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# Impact of climatic changes on the montane and alpine lake ecosystems (High Tatras, Western Carpathians)

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**Abstract.** Climate models show that the frequency of irregular climatic phenomena (extreme rains and hot days) are increasing in mountain environments. 1. Extreme precipitation causing irregular (but more frequent) floods and impacting the leaching of dissolved organic material from the water column. Due to the morphology and poor vegetation of high-mountain lakes, there is no return of allochthon organic matter. However, the process mentioned above is responsible for faster recovery (after flood) of lower altitude lakes with plenty of surrounding vegetation. The second effect brings increased mobilization of elements and metals within the ecosystem, triggering the leaching of heavy metals from the littoral zone to the aquatic environment and changing their levels in surrounding vegetation. 2. Changes in the characteristically low temperatures at higher-altitude lakes are due to the sensitivity towards increasing and fluctuating air temperatures. All processes and interactions in lake ecosystems are influenced by the high altitudinal gradient and differences between dystrophic (below the tree-line) and oligotrophic alpine lakes.

*Key words:* impact of flood, organic matter, mobilization of elements and metals, recovery, warming, water temperature

## Introduction

Lake ecosystems are often considered closed ecosystems without a significant impact from the surrounding terrestrial environment ("The Lake as a Microcosm" Forbes 1887). However, research showed that the littoral zone considerably contributes to organic matter turnover compared to the central part of the lake (Sala and Gude 2006). Most lakes are dependent on the supply of organic matter from the catchment area (Sobek *et al.* 2007).

It is the content of nutrients and organic matter that affects the entire ecosystem (Beracko *et al.* 2014). Two primary sources of organic matter are autochthons (from the lake) and allochthons (from the catchment area). Allochthons are responsible for most organic matter in the lake. The amount of organic matter depends on multiple physical (elevation, outflow, orientation, etc.) and climatic parameters (rainfall, droughts, etc.) (Hood *et al.* 2003; Sobek *et al.* 2007). Several parameters are used to indicate organic pollution, water quality, oxygen demand, and the total amount of organic matter (direct – COD, BOD and indirect – DOC, TOC)[1] (Seong-Tae *et al.* 2013).

Lakes located below the tree-line have significantly higher concentrations of dissolved organic matter than others situated above it (Hood *et al.* 2003). The lowest average amount of organic matter was recorded in alpine lakes (Sobek *et al.* 2007; Beracko *et al.* 2014). Such low values are due to the poorly drained soil and almost complete absence of vegetation (Kochjarová and Hrivnák 2017). On the contrary, an essential feature of lower located peat lakes is the high concentration of dissolved organic matter (Mladenov *et al.* 2005; Kapusta *et al.* 2018).

Water level fluctuation (WLF) is a neutral term commonly used to describe multimodal and predictable events. One example is snow melting in the springtime, which causes prolonged floods and changes in regularly flooded areas or periodic lakes. However, current climate models show that the frequency of these common hydrological phenomena is declining, while irregular climatic phenomena (extreme rains, prolonged droughts) are increasing (IPCC 2001; Christensen and Christensen 2003). These are responsible for unpredictable and drastic changes in water level fluctuation (for example, large-scale floods). Floods have proved to rapidly mobilize large amounts of organic matter and terrestrial organic inputs in water (Mladenov *et al.* 2005; Steven and Melack 2012). This displays an interesting relationship between leaching and the transfer of organic matter from the water column to ATTZ2 (Nogueira *et al.* 2002; Wantzen *et al.* 2008) and subsequent reverse washing out/leaching of organic matter from vegetation and soil, which increases nutrient content in water (Sobek *et al.* 2007; Steven and Melack 2012).

Floods represent hot biochemical events (McClain *et al.* 2003) for heavy metal concentration / accumulation in soil and sediments of aquatic environments (Szabó *et al.* 2008; Du Laing *et al.* 2009). They can trigger the leaching of heavy metals into the water environment within ecosystems (Chrastný *et al.* 2006). Water level fluctuation (also due to floods) has an undoubted impact on the ecology of aquatic habitats. Researchers have mainly observed this phenomenon in rivers (Junk *et al.* 1989; Tockner *et al.* 2000; Junk and Wantzen 2004), their deltas, swamps, regularly flooded areas, and lower altitude lakes tightly surrounded by vegetation (Coops *et al.* 2003; Mladenov *et al.* 2005; Mooij *et al.* 2005).

Attention has also been paid to recovery processes following this type of events (Sparks *et al.* 1998; Schiemer *et al.* 1999). How do floods affect specific aquatic alpine ecosystems with shallow coastal zone, little to no vegetation, and underdeveloped ATTZ? What is their recovery process?

Water temperature is a highly significant factor and environmental variable (Šporka *et al.* 2006). The surface water temperature fluctuates during the year depending on the season and current weather (Hrivnáková 2019). Alpine lakes show low water temperatures throughout most of their annual cycle (Šporka *et al.* 2006; Hrivnáková 2019). Temperature is important in the evaluation of oxygen ratios, the rate of degradation of organic matter, the acidity of the lake and the suitability of the environment for the occurrence of aquatic organisms, but also for entire lake ecosystems (Doláková and Janýšková 2012).

We consider ecosystems of High Tatra lakes to be ideal and critical locations for the study of global climate changes in the environment.

[1] COD (chemical oxygen demand) – non-specific and complex indicative measure (Judová *et al.* 2015), BOD (biochemical oxygen demand), DOC (dissolved organic carbon), TOC (total organic carbon) (Seong-Tae *et al.* 2013).
[2] ATTZ (The Aquatic-Terrestrial Transition Zone) – represents a flood zone in the event of level fluctuations (even in flood situations) between water and land.

## Material and Methods

### *Study area*

The High Tatras are a small mountain range (26 km long) with a typical alpine relief formed by glaciation (Kapusta *et al.* 2018). The youngest natural formations, "plesá" (glacial lakes), represent an important and characteristic component of these high mountains, and were precisely formed due to this glaciation (Lackovič 2015; Kapusta *et al.* 2018). Often, we mistakenly include lakes that were formed in the interglacial period which had nothing to do with glaciation. Nevertheless, even these lakes are still ecologically significant (Lackovič 2015). They represent more than 90 % of all lakes in Slovakia (Štefková and Šporka 2001). The High Tatras lakes lie on a relatively large altitude gradient, and the forest boundary is the most important ecological divide (Krno *et al.* 2010). The vast majority of lakes are located above the forest border, in the alpine zone (Štefková and Šporka 2001; Beracko *et al.* 2014). They are transparent, deep lakes with a shallow coastal area (Hanušin 2009) and a characteristic low content of nutrients (oligotrophic) due to insufficient supply of organic material from the subsoil and a lack of vegetation in the area (pH ≤ 7) (Beracko *et al.* 2014). Low temperatures are also typical during most of the annual cycle (Hrivnáková 2019).

The second type of formation are montane, shallow lakes, which gradually overgrow the surrounding vegetation (Kapusta *et al.* 2018). Their water is mostly colored brown from humic acids (leached from peat), and we classify them as dystrophic lakes (pH = 3.5-5.5) (Górniak *et al.* 1999; Slovenská lesnícka spoločnosť 2012a; Beracko *et al.* 2014). They have higher temperatures, especially during summer (Kochjarová and Hrivnák 2017).

### *Climatic conditions of the area*

The climate of the High Tatras has a specific set of characteristics that significantly influence the existing ecosystems and environmental phenomena, as well as their further development. In terms of temperature and precipitation, the Tatras region is characteristic of its extreme and unstable weather (compared to other regions within Slovakia). The most general sign of this is a decrease in air temperature with increasing altitude (it decreases by 0.6 ˚ C every 100 m upward). In the future, this effect could be disrupted by gradual warming and dry seasons. Total precipitation increases at higher altitudes. Relatively high precipitation and low evaporation cause excessive outflow. Therefore, in the months with the highest precipitation and most significant storm activity, there is an increase in water levels, bringing more extensive erosion and devastating floods. All these extreme environmental phenomena can trigger (as they did in the past) significant changes in the ecosystems of mountain lakes (Slovenská lesnícka spoločnosť 2012a; Lacika 2020).

[3] Summer floods (1662; August 1813; July 1845; June 1958; July 2001; August 2008, 2011, 2019). Spring floods from melting snow are rather exceptional and do not cause as much damage as summer ones (Slovenská lesnícka spoločnosť 2012a).

### *Fieldwork*

This study was part of a larger study of the Ecosystems of the High Tatras mountain lakes as environmental indicators. Fieldwork and sampling took place during the summer seasons (from May to October) of 2019 and 2020. Due to the longer summer, without snow or frost, we continued sample collection into November 2020.

To the extent possible, we aimed to collect samples from different lakes in the same weather conditions during warm and sunny days without any fluctuations or extremes, so that changes in climate would not influence content. We successfully collected samples from 101 lakes located in 14 valleys of the High Tatras, 17 in northern reaches and 84 in their southern counterparts (Appendix 1 and 2). Independent work taking place directly at the location of individual lakes consisted of water, (top) sediment from littoral zone4 , and bryophyte5 sampling (details in Table 1) followed by in situ measurements of physical parameters (Fig. 1).

[4] The littoral zone and its differences between alpine lakes and lakes under tree-line are characterized in the Study area. Due to the rocky subsoil and the absent littoral zone, it was not possible to take sediment samples at some of the above-located lakes. Conversely, in some significantly overgrown lower lakes, it was also impossible to take sediment samples from the littoral peat zone.
[5] We took one or two different samples of bryophytes near the water surface, mainly in the littoral and limouse ecophase and in the terrestrial ecophase (sensu Hejný 1960) from each locality.

| | | Container | Amount | Method |
|---|---|---|---|---|
| **WATER** | measurement of physical parameters:<br><br>• water temperature [° C];<br>• pH;<br>• electrical voltage [mV];<br>• salinity;<br>• conductivity [μS/cm];<br>• resistance [kΩ*cm];<br>• TDS (Total Dissolved Solids) [mg/l];<br>• dissolved oxygen [mg/l ,%, mbar]; | in situ | | from the lake shore with a portable WTW 3430 multimeter (Geotech, Weiheim; Germany) with compatible probes:<br><br>• IDS pH electrode Sen TixR 940-3;<br>• conductivity electrode TetraCon 925-3;<br>• optical oxygen electrode FDO 925-3; |
| | sampling for subsequent analysis | plastic bottle | 0.7 l | from the lake shore |
| **(TOP) SEDIMENTS** | sampling for subsequent analysis | plastic vial | 0.05 l | from the lake shore, surface sediments up to 15 cm from littoral zone[4] |
| **BRYOPHYTES** | sampling for subsequent analysis | plastic Zip Lock bag | 1 - 2 sp. | on the shore of a lake, near a body of water, or in water[5] |

**Table 1.** *Fieldwork:* sample collection (sample type, sample containers, sample quantity, sampling method).
Note: All containers were properly disinfected and labeled prior to sample collection. We made sure that the intervals between transport, storage, and subsequent analysis in the laboratory were as short as possible.

**Fig. 1.** Litvorové pleso and Zelené Kačacie pleso (no.96 and 97) (North 1860 and 1675 m asl..) are permanent lakes belonging to the oligotrophic category. Litvorové pleso is a kettle lake in the alpine zone, while Zelené Kačacie pleso is a transitional type of lake in the zone of mountain pine. (31.8.2020 side valleys of Bielovodská dolina – Litvorová and Kačacia dolina (photo: K. Hrivnáková).

*Laboratory work*

*Chemical oxygen demand (COD) – Permanganate (Kubel) method*

COD is a complex indicator of organic pollution, whether biodegradable or not (Judová *et al.* 2015). It is defined as the amount of oxygen consumed under specified conditions to oxidate organic matter in water by a potent oxidizing agent. This nonspecific parameter is expressed in mg/l (Diviš 2008).

We made measurements in three boiling flasks with the same sample (100 ml of lake water) to average the result for a more accurate measurement. The method used is based on the oxidation of organic substances with 20 ml of a solution of potassium permanganate ($K_2MnO_4$) (0.002 mol/l) in 5 ml of sulfuric acid ($H_2SO_4$, 96 %) diluted (1 : 2) at boiling for 10 minutes. We placed 4-5 cooking stones in each boiling jar and covered the jar with a watch glass. Oxidation occurs when there is an excess of permanganate. After oxidation is complete, unreacted $KMnO_4$ is reduced with an excess of standard oxalic acid $(COOH)_2$ solution (0.005 mol/l), which is added in the exact amount (20 ml) to the sample. The solution is then decolorized to a clear solution. It is back – titrated with potassium permanganate (0.002 mol/l) to $KMnO_4$ until it turns a faintly pink colour. Consumption during titration shows the consumption of manganese for the oxidation of organic substances.

Equations:

$MnO_4^- + 5e^- + 8H^- \rightarrow Mn^{2+} + 4H_2O$
$2MnO_4^- + 5(COO)_2^{2-} + 16H^+ \rightarrow 2Mn^{2+} + 10CO_2 + 8H_2O$

*Photometry*

To determine other chemical indicators of water quality ($Cl^-/NaCl/CaCO_3$, $S/SO_4^{2-}$, ammonia $N/NH_3/NH_4$, $N/NO_3^-$, $P/PO_4^{3-}$ and the content of total water hardness $CaCO_3$) in the lakes, we used optical-analytical method – photometry. The essence of photometric methods is the passage of a substance and absorption by a portion of the light spectrum. The area of light absorbed by the substance and the absorption intensity measured by the photometer depends on the particular substance. Concentrations of ions in our samples were determined by the YSI EcoSense 9500 (YSI, USA) photometer and accessories compatible with this water analyser. Test procedures require different specific reagents for every chemical parameter. Measurement with this optical – analytical method was performed using the YSI EcoSense 9500 (YSI, USA) measurement instructions.

*Drying of samples*

Sediment and bryophytes samples were dried separately on Petri dishes (3 hours at 60° C) in an IF 160 Plus dryer (Memmert, Germany). Samples of bryophytes were divided into upper (photosynthetic) and lower parts, contaminated with soil, before drying. Only the top sections were used for further analysis.

*Milling of samples*

For further processing of the samples, it was important to homogenize it into one common sample – removing the pieces from stones, roots and grinding them. A ceramic mortar (used in some bryophytes) can be used for initial homogenization and grinding. We used a cryogenic ball mill (CryoMill Retsch, Germany), which uses ball impact and continuous cooling, grinding vessels, and liquid nitrogen – maintaining a temperature of –196 ° C.

- We ground the upper parts of the bryophytes for 30 seconds to 1 minute with a frequency of 20 Hz.
- The sediments were ground for 1 minute at a frequency of 30 Hz.
- Subsequently, we analysed the correctly homogenized samples for surface contamination by two methods.

*X-ray spectrometry analysis*

We used X-ray spectrometry to determine the values of some chemical elements (trace elements) in homogenized sediments (milled) and upper parts of bryophytes (milled). We used a DELTA handle ED–XRF spectrometer (Bas, Rudice, Czech Republic) with a Delta XRF Portable WorkStation tripod (Olympus, Innov-x Systems, USA). Samples were analysed in plastic cuvettes with plastic foil at the bottom for 240 s in three 80 s intervals, from which the average was calculated. The analytical methods and calibration procedures used in the laboratory shall comply with internationally accepted standards (Spectrapure Standards, Norway).

The detection limits were determined continuously for each measurement and each element by software using the Compton Normalization method.

*Mercury analysis*

Mercury concentrations in the upper parts of bryophytes (unmilled) and sediments (milled) were analysed with a DMA-80 mercury analyser (Milestone, USA) using nickel boats which were always cleaned after six measurements. The cleaning procedure was repeated until absorbance was stable and lower than 0.001. A dried sample is taken at a high temperature (650 degrees) and burned for decomposition in the apparatus. The temperature level guarantees complete decomposition and release of mercury. During and after decomposition, pyrolytic gases are blown by a stream of oxygen into the amalgamator. The mercury is retained in the amalgamator, and all other gases are eliminated from the system before measurement. The device provides us with an accurate measurement of the mercury content. The mercury concentration, measured in mg/kg, is calculated automatically when the measurements are calibrated to the weight of each sample. The accuracy of this method was determined by analysis of the reference material (tobacco leaves) (ICHTI, Poland). The determined value of tobacco leaves agreed well with the certified value of 0.0232 mg/kg (tobacco leaves) and fell within the limit of uncertainty specified for the material.

*Statistics*

The potential synergistic effect of the individual measured variables was evaluated by principal component analysis (PCA). Correlation between component (PC 1) value with elevation was plotted using polynomial regression curves and verified by F test ($p < 0.05$). One–way ANOVA and its nonparametric equivalent the ANOVA – Kruskal – Wallis test (and their graphical representation) were used for statistical comparison of individual factors with the properties of observed ecosystems and seasonality, with a disproportionate number of samples at a 95 % confidence level ($p < 0.05$). Some measured variables were summarized by means of standard deviation. Statistica 8 software for Windows (TIBCO, USA) was used for data analysis.

## Results

In addition to the monitored physical and chemical parameters of water in lakes and mercury in bryophytes and sediments, we recorded ten elements in bryophytes and twelve elements in surface sediments of the littoral zone above the detection limit by X-ray spectrometry (Table 2).

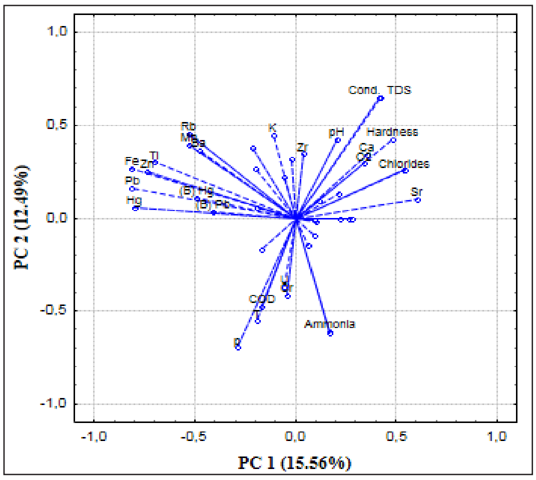
The analysis of the main components revealed 45 components, of which only seven factors explain more than 5 % of the interpretable variability[6].

[6] Our work focused on interpreting the first two components (PC 1 and PC 2) with the greatest interpretable variability due to the enormous scope of work.

The first component (PC 1), with the largest interpretable variability of 15.56 %, describes to us the combined relationship of descent/ascent of measured metals (Pb, Hg, Fe, Zn, Ti, Rb, Mn, Ba) in the upper layers of the littoral zone versus ascent/descent of dissolved substances (TDS, salts and chlorides) in the water in addition to hardness and conductivity (Fig. 2). The first factor also describes the interdependence of Hg and Pb concentrations in sediments and the concentration of these elements in bryophytes. The second factor (PC 2 – 12.49 %) is a bipolar vector that describes an increase/decrease in dissolved organic matter, ammonia, temperature, and water resistance, compared to changes in the number of solutes in water (TDS), its pH, conductivity, and hardness along with some elements in sediments (mostly cations) (Fig. 2).

*Impact of vertical distribution*

As the elevation increases, the content of dissolved substances (TDS, salts, and chlorides) in the lake




° Active

**Fig. 2.** Projection of the variables on the factor – plane (PC 1 x PC 2).
The graph shows the correlated structures between the variables of the first and second components. Variables located along the same directional axis are positively correlated with each other. The variables located on the opposite side of the graph are negatively correlated with each other. The variables placed in the middle are weak predictors, and their descriptions have been omitted for clarity. The horizontal axis explains 15.56 % and the vertical 12.49 % of the total variability.

| Variable | PC 1 | PC 2 |
|---|---|---|
| (B) Hg | **-0.49** | **0.11** |
| (S) Hg | **-0.80** | 0.06 |
| (W) COD | -0.17 | **-0.47** |
| (W) t | -0.19 | **-0.55** |
| (W) pH | 0.20 | **0.42** |
| (W) U | -0.05 | -0.37 |
| (W) Concent. $O_2$ | 0.34 | 0.29 |
| (W) $O_2$ | 0.22 | -0.01 |
| (W) Satur. $O_2$ | 0.09 | -0.09 |
| (W) Conduct. | **0.42** | **0.65** |
| (W) TDS | **0.42** | **0.65** |
| (W) p | -0.29 | **-0.70** |
| (W) $Cl^-$ | **0.54** | 0.26 |
| (W) NaCl | **0.54** | 0.26 |
| (W) Tot. hardness $CaCO_3$ | **0.48** | **0.43** |
| (W) Chlorides $CaCO_3$ | **0.54** | 0.26 |
| (W) $SO_4^{2-}$ | 0.27 | 0.00 |
| (W) S | 0.28 | 0.00 |
| (W) Ammonia N-$NH_4$ | 0.17 | -0.61 |
| (W) Ammonia N | 0.17 | -0.62 |
| (W) Ammonia N-$NH_3$ | 0.17 | -0.62 |
| (W) $PO_4^{3-}$ | 0.10 | -0.02 |
| (W) P | 0.10 | -0.01 |
| (B) S | -0.17 | -0.17 |
| (B) K | 0.12 | 0.09 |
| (B) Ca | 0.22 | 0.13 |
| (B) Cr | 0.07 | -0.14 |
| (B) Mn | -0.22 | 0.38 |
| (B) Fe | -0.20 | 0.27 |
| (B) Zn | -0.19 | 0.06 |
| (B) Rb | -0.02 | 0.32 |
| (B) Ba | -0.05 | 0.22 |
| (B) Pb | **-0.41** | 0.03 |
| (Ls) K | -0.11 | **0.45** |
| (Ls) Ca | 0.35 | 0.34 |
| (Ls) Ti | **-0.70** | 0.30 |
| (Ls) Cr | -0.04 | **-0.41** |
| (Ls) Mn | **-0.53** | **0.40** |
| (Ls) Fe | **-0.81** | 0.27 |
| (Ls) Zn | **-0.74** | 0.26 |
| (Ls) Rb | **-0.53** | **0.45** |
| (Ls) Sr | **0.60** | 0.10 |
| (Ls) Zr | 0.04 | 0.35 |
| (Ls) Ba | **-0.48** | 0.36 |
| (Ls) Pb | **-0.81** | 0.16 |
| Variance % | 15.56 | 12.49 |

**Table 2.** Weights of PC 1 and PC 2 with the percentage of variation in principal component analysis of the physical and chemical variables of water (W), 13 elements in the littoral (surface) sediments (Ls), and 11 elements in the bryophytes (B) (N = 83). Significant correlations are bold.

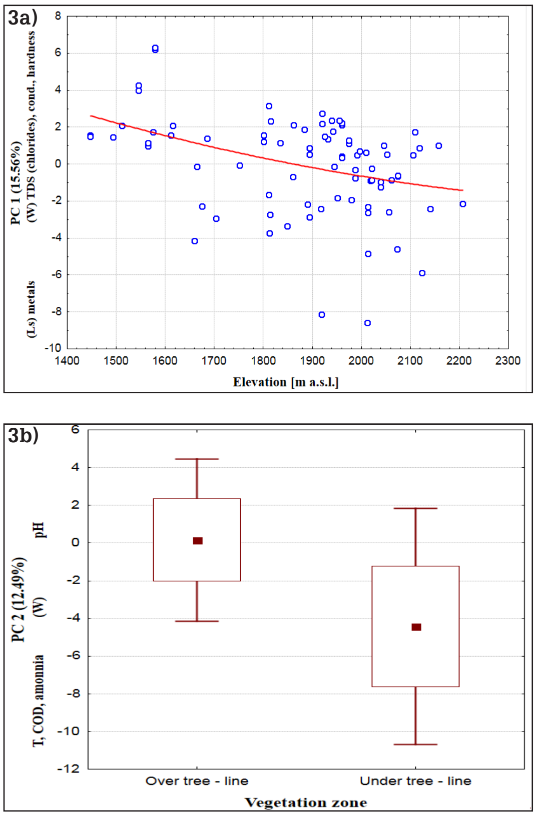



**Fig. 3.** Principal components PC 1 and PC 2 in relation to the vertical division. **3a)** Metal levels in the upper sediments of the littoral zone increase with elevation, while dissolved ion concentrations decrease. PC 1 = 19.4027 – 0.0157 * Elevation + 2.8425 $E^{-6}$ * Elevation$^2$; r = – 0.3771, p = 0.001. **3b)** The higher temperature, the amount of dissolved organic material and ammonia in the water, and the lower pH are in the lakes below the tree-line than above the tree-line. PC 2: VEGETATION ZONE: KW ANOVA – H (1, 83) = 5.4857; p = 0.019. (means, box - mean SD, whisker - ±1,96*SD).

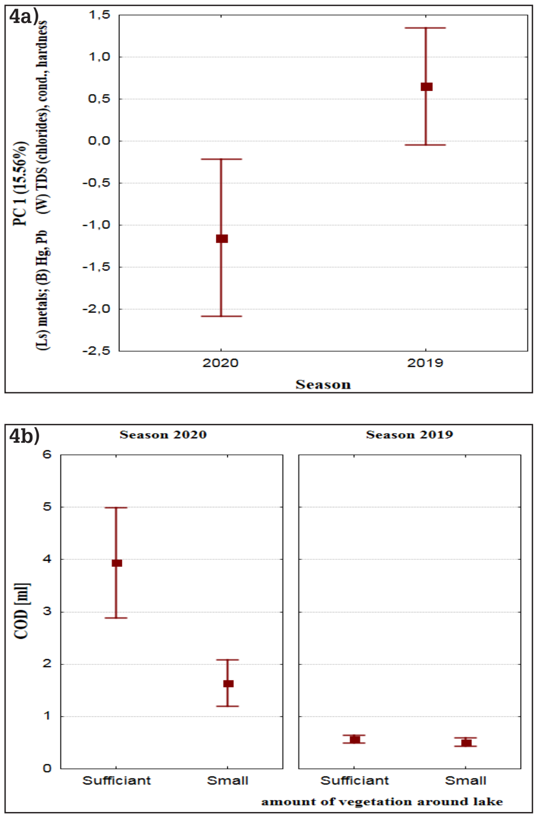



**Fig. 4.** Differences in concentrations of observed variables between the two seasons 2019 and 2020 for factors PC 1 and PC 2 denoted by Means with standard errors at 0.95 confidence interval. **a)** Levels of conductivity, dissolved substances (salts, chlorides), and water hardness decrease in 2020 compared to the rise of metals in the upper sediments and Hg, Pb in bryophytes. PC 1 : SEASON 2019/2020: KW Anova – H (1,83) = 10.448, p = 0.001. **b)** The levels of pH, conductivity, solutes (salts, chlorides), and water hardness together with cations in the upper sediments decreased in 2020 compared to the increase in the amount of organic matter, water temperature, voltage, resistance, and ammonia in the water. PC 2: Season 2019/2020: KW Anova – H (1,83) = 4.000, p = 0.046.

water decreases, along with its conductivity and hardness. However, in these higher-altitude lakes, the metal concentrations in the upper layers of the littoral zone are more pronounced (Fig. 3a; r = 0.38, p = 0.001). Lakes below the tree-line have higher temperatures and dissolved organic matter content (higher ammonia levels) in the water but lower pH values than alpine lakes (Fig 3b; p = 0.019).

*Impact of seasonality*

Even though the research took place during the summer season, we cannot rule out the influence of individual months. The effect was significant for both factors:
PC 1 – H (5, 83) = 14.9552, p = 0.001;
PC 2 – H (5, 83) = 30.0844, p = 0.000;

With both factors, the content of dissolved substances (salts, chlorides) increased in the autumn months.

However, in addition to the effect of individual months, we observed a surprising difference between the summer seasons of 2019 and 2020. This impact was also observed for the first (PC 1) and the second component (PC 2) (Table 2). The first component (PC 1) mainly describes an increase in water-soluble substances (cations and anions), their conductivity, and water hardness in 2019 against the growth of metal concentrations in the littoral zone of lakes and the growth of metals (Hg, Pb) in bryophytes in 2020 (Fig. 4a; p = 0.001). While the second factor (PC 2) is important to demonstrate the significant difference in water parameters between the two seasons. In addition to the already mentioned differences in conductivity, TDS (cations), and water hardness, the second factor shows us the relationship of the decrease in pH in 2019 antagonistically to the increase of COD levels, temperature, voltage, resistance, and ammonia in water in the 2020 season (Fig. 4b; p = 0.046). An important finding (Fig. 5), shows the effect of the amount of vegetation around the lake on the COD values in 2020, while we did not demonstrate this effect in 2019.

The average COD value for all monitored lakes for both summer seasons (2019 – 2020) was 1.82 (± 2.32) ml (Table 3). In 2019 it was around 0.55 (± 0.20) ml, and in 2020 the average COD value increased to 3.14 (± 2.58) ml. The minimum measured value of COD (0.22 ml) was measured in the summer season of 2019 (October), at the highest stand-

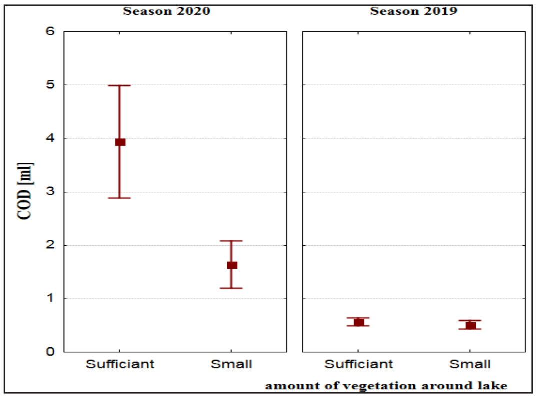



**Fig. 5.** The effect of the amount of vegetation on the growth of dissolved organic material in lakes in the 2020 season denoted by Means with standard errors at 0.95 confidence interval. In 2020, enough vegetation around the lake impacted the growth of the organic composition in the water and the decrease in pH. Season 2020 COD: amount of vegetation: KW Anova – H (1, 46) = 7.878, p = 0.005; Season 2019 COD: amount of vegetation: KW Anova – H (1, 53) = 0.913, p = 0.340.

ing lake, Modré pleso (no. 76). This year did not exceed the maximum value of 1.15 ml (September). There was only one such high COD value this season, measured in Slavkovské pliesko. In the summer season of 2020, there was a significant increase in the COD value, and some lakes had values exceeding 10 ml (the max. value set by us). This included both lakes under the tree-line zone (lakes Čierne pleso, no. 41 in valley Motykova dolina and Žabie pliesko, no. 52), as well as also alpine lakes (Prostredné Spišské pleso, no. 72 and Skalnaté oko, no. 78). However, throughout the study of alpine lakes, the mean COD values were significantly lower, at 1.55 (± 1.99) ml than for lakes under the tree-line zone 5.31 (± 3.52) ml. The average pH values in both seasons (Table 3), for both alpine lakes and lakes under tree-line zone, ranged from 7.337 to 7.765. The lowest measured pH (94.634), was measured in Trojrohé pleso (June 2020), while the highest measured pH (9.084) was measured in lake Skalnaté pleso, no. 77 (September 2020). The average value of the water temperature in the monitored lakes was 8.6° C. The lakes located in the alpine have an average temperature of 8.1° C in the summer season (with a minimum value of 0.9° C), while the lakes located under the tree-line zone reached an average value of up to 14.8 ° C (with a minimum value of 11.8° C) (Table 3). The highest measured temperature (20.1° C) of water was found in Čierne pleso, no. 41, in Motykova dolina (May 2020). However, some alpine lakes also reached high temperatures – up to 18.9° C - as was the case with Nižné studené pleso, no. 64 (August 2019).

## Discussion

The interaction between individual components of aquatic mountain ecosystems (Table 2; Fig. 2) is a process sensitive to external changes and factors (Wathn *et al.* 1995).

The first crucial factor, explained by the first component (PC 1 – 15.56 %), that influences the environmental condition of mountain lakes is their mineralization. This relationship between concentrations of ions within the water column is expressed by the amount of total dissolved solids (TDS) in the form of cations and anions, as well as their connectivity (Tölgyessy *et al.* 1984; Judová *et al.* 2015) or inputs from the bedrock (in this case, elements from the littoral zone) (Du Laing *et al.* 2009). Higher TDS values and connectivity indicate higher solubility of substances in water, which are influenced by increased water hardness (mainly cations) (Diviš 2008) (Table 2).

This process and changes within aquatic ecosystems are also a result of influences from surrounding vegetation, which affect the amount of organic matter in lakes (Du Laing *et al.* 2009). More vegetation around the lakes affects the build-up of organic matter that increases the acidity of the water (Kopáček *et al.* 2006; Beracko *et al.* 2014) and ammoniacal nitrogen concentration (Doláková and Janýšková 2012) (Table 2; Fig. 2) The second component (PC 2) explains 12.49 % of the total variability.

### *Impact of vertical distribution*

Vertical distribution undoubtedly impacts the factors mentioned above (Fig. 3a, b). The high altitudinal gradient of the High Tatras and tree-line de-

| | | COD [ml] | | | pH | | | t [° C] water temperature | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Arithmetic Mean ± SD | Min. | Max. | Arithmetic Mean ± SD | Min. | Max. | Arithmetic Mean ± SD | Min. | Max. |
| **2019 + 2020** | 99 | 1.82 ± 2.318 | 0.22 | >> 10 | 7.624 ± 0.784 | 4.634 | 9.084 | 8.6 ± 4.231 | 0.9 | 20.1 |
| **2019** | 53 | 0.55 ± 0.196 | 0.22 | 1.15 | 7.765 ± 0.654 | 5.444 | 8.990 | 7.9 ± 4.064 | 1.7 | 18.9 |
| **2020** | 46 | 3.14 ± 2.581 | 0.56 | >> 10 | 7.462 ± 0.891 | 4.634 | 9.084 | 9.4 ± 4.329 | 0.9 | 20.1 |
| **Lakes over tree-line** | 92 | 1.55 ± 1.991 | 0.22 | >> 10 | 7.645 ± 0.780 | 4.634 | 9.084 | 8.1 ± 3.939 | 0.9 | 18.9 |
| **Lakes under tree-line** | 7 | 5.31 ± 3.518 | 0.40 | >> 10 | 7.337 ± 0.844 | 5.777 | 8.138 | 14.8 ± 3.031 | 11.8 | 20.1 |

**Table 3.** The average values (± with standard deviations) of mercury and lead in bryophytes and upper sediments of lakes with mutual correlations. (Ls) – littoral (surface) sediments, (B) – bryophytes.

termining the amount of vegetation, as well as lake morphology, play an essential role in these processes (Krno *et al.* 2010; Beracko *et al.* 2014). In higher altitude lakes, their shallow coastal zone (Hanušin 2009) with a lack of vegetation affects the supply of organic matter (Beracko *et al.* 2014) and causes shortages of dissolved organic material in the water. The highest located permanent lake, Modré pleso (no. 76; 2189 m asl.), with a minimum value of 0.22 ml (2019), and Zamrznuté pleso (no. 92; 2040 m asl.), having a minimum value of 0.56 ml (2020), function as examples. These are typically clean, oligotrophic lakes with low concentrations of solutes in the water column (Beracko *et al.* 2014), and higher levels of metals stored in the littoral zone (Fig. 3a). However, this depends on the bedrock composition, its weathering, and inputs from the atmosphere (Slovenská lesnícka spoločnosť 2012b).

Although the majority of the High Tatra lakes are located in the alpine or sub-alpine zone (Štefková and Šporka 2001; Beracko *et al.* 2014) (Table 2), even the ones found under the tree-line (in the montane zone) are ecologically significant (Lackovič 2015). We mostly talk about shallower lakes with intended coastal zones and plenty of vegetation by which they are often gradually overgrown (Beracko *et al.* 2014; Kapusta *et al.* 2018). Their higher temperatures (Kochjarová and Hrivnák 2017) and surrounding vegetation cause higher concentrations of organic matter (Górniak *et al.* 1999; Hood *et al.* 2013) and more acidic environments (Kopáček *et al.* 2006) (Table 2; Fig. 3b). We classify them as dystrophic lakes while observing that many are on their way to extinction (Lackovič 2015; Kapusta *et al.* 2018). Examples of this include Trojrohé pleso (no. 83; 1611 m asl.), which had the lowest pH (4.634), and Jamské pleso (no. 5; 1447 m asl.), overgrown by mountain pine, with a pH of 5.777.

