ACTA BIOLOGICA SLOVENICA

VOL. 58 ŠT. 2 LJUBLJANA 2015

prej/formerly BIOLOŠKI VESTNIK

ISSN 1408-3671
UDK 57(497.4)

izdajatelj/publisher
Društvo biologov Slovenije

ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 1–98

Acta Biologica Slovenica
Glasilo Društva biologov Slovenije – Journal of Biological Society of Slovenia

Izdaja – Published by
Društvo biologov Slovenije – Biological Society of Slovenia

Naslov uredništva – Address of Editorial Office
Acta Biologica Slovenica, Večna pot 111, SI-1001 Ljubljana, Slovenija
http://bijh.zrc-sazu.si/abs/

Zasnova oblikovanja – Design
Žare Vrezec

ISSN 1408-3671 UDK 57(497.4)

Natisnjeno – Printed on: 2015
Tisk – Print: Tiskarna Pleško d.o.o., Ljubljana
Naklada: 400 izvodov

Cena letnika (dve številki): 15 € za posameznike, 42 € za ustanove

Številka poslovnega računa pri Ljubljanski banki: 02083-142508/30

Publikacijo je sofinancirala Javna agencija za raziskovalno dejavnost Republike Slovenije

Acta Biologica Slovenica je indeksirana v – is indexed in: CAB Abstracts, Web of Knowledge – Thomson Reuters

ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 3–11

Relationship of nuclear genome size, cell volume and nuclei volume in endosperm of *Sorghum bicolor*

Razmerje velikosti jedrnega genoma, prostornine celic in prostornine jeder v endospermu sirka (*Sorghum bicolor*)

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Abstract: Endosperm cells of *Sorghum bicolor* undergo several rounds of endoreplication during seed development, resulting in somatic endopolyploidy with cells containing 3 C to 96 C nuclei (1 C represents the amount of DNA in an unreplicated haploid genome). Cells with higher DNA content are larger and contain larger nuclei. The function of large endosperm cells in *Sorghum bicolor* is storage of starch that will be used in germination. We analysed the ratios of nuclear genome size and volume of nuclei and cells to determine if karyoplasmic ratio is constant in cells of different endopolyploidy levels. Interestingly, the volume of cells and nuclei increases more than can be expected from the increase in genome size alone. Instead, a constant ratio was observed between genome size and surface of cells and nuclei. However, an isometric relationship was found between volume of nuclei and volume of cells, indicating that karyoplasmic ratio is constant in sense of dimensions of cellular compartments, rather than with nuclear genome size alone.

Keywords: cell volume, endopolyploidy, endoreplication, endosperm, nuclear genome size

Izvleček: Tekom razvoja semena sirka (*Sorghum bicolor*) v celicah endosperma poteče več ciklov endoreplikacije, kar se kaže v somatski endopoliploidiji tkiva, ki vsebuje celice s 3 C do 96 C jedri (1 C predstavlja količino DNA v nepodvojenem haploidnem genomu). Celice z večjo količino DNA so večje in vsebujejo večja jedra. Vloga velikih celic v endospermu sirka je shranjevanje založnega škroba, ki bo porabljen med kalitvijo. Analizirali smo razmerje med velikostjo jedrnega genoma in prostornino jeder ter celic, da bi preverili konstantnost karioplazemskega razmerja v celicah z različno stopnjo endopoliploidije. Zanimivo, prostornina celic in jeder se povečuje bolj kot bi pričakovali samo zaradi povečevanja velikosti genoma. Namesto tega smo opazili konstantno razmerje med velikostjo genoma in površino celic in jeder. Poleg tega smo pokazali, da obstaja izometrično razmerje med prostornino jeder in prostornino celic, kar kaže na to, da je karioplazemsko razmerje konstantno v smislu dimenzij celičnih sestavnih delov, ne pa glede na velikost jedrnega genoma.

Ključne besede: endopoliploidija, endoreplikacija, endosperm, prostornina celic, velikost jedrnega genoma

Introduction

Polyploidization in cell differentiation to produce large cells is a widespread developmental strategy throughout the animal and plant kingdoms (Orr-Weaver 2015). Endoreplication is a variation of a cell cycle, where DNA replication in the S-phase is not followed by mitotic division and sister chromatids are not separated into daughter cells. Repeated endoreplication cycles generate chromosomes with an exponentially growing number of chromatids (Joubès and Chevalier 2000, Sugimoto-Shirasu and Roberts 2003). Occurrence of endoreplication is common in plants, but is not related to the initial genome size, it is more correlated to the life strategy and phylogeny of plant groups (Barow and Meister 2003). The significance of endoreplication in organisms may not be due to any specialized function that is supported by endopolyploidy, but rather in the consequences of the endopolyploidy state that makes the polyploid cells different from diploid cells, such as the tissue growth with absence of mitosis, changed surface to volume ratio and differences in gene expression or growth factor gradients (Barlow 1978, Joubès and Chevalier 2000). However, the precise functions of polyploidization is still elusive today (Orr-Weaver 2015). Recently, Bourdon et al. (2012) provided the direct evidence that endopolyploidy increased transcription of rRNA and mRNA on a per-nucleus basis. The positive correlation between ploidy levels and cell size indicates that endopolyploid nuclei might be required for the formation of large cells (Kondorosi et al. 2000), but under certain conditions, the final size of cells and organs can be uncoupled from endoreplication (Cookson et al. 2006). The positive relationship between size of nuclei and size of cells was shown widely throughout the eukaryotes, for example the size of nuclei was correlated to cell volume in yeast (Jorgensen et al. 2007). The constant ratio of nucleus and cell volume was observed already a century ago and led to the hypothesis of a "karyoplasmic ratio" (Wilson 1925). Closely related observations, but should not be considered equivalent to the volume of nuclei, is that variations in genome size is related to cell size (Cavalier-Smith 2005).

Cereal endosperm is a storage tissue that is comprised of cells with different endopolyploidy levels. The function of endosperm cells is accumulation of storage compounds, mainly starch (Kowles et al. 1992). Maize endosperm contains endoreplicated nuclei with at least 192 C DNA content (Vilhar et al. 2002). The endopolyploid state evolved before domestication, since the wild relative of maize, teosinte, contains nuclei with up to 96 C (Dermastia et al. 2009). The initial genome size of maize endosperm cells is 3 C, therefore endoreplication generates 6 C, 12 C, 24 C, etc. cells. The extent of endopolyploidy correlates with the yield of maize grain (Kowles et al. 1992).

The aim of this study was to re-evaluate the data measured by Kladnik et al. (2006) to examine the increase in volume of cells and nuclei of *Sorghum bicolor* with increase in nuclear genome size due to endoreplication and test whether the increase in volume deviates from isometry. Moreover, we examined the ratios of volume and surface of cells and nuclei with nuclear genome size. At last, but not least, we analysed the relationship of nuclear volume with cell volume to test the classical karyoplasmic ratio hypothesis.

Materials and methods

Tissue sections and staining

Developing caryopses of sorghum (*Sorghum bicolor* (L.) Moench) were sampled 5 to 16 days after pollination, fixed in FAA (3.7 % formaldehyde, 5 % acetic acid, 50 % ethanol), embedded in Paraplast Plus (Sherwood Medical Co., USA) and sectioned longitudinally to 12-20 μm thick sections as described in detail by Kladnik et al. (2006). Sections were stained for starch using an aqueous solution of 2 % iodine and 3 % potassium iodide (I_2/KI). Nuclear DNA was stained with Feulgen reagent according to Dolenc Koce et al. (2003) and Kladnik et al. (2006). The Feulgen reaction is quantitative for DNA if the only aldehydes remaining in the cell are those produced from the hydrolysis of DNA (Feulgen and Rossenbeck 1924). Sections were observed on an Axioskop 2 MOT microscope (Carl Zeiss, Germany) and images were acquired with an AxioCam MRc digital camera (Carl Zeiss Vision, Germany).

Measurement of genome size, cell volume and nuclei volume

Nuclear genome size was measured on Feulgen stained median longitudinal sections of caryopses at 5, 8, 10, 12 and 16 days after pollination (DAP) in three replicates. The nuclear DNA amount was measured by image densitometry using the interphase-peak method adapted for use with tissue sections (Vilhar et al. 2002, Dermastia et al. 2009). Integrated optical density (a measure of relative DNA amount), size and positions of the nuclei were measured in the whole endosperm transect. The amount of nuclear DNA was expressed in C-value units, with 1 C representing the nuclear DNA content of a non-replicated haploid genome. The volume and surface area of a nucleus was estimated as a sphere based on the area of the nucleus section in the acquired image. The size of cells was measured by outlining the cell walls, visible due to their autofluorescence in UV. Volume and surface of cells was estimated as a sphere based on the area of the cell transects in the image. In total, we measured nuclear DNA content and size of cells and nuclei for 995, 1651, 2711, 2470 and 2587 cells in 5, 8, 10, 12 and 16 DAP samples, respectively.

Data analysis

Data was analysed using R version 3.1.2 (R Core Team 2014) in RStudio version 0.98.1102 and GraphPad Prism version 6.01 for Windows (GraphPad Software, USA). The ratios of nuclear genome size with different cell size parameters were calculated by dividing measured relative DNA amount, normalized to C-units, with respective cell size parameter, measured in µm. Variation of volumes and ratios with respect to nuclear genome size was analysed using linear model on logarithmically transformed data in R and calculating R^2 value to express the portion of the variance explained by variation in nuclear genome size. The relationship between cell volume, nuclei volume and different ratios with genome size was analysed by calculating medians in all endopolyploidy classes (3 C to 96 C) and performing linear regression in Prism, thus obtaining the slope of the log-log relationship.

Results

The endosperm of *Sorghum bicolor* is composed of cells with variable sizes, containing nuclei

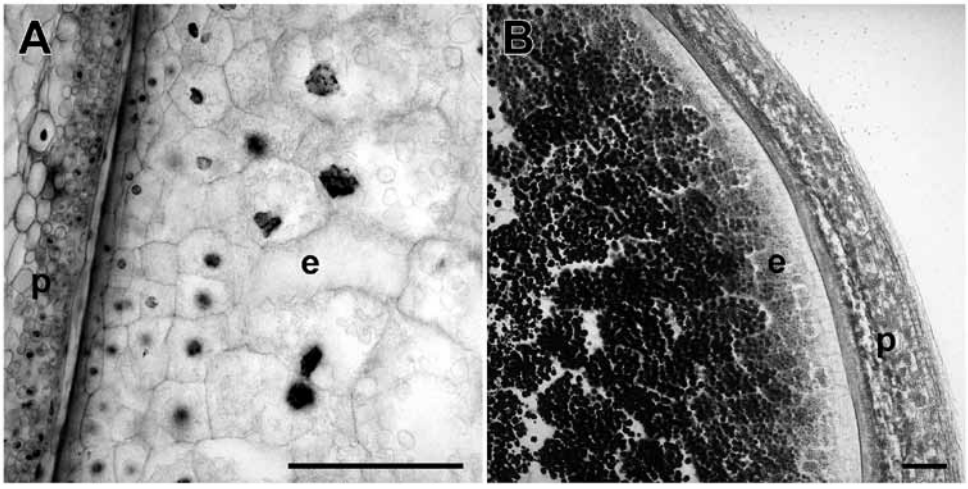

Figure 1: Endosperm of *Sorghum bicolor* 16 days after pollination. A - Feulgen stained tissue section, showing size of nuclei and cells of different endopolyploidy levels. B - Starch deposition in endosperm cells. Labels: e, endosperm; p, pericarp. Bar represents 100 µm.

Slika 1: Endosperm sirka (*Sorghum bicolor*) 16 dni po oprašitvi. A - Tkivna rezina barvana po Feulgen-u, ki prikazuje velikost jeder in celic različnih endopoliploidnih stopenj. B - Nalaganje škroba v celicah endosperma. Oznake: e, endosperm; p, perikarp. Merilo predstavlja 100 µm.

with different amounts of DNA that is replicated in multiple endocycles (Fig. 1A). The endosperm cells contain large amounts of starch in the form of starch grains, with exception of the smallest cells in the outermost layer of the endosperm (Fig. 1B). The cells in the sorghum endosperm undergo several rounds of endoreplication, resulting in nuclei with DNA content up to 96 C (Kladnik et al. 2006).

We have tested the effects of nuclear genome size variation on variations in volume of cells and nuclei. Duplication of DNA during endoreplication cycles and growth of cells and nuclei are exponential processes by nature, so all relationships were plotted on log-log graphs. Scatterplots (Fig. 2) represent all measured data for samples 10 to 16 days after pollination (DAP), where all possible endopolyploidy classes were present (3 C to 96 C). Five and eight DAP samples lacked the highest endopolyploidy levels (Kladnik et al. 2006). The volume of cells increases with increasing nuclear genome size (Fig. 2A), with 65 % of variation explained by variation in genome size ($R^2 = 0.65$). Volume of nuclei is tightly related to nuclear DNA amount (Fig. 2B), with 95 % of variation explained by genome size ($R^2 = 0.95$). Furthermore, we examined the ratio of nuclear genome size with cell dimensions. The ratio of genome size with cell volume (Fig. 2C), expressed as C-units per unit of cell volume, shows a slight negative correlation with genome size with only 13 % of its variation explained by genome size ($R^2 = 0.13$). The ratio of genome size with nuclei volume (Fig. 2D), expressed as C-units per unit of nuclei volume, shows a negative correlation, with 64 % of variation accounted for genome size ($R^2 = 0.64$). However, when we calculate a ratio of genome size versus cell surface (Fig. 2E) or nuclei surface (Fig. 2F), the variation in the ratio is no longer dependent on variation in nuclear genome size ($R^2 = 0.01$ and 0.02, respectively).

To test if increase in volume of cells and nuclei, related to increase in genome size, follows a power law ($y = bx^a$), that is if their relationship is allometric, we calculated median values of volumes of cells and nuclei belonging to different endopolyploidy classes, and plotted volume versus C-value on a log-log scale (Fig. 3A). If the relation between both is exponential, the data would be distributed on a straight line. The slope of the fitted line (exponent *a* in the above equation) indicates the type of allometry (Barow 2006). Both for cell volume and nuclei volume, the slope was larger than 1, indicating positive allometry, 1.44 and 1.34, respectively (Tab. 1). The volume of cells and nuclei increases with a higher rate, than can be attributed to the increase in genome size alone.

