organization that you think is responsible for preventing the penetration and spread, as well as the control of invasive alien species in Bulgaria?

More than 73% of the surveyed professionals indicate a structure/organization that is responsible for preventing the penetration, dissemination and control of IAS in Bulgaria. In the general public, the percentage is approximately twice as low. More than 60% of professionals believe that the term 'invasive species' should refer to organisms that expand their range, regardless of their impact, and that IASs are one of the main threats to biodiversity. On the other hand, for non-professionals this share is about 15 points lower and is in the range between 44-46%.

Just over 50% of professionals can indicate one or more IAS relevant to the EU for non-professionals, this percentage is about 30 points lower. This is a clear sign of the low awareness of both the general public and the experts regarding the IAS in Bulgaria. Unfortunately, just over 46% of the surveyed professionals have found information about invasive species in various information sources, this share is more than 20 points lower among non-professionals. The results show the insignificant awareness of both experts and the general public regarding the important issue of IAS in Bulgaria. The lack of well-structured information campaigns aimed at both experts and the general public is clearly evident in the results obtained. Despite the fact that IAS have a strong media coverage and this issue is not attractive and does not represent journalistic and media interest, their role in terms of biodiversity has been proven. This gives us reason to look for errors in terms of awareness on this issue, both in terms of professionals and the general public. The survey clearly outlined the gaps and white spaces in this direction. Less than 40% of professionals and respectively less than 20% of non-professionals are familiar with the adopted regulation regarding IAS in the EU, this share is extremely small. Compared to other European countries, and here comes the role of CS, through the transfer in an accessible way of knowledge and competencies from experts to the general public. Despite the lower awareness of both experts and non-professionals regarding the IAS. With regard to the question of who we need to inform, if we notice a mass multiplication of plant and animal species in nature. Both groups of respondents put in the first place the structures of the Ministry of Environment and Water, environmental NGOs, the structures of the Ministry of Agriculture, Food and Forestry, including the Executive Forest Agency and others. etc. In this respect, there are no significant differences in the answers of both professionals and non-professionals. Both groups of respondents know the roads and are clear about the principle of alerting the competent authorities in the country in case of environmental problems.

Regarding the next section of the survey, concerning the level of awareness of the existing IAS initiatives in the UK, no significant differences in responses were observed (Fig. 3). An exception, according to experts, according to 2/3, of which the initiatives to promote CS, means conducting activities to promote science among the general public. In contrast, among non-professionals this share is insignificant, less than 15% of respondents answer positively.

Logically, in terms of experience in the observation or registration of plants and animals, experts and professionals answer a higher percentage of positive questions. It is noteworthy, however, that the general public, despite the fact that it has not participated in such initiatives and rarely receives information about such events and actions, readily responds that it would participate in the implementation of such activities (Fig. 4).

A significant part of the study is the issues related to motivation and appropriate ways of participation of both experts and the general public in initiatives related to CS and in particular IAS. Despite the fluctuations in the answers of the two groups of respondents, Factors that would motivate them to participate in CS-related initiatives are similar (Fig. 5) In the first place, both groups outline the care for nature, followed by the commitment to contribute to nature conservation, etc.

The two leading reasons that limit the participation of respondents in CS-related initiatives are, respectively, the lack of time and the lack of sufficient information about similar opportunities and actions related to citizen initiatives for IAS (Fig. 6).

The importance of scientific institutions and the scientific community for initiating activities related to CS is clearly outlined in the answer to the question related to the most appropriate and convenient way to participate in such activities (Fig. 7). More than half of the respondents from both groups outline the leading role of research institutions in organizing CS-related initiatives.

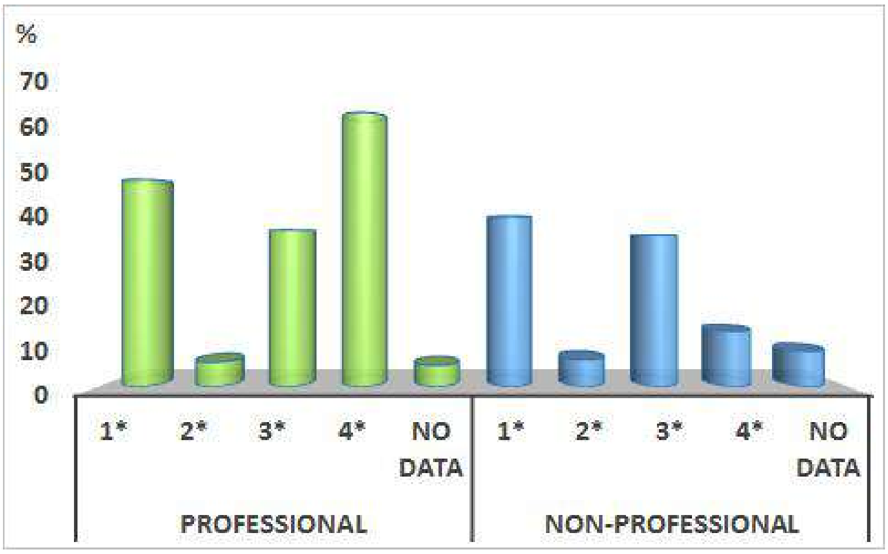

Fig. 3. Answers to the question "What do you think the term" citizen science "means?".

Legend:
1*. Transfer of knowledge from professionals to non-professionals (trainings, information campaigns).
2*. Sharing information on Facebook forums.
3*. Voluntary collection of information and its provision for subsequent analysis and use.
4*. Initiatives to promote science among the general public.

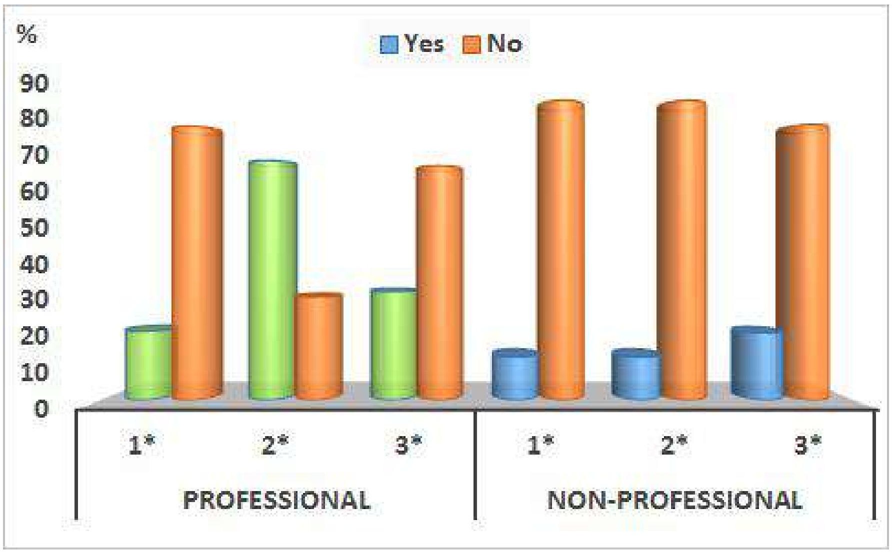

Fig. 4. Level of awareness of existing IAS CS initiatives in Bulgaria and experience of IAS monitoring or registration.

Legend:
1*. Do you have information about a project/website or a CS initiative related to biodiversity?
2*. Have you participated independently or in an organized manner in the registration/monitoring of plant and animal habitats?"
3*. Have you been involved in reporting (and sending data) on plant and animal habitats?

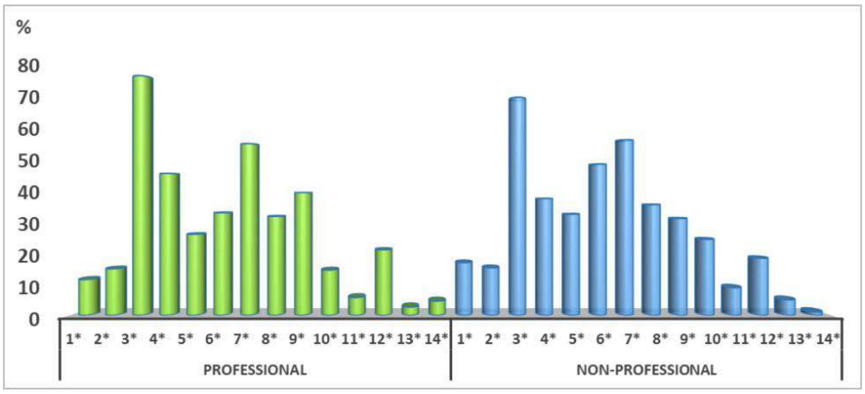

Fig. 5. Answers to the question "Indicate what would motivate you to participate in citizen science initiatives".

Legend:
1*. Loss prevention for my business.
2*. Prevent damage to my hobby.
3*. Caring for nature.
4*. Engaging the younger generation with the topic of threats to biodiversity.
5*. To be connected with nature.
6*. To learn more about nature.
7*. To contribute to nature conservation.
8*. To spend more time in nature.
9*. To contribute to science.
10*. To notice as many species as possible.
11*. It gives meaning to my life.
12*. To work in a team with other nature lovers.
13*. Others.
14*. No answer.

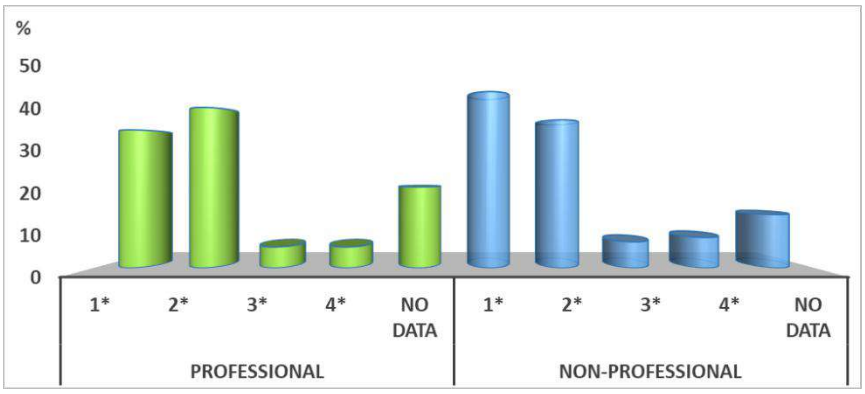

Fig. 6. Answers to the question "Give a reason why you would not participate in CS initiatives".

Legend:
1*. I have no time
2*. I do not have enough information about such a possibility
3*. I do not want to share information
4*. Other
No answer

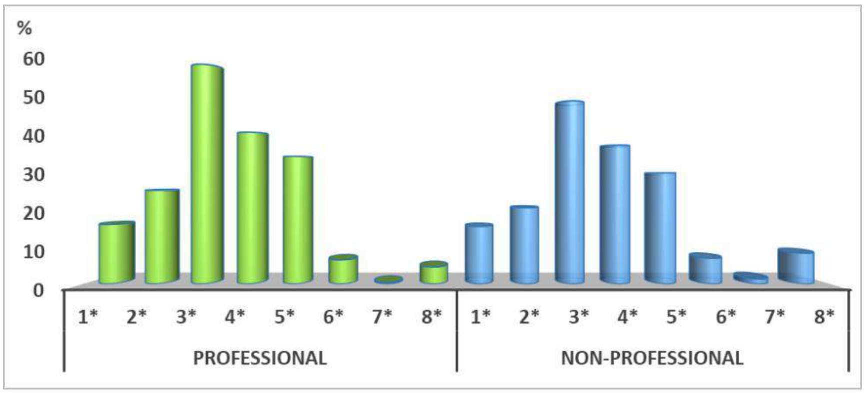

Fig. 7. Answers to the question "Indicate the most appropriate/convenient way for you to participate in citizen science initiatives".

Legend:
1*. Independently.
2*. Organized by NGOs.
3*. Organized by a scientific institution.
4*. Using mobile applications for smartphones.
5*. Submitting data to internet forums/sites.
6*. Filling in paper forms.
7*. Other…
8*. No answer.

The study focuses on young people, on whom the solution of environmental problems will depend in the future, including as a result of the IAS. Respondents say that the main motive for their participation in CS-related initiatives would be their personal contribution to environmental protection, as well as the opportunity to spend time in nature. The "new reality" that has emerged in recent years has contributed to the reduction of group initiatives, so participation in many projects is done independently, whether in online projects or environmental monitoring. This combined with a lack of awareness to deter potential participants who want to meet like-minded people and join the CS volunteer community.

Conclusions

The main reasons for the low participation of citizens in CS initiatives are the lack of available information on the negative impact on biodiversity of the IAS, as well as the lack of tools and initiatives on these issues, which are well promoted in Bulgaria. For most of the questions, the survey showed a similar attitude and motivation among both professionals and non-professionals.