*Temperature as an essential factor*

The average temperature of examined lakes was 8.6° C. Decreasing air temperature with increasing altitude is the standard (it falls by 0.6° C every 100 m upward) (Slovenská lesnícka spoločnosť 2012a). In summer, alpine lakes represented by the highest located lake, Modré pleso (no. 76, 2189 m asl. – October 2019), reached an average temperature of 8.1° C (with a low of 0.9° C). The lakes located under the tree-line reached an average temperature of 14.8° C (with a low of 11.8° C). These mountain lakes (dystrophic) have significantly higher water temperatures than their higher-altitude counterparts (oligotrophic) (Fig. 3b). The highest temperature (20.1° C) within the examined lakes was recorded in the lowest-altitude dystrophic lake - Čierne pleso, in Motyková dolina (no. 41; 1235 m asl. – May 2020). Some alpine lakes also reached high values (18.9° C), as was the case of Nižné studené pleso (no. 64; 1811 m asl. – August 2019). However, this particular lake often dries out due to its shallowness (Lackovič 2015), and the "extremely" high water temperature was undoubtedly influenced by hot weather on the day when measuring took place.

Mountain lakes are sensitive to air temperature and react to it immediately, especially in the summer months (Šporka *et al.* 2006; Hrivnáková 2019). This will likely continue into the future, due to gradual warming and droughts (Slovenská lesnícka spoločnosť 2012a; Vido *et al.* 2015). Moreover, water temperature plays a significant role in evaluating oxygen ratios, acidity, water voltage, and organic matter degradation (Judová *et al.* 2015), which is apparent when looking at the correlations between these variables (Fig. 2).

Some effects mentioned above can be expected, based on the influence of seasonality. Increasing amounts of chloride (solute content) and organic matter (due to the leaching of decaying vegetation in autumn) are present with both factors (Mikuš 2012) (Results – Impact of seasonality). However, the contrast between the two summers is surprising (Fig. 4a,b). Even if the difference might seem insignificant, especially with such low water levels and concentrations, it should not be omitted.

*Impact of floods on the mountain lakes and following recovery process*

The chemical oxygen demand (COD), as a complex non-specific indicator of organic pollution (Judová *et al.* 2015), was low in the examined High Tatra lakes (Table 3). The average COD during both summers of our fieldwork was 1.82 (± 2.32) ml, reaching a low of 0.55 (± 0.20) ml in 2019 and subsequently increasing to 3.14 (± 2.58) ml in 2020. We can find exceptions similar to the previous chapter if we look at some examples from dystrophic lakes. In 2019, the maximum COD value of water in Slavkovské pliesko, (no. 51) did not exceed 1.15 ml (Table 3). The value was significantly low (Górniak *et al.* 1999), despite the dystrophic lake being on its way to extinction, overgrown by vegetation and mountain pine (Lackovič 2015). Even for such clean lakes, all other values during the season were surprisingly below 1 ml (Hrivnáková 2019). During the summer of 2020, there was a significant increase in COD (Fig. 4b), in some cases reaching values that exceeded 10 ml (which we set as our maximum). These included lakes under the tree-line (Čierne pleso, no. 41 in valley Motyková dolina and Žabie pliesko, no. 52), as well as alpine lakes (Prostredné spišské pleso, no. 72 and Skalnaté oko, no. 78) (Table 3). Organic matter concentration, as mentioned above, depends on lake water and its acidity (which functions as an antagonist to water voltage). The average water reaction value was 7.624 ± 0.784 for both summers, and fell with the increase in COD and water temperature in 2020 (Kopáček *et al.* 2006; Hrivnáková 2019) (Table 3; Fig. 4b).

But what is the cause of this? Here we need to consider the climatic conditions of Tatras, an area of extreme precipitation and intensive storm activity in summer (from July to August). This period is characterized by an increase in water levels and devastating floods (Slovenská lesnícka spoločnosť 2012a). During this seasonal research at Kolové pleso from 2017 until 2018 (Hrivnáková 2019), we recorded such extreme precipitation in July 2018. It triggered extensive floods throughout the High Tatras, which had a significant impact on the organic composition and acidity of the lake mentioned above, causing a COD decrease and pH increase from the exact day that flooding started (Hrivnáková *et al.* 2020). It has been concluded that floods are responsible for the rapid accumulation of organic matter in the lake (McClain *et al.* 2003; Mladenov *et al.* 2005; Steven and Melack 2012), while in similarly pristine aquatic ecosystems, their pH is significantly influenced by precipitation (Judová *et al.* 2015; Hrivnáková *et al.* 2020). Floods cause the leaching of dissolved organic matter into ATTZ (Wantzen *et al.* 2008), which is immediately followed by reverse and more effective washing out of allochthonous organic matter from the catchment area to the lake. Therefore, the levels of organic matter increase after the flood (Coops *et al.* 2003; Steven and Melack 2012).

While researching for this thesis and previously working at Kolové pleso, (no. 86) (Hrivnáková 2019), we observed that flooding affected all lake ecosystems in the High Tatras by extreme long-term reduction of COD (Hrivnáková *et al.* 2020, unpublished data from the ongoing study of Kolové pleso). The exact opposite effect was recorded in lakes surrounded by enough vegetation, where the COD value increases during floods (Steven and Melack 2012). However, this may be a result of one extreme value being recorded in the dystrophic and gradually disappearing Slavkovské pliesko, (no. 51) (Table 3) in 2019. It had a low level of COD, but was most likely influenced by washing out of allochthonous organic matter from the catchment area (Coops *et al.* 2003).

It is interesting to monitor the recovery of lakes from floods, which we compare to ongoing research of Kolové pleso, (no. 86). In the summer of 2020 (from August to September), we recorded changes and a subsequent increase in COD (unpublished data from the ongoing study of Kolové pleso). Our research shows the recovery of organic composition two years after the flooding occurred, thanks to COD increase, pH decrease, and changes in correlated parameters (Fig. 4b). We can state that according to our data from 2019, the COD values fell in all the lakes (average being 0.55 ± 0.196 ml). Due to the washing out of organic matter during the flood in 2019, we did not observe any demonstrable distinction between the lakes with enough and minimum vegetation. However, there have been apparent differences during the lake's recovery in 2020 (Fig. 5). Some of the lakes showed low COD, but others recorded the highest values observed (similar effect with pH). These were not just lakes below the tree-line with large amounts of organic matter.

The aforementioned lake morphology of higher-altitude lakes with shallow or missing ATTZ and a lack of vegetation plays an essential role (Mladenov *et al.* 2005). The amount of vegetation initially did not affect organic matter levels but (in the long term) plays a vital role in lake recovery. An increase (pH – KW Anova – $H (1,46) = 4.150$, $p = 0.042$) in COD was significantly faster (Fig. 5; KW Anova – $H (1,46) = 7.878$, $p = 0.005$) inside the lakes with enough surrounding vegetation, while their counterparts with less flora seemingly took longer to recover. We assume that the lowest-altitude dystrophic lakes with wide ATTZ and a lot of vegetation are fastest in their recovery, followed by lakes (even in higher altitudes) with enough surrounding flora and shallower coastal zones. The highest-altitude lakes are characterised by the slowest recovery due to inadequate vegetation and low organic input as a result of the limited (sometimes absent) coastal zone. This has an apparent impact on lake ecosystems.

Among other things, floods are responsible for the increased mobilization of elements and accumulation of heavy metals (Bradley and Cox 1990; Szabó *et al.* 2008; Chrastný *et al.* 2006; Hrivnáková *et al.* 2020). This effect is partially displayed in the first and second factors (Table 2; Fig. 4a). However, during floods, the mobility and speciation of elements/metals within ecosystems are influenced by different factors or processes (Chrastný *et al.* 2006; Du Laing *et al.* 2009). It is the organic matter that directly affects the increase or decrease in metal mobility (Du Laing *et al.* 2009). Similarly, pH that immediately rises due to floods (Hrivnáková *et al.* 2020) speeds up the transfer of trace elements as well as metals from water to sediments and vice versa; demonstrably, with metals like Pb, Hg, Zn, Cr (Salomons *et al.* 1987; Gambrell *et al.* 1991; Calmano *et al.* 1993). Therefore, floods can also change the solute content by dissolving chloride complexes (Hahne and Kroontje 1973) and increase the concentrations of major cations competing with heavy metal absorption sites (Tam and Wong 1999). These affect their toxicity and specialization, while Pb (and Cd) have the strongest effect (Du Laing *et al.* 2009).

Another necessary process is binding heavy metals to oxides and hydroxides, which are the primary carriers for these elements (especially Cd and Zn) (Salomons *et al.* 1987; Bradley and Cox 1990; Chrastný *et al.* 2006; Du Laing *et al.* 2009).

These after-flood processes caused the mobilization of metals in the littoral zone by decreasing their levels in 2019 (probably since 2018), except for the increase of cation concentrations (Ca, K,..). Subsequently, the number of total dissolved solids (TDS) and the number of chloride complexes improved, followed by increased connectivity and hardness of the water (Table 2; Fig. 4a). The affinity of metals for organic matter became evident during the after-flood recovery in 2020 by increasing their levels in the littoral zone and the amount of organic material in the water (Table 2) (Davis 1984; Grba *et al.* 2016).

In 2018, our research at Kolové pleso (Hrivnáková *et al.* 2020) showed that such catastrophic and unpredictable events could trigger the leaching of heavy metals from lake ecosystems, contaminating the aquatic environment (Chrastný *et al.* 2006; Du Laing *et al.* 2009). Therefore, the heavy metal concentrations in bryophytes should follow the rising tendency of metals in the littoral zone in 2020 (Fig. 4a). However, this was not the case for all species. In species that showed lower metal levels – *Sphagnum russowi, Sphagnum girgensohnii* and *Polytrichum commune*, recorded concentrations were higher in 2019 than in 2020. It seems that they absorbed these metals following their leaching from lake sediments. The surrounding vegetation (especially the macrophytic) of lakes can be significantly affected by floods (Wantzen *et al.* 2008). Fortunately, there already seems to be measurable recovery after two years, accompanied by an increased metal content in the littoral zone, reduced mineralization, and stabilization of the aquatic environment in 2020.

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## Appendix 1: Sampling sites

The following abbreviations have been used in the list of sites for the names of valleys: **KD** – Kôprová dolina, **VD** – Važecká dolina, **FD** –Furkotská dolina, **MD** – Mlynická dolina, **MeD** – Mengusovská dolina, **ZD** – Zlomiská dolina, **BaD** – Batizovská dolina, **VeD** – Velická dolina, **SlD** – Slavkovská dolina, **VSD** – Veľká Studená dolina, **MSD** – Malá Studená dolina, **SD** – Skalnatá dolina, **DKBV** – Dolina Kežmarskej Bielej vody, **JD** – Javorová dolina, **BD** – Bielovodská dolina. All samples were collected by author.

Way of registering the site:
**Lake: valley – side valley, elevation, orientation, GPS coordinates of sampling, type of shore, bottom type: nutrient content; date of sampling;**

**1. Nižné Temnosmrečinské pleso:** KD – Temnosmrečinská dolina, 1677 m asl., S, 49° 11'40.6"N, 20° 01'47.0"E, rocky – overgrown shore, rocky bottom: oligotrophic; 7.11.2020;
**2. Vyšné Temnosmrečinské pleso:** KD – Temnosmrečinská dolina, 1725 m asl., S, 49° 11'22.0"N, 20° 02'11.7"E, rocky shore, rocky bottom: oligotrophic; 7.11.2020;
**3. Nižné Terianske pleso:** KD – Nefcerská dolina, 1940 m asl., S, 49° 10'08.1"N, 20° 00'43.5"E, rocky – overgrown shore, rocky bottom: oligotrophic; 28.10.2020;
**4. Vyšné Terianske pleso:** KD – Nefcerská dolina, 2124 m asl., S, 49° 10'02.6"N, 20° 01'15.7"E, rocky shore, rocky bottom: oligotrophic; 28.10.2020;
**5. Jamské pleso:** VD – Jamy, 1447 m asl., S, 49° 08'01.0"N, 20° 00'42.1"E, overgrown shore, gravely – sandy bottom: dystrophic; 20.5.2020; KH.
**6. Malé kriváňske pliesko:** VD – Zadný Handel, 2004 m asl., S, 49° 09'26.9"N, 20° 00'23.3"E, shore – under snow, rocky bottom: oligotrophic; 30.10.2020;
**7. Zelené kriváňske pleso:** VD – Zadný Handel, 2012 m asl., S, 49° 09'27.1"N, 20° 00'25.9"E, shore – under snow, rocky bottom: oligotrophic; 30.10.2020;
**8. Vyšné rakytovské pleso:** FD – Rakytovec, 1307 m asl., S, 49° 07'35.6"N, 20° 01'29.0"E, overgrown shore, peat bottom: dystrophic; 20.5.2020;
**9. Nižné rakytovské pleso:** FD – Rakytovec, 1323 m asl., S, 49° 07'30.2"N, 20° 01'34.2"E, overgrown shore, peat bottom: dystrophic; 20.5.2020;
**10. Vyšné smrekovické pliesko:** FD – Smrekovica, 1355 m asl., S, 49° 07'31.9"N, 20° 02'05.9"E, overgrown shore, peat bottom: dystrophic; 20.5.2020;
**11. Nižné smrekovické pliesko:** FD – Smrekovica, 1350 m asl., S, 49° 07'31.2"N, 20° 02'08.5" E, overgrown shore, peat bottom: dystrophic; 20.5.2020;
**12. Prvé Sedielkové pliesko:** FD, 1876 m asl., S, 49° 09'03.9"N, 20° 01'32.6"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 20.10.2020;
**13. Nižné Wahlenbergovo pleso:** FD, 2053 m asl., S, 49° 09'33.0"N, 20° 01'35.4"E, rocky shore, rocky bottom: oligotrophic; 1.9.2019; .
**14. Soliskové pliesko:** FD, 2073 m asl., S, 49° 09'38.2"N, 20° 01'31.4"E, rocky shore, rocky bottom: oligotrophic; 28.10.2020;
**15. Vyšné Wahlenbergovo pleso:** FD, 2157 m asl., S, 49° 09'50.4"N, 20° 01'39.4"E, rocky shore, rocky bottom: oligotrophic; 1.9.2019;
**16. Štrbské pleso:** MD, 1347 m asl., S, 49° 07'25.2"N, 20° 03'13.2"E, overgrown shore, gravely – sandy bottom: dystrophic; 20.5.2020;
**17. Pliesko pod Skokom:** MD, 1685 m asl., S, 49° 09'06.5"N, 20° 02'49.5"E, overgrown shore, gravely – sandy bottom: oligotrophic; 1.9.2019;
**18. Pleso nad Skokom:** MD, 1801 m asl., S, 49° 09'16.8"N., 20° 02'43.0"E, ocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 1.9.2019;
**19. Vyšné Volie pliesko:** MD, 1980 m asl., S, 49° 09'42.3"N, 20° 02'32.2"E, rocky – overgrown shore, rocky bottom: oligotrophic; 1.9.2019;
**20. Malé Kozie pleso:** MD, 1932 m asl., S, 49° 09'44.2"N, 20° 02'30.5"E, rocky shore, rocky bottom: oligotrophic; 1.9.2019;

**21. Nižné Kozie pleso:** MD, 1942 m asl., S, 49° 09'48.0"N, 20° 02'37.4"E, rocky – overgrown shore, rocky bottom: oligotrophic; 1.9.2019;
**22. Vyšné Kozie plesá:** MD – Kozí kotol, 2109 m asl., S, 49° 10'03.3"N, 20° 02'41.6"E, rocky shore, rocky bottom: oligotrophic; 1.9.2019;
**23. Capie pleso:** MD, 2075 m asl., S, 49° 10'04.5"N, 20° 02'16.4"E, rocky shore, rocky bottom: oligotrophic; 1.9.2019;
**24. Okrúhle pleso:** MD, 2105 m asl., S, 49° 10'12.2"N, 20° 02'07.9"E, rocky shore, rocky bottom: oligotrophic; 1.9.2019;
**25. Popradské pleso:** MeD – Beginning of the Zlomiská dolina, 1494 m asl., S, overgrown shore, gravely – sandy bottom: dystrophic; 4.9.2019;
**26. Ľadové pleso in valley Zlomiská dolina:** MeD – ZD (Ľadová kotlina), 1925 m asl., S, 49° 09'48.7"N, 20° 06'18.2"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 4.9.2019;
**27. Dračie pleso:** MeD – ZD (Dračia dolinka), 2019 m asl., S, 49° 09'56.7"N, 20° 05'16.7"E, rocky shore, rocky bottom: oligotrophic; 4.9.2019;
**28. Malé Dračie pleso:** MeD – ZD (Dračia dolinka), 1951 m asl., S, 49° 09'55.3"N, 20° 05'27.6"E, rocky shore, rocky bottom: oligotrophic; 4.9.2019;
**29. Dračie oko:** MeD – ZD (Dračia dolinka), 2020 m asl., S, 49° 09'55.3"N, 20° 05'22.3"E, rocky shore, rocky bottom with an organic layer of sediment: oligotrophic; 4.10.2019;
**30. Rumanovo pleso:** MeD – ZD (Rumanova dolinka), 2090 m asl., S, 49° 10'09.9"N, 20° 06'00.9"E, rocky shore, rocky bottom: oligotrophic; 4.9.2019;
**31. Nižné Rumanovo pliesko:** MeD – ZD (Rumanova dolinka), 2088 m asl., S, 49° 10'07.9"N, 20° 06'00.0"E, rocky shore, rocky bottom: oligotrophic; 4.9.2019;
**32. Veľké Žabie pleso:** MeD – Žabia dolina (Žabia kotlinka), 1921 m asl., S, 49° 10'21.7"N, 20° 04'34.9"E, rocky shore, rocky bottom: oligotrophic; 14.7.2020;
**33. Malé Žabie pleso:** MeD – Žabia dolina, 1919 m asl., S, 49° 10'22.4"N, 20° 04'34.2"E, rocky shore, rocky bottom: oligotrophic; 14.7.2020;
**34. Predné Žabie pleso:** MeD – Žabia dolina, 1917 m asl., S, 49° 10'20.9"N, 20° 04'25.6"E , rocky – overgrown shore, rocky bottom: oligotrophic; 14.7.2020;
**35. Vyšné Žabie pliesko:** MeD – Žabia dolina (Volia kotlinka), 2046 m asl., S, 49° 10'31.4"N, 20° 04'23.8"E, rocky shore, rocky bottom: oligotrophic; 14.7.2020;
**36. Malé Satanie pliesko:** MeD – Satania dolinka, 1894 m asl., S, 49° 10'11.6"N, 20° 03'43.9"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 14.7.2020;
**37. Satanie pleso:** MeD – Satania dolinka, 1894 m asl., S, 49° 10'12.0"N, 20° 03'39.7"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 14.7.2020;
**38. Hincové oká:** MeD – Hincová kotlina, 1 940 m asl., S, 49° 10'31.5"N, 20° 03'47.3"E, rocky shore, rocky bottom: oligotrophic; 14.7.2020;
**39. Malé Hincovo pleso:** MeD – Hincová kotlina, 1921 m asl., S, 49° 10'28.2"N 20° 03'28.8"E, rocky – overgrown shore, rocky bottom: oligotrophic; 14.7.2020;
**40. Veľké Hincovo pleso:** MeD – Hincová kotlina, 1945 m asl., S, 49° 10'35.0"N, 20° 03'41.3"E, rocky – overgrown shore, rocky bottom: oligotrophic; 14.7.2020;
**41. Čierne pleso in valley Motyková dolina:** HT, BaD – Motyková dolina, 1235 m asl., S, 49° 07'36.5"N, 20° 07'47.6"E, overgrown shore, peat bottom: dystrophic; 20.5.2020;
**42. Batizovské pleso**: BaD – Nižná Batizovská roveň, 1883 m asl., S, 49° 09'05.5"N, 20° 07'46.4"E, rocky shore, rocky bottom: oligotrophic; 23.10.2019;
**43. Malé Batizovské pleso:** BaD – Nižná Batizovská roveň, 1920 m asl., S, 49° 09'11.0"N, 20° 07'27.5"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 23.10.2019;
**44. Pliesko pod Kostolíkom**: BaD – Vyšná Batizovská roveň, 2075 m asl., S, 49° 09'38.1"N, 20° 07'15.8"E, rocky shore, rocky bottom: oligotrophic; 23.10.2019;
**45. Velické pleso:** VeD, Velické pleso, 1665 m asl., S, 49° 09'31.4"N, 20° 09'22.1"E, rocky – overgrown shore, rocky bottom: oligotrophic; 11.9.2019;
**46. Kvetnicové pliesko (1):** VeD – Kvetnica, 1815 m asl., S, 49° 09'44.9"N, 20° 09'12.6"E, overgrown shore, gravely – sandy bottom: oligotrophic; 11.9.2019;
**47. Kvetnicové pliesko (2):** VeD – Kvetnica, 1890 m asl., S, 49° 09'52.9"N, 20° 08'44.3"E, rocky – overgrown shore, gravely – sandy bottom: oligotrophic; 23.10.2019;
**48. Dlhé pleso in valley Velická dolina:** VeD – Horná Kvetnica, 1939 m asl., S, 49° 09'55.0"N, 20° 08'40.9"E, rocky – overgrown shore, rocky bottom: oligotrophic; 23.10.2019;
**49. Vyšné Velické pliesko – dolné:** VeD – Velická kotlina, 2118 m asl., S, 49° 10'20.4"N, 20° 08'13.2"E, rocky shore, rocky bottom: oligotrophic; 23.10.2019;
**50. Vyšné Velické pliesko – horné:** VeD – Velická kotlina, 2141 m asl., S, 49° 10'20.7"N, 20° 08'09.1"E, rocky shore, rocky bottom: oligotrophic; 23.10.2019;
**51. Slavkovské pliesko:** SlD – under Senná kopa, 1676 m asl., S, 49° 09'09.0"N, 20° 10'59.6"E, overgrown shore, rocky – peat bottom: dystrophic; 11.9.2019;
**52. Žabie pliesko:** SlD – over Starý Smokovec, 1050 m asl., S, 49° 08'41.0"N, 20° 13'02.5"E, overgrown shore, peat bottom: dystrophic; 3.10. 2020;
**53. Vareškové pleso:** VSD – Varešková kotlina, 1834 m asl., S, rocky – overgrown shore, rocky bottom: oligotrophic; 21.10.2019;
**54. Dlhé pleso in valley Veľká Studená dolina:** VSD, 1894 m asl., S, 49° 10'30.0"N, 20° 10'10.3"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 21.10.2019;
**55. Nižné Sesterské pleso:** VSD – Zbojnícka pláň, 1974 m asl., S, 49° 10'38.6"N, 20° 09'59.4"E, rocky – overgrown shore, rocky bottom: oligotrophic; 21.10.2019;
**56. Starolesnianske pleso:** VSD – Zbojnícka pláň, 1988 m asl., S, 49° 10'48.9"N, 20° 09'58.4"E, rocky – overgrown shore, rocky bottom: oligotrophic; 29.9.2019;
**57. Nižné zbojnícke pleso:** VSD – Zbojnícka pláň, 1955 m asl., S, 49° 10'38.8"N, 20° 09'41.6"E, rocky – overgrown shore, rocky bottom: oligotrophic; 29.9.2019;
**58. Prostredné zbojnícke pleso:** VSD, 1960 m asl., S, 49° 10'42.6"N, 20° 09'37.6"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 12.8.2019;
**59. Zbojnícke pleso:** VSD, 1960 m asl., S, 49° 10'41.3"N, 20° 09'40.6"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 29.9..2019;
**60. Vyšné (Veľké) zbojnícke pleso:** VSD, 1962 m asl., S, 49° 10'44.0"N, 20° 09'32.0"E, rocky – overgrown shore, rocky bottom: oligotrophic; 12.8.2019;
**61. Ľadové (zbojnícke) pleso:** VSD – Rovienková kotlina, 2057 m asl., S, 49° 11'00.2"N, 20° 09'39.9"E, rocky shore, rocky bottom: oligotrophic; 12.8.2019;
**62. Pusté pleso:** VSD – Rovienková kotlina, 2056 m asl., S, 49° 10'54.5"N, 20° 09'15.7"E, rocky – overgrown shore, rocky bottom: oligotrophic; 12.8.2019;
**63. Malé pusté pleso:** VSD – Rovienková kotlina, 2061 m asl., S, 49° 10'59.7"N, 20° 09'17.6"E, rocky shore, rocky bottom: oligotrophic; 12.8.2019;
**64. Nižné studené pleso:** VSD – Strelecká plošina, 1811 m asl., S, 49° 10'44.7"N, 20° 10'39.8"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 12.8.2019;
**65. Vyšné studené pleso:** VSD – Strelecká plošina, 1812 m asl., S, 49° 10'47.8"N, 20° 10'35.0"E, rocky bottom, rocky bottom with an organic layer of sediment: oligotrophic; 12.8.2019;
**66. Nižné sivé pleso:** VSD – Ostrý kotol, 2012 m asl., S, 49° 10'59.8"N, 20° 10'31.2"E, rocky – overgrown shore, rocky bottom: oligotrophic; 12.8.2019;
**67. Prostredné sivé pleso (Sivé pleso):** VSD – Ostrý kotol, 2013 m asl., S, 49° 11'02.4"N, 20° 10'29.5"E, rocky shore, rocky bottom: oligotrophic; 12.8.2019;
**68. Tretie Nižné strelecké pliesko:** VSD – Strelecká kotlina, 2021 m asl., S, 49° 11'02.3"N, 20° 10'52.5"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 30.8.2020;

**69. Prvé Nižné strelecké pliesko:** VSD – Strelecká kotlina, 2013 m asl., S, 49° 11'03.2"N, 20° 10'59.3"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 30.8.2020;

**70. Malé spišské pleso:** MSD – Kotlina Piatich spišských plies, 1997 m asl., S, 49° 11'24.8"N, 20° 12'00.6"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 7.8. 2020;

**71. Nižné spišské pleso:** MSD – Kotlina Piatich spišských plies, 1992 m asl., S, 49° 11'25.8"N, 20° 11'48.6"E, rocky – overgrown shore, rocky bottom: oligotrophic; 7.8.2020;

**72. Prostredné spišské pleso**: MSD – Kotlina Piatich spišských plies, 2010 m asl., S, 49° 11'27.8"N, 20° 11'54.1"E, rocky – overgrown shore, rocky bottom: oligotrophic; 7.8.2020;

**73. Veľké spišské pleso:** MSD – Kotlina Piatich spišských plies, 2013 m asl., S, 49° 11'38.9"N, 20° 11'43.7"E, rocky shore, rocky bottom: oligotrophic; 7.8.2020;

**74. Vyšné spišské pleso:** MSD – Kotlina Piatich spišských plies, 2018 m asl., S, 49° 11'41.0"N, 20° 11'45.0"E, rocky shore, rocky bottom: oligotrophic; 7.8. 2020;

**75. Baranie pliesko:** MSD – Kotlina pod Baraními rohmi, 2207 m asl., S, 49° 11'55.1"N, 20° 11'42.4"E, rocky shore, rocky bottom: oligotrophic; 7.8.2020;

**76. Modré pleso:** MSD – Dolina pod Sedielkom, 2189 m asl., S, 49° 11'31.7"N, 20° 11'07.0"E, rocky shore, rocky bottom: oligotrophic; 22.10.2019;

**77. Skalnaté pleso:** SD, 1751 m asl., S, 49° 11'18.7"N, 20° 13'54.6"E, rocky – overgrown shore, rocky bottom: oligotrophic; 20.9.2020;

**78. Skalnaté oko:** SD, 1546 m asl., S, 49° 11'10.1"N, 20° 13'47.1"E, rocky – overgrown shore, rocky bottom: oligotrophic; 1.11.2020;

**79. Zelené (Kežmarské) pleso:** DKBV – Dolina Zeleného plesa, 1546 m asl., S, 49° 12'35.1"N, 20° 13'17.2"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 29.9.2019;

**80. Čierne pleso:** DKBV – Dolina Zeleného plesa, 1579 m asl., S, 49° 12'27.8"N, 20° 13'28.5"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 29.9.2019;

**81. Červené pleso:** DKBV – Červená dolinka, 1811 m asl., S, 49° 12'47.5"N, 20° 12'50.8"E, rocky – overgrown shore, rocky bottom: oligotrophic; 29.9.2019;

**82. Belasé pleso:** DKBV – Červená dolinka, 1862 m asl., S, 49° 12'53.8"N, 20° 12'41.6"E, rocky shore, rocky bottom: oligotrophic; 29.9.2019;

**83. Trojrohé pleso:** DKBV – Dolina Bielych plies, 1611 m asl., S, 49° 13'09.9 N, 20° 13'45.6 E, overgrown shore, peat bottom: dystrophic; 24.6.2020;

**84. Veľké Biele pleso:** DKBV – Dolina Bielych plies, 1615 m asl., S, 49° 13'17.1"N, 20° 13'51.1"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 24.6.2020;

**85. Malé Biele plesá**: DKBV – Dolina Bielych plies, 1660 m asl., S, 49° 13'28.3"N, 20° 13'10.9"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 24.6.2020;

**86. Kolové pleso:** JD – Kolová dolina, 1565 m asl., N, 49° 13'15.7"N, 20° 11'33.5"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 1.8.2019;

**87. Zelené Javorové pleso:** JD – Zelená Javorová dolina, 1815 m asl., N, 49° 12'21.6"N, 20° 08'31.7"E, overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 22.10.2019;

**88. Zelené Javorové oko (väčšie):** JD – Zelená Javorová dolina, 1814 m asl., N, 49° 12'23.5"N, 20° 08'34.9"E, overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 22.10.2019;

**89. Predné rígľové oko:** JD, 1512 m asl., N, 49° 11'40.1"N, 20° 09'29.9"E, rocky – overgrown shore, gravely – sandy bottom: oligotrophic; 22.10.2019;

**90. Malé Žabie Javorové pleso:** JD – Žabia Javorová dolina, 1704 m asl., N, 49° 12'09.0"N, 20° 08'57.8"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 22.10.2019;

**91. Žabie Javorové pleso:** JD – Žabia Javorová dolina, 1878 m asl., N, rocky shore, rocky bottom: oligotrophic; 22.10.2019;

**92. Zamrznuté pleso:** BD – Zamrznutý kotol, 2040 m asl., N, 49° 10'34.0"N, 20° 08'14.6"E, rocky – overgrown shore, rocky bottom: oligotrophic; 31.8.2020;

**93. Zamrznuté oká:** BD – Zamrznutý kotol, 2056 m asl., N, 49° 10'39.1"N, 20° 08'14.5"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 31.8.2020;

**94. Hrubé pleso:** BD – Svišťová dolina, 1929 m asl., N, 49° 10'53.1"N, 20° 08'01.4"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 31.8.2020;

**95. Svišťové plieska:** BD – Svišťová dolina, 1929 m asl., N, 49° 10'53.9"N, 20° 08'01.0"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 31.8.2020;

**96. Litvorové pleso:** BD – Litvorová dolina, 1860 m asl., N, 49° 10'39.8"N, 20° 07'47.9"E, rocky – overgrown shore, rocky bottom: oligotrophic; 31.8.2020;

**97. Zelené Kačacie pleso:** BD – Kačacia dolina, 1575 m asl., N, 49° 10'40.4"N, 20° 06'59.6"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 31.8.2020;

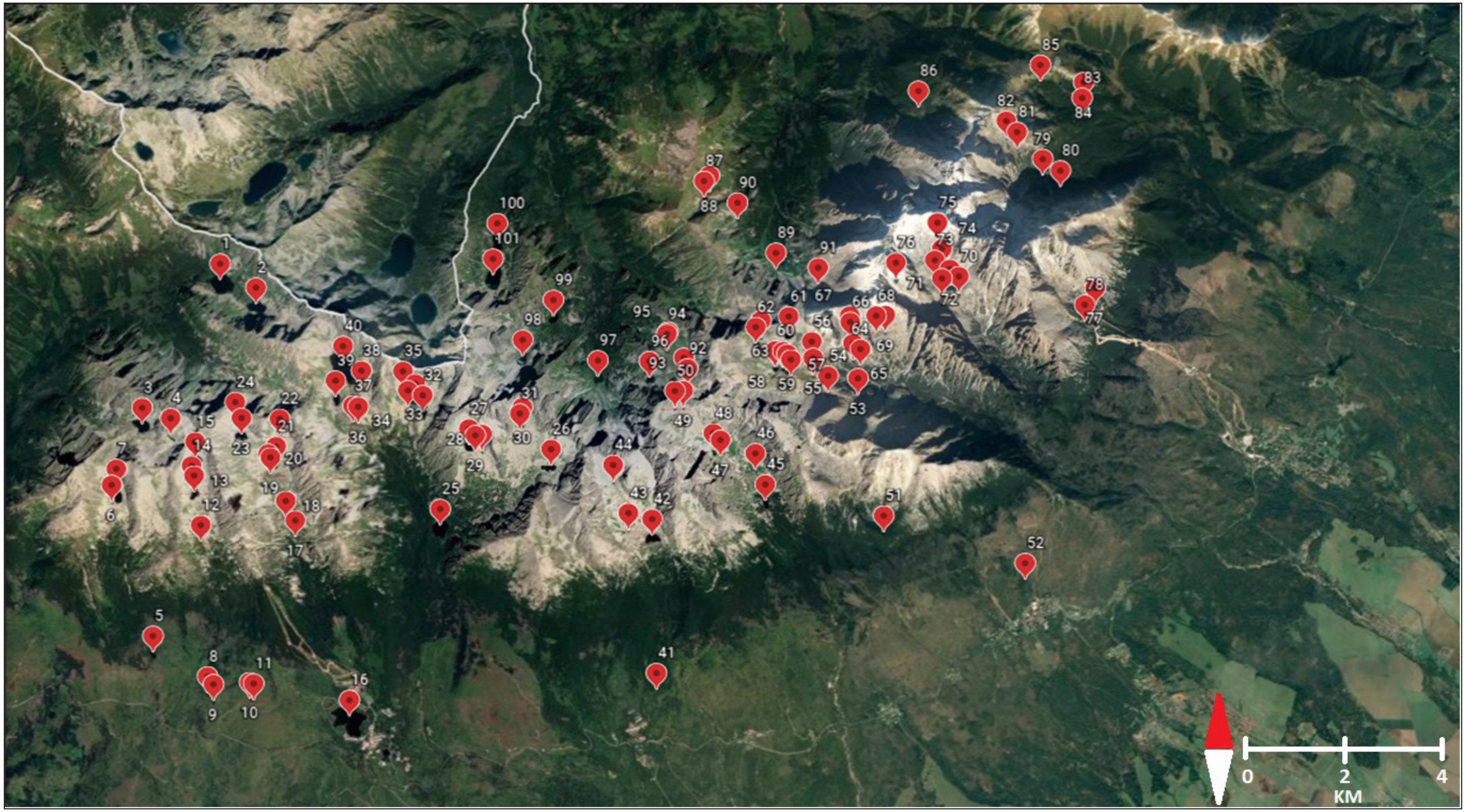
**98. Zmrzlé pleso:** BD – Ťažká dolina, 1762 m asl., N, 49° 10'53.2"N, 20° 06'02.5"E, rocky shore, rocky bottom: oligotrophic; 31.8.2020;

**99. Ťažké pleso:** BD – Ťažká dolina, 1611 m asl., N, 49° 11'11.6"N,m20° 06'26.0"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 31.8.2020;

**100. Nižné Žabie Bielovodské pleso:** BD – Žabia Bielovodská dolina, 1675 m asl., N, 49° 11'53.8"N, 20° 05'41.1"E, rocky – overgrown shore, rocky bottom with an organic layer of sediment: oligotrophic; 4.10.2020;

**101. Vyšné Žabie Bielovodské ples**o: BD – Žabia Bielovodská dolina, 1699 m asl., N, 49° 11'46.3"N, 20° 05'37.9"E, rocky – overgrown shore, rocky bottom: oligotrophic; 4.10.2020.