Maintenance of karyoplasmic ratio can be examined by calculating ratio of nuclear genome size versus cell volume, thus obtaining the amount of genome (C-units) per unit of cell volume (Fig. 3B). The ratio is related to different endoploidy levels; on log-log scale their relationship is linear with a negative slope (Tab. 1), indicating that progressively less genome units are found in a unit of cell volume. Similarly, the ratio of genome size per volume of nuclei also shows a negative linear relationship with increasing ploidy level (Fig. 3B, Tab. 1). However, when calculating the ratio between genome size and surface area of cells or nuclei, and comparing it to ploidy level, the ratio is constant in the whole range of endoploidy levels (Fig. 3B). The slope of the linear relationship between genome size to surface area on a log scale is not significantly different from zero (Tab. 1). Finally, if using nuclear genome size only to separate cells into different endoploidy classes, the relationship between volume of nuclei and volume of cells is isometric, with a slope not significantly different from 1 (Fig. 3C, Tab. 1).

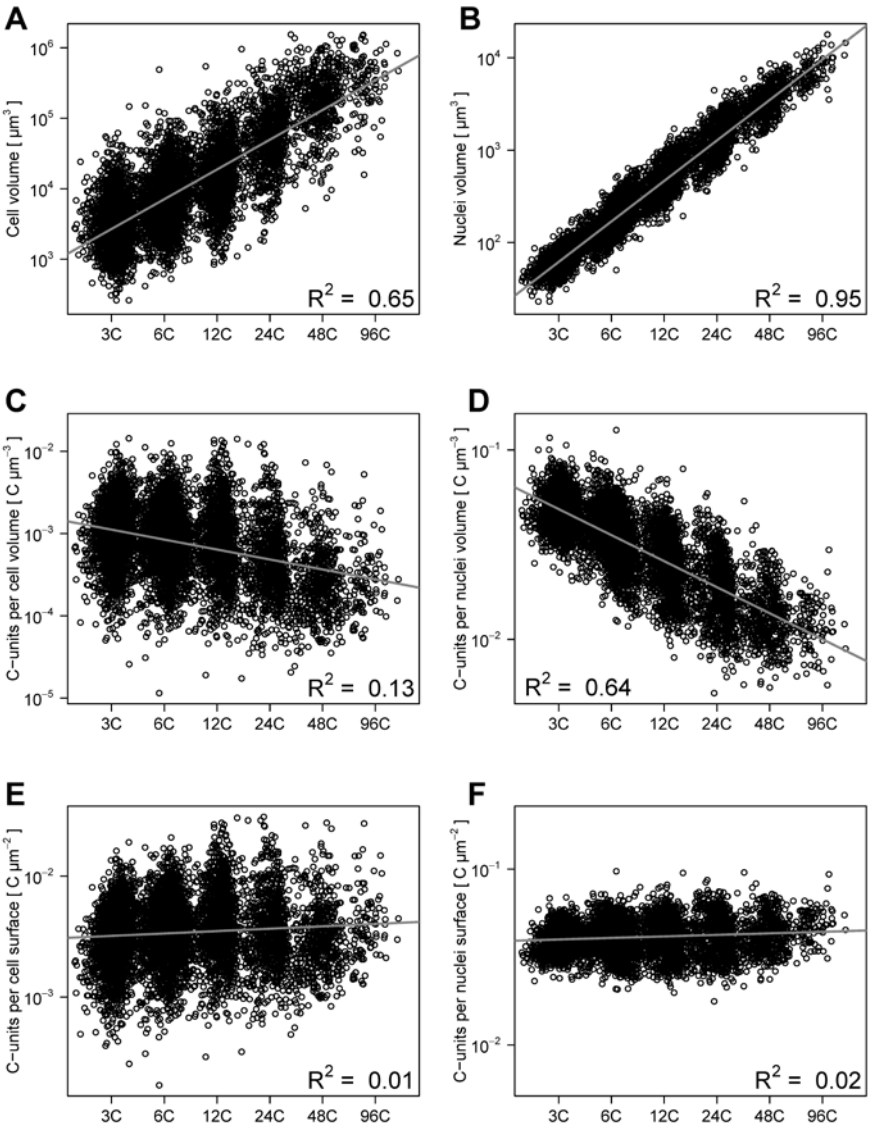

Figure 2: Volume of cells and nuclei of different endopolyploidy levels in endosperm of *Sorghum bicolor* and ratios of nuclear genome size to cell size parameters. A - cell volume, B - nuclei volume, C - ratio of nuclear genome size per cell volume, D - genome size per nuclei volume, E - genome size per cell surface area, F - genome size per nuclei surface area. The range of observed nuclear genome sizes was 3 C to 96 C. Grey line represents a linear fit applied to logarithmically transformed data, R^2 value represents the percentage of variation in the examined parameter explained by the variation in nuclear genome size.

Slika 2: Prostornina celic in jeder različnih stopenj endopoliploidnosti v endospermu sirka in razmerja med velikostjo genoma in parametri velikosti celic. A - prostornina celic, B - prostornina jeder, C - velikosti genoma na prostornino celic, D - velikost genoma na prostornino jeder, E - velikost genoma na površino celic, F - velikost genoma na površino jeder. Razpon velikosti jedrnega genoma je 3 C do 96 C. Sive črte predstavljajo linearni model na logaritmiranih podatkih, R^2 vrednost je delež variance v preučevanem parametru, pojasnjen z endopoliploidijo.

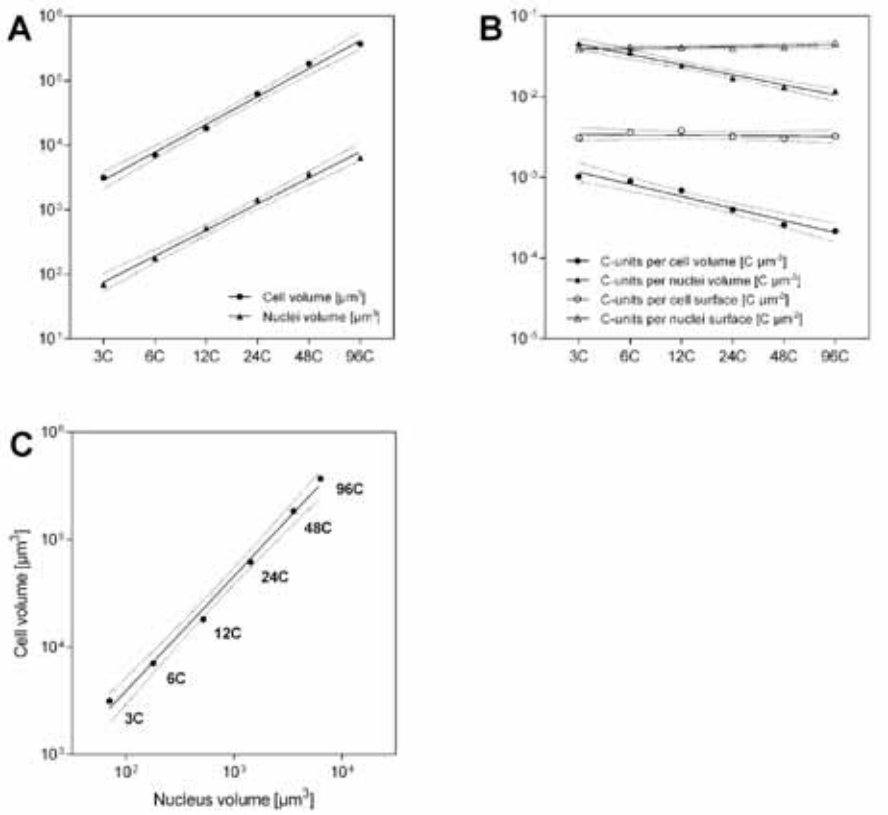

Figure 3: Relationship between endopolyploidy level and size of cells and nuclei. A - Volume of cells and nuclei of different endopolyploidy levels (3 C to 96 C). B - Ratios of nuclear genome size with cell size parameters (volume of cells and nuclei, surface of cells and nuclei). C - Relationship between volume of nuclei and volume of cells of different endoploidy levels. Data presented are median values calculated for individual endopolyploidy classes. Solid lines represent linear regressions with 95 % confidence intervals (dashed lines).

Slika 3: Razmerje med stopnjo endopoliploidnosti in velikostjo celic in jeder. A - Prostornina celic in jeder različnih stopenj endopoliploidnosti (3 C do 96 C). B - Razmerja med velikostjo jedrnega genoma in parametrov velikosti celic (prostornina celic in jeder, površina celic in jeder). C - Razmerje med prostornino jeder in prostornino celic različnih stopenj endopoliploidnosti. Predstavljeni podatki so mediane vrednosti izračunane za posamezne razrede endopoliploidnosti. Polne črte predstavljajo linearno regresijo s 95 % intervalom zaupanja (črtkane črte).

Table 1: Relationship between endopolyploidy and cell size parameters. Slope of log-log relationship calculated from median values for individual endopolyploidy classes (Fig. 3A, B) or log-log relationship between cell volume and nucleus volume (Fig. 3C). Slope = 1 indicates isometry, slope > 1 indicates positive allometry (for volumes), slope = 0 indicates no relationship. Slope error based on 95% confidence intervals, * - deviation from zero is significant ($p < 0.001$), ns0, ns1 - deviation from zero or 1 not significant ($p > 0.05$); R^2 value is a portion of variance in the examined parameter explained by endopolyploidy (Fig. 2) or nucleus volume ($p < 0.001$).

Tabela 1: Razmerje med endopoliploidijo in parametri velikosti celic. Naklon log-log razmerja, izračunanega iz medianih vrednosti za posamezne razrede endopoliploidnih celic (Fig. 3A, B) ali log-log razmerja med prostornino celic in prostornino jeder (Fig. 3C). Naklon = 1 pomeni izometrijo, naklon > 1 pomeni pozitivno alometrijo (za prostornine), naklon = 0 pomeni, da ni odvisnosti. Napaka naklona izvira iz 95 % intervala zaupanja, ns0, ns1 - odklon od 0 oz. 1 ni statistično značilen ($p > 0.05$); R^2 vrednost je delež variance v preučevanem parametru, pojasnjen z endopoliploidijo (Fig. 2) ali prostornino jedra ($p < 0.001$).

	Slope	R^2 (linear model)
Cell volume [μm^3]	$1.44 \pm 0.05^{*}$	0.65
Nuclei volume [μm^3]	$1.34 \pm 0.05^{*}$	0.95
C-units per cell volume [C μm^{-3}]	$-0.50 \pm 0.05^{*}$	0.13
C-units per nuclei volume [C μm^{-3}]	$-0.42 \pm 0.03^{*}$	0.64
C-units per cell surface [C μm^{-2}]	-0.02 ± 0.03^{ns0}	0.01
C-units per nuclei surface [C μm^{-2}]	0.03 ± 0.01^{ns0}	0.02
Cell volume by nuclei volume	1.07 ± 0.04^{ns1}	0.68

Discussion

The early observations of a constant relationship between volume of nuclei and volume of cells led to the "karyoplasmic ratio" hypothesis (Wilson 1925). The exponential nature of this relationship was reported already by Sinnot and Trombetta (1936). Numerous studies reported on a tight relationship between volume of cells and nuclei and genome size (Cavalier-Smith 2005, Barow 2006). A clear relationship between nuclear genome size (endopolyploidy level) and volume of nuclei and cells was shown in *Sorghum bicolor* endosperm. The large endoreplicated endosperm cells are associated with deposition of starch (Kladnik et al. 2006). In the present study, the relationship was further characterized. The volume of cells is increasing by a higher rate than would be proportional with doubling of the ploidy level, since the slope of their log-log relationship is higher than 1, indicative of positive allometry (Barow 2006). The volume of cells more than doubles with each endocycle, and this in turn influences the ratio of genome size per cell volume that shows a negative slope in relation to ploidy level, although with a low R^2 value. The motivation for calculating a ratio of genome size with cell volume is to express the amount of genome that is in charge of a unit of tissue volume, or simply "genome concentration". Surprisingly, the volume of nuclei also shows a similar positive allometric relationship with nuclear genome size and a negative slope for the genome size to nuclei volume ratio. This indicates that the volume of nuclei increases with a higher rate as can be expected from the duplication of DNA alone. A similar positive allometry for nuclei has been observed in mesocarp cells of *Cucumis melo* (Kladnik, unpublished observation).

Of great interest is an observation that genome size to surface ratio is constant both for cells and nuclei. The possible explanation for both is however different. Nucleus volume increases with increasing endopolyploidy level at a rate higher than 1, but the genome to surface ratio remains constant throughout the endopolyploidy range. This indicates that the surface area of the nucleus (nuclear envelope) is crucial for the function of nucleus. Indeed, increased nucleus surface

in endopolyploid cells was observed in tomato pericarp, where large endopolyploidy nuclei show deep invaginations (Bourdon et al. 2012). The authors hypothesize that nuclear exchange ability is maintained by keeping the ratio between nuclear envelope area with nuclear volume constant. Another possibility for maintenance of nuclear exchange ability is peripheral distribution of DNA in nuclei of higher endopolyploidy levels, observed in mesocarp of *Cucumis melo* (Kladnik, unpublished observation). In the present analysis, a higher rate of nuclear volume increase in endopolyploid nuclei of sorghum endosperm as can be expected from duplication of DNA, compared to a constant ratio of genome size to nuclear surface, indicates that the nuclear exchange ability is most likely the important factor that needs to be maintained with increase of nuclear genome size in endoreplication. However, the explanation for a higher rate of cell volume growth is not so straightforward. The positive allometric relationship between cell volume and nuclear genome size is seemingly not in accordance with a hypothesis of a constant karyoplasmic ratio. As noted by Edgar et al. (2014), expansion of plant cells is often driven by increasing the size of the fluid-filled vacuole and it is not necessarily accompanied by increased mass of proteinaceous cytoplasm. Here, the endopolyploid endosperm cells are filled with starch grains and we could speculate that the volume of cytoplasm is related more to the surface area of the cell than with the volume of the whole cell. On the other hand, we have shown an ideal karyoplasmic ratio if we compare cell volume to the volume of the nucleus, not to the size of the genome it contains. Therefore, we can conclude that in this case, the main factor influencing endosperm cell size is not its DNA content, but rather the nuclear exchange ability that is limiting gene expression and cell metabolism.