Acknowledgments

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Grassland Habitats of Community Importance on the Territory of Godech Municipality, West Bulgaria

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Abstract. The investigated territory is located in the Western part of Bulgaria. The aim of the presented research is to reveal the diversity of grassland habitats in Godech Municipality. Following the Braun-Blanquet approach, 418 relevés were collected during the 2019-2020 field seasons and the grassland habitats were verified at 3422 field points, as well. The latter represent terrain samplings that prove or disprove preliminary habitat interpretation. The following habitat types (eight in total): 6210, 6230, 62A0, 62D0, 6410, 6430, 6510 and 6520 were found on field, according to Directive 92/43/EEC. The main threats are modification of the habitats into shrublands and the invasion of plants species. Some of the habitats are being ploughed and turned into agricultural areas. The results obtained may be used as a basis for a territorial expansion of the study in the neighboring municipalities.

Key words: vegetation, NATURA 2000, GIS, mapping.

Introduction

Habitat identification is a matter of a wide present interest among scientists and environmentalists. The conservation of natural habitats of Community importance is an essential part of Council Directive 92/43/EEC of the European Union.

According to the official national sources (EEA, 2021) over 30% of the investigated area is covered by habitats, which are a part of two Sites of Community Interest: the smaller one is Dragoman (BG0000322), located to the south and the bigger one is Western Balkan Range and Forebalkan (BG0001040). The authors expect that this percent will prove out to be even bigger.

Grassland vegetation in different areas of Godech Municipality was investigated by a number of authors. Iordanov (1924) focuses his study on the phytogeography of Western Stara Planina Mountain, while Tashev et al. (2010) contributed with a study, regarding the habitat diversity in the mountain. Pedashenko et al. (2010) studied the local occurrence of *Artemisia*

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chamaemelifolia Vill. Velev et al. (2011 a, b) published two research papers focused on the investigation of *Cynosurus cristatus* grasslands and alliance *Arrhenatherion elatioris* in West Bulgaria. Vassilev et al. (2011) studied the effect of land abandonment on the vegetation of upland semi-natural grasslands. Vassilev et al. (2012a) studied the class *Festuco-Brometea* with an emphasis on alliance *Cirsio-Brachypodion pinnati*. Velev & Vassilev (2014) focused on the management regimes within syntaxa of semi-natural grasslands. Several authors contributed with papers focused on Ponor Special Protection Area (Natura 2000). Among them are Dimitrov & Petrova (2014) who investigated forest habitats, Pedashenko & Vassilev (2014) studied the flora in the same area, Tzonev et al. (2014) did a research on scrub, grassland and rocky habitats and Vassilev et al. (2014) studied grassland vegetation.

The aim of the present study is a complete investigation and mapping of grassland habitats of Community importance on the territory of Godech Municipality, West Bulgaria.

Materials and Methods

Study area

Godech Municipality covers around 375 km^2. The two Sites of Community Interest cover ca. 76% of the municipality territory. Over 50% of the territory has an elevation between 1000 and 1600 m, while territories with an elevation between 600 and 1000 m account for 45.78 %. Almost 48% of the area is build up by the rocks of Iskar Carbonate Group, represented mainly by limestones, dolomitic limestones, dolomites and less shales, sandstones, siltstones. Together with the West Balkan Carbonate Group (7.52%), which covers the northern slopes of Chepan Mountain, they are a basis for karstification. The Petrohan Terrigenous Group (15.38%) includes breccia, conglomerates and sandstones and it can be found along the famous road. Quaternary alluvial deposits mainly cover the floodplains and the river terraces along the municipality (Bonchev, 1910).

The main river in the municipality is Nishava and its main tributaries are Glutnitsa and Arakul Rivers. The main soil types are *rendzic Leptosols, LPk* and *eutric Cambisols, CMe* (Ninov, 2002).

Habitat investigation and mapping

The habitat and vegetation sampling was conducted during the 2019 and 2020 field seasons, following the Braun-Blanquet approach (Braun-Blanquet, 1965; Westhoff & van der Maarel, 1973). A total of 418 relevés were collected. They were contributed to the Balkan Vegetation Database (Vassilev et al., 2020) and Balkan Dry Grassland Database (Vassilev et al., 2012b). All relevés were plotted in the homogenous areas of grassland communities and were subsequently assigned to relevant habitat types. Habitat types were determined according to Directive 92/43/EEC (Interpretation Manual of European Union Habitats, 2013; Kavrakova et al., 2009) and subsequently related to the revised version of the EUNIS system (Chytrý et al., 2020) and to the Palearctic habitat classification (Devillers & Devillers-Terschuren, 1996). Additionally, data was collected from 3422 field verification points, evenly set on the whole territory of the municipality. All the data collected in the field was applied in order to build a precise habitat map of the area.

Mapping was done using the ArcGIS 10.0 software package (ESRI 2011). Spatial data was collected in the field using GPS devise Juno BS by Trimble and was later laid over the most recent orthophoto images available. The habitat map was created by the help of the "Intersect" tool by combining the layers, containing forestry data from Forestry Management Plans, as well as data about agricultural areas and habitat data from habitat mapping of NATURA 2000 in Bulgaria. Later, the "Cut polygon" tool was used in order to modify polygon geometry. All the polygons were outlined manually using all the field collected data as well as the orthophoto images. The habitat map was elaborated in scale 1:5000.

Results

Habitat diversity

As a result of the investigation eight grassland habitat types were established: 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (* important orchid sites), 6230 * Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas, in Continental Europe), 62A0 Eastern sub-mediteranean dry grasslands (*Scorzoneratalia villosae*), 62D0 Oro-Moesian acidophilous grasslands, 6410 *Molinia* meadows on calcareous, peaty or clayey-siltladen soils (*Molinion caeruleae*), 6430 Hydrophilous tall herb fringe communities of plains and the montane to alpine levels, 6510 Lowland hay meadows (*Alopecurus pratensis, Sanguisorba officinalis*), and 6520 Mountain hay meadows included in Directive 92/43/EEC. They cover a total of 121.31 km² or 32.3% of the whole territory of Godech municipality (Fig. 1).

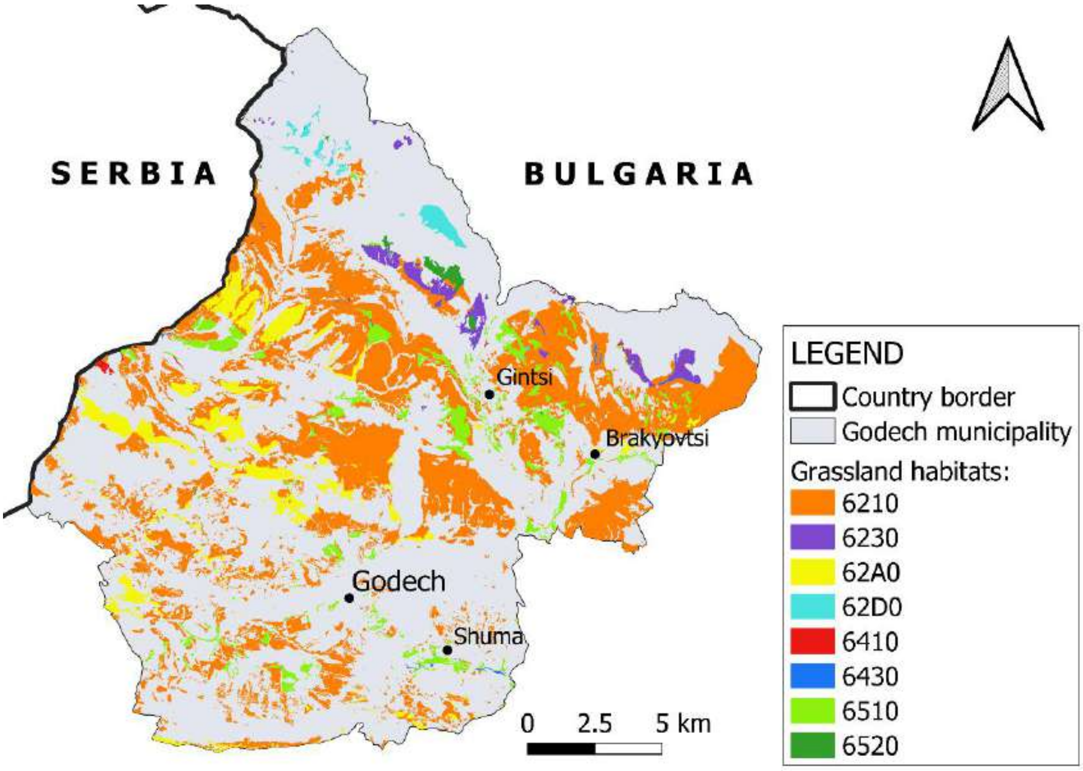

Fig. 1. Habitat map of Godech Municipality.

6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) (important orchid sites)*

EUNIS: E1.222 Moesio-Carpathian steppes, E1.234 Moesio-Carpathian meadow steppes; PAL. CLASS.: 34.3161 Moesio-Carpathian steppes, 34.3163 Moesio-Carpathian meadow-steppes.

Abiotic characteristic: It was the most widespread habitat type, covered an area of 92.7 km^2 and represented by 1644 polygons. It was equally distributed in the range between 600 and 1600 m a.s.l. Stands covered slopes with different exposition. Terrains were predominantly flat or slightly inclined (up to 20°) and rarely steep (up to

40-50°). Soils were shallow to moderately deep. The bedrock types were diverse – sediments, silicates and metamorphic rocks. The vegetation was used for haymaking and as pastures.

Vegetation structure: The phytocoenoses of this habitat type had semi-open to closed horizontal structure with total cover of 90-100%. The vertical vegetation structure consisted of two well developed layers. The first layer reached a height of 40-120 cm and was composed of grasses such as *Chrysopogon gryllus, Festuca dalmatica, F. pseudodalmatica, Dichanthium ischaemum, Stipa capillata, S. eriocaulis, Danthonia alpina, Poa angustifolia, Agrostis capillaris, Brachypodium pinnatum, Briza media* as well as some herbs such as *Filipendula vulgaris, Agrimonia eupatoria.* The second layer was more species-rich and included all other species of the phytocoenoses. Species of higher cover and abundance were *Trifolium repens, T. alpestre, Leontodon hispidus, Dorycnium herbaceum, Lotus corniculatus, Astragalus onobrychis, Medicago falcata, Eryngium campestre,* etc. In some stands, rich of lichens and bryophytes, a third layer of cryptogams was formed above the ground.

This habitat type included two ecologically different grassland vegetation types – xerophilic grasslands of *Festucion valesiacae* alliance and xero-mesic grasslands of *Cirsio-Brachypodion pinnati* and *Chrysopogono-Danthonion* alliances (Vassilev, 2012; Vassilev et al., 2012a). Xerophilic grasslands were dominated by tussock-forming grasses such as *Stipa capillata, S. eriocaulis, Festuca dalmatica, Dichanthium ischaemum, Poa angustifolia* and *Chrysopogon gryllus.* Other species of higher cover and abundance were *Medicago falcata, Eryngium campestre, Coronilla varia, Astragalus onobrychis, Ononis arvensis.* They were widespread on the whole territory of the municipality at lower altitudes (up to 1000-1200 m) within the oak and beech forest belts. This vegetation was adjacent to or formed mosaics (complexes) with phytocoenoses of habitat 62A0 Eastern sub-Mediteranean dry grasslands and alliance *Saturejion montanae* (northern slopes of Chepan Mts).

The xero-mesic grasslands were found generally at higher altitudes (800-1500 m) or at lower altitudes but on slopes with northern or western expositions. Sometimes they were found as strip vegetation in the ecotone zone, beside or within forests, where remnants of abandoned meadows were present. Most of this vegetation belonged to alliance *Chrysopogono-Danthonion* and association *Agrostio-Chrysopogonetum,* where the bedrock type was silicate. Species of higher cover and abundance were *Festuca pseudodalmatica, Agrostis capillaris, Briza media, Danthonia alpina, Filipendula vulgaris, Anthoxanthum odoratum, Dorycnium herbaceum.* This vegetation had transitional species composition with the communities of alliance *Cynosurion cristati* of class *Molinio-Arrhenatheretea* and share some common species such as *Leontodon hispidus, Lotus corniculatus, Trifolium repens, T. pratense, Cynosurus cristatus, Leucanthemum vulgare, Rhinanthus rumelicus, R. angustifolius,* etc. On the other hand, communities of alliance *Cirsio-Brachypodion pinnati* were restricted to calcareous bedrock with soils shallow to moderately-deep. This vegetation type had widest distribution on the territory of Ponor Mt, where it was presented by two associations – *Hieracio pilosello-Festucetum dalmaticae* Vassilev et al. 2012 and *Galio lovcense-Artemisietum chamaemelifoliae* 2010 (Pedashenko et al., 2010; Vassilev et al., 2012a). The dominant species was *Brachypodium pinnatum.* Other species of higher cover and abundance were *Sesleria latifolia, Thymus longicaulis, Briza media, Artemisia chamaemelifolia.*

Threats: Invasion of shrubs and trees, natural succession of pastures and meadows to shrub and forest vegetation, abandonment of pastures and meadows, ruderalization.