**Appendix 2.**
Map with the exact location of examined High Tatras lakes (101 in total - Appendix 1).
Source: Google Earth – Maxar Technologies © 2021 CNES / Airbus Modified by Hrivnáková K.



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# Orchid diversity in forest habitats of the Strážovské and Súľovské Vrchy Mountains

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**Abstract.** The work presented here is the culmination of four years of intensive monitoring of orchids in all forest and non-forest habitats of the Strážovské and Súľovské vchy Mountains and vicinity between 2017 and 2020. It is a direct follow-up to Ruček, 2019, and the list of re-corded species from the vegetation period between 2019 and 2020 is supplemented. In total, more than 12,000 individuals of 61 taxa were recorded in 2865 populations. From these data, diversity in non-forest, deciduous, coniferous and mixed forests was calculated, while the highest diversity was found in deciduous forests. The relationship of altitude to the abundance of species and populations was also confirmed. The greatest emphasis was placed on the genus *Epipactis*, which is typical for forest habitats. Records for 17 species of this genus and other Central European species with potential occurrence in the studied area were evaluated in detail. Distribution maps in the Central European square network, maps with the exact location of the population and detailed photographs of the studied plants were prepared.

*Key words: Orchidaceae, Epipactis*, diversity, forest, Slovakia

## Introduction

Humanity is facing one of the biggest ecological and climate crises in history. Our activities degrade all ecosystems and reduce biodiversity of flora and fauna. The greatest threat to biodiversity is the destruction or reshaping of natural habitats, including through mass deforestation, wetland drying, the expansion of arable and pasture land, continued urbanization and extensive exploitation of natural resources. In Slovakia, biodiversity is relatively well conserved compared to neighbouring countries. More than 11,000 species of plants, fungi and algae, and about 28,800 different animal species were recorded in Slovakia (www.cbd.int 2014). Wetlands and forests are among the most damaged ecosystems both in Slovakia as well as globally. Many species dependant on these ecosystems are in danger of extinction. Orchids are also affected, particularly species that are considered umbrella species for specific habitats. The limestone mountains of western Slovakia are significant to the occurrence of orchids on a global scale; an extraordinary abundance and diversity of forest orchids have developed, especially in the genus *Epipactis* Zinn. This genus represents about 17 known species as well as others waiting to be studied. Forest orchids are less well-known, easily overlooked and sometimes also difficult to identify. For example, the genus *Epipactis*, which represents typical forest orchids, has not been sufficiently explored to date. Currently, the only existing studies were conducted by Mereďa (1996a, 1996b, 2000, 2002a, 2002b, 2010) and Ruček (2019). Only three new species of *Epipactis* were described in the Strážovské vrchy Mts only during the 1990s.

The *Epipactis* genus is a typical group of orchids occurring mainly in the forest, with the exception of *E. palustris* (L.) Crantz, which is a distinctly heliophilic species inhabiting wetland habitats (springs, fens, peat bogs, waterlogged meadows, banks of water bodies, damp road ditches, waterlogged areas of abandoned quarries or terraces of orchards and vineyards). *E. microphylla* (Ehrh.) Sw. occurs in shady forests, particularly in the beech and oak-hornbeam forests; *E. atrorubens* (Hoffm.) Besser is found in light pine forests, sparse grassy forest edges, brushed hillsides, and sometimes light spruce forests or sunbathing terraces of abandoned quarries, roadsides and limestone debris; *E. purpurata* Sm. Is found in oak-hornbeam forests, up to beech forests of lower and middle fields, sometimes descending into alluvial forests; *E. pseudopurpurata* Mereďa occurs in shady beech forests; *Epipactis helleborine* (L.) Crantz subsp. *helleborine* is of wide ecological amplitude, it grows in deciduous and coniferous forests from floodplain forests to spruce forests, on grassy forest edges, in shrubbery, in abandoned quarries, on piles, around buildings and roads, in fields, and rarely, on the edges of wetlands; *E. greuteri* H. Baumann et Künkele is a species with a narrow ecological amplitude. It grows in damp shady fir-beech and spruce forests; *E. muelleri* Godfery is found in light deciduous and coniferous forests in warmer areas, shrubs and forest trimmings, and less so on sunny hillsides and secondary habitats; *E. leutei* Robatsch occurs in shady places in flowery beech forests and oak-hornbeams; *E. neglecta* (Kümpel) Kümpel and *E. leptochilla* (Godfery) Godfery grows in shaded and semi-shaded oak-hornbeams or flowery beech forests; *E. futakii* Mereďa et Potůček is found in shaded oak-hornbeams with scattered beech trees; *E. pontica* Taubenheim is in semi-shady to shady mesophilic oak-hornbeams and beech forests; *E. albensis* Nováková et Rydlo is in shady alluvial hardwood forests and riverside streams, or less often, in oak-hornbeams and beechwoods on the banks of a stream, around slope springs, or on forest roads. It is most common among woody species such as poplar, willow and linden. *E. tallosii* Molnár et Robatsch is found in shady alluvial forests and shoreline stands of poplars, ash trees and willows, or less frequently, in oak-hornbeams on the banks of streams and in damp terrain depressions; *E. placentina* Bongiorni et Grünanger is found in light beech or oak-hornbeam forests (Batoušek and Kežlínek 2012).

Other species that also occur in forest habitats are *Anacamptis pyramidalis* (L.) Rich. that can be found on forest edges and in light forests; *Cephalanthera damasonium* (Mill.) Druce, found in light and shady deciduous forest, but rare in coniferous forests, forest edges and brushed hillsides; *C. longifolia* (L.) Fritsch in light deciduous forest (beech and oak), but very rare in coniferous, woodland edges and bushes; *C. rubra* (L.) Rich. grow in deciduous forest, are rarer in coniferous forests, forest edges and shrubs; *Corallorhoza trifida* Châtel. grow in shady beechwoods, and more rarely in spruce forests; *Cypripedium calceolus* L. are found in deciduous forest (beech, oak), but are rare in coniferous (also secondary) forests, in the bushes and forest edges; *Dactylorhiza fuchsii* (Druce) Soó subsp. *fuchsii*, *D. fuchsii* subsp. *sooiana* (Borsos) Borsos and *D. sambucina* (L.) Soó are found in forest edges; *D. viridis* (L.) R. M. Bateman, A. M. Pridgeon et M. W. Chase grow in the forest and near forest roads; *Epipogium aphyllum* (F. W. Schmidt) Sw. are found in shady deciduous and coniferous (also secondary) forests, often near forest springs and on wet slopes; *Goodyera repens* (L.) R. Br. gsrow in shady coniferous (also secondary) forests; *Gymnadenia conopsea* (L.) R. Br. are found in bushes and sparse forests; *G. odoratissima* (L.) Rich. are found in rocky places; *Herminium monorchis* (L.) R. Br. grow in forest edges; *Himantoglossum adriaticum* H. Baumann are found in forest steppes, light forests, forest edges and brushed hillsides; *Limodorum abortivum* (L.) Sw. are found in light deciduous forest, bushes, and forest steppes; *Listera ovata* (L.) R. Br. grow in coniferous and deciduous forest, bushes, and forest edges; *Malaxis monophyllos* (L.) Sw. are found in wet forests, bushes, forest edges, and near forest roads; *Neotinea tridentata* are found in bushes, forest edges, and rarely in light forests; *Neotinea ustulata* (L.) R. M. Bateman, A. M. Pridgeon et M. W. Chase subsps. *aestivalis* (Kümpel) Jacquet et Scappat. grow in forest edges, and are rare in light forests; *Neottia nidus-avis* (L.) Rich. are found in shady forests, mixed and coniferous forests, and bushes; *Ophrys apifera* Huds. grow in bushy slopes, sparse pine and oak forests; *O. holubyana* András. are found in bushes, and on the forest's edge; *O. insectifera* L. grow in skeletal biotopes, and on forest edges; *Orchis mascula* (L.) L. subsp. *signifera* (Vest) Soó are found in scrubs, on the borders of forests, and in clear broadleaved forests; *O. militaris* L. are found on the borders of forests, and in clear broadleaved forests; *O. pallens* L. grow in clear forests, scrubs, and on forest edges; *O. purpurea* Huds. are found in forest edges, meadows, scrub slopes, and in clear broadleaved forests; *O. spitzelii* Saut. Ex W. D. J. Koch grow in beech forest, and in calcareous soils; *Platanthera bifolia* (L.) Rich. are found in clear forest, and scrubs; *P. chlorantha* (Custer) Rchb. grow in clear and also shady forests, alluvial forests, and scrubs (Vlčko *et al.* 2003).

*Potential occurrence of other species of genus Epipactis in the studied area*

In Slovakia, 5 recorded described species were found outside the studied area: *Epipactis distans* Arvet-Touvet, *E. gracilis* B. et H. Baumann, *E. leptochila* s. str., *E. moravica* Batoušek, *E. voethii* Robatsch (Vlčko *et al.* 2003). Due to the special requirements of the species for biotic and abiotic components of the environment, there is a different probability of occurrence.

Although *E. distans* is reported in the territory, its occurrence is not confirmed (Ruček 2019; Ujházyová *et al.* 2007). Individuals with an overall appearance similar to this taxon were recorded in the area of Jankov vŕšok, but they were not studied in detail (Mereďa Jr. 2010). The nearest occurrence is from the higher mountains: Malá Fatra, ~22 km away (Vlčko *et al.* 2003), Chočské vrchy (Kolník ined.), Spišská Magura (Jasík 2012). Typical habitats, including light pine forests and their edges (Průša 2019) are also in the studied area.

*E. gracilis* (syn. *E. exilis* P. Delforge, *E. baumanniorum* Ströhle). The nearest location of occurrence is in the Hungarian mountains Kőszeg (AHO 2011), 200 km away. In Slovakia, the nearest locality is in the Slanské vrchy Mts and the Nízke Beskydy Mts, 210 km - 220 km away (Kolník ined.; Vlčko *et al.* 2003). according to Batoušek and Kežlínek (2012), it may also occur in the Moravský kras Karst, 120 km away. It occurs in beech and beech-hornbeam forests, in aluvium streams or springs, on flysch, and in the submountain altitudinal zone (Vlčko *et al.* 2003). Distribution in Europe is not precisely known. This species does not only occur on flyschs as stated in Slovakia. The assumed northern border can also cross the studied area. There may be more suitable habitats, but not as compact as elsewhere.

*E. leptochila* s. str. is reported in older records, but may be a species not described at the time (Mereďa Jr. 2010). The nearest known site is in the southern part of the Veľká Fatra Mts, 33 km away (Kolník ined.). Our sighting was in the Chočské vrchy Mts, 43 km away. There are extensive suitable habitats with a high probability of occurrence in the studied area, such as beech-hornbeam forests, beech, and fir-spruce forests on calcareous soils (Vlčko *et al.* 2003).

*E. moravica* was described in 2004 near Uherské Hradiště in the Morava Region, with the same latitude as the studied area. Due to the fact that it was described only recently, it is possible that its habitat will be larger (Průša 2019). In addition to the Czech Republic, it also occurs in Slovakia (Kolník 2005), Hungary and allegedly in Italy (Průša

2019). The nearest locality is in the Morava Region near Suchá Loz Village, 25 km west of the border of the studied area (Popelářová 2012). The nearest Slovak locality is in the Malé Karpaty Mts near Čachtice and Hrachovište Village, also 25 km away. It has similar ecological requirements as *E. tallosi*. It is a lowland floodplain species and may have its northern limit in the southern part of the study area.

*E. voethii* was described near Vienna City in Austria in 1993 by Robatsch (1993). The distribution area is still known from Austria, Czech republic, Hungary and Slovakia (Batoušek and Kežlínek 2003; Průša 2019; Vlčko *et al.* 2003). In Slovakia, it is recorded in Slovenský kras Mts, and Myjavská pahorkatina Hills (Figura 2013, 2014). The nearest locality is the surroundings of Krajné Village in the Myjava District, 34 km away from the border of the studied area. It occurs in warm oak-hornbeam forest on calcareous soils in collin (Vlčko *et al.* 2003). The logical continuation of the expansion area is the valley of Váh River to the north. The potential occurrence may be in the peripheral parts of Ilavská and Bytčianská kotlina Basin and Podmanínska pahorkatina Hills. Respectively, the foothill area in the west of the Strážovské and Súľovské vrchy Mts.

Other species of orchids of the genus *Epipactis* are recorded in surrounding countries or new species are described in aggregate that occur in Slovakia as well. It is not impossible that some of these taxa may occur in Slovakia at present: *Epipactis bugacensis* Robatsch, *E. lapidocampi* Klein et Laminger, *E. moratoria* Reich et Zirnsack, *E. nordeniorum* Robatsch, *E. peitzii* Neumann et Wucherpfennig. *E. nordeniorum* (115 km away) and *E. bugacensis* (110 km away) are located near the Slovak border. This is a lowland species occurring in floodplain forests. The highest probability of occurrence is in the Podunajsko Region which may be their northern border. There are a few suitable habitats in the studied area, including moist forests (oak, hornbeam or poplar, willow) on sandy soil (AHO 2011; Kleesadl 2008; Robatsch 1991). *E. peitzii* is a species from the leptochila aggregate, which has similar ecological requirements as *E. leptochilla* s. l. in the studied area (Gévaudan and Delforge 2002). It was rare in Germany, but has also been reported in Hungary, 140 km away (Lajos *et al.* 2016). Other newly described species are *E. moratoria* from the group of *E. helleborine* agg. (Lipovšek *et al.* 2017) and *E. lapidocampi* (176 km away) from *E. muelleri* agg. (Klein and Laminger 2004). According to Aho (2011), *E. moratorium* is reported in the studied area near Trenčianske Teplice Town. It could be necessary to revise individuals in the orchid populations listed as *E. helleborine* s. str. and *E. muelleri* s. str. However, both species are of questionable taxonomic value (AHO 2011).

## Material and Methods

*Definition of the study area*

The territory is bounded by Váh River in the west, Rajčianka River and the Malá Fatra Mts. in the east. The southern border crosses the cadastral municipality of Trenčín, Soblahov, Mníchova Lehota, Timoradza, Kšinná, Čavoj and Kľačno (Fig. 1).

*Monitoring and data processing*

Monitoring of orchids took place during growing seasons between 2017 and 2020 in all suitable habitats. Species determination was carried out directly at the site by determining key and detailed descriptions according to Batoušek and Kežlínek (2012), Baumann *et al.* (2009), Vlčko *et al.* (2003), Potůček and Čačko (1996), Mereďa (1999) and the AHO-Bayern e.V. website (AHO 2011). Photo documentation of whole plants, flower organs and their habitat were prepared. Each population or individual was recorded in WGS84 geographical coordinates with corresponding altitudes using a Garmin Etrex 30 device. The exact coordinates are stored in the au-

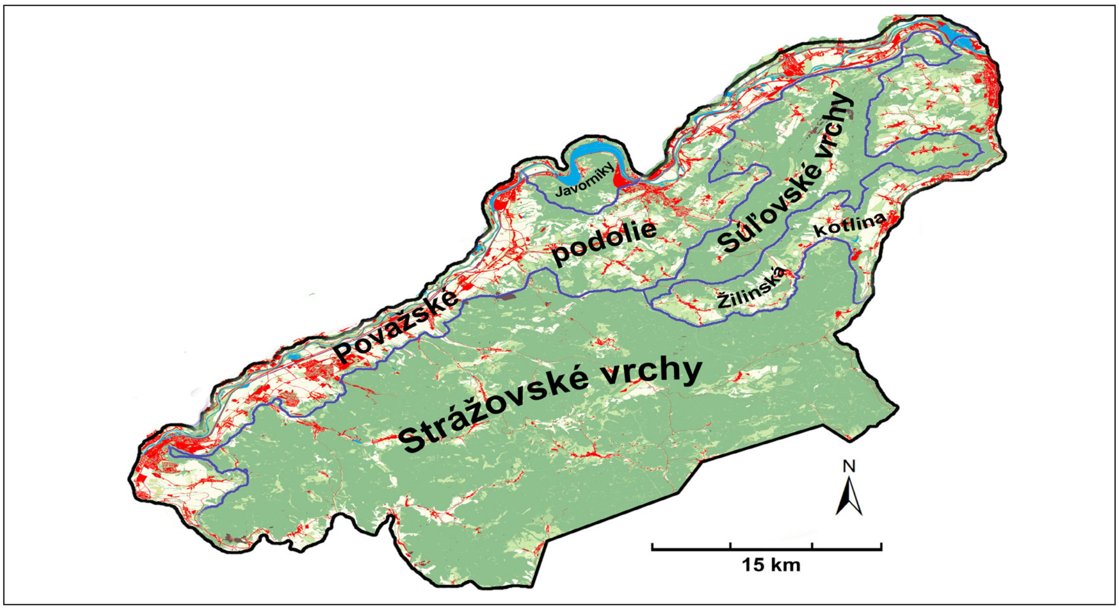


**Fig. 1.** Boundaries of the studied area and geomorphological division.

thor's personal archive and some are embedded in the Comprehensive Information and Monitoring Database (Štátna ochrana prírody SR 2014).

In ArcMap (Esri, USA), forest stand maps (NLC Zvolen 2018) were extracted attributes to the list of the coordinates of the botanical records. In this software a map was drawn of the expansion in the Central European square map (Niklfeld 1971) as well as map with the exact location of the orchid populations.

Thanks to the Arcmap software, each population had details of the forest in which it was located, presented as a ratio of the percentage of tree composition. From this, 4 categories were subsequently created: deciduous, coniferous, mixed forests, and non-forest areas. Deciduous and coniferous forests were defined as a 75 - 100 % share of deciduous or coniferous trees (Bravo-Oviedo *et al.* 2014). The Shannon Diversity Index (Shannon 1948) was calculated for these four categories. The equations for the Shannon index:

$$\mathbf{H=\Sigma[(pi)\times ln(pi)]}$$

Where, p is the proportion (*n*/N) of individuals of one particular species found (*n*) divided by the total number of individuals found (N), ln is the natural log, Σ is the sum of the calculations, and s is the number of species (Shannon 1948).

A graph of species abundance and population with respect to altitude at well-defined intervals was also developed. Input data for the calculation of the diversity index are taken from work by Ruček (2019), from the vegetation period in 2017-2018, as well as from reliable sources stored in the Comprehensive Information and Monitoring System of the State Nature Conservation of the Slovak Republic. This data contains 3,699 floristic records. The data set was supplemented by new records collected from the vegetation period during 2019-2020. Taxa nomenclature is assigned according Vlčko *et al.* (2003) and Batoušek and Kežlínek (2012). Behind the name is the abbreviation for the category of threats according to IUCN identified by Eliáš Jr. *et al.* (2015). The localities are arranged from south to north. Topographical names are taken from publicly available online maps licensed by the OpenStreetMap Foundation. Locality and taxon information are listed in the following symbols and senquences. It is inspired by the works Kolník (2004) and Kučera (2005):

1. Name of cadastral municipality,
2. more precise localization,
3. altitude (meters above sea level),
4. terrain slope: P – plain, S – slight, M – medium, G – great inclination,
5. slope orientation: 0 – indefinite , W – west, N – north, E – east, S – south,
6. number of individuals: O – to 10 pcs, T – tens, H – hundreds, or exact number is given,
7. density: S – sparsely, scattered over a larger area, G – group, several isolated groups, I – isolated one group,
8. date,
9. quadrant Code of the Central European Network Mapping (Niklfeld 1971),
10. name of the mapper,
11. author's comments.

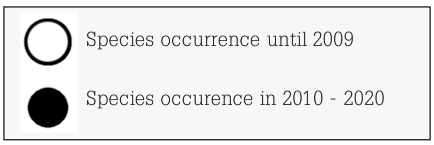


**Fig. 2.** Legend for the map in the Central European Network Mapping.

The individual information is separated by a symbol „ ; ", if information is missing, in its place it is " / ", records of findings are separated by a symbol „ * ". Other record authors are listed in a separate section. Behind the author's name is the cadastral area, year of record and number of base field and quadrant of Central European Network Mapping.

All orchids of the genus *Epipactis* occurring in or near the study area are processed. The description of the species is divided into two parts: 1) information from the studied area, recorded occurrence, potential occurrence; 2) current distribution of the taxon in the Central European Network Mapping (Appendix 1, 2, 3, 4), full circles represent recent data from 2010 to 2020, empty circle older data by 2009 (Fig. 2).

## Results

*Detailed list of taxa*

*Cephalanthera damasonium*, NT
Valaská Belá; saddle under Homôlka hill, under military monument; 765; S; N; 1; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; 0,5 km NE of Homôlka Peak; 770; M; S; O; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; 0,7 km NE of Homôlka Peak; 795; M; S; 1; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; near a forest road, 0,7 – 1,2 km NW of Šenkovci Settlement; 705-765; M; E; T; G; 23.6.2019; 7076c; V. Ruček; / * Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 715-805; M, G; S; O; G; 23.7.2020; 7075d; V. Ruček; 3 micro-localities * Horná Poruba; Kržlenica Locality, 0,8 km NNE of Hoľazne Top; 835; S; N; 1; I; 23.7.2020; 7075d; V. Ruček; / * Horná Poruba; near the red-marked hiking trail, above the second class road; 560; S; E; 1; I; 23.7.2020; 7075d; V. Ruček; / * Dubnica nad Váhom; Matejovská Locality, near the paved forest road, 0,5 km east of Krásna Hôrka Peak ; 445; M; W; O; I; 23.7.2020; 7075d; V. Ruček; / * Košecké Podhradie; 0,4-1 km SW of Kopec Village; 480-605; S-M; N; O; S; 15.6.2019; 7076c; V. Ruček; 3 micro-localities * Košecké Podhradie; Suchá Valley, near the Kopec Village, 1 km from the mouth of the valley; 475; M; N; O; I; 21.5.2020; 7076a; V. Ruček; / * Veľké Košecké Podhradie; Podhradská Valley, foot of Michalová Hill; 380; M; S; 1; I; 3.7.2020; 7076a; V. Ruček; / * Veľké Košecké Podhradie; Mraznica Locality, 1,3 km east of Stupičie Peak; 600; M; E; 1; G; 19.8.2020; 7076a; V. Ruček; / * Veľké Košecké Podhradie; Mraznica Locality, 0,4 km south of abandoned grange; 485; S; W; 1; I; 2.6.2020; 7076a; V. Ruček; / * Veľké Košecké Podhradie; xerothermic slope under the southern view of Rohatá Hill; 525-715; M, G; S; 14; G; 2.6.2020; 7076a; V. Ruček; 3 micro-localities * Mojtín; west of Mojtín Village, near the surface quarry; 645; S; E; 3; I; 4.6.2019; 7076a; V. Ruček;

/ * Košeca; Košecká Valley, south-facing slopes; 325-455; M; S; 16; S; 20.7.2020; 6975d; V. Ruček; 5 micro-localities * Podskalie; border of Podskalský Roháč NNR; 415-475; M; S; O; G; 4.5.2019, 24.6.2019; 6976d; V. Ruček; 2 micro-localities * Podskalie; 0,5 km west of the peak of Tŕnie Hill; 605; M; S; 1; I; 24.6.2019; 6976b; V. Ruček; / * Beluša; valley between Bukovina and Tlstá hora Hill, near the forest road and cottage area; 355-465; S; S; 9; G; 6.6.2019; 6976c; V. Ruček; 3 micro-localities * Beluša; valley between Bukovina and Tlstá hora Hill, near the forest road and cottage area; 355-465; S; S; 9; G; 6.6.2019; 7075d; V. Ruček; 3 micro-localities * Ladce; N-NW of Kalište Peak; 385; S; N; 1; I; 9.7.2019; 6975d; V. Ruček; / * Hloža-Podhorie; 1-1,4 km NNE of Butkov Peak; 440-485; M; N; 3; S; 6.8.2020; 6976c; V. Ruček; 3 micro-localities * Beluša; below the Jelenia skala Locality; 335; M; S; 1; I; 19.7.2019; 6976c; V. Ruček; / * Beluša; forest northeast of Čerencové Settlement; 315-350; M; S; 9; G; 2.7.2020; 6976c; V. Ruček; 2 micro-localities * Beluša; Vŕšok Hill; 395-430; M; N, S, E; 16; G; 22.7.2020; 6976c; V. Ruček; 4 micro-localities * Visolaje; near the road along Markov Stream; 300-330; S; W; 5; G; 19.6.2020; 6976a; V. Ruček; 2 micro-localities * Horný Moštenec, Zemianská Závada; near the educational trail to Temné Caves; 520-620; S, M; W, S; 11; G; 4.6.2020; 6976b; V. Ruček; 3 micro-localities * Kardošová Vieska; northern ridge and southern slopes above Kobylia Valley; 560-585; M; S; O; G; 25.7.2019; 6977a, 6977c; V. Ruček; 2 micro-localities * Rajec; 0,2 km north and 0,4 km SW of Baby Peak; 575-610; G; N, E; O; G; 25.7.2019; 6977b; V. Ruček; 2 micro-localities * Rajec, Malé Lednice; Veľký háj Locality, 2,5 km east and NEE of Malé Lednice Village; 505-580; S, M; W, S; O; S; 25.7.2019; 6977b; V. Ruček; 4 micro-localities * Horné Kočkovce; 1,1 km NW of Kozinec Peak; 395; M; N; 1; I; 21.6.2020; 6876c; V. Ruček; / * Horné Kočkovce; 0,9 km NE of Kozinec Peak; 510; S; S; 1; I; 17.7.2019; 6876c; V. Ruček; / * Nosice; near the peak of Hradisko Hill; 555; M; N; 1; I; 17.7.2019; 6876c; V. Ruček; / * Považská Bystrica; 1 km NW of Šurabová Settlement; 295-365; S; S; 10; G; 2.6.2019; 6876d; V. Ruček; 2 micro-localities * Považská Teplá; eastern foothill of Malý Manín Hill; 570-585; M; E; O; G; 22.7.2019; 6877a, 6877c; V. Ruček; 5 micro-localities * Kostolec; near the road to Kresania Settlement; 450; S; S; 1; I; 20.8.2020; 6877c; V. Ruček; / * Jablonové; south slopes of the nameless hill 2,2 km east of Jablonové Village; 620-635; M; S; O; G; 16.7.2019, 4.8.2020; 6877b; V. Ruček; 2 micro-localities * Jablonové; south slopes of the nameless hill 2,2 km east of Jablonové Village; 635; M; S; 1; I; 4.8.2020; 6877b; V. Ruček; /.

*Cephalanthera rubra*, NT
Valaská Belá; 0,5 km east of peak with an elevation of 841,5 m asl., near a forest road; 715; M; S; T; I; 23.6.2019; 7075d; V. Ruček; / * Horná Poruba; Kršlenica, 0,5 km northeast of Hoľazne Peak; 850; M; E; 10; I; 23.7.2020; 7075d; V. Ruček; / * Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 760-800; M, G; S; 21; G; 23.7.2020; 7075d; V. Ruček; 3 micro-localities * Horná Poruba; 0,4 km west of Suchá hora Peak; 950; M; S; O; I; 18.7.2019; 7076c; V. Ruček; / * Košecké Podhradie; 1 km north of Kopec Village, near the third class road; 405; M; E; 1; I; 15.6.2019; 7076a; V. Ruček; / * Ladce; Obesenec Locality, 1 km west of Strúčkova Settlement; 450; S; W; 1; I; 20.7.2020; 6975d; V. Ruček; / * Ladce; north of Kalište Peak; 375; S; N; O; I; 9.7.2019; 6975d; V. Ruček; / * Podskalie; border of Podskalský Roháč NNR; 475; S; E; 1; I; 24.6.2019; 6976d; V. Ruček; / * Podskalie; 0,5 km west of the peak of Tŕnie Hill; 605-630; M; S; 2; S; 24.6.2019; 6976b; V. Ruček; 2 micro-localities * Hloža-Podhorie; under Kavčia Rock, Prvé vráta Gorge; 380-480; M, G; N; O; G; 23.6.2020; 6976c; V. Ruček; 2 micro-localities * Beluša; Ostrá Malenica Hill, near the old hunting forest trail to the southern peak; 735; M; W; 1; I; 1.8.2020; 6976c; V. Ruček; / * Beluša; 0,6 km west of the top of Kamenica Hill; 325; M; S; 1; I; 30.6.2020; 6976a; V. Ruček; / * Visolaje; 0,8 km SWW of Markov Settlement; 315; M; S; 10; I; 19.6.2020; 6976a; V. Ruček; / * Zemianská Závada; near the educational trail to Temné Caves; 460; M; S; 1; I; 14.6.2020; 6976b; V. Ruček; / * Počarová; near the third class road to Zemianská Závada Village; 445; M; S; 1; I; 14.6.2020; 6977a; V. Ruček; / * Rajec; 0,2 km NE and 0,4 km SW of Baby Peak; 610; G; E; O; G; 25.7.2019; 6977b; V. Ruček; 2 micro-localities * Rajec; 0,7 km north of Srniak Peak; 640; M; N; O; I; 25.7.2019; 6977b; V. Ruček; / * Rajec; Veľký háj Locality, 2,4 km NEE of Malé Lednice Village; 535; S; W; O; I; 25.7.2019; 6977b; V. Ruček; / * Nosice; near the peak of Hradisko Hill; 560; G; N; 1; I; 17.7.2019; 6876c; V. Ruček; / * Jablonové; south slopes of the nameless hill 2,2 km east of Jablonové Village; 640; M; S; 8; I; 4.8.2020; 6877b; V. Ruček; /.

*Corallorhiza trifida*, LC
Omšenie; near the yellow hiking trail; 525-630; S; W; O; S; 14.6.2019; 7075c; V. Ruček; / * Valaská Belá; 0,5 km east of peak with an elevation of 841,5 m asl., near a forest road; 735; M; S; T; I; 23.6.2019; 7075d; V. Ruček; / * Beluša; 0,2 km west of the top of Vŕšok Hill; 415; M; W; 1; I; 22.7.2020; 6976c; V. Ruček; / * Považská Teplá; Manínska Gorge; 570; G; N; 1; I; 13.6.2020; 6877c; V. Ruček; verified older data by Urbanová from 1991 * Súľov-Hradná; near the green-marked hiking trail from Jabloňové Village to Súľov Castle; 440; M; W; 1; I; 16.7.2019; 6877a; V. Ruček; /.

*Dactylorhiza fuchsii* subsp. *fuchsii*, NT
Košecké Podhradie; 0,5 km NE of Vápeč Peak; 660; S; N; 2; S; 15.6.2019; 7075d; V. Ruček; / * Nosice; Za hájom Locality, 1,2 km south of Nosice Village; 465; P; 0; 2; I; 21.6.2020; 6876c; V. Ruček; /.

*Dactylorhiza fuchsii* subsp. *sooiana*, NT
Košecké Podhradie; 0,5 km NE of Vápeč Peak; 670; S; N; 5; S; 15.6.2019; 7075d; V. Ruček; / * Nosice; Za hájom Locality, 1,2 km south of Nosice Village; 465; P; 0; 1; I; 21.6.2020; 6876c; V. Ruček; /.
Record of occurence according to P. Bagin (Eliáš Jr. 2020): Malé Košecké Podhradie (2010, 7075b).

*Dactylorhiza majalis*, NT
Dolná Poruba; 0,6 km west of Homôlka Peak; 660; S; W; 1; I; 20.5.2019; 7075d; V. Ruček; / * Dolná Poruba; west part of Pod Homôlkou NR; 685; S; W; 4; I; 20.5.2019; 7075d; V. Ruček; / * Valaská Belá; saddle under Homôlka hill, near the pond under military monument; 765; S; E; 3; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; 0,4 km NE of Homôlka

Peak; 755; P; E; O; I; 20.5.2019; 7075d; V. Ruček; / * Pružina; Radotiná Valley, south of Mlynište Settlement; 415; S; N; 2; I; 10.6.2019; 6976d; V. Ruček; / * Beluša; Kráľové Locality, 1 km NW of Rohatín Peak; 434; S; W; 1; I; 17.5.2019; 6976c; V. Ruček; / * Horné Kočkovce; 0,9 km NNE of Kozinec Peak; 440; S; N; 3; I; 21.6.2020; 6876c; V. Ruček; verified older data by Fajmonová from 2003.

*Dactylorhiza viridis*, NT
Pružina; meadow below the Hrubá Kačka Peak; 1020; P; 0; 1; I; 2019; 7077d; V. Ruček; /.

*Epipactis albensis*, NT
Košeca; Košecká Valley, 3,5 km from the mouth of the valley; 350; P; /; 9; I; 9.8.2020; 6975d; V. Ruček; / * Visolaje; confluence of streams, south of Jančekovica Hill; 315; P; 0; 7; I; 17.8.2020; 6976a; V. Ruček; /.

*Epipactis atrorubens*, LC
Omšenie; 0.3 km S-SW of Omšenská baba Peak; 525; G; S; O; I; 14.6.2019; 7075c; V. Ruček; / * Kopec; 1 km north of Kopec Village, near the third class road; 410; M; E; O; G; 15.6.2019; 7076a; V. Ruček; 2 micro-localities * Zliechov; north of Košecké Rovné Village, 0,5 km SW of Gábrišské vrchy Peak; 717; M; S; 1; I; 29.7.2019; 7076a; V. Ruček; / * Beluša; Ostrá Malenica Hill, near the old hunting forest trail to the southern peak; 775-880; M; W; O; G; 1.8.2020; 6976c; V. Ruček; 2 micro-localities * Pružina; Rečica Valley, 0,5 km SW of Bukovina Peak; 555-587; S; W; O; S; 28.6.2019; 6977c; V. Ruček; / * Pružina, Čelková Lehota; SE of Briestenné Settlement, foothill of Bukovina Hill; 528-604; M; N; O; S; 28.6.2019; 6977c; V. Ruček; / * Zemianská Závada; 0,5 km north of the peak of Tínie Hill; 720; G; E; 1; I; 24.6.2019; 6976b; V. Ruček; / * Čelková Lehota; valley south of Čelková Lehota Village; 854-859; M; N; O; S; 29.6.2019; 6977c; V. Ruček; / * Visolaje; near the road to Markov Settlement; 285; S; N; 1; I; 19.6.2020; 6976a; V. Ruček; / * Kardošová Vieska; 0,9 km NEE of Kardošová Vieska Village; 480; S; W; O; G; 25.7.2019; 6977a; V. Ruček; /2 micro-localities * Malé Lednice; the main ridge of Sádecké vrchy Mts, 0,7 - 1,1 km NW of Srniak Peak; 700-730; M; S; O; G; 25.7.2019; 6977d; V. Ruček; 2 micro-localities * Považská Teplá; NNR Manínska Gorge; 405-611; M, G; N, S; /; S; 21.6.2019; 6877c; V. Ruček; verification of older data recorded by J. Smatanová from 2002 * Hlboké nad Váhom; calvary, north of the village; 355; M; S; 1; I; 7.5.2019; 6777d; V. Ruček, M. Jánoš; /.