Summary

The data on sorghum endosperm cell volume, nuclei volume and nuclear genome size (Kladnik et al. 2006) was re-evaluated to examine the relationship between cell parameters. Endosperm cells contain endoreplicated nuclei with DNA content from 3 C to 96 C and the volume of cells and nuclei is positively correlated with nuclear genome size. The larger cells with higher DNA content are located in the central part of endosperm and accumulate starch. The relationship of cell and nuclei volume with genome size is positively allometric, ie. the volume increases by a higher rate than can be expected from DNA duplication alone. Instead, a constant ratio was observed between genome size and surface of cells and nuclei. Finally, an isometric relationship was shown between the volume of nuclei and volume of cells, indicating that a constant karyoplasmic ratio is related to the volume of nucleus, not to its DNA content.

Povzetek

Podatke o prostornini celic in jeder, ter velikosti jedrnega genoma v endospermu sirka (Kladnik et al. 2006) smo dodatno analizirali, da bi ovrednotili razmerja med celičnimi parametri. Celice endosperma vsebujejo endoreplicirana jedra, ki vsebujejo 3 C do 96 C DNA. Prostornina celic in jeder je v pozitivni korelaciji z velikostjo jedrnega genoma. Večje celice z višjo vsebnostjo DNA so v osrednjem delu endosperma in kopičijo založni škrob. Razmerje prostornine celic in jeder z velikostjo genoma je pozitivno alometrično, kar pomeni, da se prostornina povečuje hitreje, kot bi pričakovali samo zaradi samega podvajanja DNA. Po drugi strani smo opazili konstantno razmerje med velikostjo genoma in površino celic in jeder. Pokazali smo tudi izometrično razmerje med prostornino jeder in prostornino celic, kar kaže na to, da je konstantno karioplazemsko razmerje povezano s prostornino jedra, ne pa s količino DNA, ki jo le-ta vsebuje.

Acknowledgement

This work was supported by the Slovenian Research Agency (ARRS) through research programme Plant Biology P1-0212.

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ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 13–21

Sivi dren *(Cornus sericea* L.*)* - nova invazivna vrsta v flori Slovenije

Red osier dogwood (*Cornus sericea* L.*)* - a new invasive species in Slovenian flora

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Izvleček: Sivi dren (*Cornus sericea* L.) je priljubljen severnoameriški okrasni grm, ki ga pogosto sadijo tudi v Sloveniji. Subspontano pojavljanje vrste v naših krajih je znano že dve desetletji, a šele v zadnjih letih opažamo, da je vrsta invazivna. Sivi dren uspeva na številnih nahajališčih v Ljubljanski kotlini, znano pa je tudi uspevanje na dveh lokalitetah na Gorenjskem. Pojavlja se v mokriščnih habitatih, posebej v bližini naselij, kjer ga gojijo kot okrasni grm. Da bi omejili njegovo širjenje, predlagamo pravočasne ukrepe. Objavljamo tudi posodobljeni ključ za določanje drenov v Sloveniji.

Ključne besede: *Cornus sericea*, sivi dren, invazivne tujerodne vrste, mokrišča, Slovenija

Abstract: The Red osier dogwood (*Cornus sericea* L.) is a popular North American ornamental shrub, often planted also in Slovenia. Subspontaneous occurrence of the species in Slovenia is known for two decades, but only in recent years, we can recognize it as an invasive species. The Red osier dogwood is currently known from numerous localities in the wider city area of Ljubljana and also from two sites in the Gorenjska region. It occurs in wetland habitats, particularly in the vicinity of settlements, where the shrub is planted for ornamental purposes. To limit the invasion of the Red osier dogwood, timely measures should be taken. We also publish the updated determination key for the genus *Cornus* in Slovenia.

Key words: *Cornus sericea*, The Red osier dogwood, invasive alien species, wetlands, Slovenia

Uvod

Namen raziskave

Sivi dren (*Cornus sericea* L.) je priljubljen okrasni grm, ki ga v naših krajih pogosto sadijo v parkih in vrtovih. Posebej je grm dekorativen pozimi, ko pridejo do izraza koralno rdeči ali živorumenozeleni odtenki lubja mladih poganjkov. Vrsta izhaja iz vzhodnega dela Severne Amerike (Fischer et al. 2008). Zaenkrat jo slovenska zbirna floristična dela (Martinčič et al. 2007, Jogan et al. 2001) ne navajajo, naša najdba je bila prvič omenjena v prilogi poročila projekta Neobiota z oceno starosti prvega pojavljanja »2000« in oceno invazivnosti »naturaliziran« (Jogan et al.

2012). Avtorji opažamo subspontano pojavljanje drena v Sloveniji že dve desetletji, vendar šele v zadnjih letih brez dvoma lahko trdimo, da se vrsta pri nas pojavlja ne le prehodno podivjano, pač pa kot invazivna vrsta, ki lokalno s popolno prevlado v grmovni plasti vegetacije predstavlja grožnjo domačim vrstam. Posebej problematično je, da se vrsta ne pojavlja le na ruderalnih rastiščih, na katerih se pot naturalizacije tujerodnih vrst pogosto začne, pač pa tudi v naravi, in sicer v mokriščnih habitatih gozdnih obronkov. Širjenje sivega drena smo v zadnjih letih opazovali v Ljubljani v jelševih grezih ob Večni poti ter ob Koseškem bajerju, pri sistematičnem popisovanju flore v Ljubljani pa smo zabeležili še več nahajališč. Namen članka je opozoriti botanično javnost na prisotnost sivega drena kot invazivne tujerodne vrste, predstaviti podatke o njeni razširjenosti v Sloveniji ter podati oceno invazivnosti vrste in perspektive.

Predlagano slovensko ime »sivi« dren, ki je že uporabljeno med drugim v omenjenem poročilu (ibid.), je zaradi razločno sivkaste spodnje strani listnih ploskev ustreznejše od drugega, ki ga prav tako zasledimo ponekod v vrtnarski literaturi: svilnati dren. Pri slednjem gre namreč za neroden prevod latinskega imena »*sericeus*«, ki pomeni svilnat, a je rabljeno lahko v smislu svilnate dlakavosti (gosta poraslost s prileglimi, vzporedno ležečimi dlakami) ali svetlo sive barve naravne svile. Ker noben del rastline sivega drena nima značilne svilnate dlakavosti, je torej smiseln prevod v slovenščino »sivi« in ne »svilnati«.

Stanje invazivnosti v sosednjih deželah in v svetu

Sivi dren je bil kot ena od izbranih vrst za presojo potencialne invazivnosti za Srednjo Evropo ocenjena kot zelo invazivna (Weber et Gut 2004). Dva mednarodna spletna portala o tujerodnih vrstah prikazujeta pojavljanje sivega drena po skoraj vsej zahodni (DAISIE: http://www.europe-aliens.org) in srednji ter severni Evropi (NOBANIS: www.nobanis.org) z izjemo južnejših predelov, vendar pa do izrecnih podatkov o pojavljanju v posamezni državi ni vedno lahko priti. Po omenjenih dveh zbirnih bazah naj bi bil sivi dren invaziven v Belgiji, Latviji, na Norveškem in Poljskem, potencialno invaziven na Nizozemskem in Irskem, naturaliziran pa še na Češkem, v Nemčiji, v evropskem delu Rusije, v Veliki Britaniji in Švici, medtem ko za Francijo, Nemčijo, Avstrijo, Madžarsko in Češko tu ni natančnejših podatkov o statusu tujerodne vrste.

Prve navedbe o pojavljanju te vrste v posameznih evropskih državah so s konca 19. stoletja (Avstrija, Češka, Belgija, Norveška, po www.nobanis.org).

Na Irskem so sivi dren sadili v vlažne habitate kot okrasni grm, vendar se je dren začel na mnogih mestih širiti in predstavlja grožnjo mokriščnim gozdovom (Kelly 1990). V Veliki Britaniji je pogosto naturaliziran po nižinah, širil naj bi se predvsem vegetativno (Stace 1991). V Franciji se navaja za nekaj območij na severu kot naturalizirana vrsta v širjenju (Tison et al. 2014), v Švici pa kot naturalizirana (Wittenberg et al. 2006). Na Češkem ga obravnavajo kot mestoma podivjano vrsto vlažnih gozdov v nižini, kjer se pojavlja v vegetaciji zveze *Alno-Ulmion* in reda *Salicetea purpureae* (Holub 1997). Po drugi svetovni vojni je več podatkov o pojavljanju v Avstriji, danes velja tam za naturalizirano vrsto na območjih Dunaja, Spodnje Avstrije in okoli Gradca, vendar z izraženo domnevo o pojavljanju tudi drugod (Walter et al. 2002) oziroma se omenja kot lokalno naturaliziran na območjih Zgornje Avstrije, Koroške, Solnograške in Severne Tirolske (Fischer et al. 2008). Pri tem je zanimiva neusklajenost navedb v dveh pomembnih monografskih obdelavah. Podobna neusklajenost se v zvezi s pojavljanjem te vrste kaže večkrat, kar po eni strani kaže na hitro zastarevanje podatkov o tujerodnih invazivnih vrstah, po drugi strani na prezrtost vrste, ki je zelo podobna nekaterim avtohtonim. Zelo verjetno se zdi, da je bila naturalizacija vrste marsikje po Evropi prezrta zaradi navidezne podobnosti z rdečim drenom, ki je izredno variabilna avtohtona vrsta.

Ekologija in razširjenost vrste v njeni domovini

Naravno območje razširjenosti vrste *C. sericea* je Severna Amerika: od Mehike na jugu do Kanade in Aljaske na severu (USDA, NRCS 2015). Uspeva na nadmorskih višinah do 2500 m n. m., na z dušikom bogatih tleh, ki so vsaj del leta namočena, kot na primer bregovi jezer in tekočih voda, ter na zamočvirjenih območjih. Zelo dobro prenaša mraz. Razmnožuje se vegetativno z ukoreninjanjem odlomljenih poganjkov in spontanim grebeničenjem ter spolno s semeni.

V Severni Ameriki se s plodovi hranijo mnoge vrste ptic in sesalcev, ki tako razširjajo semena (USDA, NRCS 2006).

Tudi v neposredni soseščini primarnega areala, npr. v južnem Quebecu, velja za invazivno vrsto, ki ima dve strategiji invazivnosti: v senčnih razmerah se razrašča bolj horizontalno in se poganjki zakoreninjajo ter ne cvetijo, v bolj presvetljenih razmerah pa bujno požene pokončne cvetoče poganjke, ki z zasenčenjem postopno izpodrinejo konkurenčne vrste, poleg tega pa razvijajo še plodove, ki jih ptice širijo dalje (Charles-Dominique et al. 2010).

V ZDA in Kanadi *C. sericea* uporabljajo za zaščito rečnih bregov pred erozijo, saj koreninski sistem dobro zadržuje prst (https://en.wikipedia.org/wiki/Cornus_sericea, Walsh 2012). Sadijo ga tudi na območja, ki jih je prizadel vetrolom (USDA, NRCS 2006).

Materiali in metode

Podatke smo zbrali iz štirih virov: pregled herbarija LJU na Oddelku za biologijo Biotehniške fakultete, sistematično kartiranje flore Ljubljane v okviru projekta Mestne občine Ljubljana »Popis flore znotraj obvoznice mesta Ljubljana s poudarkom na tujerodnih invazivnih rastlinskih vrstah« v letu 2015, lastna terenska opažanja avtorjev in podatkovna zbirka Centra za kartografijo favne in flore (CKFF).

Slika 1: List sivega drena (*Cornus sericea*). Foto: N. Jogan
Figure 1: Leaf of the red osier dogwood (*Cornus sericea*). Photo: N. Jogan

Sistematično kartiranje flore Ljubljane je potekalo v vegetacijski sezoni 2015 tako, da je bilo območje razdeljeno na kvadrate velikosti 1 km^2, s tem je bilo 70 km^2 mestne občine solidno skartirano z vsaj dvema terenskima dnevoma na kvadrat, kar za vrste, ki so vse leto prepoznavne, da dobre rezultate.

Vrsto smo določili s pomočjo določevalnih ključev Fitschen (2002), Fischer et al. (2008) in Lauber et Wagner (2007). Razlikovalne znake smo preverjali na svežem in suhem materialu.

Rezultati z diskusijo

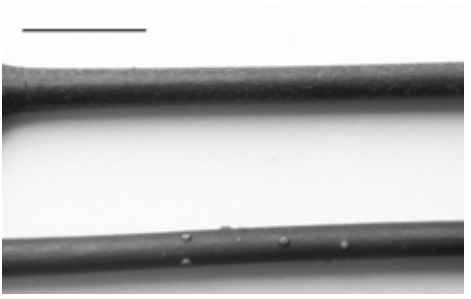
Prepoznavanje in določanje vrste

Vrsta je lahko prepoznavna, tako med rastno sezono kot tudi pozimi, ko listi odpadejo. Od domorodnih vrst ji je po videzu vej, socvetja in listov ter po ekologiji še najbolj podoben rdeči dren (*C. sanguinea*).

V rastni sezoni je sivi dren že od daleč opazen in prepoznaven po velikih listih (8 – 12 cm), posebej na enoletnih vejah (tudi čez 10 cm), njihovi jajčastosuličasti obliki (Sl. 1) in sivozeleni spodnji strani. Posebej naj poudarimo, da k sivozeleni obarvanosti spodnje površine listov ne prispeva svilnata dlakavost, kot bi pričakovali glede na ime »svilnati« oz. »*sericea*« in kot jo srečamo na primer pri beli vrbi. Laski so sicer prisotni, a so redki in po obliki izključno kompasni, podobno kot pri *C. sanguinea* ssp. *australis*. Plodovi so beli do svetlosivomodrikasti in torej zelo drugačni od črnovijoličnih plodov rdečega drena (Sl. 2).