62A0 Eastern sub-Mediteranean dry grasslands (Scorzoneratalia villosae)

EUNIS: R1A1 Helleno-Balkanic *Satureja montana* steppes; PAL. CLASS.: 34.311 Helleno-Balkanic savory steppes.

Abiotic characteristic: This habitat type was presented by 213 polygons and covered 13.9 km^2. It was found predominantly on eastern and southern facing slopes of Vidlich hill, Ponor Mts and Mala planina Mts, and northern slopes of Chepun Mt. up to 1000 m a.s.l. Terrains were flat to moderately inclined – up to 25-30°. The bedrock type was of carbonate and dolomite rocks. Soils were predominantly shallow and rarer moderately deep, dry with rough microreleif and calcareous outcrops. The vegetation was used for grazing only at some localities, situated close to the settlements. Most of the distant localities of this habitat type were abandoned and not managed any longer.

Vegetation structure: Species-rich communities with semi-open horizontal structure and total cover between 75-95%. The cover of rocky outcrops was on average 10-15%. Mosses and lichens were well presented in most phytocoenoses and had a cover in the range 8-20%. In the species composition, no prominent dominant was found. Subdominant species were *Festuca dalmatica, Artemisia alba, Satureja montana* subsp. *kitaibelii, Potentilla cinerea, Dichanthium ischaemum, Thymus callieri, Stipa capillata.* Some species of conservation value were also found, such as *Chamaecytisus jankae, Ch. calcareous, Himantoglossum jankae, Hypericum rumeliacum, Astragalus wilmottianus, Achillea clypeolata, Tragopogon balcanicus, Edraianthus serbicus, Festuca stojanovii,* etc. Many obligate calciphiles (such as *Anthyllis montana, A. vulneraria, Inula oculus-christi, Koeleria nitidula, Rhodax canus, Sesleria latifolia, Trigonella gladiata*) and facultative calciphiles (such as *Chamaecytisus jankae, Hypericum rumeliacum, Achillea clypeolata, Aethionema saxatile, Amygdalus nana, Asyneuma anthericoides, Corothamnus procumbens, Hyacintella leucophaeae, Thymus striatus,* etc.) were also distributed (Velchev 1998). The vegetation of this habitat type was referred to alliance *Saturejon montanae* and class *Festuco-Brometea.*

Threats: Invasion of shrubs and trees, natural succession to shrub and forest vegetation, abandonment of pastures, development of quarry activities.

*6230 * Species-rich Nardus grasslands, on siliceous substrates in mountain areas*

EUNIS: R4318 Oro-Moesian mat-grass swards; PAL. CLASS.: 36.318 Oro-Moesian mat-grass swards.

Abiotic characteristic: This habitat type was found in the central and northern parts of the municipality (the northern parts of Vidlich Ridge, Berkovska and Ponor Mountains) in the oak and beach belts. It was represented by 49 polygons, mainly distributed in Berkovska Mt. (the southern slopes of Kom Peak and Malak Kom Peak).

In the valley between Vidlich Ridge and Berkovska Mt., the habitat was found in some small areas only. The habitat occurred on flat or slightly inclined terrains up to 10-15°. Slope exposition varied. The most frequent rock types were silicates and limestones. These grasslands were used as pastures with this intensity significantly declined in the past three decades.

Vegetation structure: Moderately species-rich communities with closed horizontal structure and total cover 95-100%. The only dominant species was *Nardus stricta.* Other species of higher cover and abundance were *Agrostis capillaris, Festuca rubra* agg., *F. airoides, Lerchenfeldia flexuosa, Chamaespartium sagittale, Thymus longicaulis.* This vegetation was related to the alliance *Violion caninae* of class *Nardetea strictae* and *Potentillo ternatae-Nardion strictae* of class *Juncetea trifidi.* The *Violion caninae* alliance was found at lower altitudes (800-1300 m a.s.l) in the beech forest belt. The stands of

Potentillo ternatae-Nardion strictae alliance (in Ponor Mt, Vidlich hill) included some subalpine species and were found at higher altitudes (1500-2016 m a.s.l.) on the territory of Berkovska Mt. As a result of pasture abandonment, the cover of *Nardus strcta* in the communities decreased whereas the cover of *Agrostis capillaris, Lerchenfeldia flexuosa, Chamaespartium sagittale, Festuca rubra* agg. increased.

Threats: Pasture abandonment and subsequent shrub encroachment.

6410 Molinia meadows on calcareous, peaty or clayey-siltladen soils (Molinion caeruleae)

EUNIS: R371 *Molinia caerulea* meadows and related communities; PAL. CLASS.: 37.31 Purple moorgrass meadows and related communities.

Abiotic characteristic: This habitat type had local distribution in the central and northern parts of Godech municipality (Godech lowland, Berkovska Mt. and Ponor Mt.). It was found between 600 and 1600 m a.s.l., covered 0.34 km^2 and was represented by 23 polygons. All the habitat polygons had small sizes and were located along river tributaries and areas with high water levels. Frequently, this habitat type was found next to habitats 6430 and 6510. Terrains were flat and soils deep, humid and clayed. Soil moisture was variable during the year and frequently dried out in July-September period. The bedrock type was predominantly silicate and rarer calcareous (Ponor Mt.). Most of these grasslands were used as pastures, which lead to eutrophication, trampling, loss of vegetation and degradation of the plant communities. At some localities, grasslands were also used for haymaking.

Vegetation structure: Moderately-species rich communities with closed horizontal structure and total cover 90-100%. Dominant species was *Molinia caerulea* with cover 60-90%. Mesic species predominated in the species composition, such as *Agrostis capillaris, Festuca pratensis, F. rubra* agg, *Serratula tinctoria, Bistorta major, Sanguisorba officinalis, Cynosurus cristatus*. This habitat frequently formed complexes with habitat 7140 or were neighboring to the latter or 6510. The vegetation belongs to alliance *Molinion caeruleae* W. Koch 1926, order *Molinietalia caeruleae* W. Koch 1926 and class *Molinio-Arrhenatheretea* Tüxen 1937.

Threats: Intensive grazing, over trampling, ruderalization.

6430 Hydrophilous tall herb fringe communities of plains and the montane to alpine levels

EUNIS: R551 Screens or veils of perennial tall herbs lining watercourses, R553 Shady woodland edge fringes, R5672 Moesian tall herb communities; PAL. CLASS.: 37.71 Watercourse veils, 37.72 Shady woodland edge fringes, 37.872 Moesian tall herb communities.

Abiotic characteristic: This habitat type had a limited distribution from 600 to 1600 m a.s.l., covered an area of 0.31 km^2 and was represented by 40 polygons. Terrains were flat to moderately inclined up to 10°. It was found in floodplain areas on rather wet and nutrient-rich soils. The latter were shallow to moderately deep. The ground water usually decreased in dry summer periods in the central part of the municipality. In some stands of subtype 37.8 along river banks they are rich of gravels and stones. Bedrock type was predominantly silicate. Some stands were used as pastures.

Vegetation structure: Species-poor communities with closed horizontal structure and total cover of 90-100%. The maximum of vegetation development was in the second half of summer. The strong shade effect led to low presence of bryophytes and the litter formed separate layer with cover about 70-90%. This habitat was presented by two subtypes: 37.7 Wet and nitrophilous tall herb edge communities, along water courses and woodland borders related to the *Glechometalia hederaceae* and the *Convolvuletalia sepium* orders (*Senecion fluviatilis, Aegopodion podagrariae, Convolvulion sepium, Filipendulion*) and 37.8 Hygrophilous perennial tall herb communities of montane to alpine levels of the *Betulo-Adenostyletea* class. The phytocoenoses of subtype 37.7 were dominated by broad-leaved tall forbs *Filipendula ulmaria* as well as *Mentha longifolia* and *Aegopodium podagraria*. In the species composition, some hygrophyte species were found (e.g. *Carex riparia, Lythrum salicaria, Epilobium hirsutum, Mentha longifolia, Angelica sylvestris*) and mesic ones (e.g. *Festuca pratensis, Agrostis stolonifera, Deschampsia caespitosa, Molinia caerulea*) also. Subtype 37.8 was dominated by *Petasites hybridus, Trollius europaeus* and *Cirsium appendiculatum*. The vegetation of this habitat subtype was related to alliances *Cirsion appendiculati* Horvat et al. 1937, *Adenostylion alliariae* Br.-Bl. 1926 and *Petasition officinalis* Sillinger 1933 and class *Mulgedio-Aconitetea* Hadač et Klika in Klika et Hadač 1944.

Threats: Grazing, trampling, eutrophication.

6510 Lowland hay meadows

EUNIS: R2232 Moesio-Thracian hay meadows; PAL. CLASS.: 38.252 Moeso-Thracian mesophile hay meadows.

Abiotic characteristic: This habitat type had wide distribution on the territory of municipality. It was represented by 443 polygons and occupied a total area of 11.53 km^2. The habitat was found on flat terrains (riverside terraces and ponors) or on slightly inclined terrains up to 10^o in the lower parts of the mountain slopes. Rocks were silicates, and soils alluvial and delluvial with high contents of clays. The vegetation was managed as pastures and meadows. These territories in Godech Valley have been periodically ploughed up for the last 20 years. There were also abandoned agricultural lands undergone a natural succession towards habitat 6510.

Vegetation structure: This habitat was represented by species rich mesic grasslands with closed horizontal structure and total cover in the range 90–100%. The vegetation was dominated by mesic grasses such as *Arrhenatherum elatius, Festuca pratensis, F. rubra* agg., *Alopecurus pratensis, Cynosurus cristatus, Poa pratensis, Trisetum flavescens, Anthoxanthum odoratum, Agrostis capillaris, Holcus lanatus, Lolium perenne*. In the species composition, other species of higher cover and abundance were *Trifolium repens, T. pratense, T. campestre, Lathyrus pratensis, Lotus corniculatus Leontodon autumnalis, L. hispidus, Convolvulus arvensis, Plantago lanceolata, Centaurea jacea, Daucus carota, Filipendula vulgaris, Betonica officinalis, Stellaria graminea*. This vegetation is related to class *Molinio-Arrhenatheretea* Tüxen 1937, order *Arrhenatheretalia elatioris* Tüxen 1931, alliances *Arrhenatherion elatioris* Luquet 1926, which included four associations (*Ranunculo repentis-Alopecuretum pratensis* (Eggler 1933) Ellmauer in Mucina & al. 1993, *Pastinaco sativae-Arrhenatheretum elatioris* Passarge 1964, *Cirsio cani-Festucetum pratensis* Májovský ex Růžičková 1971 and *Ranunculo bulbosi-Arrhenatheretum elatioris* Ellmauer in Mucina & al. 1993) and *Cynosurion cristati* Tüxen 1947 presented by two associations (*Bromo racemosi-Cynosuretum cristati* Horvatić (1930) 1958 and *Festuco rubrae-Agrostetum capillaris* Horvat 1951).

Threats: Abandonment, lack of mowing, overgrazing, plowing.

6520 Mountain hay meadows

EUNIS: R1M2 *Agrostis-Festuca* grassland, R235 Balkan mountain hay meadows; PAL. CLASS.: 35.12 *Agrostis-Festuca* grasslands, 38.3 Mountain hay meadows.

Abiotic characteristic: This habitat type had a local distribution on the territory of Berkovska Mt. and Ponor Mt. over 1000 m a.s.l. It was presented by 10 polygons only and occupied a total area of 0.99 km^2. Terrains were flat to moderately inclined up to 10°. The slope expositions were predominantly northern and western. Soils were moderately deep and moist during the year. The climate was temperate with typical high air humidity and small amplitudes during the vegetation season. The bedrock was of silicate origin. The vegetation was used for mowing and as pastures.

Vegetation structure: Moderately species-rich communities with closed horizontal structure and total cover 90-100%. Dominant species were *Agrostis capillaris, Festuca rubra* agg., *Arrhenatherum elatius*. Other species of higher cover and abundance (up to 5-10%) were *Cynosurus cristatus, Festuca pratensis, Holcus lanathus, Trifolium alpestre, T. montanum, T. medium, Veratrum lobelianum, Hypericum maculatum, Pastinaca hirsuta, Filipendula vulgaris*. This vegetation represented secondary grasslands, found at the areas of former beech, common hornbeam and oak forests. In the species composition were found some species from classes *Festuco-Brometea* (such as *Festuca pseudodalmatica, Danthonia alpina, Briza media,* etc.) and *Nardetea strictae* (*Nardus stricta, Chamaespartium sagittale, Lerchenfeldia flexuosa*). These phytocoenoses were related to alliance *Cynosurion cristati,* order *Arrhenatheretalia elatioris* and class *Molinio-Arrhenatheretea*.

Threats: Abandonment, lack of mowing, overgrazing.