*Epipactis helleborine* subsp. *helleborine*, LC
Valaská Belá; near a forest road, 0,4 – 1,1 km NW of Šenkovci Settlement; 710-780; M; E; O; S; 23.6.2019; 7076c; V. Ruček; / * Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 735-840; M, G; S; 68; G; 23.7.2020; 7075d; V. Ruček; / * Horná Poruba; 0,4 - 1,8 km NW-W of Suchá hora Peak on the ridge; 840-965; M; S; O; S; 18.7.2019; 7076c; V. Ruček; verification of older record recorded by Grulich from 2003 (Mertanová and Smatanová 2006) * Horná Poruba; near the red-marked hiking trail under the second class road; 490; S; E; 1; I; 23.7.2020; 7075d; V. Ruček; / * Dubnica nad Váhom; 0,8 km NW of Hoľazne Top; 795; S; W; 1; I; 23.7.2020; 7075d; V. Ruček; / * Dubnica nad Váhom; 1,2 km south of Beňová skala Peak; 675; M; S; 1; I; 23.7.2020; 7075d; V. Ruček; / * Košecké Podhradie; 0,4 km SW of Kopec Village; 475; S; E; 1; I; 15.6.2019; 7076c; V. Ruček; / * Beluša; valley between Bukovina and Tlstá hora Hill, near the forest road and cottage area; 380; S; S; 1; I; 6.6.2019; 6976c; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes; 345-430; M; S; 10; S; 20.7.2020; 6975d; V. Ruček; 6 micro-localities * Ladce; east of Horné Ladce Settlement, above Lúčkovský potok Stream; 260-330; M; W; 12; G; 9.7.2019; 6975d; V. Ruček; / * Ladce; northern foot of Kalište Hill; 335-385; M; N; 7; G; 9.7.2019; 6975d; V. Ruček; 2 micro-localities * Beluša; Jelenia skala Locality; 424-448; M; S; T; G; 19.7.2019; 6976c; V. Ruček; / * Hloža-Podhorie; 1 km NNE of Butkov Peak; 495; G; N; 1; I; 11.8.2020; 6976c; V. Ruček; / * Hloža-Podhorie; Druhé Kamenné vráta Gorge, near the third class road; 442; G; S; 2; I; 26.8.2020; 6976c; V. Ruček; / * Beluša; Ostrá Malenica Hill, near the old hunting forest trail to the southern peak; 635-880; M; W; 5; S; 1.8.2020; 6976c; V. Ruček; 3 micro-localities * Slopná; Ostrá Malenica Hill, near the top of the hill; 884; G; W; 1; I; 2.8.2020; 6976c; V. Ruček; / * Pružina; Hrubá Kačka, between the Samostrel Meadow and meadow below the top, near the green-marked hiking trail, below the top and 0,5-0,8 km north of Hrubá Kačka Peak; 909-1019; M; W, N; 16; S; 11.7.2019; 7077a; V. Ruček; 3 localities * Pružina; 0,2 km NE of Priepasť medzi Kačkami Cave; 824; S; N; 1; I; 28.6.2019; 7077a; V. Ruček; / * Pružina; near the Dúpna Cave; 613; G; N; O; I; 30.6.2019; 7077a; V. Ruček; / * Pružina; Rečica Valley, 0,9 km SE of Bukovina Peak; 539; S; W; 3; I; 1.7.2019; 7077a; V. Ruček; / * Pružina; SE of Briestenné Settlement, foothill and northwest slope of Bukovina Hill; 528-604; M; N; O; S; 28.6.2019; 6977c; V. Ruček; / * Podskalie; border of Podskalský Roháč NNR; 460; M; S; 1; I; 20.6.2019; 6976d; V. Ruček; / * Beluša; under the rock cliff of Kamenica Hill; 325-375; M; S; 3; S; 30.6.2020; 6976a; V. Ruček; / * Beluša; Víšok Hill; 400-435; M; N, E, S; 30; G; 22.7.2020, 3.8.2020; 6976a, 6976c; V. Ruček; 5 micro-localities * Visolaje; near Markov Settlement; 310; S; W; 1; I; 19.6.2020; 6976a; V. Ruček; / * Kardošová Vieska; Boria Locality, 1,5 km NEE of Kardošová Vieska Village; 575; S; N; 1; I; 25.7.2019; 6977a; V. Ruček; / * Malé Lednice; Srniak Peak and the main ridge of Sádecké vrchy Mts; 705-790; M; W; O; G; 25.7.2019; 6977b, 6977d; V. Ruček; 2 micro-localities * Rajec; 0,2 km north of Baby Peak; 575; G; N; O; I; 25.7.2019; 6977b; V. Ruček; / * Rajec; 0,7 km north of Srniak Peak; 610; M; N; O; I; 25.7.2019; 6977b; V. Ruček; / * Rajec; Veľký háj Locality, 2,3 km NEE of Malé Lednice Village; 525; S; W; 4; I; 25.7.2019; 6977b; V. Ruček; / * Horné Kočkovce, Nosice; near the peak of Hradisko Hill; 470-565; M, G; N, E, S, W; 21; S; 17.7.2019, 21.6.2020; 6876c; V. Ruček; 3 micro-localities * Považská Teplá; NNR Manínska Gorge; 375-525; M, G; N, E; O; S; 13.6.2020, 6.7.2020; 6877c; V. Ruček; 3 micro-localities * Súľov-Hradná; 0,8 NNE of Súľov Castle; 575; M; S; 1; I; 16.7.2019; 6877b; V. Ruček; / * Jablonové; the ridge in Deliška Localition; 520; M; S; 1; I; 4.8.2020; 6877a; V. Ruček; /

* Súľov-Hradná; Brada Locality; 625-710; S, M, G; S, E; T; S; 16.7.2019; 6877b; V. Ruček; 8 micro-localities * Jablonové; east slopes of the nameless hill 2,2 km east of Jablonové Village; 640; M; E; 1; I; 16.7.2019; 6877b; V. Ruček; /.

*Epipactis komoricensis*, NT
Súľov-Hradná; 0,3 km SSW of Brada Peak; 680; M; S; 33; I; 16.7.2019; 6877b; V. Ruček; / * Hrabové; 0,9 km SE of Hrabové Village, near the blue-marked hiking trail; 410; S; N; 1; I; 16.7.2019; 6777c; V. Ruček; /.

*Epipactis leptochila* s.l.
Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 730-805; M, G; S; 7; S; 23.7.2020; 7075d; V. Ruček; 3 micro-localities * Horná Poruba; 0,8 km NW-W of Suchá hora Peak; 950; M; S; 1; I; 18.7.2019; 7076c; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes, 2 and 5 km from the mouth of the valley; 375; M; S, E; 2; S; 20.7.2020; 6975d; V. Ruček; 2 micro-localities * Ladce; N-NW of Kalište Peak; 380; S; N; 4; G; 9.7.2019; 6975d; V. Ruček; 2 micro-localities * Pružina; near the Dúpna Cave; 608; M; N; 10; G; 30.6.2019; 7077a; V. Ruček; / * Nosice; near the peak of Hradisko Hill; 550-560; M, G; N; 4; I; 17.7.2019; 6876c; V. Ruček; / * Považská Teplá; NNR Manínska Gorge; 420; G; N; 1; I; 6.7.2020; 6877c; V. Ruček; / * Považská Teplá; eastern foothill of Malý Manín Hill; 585-625; M, G; E; 6; S; 22.7.2019; 6877c; V. Ruček; 5 micro-localities * Súľov-Hradná; near the green-marked hiking trail from Jabloňové Village to Súľov Castle; 400; M; N; 3; G; 4.8.2020; 6877a; M. Jánoš; / * Súľov-Hradná; 0,7 km NNE of Súľov Castle; 590; M; S; 1; I; 16.7.2019; 6877b V. Ruček; / * Súľov-Hradná, Hrabové; Brada Locality; 690; M; W, E; 50; G; 16.7.2019, 4.8.2020; 6877b V. Ruček; 2 micro-localities * Jablonové; south slopes of the nameless hill 2,2 km east of Jablonové Village; 625-665; M, G; S; 16; S; 16.7.2019, 4.8.2020; 6877b V. Ruček; 6 micro-localities * Hrabové; 1,6 km NW of Brada Peak, near the blue-marked hiking trail; 525; M; N; 1; I; 16.7.2019; 6877a V. Ruček; /.

*Epipactis leutei*, EN
Beluša; Víšok Hill; 350-380; S, M; N; 30; G; 22.7.2020, 3.8.2020; 6976a, 6976c; V. Ruček; 3 micro-localities * Horné Kočkovce; 0,2 km SE of Hradisko Hill; 535; M; S; 1; I; 17.7.2019; 6876c; V. Ruček; / * Považská Teplá; 0,7 km east of Malý Manín Peak; 580; G; E; 1; I; 22.7.2019; 6877c; V. Ruček; / * Súľov-Hradná; Brada Locality; 645; S; E; 3; G; 16.7.2019; 6877b V. Ruček; 2 micro-localities.

*Epipactis microphylla*, LC
Omšenie; near the yellow hiking trail under ; 490-590; M; W; O; G; 14.6.2019; 7075c; V. Ruček; / * Valaská Belá; 0,2-0,5 km west of Šenkovci Settlement; 785; M; S; T; G; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; near a forest road, 0,6 – 1,2 km NW of Šenkovci Settlement; 705-755; M; E; T; G; 23.6.2019; 7076c; V. Ruček; / * Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 750-850; M, G; S; T; G; 23.7.2020; 7075d; V. Ruček; 5 micro-localities * Dubnica nad Váhom; 1,3 km south of Beňová skala Peak; 665; G; S; O; I; 23.7.2020; 7075d; V. Ruček; / * Dubnica nad Váhom; 1,3 km SW of Beňová skala Peak; 595; G; W; 6; I; 23.7.2020; 7075d; V. Ruček; / * Horná Poruba; Vápeč NNR; 795; M; W; O; I; 24.6.2019; 7075d; V. Ruček; 2 micro-localities * Veľké Košecké Podhradie; Mraznica Locality, 1,1 km east of Stupičie Peak; 670; M; E; 1; I; 19.8.2020; 7076a; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes; 315-455; M; S; 24; S; 20.7.2020; 6975d; V. Ruček; 5 micro-localities * Beluša; valley between Bukovina and Tlstá hora Hill, near the forest road and cottage area; 365-380; S; S; 4; G; 6.6.2019; 6976c; V. Ruček; 2 micro-localities * Ladce; east of Horné Ladce Settlement, above Lúčkovský potok Stream; 340; M; S; 1; I; 9.7.2019; 6975d; V. Ruček; / * Ladce; north of Kalište Peak; 385; S; N; 2; I; 9.7.2019; 6975d; V. Ruček; / * Podskalie; border of Podskalský Roháč NNR; 480; M; E; 2; S; 24.6.2019; 6976d; V. Ruček; 2 micro-localities * Podskalie; 0,5 km west of the peak of Týnie Hill; 590; M; S; 1; I; 24.6.2019; 6976b; V. Ruček; / * Beluša; Jelenia skala Locality; 383-434; M; S; O; G; 19.7.2019; 6976c; V. Ruček; 2 micro-localities * Beluša; forest northeast of Čerencové Settlement; 315-355; M; S; 7; G; 2.7.2020; 6976c; V. Ruček; 2 micro-localities * Beluša; under the rock cliff of Kamenica Hill; 320-400; M; S; 14; S; 30.6.2020; 6976a; V. Ruček; / * Beluša; Víšok Hill; 400-435; M; N, E, S; 27; G; 22.7.2020; 6976c; V. Ruček; 3 micro-localities * Horné Kočkovce; 0,6 km NE of Kozinec Peak; 485; S; W; 1; I; 17.7.2019; 6876c; V. Ruček; / * Horné Kočkovce, Nosice; near the peak of Hradisko Hill; 527-565; M, G; N, E, S; 11; S; 17.7.2019; 6876c; V. Ruček; 2 micro-localities * Považská Teplá; NNR Manínska Gorge; 365-575; M, G; N, S; 6; G; 22.7.2019, 6.7.2020; 6877c; V. Ruček; 3 micro-localities * Považská Teplá; eastern foothill of Malý Manín Hill; 545-615; M, G; E; 28; G; 22.7.2019; 6877a, 6877c; V. Ruček; 5 micro-localities, verification of the older record (Ujházyová *et al.* 2007) * Súľov-Hradná; near the red-marked hiking trail from Súľov Village to Brada Localitions; 535-670; S, M; E; O; S; 16.7.2019, 4.8.2020; 6877b; V. Ruček; / * Jablonové; south slopes of the nameless hill 2,2 km east of Jablonové Village; 630-650; M; S; T; G; 16.7.2019, 4.8.2020; 6877a, 6877b; V. Ruček; 3 micro-localities.

*Epipactis muelleri*, NT
Valaská Belá; 0,3 km east of Homôlka Peak; 780; S; E; 1; I; 23.6.2019; 7075d; V. Ruček; / * Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 755-830; M, G; S; 4; S; 23.7.2020; 7075d; V. Ruček; / * Horná Poruba; near the red-marked hiking trail around the second class road; 505-565; S; E; 6; G; 23.7.2020; 7075d; V. Ruček; 2 micro-localities * Veľké Košecké Podhradie; Podhradská Valley, Michalová, Malá Šimerka Locality; 385-470; M; S; 8; G; 3.7.2020; 7076a; V. Ruček; 3 micro-localities * Veľké Košecké Podhradie; Mraznica Locality, 1,3 km east of Stupičie Peak; 610; M; E; 3; I; 19.8.2020; 7076a; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes, 3-4 km from the mouth of the valley; 345-410; M; S; 2; S; 20.7.2020; 6975d; V. Ruček; 2 micro-localities * Beluša; Ostrá Malenica Hill, near the old hunting forest trail to the southern peak; 745; M; W; 1; I; 1.8.2020; 6976c; V. Ruček; / * Čelková

Lehota; 0,4 km SE-E of Briestenné Settlement; 497; S; E; 1; I; 28.6.2019; 6977c; V. Ruček; / * Beluša; forest northeast of Čerencové Settlement; 305-365; S, M; S; 3; S; 2.7.2020; 6976c; V. Ruček; / * Beluša; Víšok Hill; 405-440; S, M; E; 5; S; 22.7.2020, 3.8.2020; 6976c; V. Ruček; 4 micro-localities * Visolaje; near the road along Markov Stream and south slope 0,8 km SWW of Markov Settlement; 310; S, M; S; 11; G; 19.6.2020; 6976a; V. Ruček; 2 micro-localities * Kardošová Vieska; northern ridge and southern slopes above Kobylia Valley; 580; M; W; O; G; 25.7.2019; 6977c; V. Ruček; 2 micro-localities * Rajec; 0,3 km north of Baby Peak; 580; G; N; O; I; 25.7.2019; 6977b; V. Ruček; / * Nosice; near the peak of Hradisko Hill; 560; M; E; 1; I; 17.7.2019; 6876c; V. Ruček; / * Súľov-Hradná; mouth of Čierny potok Valley; 370; M; N; 1; I; 21.6.2019; 6877a; V. Ruček; / * Súľov-Hradná; near the red-marked hiking trail from Súľov Village to Brada Locality; 485-545; S; E; 1; I; 16.7.2019; 6877b; V. Ruček; / * Jablonové; the ridge in Deliška Localition; 505; M; S; 1; I; 4.8.2020; 6877a; V. Ruček; / * Jablonové; south and west slopes of the nameless hill 2,2 km east of Jablonové Village; 625-655; M; S; 4; S; 16.7.2019, 4.8.2020; 6877a, 6877b; V. Ruček; 3 micro-localities * Hrabové; Makovce Locality, near the blue-marked hiking trail; 475; M; N; 1; I; 16.7.2019; 6877a; V. Ruček; / * Hlboké nad Váhom; calvary, north of the village; 390; M; S; 1; I; 7.5.2019; 6777d; V. Ruček; /.

*Epipactis neglecta*, VU
Považská Teplá; 1,1 km NE of Malý Manín Peak; 595; G; E; 1; I; 22.7.2019; 6877a; V. Ruček; verification of the older record from 1999 (Mereďa 2002).

*Epipactis palustris*, NT
Horná Poruba; Pod Hoľaznami Locality; 655; S; E; 4; I; 23.7.2020; 7075d; V. Ruček; there were 2 micro-locality of occurrence according to Fajmonová from 1990 * Horná Poruba; near the red-marked hiking trail under the second class road; 520; S; E; 9; I; 23.7.2020; 7075d; V. Ruček; /.

*Epipactis pontica*, LC
Beluša; Jelenia skala Locality; 455; M; S; 2; I; 19.7.2019; 6976c; V. Ruček; / * Dolné Kočkovce; 0,9 km SW of Kozinec Peak; 440-460; S, M; W, E; 30; G; 17.7.2019; 6876c; V. Ruček; 3 micro-localities * Súľov-Hradná; 0,4 km SSW of Brada Peak; 660; S; S; 4; I; 16.7.2019; 6877b; V. Ruček; /.

*Epipactis pseudopurpurata*, VU
Horná Poruba; Hoľazne, southern slopes under the rock Ničová; 780-790; M; S; 4; I; 23.7.2020; 7075d; V. Ruček; / * Veľké Košecké Podhradie; Mraznica Locality, 1-1,3 km east of Stupičie Peak; 600-670; M; E; 5; G; 19.8.2020; 7076a; V. Ruček; 2 micro-localities * Košeca; Košecká Valley, south-facing slopes; 345-485; S, M; S; 9; S; 20.7.2020; 6975d; V. Ruček; 5 micro-localities * Beluša; 1 km west of the top of Ostrá Malenica Hill; 595; M; N; 1; I; 2.8.2020; 6976c; V. Ruček; /.

*Epipactis purpurata*, NT
Valaská Belá; 0,3 km SE of Homôlka Peak; 770; S; S; O; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; 0,7 km SE-E of peak with an elevation of 841,5 m asl., near a forest road; 735; S; E; 1; I; 23.6.2019; 7076c; V. Ruček; / * Horná Poruba; near the red-marked hiking trail, Pod Hoľaznami Locality; 585; S; E; 1; I; 23.7.2020; 7075d; V. Ruček; / * Ilava; between Kohútky (Sokolia) and Belanové lazy Locality; 645; M; N; 1; I; 2.9.2020; 7075d; V. Ruček; / * Hloža-Podhorie; 0,5 km north of Rohatá Peak; 490; S; W; 1; I; 19.8.2020; 7076a; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes; 325-475; S, M; S; T; G; 20.7.2020; 6975d; V. Ruček; 4 micro-localities * Pružina; above Babirátka Cave; 450; S; N; 3; I; 2019; 6976d; V. Ruček; / * Ladce; NW of Kalište Peak; 370; M; N; 1; I; 9.7.2019; 6975d; V. Ruček; / * Hloža-Podhorie; near Maják Recreational facility; 342; S; N; 15; I; 6.8.2020; 6976c; V. Ruček; / * Beluša; Jelenia skala Locality and 0,3 km west of Panský háj Peak; 428-453; S; S; 24; G; 19.7.2019; 6976c; V. Ruček; 2 micro-localities * Beluša; 1,2 km west of the top of Prašnica Hill; 400; P; /; 6; S; 9.12.2020; 6976c; V. Ruček; / * Beluša; Víšok Hill; 310-410; S; N, E; 36; G; 22.7.2020, 3.8.2020; 6976a, 6976c; V. Ruček; 6 micro-localities * Horné Kočkovce; 0,9 km NE of Kozinec Peak; 510; S; S; 1; I; 17.7.2019; 6876c; V. Ruček; / * Nosice; 0,6 km west of Hradisko Peak; 470; S; W; 1; I; 21.6.2020; 6876c; V. Ruček; / * Považská Teplá; 1,6 km NW of Veľký Manín Peak, foot of the hill; 440; S; W; 20; I; 20.8.2020; 6876d; V. Ruček; / * Považská Teplá; NNR Manínska Gorge, southeastern and eastern foothill of Malý Manín Hill; 490-595; M; S, E; 17; G; 22.7.2019; 6877c; V. Ruček; 5 micro-localities.

*Goodyera repens*, NT
Súľov-Hradná; near the red-marked hiking trail, 0,3 km east of Súľov Castle; 535; S; E; O; I; 4.8.2020; 6877b; V. Ruček, J. Smatanová; /.

*Gymnadenia conopsea*, LC
Valaská Belá; 0,3 km east of Homôlka Peak; 765; S; E; H; I; 23.6.2019; 7075d; V. Ruček; / * Horná Poruba; Pod Hoľaznami Locality; 645; S; E; 4; I; 23.7.2020; 7075d; V. Ruček; nearby are 3 micro-locality of occurrence according to Smatanová from 2010 * Horná Poruba; Srvátková lúka Locality; 775; S; W; 1; I; 23.6.2019; 7075d; V. Ruček; / * Mojtín; 0,3 km west of Gabrišovci Settlement; 711; M; N; O; I; 15.9.2019; 7076a; V. Ruček; / * Pružina; Špicov lán Locality; 400-425; S, M; S; 153; I; 17.6.2019; 6976d; V. Ruček; with the occurrence of albino * Visolaje; near the road to Markov Settlement; 295; S; N; 2; I; 19.6.2020; 6976a; V. Ruček; / * Jablonové; 0,9 km SWW of Brada Peak, near the blue-marked hiking trail; 620; S; E; 1; I; 16.7.2019; 6877b; V. Ruček; /.

*Gymnadenia densiflora*, NT
Kopec; the mouth of Kopčianská Valley; 375; P; /; 1; I; 16.6.2020; 7076a; V. Ruček; /

*Gymnadenia odoratissima*, NT
Súľov-Hradná; mouth of Čierny potok Valley; 370; M; N; 1; I; 21.6.2019; 6877a; V. Ruček; / * Hrabové; 0,2 km SW of Brada Peak; 725; G; W; 1; I; 16.7.2019; 6877b; V. Ruček; /.

*Listera ovata*, LC
Valaská Belá; 0,3 km east of Homôlka Peak; 765; S; E; 1; I; 23.6.2019; 7075d; V. Ruček; / * Košecké Podhradie; 0,2 km SW of Kopec Village; 446; S; E; T; I; 15.6.2019; 7076c; V. Ruček; / * Košecké

Podhradie; Suchá Valley, near the Kopec Village, 1 km from the mouth of the valley; 445; S; N; T; I; 21.5.2020; 7076a; V. Ruček; / * Hloža-Podhorie; 0,8 km SEE of Dielec Peak; 415; M; N; 10; I; 7.5.2020; 6976c; V. Ruček; / * Beluša; valley between Bukovina and Tlstá hora Hill, near the forest road and cottage area; 380; S; S; 9; I; 6.6.2019; 6976c; V. Ruček; / * Beluša; 0,9 km NW of Hradište Peak, near the yellow-marked hiking trail; 315; S; W; T; I; 9.6.2020; 6976c; V. Ruček; / * Sádočné; surroundings of the peak of Ostrá Kačka; 895; M; N; O; S; 12.6.2019; 7077a; V. Ruček; / * Visolaje; near the road along Markov Stream and south slope 0,8 km SWW of Markov Settlement; 305-340; S; S, W; 6; G; 19.6.2020; 6976a; V. Ruček; 3 micro-localities * Nosice; Za hájom Locality, 1,4 km south of Nosice Village; 460; S; W; 1; I; 21.6.2020; 6876c; V. Ruček; / * Hlboké nad Váhom; Boky Locality, 0,7 km NW of Hlboké nad Váhom Village; 335; M; N; 10; I; 7.5.2019; 6777c; V. Ruček; / * Hlboké nad Váhom; calvary, north of the village; 385; M; S; 1; I; 7.5.2019; 6777d; V. Ruček; /.

*Malaxis monophyllos*, NT
Považská Teplá, NNR Manínska Gorge; 420; M; N; 5; I; 6.7.2020; 6877c; V. Ruček; verification of older data recorded by G. Runkovič from 1990.

*Neottia nidus-avis*
Dolná Poruba; 0,7 km SW-W of Homôlka Peak; 635; S; W; 7; I; 20.5.2019; 7075d; V. Ruček; / * Valaská Belá; 0,7 km NE of Homôlka Peak; 790; M; S; 1; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; 0,5 km east of peak with an elevation of 841,5 m asl., near a forest road; 710; M; S; 1; I; 23.6.2019; 7075d; V. Ruček; / * Valaská Belá; Srvátková lúka Locality; 760; P; 0; 1; I; 23.6.2019; 7075d; V. Ruček; / * Košecké Podhradie; 0,4 km SW of Kopec Village; 480; S; E; 1; I; 15.6.2019; 7076c; V. Ruček; / * Košecké Podhradie; around of Malá Zliezajňa and Šivarina Peak; 850-920; M, G; N; O; G; 20.5.2020; 7076c; V. Ruček; 2 micro-localities * Košecké Podhradie; Suchá Valley, near the Kopec Village, 1 km from the mouth of the valley; 475; M; N; 2; I; 21.5.2020; 7076a; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes, 4,5-5,5 km from the mouth of the valley; 345-410; M; S; 4; S; 20.7.2020; 6975d; V. Ruček; 2 micro-localities * Podskalie; border of Podskalský Roháč NNR; 450; M; S; 10; I; 4.5.2019; 6976d; V. Ruček; / * Beluša; valley between Bukovina and Tlstá hora Hill, 0,8 – 1 km east of Podlavičky Settlement; 400; S; S; O; G; 6.6.2019; 6976c; V. Ruček; 2 micro-localities * Hloža-Podhorie; 1 km NNE of Butkov Peak; 460-500; M; N; 6; S; 8.6.2020; 6976c; V. Ruček; / * Hloža-Podhorie; northern slope of Hradište Hill; 315-415; M; N; 17; G; 9.6.2020; 6976c; V. Ruček; 2 micro-localities * Beluša; Ostrá Malenica Hill, near a hiking trail below the middle top of the hill; 880; M; W; 50; I; 1.8.2020; 6976c; V. Ruček; / * Beluša; forest northeast of Čerencové Settlement; 325-355; S, M; S; 2; S; 2.7.2020; 6976c; V. Ruček; / * Sádočné; surroundings of the peak of Ostrá Kačka; 860-878; S; N; O; S; 12.6.2019; 7077a; V. Ruček; / * Sádočné; 0,5 km NW-W of Sádocký vrch Peak; 890; M; N; 20; G; 12.6.2019; 7077a; V. Ruček; / * Visolaje; 0,9 km SW of Markov Settlement, near the road along Markov Stream; 305; S; W; 1; I; 19.6.2020; 6976a; V. Ruček; / * Horný Moštenec, Zemianská Závada; near the educational trail to Temné Caves; 520-630; S, M; W, N, E; 14; S; 4.6.2020, 14.6.2020; 6976b; V. Ruček; / * Malé Lednice; 0,3 km NW of Srniak Peak; 690; M; N; O; I; 25.7.2019; 6977b; V. Ruček; / * Horné Kočkovce; 0,4 km SW of Hradisko Peak; 470; M; S; 3; I; 21.6.2020; 6876c; V. Ruček; / * Horné Kočkovce; Dubový háj Locality; 395; S; W; 1; I; 21.6.2020; 6876c; V. Ruček; / * Nosice; Za hájom Locality, 1,3 km south of Nosice Village; 480; P; 0; 9; I; 21.6.2020; 6876c; V. Ruček; / * Považská Bystrica; 1 km NW of Šurabová Settlement; 360-375; M; S; 26; I; 2.6.2019; 6876d; V. Ruček; / *.

*Ophrys insectifera*, NT
Visolaje; 0,8 km SWW of Markov Settlement; 310; M; S; 11; I; 19.6.2020; 6976a; V. Ruček; / * Hlboké nad Váhom; calvary, north of the village; 385; M; S; 1; I; 7.5.2019; 6777d; M. Jánoš, V. Ruček, J. Smatanová; /.

*Orchis mascula* subsp. *signifera*, NT
Dolná Poruba; 0,6 km west of Homôlka Peak; 665; S; W; 150; G; 20.5.2019; 7075d; V. Ruček; / * Dolná Poruba; east part of Pod Homôlkou NR; 685; S; W; H; G; 20.5.2019; 7075d; V. Ruček; /.

*Orchis pallens*, NT
Košecké Podhradie; Šivarina Peak; 915; S; W; 1; I; 20.5.2020; 7076c; V. Ruček; / * Beluša; valley between Bukovina and Tlstá hora Hill, near the forest road and cottage area; 380; S; S; 12; G; 6.6.2019; 6976c; V. Ruček; 2 micro-localities * Beluša; forest northeast of Čerencové Settlement; 355; M; S; 1; I; 2.7.2020; 6976c; V. Ruček; / * Prečín; Líčšia Locality, 0,6 km SW of Hradište Peak; 450; M; E; 2; I; 21.4.2020; 6977ac; V. Ruček; / * Podmanín, Praznov; near Pechov Settlement; 475-535; S; S; 22; G; 22.4.2020; 6877c; V. Ruček; 2 micro-localities * Vrchteplá; north of Vrchteplá village, near the red-marked hiking trail; 585; M; E; 25; I; 22.4.2020; 6877c; V. Ruček; verification of data recorded by P. Smatanová from 2015 * Súľov-Hradná; 0,2 km SSE of Brada Peak, near the red-marked hiking trail; 735; M; S; O; I; 23.4.2020; 6877b; V. Ruček; / * Hlboké nad Váhom; calvary, north of the village; 430; S; S; 1; I; 7.5.2019; 6777d; V. Ruček; / * Hlboké nad Váhom; 0,3 km NW of Veľký Ostrý Hill; 492; S; N; T; G; 7.5.2019; 6777d; M. Jánoš; /.

*Orchis ×loreziana*
Hlboké nad Váhom; 0,3 km NW of Veľký Ostrý Hill; 490; S; N; O; I; 7.5.2019; 6777d; M. Jánoš; /.

*Platanthera bifolia*, LC
Valaská Belá; 0,5 km east of peak with an elevation of 841,5 m asl., near a forest road; 710; M; S; O; G; 23.6.2019; 7076c; V. Ruček; / * Horná Poruba; near the red-marked hiking trail above the second class road; 540; S; E; 1; I; 23.7.2020; 7075d; V. Ruček; / * Kopec; the mouth of Kopčianská Valley; 370; P; /; 7; I; 16.6.2020; 7076a; V. Ruček; / * Košecké Podhradie; 0,3 km SW of Za Rohatou Peak; 860; S; W; O; I; 4.6.2019; 7076a; V. Ruček; / * Košeca; Košecká Valley, south-facing slopes, 4,5-5,5 km from the mouth of the valley; 365-400; S, M; S; O; S; 20.7.2020; 6975d; V. Ruček; 3 micro-localities * Visolaje; 0,9 km SW of Markov Settlement, near the road along Markov Stream; 305; S; W; 1; I; 19.6.2020; 6976a; V. Ruček; / * Horný

Moštenec, Zemianská Závada; near the educational trail to Temné Caves; 490-625; M; W; 7; S; 4.6.2020, 14.6.2020; 6976b; V. Ruček; / * Kardošová Vieska; northern ridge above Kobylia Valley; 640; S; S; 1; I; 25.7.2019; 6977d; V. Ruček; / * Kardošová Vieska; Boria Locality, 1,3 km NE of Kardošová Vieska Village; 540; S; W; 1; I; 25.7.2019; 6977a; V. Ruček; / * Malé Lednice; Srniak Peak; 750; M; S; 1; I; 25.7.2019; 6977b; V. Ruček; / * Rajec; 0,7 km north of Srniak Peak; 620; M; N; O; I; 25.7.2019; 6977b; V. Ruček; / * Nosice; Za hájom Locality, 1,2 km south of Nosice Village; 455; S; N; 1; I; 21.6.2020; 6876c; V. Ruček; /. Record of occurence according to P. Bagin (Eliáš Jr. 2020): Dubnica nad Váhom (2009, 7075a).

*Platanthera chlorantha*, NT
Valaská Belá; 0,3 km east of Homôlka Peak; 780; S; E; 1; I; 23.6.2019; 7075d; V. Ruček; / * Kopec; the mouth of Kopčianská Valley; 375; P; /; 1; I; 16.6.2020; 7076a; V. Ruček; / * Veľké Košecké Podhradie; Podhradská Valley, Michalová, Veľká Šimerka Locality; 475; M; S; 2; I; 3.7.2020; 7076a; V. Ruček; / * Beluša; forest northeast of Čerencové Settlement; 315-350; M; S; 3; S; 2.7.2020; 6976c; V. Ruček; / * Beluša; Víšok Hill; 435; M; E; 1; I; 22.7.2020; 6976c; V. Ruček; / * Visolaje; near the road along Markov Stream and south slope 0,8 km SWW of Markov Settlement; 240-335; P, S, M; S; 16; G; 19.6.2020; 6976a; V. Ruček; 4 micro-localities * Nosice; Za hájom Locality, 1,2 km south of Nosice Village; 455; S; N; 1; I; 21.6.2020; 6876c; V. Ruček; /.

*Platanthera × hybrida*
Beluša; forest northeast of Čerencové Settlement; 310; M; N; 1; I; 2.7.2020; 6976c; V. Ruček; /.

*Traunsteinera globosa*, NT
Košecké Podhradie; 0,5 km SW of Kopec Village; 490; S; E; 1; I; 15.6.2019; 7076c; V. Ruček; /.

*Orchid diversity*

The Shannon Diversity Index was calculated for three forest categories: deciduous, coniferous and mixed forest; the fourth category was non-forest habitats (Table 1; Fig. 3). The graph represents the richness of the species and the number of populations with respect to altitude (Fig. 4).

## Discussion

The genus *Epipactis* belongs to the most species-rich genera of the Orchidaceae family. The studied area is exceptional in terms of the number of species available. It was found by Mereďa Jr. (1996a, 1996,b, 2002a, 2010; Mereďa Jr. and Potůček 1998), where he contributed to the discovery and description of three new species. This last complex work is from the southern part of the Strážovské vrchy Mts, which extends slightly into the studied area. Research on forest orchids in the study area has not taken place in the last two decades. It is possible to follow up on the research of Mereďa Jr. (2002a) and Ruček (2019).

In total, 16 species of the genus *Epipactis* and one undescribed species were recorded

| | number of species (S) | number of individuals (p) | Shannon diversity index (H) | $H_{max}$ (ln(S)) | Equitability ($H/H_{max}$) |
|---|---|---|---|---|---|
| **Deciduous forest** | 45 | 4941 | 2.945 | 3.807 | 0.77 |
| **Mixes forest** | 27 | 973 | 2.569 | 3.296 | 0.78 |
| **Coniferous forest** | 31 | 805 | 2.717 | 3.434 | 0.79 |
| **Non-forest areas** | 47 | 5361 | 2.670 | 3.850 | 0.69 |

**Table 1.** Data from records of orchids in the studied area are divided into 4 categories: deciduous, mixed, coniferous forests and non-forest areas. Equitability represents the ratio between the maximum possible diversity ($H_{max}$) in a category and the actual diversity (H).

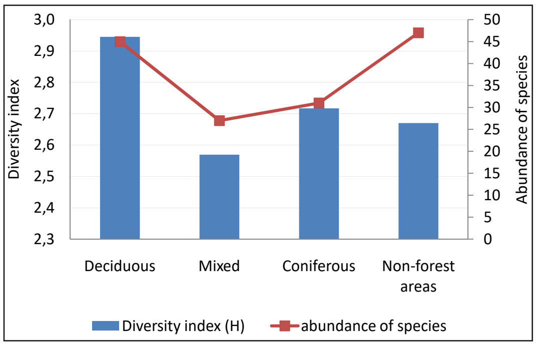


**Fig. 3.** A graphical expression of the index of diversity and abundance of species. The highest diversity is recorded in deciduous forests and the lowest in mixed forests. Although non-forest areas have a high number of species, diversity is low. Because there are several large populations of the *Dactylorhiza majalis*, *Gymnadenia conopsea*, *Listera ovata* and *Orchis mascula* subsp. *signifera*. These species significantly outnumber other species.