Pozno jeseni in pozimi, ko listi odpadejo, sivi dren najlažje ločimo od rdečega po prisotnosti lenticel na lubju mladih poganjkov (Sl. 3) in po razrasti.

Slika 2: Plodovi sivega drena (*Cornus sericea*). Foto: S. Strgulc Krajšek
Figure 2: Fruits of the red osier dogwood (*Cornus sericea*). Photo: S. Strgulc Krajšek



Slika 3: Primerjava vejic rdečega (*C. sanguinea*, zgoraj) in sivega drena (*C. sericea*, spodaj) (črtica predstavlja 1 cm).
Figure 3: The comparison of twigs in *C. sanguinea* (up) and *C. sericea* (down) (the line represents 1 cm).

Na tem mestu podajamo **ključ za razlikovanje vrst znotraj rodu *Cornus***, prirejen po Martinčič (2007) in Fischer et. al. (2008):

1 Venec rumen, cvetovi v kobulih, razvijejo se pred cvetenjem, listi zgoraj bolj ali manj bleščeči, spodaj v kotičkih žil s šopi dlačic; luskolista pri dnu enoletnega poganjka zelo obstojna, srpasto ukrivljena, poganjki vsaj pri vrhu razločno četverorobi. Plodovi viseči, podolgastojajčasti (približno 2x tako dolgi kot široki), viseči (»drnulje«), zreli temnordeči do skoraj črni. *Cornus mas* L.

1* Venec bel, cvetovi v češuljah, razvijejo se po olistanju, listi zgoraj niso bleščeči, spodaj v žilnih kotih niso dlakavi; luskolisti zgodaj odpadejo, poganjki v prerezu okrogli. Plodovi pokončni, kroglasti, zreli črnovijolični, beli ali svetlosivomodrikasti. 2

2 Listi po obeh straneh zeleni, s 3 – 4 pari stranskih žil, pri vrhu naglo zoženi v topo konico, po spodnji strani z dvokrakimi laski, ki imajo navadno vsaj en krak štrleč in ukrivljen; lubje mlajših vej brez lenticel; zrel plod črnovijoličen. Grebeničenja ni. *C. sanguinea* L.

2* Listi zgoraj zeleni, spodaj sivi do sivozeleni, torej zgoraj in spodaj različne barve, s 5 – 7 pari žil, pri vrhu postopno zoženi v konico, po spodnji strani s prileglimi dvokrakimi laski (»kompasni« laski), veje z lenticelami, zrel plod bel do svetlosivomodrikast. Rastlina s številnimi olesenelimi pritlikami, spontano grebeničenje predvsem v senčnih razmerah. *C. sericea* L.

Sivemu drenu je podoben beli ali tatarski dren (*Cornus alba* L.), ki ga prav tako gojijo kot okrasni grm, izhaja pa iz severne Azije. Beli dren ima manjše, kratko koničaste liste (listi 4 – 8 cm dolgi), mladi poganjki pa imajo modrikast poprh (Fischer et al. 2008). V preteklosti razlikovanje med vrstama ni bilo vedno jasno, tako je eden od sinonimov za sivi dren tudi *C. alba* auct.

Razširjenost sivega drena v Sloveniji

Herbarijski material iz LJU:

9852/4 Slovenija: Ljubljana: Brod, ob mostu gorenjske avtoceste čez reko Savo, desni breg Save, mejica. Leg. & det. S. Strgulc Krajšek, 28. 9. 2011 (LJU1014162)

9953/2 Slovenija: Ljubljana: Zalog, vznožje Debnega vrha, pri prvem podhodu pod železnico, od table konec Ljubljane, v smeri iz Ljubljane. Leg. B. Podvršič, 1. 6. 2000, det. N. Jogan, l. 2000 (LJU10015631)

9651/4 Slovenija: Gorenjska: okolica Tržiča, na desni strani ceste, za vasjo Golnik, ob cesti, rob gozda. 520 m. n. m., leg. & det. U. Bidovec, 20. 6. 1995 (LJU10015630)

9749/1 Slovenija: Gorenjska, Bohinjsko jezero, ob vzhodnem bregu, podivjano. Leg. B. Pipan, det. N. Jogan, 1995 (LJU10015628)

V podatkovni zbirki CKFF (dostop: oktober 2015) je en sam podatek o domnevno spontanem pojavljanju te vrste, in sicer podatek Špele Štrekelj iz leta 1998 za Ljubljano (Šiška, kvadrant 9952/2; zbirka: Študentski herbariji). Ta podatek je dokumentiran s primerkom v herbariju LJU (LJU10015629), vendar je iz etikete razvidno, da gre za kultiviran grm (»Ljubljana - Šiška: 1,5 m visok grm, ki tvori živo mejo pri hiši ob Celovški cesti, 300 m. n. m., leg. Š. Štrekelj, 24. 5. 1998, det. N. Jogan«). Nekaj nadaljnjih podatkov v omenjeni bazi se nanaša na sivi dren kot gostiteljsko rastlino nekaterih nevretenčarjev, v teh primerih pa gre pogosto ali izključno tudi za gojene rastline, tako da jih v zvezi s subspontanim širjenjem sivega drena ne moremo upoštevati.

Podatki iz sistematičnega kartiranja flore Ljubljane v okviru projekta Mestne občine Ljubljana v letu 2015 in lastna terenska opažanja avtorjev:

9952/2 Slovenija: Ljubljana, Koseze, Koseški bajer z vlažnim gozdom proti Mostecu. Območje MOL: 96. Leg. Katarina Šoln, 18. junij 2015

9952/2 Slovenija: Ljubljana, med Dravljami in Podutikom, urbano in gozdni rob. Območje MOL: 77. Leg. Barbara Nemec, 6. maj 2015

9952/2 Slovenija: Ljubljana, Podutik, močvirno območje med cerkvijo in Krivcem. Območje MOL: 77. Leg. Nejc Jogan, 28. september 2015

9952/2 Slovenija: Ljubljana, pri Podutiku med Pržancem in Glinščico, rob travnika. Območje MOL: 95. Leg. Simona Strgulc Krajšek, 25. maj 2015

9952/2 Slovenija: Ljubljana, Rožna dolina, Večna pot, okolica BF, rob vlažnega gozda. Območje MOL: 129. Leg. Nejc Jogan, 15. junij 2015

9952/2 Slovenija: Ljubljana, Šiška, Dravlje, grmovje ob cesti pri nadvozu nad obvoznico. Območje MOL: 78. Leg. Nejc Jogan, 29. september 2015

9952/2 Slovenija: Ljubljana, Šiška, ob cesti Pod hribom, obronki Šišenskega hriba, rob gozda. Območje MOL: 97. Leg. Nejc Jogan, 11. september 2015

9952/2 Slovenija: Ljubljana, Zgornja Šiška, zapuščeno gradbišče blizu obvoznice. Območje MOL: 79. Leg. Nejc Jogan, 21. september 2015

9952/4 Slovenija: Ljubljana, Brdo-Bokalce. Območje MOL: 127. Leg.: Tinka Bačič & Simona Strgulc Krajšek, 29. september 2015

9952/4 Slovenija: Ljubljana, Brdo. Območje MOL: 128. Leg.: Nejc Jogan, 20. september 2015

9952/4 Slovenija: Ljubljana, Kolezija, urbani mozaik. Območje MOL: 148. Leg. Filip Küzmič, 14. julij 2015

9952/4 Slovenija: Ljubljana, Vič-Murgle, urbano okolje. Območje MOL: 147. Leg. Teja Bizjak, 15. junij 2015

9953/1 Slovenija: Ljubljana, Bežigrad, Savlje, okolica Mercator EMBA. Območje MOL: 65. Leg. Nejc Jogan, 5. oktober 2015.

9953/1 Slovenija: Ljubljana, Savsko naselje, urbano okolje ob glavnih cestah in urbani gozdiček. Območje MOL: 100. Leg. Teja Bizjak, 5. september 2015

9953/1 Slovenija: Ljubljana, Štepanjsko naselje-Nove Fužine. Območje MOL: 118. Leg.: Aljaž Jakob, 9. september 2015

9953/1 Slovenija: Ljubljana, Stožice, BS3, mejice med travniki. Območje MOL: 82. Leg. Nejc Jogan, 3. junij 2015

9953/3 Slovenija: Ljubljana, južno od TC Rudnik. Območje MOL: 195. Leg.: Tinka Bačič & Simona Strgulc Krajšek, 1. september 2015

9953/3 Slovenija: Ljubljana, opuščena nasipališča TC Rudnik. Območje MOL: 183. Leg.: Nejc Jogan, 12. oktober 2015

9953/3 Slovenija: Ljubljana, Rakova Jelša-Ilovica. Območje MOL: 166. Leg.: Nejc Jogan, 1. oktober 2015

9953/3 Slovenija: Ljubljana, Sibirija-Rakova Jelša. Območje MOL: 165. Leg.: Nejc Jogan, 26. julij 2015

9953/3 Slovenija: Ljubljana, Sp. Hrušica, vznožje Golovca. Območje MOL: 152. Leg.: Nejc Jogan, 11. oktober 2015

9953/3 Slovenija: Ljubljana, Trnovo-Galjevica, ob Hladnikovi. Območje MOL: 149. Leg. Filip Küzmič, 11. junij 2015

9953/3 Slovenija: Ljubljana, Zgornja Hrušica, opuščena drevesnica podjetja Rast. Območje MOL: 135. Leg. Aljaž Jakob, 18. junij 2015

Vrsta je zaenkrat znana iz Ljubljanske kotline in Gorenjske (Sl. 4), iz 8 MTB kvadrantov. Slika 5 prikazuje natančnejšo razširjenost vrste v Ljubljani (območje MOL znotraj obvoznice). Prepričani smo, da je nahajališč še mnogo več, a je pojavljanje prezrto. Vrsto pričakujemo vzdolž rek, npr. Save, Ljubljanice, in različnih mokriščnih habitatov v bližini naselij, kjer vrsto sadijo v okrasne namene. Na vrsto je treba biti še posebej pozoren na zavarovanih območjih, kjer jo je v začetni fazi invazije mogoče brez velikih stroškov uspešno odstraniti in s tem preprečiti ogrožanje avtohtonih mokriščnih vrst.

Načini širjenja vrste, njena invazivnost, načini odstranjevanja

Vrsta se širi vegetativno s pritlikami (kar nam pove tudi njegov sinonim *C. stolonifera* Michx.), s semeni pa se razširja s pomočjo ptičev. Na terenu smo opažali, da divjerastoči grmi uspevajo precej razmaknjeno eden od drugega, tako da ne gre le za poleganje in zakoreninjanje poganjkov. Kelly (1990) piše, naj bi se grm na Irskem razširjal le vegetativno, a ne le s poleganjem poganjkov in njihovim vkoreninjanjem (spontanim grebeničenjem), pač pa tudi z raznašanjem odlomljenih vej, ki se lahko vkoreninijo na oddaljenem mestu. Menimo, da se pri nas grm razširja prav na slednji način. Sajene grme redno obrezujejo, odrezane kose vej pa lahko zanese tudi v bližnje naravne habitate, kjer se nekateri uspejo vkoreniniti. Grme sivega drena najdemo pri nas tudi v podrasti živih mej, kar pa bi morda lahko nakazovalo, da se grm vendarle širi tudi s semeni, s pomočjo ptičev, ki se radi zadržujejo v gostem grmovju. Zanesljive potrditve o razširjanju s semeni v Sloveniji zaenkrat nimamo.

Tako v njeni domovini kot tudi na tujem vrsti ustrezajo težka, vlažna, namočena oz. zamočvirjena tla, vrsta se zato pojavlja podivjano v različnih tipih obrežne vegetacije, v močvirjih, jelševih grezih in v podobnih vlagoljubnih združbah. Na takšnih rastiščih sivi dren s svojim agresivnim vegetativnim razraščanjem in senčenjem škodi domorodnim vrstam in s tem predstavlja grožnjo biodiverziteti teh habitatnih tipov, ki so naravovarstveno pomembni, saj v njih najdemo mnoge ogrožene in ranljive vlagoljubne vrste. Iz tega razloga moramo sivi dren pri nas obravnavati kot invazivno vrsto na začetku širjenja in načrtovati ukrepe za odstranjevanje. Zavedamo se, da bo težko o tem prepričati javnost, saj vrsta človeku ni neposredno škodljiva, tako kot sta na primer pelinolistna žvrklja ali orjaški dežen.

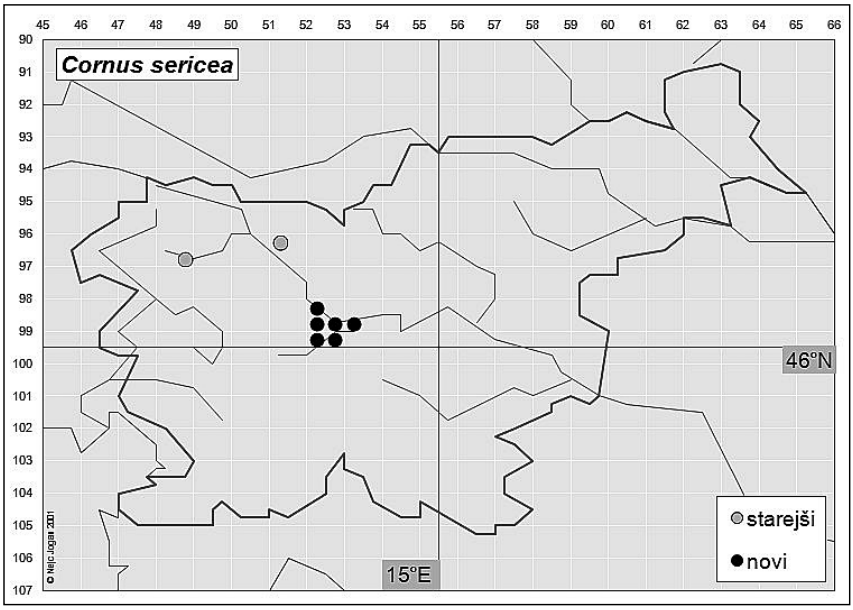
Grme sivega drena bi bilo treba mehansko odstranjevati iz naravnih združb, saj uporaba herbicidov ob vodah ni dopustna. Pomemben ukrep proti širjenju pa je previdno ravnanje z odrezanimi vejami sivega drena. Odložene morajo biti na ustrezno mesto, po možnosti uničene in vsekakor ne smejo biti puščene vnemar.