Discussion

This is the second municipality in Bulgaria where all habitat types, protected by the Council Directive 92/43/EEC, are studied and mapped, using the scale of 1 : 5000. Grigorov et al. (2021) conducted similar research on grassland habitats in Dragoman Municipality where six grassland habitat types were distinguished. Grassland habitats in Godech Municipality have also been strongly influenced by anthropogenic activities. Three of the habitat types cover the largest proportion of the territory – 6210, 62A0 and 6510. Habitat 6210 has both xero-mesic and xeric communities located mainly in the central and northern municipality areas. Habitat 62A0 is characterized by xeric grasslands on carbonate rocks to the west and habitat 6510 consists of mesic plant communities that can be discovered on flat terrains or on slopes with up to 5-6^0 inclination in Vuchibaba hill and Ponor Mts.

The NATURA 2000 ecological network plays its important role for the conservation and condition improvement of the habitats, partially because of the subsidies for agriculture. In the same time, areas where agricultural activities have occurred in the past, are now turning into grasslands. The three main grassland habitats (6210, 62A0 and 6510) in Godech Municipality were abandoned in the years followed the end of the socialist period in Bulgaria (1990 year). Similar problems were observed by Vassilev et al. (2011). Wherever grazing occurs, the problem of overgrazing takes its toll. Another problem is tied to the shrub encroachment into grasslands. Habitats that are located around the depopulated boundary areas are not in a good condition. Some meadows and pastures in the flat terrains of the Godech hollow are also plowed and transformed into agricultural lands. They are turning into shrublands and the issue with invasive species should not be taken lightly as well. Invasion of *Robinia pseudoacacia* from its plantations to the grasslands nearby also occurs.

Knowledge on the habitat distribution and condition in the studied area is of a particular importance for their protection by the Council Directive 92/43/EEC within the Natura 2000 sites of Dragoman (BG0000322) and Western Balkan Range and Forebalkan (BG0001040).

Conclusions

This research explores the diversity of grassland habitats of Community importance in Godech Municipality (West

Bulgaria), protected by the Directive 92/43/EEC. Two Sites of Community Interest are present on the municipality territory – BG0000322 and BG0001040 as part of the Natura 2000 network. The study is based both on 418 original phytocoenological relevés and on 3422 field point observations for habitat type verification. A total of eight grassland habitat types was established in the studied area – 6210, 6230, 62A0, 62D0, 6410, 6430, 6510 and 6520. All grassland habitat types considered according to the Interpretation Manual of European Union Habitats (2013) and additionally mapped in scale 1 : 5000.

The intensive human activities or the lack of any negatively impact the grassland habitats and define more of the threats. Among the main grassland habitat threats observed on field could be mentioned: abandonment, lack of mowing, shrub encroachment, and intensive grazing, over trampling, plowing, eutrophication and subsequent ruderalization.

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Ecological Aspects of the Development of Harmful Objects on Plants of Genus Chaenomeles (Rosaceae)

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Abstract. Currently, the cultivation of environmentally friendly products in agriculture is of great importance in the world. This article is devoted to the study of new cultivars of genus *Chaenomeles* in order to identify the ones most resistant to harmful organisms for cultivation in private and farm gardens. The pathogenic microflora and entomofauna were studied on the *Chaenomeles* Lindl. cutivars (Maloideae, Rosaceae), obtained at the Michurinsky State Agrarian University. In central Russia (Moscow and Tambov Region), many years of systematic monitoring of pathogens and phytophages were spent for plants *Ch. japonica*, *Ch. cathayensis*, *Ch. × superba*. Among the identified pathogens, the micromycetes belonging to genera *Botrytis, Cytospora, Diplocarpon, Entomosporiu*m, *Gloeosporium, Monilia, Neonectria, Penicillium, Pestalotia, Phomopsis, Phyllosticta, Septoria* and *Sphaeropsis* were found to be injurious. With age, the accumulation of infectious background, including viral diseases and the damaging entomofauna, was noted in the plantings of fruit crop *Chaenomeles*. The species composition of phytophages is mainly represented by autochthonous species (polyphages and oligophages), preferring plants of the Rosaceae family. Despite the abundance of species in the phytophage complex, it has a little effect on the decorative effect of *Ch. japonica, Ch. cathayensis* and *Ch. × superba*. No outbreaks of epiphytotics and epizootics were observed in the collections of *Chaenomeles* in different ecological growing conditions over a long period of time, and, in general, all plants were in a satisfactory condition. Entomophytopathological monitoring made it possible to identify environmentally safe *Chaenomeles* cultivars resistant to harmful objects - such as Albatross, Allure, Voskhod, Michurinsky Vitamin, Flagship and Charm.

Key words: Chaenomeles, microflora, entomofauna, cultivar.

Introduction

Species of genus *Chaenomeles* Lindl. (Maloideae, Rosaceae*)* come from Japan and China and are valued in decorative gardening. To obtain flowering cultivars, such cultivars as *Ch. speciosa* (Sweet) Nakai, *Ch. cathayensis* (Hemsl.) C.K. Schneid., *Ch. japonica* (Thunb.) Lindl. ex Spach, as well as garden hybrids - *Ch.* × *superba* (Frahm) Rehder (Ch. *Japonica* × *Ch. speciosa*) and others were used (Garkava et al., 2000; Norin et al., 2002).

In the climatic conditions of Russia, the most stable species is Japanese Chaenomeles called "Japanese quince" - *Ch. japonica* (Thunb.) Lindl. ex Spach; it blooms with bright orange flowers and safely winters under a snow cover. This species is interesting for its highly vitaminized fragrant fruits and attracts breeders in Sweden, Finland, Poland, Spain, Baltic countries, Moldova, Belarus, Ukraine (Rumpen, 2002; Mezhinsky, 2004; 2010) and Russia. As an unconventional fruit crop *Ch. japonica* is tested in central Russia, the Middle Volga region, in the Crimea and Western Siberia (Saveliev et al., 2009; Fedulova, 2009; Komar-Dark, 2015; Kuklina et al., 2016, 2017).

Although it is believed that *Chaenomeles* species are resistant to diseases and pests, it is known from literature that, in China, the genus representatives *Phoma sp.* and *Phyllosticta sp.* (Yu & Bai, 1995) settle on bushes. The following pathogens were detected on plants of genus *Chaenomeles*: *Monilinia fructicola* (G. Winter) Honey, *M. fructigena* Pers., *M. laxa* (Aderh. & Ruhland) Honey, *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers., *Phyllosticta chaenomeli*na Thüm., *Cytospora piricola* Westend., *Septoria cydoniae* Fuckel, *Diplodia cydoniae* Schulzer, *Phoma chaenomeles* Brunaud, *Ph. pomorum* Thüm., *Ph. herbarum* Westend., *Ph. exigua* Desm. *Penicillium expansum* Link, *Sphaeropsis lichenoides* Sacc., *Ulocladium botrytis* Preuss, *Thubercularia vulgaris* Tode, *Ramularia sp.*, *Cladosporium sp.*, *Fusarium sp.*, *Asteromella sp.*, as well as phytophages *Aphis pomi* Deg., *A. fabae* Soper. Kalt., *Phyllobius sp.*, *Operophtera sp.*, *Yaponomeuta sp.*, *Caliroa sp.* in Europe (Rumpen, 2002) and *Aphis pomi* in Iran (Madachi & Sahragard, 2012).

For the successful breeding and a further expansion of this culture, information about diseases and pests that threaten nontraditional fruit crops is relevant. The purpose of this study was to research and identify representatives of pathogenic microflora and harmful entomofauna on the *Chaenomeles* cultivars in the regions of central Russia.

Material and Methods

The study of phytopathogens and phytophages on the *Chaenomeles* crops was carried out in the Michurinsk City, Tambov Region (Michurinsk State Agrarian University), Moscow Region (Orekhovo-Zuevo District, urban plantings) and in the arboretum of N.V. Tsitsin Main Botanical Garden of the Russian Academy of Sciences (Moscow, MBG RAS). The plantings of *Ch. japonica, Ch. cathayensis, Ch.* × *superba* were surveyed, as well as 25 selected Chaenomeles forms and 6 cultivars (Flagman, Voshod, Sharm, Michurinsky Vitamin, Alyur, Albatros) of the Michurin selection.

In 2007-2009, in the Tambov region, endophytic microflora was tested on cultivars, forms and types of *Ch. japonica* and *Ch. cathayensis*. Crops taken from annual shoots were placed in sterile tubes and Petri dishes with 2 variants of nutrient media from agar-agar (15 g): in the 1st variant with intoxicating wort (1 L) and in the 2nd - with potato (1 L). The analysis and registration of the fungal and bacterial microflora colonies was carried out under the microscope "Biomed-4" and expressed in (%) of the total number of tests (Fedulova, 2009).

In 2010-2017, in field conditions, the monitoring was carried out, including analysis of leaves and fruits on *Chaenomeles* samples with symptoms of phytopathogen lesions and phytophagous damage. The identification of fungi was performed by standard methods (Khokhryakov & Potlaychuk, 1984; Treivas & Kashtanova, 2014) and listed on Index Fungorum (2022). The species composition of arthropods (Arthropoda) is determined by damage, larvae and imago (Savkovsky, 1990; Isaeva & Shestopal, 1991), and the Fauna Europaea (2022) is also given. The affection of cultivars by diseases was estimated on a 5-point scale: 0 - there are no lesions on leaves and fruits; 1 - lesion area ≥10 %; 2 - lesions on the area is in the range of 11-30 %; 3 -

damages in the area is in the range of 31-70 %; 4 - damaged ≥70 % of the area, and shoots die off.

Results and Discussion

Since 2003, on the basis of Michurinsk State Agrarian University in the Tambov region, the breeding of *Chaenomeles* has been carried out (Fedulova, 2009). The cytological study of the first haploid mitosis in the pollen from selected forms of *Chaenomeles* showed that *predatirovnaiya?* pollen grains can be formed in flowers. This development of anthers provides an additional opportunity to obtain meiotic amphidiploids (Fedulova & Pimkin, 2016), potentially promising in further selection.

Stability of Russian Chaenomeles cultivars

New cultivars Flagman, Voshod (Fig. 1), Sharm (Fig. 2), Michurinsky Vitamin, Alur and Albatros are included in the State Register of the Russian Federation. They have the flowers of the original color, corolla diameter being 3.5-5.8 cm, and their shoots without spikes (Table 1).

These *Chaenomeles* cultivars are characterized by a universal purpose and can be considered as a fruit crop. Their fruits weigh 60-75 g with thick pericarps (1.3-1.9 cm), the pulp fraction is up to 88-92 % (Fig. 3). They (fruits) are saturated with vitamin C (110-350 mg %), contain 450-750 mg % of catechins, up to 120 mg % of leucoanthocyanins, about 5% of organic acids and 3.5 % of sugars, and are characterized by a low sugar-acid index, contain up to 12 % of pectin; so, they are suitable for various types of processing and are recommended for therapeutic and preventive nutrition (Kuklin & Fedulova, 2015; 2017).

Fig. 1. Bush of cultivar Voshod. Photo by Y. Fedulova.

Fig. 2. Inflorescences of cultivar Sharm. Photo by A. Kuklina.

Table 1. Characteristics of *Chaenomeles* cultivars.

Cultivar	Average bush height, cm	Corolla color	Average corolla diameter, mm	Average fruit diameter, mm	Average pericarp thickness, mm
Voshod	90	Pale- yellow	43	50	19
Flagman	40	Bright-crimson	35	50	20
Sharm	70	Light- orange	40	48	15
Michurinsky Vitamin	65	Creamy orange	45	42	20
Alyur	80	Orange	58	55	18
Albatros	70	White	38	45	14

Fig. 3. Fruits of cultivar Sharm. Photo by Y. Fedulova.

The selected *Chaenomeles* forms and cultivars, tested in the Tambov region for the resistance to endophytic microflora, showed a high plant viability degree (Chesnokova et al., 2010). The presence of bacterial and fungal microflora in different proportions, depending on the date of testing, was revealed on the shoots. Bacterial microbiota prevailed and varied greatly in color, size, shape of colonies, the nature of surface and the marginal zone. Fungal microflora, during this testing, was less abundant and was represented by the species of genera Alternaria (Fig. 4), *Penicillium sp.*, *Stemfillium sp.*, *Cladosporium sp.*, *Fusarium sp.*; some cultivars were absent in both media. Negative tests were recorded on the potato culture medium in cultivars Flagman, Sharm and Michurinsky Vitamin. Such cultivars as Alyur, Albatros and Voshod have only 10% of micromycetes, and that confirmed a good adaptability of these plants (Fig. 5).

Long-term monitoring of 6 *Chaenomeles* cultivars in the Michurin breeding under field conditions revealed 4 types of phytopathogens with a minimal plant susceptibility degree (Table 2).

Fig. 4. Pure culture *Alternaria* sp. in a Petri dish on a wort nutrient medium. Photo by Y. Fedulova.

Table 2. Affection of *Chaenomeles* cultivars by the pathogenic microflora during 2010-2016, points.