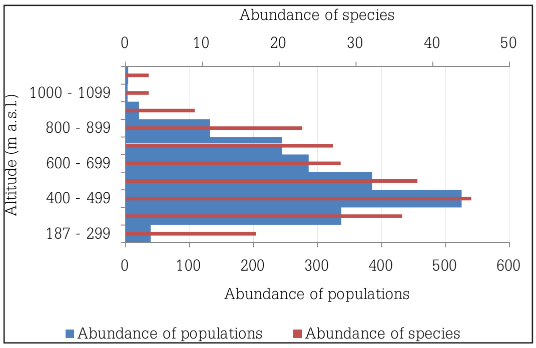


**Fig. 4.** Species richness and number of populations in relation to altitude with a defined interval. Data were used from a private list of populations with assigned measured altitudes.

in the studied area: *Epipactis albensis*, *E. atrorubens*, *E. futakii*, *E. greuteri*, *E. helleborine*, *E. komoricensis*, *E. leutei*, *E. microphylla*, *E. muelleri*, *E. neglecta*, *E. palustris*, *E. placentina*, *E. pontica*, *E. pseudopurpurata*, *E. purpurata* and *E. tallosii*; and one undescribed species: *Epipactis* sp. „karpatský". There are probably other taxa of the *E. leptochila* aggregate in the area that have not been published yet (Mereďa Jr. 2010), but which occurrence of has not been confirmed. Some species are very difficult to determine, mainly due to the great variability, and uncertain determining features. Additionally, dry weather can cause imperfect plant development, making it even more difficult to correctly identify some species.

In terms of categories of Conservation status: *E. futakii, E. greuteri, E. leutei*, and *E. placentina* are Endangered (EN); *E. neglecta*, and *E. pseudopurpurata* are Vulnerable (VU); *E. albensis, E. komoricensis, E. muelleri, E. palustris, E. purpurata*, and *E. tallosii* are Near Threatened (NT); and *E. atrorubens*, *E. microphylla*, and *E. pontica* are of Least Concern (LC) (Eliáš Jr. *et al.* 2015).

*Evaluation of taxa*

*Epipactis albensis*
This species was searched for along streams and rivers in alluvial softwood forest with poplar occurrence. The first mention of this species is from Slatinský Stream near Beluša Village (Mereďa 2002). Occurrence was confirmed in this area, but not precisely at the original site, as it was disrupted by the construction of a highway, a rest area and the excavation of riparian vegetation in the area. 12 new localities were discovered: primarily around the river Váh (6), near the foothill streams in Strážovské vrchy (4) (Podhradský, Košecký and Slatinský Stream), in Podmanínská pahorkatina Hills (1) (Visolajský Stream), in Súľovské vrchy (1) (Hradnianka Stream), and in Rajecká kotlina (a) (Rajčianska Stream). The first targeted mapping of this taxon in the studied area took place during field surveys for this work (Ruček 2019). Based on the discovery of 10 new localities in 2018, the probability of additional localities being discovered is high. The total number of exemplars/number of localities (year) is: 100 ex./ 10 loc. (in 2018), 16 ex./ 2 loc. (in 2020). In the near future, a survey of preserved alluvial softwood forests in the Žilinská kotlina Basin, southwestern Považské podolie Valley and side valleys with should be carried out. A high presumption of further findings exists in the Rajecká kotlina, and Považské podolie valleys in highland areas, with the exception of the central Strážovské vrchy Mts. The average altitude of these sites is 320 m asl., while the maximum is 467 m. The locality near Bytča has shifted the northern border of distribution in Slovakia, with the assumption that species may occur further north, in the Kysuce Region, as there are many northern locations in the Czech Republic, Poland, Germany and Lithuania.

*Epipactis atrorubens*
The typical habitat of *E. atrorubens* is on drier calcareous to mesophilic soil (Batoušek and Kežlínek 2012). Such soils are typical for the studied area. It is one of the most widespread species of *Epipactis* in the area, recorded at 36 localities with 105 micro-localities and 512 exemplars between 2017 and 2020. The most common occurrence is on south-facing slopes with a medium to high incline, and terrain with an altitude of 285 m to 840 m. The species is widespread in the mountainous part of the Strážovské and Súľovské vrchy Mts. In the vicinity of the Váh River, Podmanínská and Žilinská Collin, where agricultural land is intensively used. It is possible that this species may also occur in limestone or dolomite quarries, embankments or road edges. Most localities were recorded near Košecké Podhradie Village on forest-stepped slopes with unstable and often naturally disturbed soil on a dolomitic base, in the vicinity of Pružina and Čelková Lehota Village on the edge of forest roads and Súľovské skály NNR.

*Epipactis futakii*
Recorded occurrence of this species is in the vicinity of Veľký Kolačín Village, Trenčianská Teplá, and Teplice Town. The next nearest sites are in the Biele Karpaty Mts and Považský Inovec Mts. Records of the last documented occurrence come from one locality near Veľký Kolačín Village in 2018 (3 ex.) and 2020 (10 ex.). This small area of occurrence is probably due a relatively evolutionarily young taxon that may be easily confused with *E. helleborine* or other cleistogamically flowering *Epipactis*. Due to the small population and low number of localities, this species is classified as endangered. Its northern border is near Veľký Kolačín Village or Vlárský průsmyk Pass in the Czech Republic. However, there are more suitable habitats in the studied area, so it is possible that this taxon may occur at higher latitudes.

*Epipactis greuteri*
In the study area there are two localities where this species occurs. However, the only successfully verified locality was near Považská Teplá Village. The locality near Lietavská Svinná Village (Mereďa Jr. 2000) was degraded by forestry and the species could not be found (ined.). In the foothills of Veľký Manín Hill near Považská Teplá Village, a total of 64 individuals were found in 7 microlocalities in 2017, 2018 (Ruček 2019) and 2020. *E. greuteri* has specific ecological requirements. It generally grows near running water in wet fir-beech and spruce forests but running water is not a requirement (Batoušek and Kežlínek 2012). The results of frequent field research is that there are not many suitable habitats for this orchid in the studied area. The result of frequent field research is the finding that there is a lack of suitable habitats for this *Epipactis* in the study area. The year-over-year population dynamics of the species are not known, so it is appropriate to verify suitable habitats every year. Genetic material can be spread from nearby localities in Javorníky Mts – Čertov Locality, Makov and Papradno Village (ined.).

*Epipactis helleborine* subsp. *helleborine*
This is the species with the highest number of localities within the genus *Epipactis* and second highest within the family Orchidaceae. in the years 2017 to 2020, there were 500 recorded individuals

in 104 micro-localities at 62 localities in all geomorphological units. According to the density of distribution, the areas are divided into eurytopic-uplands to highlands and stennotopic plains to hill lands. The low-density area is the valley of the Vah River and its basins. These are anthropogenically created habitats. As an apophyte, *Epipactis helleborine* can also occur in such a habitat. There have also been very few botanical surveys in this habitat. Older records were not used to create the extension map, as there is a high probability of misidentification. The oldest herbarium item of this species is from Klepáč Hill in 1898 with literature referencing the occurrence prior to 1984 (Brancsik 1884; Mereďa Jr. 2010).

*Epipactis komoricensis*
This species is widespread predominantly in the northern and southern parts of the study area. Many suitable habitats exist in the central part of the study area as well, but there have been fewer field surveys. A total of 22 localities were discovered, including 5 in 2018 and 2019 (Mereďa Jr. 2002a; Mereďa Jr. 2010; Ruček 2019). The most numerous population was observed in the Pod Bradou Locality in Súľovské skaly NNR (33 individuals) and in Vápeč Hill (about 50 individuals). The highest concentration of localities is in the wider vicinity of Omšenie, Kostolec and Súľov Village. This orchid prefers beech forests. Occurrence localities are on average 84 % beech and 6 % spruce. The potential to find new localities is great, as 41 % of the total area studied is beech forests.

*Epipactis leptochilla* s. l.
A nominal subspecies of *Epipactis leptochila* (Godfery) Godfery subsp. *leptochila* has not yet been discovered in the study area. It is likely that all records of *E. leptochila* relate to species not yet described from the *E. leptochila* agg. or *E. neglecta* agg. groups. The nearest localities of *E. leptochila* s. str. are most likely in the Veľká Fatra Mts and the Chočské vrchy Mts (Mereďa Jr. 2002a; Mereďa Jr. 2010). The only recorded species from this aggregate is undescribed *Epipactis* sp. "karpatský". 39 new micro-localities were discovered at 22 localities with 144 individuals at an altitude ranging from 375 to 950 meters. Populations are usually small. The largest number of individuals (50 ex.) was recorded at the Pod Bradou Locality in Súľovské skaly NRR. The distribution of localities is even throughout the studied area. Therefore, it is assumed that the density of discovered sites may increase in the future. Its preferred habitat is on slopes with a medium to large inclination. 51 % of the population is found in deciduous forests with 90 to 100 % representation of *Fagus sylvatica*. Other localities include mixed forest stands with a 49 % share of *Fagus sylvatica*, 25 % share of *Pinus* sp., 11 % share of *Quercus* sp. and 9 % *Picea abies*.

*Epipactis leutei*
Individuals from the studied area are most similar in their flower structure to *E. leutei*, although they are characterized by minor differences. No record has been published in the territory yet. The first published data on this taxon are in Ruček (2019) and in this work. 21 new microlocalities were discovered at 11 localities. 21 new micro-localities were discovered at 11 localities in the Strážovské vrchy Mts, the Súľovské vrchy Mts, the Považské podolie Unit and in the Javorníky Mts at an altitude ranging from 340 to 850 meters. 61 % of sites are located in beech forests. The others are located in various forest stands, including beech-oak, beech-spruce, pine-spruce, pine-oak-hornbeam-fir-spruce.

*Epipactis microphylla*
The oldest mention of this species is from the vicinity of Trenčianske Teplice Town in 1881 (Holuby 1881; Mereďa Jr. 2010). Within the studied area it occurs abundantly in mountainous regions. There were none recorded in the basins. A total of 87 micro-localities were recorded in 44 localities in the Javorníky Mts, the Strážovské and the Súľovské vrchy Mts at altitudes from 314 (Košecká dolina Valley) to 937 meters (Strážov Hill). 49 % of localities are located in beech forests, and 11 % are located on the edge of forest stands. Other sites are found in mixed forests in the presence of *Pinus, Quercus, Carpinus betulus, Picea abies* and more. Its preferred habitat is on the slopes with a medium to high inclination.

*Epipactis muelleri*
This species occurs scattered over all geomorphological units. 109 individuals from 47 microlocalities were recorded in 34 localities at an altitude between 307 and 836 meters. 22 % of the localities are in beech forests, 15 % are in pine or pine-beech forest, 11 % are in mixed spruce forests and 26 % occur outside of forest stands. Populations are small in number; on average three plants in each locality. They were most often recorded on flat terrain and on slopes with a medium inclination.

*Epipactis neglecta*
This is a highly variable taxon, divided into several character-specific populations (Mereďa Jr. 2010). During he field survey, several individuals were found, but they were not possible to determine unambiguously. The plants were affected by prolonged drought, defective development of flower organs, atypically coloured petals and epichilus, or damaged by insects of the superfamily Aphidoidea. Only one individual was found on Malý Manín Hill near Záskalie Village - a verified older locality from 1999 (Mereďa Jr. 2002a). According to Mereďa Jr. (2002a, 2010), older records are from Markovica Hill, Vápeč Hill, Súľovské skaly NRR and from the southern part of the Strážovské vrchy outside the studied area. In 2016, P. Novosádová confirmed their occurrence in Súľovské skaly NRR in two localities (Štátna ochrana prírody SR 2014). The nearest localities are from the vicinity of Trenčianske Bohuslavice and Nová Bošáca Village in the Biele Karpaty Mts. and the south Moravia Region in the Czech Republic.

*Epipactis palustris*
As a heliophilic and wetland species, *Epipactis palustris* prefers illuminated areas and wetland habitats. As the field survey was mainly conducted in forest habitats, its occurrence was recorded in only 10 localities among springs, fens, road ditches and the banks of a watercourse. The largest populations were found in the locality near

Jasenová Village (hundreds of individuals), and near the villages of Omšenie, Zliechov and Domaniža (tens of individuals). *Epipactis palustris* is endangered due to declining wetland habitats, as a result of drainage; shading of the habitat by over grown trees; succession by competitively resistant plants; and storage of wood after logging.

*Epipactis placentina*
In the studied area this species occurs in only one locality in the territory of Dubnica nad Váhom City in the oak-beech forest. According to P. Mereď Jr. and P. Mereďa Sr. , the population has a declining trend. In 2018, 9 individuals were recorded here. Forest harvesting took place near and illuminated the site, which can be one of the causes of the decline of flowering individuals. However, the most likely reason is prolonged drought. The Italian orchid population along the Apennines faces a similar problem (Magrini *et al.* 2012). This orchid is very inconspicuous, so it is very difficult to find. It may ultimately be found in other localities, as it occurs in the nearby Biele Karpaty Mts as well as in the southern part of the Strážovské vrchy Mts (Mereďa Jr. 2010).

*Epipactis pontica*
Between 2017 and 2020, 5 localities were recorded; three of them are new (Javorníky, Strážovské and Súľovské vrchy Mts). The most numerous populations were found in Kozinec Hill near Dolné Kočkovce (30 individuals, new site); in Veľký Manín Hill near Považská Teplá Village (37 individuals) and at Butkov Hill near Belušské Slatiny Village (50 individuals). The habitats were at an altitude ranging between 434 and 660 meters in beech-oak forests with an admixture of *Pinus* sp., *Picea abies*, *Abies alba* and *Carpinus betulus*. A total of 20 localities were recorded in the studied area (Mereďa Jr. 2002a, 2010; Ruček 2019). The nearest localities are in the northern part of the Biele Karpaty Mts and from Udiča Village in the Javorníky Mts (Mereďa Jr. 2002a). There is a presumption that this species may also be discovered in other localities in the foothills of the Považie Region and basins.

*Epipactis pseudopurpurata*
With regard to this species, the highest frequency and most continuous area of occurrence is in the Strážovské vrchy Mts at 35 localities. In the Súľovské vrchy Mts, There are only 3 localities. During 2018 to 2020, 11 new sites were discovered at an altitude of 346 (Košecká dolina Valley) to 793 meters (near Košecké Rovné Village), mostly in beech forests. The populations were small; 54 individuals were found on slopes with a slight to medium inclination.

*Epipactis purpurata*
This species has numerous taxa with different population sizes. It likely occurs throughout the whole studied area. Between 2017 and 2020, 473 individuals were recorded in 60 micro-localities at 34 localities in the Javorníky Mts, the Strážovské and Súľovské vrchy Mts and in the foothill areas in the Považské podolie Valley, at an altitude of 309 (near Beluša) to 803 meters (near Mojtín). The species occurs in forests with different wood composition. 18 % of the population was located in beech forest, 8 % in coniferous forests of spruce and spruce-pine; 15 % were outside the forest; and the others were found in mixed forest with different proportions of wood species including *Larix decidua, Picea abies, Quercus* sp., *Pinus* sp., *Fagus sylvatica*, *Carpinus betulus*, and others.

*Epipactis tallosii*
The only recent data from the studied area is from the vicinity of Dubnica nad Váhom City. This location is no larger than 10 x 25 meters. Population dynamics is significant in the number of plants between seasons (Mereďa Jr. 2002b). In 2018, more than 50 individuals were recorded, but none were recorded in 2020. According to Mereďa Jr. (2002b), unstable population dynamics is rather frequent, and is likely the reason other sites have not been discovered. The site near Borčice Village was verified, but occurrence was not confirmed. There was a significant human intervention in the floodplain forest. According to the model of potentional distribution by Ljubka (2018), there is a boundary between high and medium probability of occurrence of *E. tallosii*. Further north, there is a medium to zero probability due to increasing altitude and associated ecological and climatic conditions. The nearest localities are from the southern part of the Strážovské vrchy Mts from Motešice Village (Mereďa Jr. 2010) to the Bošácká dolina Valley in the Biele Karpaty Mts (Kolník 2003).

*Diversity*

According to the Shannon Diversity Index (Table 2), the highest diversity of orchids should be in a non-forest environment ($H_{max}$), but the actual diversity (H) here reaches only 69 % ($100*H/H_{max}$). The highest recorded diversity is in deciduous forests, where it reaches 77 % of the maximum possible diversity. Although non-forest areas have a high number of species, diversity is low, as there are several large populations of *Dactylorhiza majalis, Gymnadenia conopsea, Listera ovata* and *Orchis mascula* subsp. *signifera*. These species significantly outnumber other species. On the contrary, mixed and coniferous forests have fewer species and less diversity, because most species prefer deciduous forests. Comparatively, 4941 individuals were recorded in deciduous forests, 973 in coniferous forests, 805 in mixed forests and 5361 in non-forest areas.

The graph of species and population richness (Fig. 4) in the studied area shows the importance of altitude for the occurrence of orchids. The highest altitude inhabited by any of these orchid species is in the range of 400 - 500 m. Populations gradually decrease with higher altitudes. Žilinská kotlina Basin and Považské Podolie Valley have altitudes up to 300 m, where the forested area is low with a higher built-up area and agricultural land (Table 1). Low abundance of species and populations at higher altitudes are due to a smaller total area for study, as there are only ten hills in the Strážovské vrchy Mts. Harsher climatic conditions in mountainous areas are also unsuitable for orchids.

The motto of the protectors is "Know and protect". Any new information can contribute to nature and landscape protection. Forest management has the greatest anthropogenic influence on forest orchids. Most of the forests in the study area are commercial forests, so it is important to inform the public about the occurrence of rare and protected plants. Many species are sensitive to changes in wood composition and surface lightening. Atmospheric conditions, which are reflected in the seasonal abundance of populations, also have a significant impact. However, further research is needed on the relationship of population dynamics to climate change, as well as on the natural expansion of populations and the migration potential of new species.

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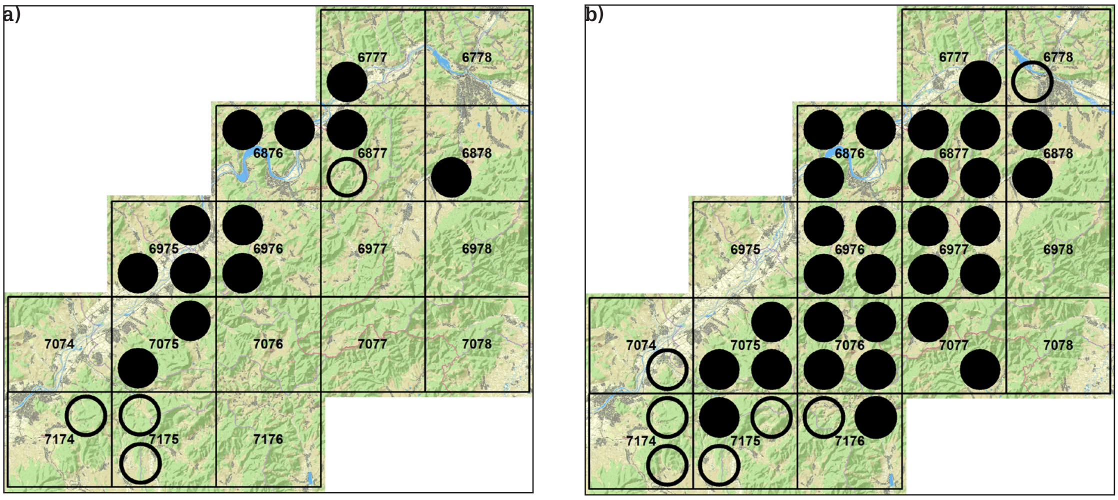
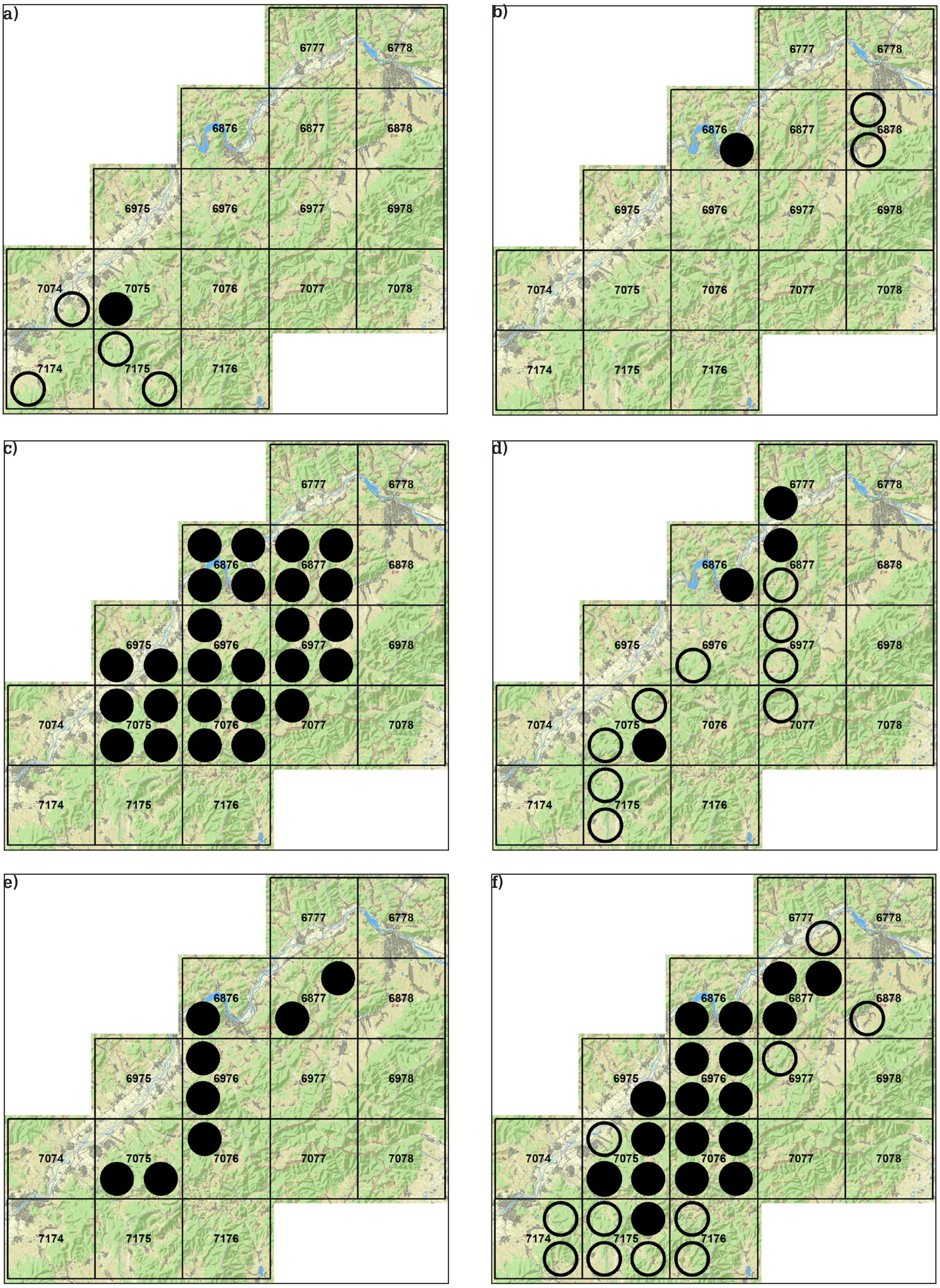
## Appendix 1



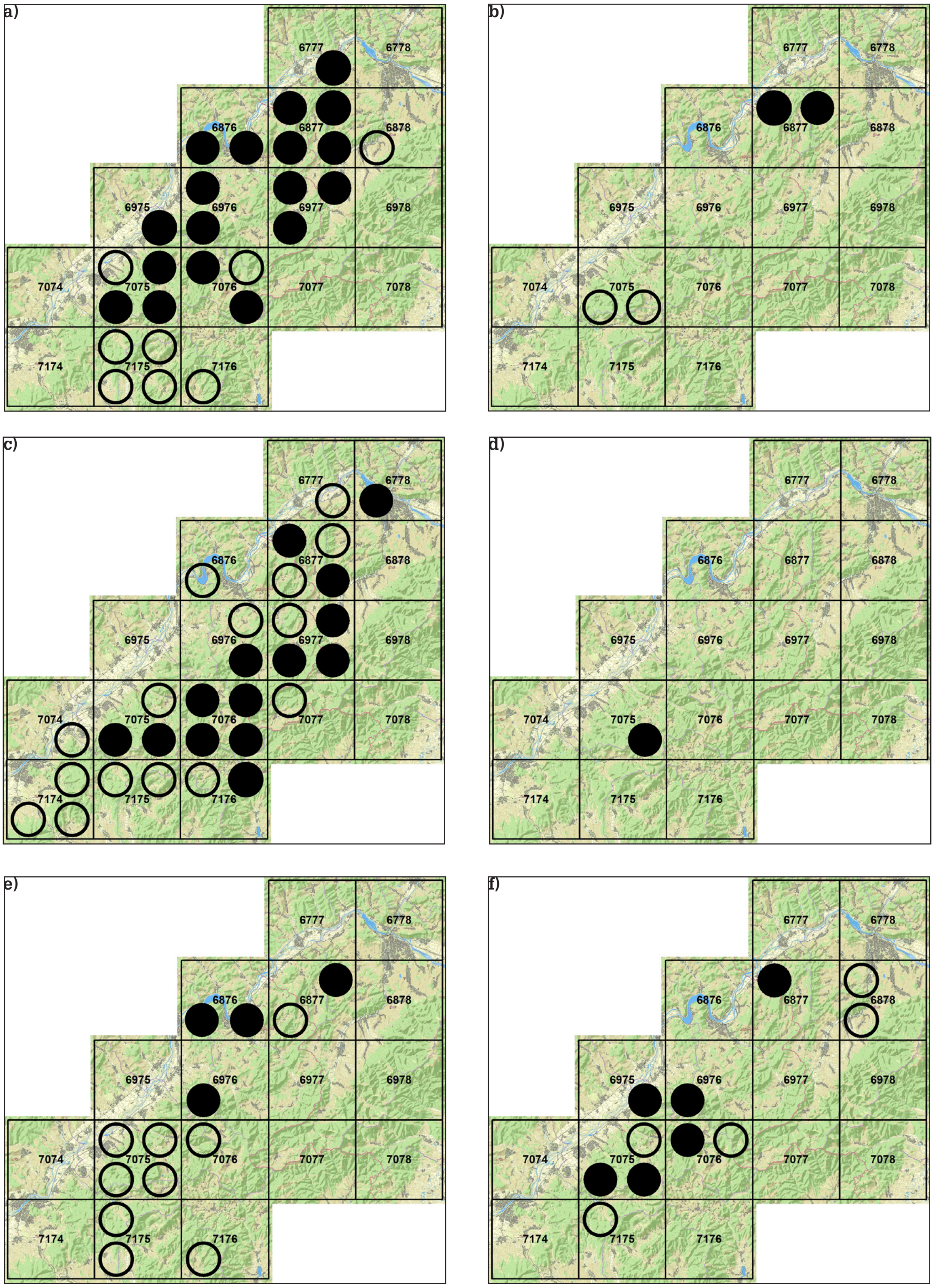

**Appendix 1. a)** *Epipactis albensis;* **b)** *E. atrorubens*

## Appendix 2



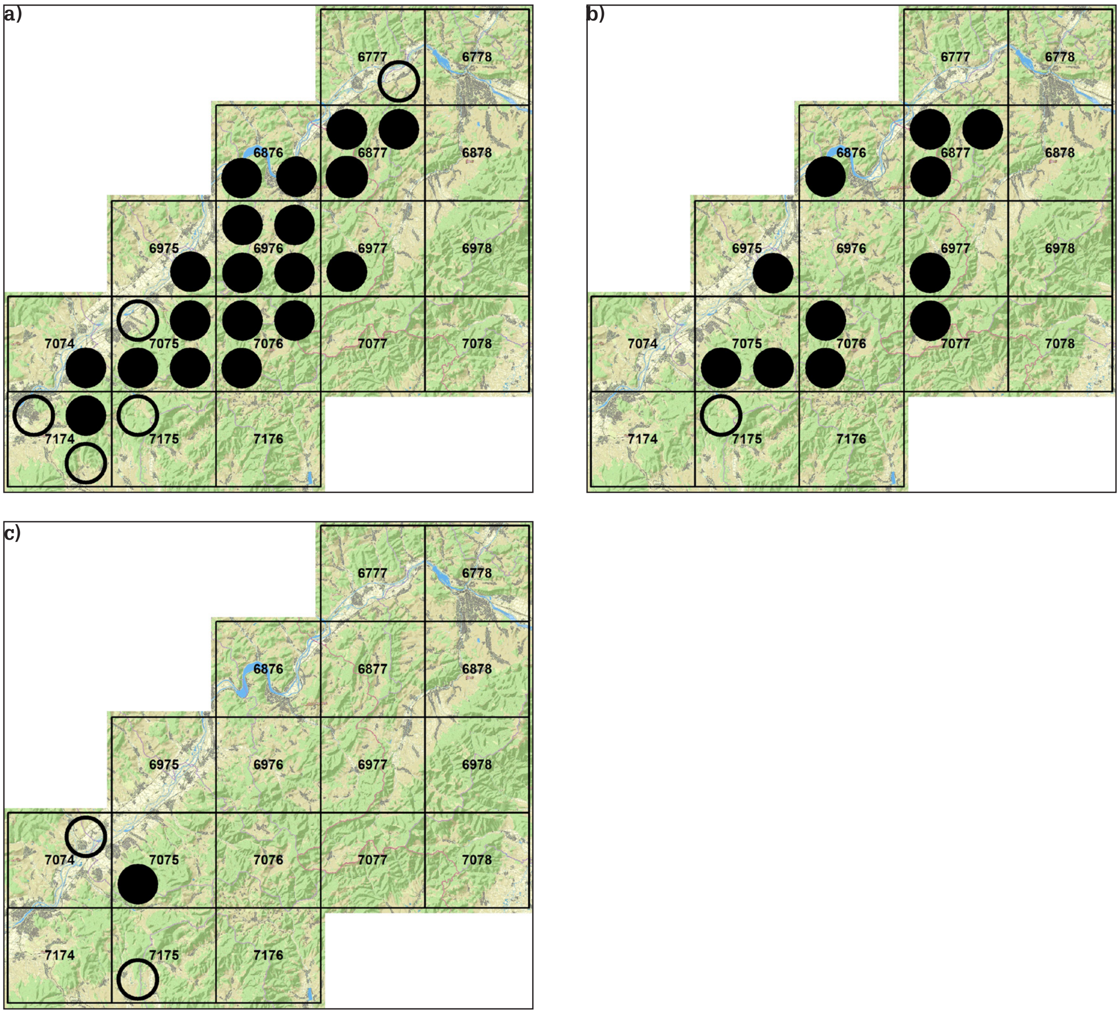

**Appendix 2. a)** *E. futakii;* **b)** *E. greuteri;* **c)** *E. helleborine* subsp. *helleborine;* **d)** *E. komoricensis;* **e)** *E. leutei;* **f)** *E. microphylla.*

## Appendix 3




**Appendix 3. a)** *E. muelleri;* **b)** *E. neglecta;* **c)** *E. palustris;* **d)** *E. placentina;* **e)** *E. pontica;* **f)** *E. pseudopurpurata.*

## Appendix 4




**Appendix 4. a)** *E. purpurata*; **b)** *E.* sp. "karpatský"; **c)** *E. tallosii*.

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# Heavy metals compounds from tailing pond sludge and their distribution to the tissues of the selected common *Poaceae* species and crop plants

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**Abstract.** This study deals with the issue of contaminant transfer from a tailings pond (Rosina-Žilina, Slovakia) to the surrounding environment. More than one hundred samples of soil and plant tissues were taken. Analysis focused to two species of the *Poaceae* family (*Phragmites australis* and *Calamagrostis* sp.) as well as common crops (corn, apple, cherry) in the vicinity of the tailings pond. All samples were analysed by x-ray spectrometry for rapid and accurate measurements in situ as well as in laboratory conditions. We assessed the risk of ash-waste contamination to the ecosystem during transfer and the related potential threat to human health long term. Arsenic content in soil samples of ash material ranged from 41 to 91 ppm, but these values did not confirm the transfer of this element into the tissues of monitored plants. Mercury levels were only detected in samples from the tailings pond where mean values in soil and stems and blooms were equal. The presence of lead in soil samples and plant tissues was also detected. The data obtained indicates possible bioaccumulation of lead, especially in *Calamagrostis* sp.. Soil samples from tailings pond contained a mean of 36 ppm of lead and individual measured tissues of *Calamagrostis* sp. contained lead levels of 13 to 18 ppm. These findings support the hypothesis that heavy metals are bio-avalible via the food chain, especially for herbivores. Heavy metals in investigated crops did not differ between sample sites. Corn leaves were more polluted than kernels, while fruit seeds were more polluted than pulp or leaves.

*Key words:* crop plants, *Phragmites australis, Calamagrostis* sp., contamination, heavy metals

## Introduction

The processing and storage of waste material from anthropogenic activities is the subject of much research as many deposited substances in landfills or in sludge disposal sites can become a long-term risk to human health and the local ecosystem (Jacob and Otte 2004; Liang *et al.* 2017; Gabbarón *et al.* 2018).

Power and thermal plants that use coal produce various forms of ash. These by-products from the combustion of coal are usually transported by water in the form of sludge and deposited in landfills called tailings ponds. These ponds are generally to be the main source of pollution in regions where they occur (Demková *et al.* 2019), because they could affect environmental components such as air, water and soil (Ettler *et al.* 2009; Hiller *et al.* 2009; Petrilean *et al.* 2014). Several studies confirm the content of arsenic and lead in the deposited ash material (Scherer *et al.* 2015). In addition to heavy metals (HM), the sludge was found to contain various organic and inorganic compounds (Ruhl *et al.* 2009; Lam *et al.* 2010). After sedimentation of the sludge, the surface of the sludge bed is formed out of small particles that could be transferred to the surrounding area, as well as over longer distances (Razo *et al.* 2004). Wind erosion plays a significant role in this process, particularly in warm weather. Generally, these small dust particles in the air reach the surface through a dry or wet deposition process and contaminate the environment with possible consequences to human health (Zanuzzi *et al.* 2009; Desouki and Feng 2012). Different heavy metals (HM) may be associated with different particle sizes (Yoo *et al.* 2002; Demková *et al.* 2017), and can be transported over greater distances. Water discharge from drainage systems may also have dangerous impacts, mainly during periods of increased precipitation (Mayes *et al.* 2011), as excess water in tailings ponds discharge to the nearest water source. Accidents caused by the rupture of a dam are particularly serious (Majerník *et al.* 2012).

Bioindicators are widely used for assessment of pollution load in environments (Fränzle 2003). Various risk assessment studies (e.g., Shahid *et al.* 2020) provide valuable information about possible pathways into the food chain for trace elements with potential health risks. It is well known that the HM accumulation capacity of plants depends on various conditions (Kabata-Pendias 2011), including type, morphology and physiology of plants, type of metal, soil conditions (Barman *et al.* 2001), as well as multiple stress factors (Vighi and Villa 2013). These differences were also found in individual parts of plants (Chaplygin *et al.* 2018). If we compare the levels of HM in one species at several localities (polluted and reference), we can achieve satisfactory estimates of environmental pollution.

Approximately 56 tailings ponds exist in Slovakia (Masarovičová *et al.* 2008). Fifteen of these are used for storage of ash and slag materials that are a byproduct of coal generated heating and power plants (Bosák 2017). Reclamation of these sites will be a major milestone in Slovakia's transition to a low carbon economy. Many published studies deal with old mining sites (e.g., Angelovičová and Fazekašová 2014; Angelovičová *et al.* 2014; Demková *et al.* 2017, 2019; Jurkovič *et al.* 2019) but studies regarding the current impacts of active tailings ponds on the environment is lacking. At this time only basic operational monitoring is conducted. This research focuses on a biomonitoring study at one of the tailings ponds in Žilina – Rosina, which is close to a populated area.