Summary

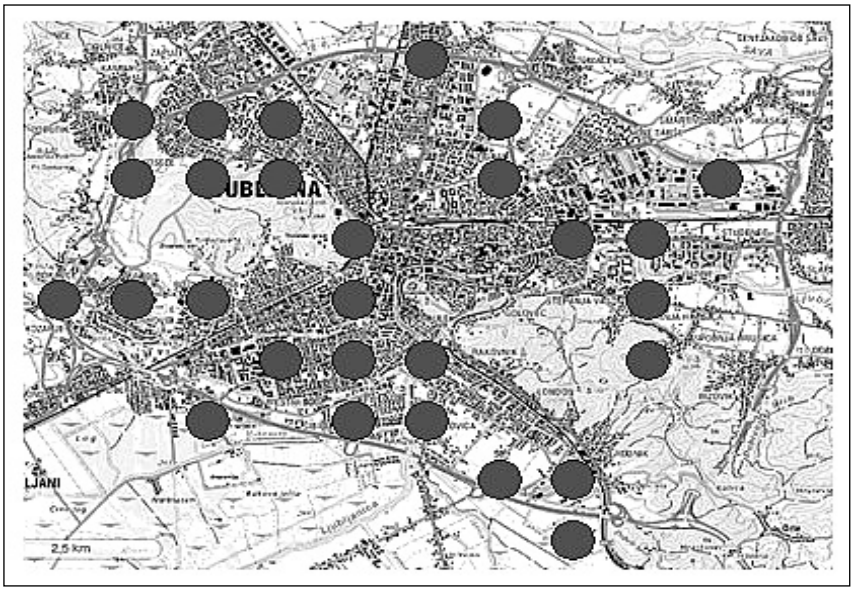
The Red osier dogwood (*Cornus sericea* L.) is a popular North American ornamental shrub, which in Slovenia is often planted in parks and gardens. Subspontaneous occurrence of the species in Slovenia is known for two decades, but only in recent years, we can reliable characterize it as an invasive species. The purpose of this article is to point out *Cornus sericea* as a new invasive species in Slovenian flora, to present its distributional data and discuss its invasiveness.

According to two major international web portals on alien species (DAISIE: http://www.europe-aliens.org, NOBANIS: www.nobanis.org), the Red osier dogwood occurs practically throughout Western, Central and Northern Europe, with the exception of southern areas. It is considered invasive in Belgium, Latvia, Norway, Poland, potentially invasive in the Netherlands and Ireland, naturalized in the Czech Republic, Germany, in the European part of Russia, the UK and Switzerland, while in France, Germany, Austria, Hungary and the Czech Republic, there is no accurate data on the status of this alien species.

To collect distributional data for Slovenia, we used four sources: Herbarium LJU in the Department of Biology at the Biotechnical Faculty (University of Ljubljana), data, collected during the systematical mapping the flora of Ljubljana in 2015, our own field observations, and the data from the biodiversity database of the Centre for Cartography of Fauna and Flora (CKFF). The distribution map was prepared and an updated determination key for *Cornus* in Slovenia was compiled.



Slika 4: Znana razširjenost sivega drena (*Cornus sericea*) v Sloveniji.
Figure 4: Known distribution of Red osier dogwood (*Cornus sericea*) in Slovenia.



Slika 5: Znana razširjenost vrste v Ljubljani.
Figure 5: Known distribution of Red osier dogwood (*Cornus sericea*) in Ljubljana.

Our results show, that the species is widely distributed in the Ljubljana basin and is also known from two localities in Gorenjska region. The species prefers heavy, wet, marshy ground, so it can be found in various types of riparian vegetation, marshes and alder stands. The species is expected along the rivers, for example Sava, Ljubljanica and in various wetland habitats in the vicinity of settlements, where the shrub is planted for ornamental purposes. In our opinion, the Red osier dogwood is much more widespread than shown by the map, but its occurrence has probably been overlooked.

The species spreads vegetatively by rooting of horizontal branches and its seeds are disseminated through birds. Humans also accidentally take part in the spread of the species: when ornamental shrubs are pruned, the cut branches are dispersed in the nearby natural habitats, where some of the branches manage to grow roots. The dogwood's aggressive vegetative tillering and shading other plants can have damaging effect on the native vegetation.

To limit the invasion of the Red osier dogwood, timely measures should be taken. Shrubs should be removed from natural communities mechanically, since the use of herbicides in wet habitats is unacceptable. As an important measure against the dispersal of the plants, we suggest carefull handling with cut branches of the ornamental shrubs. They should be destroyed or deposited adequately. Special attention should be paid towards presence of the Red osier dogwood in protected areas, from where in the early stages of invasion can be successfully removed without large costs.

Zahvala

Avtorji se zahvaljujemo vsem študentom, ki so pomagali kartirati floro Mestne občine Ljubljana v letu 2015, MOL, ki se je odločila podpreti projekt »Popis flore znotraj obvoznice mesta Ljubljana s poudarkom na tujerodnih invazivnih rastlinskih vrstah«, kot tudi botanikom, ki so prispevali svoje herbarijske pole v herbarij LJU. Zahvala velja tudi dr. Igorju Dakskoblerju za koristne informacije pri zbiranju podatkov pri pripravi tega članka.

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21Bačič al.: Sivi dren - nova invazivna vrsta v flori Slovenije

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ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 23–34

Presence and abundance of macrophytes in Lake Slivniško jezero

Prisotnost in pogostost makrofitov v Slivniškem jezeru

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Abstract: Macrophytes are an important part of the lake biota. They are also bioindicators of environmental conditions. The goal of the present research was to determine species richness and abundance as well as longitudinal and depth distribution of macrophytes in Lake Slivniško jezero. A survey of macrophytes in the whole lake littoral was made, the minimum and maximum depth of taxa were measured and their abundance was estimated as well. We also assessed selected environmental parameters of the littoral and catchment. 22 macrophyte taxa: 9 emergent, 9 submerged and 4 natant macrophytes were determined. The most frequent species were *Phragmites australis*, *Najas marina*, *Myriophyllum spicatum* and *Potamogeton nodosus*. The maximum depth of colonisation was achieved by *Nymphaea alba* (to 2.4 m), while *M. spicatum* and *N. marina* grown to the depth of 1.9 m. According to CCA the distribution of macrophytes was significantly influenced by exposition, bottom slope, sediment type, slope of riparian zone, macroalgae abundance, type of riparian vegetation, completeness of riparian zone, land-use beyond the riparian zone and water turbidity.

Key words: macrophytes, Lake Slivniško jezero, species composition, environmental assessment

Izvleček: Makrofiti igrajo pomembno vlogo pri kroženju snovi in pretoku energije v jezerskih ekosistemih. Že dolgo je znano, da so pokazatelj stanja okolja, kjer uspevajo. Cilj raziskave je bil ugotoviti vrstno zastopanost, razporeditev, pogostost in globino uspevanja makrofitov v litoralu Slivniškega jezera. Na posameznih odsekih smo ocenili določene značilnosti litorala in zaledja Slivniškega jezera. Popisali smo 22 taksonov: 9 emerznih, 9 submerznih in 4 natantne. Najpogosteje zastopane vrste so bile *Phragmites australis*, *Najas marina*, *Myriophyllum spicatum* in *Potamogeton nodosus*. Najglobje je uspevala vrsta *Nymphaea alba* (povprečno do 2,4 m), do globine 1,9 m sta rastli tudi vrsti *M. spicatum* in *N. marina*. CCA analiza je pokazala, da na razporeditev makrofitov statistično značilno vplivajo naslednji okoljski dejavniki: osončenost, naklon dna, tip sedimenta, naklon brega nad vodo, prisotnost makroalg, vegetacija obrežnega pasu, sklenjenost z lesnatimi ali močvirskimi rastlinami poraslega obrežnega pasu, izraba tal v zaledju in kalnost vode.

Ključne besede: makrofiti, Slivniško jezero, okoljska ocena, vrstna sestava

Introduction

Macrophytes present a base of aquatic food-chains and services in freshwater ecosystems (Scheffer and Jeppesen 2007, Smith 2011). Their basic structural and physiological characteristics are in accordance with the ecological conditions and resources of the aquatic environment (Vukov et al. 2012). Aquatic macrophytes also act as important bioindicators of environmental conditions and long-term ecological changes in water quality (Solimini et al. 2006, Pall and Moser 2009, Dar et al. 2014). It is known that macrophytes can be successfully used as indicators of changes in freshwaters at narrow and wider scales, as they integrate temporal, spatial, chemical, physical and biological qualities of the ecosystem (Balaži et al. 2014). Macrophyte communities in aquatic habitats are characterized by a high spatial and temporal variation of species composition, richness and environmental conditions (Hrivnak et al. 2012). Due to the lower self-purification ability the standing inland waters are more vulnerable to pollution as running waters. The state of each lake or reservoir depends on the hydrological and morphological characteristics, especially on the input of various substances (Remec Rekar 2003). Water storage and flood protection reservoirs have been built worldwide for at least 4000 years in countries without large water bodies (Krolova et al. 2013). Artificial water bodies are mostly built for water supply, to increase flood safety, for the hydro-power generation, and for recreation. Reservoirs differ from natural lakes however studies show that there are also similarities in the operation of the two types of water bodies (Wetzel 2001). Compared to natural lakes, reservoirs have a low residence time and regular water level fluctuations (Alaoui et al. 2013). Problems of reservoirs are communal and industrial waste water and the run-off of nutrients from agricultural areas. Discharge is also very important for the reservoir characteristics and depends on the management of the reservoir. Presence of aquatic macrophytes in a pond alters the physicochemical environment of water (Reddy 1982). On the other hand, water level fluctuations also strongly affect macrophyte growth through erosion and degradation of the substrate due to the washing out of fine particles and nutrient-rich substances (Furey et al. 2004). The aim of our study was (1) to examine the presence and abundance of different macrophyte taxa, as well as their longitudinal and depth distribution in Lake Slivniško jezero, and (2) to describe prevailing abiotic habitat characteristics of macrophytes.

Materials and methods

Study area

Lake Slivniško jezero was created by damming the Ločnica watercourse by Tratna in 1976, mainly due to protection against flooding of the town Celje and the need for technological water for Ironworks Štore (Štraus 2006). It is eutrophic reservoir and its great advantage over other reservoirs in central and eastern Slovenia is that it is overgrown with seed plants (ARSO 2005). The lake is located southeast from Šentjur at the altitude of 294 m. It is surrounded by hills Rakitovec, Lipovški hrib, Požgani hrib, Gradišce and damm Tratna. Inflows into the lakes are Ločnica and Tratna streams and outflow is Voglajna River. The surface of the reservoir comprises 0.84 km^2, and it accumulates cca 4 million m^3 of water. Maximum depth is 14.5 m, while the average is about 4.8 m (ARSO 2005). The coast around the lake is 7.5 km long, and the size of the catchment areas is estimated to 30 km^2. The length of the lake is cca 5 km, while its width is from 250 to 500 m (Štraus 2006). Because of the accumulation of silt due to riparian zone overgrown with the macrophytes and due to leaching of soil into the reservoir due to erosion and landslides banks of the lake in recent years, the depth of the reservoir is decreasing (Gobec 2001, Videc 2010).

Lake Slivniško jezero is a popular place of sport fishing because it is very rich in fish populations. Fish, found in the lake, are carp, catfish, roach, pike, perch, tench, nose carp, asp, chub and others. As much as 112 species of birds nest at the lake, mostly wild ducks. In 1992, 35 ha wetland area of the lake was protected (Štraus 2006). Transparency of the lake, water is low, the average value being around 1.1 m.

The survey of macrophytes was carried out in entire littoral (Fig. 1). The surveys were performed from a boat using a rake with hooks to sample plants. Macrophyte species abundance

was estimated using a five-degree scale: 1 = very rare; 2 = infrequent; 3 = common; 4 = frequent; 5 = abundant, predominant (Kohler 1978, Kohler and Janauer 1995) and the relative abundance as well as abundance indices were calculated following the methodology proposed by Pall and Janauer (1995).

Environment assessment

The environmental condition of the lake was assessed in the same segments as macrophytes. We assessed 6 parameters, each describing 4 levels of environmental quality gradient. The parameters included bank structure, slope of riparian zone, its width and completeness and land-use type beyond the riparian zone. The parameter "Bank structure" includes the following categories: no modifications (1), modifications by wood (2), modifications by stones (3) and modifications by concrete (4). Slope of the littoral can be gentle (1), a medium steep (2), a very steep (3), or rectangular or hardened (4). Vegetation of the riparian zone can be forest or wetland species (1), pioneer woody vegetation (2), herbaceous plants (3) no vegetation (4). Width of the riparian can be more than 30 m (1), between 5 and 30 m (2), less than 5 m an without riparian vegetation (4). The parameter "Completeness of the riparian zone" includes the following categories: without disturbances (1), disturbances every 50 m (2), riparian cone strongly disturbed in all surveyed length of the shore (3) riparian cone without woody or wetland vegetation (4). The parameter "Land use" includes the following categories: catchment is overgrown with forests or wetlands (1), mosaics mown meadows/pasture with a little arable land (2), catchment dominated by arable land, individual houses (3), urban area (houses, factories) (4). In addition we examined the slope of the bank above water surface, slope of the littoral bottom, reach exposition, the presence of filamentous algae and water transparency.