Cultivars and forms	*Septoria cydoniicola*	*Alternaria alternata*	*Phyllosticta cydoniae* var. *cydoniaecola*	*Diplocarpon mespili*
Voshod	0	0	1	0
Flagman	1	1	0	0
Sharm	1	0	0	0
Michurinsky Vitamin	1	0	0	0
Alyur	1	1	1	0
Albatros	1	0	0	1

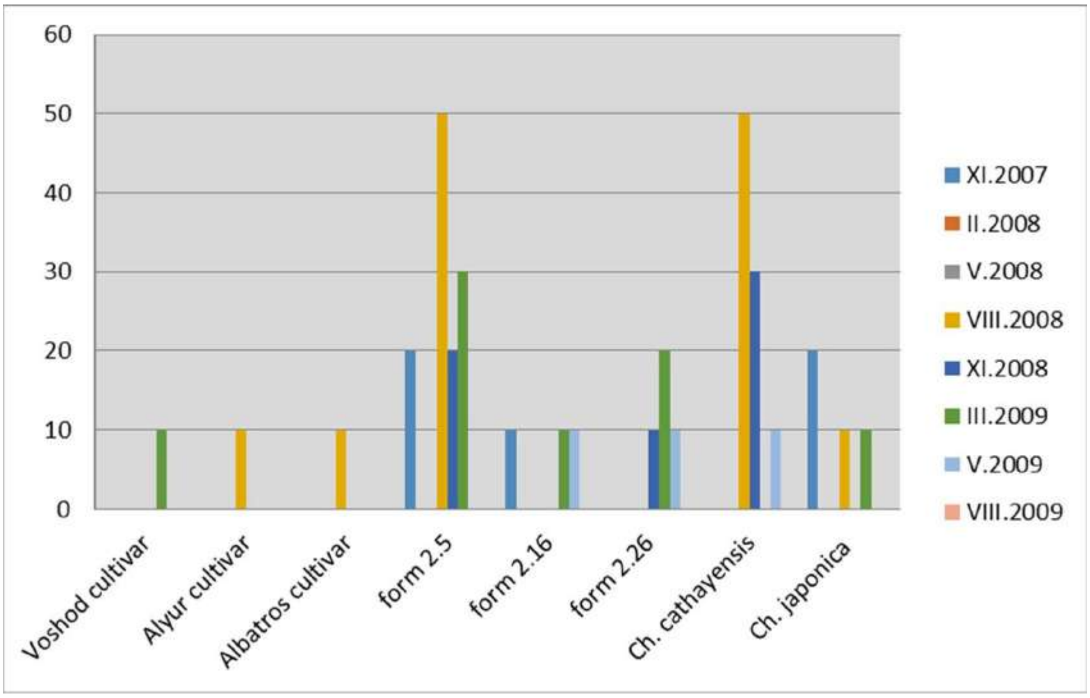

Fig. 5. Concentration of endophytic microbiota in *Chaenomeles* cultivars on a potato nutrient medium, %.

Septoria cydoniicola Thüm was most commonly found in *Chaenomeles* cultivars (*Mycosphaerellaceae*). At the beginning of summer, numerous whitish-gray round spots with a dark rim are formed on the leaves (Fig. 6). Cultivars were affected by the disease at 1 point, but Voshod turned to be the most resistant cultivar.

Drying and premature falling of leaves with brown spots with a light middle causes the appearance of *Phyllosticta cydoniae* var. *cydoniicola* (Allesch.) Cif. (*Phyllostictaceae*). The cause of the appearance of brown spots in new cultivars Flagman, Voshod (Fig. 1), Sharm (Fig. 2), Michurinsky Vitamin, Alu can also be entomosporiosis - the causative agents of *Entomosporium eriobotryae* S. Takim. and *Diplocarpon mespili* (Sorauer) B. Sutton, syn. *Entomosporium maculatum* forma *maculatum* Lev. (Dermateaceae), identified in cultivar Albatros (1 point).

More rarely, *Alternaria alternata* (Fr.) Keissl is encountered in *Chaenomeles* cultivars (*Pleosporaceae*). At the end of spring, small rounded dark brown spots with a dark purple-red border are visible on the leaves (Fig. 7), and, later, the spots merge. In the course of the disease development, fruit rot (Fig. 8) develops heart rot. The most resistant to altarnariosis cultivars are Voshod, Sharm, Albatros and Michurinsky Vitamin.

The assortment of fruit culture of *Chaenomeles*, unconventional for the *Chaenomeles* fruit crop of Russia, which is resistant to phytopathogens obtained in the Michurinsk State Agrarian University, can serve as basis for further breeding activities.

Fig. 6. *Septoria cydoniicola* and *Phyllosticta cydoniae* var. *Cydoniicola* on a *Chaenomeles* leaf. Photo by Y. Fedulova.

Fig. 7. *Alternaria alternata* on *Chaenomeles* leaves. Photo by Y. Fedulova.

Fig. 8. *Alternaria alternata* on the *Chaenomeles* fruit. Photo by Y. Fedulova.

Potentially dangerous microflora

According to our observations, the pathogenic mycoflora on *Chaenomeles* plants is represented much more widely and is capable of damaging leaves, flowers, fruits, causing the drying of branches and death of entire bushes. *Monilia fructigena* (Sclerotiniaceae), which is most often noted in the collection plantings of the Moscow Botanical Garden (MBG RAS), affects *Ch. japonica* and *Ch. cathayensis* (2-3 points). During the disease development, brownish-yellow pads of conidia sporification appear on the fruit surface (Fig. 9). Later, the fruit loses its taste and nutritional qualities, and its mummification comes. The causative agent of moniliosis can be *M. cydoniae* Schell., from which leaves, flowers and ovaries fall off in summer. Old *Ch. japonica* bushes are affected by 2 points.

As a result of anthracnose quince, the causative agent of *Gloeosporium cydoniae* Mont. (*Dermateaceae*), dark brown spots appear (3 points) on the leaves of *Ch. japonica*. The premature yellowing and leaf fall (2-3 points) cause *Pestalotia breviseta* Sacc. (*Pestalotiopsidaceae*). To a lesser extent (1 point), the fruit crop is affected by *Phyllosticta velata* Bubák. (*Phyllostictaceae*).

On the old *Chaenomeles* bushes, the following diseases are often encountered (1-2 points). Quince cytosporosis is the causative agent of *Cytospora cydoniae* Bubák & Kabát. (Valsaceae) that makes bush branches shrink. Tubercular necrosis is the causative agent of *Nectria cinnabarina* Fr. (Nectriaceae), begins with the bark necrosis on the shoots and branches, but leads to the entire plant death. European cancer, the causative agent of *Neonectria galligena* (Bres.) Rossman & Samuels, syn. *Nectria galligena* Bres. (Nectriaceae), is rare, and it causes the death of leaves, fruits and whole branches. Black quince cancer - the causative agent of *Sphaeropsis cydoniae* Cooke & Ellis (*Aplosporellaceae*) - damages the fruits of *Ch. japonica* and *Ch. cathayensis*, and it leads to the cracking and drying of branches bark.

Part of the *Chaenomeles* harvest may be lost (1-2 points) as a result of botrytiosis, penicillosis and fomopsis, which are noticeably manifested during the storage of fruits. Botrytiosis - the causative agent of *Botrytis cinerea* (Sclerotiniaceae), which is found everywhere, - affects the flowers and fruits of *Chaenomeles*. First, brown spots with a clear reddish edge appear, and then the fruits rot and fall premature. The fallen fruits rotting is accelerated by a contact with soil, since *Penicillium expansum* Link and *P. cyclopium* Westling (Trichomaceae) penetrate their surface. *Phomopsis mali* Roberts, *Ph. perniciosa* Grove and *Ph. ambigua* (Nitschke) Traverso (Diaporthaceae) are phomopsis pathogens, and their presence is accompanied by the appearance of dark brown spots on the leaves, leaf fall and the rotting of *Chaenomeles*.

Only the tomato annular blotch virus (ToRSV), the symptoms of which are manifested in the form of chlorosis, wrinkling and necrotic leaf blotch with a characteristic alternation of dark and light areas, was observed in *Chaenomeles* (Fig. 10).

Fig. 9. *Monilia frutigena* with conidial sporulation pads on the fruit of *Chaenomeles japonica.* Photo by L. Treyvas.

Fig. 10. ToRSV virus on a *Chaenomeles japonica* bush. Photo by L. Treyvas.

Damaging entomofauna complex

A complex of entomofauna representatives was revealed in *Chaenomeles* plants in the regions of central Russia (Table 3). It should be noted that almost all groups of Insecta are rare and in a single amount (1 point), except for aphids forming colonies (1-2 points).

The most dangerous are migratory species *Aphis fabae*, black insect (Fig. 11), and the nonmigrating species *A. pomi* (Homoptera: Aphididae), which is common for Rosaceae fruit crops. Although they are not noted every year in *Chaenomeles*, they are a carrier of viral diseases. The complex of sucking species in the Moscow region is presented by the polyphage *Palomena prasina* (Homoptera: Pentatomidae) and the phytophage fam. Rosaceae - *Edwardsiana rosae* L. (Homoptera: Cicadellidae). In both regions, the polyphagous bug *Acanthosoma haemorrhoidalis* L. (Hemiptera: Acanthosomatidae) was found, usually feeding on deciduous plants of families Rosaceae, Betulaceae, Salicaceae, Tiliaceae, etc.

Gnawing phyllophages are diverse, although they appear sporadically, but they are capable of reducing the decorative appearance of a bush. At the beginning of summer, the gray-green caterpillars *Cladius pallipes* Lep., Syn. *Priophorus padi* L. (Hymenoptera: Tenthredinidae), capable of skeletonizing the leaves of *Chaenomeles*, appear.

Archips variegana Schiff stands out from leafworms (Lepidoptera: Torticidae) and *A. rosana* L., syn. *Ccoecia rosana* L., feeding on deciduous plants, including those from this Rosaceae family. The second type not only turns the leaves into a tube, cigar-shaped or lumpy, but also damages the ovary and fruit, gnawing the fossa in the pulp, to the seed chamber (Fig. 12).

An especially active development of caterpillars *Acleris variegana* Den. et Schiff. was noted by us in 2016 in the Tambov region on the Albatross cultivar, when they were eating out fruit buds, skeletonizing the leaves, and wrapping them into a bundle of cobwebs. Green brown head caterpillar *Exapate congelatella* Cl. also gnaws into the buds and leaves, thus, forming a lump. The

Fig. 11. *Aphis fabae* on young shoots of *Chaenomeles japonica.* Photo by A. Kuklina.

small wormhole, the crenellate lunate - *Ancylis selenana* Gn., common on Rosaceae fruit crops, was noted during the monitoring on *Ch. japonica* in the arboretum of the MBG RAS, and in the summer of 2016 it was noted in Prague (Czech Republic) at *Ch.* × *superba* (1-2 points). Greenish-yellow caterpillars (up to 1 cm long) skeletonize leaves, fold them in halves, along the central vein, and then gnaw them.

Gnawing phytophagous *Chaenomeles* includes the blue-headed scoop *Diloba coeruleocephala* L., syn. *Episema coeruleocephala* L. (Lepidoptera: Noctuidae) and alfalfa skosar - *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae), which feeds on buds and gnaws young leaves.

Fig. 12. *Chaenomeles* leaf*,* cigar-shaped swirling *Archips rosana.* Photo by A. Kuklina.

On the *Chaenomeles*, the beetle *Agriotes obscurus* L. (Coleoptera: Elateridae), usually preferring leaves of cereals, is found. Small beetle *Phyllobius urticae* Deg., syn. *Phyllobius pomaceus* Gyllenhal (Coleoptera: Curculionidae) is a polyphage found on the plants of Urticaceae and Rosaceae families.

Among phyllophages, *Nepticula malella* Stainton (syn. Stigmella malella Stt.), leaving serpentine mines extended in the middle, is marked; characteristic thin mines are typical of *Stigmella pomella* Vaugh. (Lepidoptera: Nepticulidae).

Chaenomeles fruits can be damaged by several carpophages characteristic of Rosaceae family. *Rhynchites bacchus* L. and *Coenorrhinus pauxillus* Germ are found everywhere (Coleoptera: Rhynchitidae), consuming young leaves and flowers of

Chaenomeles. The caterpillars *Laspeyresia pomonella* L., syn. *Carpocapsa pomonella* L. (Lepidoptera: Torticidae), gnawing ripe fruits, are occasionally observed.

Seed chambers in the fruits of *Ch. japonica* are destroyed by *Hoplocampa testudinea* Clug. (Hymenoptera: Tenthredinidae) frequently encountered on apple trees. European hornet - *Vespa crabro* L. (Hymenoptera: Vespidae) - usually feeds on flower nectar, fruit juice and pulp. Adult individuals eat the flesh of ripe *Chaenomeles* fruits (Fig. 13), and that accelerates their rotting.

Table 3. Occurrence of entomofauna representatives in the regions of central Russia.