The main aim of our study is the determination, using x-ray, of selected heavy metal content (Pb, Hg, As and Cd) in plant substrates and tissues, including an emphasis on the *Poaceae* family. These analyses may help indicate which parts of plants are most polluted and help estimate the potential heavy metals pose to the trophic chain, particularly for herbivores living and feeding close to tailings ponds. In addition, we analysed three crops to estimate the pollution load that may be present in the human food chain.

## Material and Methods

The study area encompassed a tailings pond in Rosina – Žilina (Slovakia), where deposited waste ash material from the local heating plant (Žilinská teplárenská, a.s.) has been present since 1985. This company uses brown coal to produce heat. The pond is located in an agricultural area characterised by a slightly undulating flat landscape with an average altitude of 400 m. asl., where quaternary fluvial and eluvio-deluvial sediments comprised of mostly with clays and gravels are dominant. The tailings pond consists of a valley dam system with a height of 22 meters. The length of the dam crown is 420 m. Waste ash (with waste from desulphurisation) is transported by water circulation through the drainage system of the tailings pond where seepage water is repeatedly used. The rest of the seepage water flows into the Bytčický potok stream. The potential accumulation volume is more than 2 milion $m^3$, and covers an area of 25 ha. This tailings pond represents an environmental burden with a high priority placed on monitoring and future remediation and reclamation (Masarovičová *et al.* 2008; EIA 2019).

For purpose of the study we selected two species of the *Poaceae* familly *Phragmites australis* and *Calamagrostis* sp. that were well represented in proximity to the tailings pond as well as within selected reference areas. Reference areas were carefully chosen based on similarity of habitats and a minimum distance of 2 km from the tailings pond. Additionally, crop samples of corn (*Zea mays*), apples (*Malus domestica*) and cherries (*Prunus avium*), were harvested from the areas immediatly surrounding the tailings pond and reference areas. Together with the *Poaceae* species, soil samples were collected from the top soil layer down to 10 cm in the place where individual plants grew up (without plant residue). All samples of selected plants were collected during 2018, between April and July, and their site distribution is shown in Fig. 1. The *Poaceae* species samples were collected directly from the tailings pond from the desimented ash layer. At the reference sites,

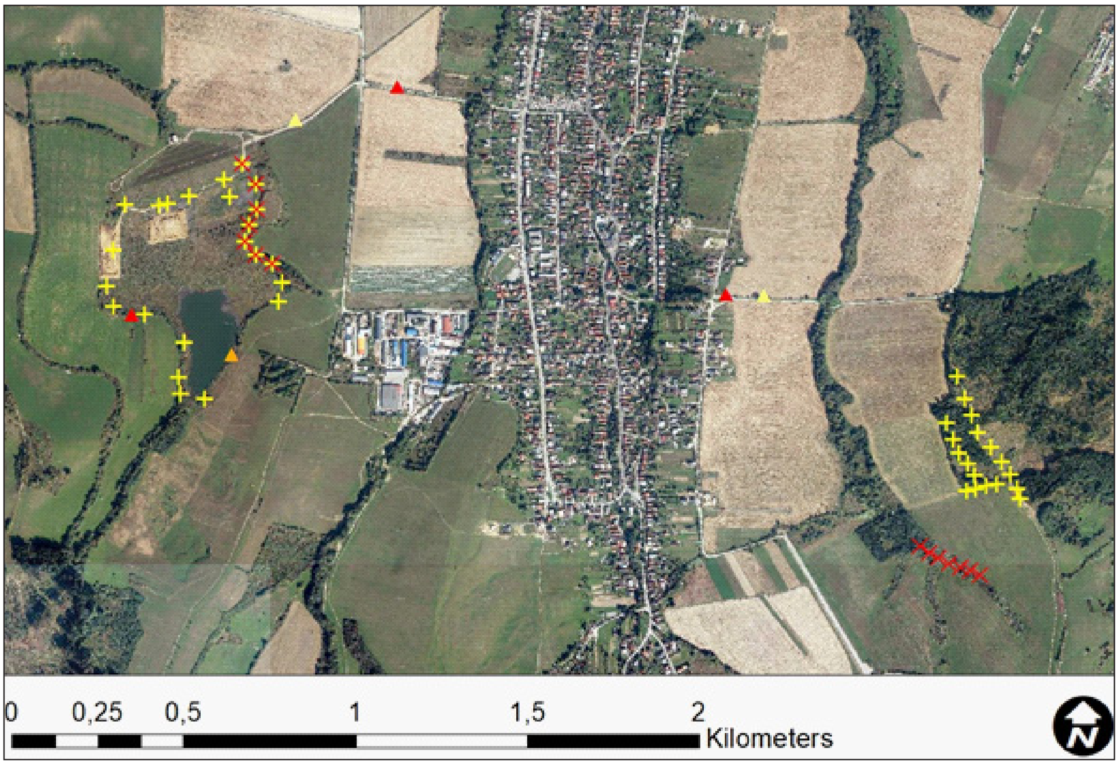


**Fig. 1.** Sampling sites (Tailing pond at left side, reference areas at right side; red x – *Phragmites australis*; yellow cross + – *Calamagrostis sp.*; yellow triangle – corn; red triangle – apple; orange triangle – cherries).

these species were collected from their natural habitats. Samples were divided into underground (roots), ground floor (sheats) and above ground (stems with blooms) parts. Crop samples (apples and cherries) were divided into leaves and fruits, and the fruits were further divided into seeds and flesh. In the case of corn, leaves and kernels were separated from the cob. Samples were not washed in order to better estimate the total potential amount of metals that may enter the organisms through the food chain.

All divided samples were dried using the Memmert IF 160 laboratory Plus dryer (Memmert, Germany) for 12 hours at 60 degrees °C and subsequently mechanically homogenized using a cryomill (Cryomill, Retsch, Germany). In the study we focused on heavy metal concentrations such as lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd). For analysis we used the ED-XRF spectrometer DELTA Premium with Portable WorkStation (Olympus, Innov-x Systems, USA), which is also used as an suitable, effective and convenient tool for determination of metal concentrations in various kinds of materials (Nganvongpanit *et al.* 2016; Buddhachat *et al.* 2018; Kompišová *et al.* 2020). Homogenized samples were measured in a plastic vial (minimum 15 mm layer). Multiple-beam measurement was used, in which every measurement consisted of 3 beams for 80 seconds, repeated three times, and then averaged. The results were given in ppm (parts per million) units. The detection limits were individual for each measured element. Pb (2-4 ppm), As (1-3 ppm), Hg (2-4 ppm) and Cd (6-8 ppm) (Innov-X Systems 2018). Standards used for basic calibration of the device were in a clean homogenous $SiO_2$ matrix without interfering elements. An additional calibration matrix was used for plant material analysis (certified plant standards INCT-PVTL-6 (ICHTI, Poland) and BCR-191), to ensure accurate measurement. NIST 1575a was used as the standard for soil measurements. Samples were randomly measured repeatedly and relative standard deviation was below 10 %. Samples with values too low or close to detectable levels were excluded.

The measured data were statistically evaluated by Statistica 12 (StatSoft, USA). The data had a normal distribution according to the Shapiro-Wilk test, but in the case of this small dataset (N < 30) we used nonparemetric statistical methods. Therefore, for differences between groups, One-way ANOVA or the Kruskal Wallis test (KW) was used with a significance level of $p < 0.05$.

## Results

In the case of the *Poaceae* family, 30 samples were analysed near the tailings pond, and an additional 30 were sampled in the reference area. The same number of idividuals, 23 *Calamagrostis* sp. and seven *Phragmites australis*, were collected at each site. Preliminary results show that levels of Hg and

| Samples | N | Mean Pb | N | Mean Hg | N | Mean As | N | Mean Cd |
|---|---|---|---|---|---|---|---|---|
| ***Reference site*** | 30 | 51 | - | - | 27 | 15 | 1 | 10 |
| *Calamagrostis* sp. | 23 | 58 | - | - | 21 | 16 | 1 | 10 |
| Soil | 23 | 58 | - | - | 21 | 16 | - | - |
| Roots | - | - | - | - | - | - | 1 | 10 |
| Sheats tissues | - | - | - | - | - | - | - | - |
| Stems and blooms | - | - | - | - | - | - | - | - |
| *Phragmites australis* | 7 | 27 | - | - | 6 | 9 | - | - |
| Soil | 7 | 27 | - | - | 6 | 9 | - | - |
| Roots | - | - | - | - | - | - | - | - |
| Sheats tissues | - | - | - | - | - | - | - | - |
| Stems and blooms | - | - | - | - | - | - | - | - |
| ***Tailing pond*** | **93** | **22** | **28** | **12** | **34** | **52** | **3** | **13** |
| *Calamagrostis* sp. | 86 | 21 | 19 | 13 | 27 | 41 | 1 | 10 |
| Soil | 23 | 36 | 10 | 13 | 21 | 48 | - | - |
| Roots | 21 | 15 | 3 | 12 | 6 | 20 | 1 | 10 |
| Sheats tissues | 21 | 13 | 2 | 10 | - | - | - | - |
| Stems and blooms | 21 | 18 | 4 | 15 | - | - | - | - |
| *Phragmites australis* | 7 | 38 | 9 | 11 | 7 | 91 | 2 | 15 |
| Soil | 7 | 38 | 7 | 11 | 7 | 91 | - | - |
| Roots | - | - | - | - | - | - | 1 | 8 |
| Sheats tissues | - | - | - | - | - | - | 1 | 21 |
| Stems and blooms | - | - | 2 | 11 | - | - | - | - |
| **Sum ∑** | **123** | **29** | **28** | **12** | **61** | **35** | **4** | **12** |

**Table 1.** Measured levels of heavy metals (Pb, Hg, As and Cd; in ppm) by x-ray method in samples of *Poaceae* family collected in tailings pond and reference areas.

Cd were not detected in the reference area, with the exception of one root sample of *Calamagrostis* sp. that exhibited Cd content (Table 1). Cd levels were detected in three samples from tailings pond; *Calamagrostis* sp. root, and two samples of roots and tissues of *P. australis*, sampled from different places in the vicinity of the pond. Seventeen of the soil samples from the tailings pond location had detactble Hg levels, including all soil samples of *P. australis* (mean 10.9 ppm) and ten soil samples of *Calamagrostis* sp. (13.4 ppm) without significant differences between these groups (KW: H (1, 17) = 0.292, p = 0.589). In the reference area, Pb and As levels were observed only in soil samples and surprisingly, Pb levels were significantly higher than in soil samples from the tailings pond (Fig. 2a). This could be due to the location of the reference site, and its proximity to other sources of pollution. However, soil samples taken from the reference area near *P. australis* were less polluted by Pb (KW: H (1, 14) = 9.887, p = 0.001) than at the pond (Fig. 2b). Generally, the levels of As were significantly higher in soil samples collected from the tailings pond (Fig. 3a, b (F (1, 53) = 22.741, p = 0.001). While soil samples of *P. australis* from the reference area had significantly lower As levels (9.3 ppm) (Fig. 3a), the opposite was true at the tailings pond site, where this species had significantly higher levels of As (Fig. 3b, 91.3 ppm) when compared to samples of *Calamagrostis* sp. (47.5 ppm). It must also be considered that the reference areas for both species were different. Reference samples of *P. australis* were taken from a terrain depression where small a stream springs, and reference samples of *Calamagrostis* sp. were taken from a forested area (sparse canopy) a top a small hill.

Reliable and detectable levels of Pb in species of *Poaceae* family, measured by x-ray, were detected only in the samples of genus *Calamagrostis* sp. (N = 21), which were taken at the tailings pond. Hg levels were detected in less than half of the samples (N = 9). It is clear that grasses of the genus *Calamagrostis* sp. accumulated much more lead at the tailings pond, although there were lower lead values found in the soil than at the reference site. We observed significant differences in Pb accumulation among investigated tissue groups of this species (F (2, 60) = 16.451, p = 0.001). Higher levels of Pb were found in samples of stems with blooms (Fig. 5a, b). There was not a significant difference in Hg levels between tissue groups of *Calamagrostis* sp. (KW: H (2, 9) = 1.625 p = 0.444) and we observed only slight mean differences with higher values in stems with blooms. Since the samples have not been washed, these results point to the total amount of metals that may be introduced to organisms through the food chain. The biggest impact is to herbivores feeding in the vicinity of the tailings pond that may consume vegetation covered in ash - dust. *P. australis* is at tall grass occurring in humid environments. Thus, in terms of biomass, this species may not demonstrate a great ability to accumulate metals.

In addition, crop samples of corn, apple, and cherry were also collected and analysed from the area by the tailings pond and the reference areas (10 samples for each crop and each site). We were able to reliably measure and detect Pb

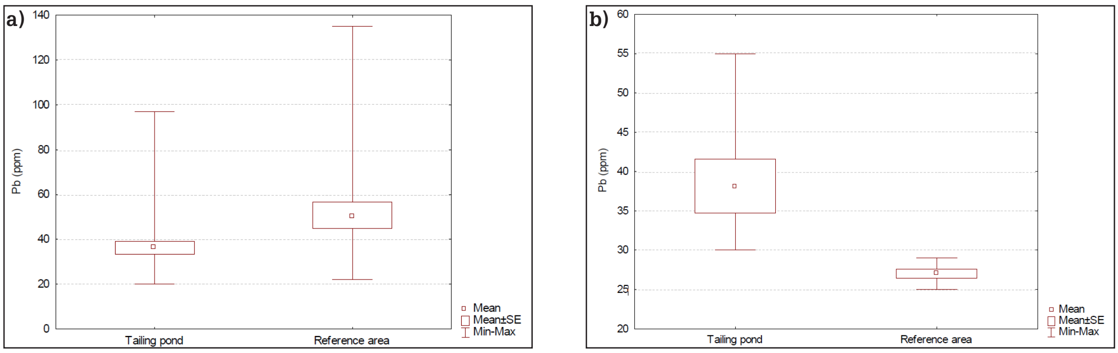


**Fig. 2.** Levels of Pb (in ppm) in soil samples. **a)** all sites (F (1, 58) = 4.796, p = 0.033); **b)** sites of *Phragmites australis* (KW: H (1, 14) = 9.887, p = 0.001).

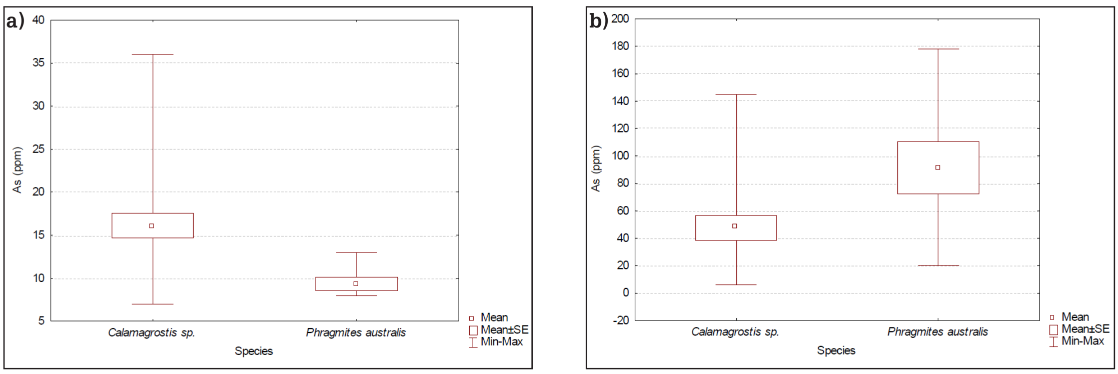


**Fig. 3.** Levels of As (in ppm) in soil samples of investigated species of *Poaceae* family. **a)** reference area (KW: H ( 1, 27) = 6.763, p = 0.009); **b)** tailings pond (KW: H (1, 28) = 4.398, p = 0.036).

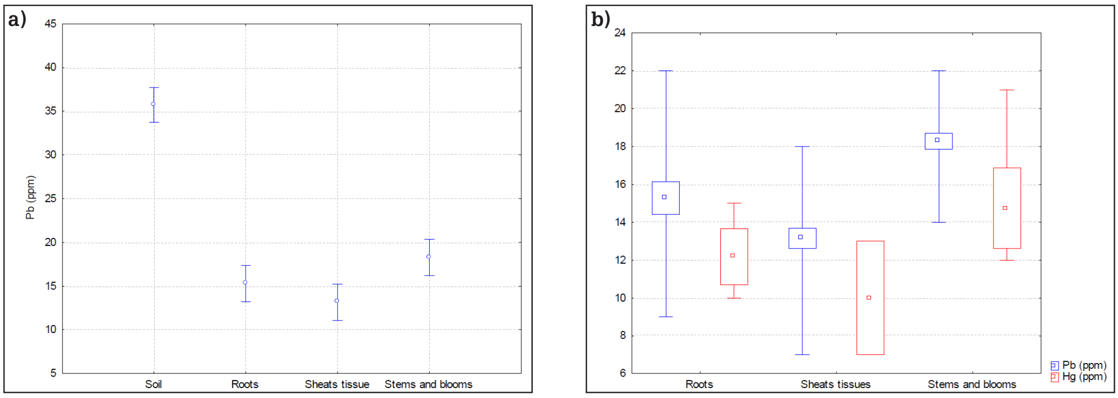


**Fig. 4. a)** – Levels of Pb (in ppm) in divided samples of *Calamagrostis sp.* (Least squares, Vertical bars denote +/- standard errors); **b)** – Comparison of Pb (F (3, 82) = 26.152, p = 0.001) and Hg (KW: H (2, 9) = 1.625, p = 0.444) levels in divided plant samples of *Calamagrostis sp.* (Mean; Box: Mean ± SE; Whisker: Min-Max).

levels using the x-ray method, as levels of As and Hg were below the detection limit and Cd levels were only detected in four samples of apple. Two of these were taken close to tailings pond (one leaf sample and one fruit sample) and the other two (one leaf sample and one fruit sample) were taken from the reference area. General tendencies were observed based on levels of Pb. Corn had significantly more polluted leaves (mean 12 ppm) than kernels (4 ppm; F = 273; p = 0.001), but a significant difference was not found between localities (Fig. 5a; p = 0.780). Apple seeds had significantly higher Pb levels than both apple pulp and leaves (Fig. 5b; F = 66; p = 0.001). However, when comparing sites, apples to the east of the tailings pond had significantly higher lead concentrations than those found near the western part of the tailings pond (F = 6.14; p = 0.003). To the east there are prevailing winds and thus increased dust levels. There is a significant interaction between factors such as site and tissue type (F = 4.7, p = 0.002). Apple samples taken along the road on the way to the east side of the tailings pond had a higher level of lead in pulp and leaves but lower levels in seeds. Samples taken from the western side of the tailings were the opposite. In the case of cherries, Pb levels were only detected in leaves. Mean lead values did not differ between sites (F (1.16) = 0.32, p = 0.570) close to the tailings pond and the reference area (Tatra region specially for this case).

## Discussion

Tailings ponds, which are generally used for the storage of power plant waste ash, sediments from chemical factories, or sludge from mining operations, can pose a burden on the environment. Sedimented sludge eroded by wind can be carried as dust into the environment (Ettler *et al.* 2009; Hiller *et al.* 2009). Increased precipitation can lead to the dangerous discharge of excess water from the tailings pond into the nearest water source. The environmental impact is mainly related to the composition of the ash and the potential for the transfer of hazardous substances into the ecosystem. Biota contamination may not be visible at first sight (Feketeová *et al.* 2016). One of the risk processes is the leaching of individual components of the sludge, which may increase concentrations of hazardous substances in water (Ugurlu 2004), including arsenic, selenium, boron, strontium, and barium (Ruhl 2010). In their study, Lokeshappa and Dikshit (2012) pointed out that the concentration of elements such as arsenic and chromium accumulates over time. In this study, the substrate analysis taken directly from the sludge sedimentation of the tailing pond had the highest level of arsenic content, at 178 mg/kg. The average arsenic concentration of all samples of sludge substrate was 55 mg/kg. In comparison, the highest arsenic measured in the substrate of the reference

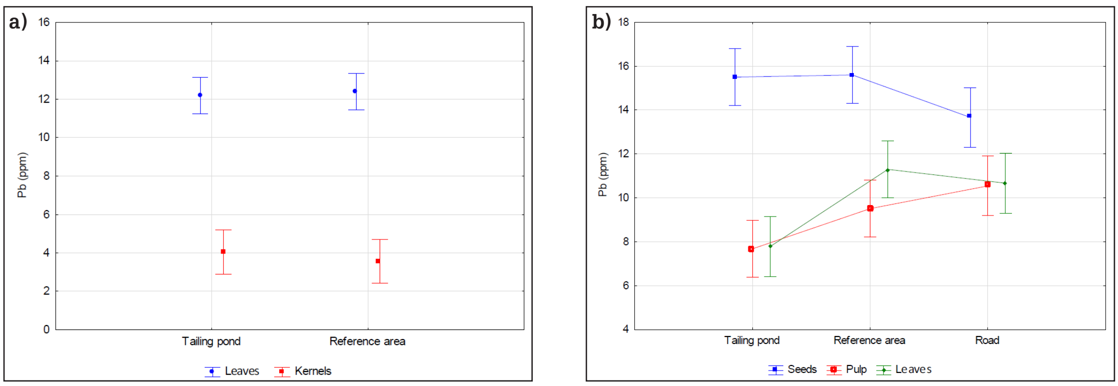


**Fig. 5. a)** Levels of Pb in divided samples of corn; **b)** Levels of Pb in divided samples of apples. (Averages with 0.95 % confidence interval).

site reached 36 mg/kg and the mean value of this element was 15 mg/kg. Comparatively, ash content from the Kingston coal-burning power plant exhibited As values averaging 75 mg/kg (Ruhl *et al.* 2009).

Plants represent a path to wider contamination of the ecosystem. Because they are the food source for herbivores, even low concentrations of pollutants in individual plant tissues are systematically accumulated in animal organs at the top of the trophic chain, where levels can reach harmfull concentrations (Peralta-Videa *et al.* 2009; Gall *et al.* 2015). For comparison, Sychra *et al.* (2011) measured cadmium, lead, and mercury concentrations in *Phragmites australis* and sediments in 21 ponds in Moravia, in the Czech Republic. They found that in the sediments of these ponds the values (in mg/l) of cadmium ranged between 0.63 - 1.42 mg/l for lead 2.52 - 46.3 mg/kg and for mercury 0.014 - 0.236 mg/kg. Values in the tissues of common reeds were much lower, e.g. cadmium ranged from 0.007 - 0.037 mg/kg, lead 0.04 - 1.19 mg/kg, and mercury 0.003 - 0.026 mg/kg. In this study, the cadmium, lead, and mercury in the *Phragmites australis* were not measured either in the tailings ponds or at the reference site. This may point to the fact that common reeds are not a good accumulator of these metals compared to other plant species (Drbal 1991; Svobodová *et al.* 1996). Cadmium was not detected in any soil samples. Mercury was detected in the 17 soil samples from the tailings pond. The highest value of mercury detected in the sample from the sludge deposit reached 28 mg/kg, while average values reached 12 mg/kg.

*Calamagrostis* grasses, which contained more lead in the tailings pond than at the reference site, offer a different perspective. Lead content in the soil at the tailings pond was found to be lower than the soil at the reference site. Higher lead concentrations in the soil at the reference site may be due to the deposition of lead from transmissions, as the reference site was situated on a windward crest. A similar windward effect of lead deposition was also shown by Klamárova and Solár (2017) in a study of the spatial distribution of trace elements near Ružomberok, as well as in many other studies (Grigoras *et al.* 2012; Da Silva 2019). The phenomenon of higher lead concentrations in grasses of the genus *Calamagrostis* in the tailings pond, may be related to plant physiology, soil moisture, pH or temperature (Lone *et al.* 2004; Suchá 2010). Translocation of heavy metals from soil to plant tissues is also significantly affected by the presence of chelating agents (Kaduková *et al.* 2006) and soil salinity (Otte 1991; Fitzgerald *et al.* 2003).

Measurable values of Pb were detected in the plant tissues of crop plants, while measurable values of As were detected in sludge deposits from the tailings pond. While it is positive that the crops did not demonstrate high levels of arsenic, this may speak more to the low bioavailability of this element that to their potential to accumulate it (Khan *et al.* 2015). Lead is usually strongly bonded to soil particles, and when absorbed, generally accumulates in the roots (Kaduková *et al.* 2006). Because lead transport from root to aerial plant parts is limited (Pourrut *et al.* 2011), it is likely that aerial plant parts accumulate high levels of heavy metals as a result of wind-born particles (Styk 2001; Suhadiyah *et al.* 2011). Wind direction, distance from the source of pollution and precipitation, and anatomical and physiological characteristics of each species affect the potential for absorption of hazardous substances. Different plant species have vastly varying tendencies to accumulate lead and heavy metals overall, as well as differing thresholds constituting toxicity, which may vary depending on both the element and plant in question (Tripathi 2009). For example, Tripathi (2009) shows that heavy metals are more likely to accumulate in vegetables than in fruits. In further scientific literature it is also mentioned that leafy crop plants (*Lactuca sativa* L., *Spinacia oleracea* L.) have the greatest capacity to accumulate heavy metals (Li *et al.* 2014). Out of the three crop plants studied, lead was detected in the leaves, seeds and pulp of both corn and apples. The highest values of lead were detected in the seeds of apples from both the tailings pond area and the reference area. Lead values in the seeds of apples were higher than in the leaves. This does not correspond with Svičeková and Havránek's study (1993), which states the highest values of heavy metals elements are found in the leaves. Primary accumulation of heavy metals in leaves (after roots) is mentioned in a number of studies (Bi *et al.* 2009; Roba *et al.* 2015), but this does not account for variations between species.

Finally, this study did not directly confirm the path of tissue contamination through the roots from contaminated soil substrate, but based on measured data it is expected that there is possible additional contamination by wind transfer of small ash particles. This phenomenon was also observed in urban and industrial areas, where pollutant concentrations correlate with the distance from the source of pollution (Simon *et al.* 2010) and wind flow (Oztas and Ata 2002). In this study, the focus was on surface distribution of hazardous elements from deposited material, but in the monitored area there is also a risk of groundwater contamination, as confirmed by Scherer *et al.* (2015). Older and deeper deposited layers of ash material may contain higher concentrations of arsenic and other heavy metals, and also due to the redox potential of eH and pH, may be transformed by heavy metal compounds into more toxic forms (Jašová *et al.* 2009). Therefore, we consider it appropriate to subject this area of research to longer-term monitoring in order to enhance the protection of human health and the health of the living ecosystem in general.

## Acknowledgements

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TECHNIQUES AND STRATEGIES

# Erythrocyte size in birds; an additional data set

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**Abstract.** Birds have an efficient respiratory system, but in exchange of respiratory gases have the most important role erythrocytes. They transport the oxygen to the cells throughout the body, and it removes waste carbon dioxide from the cells. The size of the red blood cells gives us some idea of the area that is provided to the gas exchange. Although the measurement of length (long diameter) and width (short diameter) is basic morphological data, we can still encounter various data for specific species. In our previous studies, we found that cell size and shape are not species-specific and undergo many changes during the year, depending on many exogenous and endogenous factors. The aim of this work is to provide a basic view of the function and structure of erythrocytes and to point out aspects that potentially affect changes in the size and shape of erythrocytes. Despite these changes, erythrocyte size data provide us with the relative predictive value of the cell size of a particular species. The aim of this work is to supplement the existing data on bird erythrocyte size with published data from works from Eurasia and data from own observation. Given the current knowledge, it is appropriate in the future to supplement the database with additional data (age, sex, number of examined individuals, geographical location, the region, altitude, the annual cycle).

*Key words:* birds, erythrocyte legnth, erythrocyte width, respiratory system

## Introduction

Birds are a unique group of vertebrates, due to their physiology. They are widespread worldwide from lowlands to really high altitudes such as the Andes and Himalayas. As a group of vertebrates adapted for flying, birds inhabit various habitats at all altitudes of the biosphere. During evolution, they adapted to many environmental conditions, including temperature, humidity, and varying degrees of radiation; testifying to the great variability of this group. They have adapted to the different requirements of the environment through their way of life, body structure, and the physiological adaptations of organ systems. Of note is their respiratory system; one of the most complex respiratory systems of all animal groups (Gill 1995). Their lungs receive a constant supply of fresh air during inhalation and expiration, thanks to lung-bags, in which some inhaled air remains and is transferred to the lungs during exhalation (Maina 2006; Laguë 2017). Thus, unlike other classes of vertebrates, gas exchange in the lungs is more efficient, allowing birds to maintain the supply of oxygen even in hypoxia, as peripheral tissues have an increased oxygen diffusion capacity as well as a high aerobic capacity (Scott 2011). Through the process of tissue oxygenation, the most important role is undoubtedly played by the erythrocytes.

Bird red blood cells (RBC), unlike those of mammals, are larger, elliptical in shape, more shape-stable and contain a centrally located nucleus (Mitchell and Johns 2008). They have a shorter lifespan than mammal erythrocytes. Generally, a mature erythrocyte has three main components: the lipid membrane, undermembrane skeleton, and fluid cytoplasm (Ivanov *et al.* 2012).

The red cell membrane contains nearly equal amounts of lipids and proteins and this composition is subject to change during its lifetime (De Oliveira and Saldanha 2010). RBC membrane content is composed of: 19.5 % water, 39.5 % proteins, 35.1 % lipids and 5.8 % carbohydrates (Yawata 2003). Of the lipids, phospholipids are the most represented (60 %), while non-sterified cholesterol represents about 30 % of the lipidic RBC composition, and the last 10 % are glycolipids (Yawata 2003). Changes in lipid composition of erythrocyte membranes resulting in an impairment of deformability may play a role in an altered blood rheological pattern (Bakan *et al.* 2006). Different cells have different shape-shifting capabilities, depending on the specific function of that cell within the organism. RBCs have the ability to deform according to blood flow and thus adapt to the lumen of the capillaries. This shape change is temporary and the return to the original form is ensured by intermediate filaments and the plasma membrane skeleton of erythrocytes. RBC shape is ultimately determined by membrane proteins, particularly the spectrin network, and also by the lipid bilayer content (Yawata 2003).

Molecules of haemoglobin, the most abundant protein in the cytoplasm of erythrocytes (Scanes 2015) are responsible for transporting oxygen and carbon dioxide to and from tissues. This is the most important role in gas exchange and a specialized feature of RBCs, however, other important processes include nitric oxide transportation and signalling (De Oliveira and Saldanha 2010). Binding between haemoglobin and breathing gases is markedly affected by organic phosphates and thus provides avian haemoglobin a higher affinity for oxygen (Weber 2007). The carrying capacity of blood and oxygen is affected by several factors, including the hematocrit, concentrations of haemoglobin (Hb) (Bouverot 1985) and allosteric modulators of Hb oxygen binding (Storz and Moriyama 2008; West 2012; Laguë 2017). Haemoglobin concentrations in particular have been thoroughly studied, and several factors are known to affect its value, including age (Kostelecka-Myrcha *et al.* 1971), hormonal level (Puerta *et al.* 1995), season (Breuer *et al.* 1995), exposure to hypoxia (Maxwell *et al.* 1990) and interspecies differences in Hb concentrations (Kostelecka-Myrcha 1997). Functional mitochondria are also found in cytoplasm (Stier *et al.* 2013).

The dimensions of erythrocytes offer us an idea of the area that is involved in the exchange of respiratory gases (Hartman and Lessler 1963). The average size varies between species but reaches an approximate length of 12.5 μm, and a width of 6.8 μm (in a mean of 364 or 362 wild birds species respectively; see Scanes 2015) and a thickness of 3.2 μm (Scanes 2015). Large cells represent a lower surface-to-volume ratio, making them inherently less efficient for gas exchange (Gregory *et al.* 2009); therefore, smaller cells more efficient at gas exchange, which can be advantageous for the metabolic demands of flight (Laguë 2017).

In general, there is a directly proportional relationship between erythrocyte size and haemoglobin content (Hawkey *et al.* 1991), and larger erythrocytes contain more haemoglobin. However, this relationship does not apply to post-embryonic development of birds In the , where during their first days the erythrocytes are large but the haemoglobin content is low (Kostelecka-Myrcha and Myrcha 1989; Kostelecká-Myrcha and Jaroszewicz 1993; Kostelecka-Myrcha 1997). Cell size plays a role in body size evolution and environmental adaptations (Czarnoleski 2018). When applying a direct relationship between erythrocyte size and haemoglobin amount, it must also be true that erythrocyte size is subject to change, based on the hypothesis that the level of haemoglobin concentration is subject to many changes of exogenous and endogenous origin. Red blood cells also undergo a change in shape during development (Barrett and Scheinberg 1972).

Our previous studies suggest that changes in size and shape are subject to age, but they also change over the course of the year (Janiga *et al.* 2017). Additionally, the size and shape of erythrocytes are impacted by pollutants. We found that the tendency of erythrocytes to change their shape from ellipsoid to smaller and round increased with an increasing amount of lead in their blood (Janiga and Haas 2019). We also observed different sizes in different populations of geographically separated populations of *Prunella collaris* from the high mountains of Kyrgyzstan, Bulgaria and Slovakia during the mouting period (Haas and Janiga 2020). Based on these factors, additional data should be added to RBC size measurements to form a more complete picture (e.g. sex number of tested samples, location, season).

As indicated, the size and shape of the RBC is subject to many modifications and reflects external environmental conditions as well as the subjective characteristics of each individual. The comparison of published data on erythrocyte measurements of many bird species, provides us with a relative predictive value of the cell size of a particular species. The most extensive is the database list of "Birds erythrocyte sizes" (www.genomesize.com). This dataset is a compilation of pilot studies of erythrocyte size from Guliver (1875), Bartsch *et al.* (1937), and Hartman and Lessler (1963). Due to the localization of the research, these are mainly bird species from the Nearctic and Neotropical regions. Unfortunately, the exact locations, altitudes, seasons of the year, and number of examined birds cannot be derived from these studies. Originally, we sought to supplement this database with data from our research (in Table 1 as "personal data"), however, Table 1 also includes supplementary data from other studies by primarily European and Asian authors (Palomeque *et al.* 1980; Glomski and Pica 2011); including studies of bird species from the Palearctic region (e.g. Irisova 1988; Kostelecka-Myrcha 1997; Kostelecka-Myrcha *et al.* 1993; Dolka *et al.* 2014).

The aim of this paper is to supplement a database (www.genomesize.com) with morphological data of RBCs (length, width) from other studies to extend the basic RBC size data for the species from other geographical areas. In future studies, it would be beneficial to supplement the database with geographical locations, regions, and altitudes, as well as the annual cycle, number of examined individuals, and their sex and age.