Statistical analysis

Canonical correspondence analysis (CCA) was used to assess the relationship between plant species composition and abundance and environmental parameters. Environmental parameters were coded numerically from 1 to 4. Forward selection

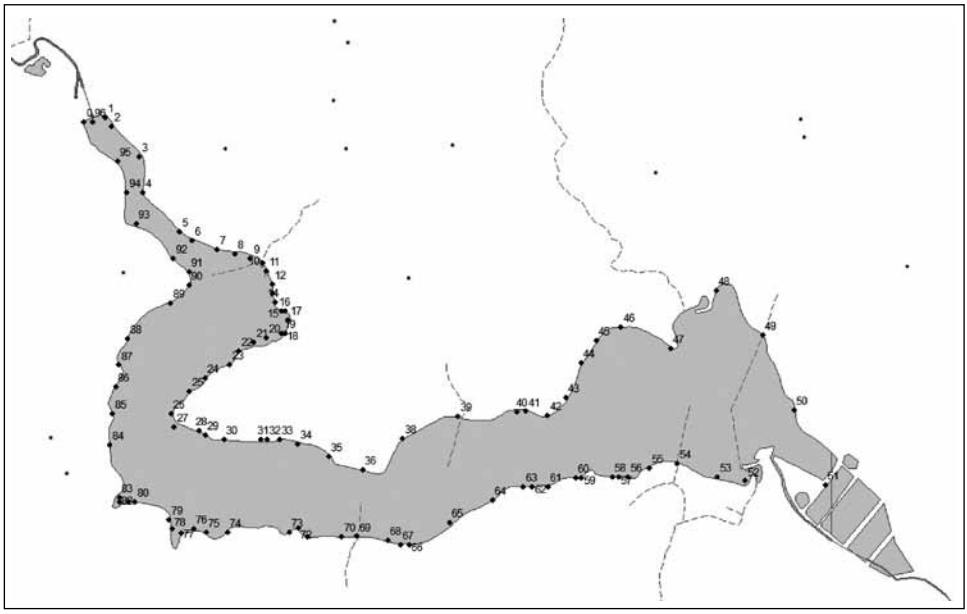

Fig. 1: Examined segments of Lake Slivniško jezero (source: Dragan Abram).
Fig.1. Tloris Slivniškega jezera z označenimi odseki (vir: Dragan Abram).

was used to determine the contribution of each parameter to the variance in species composition. The statistical significance of environmental parameters was tested by the Monte Carlo permutation test. Analyses were performed using Canoco for Windows Version 4.5.

Results

On the whole littoral of Lake Slivniško jezero we recorded 22 taxa of macrophytes of different growth forms (Table 1).

In the most sections we found a high number of macrophytes. A small number of species was determined in the sections where the bank of the water drops sharply and the steepness of the lake bottom is high (segments 2, 4, 6, 82, 83, 84). In sections 51, 52 and 53, we found only one taxa of the macrophyte with high abundance (*Phragmites australis*). The greatest abundance to the majority of sections was given to species *Myriophyllum spicatum*, *Potamogeton nodosus* and *Najas marina* (Figures 2 and 3).

Table 1: The list of the macrophytes in Lake Slivniško jezero and their growth forms.
Preglednica 1: Seznam v Slivniškem jezeru najdenih makrofitov in njihove rastne oblike.

Latin name	**Abbreviation**	**Growth form**
Alisma plantago-aquatica L.	Ali pla	he
Caltha palustris L.	Cal pal	he
Ceratophyllum demersum L.	Cer dem	sp
Eleocharis palustris (L.) Roem et Schult	Ele pal	he
Equisetum palustre L.	Equ pal	he
Iris pseudacorus L.	Iri pse	he
Lamprothamnium longifolium	Lam lon	sa
Lemna minor L.	Lem min	ap
Lysimachia nummularia L.	Lys num	he
Mentha aquatica L.	Men aqu	he
Myriophyllum spicatum L.	Myr spi	sa
Najas marina L.	Naj mar	sa
Najas minor All.	Naj min	sa
Nymphaea alba L.	Nym alb	fl
Phragmites australis (Cav.) Trin ex Steud.	Phr aus	he
Potamogeton berchtoldii Fieber.	Pot ber	sa
Potamogeton nodosus Poir.	Pot nod	fl
Potamogeton pectinatus L.	Pot pec	sa
Trapa natans L.	Tra nat	fl
Typha latifolia L.	Typ lat	he
Utricularia australis R. Br.	Utr aus	sa
Utricularia vulgaris L.	Utr aus	sa

Legend: he - helophytes, sp - submerged unrooted macrophytes, sa - submerged rooted macrophytes, ap – natant unrooted macrophytes, fl- natant rooted macrophytes, am - amphibian plants
Legenda: he - helofiti, sp - potopljeni neukoreninjeni makrofiti, sa - potopljeni ukoreninjeni makrofiti, ap - plavajoči neukoreninjeni makrofiti, fl- plavajoči, ukoreninjeni makrofiti, am - amfibijske rastline

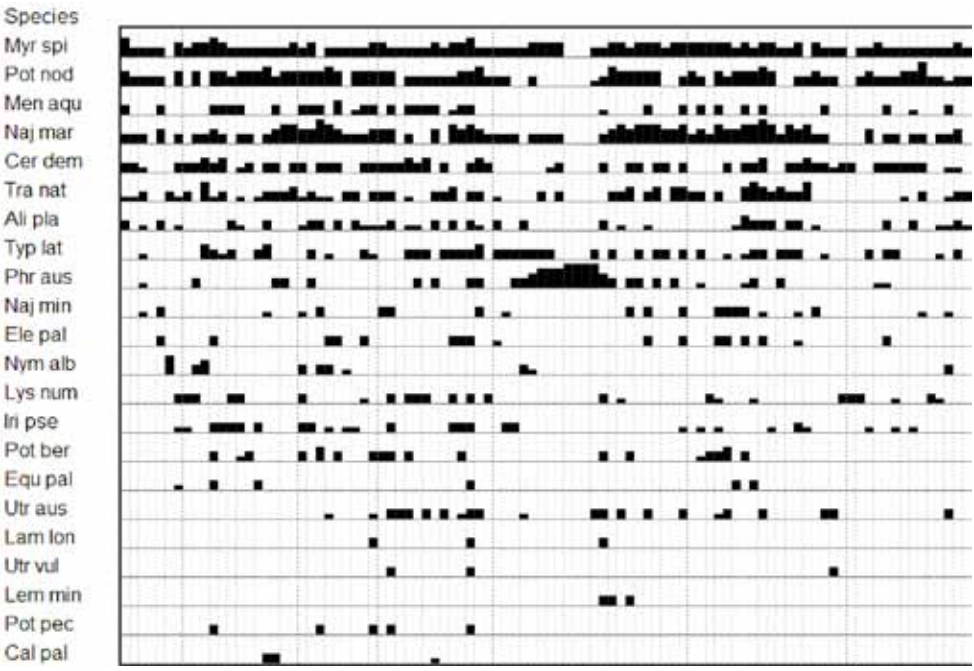

Figure 2: Distribution and abundance of macrophytes in the littoral of Lake Slivniško jezero from the 1st to 96th reach.
Slika 2: Razporeditev in pogostost makrofitov v litoralu Slivniškega jezera od 1. do 96. odseka.

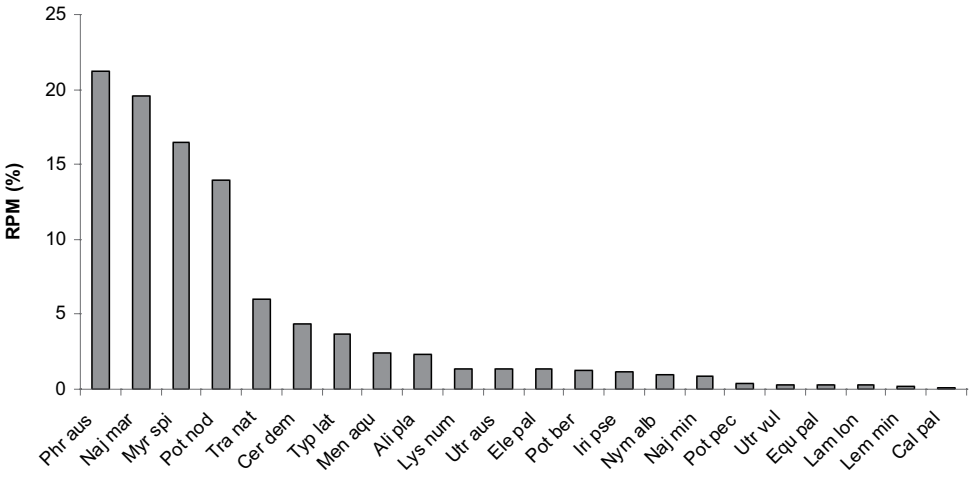

Figure 3: Relative abundance of macrophytes in Lake Slivniško jezero.
Slika 3: Relativna zastopanost makrofitov v Slivniškem jezeru.

The highest relative abundance (RPM) was reached by *Phragmites australis*, and the lowest by *Caltha palustris*. High RPM was reached also by *Najas marina*, *Myriophyllum spicatum* and *Potamogeton nodosus* (Figure 3).

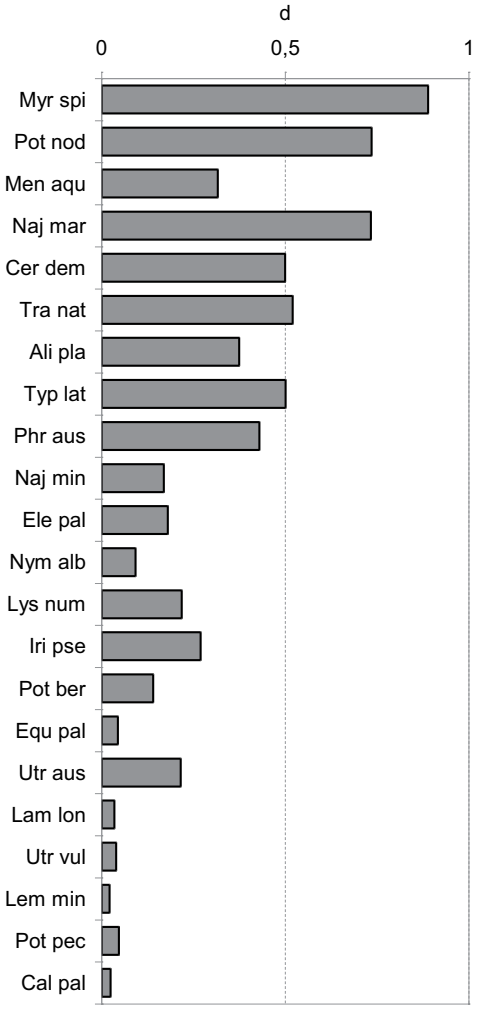

Figure 4: Ratio between relative abundance of single macrophyte per littoral reaches with this species and relative abundance of single macrophyte species per whole littoral length (d) in Lake Slivniško jezero.

Slika 4: Razmerje med relativno zastopanostjo posamezne vrste makrofitov v odsekih z vrsto in njeno relativno zastopanostjo glede na vse odseke (d) v Slivniškem jezeru.

Myriophyllum spicatum thrived on a larger share of littoral (d = 0.89), followed by *Potamogeton nodosus* (d = 0.74) and *Najas marina* (d = 0.73) (Figure 4). On about 50% of the length of the littoral we also found species *Ceratophyllum demersum*, *Trapa natans* and *Typha latifolia*. *Lamprothamnium longifolium*, *Caltha palustris* and *Lemna minor* had the lowest values of d, which means that they occupied the lowest proportion of the length of the littoral (Fig. 4).

Average depth of the macrophytes growth

The highest average maximum depth reached *Nymphaea alba* (2.4 m), which had also the highest average minimum depth of growing (1.6 m). A very wide range of depth of thriving had *Myriophyllum spicatum* (av. min. depth 0.5 m, av. max. depth 1.9 m), *Najas marina* (av. min. depth 0.4 m, av. max depth 1. 9 m) and *Potamogeton nodosus* (av. min. depth 0.4 m, av. max. depth 1.5 m). Helophytes grew on average to the maximum depth to 0.5 m (*Phragmites australis*). Smaller rooted, submerged macrophyte species, such as *Potamogeton berchtoldii* and *P. pectinatus*, thrived on average depth from 0.2 m to 0.4 m, and *Najas minor* even deeper (up to 0.6 m). Submerged rooted species *Utricularia australis* and *U. vulgaris* grew to an average depth of 0.2 m.

Environmental assessment of littoral and the catchment of Lake Slivniško jezero

The proportion of the littoral, presented by certain status of selected parameter of a wider ecological assessment of the Lake Slivniško jezero is presented in Table 3.

A 99% of the length of the lake shore is in natural state. Shore of the lake is mostly with gentle (42.5%) or medium slope (36.9%). At 40.8%, which is approximately 2960 meters long, the riparian zone is covered by forest or wetland plants. 33.2% (2412 m) of riparian zone is grown by pioneer woody vegetation. More than 50 m wide riparian zone with woody or wetland plants extends to 36.9% (2681 m) length of the lake. From 5 to 30 m wide riparian zone with woody or wetland plants extends to 28.6% (2077 m) length of the lake. At 49.9% (3625 m) length of the entire riparian zone with forest or wetland vegetation is

complete without disturbances. 53.3% of the land beyond the riparian zone overgrown with forests or wetlands. At 31.5%, the mosaics mown meadows/pasture with a little arable land is spread, 15.2% is agricultural land, where there is arable land and few individual houses.

Table 3: The proportion of different quality classes (%) of the environmetal parameters along the entire littoral in Lake Slivniško jezero.

Tabela 3: Delež litorala, ki predstavlja posamezne kakovostne razrede določenega ekološkega dejavnika Slivniškega jezera.

Parameter / Quality class	% of the whole length of the littoral belt			
	1	2	3	4
Bank structure	99.0	0.3	0.7	0
The slope of riparian zone	42.5	36.9	19	1.6
Vegetation of the riparian zone	40.8	33.2	24.8	1.2
Width of the riparian zone	36.9	28.6	12.3	22.2

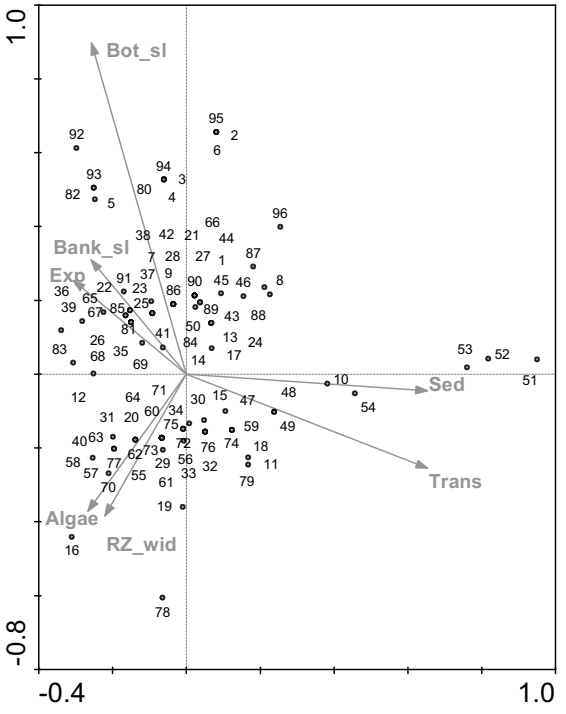

Figure 5: CCA ordination plot showing the relationship between environmental parameters and locations of littoral in Lake Slivniško jezero.