Insecta orders	Moscow region	Tambov region
Homoptera	*Aphis fabae, A. pomi, Palomena prasina, Edwardsiana rosae*	*Aphis fabae*
Hemiptera	*Acanthosoma haemorrhoidalis*	*Acanthosoma haemorrhoidalis*
Hymenoptera	*Cladius pallipes, Hoplocampa testudinea, Vespa crabro*	
Lepidoptera	*Nepticula malella, Stigmella pomella Archips variegana, A. rosana, Laspeyresia pomonella, Exapate congelatella, Ancylis selenana, Diloba coeruleocephala*	*Acleris variegana, Exapate congelatella, Stigmella pomella*
Coleoptera	*Rhynchites bacchus, Coenorrhinus pauxillus, Phyllobius urticae, Agriotes obscurus*	*Rhynchites bacchus, Coenorrhinus pauxillus, Otiorrhynchus ligustici*

Fig. 13. Damage of *Vespa crabro* on the fruit of *Chaenomeles japonica.* Photo by A. Kuklina.

Conclusion

In central Russia, many years have been spent for the systematic monitoring of pathogens and phytophages on plants *Ch. japonica, Ch. cathayensis, Ch.* × *superba* and multipurpose cultivars. Among the identified pathogens, the most harmful pathogens are *Alternaria alternata, Septoria cydoniicola, Entomosporium eriobotryae, Phyllosticta cydoniae* var. *cydoniicola, Diplocarpon mespili, Gloeosporium cydoniae, Monilia cydoniae, M. fructigena*; less harmful are *Pestalotia breviseta* and *Phyllosticta velata, Penicillium expansum* and *P. cyclopium.* Filoplan mushrooms are widely spread. With age, the increase in the infectious background and the accumulation of damaging phytophages (*Botrytis cinerea, Phomopsis mali, Ph. perniciosa* and *Ph. ambigua*), including viral disease (ToRSV), is noted in plantings. *Cytospora cydoniae, Sphaeropsis cydoniae, Nectria cinnabarina* and

Neonectria galligena are marked on country-aged stands, causing the drying of branches and death of entire bushes. For a long period of observation of Disease outbreaks were not observed for a long period in the collections of *Chaenomeles*, and in general, all plants are in a satisfactory condition. As a result of the phytopathological monitoring, the following disease-resistant *Chaenomeles* cultivars were noticed: Albatros, Alyur, Voshod, Michurinsky Vitamin, Flagman and Sharm.

Chaenomeles plants have a complex of sucking, gnawing and mining phytophages. Among the representatives of the entomofauna prevalent chewing insects are: phyllophagous - *Archips variegana, A. rosana, Acleris variegana, Exapate congelatella, Cladius pallipes, Ancylis selenana, Diloba coeruleocephala, Agriotes obscurus, Phyllobius urticae, Otiorrhynchus ligustici* and karpovagous - *Rhynchites bacchus, Coenorrhinus pauxillus, Laspeyresia pomonella, Hoplocampa testudinea, Vespa crabro.* Sucking insects *(Palomena prasina, Edwardsiana rosae, Acanthosoma haemorrhoidalis*) are dangerous, especially *Aphis fabae* and *pomi*, which are carriers of phytoviruses. Phytophagous miners (*Nepticula malella* and *Stigmella pomella*) are few in number and have almost no effect on the decorativeness of bushes. Occasionally Snail-kamnetes *Helicigona lapicida* L. (Gastropoda: Helicidae), eating in dense plantings with leaves, less often with fruits, is occasionally found on *Ch. Japonica* plants.

Analysis of the phytophages composition shows that these are mainly autochthonous species, both polyphages and oligophages, preferring plants of Rosaceae family. Probably, this set is due to the close proximity of the experimental *Chaenomeles* plants with plantings of fruit crops of Rosaceae family, which facilitates the expansion of food links and favors the creation of new potentially dangerous "introduced species-pathogen" complexes.

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Short note

First Record of Heteropterus morpheus (Pallas, 1771) (Lepidoptera: Hesperiidae) from the Republic of Kosovo

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Abstract. The first record of the Large Chequered Skipper butterfly *Heteropterus morpheus* (Pallas, 1771) from the Republic of Kosovo is presented. This species was recorded in two localities during our butterfly surveys in “Bjeshket e nemuna” National Park in 2019-2021.

Key words: skipper, “Bjeshket e nemuna” National Park, Kosovo, Balkan Peninsula.

Introduction

The Large Chequered Skipper (*Heteropterus morpheus)* is the single member of the monotypic genus *Heteropterus* Duméril, 1806. *H. morpheus* ranges from Northern Spain, much of Central Europe, Italy, Denmark, Sweden, Lithuania, Balkans, SE and North Bulgaria, North west Turkey, Central Asia, Amur and Korea (Tomas & Levington, 2008).

The species is currently known from most of the Balkans and South-eastern European countries: Albania (Cuvelier et al., 2018; Micevski et al., 2015; Šašić et al., 2015; Verovnik & Popović, 2013), Bosnia and Herzegovina (Đug, 2013; Koren & Kulijer, 2016; Lelo, 2016), Bulgaria (Abadjiev, 2001; Abadjiev & Beshkov, 2007; Hristova & Beshkov, 2017; Kolev 2017; Kolev & Shtinkov, 2016; Kolev & Tsvetanov 2018; Langourov & Simov, 2014), Montenegro (Franeta, 2018; Švara et al., 2015), Serbia (Jakšić, 2003; Popović et al., 2017; Popović & Verovnik, 2018), Romania (Rákosy, 2013, Vlad Dincă (pers. comm.), Korb & Bolshakov, 2016; Székely, 2008; Wiemers et al., 2018); Slovenia (Verovnik et al., 2012); Croatia (Šašić et al., 2015; Šašić & Mihoci, 2011). The only report from North Macedonia dates from Dimitrov (1909) who reported *Heteropterus morpheus* for the village Krustofor, now Krstoar, few kilometers S from Bitola Town.

Material and Methods

Field survey was conducted during the years 2019-2021 in Peklen Peak and Gryka e Rugoves Gorge in “Bjeshket e Nemuna” National Park. Peklen Peak is located at 1376 m a.s.l. in the northern part of “Bjeshket e Nemuna” NP, whereas Gryka e Rugoves (Rugova Gorge) with an area from 4,301 ha is one of the most beautiful parts of the national park and since 1985 has a status of

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Monument of Nature. During the survey an entomological net was used to collect butterfly specimens. After being identified and photographed, captured butterflies were released and only one specimen was taken and preserved as the evidence of its presence.

Results

In total four male specimens were recorded. The first specimen, a male of the Large Chequered Skipper butterfly *Heteropterus morpheus* (Pallas, 1771) was registered in a grassland habitat roadside, on 29 June 2019 in Peklen Peak (42°40'30.79"N, 20°14'11.48"E, 1310 m a.s.l.) (Fig. 1, 3). Next year, on 26 June 2020, at the same place, were registered two males, whereas the fourth one has occurred on 16 June 2021 in a moist habitat near the three water springs in Rugova Gorge (42°41'32"N, 20°10'07"E, 1310 m a.s.l.) (Fig. 2, 3). Prove material is located at the collection of the Department of Biology (Faculty of Mathematics and Natural Sciences in Pristina, Kosovo).

Fig. 1. Peklen Peak (1376 m).

Although the survey in the period from 2019-2021 were quite intensive, the number of *Heteropterus morpheus* (Fig. 4, 5) specimens was quite small, which confirms that the populations are isolated.

H. morpheus is in flight from late June to July.The larvae feed on *Eriophorum, Poa annua, Calamagrostis canescens, Brachypodium sylvaticum and Molinia* species, including *Molinia coerulea* (Tolman & Lewington, 2008). Most of these plant species are common in Kosovo (*Calamagrostis canescens, Brachypodium sylvaticum* and *Molinia grasses)* and were present in Peklen Peak, one of the localities where *H.morpheus* was recorded

Fig. 2. Rugova Gorge.

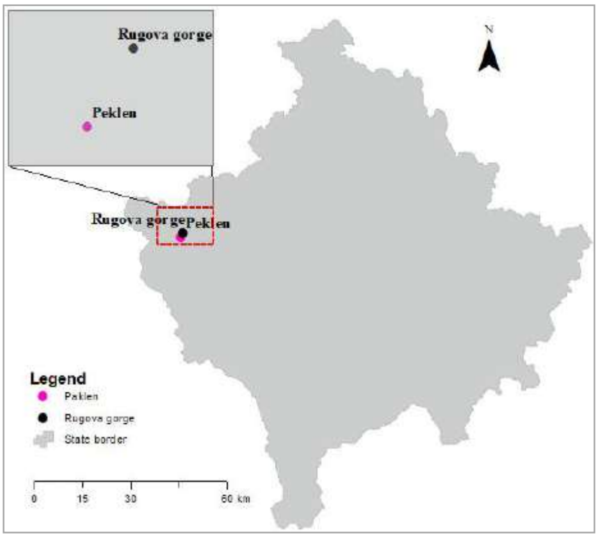

Fig. 3. The localities in Kosovo where *Heteropterus morpheus* was observed.

Fig. 4. *Heteropterus morpheus* – preserved male.

Fig. 5. A male *Heteropterus morpheus* photographed on 16 June 2021 in the Rugova Gorge (Photo: Nikola Micevski, Macedonian Entomological Society).

Discussion

Based on the existing data, *H. morpheus* is distributed in almost all Balkan countries. However, its status according to IUCN threat categories varies across the Balkans. While it has a "Least Concerned (LC)" status in Serbia (Jakšić, 2003, Popović et al., 2017; Popović & Verovnik, 2018), Bosnia and Herzegovina (Koren & Kulijer, 2016; Lelo, 2016), Slovenia (Verovnik et al., 2012) and Hungary (Ádám Kőrösi & Ádám Gór (pers. comm.), Gergely et al., 2017; Sáfián et al., 2012; Varga, 2012), in Bulgaria it is "Vulnerable" (Hristova & Beshkov, 2017), whereas in Croatia (Šašić et al., 2015; Šašić & Mihoci, 2011) and Romania it is a "Near Threatened (NT)" species (Rákosy, 2013; Vlad Dincă (pers. comm.), Korb & Bolshakov, 2016; Székely, 2008; Wiemers et al., 2018). In Albania *H. morpheus* is "Not Evaluated (NE)" according to the IUCN criteria. In Europe as well as in EU, it is evaluated as LC (van Swaay et al. 2010; Wiemers et al. 2018).

Although in this paper we present the first record of *Heteropterus morpheus*, in Kosovo, it should be stated that the threats this species is undergoing are already registered. Unfortunately, during our survey in 2021 in Peklena Peak, a new asphalt road was under construction in the vicinity of the place where the *H. morpheus* specimens were recorded two years earlier, which presents a potential threat for *H. morpheus* host plants and this butterfly species itself. Despite the fact that the area, where *H. morpheus* is registered is a protected area within the "Bjeshket e Nemuna" National Park, due to many visitors this area is under various human impacts, which affect and threaten its flora and fauna. Based on the numbers of *H. morpheus* specimens we registered during a three year survey, we can conclude that this species populations in "Bjeshket e Nemuna" National Park are small and fragmented. Further surveys in the national park and in other parts of the country would be necessary in order to have more data on the presence and distribution of *H. morpheus* in the Republic of Kosovo.

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Short note

Pluteus fenzlii (Pluteaceae, Agaricales) Rediscovered in the Balkan Peninsula after over 150 Years Gap

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Abstract. The authors present a Bulgarian collection of *Pluteus fenzlii,* a species described by Stephan Schulzer von Müggenburg over one and a half century ago on material from Serbia and never seen in the Balkan Peninsula ever since. Condensed description and illustrations of the Bulgarian specimen are provided.

Key words: biogeography, Bulgarian mycota, pink-spored agarics, Southeastern Europe.

Introduction

Pluteus fenzlii (Schulzer) Corriol & P.-A. Moreau is an uncommon member of the genus, rarely featured in mycological literature as evident from the accounts in Corriol & Moreau (2007), Malysheva et al. (2007) and Holec et al. (2018). A rare species in Europe, this fungus was evaluated as "*vulnerable*" for the IUCN Red List of Threatened Species (Brandrud, Krisai-Greilhuber & Kunca, 2019). It was described in 1866 as *Agaricus fenzlii* Schulzer (Schulzer von Müggennburg et al., 1866) on specimens from Serbia and never recorded later either in this country or in the rest of the Balkan Peninsula. In 2018 and 2019 one of us observed and collected the species in Northeastern Bulgaria after over 150 years gap after Schulzer's discovery. In this paper the authors aim to document those collections, providing description and illustrations.

Material and Methods

The specimens were documented at the time of collection and are deposited in air-dried state in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF). The colours in the description below refer as closely as possible to the Flora of British Fungi Colour Identification Chart (Royal Botanic Garden, 1969). The microscopic study was held with an AmScope T360B light microscope, equipped with AmScope MU900 digital camera. The microscopic observations were held on slides from dried material after rehydration with 5% KOH for 2 min., staining in Congo red in ammonia for 2 min. and final mounting in 5% KOH. Spores were observed in preparations with mixture of 5% KOH and Congo red in ammonia (50:50, v/v). Of each studied collection 30 random, normally developed, mature basidiospores were assessed. In the description the spore measurements are presented by the minimum and maximum values of length, width and quotient (Q), followed by the average values for spore length (L_{av}), width (W_{av}) and quotient (Q_{av}). All measurements of microscopic structures were obtained from digital photographs with the aid of "Piximètre" software v. 5.10 (©A. Henriot & J.-L. Cheype). The background relief map was generated on "Maps-for-Free".