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## Appendix

A list of bird species with the size of erythrocytes and their nuclei according to literature data and own observations. EL - erythrocyte length, EW - erythrocyte width, NL - nucleus length, NW - nucleus width (all dimensions are given in μm). The taxonomic classification of birds is made according to Kovalik *et al.* 2010.

| Order | Family | Species | EL | EW | NL | NW | References |
|---|---|---|---|---|---|---|---|
| CASUARIIFORMES | Dromaiidae | *Dromaius novaehol-landiae* | 15.00 | 8.40 | | | www.genomesize.com 2015 |
| TINAMIFORMES | Tinamidae | *Crypturellus soui* | 14.50 | 7.60 | | | www.genomesize.com 2015 |
| | | *Rhynchotus rufescens* | 13.70 | 7.60 | 4.80 | 2.40 | www.genomesize.com 2015 |
| ANSERIFORMES | Anatidae | *Dendrocygna viduata* | 14.20 | 7.10 | | | www.genomesize.com 2015 |
| | | *Dendrocygna arborea* | 13.20 | 6.80 | | | www.genomesize.com 2015 |
| | | *Dendrocygna autumnalis* | 13.30 | 6.70 | | | www.genomesize.com 2015 |
| | | *Cygnus olor* | 13.90 | 7.20 | 6.40 | 2.30 | Dolka *et al.* 2014 |
| | | *Cygnus atratus* | 14.10 | 6.90 | | | www.genomesize.com 2015 |
| | | *Branta sandvicensis* | 13.60 | 6.60 | | | www.genomesize.com 2015 |
| | | *Branta canadensis* | 13.80 | 6.80 | | | Glomski and Pica 2011 |
| | | *Cereopsis novaehol-landiae* | 14.80 | 6.90 | | | www.genomesize.com 2015 |
| | | *Chloephaga picta* | 13.60 | 6.60 | | | www.genomesize.com 2015 |
| | | *Anas penelope* | 13.60 | 5.80 | | | www.genomesize.com 2015 |
| | | *Anas acuta* | 12.70 | 6.60 | | | www.genomesize.com 2015 |
| | | *Anas acuta* | 10.88 | 6.44 | | | Irisova 1988 |
| | | *Anas querquedula* | 12.60 | 6.60 | | | www.genomesize.com 2015 |
| | | *Anas crecca* | 12.30 | 5.50 | | | www.genomesize.com 2015 |
| | | *Anas platyrhynchos* | 12.50 | 6.80 | | | Glomski and Pica 2011 |
| | | *Anas platyrhynchos* | 11.49 | 6.82 | | | Irisova 1988 |
| | | *Anas undulata* | 12.50 | 7.40 | | | Glomski and Pica 2011 |
| | | *Anas erythrorhyncha* | 12.50 | 7.50 | | | Glomski and Pica 2011 |
| | | *Aythya ferina* | 12.50 | 6.60 | | | Glomski and Pica 2011 |
| | | *Oxyura maccoa* | 12.30 | 6.20 | | | Glomski and Pica 2011 |
| | | *Plectropterus gambensis* | 12.90 | 7.80 | | | Glomski and Pica 2011 |
| | | *Aix galericulata* | 13.10 | 7.40 | | | www.genomesize.com 2015 |
| | | *Aix sponsa* | 12.70 | 6.20 | | | www.genomesize.com 2015 |
| | | *Alopochen aegyptiaca* | 13.60 | 6.60 | | | www.genomesize.com 2015 |
| | | *Somateria mollissima* | 12.70 | 7.90 | | | www.genomesize.com 2015 |
| GALLIFORMES | Cracidae | *Penelope purpurascens* | 13.40 | 7.00 | | | www.genomesize.com 2015 |
| | | *Pipile pipile* | 13.40 | 7.00 | | | www.genomesize.com 2015 |
| | | *Chamaepetes unicolor* | 13.90 | 7.50 | 6.50 | 2.80 | www.genomesize.com 2015 |
| | | *Mitu mitu* | 12.70 | 7.30 | | | www.genomesize.com 2015 |
| | | *Crax rubra* | 12.70 | 6.90 | | | www.genomesize.com 2015 |
| | Ondoto-phoridae | *Colinus virginianus* | 11.50 | 6.40 | | | www.genomesize.com 2015 |
| | | *Odontophorus guttatus* | 11.90 | 6.80 | 5.20 | 2.30 | www.genomesize.com 2015 |
| | Numididae | *Numida meleagris* | 12.40 | 5.80 | | | www.genomesize.com 2015 |
| | Phasianidae | *Pavo cristatus* | 13.80 | 7.10 | | | www.genomesize.com 2015 |
| | | *Pavo muticus* | 13.80 | 7.10 | | | www.genomesize.com 2015 |
| | | *Francolinus natalensis* | 12.50 | 7.50 | | | Glomski and Pica 2011 |
| | | *Francolinus afer* | 12.60 | 7.60 | | | Glomski and Pica 2011 |
| | | *Francolinus francolinus* | 12.10 | 6.30 | | | www.genomesize.com 2015 |
| | | *Gallus gallus* | 12.10 | 7.30 | 4.00 | 3.20 | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|---|
| | | *Coturnix coturnix* | 11.90 | 6.70 | | | Kostelecka-Myrcha *et al.* 1993 |
| | | *Alectoris rufa* | 13.00 | 6.00 | | | Glomski and Pica 2011 |
| | | *Alectoris gracea* | 11.30 | 6.40 | | | Glomski and Pica 2011 |
| | | *Alectoris chukar* | 10.62 | 7.18 | | | Irisova 1988 |
| | | *Tetraogallus caucasicus* | 13.20 | 7.30 | 5.60 | 2.80 | www.genomesize.com 2015 |
| | | *Tetraogallus himalayensis* | 12.11 | 6.97 | | | Irisova 1988 |
| | | *Perdicula argoondah* | 10.80 | 7.30 | | | www.genomesize.com 2015 |
| | | *Meleagris gallopavo* | 12.40 | 7.10 | | | www.genomesize.com 2015 |
| | | *Lagopus muta* | 11.00 | 6.50 | 5.00 | 2.40 | Glomski and Pica 2011 |
| | | *Lagopus muta* | 12.27 | 6.91 | | | Irisova 1988 |
| | | *Lagopus lagopus* | 10.96 | 6.32 | | | Irisova 1988 |
| | | *Lyrurus tetrix* | 10.70 | 6.80 | | | www.genomesize.com 2015 |
| | | *Tetrao urogallus* | 11.30 | 6.60 | | | www.genomesize.com 2015 |
| | | *Phasianus colchicus* | 11.70 | 7.00 | 4.50 | 3.60 | www.genomesize.com 2015 |
| | | *Lophura nycthemera* | 13.50 | 7.30 | 6.40 | 3.20 | www.genomesize.com 2015 |
| | | *Ammoperdix griseogularis* | 13.10 | 7.70 | 5.60 | 2.40 | www.genomesize.com 2015 |
| | | *Rhizothera longirostris* | 12.40 | 6.70 | | | www.genomesize.com 2015 |
| | | *Chrysolophus pictus* | 11.50 | 7.00 | | | www.genomesize.com 2015 |
| PHOENICOPTERIFORMES | Phoenicopteridae | *Phoenicopterus ruber* | 14.50 | 8.20 | | | Glomski and Pica 2011 |
| | | *Phoenicopterus roseus* | 14.50 | 8.00 | | | Glomski and Pica 2011 |
| | | *Phoenicopterus chilensis* | 15.20 | 8.00 | | | Glomski and Pica 2011 |
| | | *Phoeniconaias minor* | 15.10 | 8.10 | | | Glomski and Pica 2011 |
| PODICIPEDIFORMES | Podicipedidae | *Tachybaptus dominicus* | 13.70 | 8.00 | 5.70 | 2.80 | www.genomesize.com 2015 |
| | | *Podilymbus podiceps* | 14.60 | 8.20 | 5.20 | 2.20 | www.genomesize.com 2015 |
| PTEROCLIDIFORMES | Pteroclididae | *Syrrhaptes paradoxus* | 10.47 | 6.59 | | | Irisova 1988 |
| COLUMBIFORMES | Columbidae | *Zenaida aurita* | 11.20 | 7.20 | | | www.genomesize.com 2015 |
| | | *Geotrygon chiriquensis* | 12.00 | 6.90 | 5.60 | 2.80 | www.genomesize.com 2015 |
| | | *Geotrygon mystacea* | 12.10 | 7.20 | | | www.genomesize.com 2015 |
| | | *Geotrygon montana* | 11.30 | 6.90 | 4.80 | 2.10 | www.genomesize.com 2015 |
| | | *Patagioenas leucocephala* | 11.90 | 7.00 | | | www.genomesize.com 2015 |
| | | *Patagioenas corensis* | 11.60 | 7.00 | | | www.genomesize.com 2015 |
| | | *Patagioenas fasciata* | 12.10 | 7.70 | 5.80 | 2.70 | www.genomesize.com 2015 |
| | | *Streptopelia turtur* | 12.70 | 7.50 | | | www.genomesize.com 2015 |
| | | *Streptopelia turtur* | 11.42 | 6.71 | | | Irisova 1988 |
| | | *Streptopelia decaocto* | 11.90 | 7.20 | | | www.genomesize.com 2015 |
| | | *Streptopelia decaocto* | 11.04 | 6.61 | | | Irisova 1988 |
| | | *Streptopelia capicola* | 14.30 | 7.10 | | | Glomski and Pica 2011 |
| | | *Streptopelia orientalis* | 12.39 | 7.05 | | | Irisova 1988 |
| | | *Spilopelia chinensis* | 12.20 | 7.00 | | | www.genomesize.com 2015 |
| | | *Spilopelia senegalensis* | 12.50 | 6.30 | | | Glomski and Pica 2011 |
| | | *Spilopelia senegalensis* | 9.94 | 6.01 | | | Irisova 1988 |
| | | *Columba livia* | 12.00 | 6.80 | | | Kostelecka-Myrcha 1997 |
| | | *Columba livia* | 12.43 | 6.33 | | | Irisova 1988 |
| | | *Columba livia* | 12.49 | 7.11 | | | Irisova 1988 |
| | | *Columba livia f. domestica* | | | | | Kostelecka-Myrcha 1997 |
| | | *Columba rupestris* | 11.91 | 6.09 | | | Irisova 1988 |
| | | *Columba guinea* | 11.70 | 6.60 | | | www.genomesize.com 2015 |
| | | *Columba palumbus* | 12.90 | 7.00 | | | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|---|
| | | *Columbina minuta* | 12.30 | 7.40 | 5.90 | 2.50 | www.genomesize.com 2015 |
| | | *Columbina talpacoti* | 11.60 | 7.00 | 6.40 | 2.40 | www.genomesize.com 2015 |
| | | *Phaps chalcoptera* | 11.50 | 6.30 | | | www.genomesize.com 2015 |
| | | *Caloenas nicobarica* | 11.90 | 6.90 | | | www.genomesize.com 2015 |
| | | *Goura cristata* | 13.00 | 7.30 | | | www.genomesize.com 2015 |
| | | *Goura victoria* | 13.70 | 8.20 | | | Glomski and Pica 2011 |
| | | *Ectopistes migratorius* | 13.30 | 5.50 | | | www.genomesize.com 2015 |
| | | *Otidiphaps nobilis* | 13.50 | 7.60 | | | Glomski and Pica 2011 |
| | | *Treron bicincta* | 13.20 | 8.00 | | | Glomski and Pica 2011 |
| PODARGIFORMES | Podargidae | *Podargus strigoides* | 13.80 | 7.90 | | | www.genomesize.com 2015 |
| NYCTIBIIFORMES | Nyctibiidae | *Nyctibius griseus* | 13.80 | 8.00 | 6.40 | 3.40 | www.genomesize.com 2015 |
| CAPRIMULGI-FORMES | Caprimulgi-dae | *Nyctidromus albicollis* | 13.60 | 8.30 | 6.30 | 3.30 | www.genomesize.com 2015 |
| | | *Caprimulgus europaeus* | 9.99 | 5.79 | | | Irisova 1988 |
| APODIFORMES | Apodidae | *Apus apus* | 12.80 | 6.60 | 6.40 | 2.40 | www.genomesize.com 2015 |
| | | *Apus apus* | 10.22 | 6.57 | | | Irisova 1988 |
| | | *Apus apus* | 13.57 | 6.91 | 6.18 | 2.24 | Palomeque *et al.* 1980 |
| | | *Apus pallidus* | 10.11 | 5.85 | | | Irisova 1988 |
| | | *Apus pallidus* | 13.80 | 6.90 | | | Glomski and Pica 2011 |
| | | *Apus pallidus* | 13.45 | 6.83 | 6.19 | 2.55 | Palomeque *et al.* 1980 |
| | | *Apus melba* | 13.81 | 6.98 | 6.26 | 2.39 | Palomeque *et al.* 1980 |
| | | *Tachymarptis melba* | 13.80 | 6.90 | | | Glomski and Pica 2011 |
| | Trochilidae | *Glaucis hirsutus* | 12.20 | 6.90 | 6.20 | 2.80 | www.genomesize.com 2015 |
| | | *Phaethornis guy* | 11.80 | 6.70 | 4.90 | 2.10 | www.genomesize.com 2015 |
| | | *Anthrocothorax nigricollis* | 10.70 | 6.40 | 5.20 | 2.60 | www.genomesize.com 2015 |
| | | *Heliodoxa jacula* | 12.30 | 6.30 | 6.00 | 2.10 | www.genomesize.com 2015 |
| | | *Archilochus colubris* | 10.80 | 5.80 | | | Glomski and Pica 2011 |
| | | *Selasphorus scintilla* | 10.70 | 6.10 | 5.70 | 2.50 | www.genomesize.com 2015 |
| | | *Campylopterus hemileu-curus* | 11.10 | 5.60 | 5.00 | 1.80 | www.genomesize.com 2015 |
| | | *Thalurania furcata* | 11.30 | 6.40 | 5.50 | 2.10 | www.genomesize.com 2015 |
| | | *Amazilia tzacatl* | 11.20 | 6.40 | 5.70 | 2.60 | www.genomesize.com 2015 |
| | | *Trochilus sp.* | 9.50 | 6.40 | | | www.genomesize.com 2015 |
| | | *Chlorestes julie* | 10.90 | 6.10 | 5.20 | 2.60 | www.genomesize.com 2015 |
| | | *Saucerottia edward* | 11.40 | 6.00 | 5.70 | 2.00 | www.genomesize.com 2015 |
| OTIDIFORMES | Otididae | *Ardeotis kori* | 13.50 | 7.30 | | | Glomski and Pica 2011 |
| | | *Otis tarda* | 14.00 | 7.90 | | | www.genomesize.com 2015 |
| | | *Chlamydotis undulata* | 14.00 | 7.90 | | | www.genomesize.com 2015 |
| CUCULIFORMES | Cuculidae | *Crotophaga major* | 12.00 | 8.10 | 5.50 | 3.40 | www.genomesize.com 2015 |
| | | *Crotophaga ani* | 12.90 | 6.90 | 5.80 | 2.90 | www.genomesize.com 2015 |
| | | *Piaya cayana* | 13.70 | 7.90 | 6.20 | 2.90 | www.genomesize.com 2015 |
| | | *Cuculus canorus* | 12.50 | 7.10 | | | www.genomesize.com 2015 |
| | | *Cuculus canorus* | 11.02 | 6.55 | | | Irisova 1988 |
| | | *Cuculus canorus* | 11.81 | 6.74 | | | Irisova 1988 |
| | | *Cuculus canorus* | 12.02 | 6.77 | | | Irisova 1988 |
| GRUIFORMES | Psophiidae | *Psophia crepitans* | 13.50 | 7.30 | | | www.genomesize.com 2015 |
| | Gruidae | *Balearica regulorum* | 13.70 | 7.30 | | | www.genomesize.com 2015 |
| | | *Balearica pavonina* | 13.70 | 6.70 | 6.40 | 2.60 | www.genomesize.com 2015 |
| | | *Grus virgo* | 13.50 | 6.80 | | | www.genomesize.com 2015 |
| | | *Grus paradisea* | 13.30 | 7.20 | | | www.genomesize.com 2015 |
| | | *Grus japonensis* | 12.60 | 9.40 | | | Glomski and Pica 2011 |
| | | *Grus grus* | 13.60 | 7.80 | | | Glomski and Pica 2011 |

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|---|---|---|---|---|---|---|---|
| | | *Grus monacha* | 12.60 | 7.10 | | | Glomski and Pica 2011 |
| | Heliornithi-dae | *Heliornis fulica* | 12.60 | 7.50 | 5.50 | 3.00 | www.genomesize.com 2015 |
| | Rallidae | *Laterallus albigularis* | 12.80 | 7.20 | 5.20 | 3.10 | www.genomesize.com 2015 |
| | | *Rallus longirostris* | 14.50 | 7.70 | 5.70 | 2.90 | www.genomesize.com 2015 |
| | | *Rallus elegans* | 14.50 | 7.70 | 5.70 | 2.90 | www.genomesize.com 2015 |
| | | *Aramides cajanea* | 12.90 | 7.20 | 5.50 | 3.30 | www.genomesize.com 2015 |
| | | *Porzana porzana* | 12.70 | 7.10 | | | www.genomesize.com 2015 |
| | | *Porphyrio porphyrio* | 15.20 | 8.30 | | | Glomski and Pica 2011 |
| | | *Porphyrio martinica* | 14.20 | 7.80 | 6.70 | 2.90 | www.genomesize.com 2015 |
| | | *Gallinula chlorpus* | 12.40 | 6.60 | | | www.genomesize.com 2015 |
| | | *Fulica atra* | 13.40 | 7.90 | | | www.genomesize.com 2015 |
| | | *Fulica americana* | 11.40 | 7.50 | 4.20 | 2.30 | www.genomesize.com 2015 |
| SPHENICIFORMES | Sphenisciidae | *Pygoscelis papua* | 14.10 | 8.20 | | | Glomski and Pica 2011 |
| | | *Pygoscelis adeliae* | 13.70 | 8.50 | | | Glomski and Pica 2011 |
| | | *Pygoscelis antarcticus* | 13.40 | 8.60 | | | Glomski and Pica 2011 |
| | Eudyptula | *Eudyptula minor* | 18.80 | 9.20 | | | Glomski and Pica 2011 |
| PROCELLARI-IFORMES | Oceanitidae | *Oceanites oceanicus* | 11.80 | 6.40 | | | Kostelecka-Myrcha *et al.* 1993 |
| CICONIIFORMES | Ciconiidae | *Ciconia nigra* | 14.10 | 7.50 | | | www.genomesize.com 2015 |
| | | *Leptoptilos crumeniferus* | 13.70 | 7.30 | | | www.genomesize.com 2015 |
| SULIFORMES | Sulidae | *Morus bassanus* | 12.10 | 6.80 | | | Glomski and Pica 2011 |
| | Phalacro-coracidae | *Phalacrocorax carbo* | 12.70 | 6.70 | | | www.genomesize.com 2015 |
| | | *Phalacrocorax auritus* | 13.60 | 7.60 | 5.20 | 2.60 | www.genomesize.com 2015 |
| | Anhingidae | *Anhinga anhinga* | 15.10 | 8.60 | 6.50 | 3.00 | www.genomesize.com 2015 |
| PELACANIFORMES | Pelecanidae | *Pelecanus ocnocrotalus* | 14.30 | 7.50 | 7.90 | 2.60 | www.genomesize.com 2015 |
| | | *Pelecanus occidentalis* | 14.20 | 7.80 | 5.90 | 2.90 | www.genomesize.com 2015 |
| ARDEIFORMES | Threskiorni-thidae | *Plegadis falcinellus* | 14.50 | 8.30 | | | Glomski and Pica 2011 |
| | | *Platalea leucorodia* | 13.70 | 7.10 | | | www.genomesize.com 2015 |
| | Ardeidae | *Tigrisoma lineatum* | 15.80 | 10.0 | 7.00 | 2.90 | www.genomesize.com 2015 |
| | | *Ixobrychus minutus* | 12.70 | 6.60 | | | www.genomesize.com 2015 |
| | | *Nycticorax nycticorax* | 14.30 | 7.10 | | | www.genomesize.com 2015 |
| | | *Bulbucus ibis* | 14.20 | 8.40 | | | Glomski and Pica 2011 |
| | | *Butorides striatus (vire-scens)* | 13.00 | 8.30 | 6.00 | 2.90 | www.genomesize.com 2015 |
| | | *Ardea cinerea* | 13.30 | 7.30 | | | www.genomesize.com 2015 |
| | | *Ardea herodias* | 14.30 | 7.40 | 5.80 | 3.00 | www.genomesize.com 2015 |
| | | *Ardea purpurea* | 14.50 | 8.20 | | | Glomski and Pica 2011 |
| | | *Ardea alba* | 15.00 | 7.90 | 6.70 | 3.30 | www.genomesize.com 2015 |
| | | *Egretta caerulea* | 12.80 | 7.30 | 6.10 | 3.00 | www.genomesize.com 2015 |
| | | *Egretta thula* | 13.10 | 7.70 | 5.20 | 2.60 | www.genomesize.com 2015 |
| | | *Egretta garzetta* | 13.60 | 8.30 | | | Glomski and Pica 2011 |
| CHARADRI-IFORMES | Burhinidae | *Burhinus oedicnemus* | 13.3 | 6.8 | | | Glomski and Pica 2011 |
| | Charadriidae | *Charadrius dubius* | 11.50 | 6.66 | | | Irisova 1988 |
| | | *Charadrius wilsonia* | 12.80 | 7.30 | 5.80 | 2.40 | www.genomesize.com 2015 |
| | Pluvialidae | *Pluvialis apricaria* | 11.89 | 6.83 | | | Irisova 1988 |
| | Recurviro-stridae | *Himantopus mexicanus* | 12.80 | 6.90 | 5.80 | 2.50 | www.genomesize.com 2015 |
| | Haemato-podidae | *Haematopus ostralegus* | 13.40 | 6.40 | 7.90 | 2.80 | www.genomesize.com 2015 |
| | Jacanidae | *Jacana spinosa* | 13.70 | 7.50 | 5.90 | 3.70 | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|---|
| | Scolopaci-dae | *Numenius phaeopus* | 13.80 | 5.70 | | | www.genomesize.com 2015 |
| | | *Limosa limosa* | 13.10 | 7.80 | | | www.genomesize.com 2015 |
| | | *Gallinago stenura* | 11.09 | 6.80 | | | Irisova 1988 |
| | | *Gallinago gallinago* | 10.81 | 6.55 | | | Irisova 1988 |
| | | *Gallinago gallinago* | 11.70 | 7.00 | | | www.genomesize.com 2015 |
| | | *Actitis hypoleucos* | 11.01 | 6.53 | | | Irisova 1988 |
| | | *Actitis hypoleucos* | 10.45 | 6.40 | | | Irisova 1988 |
| | | *Actitis hypoleucos* | 11.43 | 6.58 | | | Irisova 1988 |
| | | *Actitis hypoleucos* | 10.75 | 6.27 | | | Irisova 1988 |
| | | *Tringa ochropus* | 11.04 | 6.52 | | | Irisova 1988 |
| | | *Tringa brevipes* | 11.27 | 6.69 | | | Irisova 1988 |
| | | *Tringa glareola* | 10.53 | 6.01 | | | Irisova 1988 |
| | | *Tringa glareola* | 10.80 | 6.74 | | | Irisova 1988 |
| | | *Calidris pugnax* | 10.58 | 6.66 | | | Irisova 1988 |
| | Stercorari-idae | *Stercorarius maccormicki* | 11.80 | 6.60 | | | Glomski and Pica 2011 |
| | Alcidae | *Alle alle* | 12.80 | 6.80 | | | Kostelecka-Myrcha *et al.* 1993 |
| | Laridae | *Chlidonias leucopterus* | 10.66 | 6.15 | | | Irisova 1988 |
| | | *Sterna paradisaea* | 12.70 | 6.60 | | | Kostelecka-Myrcha *et al.* 1993 |
| | | *Sterna paradisaea* | 12.11 | 6.76 | | | Irisova 1988 |
| | | *Sterna vittata* | 12.10 | 6.60 | | | Kostelecka-Myrcha *et al.* 1993 |
| | | *Chroicocephalus ridi-bundus* | 12.10 | 6.40 | | | www.genomesize.com 2015 |
| | | *Hydrocoloeus minutus* | 12.54 | 6.09 | | | Irisova 1988 |
| | | *Larus canus* | 12.90 | 6.60 | 7.10 | 2.40 | www.genomesize.com 2015 |
| | | *Larus dominicanus* | 12.60 | 6.60 | | | Glomski and Pica 2011 |
| | | *Larus marinus* | 12.40 | 7.10 | | | Glomski and Pica 2011 |
| | | *Larus argentatus* | 11.80 | 7.64 | | | Irisova 1988 |
| | | *Larus argentatus* | 11.90 | 6.80 | | | Glomski and Pica 2011 |
| CATHARTI-FORMES | Cathartidae | *Cathartes aura* | 14.00 | 7.50 | 6.00 | 2.40 | www.genomesize.com 2015 |
| | | *Coragyps atratus* | 14.00 | 7.70 | 6.30 | 2.60 | www.genomesize.com 2015 |
| | | *Sarcorhamphus papa* | 14.30 | 8.10 | 6.50 | 3.20 | www.genomesize.com 2015 |
| | | *Vultur gryphus* | 14.40 | 6.50 | | | www.genomesize.com 2015 |
| ACCIPITRO-FORMES | Sagitariidae | *Sagittarius serpentarius* | 14.80 | 7.70 | | | www.genomesize.com 2015 |
| | Accipitridae | *Gypohierax angolensis* | 15.10 | 8.00 | | | www.genomesize.com 2015 |
| | | *Gypaetus barbatus* | 13.30 | 7.40 | | | www.genomesize.com 2015 |
| | | *Torgos tracheliotus* | 13.80 | 7.30 | 6.40 | 2.40 | www.genomesize.com 2015 |
| | | *Gyps fulvus* | 13.90 | 7.50 | | | www.genomesize.com 2015 |
| | | *Gyps coprotheres* | 14.20 | 7.60 | | | www.genomesize.com 2015 |
| | | *Terathopius ecaudatus* | 13.40 | 7.30 | | | www.genomesize.com 2015 |
| | | *Aquila rapax* | 14.30 | 7.70 | | | Glomski and Pica 2011 |
| | | *Aquila adalberti* | 14.90 | 8.00 | | | Glomski and Pica 2011 |
| | | *Aquila chrysaetos* | 14.00 | 6.60 | | | www.genomesize.com 2015 |
| | | *Aquila audax* | 13.70 | 7.30 | | | www.genomesize.com 2015 |
| | | *Circus aeruginosus* | 13.80 | 8.00 | | | Glomski and Pica 2011 |
| | | *Accipiter nisus* | 12.70 | 7.10 | 7.90 | 2.80 | www.genomesize.com 2015 |
| | | *Accipiter cooperii* | 14.30 | 8.10 | 6.20 | 2.40 | www.genomesize.com 2015 |
| | | *Accipiter gentilis* | 12.50 | 6.20 | | | Glomski and Pica 2011 |
| | | *Milvus milvus* | 13.20 | 6.90 | | | www.genomesize.com 2015 |
| | | *Milvus migrans* | 14.20 | 8.00 | | | Glomski and Pica 2011 |

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|---|---|---|---|---|---|---|---|
| | | *Haliaeetus albicilla* | 13.90 | 7.50 | | | www.genomesize.com 2015 |
| | | *Haliaeetus leucocephalus* | 13.30 | 7.50 | | | www.genomesize.com 2015 |
| | | *Geranoaetus melano-leucus* | 14.10 | 7.10 | | | www.genomesize.com 2015 |
| | | *Buteo platypterus* | 13.60 | 7.70 | 5.90 | 2.70 | www.genomesize.com 2015 |
| | | *Buteo swainsonii* | 13.70 | 6.90 | | | www.genomesize.com 2015 |
| | | *Buteo jamaicensis* | 13.50 | 6.60 | 5.90 | 2.50 | www.genomesize.com 2015 |
| | | *Buteo buteo* | 14.30 | 7.50 | | | Glomski and Pica 2011 |
| | | *Buteo lagopus* | 13.70 | 6.90 | | | www.genomesize.com 2015 |
| STRIGIFORMES | Tytonidae | *Tyto alba pratincola* | 14.10 | 7.80 | 6.30 | 2.50 | www.genomesize.com 2015 |
| | | *Tyto capensis* | 12.60 | 7.60 | | | Glomski and Pica 2011 |
| | Strigidae | *Athene noctua* | 13.42 | 6.55 | | | Irisova 1988 |
| | | *Athene cunicularia* | 14.10 | 7.80 | 6.30 | 2.70 | www.genomesize.com 2015 |
| | | *Glaucidium passerinum* | 13.50 | 7.10 | | | www.genomesize.com 2015 |
| | | *Otus scops* | 13.90 | 7.50 | | | www.genomesize.com 2015 |
| | | *Pseudoscops clamator* | 13.70 | 7.70 | 5.90 | 3.00 | www.genomesize.com 2015 |
| | | *Megascops choliba* | 13.30 | 7.40 | 5.50 | 2.80 | www.genomesize.com 2015 |
| | | *Megascops atricapillus* | 14.80 | 7.10 | | | www.genomesize.com 2015 |
| | | *Strix nebulosa* | 13.40 | 7.90 | | | www.genomesize.com 2015 |
| | | *Strix aluco* | 13.20 | 6.70 | | | www.genomesize.com 2015 |
| | | *Strix varia* | 13.70 | 7.60 | 5.70 | 2.50 | www.genomesize.com 2015 |
| | | *Bubo scandiaca* | 16.30 | 6.30 | 7.90 | 2.40 | www.genomesize.com 2015 |
| | | *Bubo virginianus* | 13.80 | 6.40 | | | www.genomesize.com 2015 |
| | | *Bubo africanus* | 12.50 | 10.0 | | | Glomski and Pica 2011 |
| | | *Asio flammeus* | 13.50 | 6.80 | 6.40 | 2.40 | www.genomesize.com 2015 |
| TROGONIFORMES | Trogonidae | *Pharomachrus mocino* | 12.20 | 7.00 | 5.80 | 2.70 | www.genomesize.com 2015 |
| | | *Trogon collaris* | 13.60 | 7.70 | 6.80 | 2.90 | www.genomesize.com 2015 |
| | | *Trogon massena* | 13.60 | 7.60 | 6.30 | 2.60 | www.genomesize.com 2015 |
| BUCEROTIFORMES | Upupidae | *Upupa epops* | 11.24 | 6.41 | | | Irisova 1988 |
| | Bucerotidae | *Tockus leucomelas* | 12.70 | 7.90 | | | www.genomesize.com 2015 |
| | | *Buceros rhinoceros* | 15.00 | 7.90 | | | www.genomesize.com 2015 |
| CORACIIFORMES | Meropidae | *Merops apiaster* | 10.41 | 6.31 | | | Irisova 1988 |
| | Coraciidae | *Coracias garrulus* | 12.70 | 7.30 | | | www.genomesize.com 2015 |
| | Alcedinidae | *Dacelo novaeguineae* | 12.00 | 7.10 | | | www.genomesize.com 2015 |
| | | *Alcedo atthis* | 12.00 | 6.90 | | | www.genomesize.com 2015 |
| | | *Chloroceryle aenea* | 12.60 | 6.70 | 6.50 | 2.90 | www.genomesize.com 2015 |
| | | *Chloroceryle amazona* | 13.90 | 7.80 | 6.50 | 2.90 | www.genomesize.com 2015 |
| PICIFORMES | Bucconidae | *Notharchus macrorhyn-chos* | 13.10 | 7.60 | 6.20 | 2.90 | www.genomesize.com 2015 |
| | Ramphasti-dae | *Aulacorhynchus prasinus* | 12.80 | 8.00 | 7.20 | 2.90 | www.genomesize.com 2015 |
| | | *Pteroglossus torquatus* | 13.90 | 8.00 | 6.60 | 2.70 | www.genomesize.com 2015 |
| | | *Ramphastos ambiguus (swainsonii)* | 13.30 | 7.70 | 5.80 | 2.30 | www.genomesize.com 2015 |
| | Picidae | *Campephilus guate-malensis* | 13.80 | 7.20 | 6.00 | 2.50 | www.genomesize.com 2015 |
| | | *Melanerpes erythro-cephalus* | 14.10 | 7.30 | 6.50 | 2.30 | www.genomesize.com 2015 |
| | | *Melanerpes formicivorus* | 12.40 | 6.60 | 6.40 | 2.80 | www.genomesize.com 2015 |
| | | *Melanerpes chrysauchen* | 11.70 | 7.30 | 6.10 | 2.10 | www.genomesize.com 2015 |
| | | *Melanerpes rubricapillus* | 13.70 | 7.30 | 6.10 | 2.40 | www.genomesize.com 2015 |
| | | *Melanerpes carolinus* | 13.60 | 6.80 | 6.40 | 2.30 | www.genomesize.com 2015 |
| | | *Dryobates pubescens* | 12.70 | 5.80 | 6.00 | 2.30 | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|---|
| | | *Dendrocopos minor* | 11.70 | 6.50 | | | www.genomesize.com 2015 |
| | | *Dendrocopos minor* | 10.46 | 6.13 | | | Irisova 1988 |
| | | *Dendrocopos leucopterus* | 12.17 | 6.73 | | | Irisova 1988 |
| | | *Dendrocopos major* | 10.71 | 6.00 | | | Irisova 1988 |
| | | *Dendrocopos major* | 10.34 | 5.38 | | | Irisova 1988 |
| | | *Leuconotopicus villosus* | 12.40 | 6.30 | 6.20 | 2.20 | www.genomesize.com 2015 |
| | | *Dryocopus lineatus* | 14.30 | 7.60 | 6.40 | 3.10 | www.genomesize.com 2015 |
| FALCONIFORMES | Falconidae | *Caracara plancus* | 14.10 | 8.70 | 6.40 | 2.70 | www.genomesize.com 2015 |
| | | *Falco rupicoloides* | 13.10 | 7.40 | | | Glomski and Pica 2011 |
| | | *Falco tinnunculus* | 13.40 | 7.30 | | | www.genomesize.com 2015 |
| | | *Falco sparverius* | 11.80 | 7.30 | 5.80 | 2.10 | www.genomesize.com 2015 |
| | | *Falco subbuteo* | 13.90 | 7.20 | | | www.genomesize.com 2015 |
| | | *Falco subbuteo* | 11.97 | 7.03 | | | Irisova 1988 |
| | | *Falco peregrinus* | 13.30 | 6.60 | | | www.genomesize.com 2015 |
| | | *Falco rusticolus* | 14.80 | 7.20 | | | Glomski and Pica 2011 |
| | | *Falco biarmicus* | 12.80 | 7.80 | | | Glomski and Pica 2011 |
| PSITTACIFORMES | Cacatuidae | *Nymphicus hollandicus* | 11.80 | 6.10 | | | www.genomesize.com 2015 |
| | | *Calyptorhynchus funereus* | 14.60 | 7.70 | | | Glomski and Pica 2011 |
| | | *Probosciger aterrimus* | 14.50 | 7.90 | | | Glomski and Pica 2011 |
| | | *Cacatua haematuropygia* | 12.90 | 6.30 | | | www.genomesize.com 2015 |
| | | *Cacatua sulphurea* | 11.50 | 7.50 | | | www. genomesize.com 2015 |
| | | *Cacatua galerita* | 13.50 | 7.10 | | | www.genomesize.com 2015 |
| | | *Cacatua alba* | 13.80 | 7.50 | | | Glomski and Pica 2011 |
| | Psittacidae | *Coracopsis vasa* | 12.40 | 6.50 | | | www.genomesize.com 2015 |
| | | *Coracopsis nigra* | 11.90 | 6.50 | | | www.genomesize.com 2015 |
| | | *Psittacus erithacus* | 13.40 | 6.40 | | | www.genomesize.com 2015 |
| | | *Myiopsitta monachus* | 11.90 | 6.30 | | | www.genomesize.com 2015 |
| | | *Brotogeris jugularis* | 12.10 | 7.70 | 6.00 | 2.90 | www.genomesize.com 2015 |
| | | *Pionus menstruus* | 12.00 | 6.90 | | | www.genomesize.com 2015 |
| | | *Pionus senilis* | 13.00 | 7.60 | 5.80 | 2.30 | www.genomesize.com 2015 |
| | | *Graydidascalus brachyurus* | 12.50 | 6.10 | | | www.genomesize.com 2015 |
| | | *Amazona leucocephala* | 12.40 | 6.80 | | | www.genomesize.com 2015 |
| | | *Amazona albifrons* | 13.20 | 6.90 | | | www.genomesize.com 2015 |
| | | *Amazona autumnalis* | 13.60 | 7.70 | 6.20 | 3.00 | www.genomesize.com 2015 |
| | | *Amazona dufresniana* | 11.20 | 7.50 | | | www.genomesize.com 2015 |
| | | *Amazona festiva* | 13.10 | 7.60 | | | Glomski and Pica 2011 |
| | | *Amazona aestiva* | 13.50 | 8.10 | | | Glomski and Pica 2011 |
| | | *Amazona ochrocephala* | 13.80 | 7.60 | | | Glomski and Pica 2011 |
| | | *Amazona amazonica* | 14.10 | 6.60 | | | www.genomesize.com 2015 |
| | | *Amazona imperialis* | 12.20 | 7.00 | | | www.genomesize.com 2015 |
| | | *Anodorhynchus hyacinthinus* | 14.00 | 7.40 | | | Glomski and Pica 2011 |
| | | *Pionites melanocephalus* | 12.70 | 6.50 | | | www.genomesize.com 2015 |
| | | *Pyrrhura hoffmanni* | 13.20 | 7.20 | 6.10 | 2.30 | www.genomesize.com 2015 |
| | | *Cyanoliseus patagonus* | 11.80 | 6.40 | | | www.genomesize.com 2015 |
| | | *Enicognathus leptorhynchus* | 12.30 | 6.50 | | | www.genomesize.com 2015 |
| | | *Aratinga solstitialis* | 11.90 | 6.40 | | | www.genomesize.com 2015 |
| | | *Ara ararauna* | 13.00 | 6.20 | | | www.genomesize.com 2015 |
| | | *Ara militaris* | 13.50 | 7.20 | | | Glomski and Pica 2011 |
| | | *Ara macao* | 13.40 | 5.30 | | | www.genomesize.com 2015 |
| | | *Ara chloropterus* | 12.70 | 7.80 | | | Glomski and Pica 2011 |