Slika 5: CCA ordinacijski diagram, ki prikazuje razmerje med okoljskimi parametri in lokacijami litorala v Slivniškem jezeru.

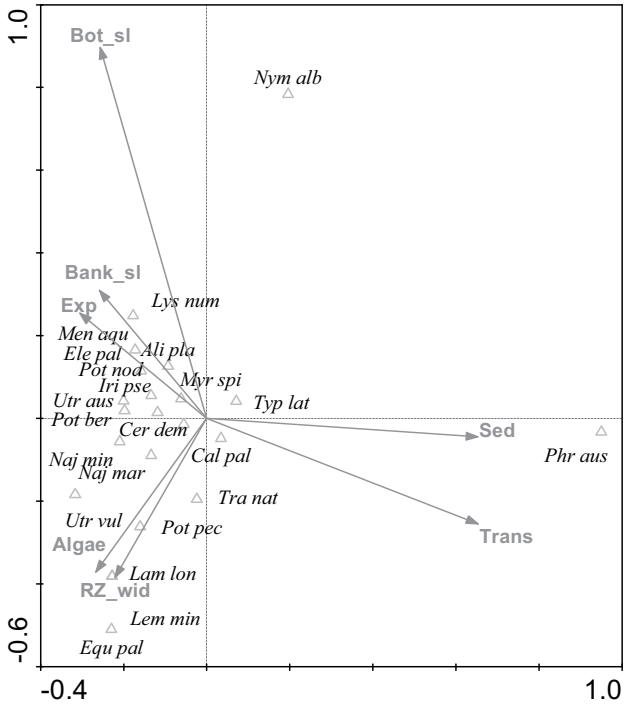

Figure 6: CCA ordination plot showing the relationship between environmental parameters and the distribution and abundance of macrophytes in Lake Slivniško jezero.

Slika 6: CCA ordinacijski diagram, ki prikazuje odnos med okoljskimi parametri ter razširjenostjo in številčnosti makrofitov v Slivniškem jezeru.

Completeness of the riparian zone	49.9	15.4	12.5	22.2
Land use	53.3	31.5	15.2	0

The effect of environmental factors on the distribution of macrophytes

Table 4: The percentage of variance, explained by each environmental parameter, using canonical correspondence analysis (CCA).

Tabela 4: Odstotek pojasnjene variance s posameznimi okoljskimi parametri s pomočjo kanonične korespondenčne analize (CCA).

Environmental parameter	Abbreviation	Explained variance	p value
Sediment	Sed	7. 5	0.001
Presence of filamentous algae	Algae	3.0	0.008
The slope of the littoral bottom	Bot_sl	3.4	0.001
Exposition	Exp	2.6	0.003
Transparency	Trans	3.4	0.024
The slope of the bank	Bank_sl	0.4	0.009
Width of riparian zone	RZ_wid	0.2	0.005

Fig. 5 shows the extent to which selected environmental factors explain the variability of the occurrence and distribution of taxa in Lake Slivniško jezero while Fig. 6 shows the locations in relation to quality gradients of environmental parameters. The proportion of explained variance of species presence and abundance is presented in the Table 4. Environmental factors with the greatest impact on the presence and abundance of macrophyte were type of the sediment, the presence of filamentous algae, the slope of the bottom, exposition and water transparency. In addition, the width of riparian zone and the slope of the bank in littoral also had an impact on the presence and abundance of macrophytes. The taxa that were grouped in the plot occurred at sites with similar environmental conditions. The majority of species were distributed around the center therefore they occupied moderate values of environmental parameters. *Potamogeton pectinatus, Utricularia vulgaris* and *Lamprothamnium longifolium* thrived better in places where riparian zone with woody or wetland vegetation was disturbed, while *Nymphaea alba* grew in places with complete riparian vegetation without disturbances. *Lemna minor* occurred more often in places overgrown with dense mass of filamentous algae.

Discussion

In Lake Slivniško jezero 22 species of macrophytes were found, namely nine species of submerged macrophytes, four natant species and nine species of emergent plants.

From emergent macrophytes *Phragmites australis* was dominated regarding relative abundance (Fig. 3). It grew mainly on the eastern part of the lake. Reed stands are among the most productive stands filtering nutrients and other substances before they reach the lake water or sediment (Wetzel 2001). *Typha latifolia* was found in areas not exposed to the winds (Hutchinson 1975). We found this species in places where the shoreline was protected by woody vegetation, or in places with steep bank.

The species *Najas marina* and *Myriophillum spicatum* achieved the highest relative abundance among submerged and natant species (Fig. 4). Both species occurred evenly throughout the littoral of the lake, and were found in more than 73% of the lake litoral. Unlike most macrophytes *Najas marina* successfully colonizes soft and unstable substrates (Germ et al. 2008). Soft, sometimes muddy bottom of the Lake Slivniško jezero provides favorable conditions for the growth of this species. *M. spicatum* is a very competitive and successful species and in many lakes it forms monospecific stands (Mazej and Germ 2008). It grows in turbid water, because it has low light compensation point and has the ability to utilize bicarbonate. It often reaches high biomass in waters rich with nitrates and in sites where the sediment is rich in organic matter (Ali and Soltan 2006). These conditions prevailed also in Lake Slivniško jezero. The coexistence of these two species is usually in favour of *M. spicatum* over *N. marina* (Ali and Soltan 2006). In Lake Slivniško jezero the two species are the most common and co-exist in most segments. On the other hand Mazej and Germ (2008) report that *N. marina* replaced the previously dense stands of *M. spicatum* and *Potamogeton crispus* in the lake Velenjsko jezero within a few years. The results suggest that the success of a species in a given aquatic ecosystem depends on the physical, chemical and geo-morphological characteristics of the water and the life strategies of the single species (Mazej and Germ 2008).

High relative abundance was also observed in *Potamogeton nodosus* (Fig. 4), which is typical species for eutrophic water bodies (Preston 2003). Two representatives of the genus *Potamogeton* in Lake Slivniško jezero were also found, *namely P. berchtoldii* and *P. pectinatus*. (Figs. 2 and 3). Both can tolerate high concentrations of phosphorus and nitrogen (Germ et al. 2008). Lehmann et al. (1997) suggest that the *P. pectinatus* grows better in shallow water bodies with muddy, organically rich sediment, which has been shown also in our research. In the stands of *M. spicatum*, *N. marina* and *P. nodosus* we often find *Ceratophyllum demersum*, which grows well in turbid water with a lot of nutrients and where the flow is slow (Šraj-Kržič 2007).

About twenty years ago *Trapa natans* expanded to the whole lake (Gobec 2001). Local policy managers regulated the water level and in a few years its abundance declined. Species was evenly distributed throughout the lake and did not form large monospecific stands.

During our study, we found two carnivorous species *Utricularia australis* and *U. vulgaris*. The appearance of these two species reflected occasional nutrient depletion in the water column due to the intensive growth of other plant species. Both species were found mainly among dense stands of other macrophytes where carnivory can bring competitive advantage, due to the low amount of nutrients in the water column (Horvat et al. 2008).

Depth distribution of macrophytes

Zonation of macrophytes in Lake Slivniško jezero reflects the differences between the species. Emergent species grow on average to a depth of 25 cm. An exception is *Phragmites australis* that colonized littoral to the depth about 45 cm. The zone of submerged rooted species such as *P. pectinatus*, *P. berchtoldii*, *Najas minor* mixed with individual specimens of *C. demersum, M. spicatum, N. marina* and *P. nodosus* reached the depth of thriving at about 50 cm. On deeper part of the litoral thrived frequently represented species in Lake Slivniško jezero namely *M. spicatum, N. marina* and *P. nodosus*. Average maximum depth of thriving of their distribution was up to 2 m.

Some specimens of *M. spicatum* and *N. marina* were found at a depth of 3 m, *N. alba* even at 4 m. However in general, the water transparency in Lake Slivniško jezero was very low, preventing a colonization of macrophytes to the deeper parts of the lake. The maximum depth of the growth of macrophytes is mostly consequence of the water transparency (Wetzel 2001).

The impact of environmental factors on macrophytes in Lake Slivniško jezero

CCA analysis showed that the distribution of macrophytes was significantly affected by the following environmental factors: insolation, the slope of the bottom, the type of the sediment, the slope of riparian zone, the presence of macroalgae in water and vegetation in the riparian zone, the completeness of woody or wetland vegetation in riparian zone, the land use beyond the riparian zone and the turbidity of water (Fig. 6). Bank structure and width of woody or wetland vegetation, did not significantly affect the distribution of macrophytes. In their macrophytes study Balaži et al. (2014) found out that water temperature, dissolved oxygen, chemical oxygen demand, and phosphorus were the main environmental variables influencing the composition of macrophyte assemblages. Hrivnak et al. (2012) also studied the effect of environmental variables on species richness of macrophytes in 39 Slovak streams. The strongest effects was exerted by the portion of artificial banks, shading by woody vegetation, flexuosity of stream course and the portion of natural land cover in the contact zone of the stream.

Conclusions

The littoral of the Lake Slivniško jezero was overgrown with aquatic plants. Reach and dense stand of plants in Lake Slivniško jezero is great advantage over other reservoirs in central and eastern Slovenia since the plants take up a large amount of nutrients. The highest relative abundance in Lake Slivniško jezero was reached by *Phragmites australis*, *Myriophyllum spicatum*, *Najas marina* and *Potamogeton nodosus*. The latter three were more or less evenly distributed throughout littoral. *P. australis* formed a huge stands at the eastern part of the lake, in other places it occurred only in small patches. The maximum average depth of macrophytes distribution in Lake Slivniško jezero was limited to about 2 m, due the low transparency of lake water. Distribution of macrophytes was significantly affected by insolation, the slope of the bottom, the type of the sediment, the slope of riparian zone, the presence of macroalgae, the type of vegetation in the riparian zone, the completeness of woody or wetland vegetation in riparian zone, the land use beyond the riparian zone and the turbidity of water.

Povzetek

Slivniško jezero je nastalo z zajezitvijo Ločnice pri Tratni leta 1976 zaradi zaščite Celja pred poplavami in za potrebe po tehnološki vodi za Železarno Štore. Na podlagi OECD kriterijev je jezero uvrščeno med evtrofne zadrževalnike.

Namen raziskave je bil ugotoviti, kateri makrofiti uspevajo v Slivniškem jezeru in kakšna je njihova pogostost in njihova razporeditev po

celotnem litoralu jezera. Ugotavljali smo tudi, kateri okoljski dejavniki vplivajo na pojavljanje, pogostost in razporeditev makrofitov v jezeru.

Litoral jezera smo razdelili na 96 odsekov na podlagi razlik v vrstni sestavi ali na podlagi sprememb okoljskih dejavnikov. Na posameznih odsekih, kjer smo popisali prisotnost in pogostost vrst, smo ocenjevali tudi okoljske dejavnike.

Slivniško jezero je na večinskem delu litorala močno poraščeno z vodnimi rastlinami. Našli smo 22 taksonov makrofitov od tega 9 submerznih vrst, 9 emerznih vrst in 4 natantne vrste. Največjo relativno zastopanost so dosegle vrste *Phragmites australis*, *Myriophyllum spicatum*, *Najas marina* in *Potamogeton nodosus*. *M. spicatum*, *N. marina* in *P. nodosus*so poleg vrste Ny*mphaea alba* dosegle tudi največjo globino uspevanja. CCA analiza je pokazala, da osončenost, naklon dna, vrsta sedimenta, naklon brega nad vodo, prisotnost makroalg, vegetacija obrežnega pasu, sklenjenost z lesnatimi ali močvirskimi rastlinami poraščenega obrežnega pasu, izraba tal v zaledju in kalnost vode, statistično značilno vplivajo na pojavljanje makrofitov v Slivniškem jezeru.

Acknowledgments

This research was financed by the Ministry of Education, Science and Sport, Republic of Slovenia, through the programme "Biology of plants" (P1-0212).

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ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 35–60

Phytosociological description of hay meadows with dominating *Trisetum flavescens* in the lower montane belt of north-western and western Slovenia

Fitocenološka oznaka travnikov s prevladujočo vrsto *Trisetum flavescens* v spodnjem gorskem pasu severozahodne in zahodne Slovenije

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Abstract: We conducted a phytosociological study into hay meadows on former fields on original sites of beech forests form the alliance *Aremonio-Fagion* in the lower montane belt of the northwestern and western Slovenia (southern Julian Alps, northern part of the Dinaric Alps) and compared them to similar, previously described meadows in Slovenia and northwestern Italy. Based on this comparison they are classified into the new association *Rhinantho freynii-Trisetetum flavescentis* and new habitat type, southeastern-Alpine-northern-Illyrian lower montane hay meadows – 38.239-S1.

Key words: secondary grasslands, synsystematics, *Trisetum flavescens*, *Arrhenatherion,* Illyrian floral province, Slovenia

Izvleček: Fitocenološko smo preučili travnike na nekdanjih njivah na izvornih rastiščih bukovih gozdov iz zveze *Aremonio-Fagion* v spodnjem gorskem pasu severozahodne in zahodne Slovenije (južne Julijske Alpe, severni del Dinarskega gorstva) in jih primerjali s podobnimi že opisanimi travniki v Sloveniji in severozahodni Italiji. Na podlagi te primerjave jih uvrščamo v novo asociacijo *Rhinantho freynii-Trisetetum flavescentis* in v nov habitatni tip jugovzhodnoalpski-severnoilirski spodnjegorski gojeni travniki – 38.239-S1.