Results and Discussion

Pluteus fenzlii (Schulzer) Corriol & P.-A. Moreau, Persoonia 19(2): 248 (2007); Bas.: *Agaricus fenzlii* Schulzer, in Schulzer, Kanitz & Knapp, Verh. Zool.-Bot. Ges. Wien 16(Abh.): 49 (1866); Syn.: *Annularia fenzlii* (Schulzer) Schulzer, in Kalchbrenner, Icon. Sel. Hymenomyc. Hung.: tab. 10, fig. 1 (1874); *Chamaeota fenzlii* (Schulzer) Singer, Sydowia 31(1-6): 198 (1979); *Pluteus fenzlii* (Schulzer) E. Malysheva, Morozova & Zvyagina, Acta Mycol. 42(2): 155 (2007) [an isonym without nomenclatural status under ICNAFP, art. 6.3, note 2, *fide* Borovička, 2008]; Fig. 1.

Macroscopic features. Pileus up to 45 mm across, obtuse-conical, campanulate to convex or irregularly convex, with broad umbo, sulfur yellow, lemon yellow to lemon chrome, in overmature specimens discolouring to pale buff, at the margin inconspicuously striate, dry, radially fibrillose and finely squamulose; squamules concolorous with the background, more or less erect towards the centre to appressed elsewhere. Stipe up to 60 × 8 mm, cylindrical or subcylindrical, usually more or less curved, smooth to fibrillose, in the upper part whitish or yellowish, in the lower part whitish to lemon yellow, with lemon yellow floccons and concolorous, flocculose, evanescent annulus. Lamellae free, more or less crowded, whitish to cream-coloured with pinkish hues in young basidiomata, in maturity pinkish; lamellulae present. Odour inconspicuous; taste not recorded.

Microscopic features. Basidiospores 5.3–7.8 × 4.7–6.3 µm, Q=1.0–1.3 (L_{av}=6.2–6.3 µm, W_{av}=5.3–5.4 µm, Q_{av}=1.2), broadly ellipsoid to subglobose, with one large central guttule, smooth, thin-walled, with inconspicuous apiculus. Basidia 4-spored, 18.3–29.9 × 5.1–8 µm clavate, thin-walled. Cheilocystidia 40.6–93.5 × 8.3–20.6 µm, abundant, covering the lamellar edge, clavate, narrowly fusoid to clavate-ventricose, fusoid-ventricose or broadly lageniform, with somewhat lengthened necks, narrow at base, thin-walled or occasionally thick-walled, sometimes with granular yellowish content. Pleurocystidia 33.7–103.5 × 14.3–27.6 µm, common, lageniform or fusoid-ventricose, often strongly inflated, with shorter or longer necks, narrowed towards the base, sometimes ampuliform, with rounded or acute apex, thin-walled. Pileipellis a trichodermium of periclinaly arranged, septate, cylindrical hyphae 3–5 µm wide, bundles of hyphae protruding in places from the main surface (corresponding to pileal scales). Clamp connections not seen in any tissue.

Specimen examined. Bulgaria, Shumen distr., in the vicinity of Pet Mogili village (*cf.* Fig. 2), 43°33'10.0"N, 27°20'56.0"E, elev. ca 285 m a. s. l., 05.06.2018, leg. D. Dimitrov (SOMF 30432); *idem*, 43°32'56.0"N, 27°20'43.0"E, 29.10.2019, leg. D. Dimitrov (SOMF 30433).

Pluteus fenzlii is a striking species, readily recognized in the field, due to the presence of annulus on stipe. The only other annulate taxon in the genus in Europe is *P. aurantiorugosus* var. *aurantiovelatus* Vizzini, nesting together with *P. fenzlii* in section *Celluloderma* Fayod (Vizzini & Ercole, 2011). The latter could be separated macroscopically by the presence of orange tinges in pileus colour, orange red floccons in the annular zone, as well as microscopically by the structure of the pileipellis, which is hymeniderm-epithelium of clavate, vesiculose, pyriform and sphaeropedunculate elements (Vizzini & Ercole, 2011), obviously distinct from the hyphal trichodermium in the species described here.

Schulzer provided a very brief diagnosis of his *Agaricus fenzlii,* which nevertheless accounts the most important distinguishing characters of the species – vivid yellow coloured fruitbodies, stipe with an annulus and pink lamellae (Schulzer von Müggennburg et al., 1866). Schulzer's collection was said to have been found on rotting stumps of *Tilia* sp. No illustration of the fungus was included in the protologue, but such appeared for the first time six years later in Kalchbrenner (1873), along with more detailed description, based on Schulzer's original materials. The morphological features of the Bulgarian collection are consistent with the original description (Schulzer von Müggennburg et al., 1866), the redescription based on Schulzer's materials and ascribed to Schulzer (Kalchbrenner, 1873), as well as to the macroscopic and microscopic features accounted in recent works (Corriol & Moreau, 2007; Malysheva et al., 2007; Holec et al., 2018).

The species was described by Schulzer (Schulzer von Müggennburg et al., 1866) from the area of "Kamenitzer Wald" (nowadays in Frushka Gora National Park, Sremska Kamenica) in Serbia (Fig. 2). No further collections have been found since in any of the Balkan countries and for the moment in Eurasia the species is known from findings in France, Poland, Serbia, Slovakia, Hungary, Russia, Georgia, and Japan (*cf.* Holec et al., 2018). The Bulgarian specimen studied by us is thus the first occurrence of *P. fenzlii* in the Balkans after a gap of over one and a half century. The new locality is situated approximately 600 km from the *locus classicus* in Serbia, which is the closest site of occurrence so far known. It is also one of the southernmost documented in Europe, surpassed only by the French sightings in the Pyrenees (Corriol & Moreau, 2007). Further on, it extends the range of the species close to the shores of the Black Sea, which fact coupled with the so far known geographic range suggests the fungus may be looked for in other countries, e. g. Romania and Ukraine. *Pluteus fenzlii* is apparently widespread, but uncommon in Europe. According to the account in Holec et al. (2018) to date the largest number of records is known from Slovakia (10 sites) and Hungary (5 localities), while in the remaining European countries only solitary findings are documented.

The Bulgarian collections were all found in a single artificial forest massif in the northeastern part of the country. The dominant tree in the stand is *Tilia* sp. (over 60% presence) and accompanying woody species are *Quercus* spp. (incl. *Q. rubra* L.), and *Acer campestre* L. It is notable that all findings of *P. fenzlii* occurred on fallen stumps of lime trees in advanced stages of decay, similarly to the first finding of the species in Serbia (Schulzer von Müggennburg et al., 1866), although throughout its documented range it is known to occur on different broadleaf trees; particularly in Central Europe it is most commonly encountered on wood of *Quercus cerris* L., and certain variance is known between different parts of the continent (for extensive discussion on this topic consult Holec et al., 2018). The species occurred in four spots in the forest patch. *Pluteus fenzlii* apparently has wide temporal range of production of basidiomata, which occur from the end of May to the last days of November, but seems more common in the beginning of the summer. The precise dates of observations of fruitings were 25.05.2018 (43°33'10.0"N, 27°20'56.0"E), 05.06.2018 (43°33'10.0"N, 27°20'56.0"E), 30.05.2019 (43°33'10.0"N, 27°20'56.0"E), 04.06.2019 (43°33'08.0"N, 27°20'56.0"E), and 29.10.2019 (43°32'56.0"N, 27°20'43.0"E).

The genus *Pluteus* Fr. is a species rich group of pink-spored agarics, attracting much attention in the last decades, with previously undescribed species being introduced continuously from Europe (see e. g. Justo et al., 2014; Ferisin & Dovana, 2019; Ferisin et al., 2019; Ševčíkova et al., 2020;

Kaysuguz et al., 2021a). It is apparently insufficiently studied in Bulgaria, where merely 18 species are recorded up to date, a number of them known by single findings (Denchev & Assyov, 2010; Assyov & Stoykov, 2011). For comparison, Orton (1986) recognized some 43 species in Britain, while 33 were listed in other southern locales like the Iberian Peninsula and the Balearic Islands (Justo & Castro, 2007), and 35 species are presently known in neighboring Turkey (Kaysuguz et al., 2019, 2021a, b). The finding of such striking and easily recognized species as *P. fenzlii* in Bulgaria confirms that the genus is worthy of further attention and deserves targeted studies in this country.

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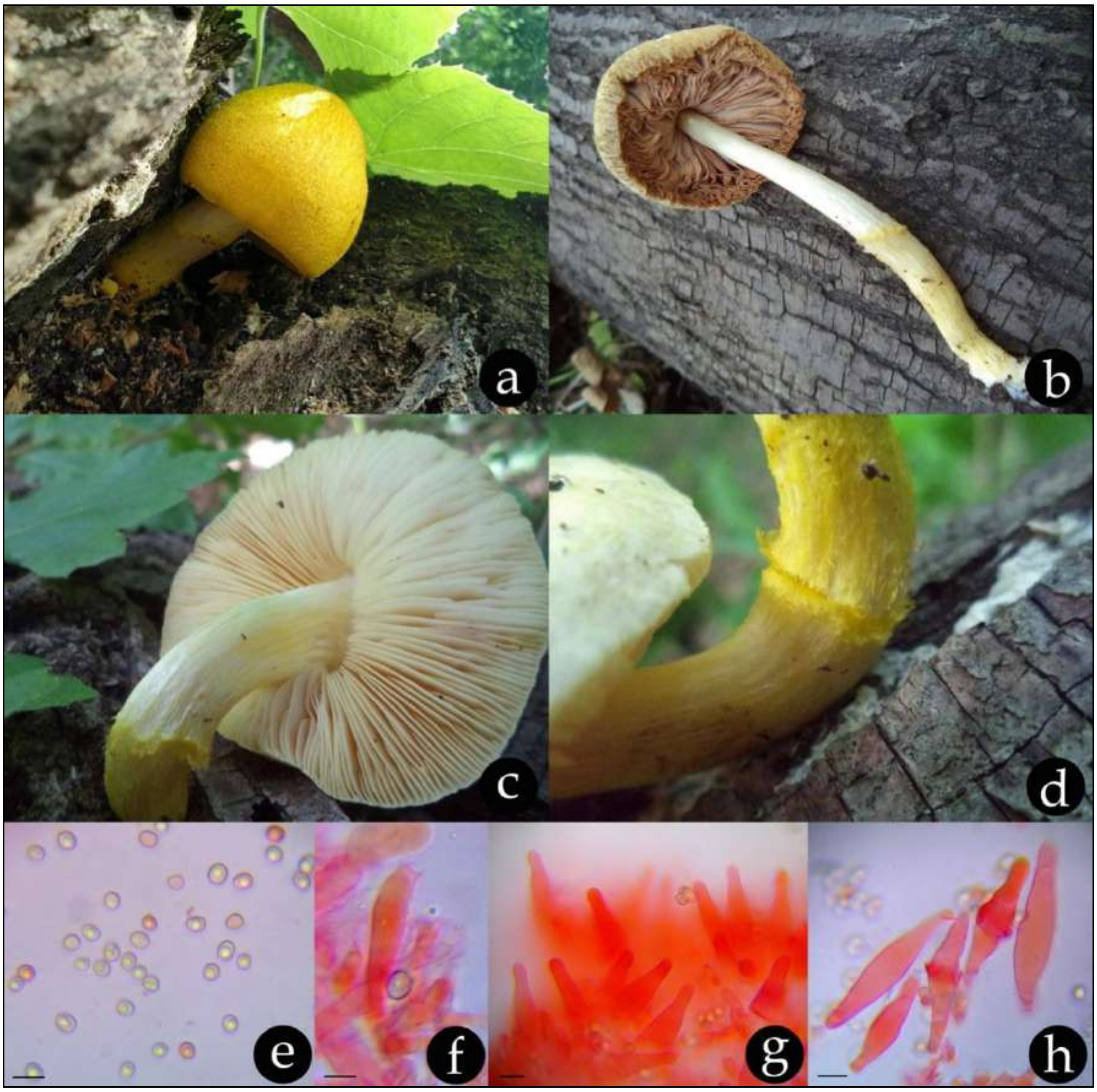

Fig. 1. Morphological features of *Pluteus fenzlii*: A,B,C,D – macromorphology of basidiomata, E – basidiospores, F – basidium, G,H – cheilocystidia. Scale bars = 10 µm (A–D not to scale).