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|---|---|---|---|---|---|---|---|
| | | *Ara severus* | 11.70 | 6.70 | | | www.genomesize.com 2015 |
| | | *Alisterus amboinensis* | 12.40 | 6.10 | | | www.genomesize.com 2015 |
| | | *Alisterus scapularis* | 12.70 | 6.30 | | | www.genomesize.com 2015 |
| | | *Tanygnathus megalo-rhynchos* | 12.10 | 6.60 | | | www.genomesize.com 2015 |
| | | *Psittacula krameri* | 11.70 | 6.50 | | | www.genomesize.com 2015 |
| | | *Psittacula cyanocephala* | 11.20 | 6.40 | | | www.genomesize.com 2015 |
| | | *Psittacula alexandri* | 12.00 | 6.50 | | | www.genomesize.com 2015 |
| | | *Cyanoramphus novae-zelandiae* | 12.00 | 6.10 | | | www.genomesize.com 2015 |
| | | *Platycercus caledonicus* | 12.00 | 6.50 | | | www.genomesize.com 2015 |
| | | *Platycercus elegans* | 12.10 | 6.50 | | | www.genomesize.com 2015 |
| | | *Platycercus eximius* | 11.60 | 6.50 | | | www.genomesize.com 2015 |
| | | *Agapornis canus* | 12.10 | 6.10 | | | www.genomesize.com 2015 |
| | | *Agapornis pullarius* | 12.10 | 6.10 | | | www.genomesize.com 2015 |
| | | *Lorius domicella* | 12.10 | 6.10 | | | www.genomesize.com 2015 |
| | | *Eos bornea* | 14.10 | 7.70 | | | Glomski and Pica 2011 |
| | | *Trichoglossus haema-todus* | 11.50 | 6.50 | | | www.genomesize.com 2015 |
| PASSERIFORMES | Pittidae | *Pitta sordida* | 10.80 | 6.10 | | | www.genomesize.com 2015 |
| | Thamnophi-lidae | *Taraba major* | 13.60 | 7.60 | 6.60 | 2.80 | www.genomesize.com 2015 |
| | Furnariidae | *Sittasomus griseicapillus* | 12.00 | 6.40 | 5.40 | 2.00 | www.genomesize.com 2015 |
| | | *Dendrocincla homochroa* | 11.80 | 6.70 | 5.40 | 2.30 | www.genomesize.com 2015 |
| | | *Xiphorhynchus guttatus* | 12.30 | 7.30 | 6.00 | 2.80 | www.genomesize.com 2015 |
| | | *Xiphorhynchus erythro-pygius* | 12.20 | 6.40 | 5.70 | 2.40 | www.genomesize.com 2015 |
| | | *Lepidocolaptes affinis* | 11.20 | 6.60 | 6.00 | 2.60 | www.genomesize.com 2015 |
| | | *Anabacerthia striaticollis* | 11.90 | 6.10 | 5.40 | 2.10 | www.genomesize.com 2015 |
| | | *Synallaxis brachyura* | 11.10 | 6.10 | 5.60 | 2.10 | www.genomesize.com 2015 |
| | Pipridae | *Corapipo leucorrhoa* | 11.10 | 6.10 | 5.10 | 2.30 | www.genomesize.com 2015 |
| | | *Manacus manacus* | 12.00 | 6.70 | 5.90 | 2.90 | www.genomesize.com 2015 |
| | Cotingidae | *Cotinga ridgwayi* | 12.20 | 6.70 | 6.20 | 2.10 | www.genomesize.com 2015 |
| | Tyrannidae | *Todirostrum cinereum* | 11.90 | 6.40 | 5.70 | 2.30 | www.genomesize.com 2015 |
| | | *Lophotriccus pileatus* | 11.50 | 6.40 | 4.70 | 2.20 | www.genomesize.com 2015 |
| | | *Attila spadiceus* | 12.30 | 6.60 | 5.70 | 1.90 | www.genomesize.com 2015 |
| | | *Myiarchus crinitus* | 11.30 | 6.70 | 5.20 | 2.30 | www.genomesize.com 2015 |
| | | *Myiozetetes cayenensis* | 10.70 | 6.30 | 5.20 | 2.50 | www.genomesize.com 2015 |
| | | *Sayornis phoebe* | 10.80 | 5.80 | 5.00 | 2.20 | www.genomesize.com 2015 |
| | | *Tityra semifasciata* | 11.70 | 6.80 | 5.60 | 2.70 | www.genomesize.com 2015 |
| | | *Mitrephanes phaeocercus* | 11.10 | 6.40 | 5.60 | 2.10 | www.genomesize.com 2015 |
| | | *Contopus virens* | 11.90 | 6.80 | 6.00 | 2.50 | www.genomesize.com 2015 |
| | | *Empidonax flaviventris* | 12.00 | 5.90 | 5.70 | 2.10 | www.genomesize.com 2015 |
| | Cracticidae | *Gymnorhina tibicen* | 12.00 | 6.50 | | | www.genomesize.com 2015 |
| | Vireonidae | *Vireo flavifrons* | 11.20 | 6.30 | 5.30 | 2.20 | www.genomesize.com 2015 |
| | | *Vireo solitarious* | 11.20 | 6.10 | 5.20 | 2.30 | www.genomesize.com 2015 |
| | | *Vireo olivaceus* | 11.00 | 6.60 | 5.90 | 2.60 | www.genomesize.com 2015 |
| | | *Cyclarhis gujanensis* | 11.80 | 6.60 | 5.90 | 2.60 | www.genomesize.com 2015 |
| | Oriolidae | *Oriolus oriolus* | 10.21 | 6.15 | | | Irisova 1988 |
| | | *Oriolus oriolus* | 11.00 | 6.44 | | | Irisova 1988 |
| | Pachyce-phalidae | *Pachycephala cinerea* | 11.70 | 6.10 | | | www.genomesize.com 2015 |
| | Laniidae | *Lanius collurio* | 11.40 | 6.50 | | | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|
| | *Lanius collurio* | 8.94 | 5.45 | | | Irisova 1988 |
| | *Lanius collurio* | 10.52 | 5.90 | | | Irisova 1988 |
| | *Lanius minor* | 10.65 | 6.15 | | | Irisova 1988 |
| | *Lanius excubitor* | 12.80 | 4.80 | | | www.genomesize.com 2015 |
| Corvidae | *Pyrrhocorax pyrrhocorax* | 10.57 | 5.35 | | | Irisova 1988 |
| | *Pyrrhocorax graculus* | 12.10 | 5.60 | | | www.genomesize.com 2015 |
| | *Pyrrhocorax graculus* | 11.10 | 5.74 | | | Irisova 1988 |
| | *Cyanocitta cristata* | 13.70 | 7.80 | 5.80 | 2.60 | www.genomesize.com 2015 |
| | *Perisoreus infaustus* | 11.47 | 6.10 | | | Irisova 1988 |
| | *Perisoreus infaustus* | 11.05 | 6.08 | | | Irisova 1988 |
| | *Cyanocorax cristatellus* | 11.90 | 6.30 | | | www.genomesize.com 2015 |
| | *Cyanocorax chrysops* | 12.40 | 6.10 | | | www.genomesize.com 2015 |
| | *Pica pica* | 13.00 | 7.50 | 6.00 | 2.30 | www.genomesize.com 2015 |
| | *Pica pica* | 10.13 | 6.26 | | | Irisova 1988 |
| | *Pica pica* | 9.96 | 6.27 | | | Irisova 1988 |
| | *Garrulus glandarius* | 12.30 | 6.50 | 6.40 | 2.40 | www.genomesize.com 2015 |
| | *Garrulus glandarius* | 11.43 | 6.57 | | | Irisova 1988 |
| | *Nucifraga caryocatactes* | 13.50 | 6.10 | | | www.genomesize.com 2015 |
| | *Nucifraga caryocatactes* | 11.36 | 6.23 | | | Irisova 1988 |
| | *Nucifraga caryocatactes* | 11.56 | 6.61 | | | Irisova 1988 |
| | *Coloeus monedula* | 11.30 | 6.10 | 6.40 | 2.40 | www.genomesize.com 2015 |
| | *Corvus brachyrhynchos* | 11.40 | 6.60 | | | Glomski and Pica 2011 |
| | *Corvus tristis* | 12.70 | 6.10 | | | www.genomesize.com 2015 |
| | *Corvus frugilegus* | 13.40 | 7.90 | 5.60 | 2.80 | www.genomesize.com 2015 |
| | *Corvus corax* | 13.00 | 6.40 | | | www.genomesize.com 2015 |
| | *Corvus albus* | 12.50 | 6.90 | | | Glomski and Pica 2011 |
| Remizidae | *Remiz pendulinus* | 11.4 | 5.41 | | | Irisova 1988 |
| Paridae | *Cyanistes caeruleus* | 11.00 | 6.20 | | | www.genomesize.com 2015 |
| | *Cyanistes caeruleus* | 10.80 | 6.00 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Cyanistes flavipectus* | 10.34 | 5.40 | | | Irisova 1988 |
| | *Parus major* | 11.50 | 6.40 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Parus major* | 9.65 | 5.74 | | | Irisova 1988 |
| | *Baelophus (Parus) bicolor* | 10.70 | 5.90 | 5.30 | 2.00 | www.genomesize.com 2015 |
| | *Periparus rufonuchalis* | 10.31 | 5.57 | | | Irisova 1988 |
| | *Poecile montanus* | 11.20 | 5.90 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Poecile carolinensis* | 10.40 | 5.60 | 4.90 | 1.90 | www.genomesize.com 2015 |
| | *Eremophila alpestris* | 10.68 | 5.46 | | | Irisova 1988 |
| Alaudidae | *Eremophila alpestris* | 10.72 | 5.52 | | | Irisova 1988 |
| | *Alauda arvensis* | 12.00 | 6.20 | 6.40 | 2.10 | www.genomesize.com 2015 |
| | *Alauda arvensis* | 10.04 | 5.89 | | | Irisova 1988 |
| Hirundinidae | *Progne chalybea* | 12.70 | 7.10 | 5.50 | 2.70 | www.genomesize.com 2015 |
| | *Stelgidopteryx ruficollis* | 12.20 | 6.60 | 5.90 | 2.10 | www.genomesize.com 2015 |
| | *Hirundo rustica* | 11.90 | 6.40 | 5.60 | 3.20 | www.genomesize.com 2015 |
| | *Hirundo rustica* | 10.80 | 5.90 | | | Irisova 1988 |
| | *Delichon urbicum* | 11.70 | 6.40 | | | www.genomesize.com 2015 |
| | *Delichon urbicum* | 10.82 | 5.82 | | | Irisova 1988 |
| | *Cecropis daurica* | 10.82 | 6.01 | | | Irisova 1988 |
| | *Pygohelidon cyanoleuca* | 11.70 | 6.40 | 5.40 | 2.10 | www.genomesize.com 2015 |
| Accrocephalidae | *Acrocephalus schoenobaenus* | 12.70 | 7.20 | | | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|
| | *Acrocephalus arundinaceus* | 9.53 | 5.68 | | | Irisova 1988 |
| Pycnonotidae | *Pycnonotus barbatus* | 12.50 | 6.90 | | | Glomski and Pica 2011 |
| Aegithalidae | *Aegithalos caudatus* | 11.90 | 5.60 | 5.30 | 2.40 | www.genomesize.com 2015 |
| | *Aegithalos caudatus* | 12.20 | 6.40 | | | Kostelecka-Myrcha *et al.* 1993 |
| Phylloscopidae | *Phylloscopus sibilatrix* | 11.00 | 5.90 | | | personal data |
| | *Phylloscopus fuscatus* | 10.66 | 5.89 | | | Irisova 1988 |
| | *Phylloscopus trochillus* | 11.20 | 5.90 | | | personal data |
| | *Phylloscopus collybita* | 10.70 | 5.90 | | | personal data |
| | *Phylloscopus collybita* | 9.74 | 6.84 | | | Irisova 1988 |
| | *Phylloscopus nitidus* | 10.15 | 5.55 | | | Irisova 1988 |
| Sylviidae | *Sylvia borin* | 10.22 | 5.99 | | | Irisova 1988 |
| | *Sylvia atricapilla* | 11.50 | 6.80 | | | Kostelecka-Myrcha *et al.* 1993 |
| Leiothrichidae | *Garrulax canorus* | 11.00 | 6.50 | | | www.genomesize.com 2015 |
| Certhiidae | *Certhia familiaris* | 11.00 | 6.40 | | | www.genomesize.com 2015 |
| | *Certhia familiaris* | 11.00 | 6.20 | | | Kostelecka-Myrcha *et al.* 1993 |
| Sittidae | *Sitta europaea* | 11.50 | 6.10 | 5.60 | 2.30 | www.genomesize.com 2015 |
| | *Sitta carolinensis* | 11.30 | 5.80 | 5.20 | 2.10 | www.genomesize.com 2015 |
| Polioptilidae | *Polioptila caerulea* | 10.00 | 6.30 | 4.50 | 2.10 | www.genomesize.com 2015 |
| Troglodytidae | *Troglodytes troglodytes* | 10.80 | 6.10 | | | www.genomesize.com 2015 |
| | *Troglodytes troglodytes* | 11.20 | 6.30 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Troglodytes aedon* | 11.10 | 6.50 | 5.40 | 2.20 | www.genomesize.com 2015 |
| | *Thryothorus ludovicianus* | 11.20 | 5.60 | 5.20 | 1.80 | www.genomesize.com 2015 |
| | *Cantorchilus modestus* | 10.80 | 6.50 | 5.60 | 2.60 | www.genomesize.com 2015 |
| | *Pheugopedius fasciatoventris* | 12.10 | 6.90 | 5.50 | 2.90 | www.genomesize.com 2015 |
| Regulidae | *Regulus regulus* | 11.10 | 6.10 | | | www.genomesize.com 2015 |
| | *Regulus regulus* | 10.70 | 6.10 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Regulus satrapa* | 9.80 | 5.00 | 4.70 | 2.00 | www.genomesize.com 2015 |
| | *Regulus calendula* | 10.20 | 5.30 | 4.80 | 2.30 | www.genomesize.com 2015 |
| Bombycillidae | *Bombycilla garrulus* | 11.90 | 6.40 | | | www.genomesize.com 2015 |
| | *Bombycilla cedrorum* | 12.20 | 6.30 | 5.70 | 2.30 | www.genomesize.com 2015 |
| Mimidae | *Toxostoma rufum* | 11.40 | 7.00 | | | www.genomesize.com 2015 |
| | *Mimus polyglottos* | 11.00 | 6.20 | 4.90 | 2.10 | www.genomesize.com 2015 |
| Sturnidae | *Gracula religiosa* | 12.20 | 6.10 | | | www.genomesize.com 2015 |
| | *Sturnus vulgaris* | 12.00 | 6.50 | 6.70 | 2.20 | www.genomesize.com 2015 |
| | *Sturnus vulgaris* | 10.41 | 6.01 | | | Irisova 1988 |
| | *Sturnus vulgaris* | 10.26 | 6.11 | | | Irisova 1988 |
| | *Pastor roseus* | 12.10 | 5.50 | | | www.genomesize.com 2015 |
| | *Pastor roseus* | 10.14 | 6.08 | | | Irisova 1988 |
| | *Acridotheres tristis* | 11.06 | 6.50 | | | Irisova 1988 |
| | *Acridotheres tristis* | 12.90 | 6.90 | | | Glomski and Pica 2011 |
| | *Spodiopsar sericeus* | 11.90 | 5.60 | | | www.genomesize.com 2015 |
| Cinclidae | *Cinclus cinclus* | 10.71 | 5.64 | | | Irisova 1988 |
| Turdidae | *Catharus guttatus* | 11.90 | 5.60 | 5.70 | 2.10 | www.genomesize.com 2015 |
| | *Turdus torquatus* | 11.70 | 6.20 | | | personal data |
| | *Turdus merula* | 12.20 | 7.00 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Turdus merula* | 12.10 | 6.00 | 4.80 | 2.80 | www.genomesize.com 2015 |
| | *Turdus ruficollis* | 11.25 | 6.01 | | | Irisova 1988 |

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|---|---|---|---|---|---|---|
| | *Turdus atrogularis* | 11.60 | 6.31 | | | Irisova 1988 |
| | *Turdus naumanni* | 12.44 | 6.96 | | | Irisova 1988 |
| | *Turdus pilaris* | 11.71 | 6.21 | | | Irisova 1988 |
| | *Turdus philomelos* | 12.50 | 7.10 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Turdus philomelos* | 11.50 | 6.10 | 6.40 | 2.60 | www.genomesize.com 2015 |
| | *Turdus viscivorus* | 11.30 | 6.40 | | | www.genomesize.com 2015 |
| | *Turdus viscivorus* | 11.15 | 6.06 | | | Irisova 1988 |
| | *Turdus plebejus* | 11.80 | 7.30 | 5.50 | 2.80 | www.genomesize.com 2015 |
| | *Turdus grayi* | 12.10 | 6.70 | 5.40 | 2.00 | www.genomesize.com 2015 |
| | *Turdus migratorius* | 11.10 | 6.10 | 5.60 | 2.20 | www.genomesize.com 2015 |
| Muscicapidae | *Muscicapa striata* | 11.52 | 6.27 | | | Irisova 1988 |
| | *Muscicapa striata* | 11.47 | 6.85 | | | Irisova 1988 |
| | *Erithacus rubecula* | 11.00 | 6.10 | | | www.genomesize.com 2015 |
| | *Erithacus rubecula* | 12.50 | 7.00 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Luscinia svecica* | 10.58 | 6.29 | | | Irisova 1988 |
| | *Luscinia svecica* | 10.74 | 6.06 | | | Irisova 1988 |
| | *Luscinia megarhynchos* | 11.35 | 5.99 | | | Irisova 1988 |
| | *Luscinia megarhynchos* | 13.40 | 5.80 | 6.40 | 2.10 | www.genomesize.com 2015 |
| | *Tarsiger cyanurus* | 10.71 | 5.75 | | | Irisova 1988 |
| | *Myophonus caeruleus* | 11.12 | 6.14 | | | Irisova 1988 |
| | *Phoenicurus caeruleocephala* | 10.52 | 5.88 | | | Irisova 1988 |
| | *Phoenicurus ochruros* | 11.50 | 6.00 | | | personal data |
| | *Phoenicurus phoenicurus* | 9.78 | 5.16 | | | Irisova 1988 |
| | *Phoenicurus erythrogastrus* | 10.52 | 5.53 | | | Irisova 1988 |
| | *Monticola saxatilis* | 10.46 | 6.29 | | | Irisova 1988 |
| | *Monticola saxatilis* | 11.10 | 5.42 | | | Irisova 1988 |
| | *Saxicola rubicola* | 10.02 | 5.34 | | | Irisova 1988 |
| | *Oenanthe isabellina* | 10.66 | 5.82 | | | Irisova 1988 |
| | *Oenanthe deserti* | 10.05 | 6.02 | | | Irisova 1988 |
| | *Oenanthe pleschanka* | 10.71 | 5.89 | | | Irisova 1988 |
| Prunellidae | *Prunella collaris* (SK) | 11.40 | 6.00 | 6.30 | 3.10 | personal data |
| | *Prunella collaris* (KG) | 11.09 | 5.66 | 5.48 | 2.58 | personal data |
| | *Prunella collaris* (BG) | 11.12 | 6.39 | 5.58 | 2.69 | personal data |
| | *Prunella himalayana* | 10.45 | 5.26 | | | Irisova 1988 |
| | *Prunella himalayana* | 10.78 | 5.66 | | | Irisova 1988 |
| | *Prunella fulvescens* | 11.66 | 6.09 | 5.47 | 2.71 | personal data |
| | *Prunella fulvescens* | 10.96 | 5.58 | | | Irisova 1988 |
| | *Prunella fulvescens* | 10.48 | 5.50 | | | Irisova 1988 |
| | *Prunella atrogularis* | 11.46 | 5.78 | 5.64 | 2.56 | personal data |
| | *Prunella modularis* | 12.10 | 6.50 | 4.10 | 2.10 | personal data |
| | *Prunella modularis* | 11.90 | 6.80 | | | Kostelecka-Myrcha *et al.* 1993 |
| Ploceidae | *Ploceus hypoxanthus* | 11.10 | 6.90 | | | www.genomesize.com 2015 |
| Viduidae | *Vidua paradisaea* | 12.70 | 6.80 | | | www.genomesize.com 2015 |
| Estrildidae | *Lonchura punctulata* | 11.30 | 8.30 | | | Glomski and Pica 2011 |
| | *Lonchura malacca* | 10.80 | 6.10 | | | www.genomesize.com 2015 |
| | *Amandava amandava* | 11.30 | 5.30 | | | www.genomesize.com 2015 |
| | *Amadina fasciata* | 12.70 | 5.80 | | | www.genomesize.com 2015 |
| | *Amadina erythrocephala* | 11.60 | 6.80 | | | Glomski and Pica 2011 |
| | *Estrilda astrild* | 11.20 | 5.40 | | | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|
| Passeridae | *Montifringilla nivalis* | 10.95 | 6.16 | | | Irisova 1988 |
| | *Montifringilla nivalis* | 10.87 | 5.06 | | | Irisova 1988 |
| | *Petronia petronia* | 10.79 | 6.18 | | | Irisova 1988 |
| | *Petronia petronia* | 11.64 | 5.65 | | | Irisova 1988 |
| | *Passer domesticus* | 11.30 | 5.60 | 5.40 | 2.30 | www.genomesize.com 2015 |
| | *Passer domesticus* | 10.46 | 6.39 | | | Irisova 1988 |
| | *Passer domesticus* | 11.60 | 6.10 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Passer melanurus* | 11.30 | 5.30 | | | Glomski and Pica 2011 |
| | *Passer montanus* | 11.50 | 6.10 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Passer montanus* | 10.63 | 5.60 | | | Irisova 1988 |
| | *Passer montanus* | 10.51 | 6.00 | | | Irisova 1988 |
| | *Passer montanus* | 10.74 | 6.07 | | | Irisova 1988 |
| Motacillidae | *Motacilla flava* | 9.63 | 5.78 | | | Irisova 1988 |
| | *Motacilla cinera* | 10.82 | 5.49 | | | Irisova 1988 |
| | *Motacilla cinera* | 10.02 | 5.45 | | | Irisova 1988 |
| | *Motacilla alba* | 11.60 | 7.10 | 6.40 | 2.40 | www.genomesize.com 2015 |
| | *Motacilla alba* | 10.36 | 5.58 | | | Irisova 1988 |
| | *Motacilla alba* | 10.03 | 5.73 | | | Irisova 1988 |
| | *Motacilla alba* | 10.64 | 5.72 | | | Irisova 1988 |
| | *Motacilla alba* | 10.11 | 5.96 | | | Irisova 1988 |
| | *Anthus trivialis* | 10.48 | 5.50 | | | Irisova 1988 |
| | *Anthus trivialis* | 10.26 | 5.78 | | | Irisova 1988 |
| | *Anthus trivialis* | 10.88 | 6.15 | | | Irisova 1988 |
| | *Anthus spinoletta* | 11.20 | 5.80 | | | personal data |
| | *Anthus spinoletta* | 10.78 | 5.66 | | | Irisova 1988 |
| Fringillidae | *Fringilla coelebs* | 11.10 | 5.70 | | | personal data |
| | *Fringilla coelebs* | 10.22 | 6.10 | | | Irisova 1988 |
| | *Fringilla coelebs* | 11.90 | 6.80 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Mycerobas carnipes* | 10.66 | 6.07 | | | Irisova 1988 |
| | *Coccothraustes cocco-thraustes* | 12.40 | 6.70 | 5.60 | 2.40 | www.genomesize.com 2015 |
| | *Coccothraustes cocco-thraustes* | 10.81 | 5.97 | | | Irisova 1988 |
| | *Pinicola enucleator* | 11.30 | 6.20 | | | www.genomesize.com 2015 |
| | *Pinicola enucleator* | 9.75 | 7.05 | | | Irisova 1988 |
| | *Pinicola enucleator* | 9.75 | 7.05 | | | Irisova 1988 |
| | *Pyrrhula pyrrhula* | 10.90 | 6.10 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Pyrrhula pyrrhula* | 10.22 | 6.10 | | | Irisova 1988 |
| | *Leucosticte brandti* | 10.35 | 5.54 | | | Irisova 1988 |
| | *Carpodacus erythrinus* | 10.97 | 5.76 | | | Irisova 1988 |
| | *Carpodacus erythrinus* | 10.03 | 5.69 | | | Irisova 1988 |
| | *Carpodacus erythrinus* | 10.59 | 5.92 | | | Irisova 1988 |
| | *Carpodacus rubicilla* | 10.85 | 5.76 | | | Irisova 1988 |
| | *Carpodacus roseus* | 11.16 | 5.05 | | | Irisova 1988 |
| | *Carpodacus purpureus* | 11.40 | 5.10 | | | Glomski and Pica 2011 |
| | *Rhodopechys sanguineus* | 10.52 | 6.16 | | | Irisova 1988 |
| | *Loxia curvirostra* | 10.70 | 6.40 | | | www.genomesize.com 2015 |
| | *Serinus canaria* | 11.40 | 7.10 | | | www.genomesize.com 2015 |
| | *Serinus pusillus* | 11.28 | 5.91 | | | Irisova 1988 |
| | *Serinus pusillus* | 10.72 | 5.52 | | | Irisova 1988 |
| | *Chloris chloris* | 11.40 | 7.10 | | | www.genomesize.com 2015 |
| | *Spinus spinus* | 11.90 | 6.40 | | | www.genomesize.com 2015 |

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|---|---|---|---|---|---|---|
| | *Spinus spinus* | 10.90 | 5.90 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Acanthis flammea* | 11.10 | 5.60 | | | www.genomesize.com 2015 |
| | *Acanthis flammea* | 10.45 | 5.78 | | | Irisova 1988 |
| | *Acanthis cabaret* | 11.18 | 5.39 | | | Irisova 1988 |
| | *Carduelis carduelis* | 11.30 | 5.30 | | | www.genomesize.com 2015 |
| | *Carduelis caniceps* | 10.34 | 5.30 | | | Irisova 1988 |
| | *Carduelis cannabina* | 10.90 | 6.70 | | | www.genomesize.com 2015 |
| Parulidae | *Seiurus aurocapilla* | 11.70 | 5.50 | 5.30 | 2.50 | www.genomesize.com 2015 |
| | *Helmitheros vermivorus* | 11.30 | 6.10 | 5.40 | 2.30 | www.genomesize.com 2015 |
| | *Parkesia motacilla* | 11.70 | 6.00 | 5.60 | 2.40 | www.genomesize.com 2015 |
| | *Mniotilta varia* | 11.20 | 6.10 | 4.90 | 2.30 | www.genomesize.com 2015 |
| | *Oreothlypis gutturalis* | 11.10 | 7.20 | 5.60 | 2.90 | www.genomesize.com 2015 |
| | *Geothlypis aequinoctialis* | 11.90 | 5.90 | 5.30 | 2.00 | www.genomesize.com 2015 |
| | *Wilsonia citrina* | 11.80 | 6.70 | 5.90 | 2.60 | www.genomesize.com 2015 |
| | *Wilsonia pusilla* | 11.70 | 5.80 | 5.60 | 2.30 | www.genomesize.com 2015 |
| | *Dendroica pensylvanica* | 11.70 | 6.50 | 5.90 | 2.20 | www.genomesize.com 2015 |
| | *Dendroica fusca* | 11.40 | 5.60 | 5.10 | 2.40 | www.genomesize.com 2015 |
| | *Dendroica magnolia* | 10.90 | 6.30 | 5.20 | 2.40 | www.genomesize.com 2015 |
| | *Dendroica caerulescens* | 11.30 | 6.30 | 5.40 | 2.60 | www.genomesize.com 2015 |
| | *Dendroica coronata* | 11.00 | 6.00 | 5.30 | 2.40 | www.genomesize.com 2015 |
| | *Dendroica virens* | 10.90 | 6.30 | 5.00 | 2.40 | www.genomesize.com 2015 |
| | *Dendroica dominica* | 11.10 | 6.10 | 5.30 | 2.40 | www.genomesize.com 2015 |
| | *Dendroica discolor* | 10.80 | 6.30 | 4.90 | 2.10 | www.genomesize.com 2015 |
| | *Dendroica pinus* | 9.70 | 5.90 | 4.80 | 2.50 | www.genomesize.com 2015 |
| | *Basileuterus rufifrons* | 11.50 | 6.00 | 5.60 | 2.60 | www.genomesize.com 2015 |
| | *Myioborus miniatus* | 11.60 | 6.10 | 5.10 | 2.30 | www.genomesize.com 2015 |
| Calcariidae | *Plectrophenax nivalis* | 11.90 | 5.40 | | | www.genomesize.com 2015 |
| Icteridae | *Dolichonyx oryzivorus* | 10.60 | 6.10 | | | www.genomesize.com 2015 |
| | *Amblycercus holoseri-ceus* | 11.60 | 6.50 | 5.70 | 3.00 | www.genomesize.com 2015 |
| | *Quiscalus quiscula* | 10.70 | 6.20 | 4.70 | 2.30 | www.genomesize.com 2015 |
| | *Psarcolius wagleri* | 11.00 | 6.70 | 4.90 | 2.20 | www.genomesize.com 2015 |
| | *Icterus mesomelas* | 13.10 | 6.90 | 5.70 | 2.80 | www.genomesize.com 2015 |
| | *Icterus galbula* | 12.00 | 6.30 | 5.30 | 2.20 | www.genomesize.com 2015 |
| Emberizidae | *Emberiza citrinella* | 11.40 | 6.40 | | | Kostelecka-Myrcha *et al.* 1993 |
| | *Emberiza citrinella* | 11.10 | 6.40 | 6.40 | 2.10 | www.genomesize.com 2015 |
| | *Emberiza leucocephalos* | 10.20 | 6.03 | | | Irisova 1988 |
| | *Emberiza leucocephalos* | 10.40 | 6.02 | | | Irisova 1988 |
| | *Emberiza cia* | 10.52 | 5.70 | | | Irisova 1988 |
| | *Emberita stewarti* | 11.65 | 5.83 | | | Irisova 1988 |
| | *Emberiza buchanani* | 12.35 | 6.05 | 5.84 | 2.79 | personal data |
| | *Emberiza hortulana* | 10.34 | 5.79 | | | Irisova 1988 |
| | *Emberiza pusilla* | 11.38 | 6.33 | | | Irisova 1988 |
| | *Emberiza aureola* | 10.64 | 5.69 | | | Irisova 1988 |
| | *Emberiza pallasi* | 11.46 | 6.47 | | | Irisova 1988 |
| | *Emberiza pallasi* | 10.70 | 4.97 | | | Irisova 1988 |
| Passerel-lidae | *Chlorospingus pileatus* | 11.40 | 6.70 | 5.90 | 2.70 | www.genomesize.com 2015 |
| | *Arremonops conirostris* | 11.40 | 6.20 | 5.50 | 2.20 | www.genomesize.com 2015 |
| | *Spizella passerina* | 10.10 | 6.00 | 5.00 | 2.30 | www.genomesize.com 2015 |
| | *Spizella pusilla* | 11.30 | 5.90 | 5.30 | 2.2 | www.genomesize.com 2015 |
| | *Spizella arborea* | 10.20 | 5.30 | 5.10 | 2.00 | www.genomesize.com 2015 |

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| | *Passerella iliaca* | 11.30 | 6.20 | 5.70 | 2.20 | www.genomesize.com 2015 |
| | *Junco hyemalis* | 11.70 | 5.90 | 5.50 | 2.30 | www.genomesize.com 2015 |
| | *Zonotrichia capensis* | 11.40 | 6.10 | 4.80 | 2.10 | www.genomesize.com 2015 |
| | *Zonotrichia albicollis* | 11.70 | 5.10 | 5.50 | 2.10 | www.genomesize.com 2015 |
| | *Melospiza melodia* | 11.10 | 6.10 | 5.30 | 2.30 | www.genomesize.com 2015 |
| | *Melospiza georgiana* | 10.90 | 5.90 | 5.10 | 2.40 | www.genomesize.com 2015 |
| | *Pipilo erythrophthalmus* | 11.40 | 6.10 | 5.20 | 2.40 | www.genomesize.com 2015 |
| Cardinalidae | *Piranga olivacea* | 11.90 | 6.20 | 5.10 | 2.40 | www.genomesize.com 2015 |
| | *Cardinalis cardinalis* | 11.90 | 6.00 | 5.60 | 2.10 | www.genomesize.com 2015 |
| | *Pheucticus tibialis* | 11.70 | 6.50 | 5.30 | 2.70 | www.genomesize.com 2015 |
| | *Passerina caerulea* | 11.10 | 6.80 | | | www.genomesize.com 2015 |
| Thraupidae | *Saltator albicollis* | 12.10 | 7.20 | 5.20 | 3.00 | www.genomesize.com 2015 |
| | *Saltator atriceps* | 12.50 | 7.50 | 5.30 | 2.90 | www.genomesize.com 2015 |
| | *Tiaris olivaceus* | 11.30 | 6.90 | 4.90 | 2.40 | www.genomesize.com 2015 |
| | *Tangara icterocephala* | 11.40 | 6.30 | 5.10 | 2.40 | www.genomesize.com 2015 |
| | *Thraupis episcopus* | 11.40 | 7.00 | 5.40 | 2.30 | www.genomesize.com 2015 |
| | *Dacnis venusta* | 11.20 | 6.00 | 4.80 | 2.10 | www.genomesize.com 2015 |
| | *Dacnis cayana* | 12.50 | 7.40 | 6.20 | 3.20 | www.genomesize.com 2015 |
| | *Ramphocelus passerinii* | 12.00 | 6.60 | 4.80 | 2.40 | www.genomesize.com 2015 |