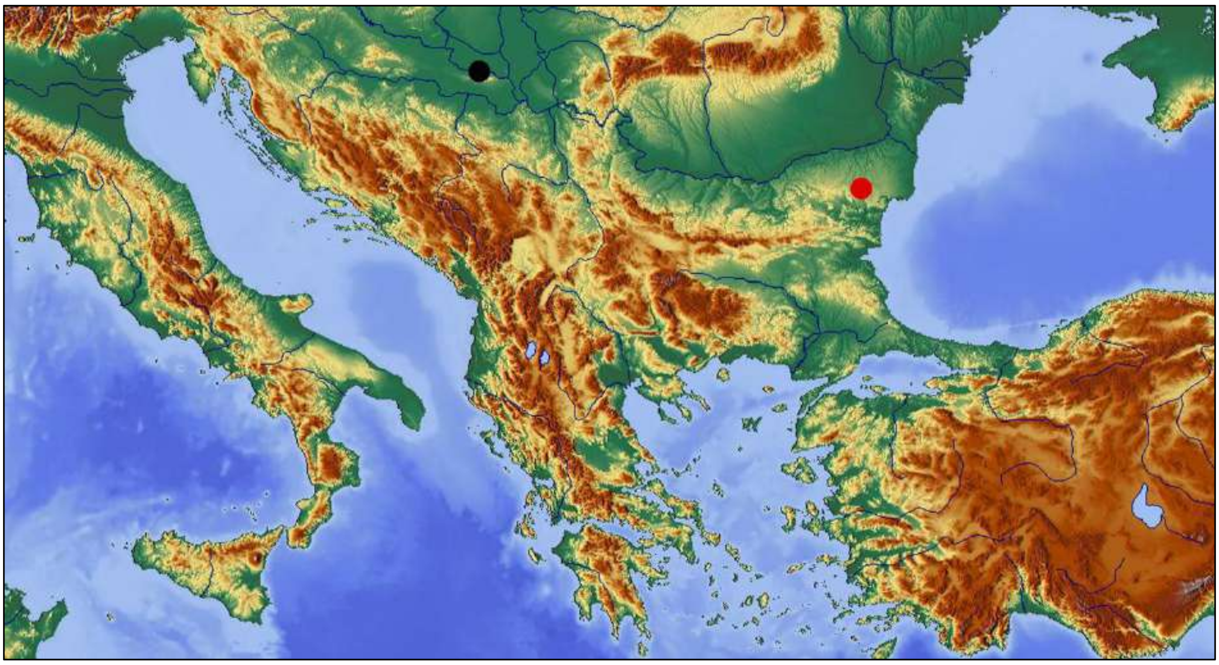

Fig. 2. Records of *Pluteus fenzlii* in the Balkan Peninsula. Black dot marks the *locus classicus* (historical occurrence) and red dot shows the locality reported in this paper.

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Short note

Age Determination of Harbour Porpoise (Phocoena phocoena relicta) from the Bulgarian Black Sea Coast

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Abstract. The following research presents the results of a pilot for Bulgaria age determination of one of the three cetacean species in the Black Sea, the harbour porpoise (*Phocoena phocoena relicta*), based on analysis of growth layers groups (GLGs). Knowledge of age structure and longevity (maximum age) are essential for modeling of marine mammal population dynamics. For small cetaceans, growth layers groups deposited in the teeth are most useful measure, because they indicate chronological age. Teeth were taken from 42 harbour porpoises (*Ph. ph. relicta*). The samples were collected from dead, stranded individuals from the Bulgarian Black Sea coast. The maximum duration of life found in this study is 10 years. The age structure shows dominance of individuals of two age groups - up to one year old and 5 years old. In the present study, no significant differences here found in the age structure in the different areas where the samples were taken, which leads to the conclusion that the age distribution of the stranded individuals *Ph. ph. relicta* in researched area is even.

Key words: Harbour porpoise, age determination, Black Sea, Bulgaria.

Introduction

One of the three cetacean species which are distributed in the Black Sea is the Harbour porpoise *Phocoena phocoena relicta* (Linnaeus, 1758). It is the second most abundant marine mammal species inhabiting the Black Sea and adjacent waters. But the Harbour porpoise is the most stranding cetacean on the Bulgarian Black Sea cost (Evtimova et al., 2016; 2018). A method of determining the age of sampled individuals is a necessity for the study of the population dynamics of any species. A previous study for age determination of the Harbour porpoise shows that 95% of specimens from the Black Sea are at the age of 11 or younger, from the Sea of Azov — 12 or younger (Gol'din, 2004). Other studies reported a life span of 10-12 years in the North Sea (Gaskin & Blair, 1977) and in the North Atlantic around the coasts of Greenland, the maximum age is 12 years in one animal and 17 years in another (Lockyer, 2001). This study aims to track the age determination of strandings harbour porpoises along the Bulgarian Black Sea Coast.

Material and Methods

A total of 42 animals, were obtained from coastal waters of the Bulgarian Black Sea

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coast, between January 2015 and November 2018. The samples are collected from dead bodies of harbour porpoises found on the sea shore during covering of transects. The selected places were located along the whole Bulgarian coastline (Table 1). The focus was mainly on relatively wild, uninhabited sandy and rocky beaches.

Table 1. The sampling sites along the Bulgarian Black Sea Coast.

Place	GPS coordinates	Place	GPS coordinates	Place	GPS coordinates
Durankulak	N 43.6771 E 28.5640	Aheloy	N 42.6390 E 27.6608	Pomorie	N 42.6039 E 27.6306
Durankulak	N 43.6809 E 28.5632	Pomorie	N 42.5821 E 27.6325	Pomorie	N 42.6062 E 27.6308
Durankulak	N 43.6929 E. 28.5641	Pomorie	N 42.6160 E 27.6321	Pomorie	N 42.5907 E 27.6317
Krapets	N 43.6579 E 28.5676	Pomorie	N 42.6213 E 27.6336	Pomorie	N 42.6018 E 27.6307
Krapets	N 43.6179 E 28.5737	Pomorie	N 42.6246 E 27.6350	Krapets	N 43.6468 E 28.5717
Ezerets	N 43.6039 E 28.5681	Pomorie	N 42.6256 E 27.6354	Aheloy	N 42.6286 E 27.6370
Ezerets	N 43.5116 E 28.5676	Pomorie	N 42.6272 E 27.6362	Krapets	N 43.7290 E 28.5734
Ezerets	N 43.5843 E 28.5743	Pomorie	N 42.6104 E 27.6311	Shabla	N 43.5582 E 28.5974
Shabla	N 43.5553 E 28.6010	Pomorie	N 42.5928 E 27.6313	Shabla	N 43.5457 E 28.6041
Shabla	N 43.5456 E 28.6441	Pomorie	N 42.5992 E 27.6307	Ravda	N 42.6413 E 27.68.08
Shkorpilovtsi	N 42.9409 E 27.9027	Shkorpilovsi	N 42.9409 E 27.9027	Shabla	N 43.5702 E 28.5851
Shkorpilovtsi	N 42.9955 E 27.8905	Ravda	N 42.6375 E 27.6710	Shkorpilovtsi	N 42.9526 E 27.8994
Kamchia	N 43.0148 E 27.8894	Ravda	N 42.6373 E 27.6776	Krapets	N 43.6181 E 28.5739
Kamchia	E 43.0130 N 27.8895	Aheloy	N 42.6262 E 27.6356	Krapets	N 43.6341 E 28.5768

Several methods have been used to determine the age of cetaceans but the examination of the tooth is at present the most reliable criterion and the most used for the Odontoceti. The teeth are collected from dead, stranded cetaceans on the coastline. Samples of teeth are collected from the central maxilla or mandibles of *Ph. phocoena.* The teeth are stored in 70% ethanol solution. The selected teeth are embedded in a polyester inclusion resin, then subjected to microtome cutting; later they are glued to a microscope slide on thin layers, colored with toluidine blue and covered with Canadian balm. The age of the Harbour porpoises was determined using dentinal Growth Layer Group (GLG) method (Boutiba, 2012) (Fig. 1).

Histological section of a tooth Odontoceti shows the following structure:

- A thin outer layer of enamel formed in the foetal period (Myrick, 1983).
- The neonatal line is the first of a series of layers of neonatal dentin, denoted

Growth Layer Group (GLG), and lies within the pulp cavity. This line is formed by the change of nutrition during the transition from foetal life to postnatal life (Nishiwaki & Yagi, 1953; Ross, 1977).

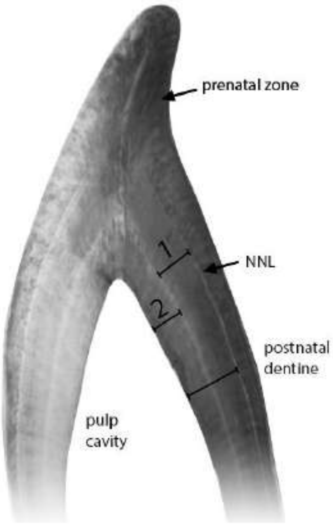


Fig. 1. Generalised longitudinal section of a porpoise tooth. *Legend:* NNL - neonatal line. 1 and 2 - Growth Layer Group (GLG).

The GLG in the secondary dentin correspond to one year in the Odontoceti.

The GLG consist of two sub-layers: a wide opaque area and a narrow translucent area.

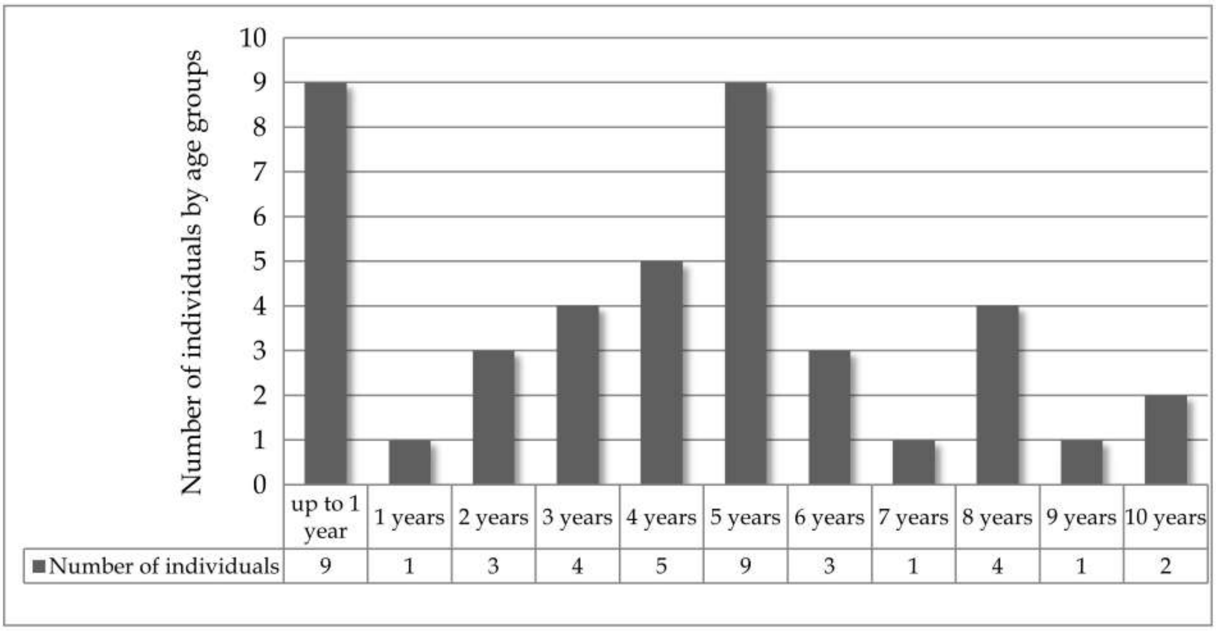
Results and Discussion

The current study present the results of a pilot for Bulgaria age determination of Cetaceans. The study was made based on samples from a total of 42 harbour porpoises.

The life span of the Harbour porpoise is up to 24 years (Reeves, 2002), but less than 5% from the animals live beyond 12 years (Koschinski, 2001), because there is high mortality in the first years of age.

The maximum age determined for harbour porpoise in our study is 10 years. Age structure shows clear dominance of individuals up to one year and those between five and six years (Fig. 2).

At this stage, it is not possible to explain the high mortality of individuals in the fifth year and to describe the reasons leading to this occurrence.



	up to 1 year	1 years	2 years	3 years	4 years	5 years	6 years	7 years	8 years	9 years	10 years
■ Number of individuals	9	1	3	4	5	9	3	1	4	1	2

Fig. 2. Distribution of the number of the studied dead harbour porpoises by age groups.

It can only be noted that this peak coincides with the middle life cycle of the Harbour porpoise (in this study), which is ten years (from birth to death). Thus, the life span of the Harbour porpoise in this study is comparable with the values reported from Gol'din (2004) - up to 11 years.

Conclusions

The maximum age determined for the Harbour porpoise in this study is 10 years.

The duration of life is studied in detail in the Harbour porpoise with two clear peaks of mortality rates at the beginning and mid-life periods.

In our study the age determination of *Phocoena phocoena* in the Black Sea does not show significant differences from previous studies on the same species in other seas or oceans.

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