Ključne besede: drugotna travišča, sinsistematika, *Trisetum flavescens*, *Arrhenatherion,* Ilirska florna provinca, Slovenija

Introduction

Phytosociology of meadows with dominant *Trisetum flavescens* in the submontane and montane belt in Slovenia was studied several years ago by Petras Sackl et al. (2012) who classified these meadows into two associations: *Astrantio-Trisetetum* Knapp et Knapp ex Oberdorfer 1957

and *Pastinaco-Arrhenatheretum* Passarge 1964. Similar meadows in Friuli Venezia Giulia are classified into the syntaxon *Centaureo carniolicae-Arrhenatheretum* Oberdorfer 1964 corr. Poldini et Oriolo 1994 f. *montana* Poldini et Oriolo 1994 (Poldini and Oriolo 1994). When mapping the habitat types in the Spodnja Dolina in Bohinj between Bitnje and Ribčev Laz in 2014 we classified the meadows with dominant *Trisetum flavescens* as habitat type 38.31 (central-European montane hay meadows), but we found this classification to be inadequate. In the following, 2015, we made a phytosociological inventory of these meadows. Similar meadows were observed and recorded also on the Banjšice plateau above the Central Soča Valley and elsewhere in the foothills of the Julian Alps and in the Trnovski Gozd plateau. We arranged these relevés in the phytosociological table and tried to provide a corresponding syntaxonomical definition for them. We also described a new habitat type 38.239-S1.

Methods

Phytosociological records of lower montane meadows were made according to the standard Central-European method (Braun-Blanquet 1964) and entered into the FloVegSi database (Seliškar et al. 2003). We transformed the combined cover-abundance values with numerical values (1–9) according to van der Maarel (1979). Numerical comparisons were performed with the SYN-TAX 2000 program package (Podani 2001). The relevés were compared by means of "(unweighted) average linkage method" – UPGMA, using Wishart's similarity ratio and Jaccard's index. The nomenclature source for the names of vascular plants is the Mala flora Slovenije (Martinčič et al. 2007) and Šilc and Čarni (2012) for the names of syntaxa. In the classification of species into phytosociological groups (groups of diagnostic species) we mainly refer to the Flora alpina (Aeschimann et al. 2004). Geographic coordinates of relevés are determined according to the Slovenian geographic coordinate system D 48 (5th zone) on the Bessel ellipsoid and with Gauss-Krüger projection.

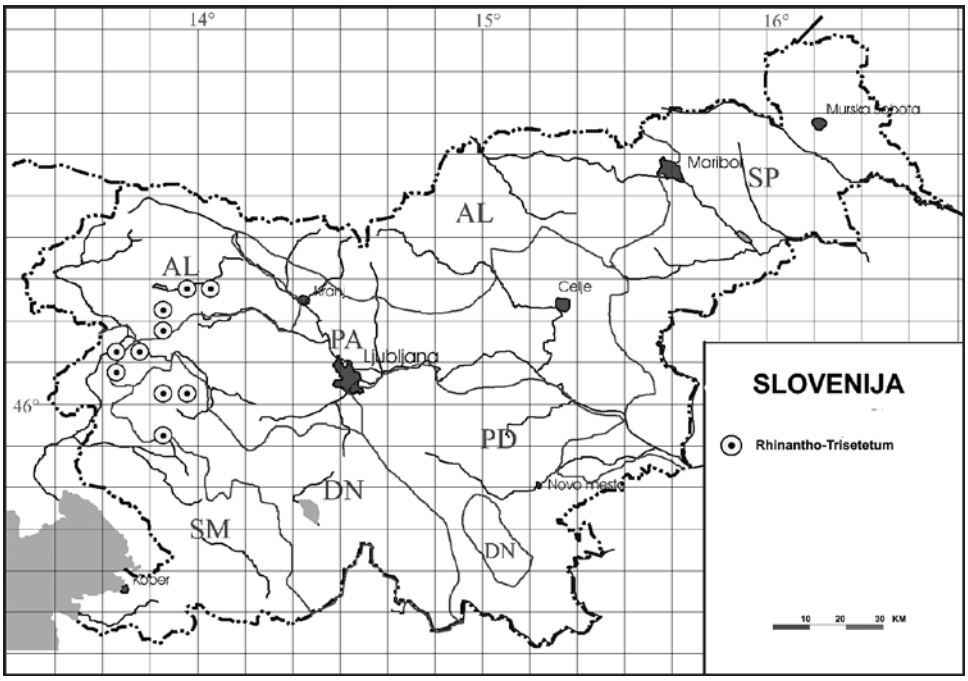

Figure 1: Approximate localities of researched hay meadows on the map of Slovenia
Slika 1: Približna lokacija popisanih travišč na zemljevidu Slovenije

Short ecological description of the study area

Figure 1 shows approximate localities of studied meadows in the southern Julian Alps (Bohinj and the Bača Valley) and in the northern part of the Dinaric Alps (Banjšice, Trnovski Gozd). They are situated in the elevation belt between (335) 500 m and 800 (1030) m, mainly on plateaus or very gentle slopes. Most of them occurred on abandoned fields. Geological bedrock is glacial material (till), limestone, limestone and flysch (or marlstone), rarely dolomite and marlstone, talus and gravel. The soil is shallow to medium deep. The predominating soil types are brown calcareous soil, eutric brown soil and rarely rendzina. The study area has a humid mountain climate. Ogrin (1998) describes it as a temperate-continental climate of western and southern Slovenia. The average annual temperature is 6–8°C (Cegnar 1998) and average annual precipitation is 2000 mm to 2500 mm (Zupančič 1998). The southwestern part of the study area (Banjšice) has a slightly warmer and less moist climate, and the same applies to the Bača Valley. In terms of climate the relevés from Bohinj are comparable with the relevés from higher elevations in the Trnovski Gozd plateau. The studied hay meadows have been cleared in the belt of Illyrian beech forests from the alliance *Aremonio-Fagion*. These are potentially the sites of associations *Anemono trifoliae-Fagetum* (Bohinj, partly also the Bača Valley), *Lamio orvalae-Fagetum, Omphalodo-Fagetum* (Trnovski Gozd), *Lamio orvalae-Fagetum* and *Ornithogalo pyrenaici-Fagetum* (Banjšice).

Results and discussion

In Table 1we arranged 24 relevés of hay meadows in the lower montane belt of northwestern and western Slovenia. Their species composition was compared to the species composition of stands from the associations *Astrantio-Trisetetum,*

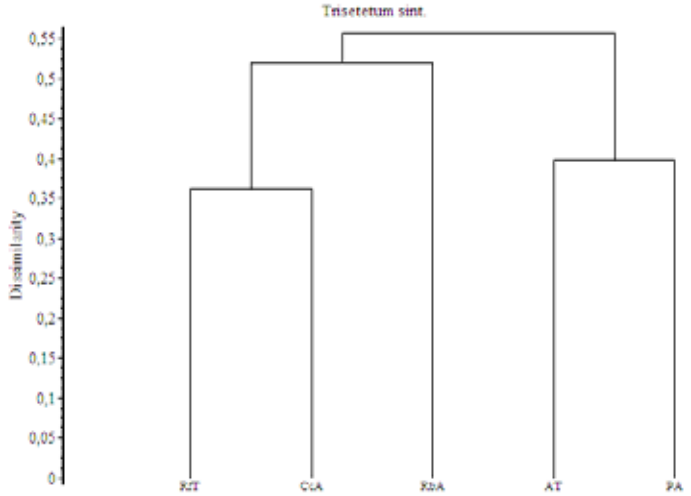

Figure 2: Dendrogram of hay meadows with dominating *Arrhenatherum elatius* and *Trisetum flavescens* in Slovenia and NE Italy (RfT – *Rhinantho freynii-Trisetetum*, CcA – *Centaureo carniolicae-Arrhenatheretum*, RbA – *Ranunculo bulbosi-Arrhenatheretum*, AT – *Astrantio-Trisetetum*, PA – *Pastinaco-Arrhenatheretum*) – UPGMA, similarity ratio

Slika 2: Dendrogram travnikov s prevladujočima vrstama *Arrhenatherum elatius* in *Trisetum flavescens* v Sloveniji in severovzhodni Italiji (RfT – *Rhinantho freynii-Trisetetum*, CcA – *Centaureo carniolicae-Arrhenatheretum*, RbA – *Ranunculo bulbosi-Arrhenatheretum*, AT – *Astrantio-Trisetetum*, PA – *Pastinaco-Arrhenatheretum*) – UPGMA, similarity ratio

Pastinaco-Arrhenatheretum (Petras Sackl et al. 2012), *Ranuncolo bulbosi-Arrhenatheretum* (Čarni 2003) and stands of the montane form of the association *Centaureo carniolicae-Arrhenatheretum* (Poldini and Oriolo 1994) in the synthetic table that was prepared for this purpose (Table 2). The comparison was conducted by means of hierarchical classification with consideration of species frequencies (Figure 2) and either presence or absence of species (Figure 3).

Considering only the presence or absence of species (Figure 3) our relevés are floristically slightly similar to the stands of associations *Pastinaco-Arrhenatheretum* and *Astrantio-Trisetetum*, whereas with consideration of species frequencies (Figure 2) we observe more similarity with the stands of associations *Centaureo carniolicae-Arrhenatheretum* and *Ranunculo bulbosi-Arrhenatheretum*. We additionally analysed the composition of compared syntaxa by groups of diagnostic species (Tables 2 and 3). This comparison indicates the following. According to this criterion the studied stands are very similar to the stands of the syntaxon *Centaureo-Arrhenatheretum* f. *montana,* but have a higher proportion of diagnostic species of dry grasslands from the class *Festuco-Brometea* and a much smaller proportion of hygrophilous tall herb species from the class *Mulgedio-Aconitetea*. Compared to the stands of the association *Ranunculo bulbosi-Arrhenentheretum* the studied stands have a considerably smaller proportion of acidophilous species from the class *Calluno-Ulicetea*, and in comparison with the stands of the association *Pastinaco-Arrhentheretum* they have a higher proportion of species of the class *Festuco-Brometea* and a smaller proportion of species from classes *Stellarietea mediae* and *Trifolio-Geranietea*. Compared to the stands of the association *Astrantio-Trisetetum* the studied stands have a considerably higher proportion of species from the class *Molinio-Arrhenathertea* and a smaller proportion of species from the order *Molinietalia* and classes *Calluno-Ulicetea*, *Trifolio-Geranietea* and *Querco-Fagetea*.

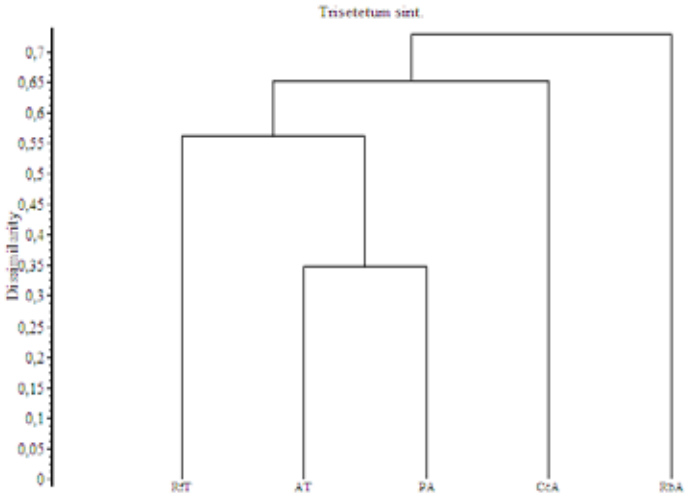

Figure 3: Dendrogram of hay meadows with dominating *Arrhenatherum elatius* and *Trisetum flavescens* in Slovenia and NE Italy (RfT – *Rhinantho freynii-Trisetetum*, CcA – *Centaureo carniolicae-Arrhenatheretum*, RbA – *Ranunculo bulbosi-Arrhenatheretum*, AT – *Astrantio-Trisetetum*, PA – *Pastinaco-Arrhenatheretum*) – UPGMA, Jaccard

Slika 3: Dendrogram travnikov s prevladujočima vrstama *Arrhenatherum elatius* in *Trisetum flavescens* v Sloveniji in severovzhodni Italiji (RfT – *Rhinantho freynii-Trisetetum*, CcA – *Centaureo carniolicae-Arrhenatheretum*, RbA – *Ranunculo bulbosi-Arrhenatheretum*, AT – *Astrantio-Trisetetum*, PA – *Pastinaco-Arrhenatheretum*) – UPGMA, Jaccard

Some of the diagnostic species of the association *Centaureo-Arrhenatheretum* (*Centaurea carniolica, Anthriscus sylvestris* and *Myosotis sylvatica*) are rare in the studied stands. Most of the diagnostic species of the association *Ranunculo bulbosi-Arrhenatheretum* (as defined by Ellmauer and Mucina 1993: 346): *Silene nutans, Clinopodium vulgare, Carlina acaulis, Carex montana, Linum catharticum*, *Trifolium montanum* and *Lychnis viscaria*, are only rarely or not at all recorded in the studied stands. Rare or absent among the diagnostic species of the association *Pastinaco-Arrhenatheretum* in the studied stands are *Pastinaca sativa* and *Geranium pratense,* and *Astrantia major, Carex montana, Linum catharticum* and *Listera ovata* are rarely recorded or absent from the diagnostic species of the association *Astrantio-Trisetetum*.

Based on mentioned analyses we came to the following conclusions. The studied stands cannot be classified into associations *Pastinaco-Arrhenatheretum* or *Astrantio-Trisetetum* due to the absence of their diagnostic species. Based on the presence of diagnostic species and (or) full floristic similarity these stands could be classified into the association *Ranunculo bulbosi-Arrhenatheretum* or into the montane form of the association *Centaureo carniolicae-Arrhenatheretum*. With consideration of the dominant species of these grasslands, *Trisetum flavescens*, their species composition and distribution in the lower montane belt we decided to name them after the dominant species and classified them into the new association *Rhinantho freynii-Trisetetum flavescentis*. Their physiognomy and species composition differ both from the meadows of the colline and submontane belt that are usually classified into the association *Ranunculo bulbosi-Arrhenatheretum*, and from the meadows of the montane and altimontane belt, which are dominated by *Trisetum flavescens*. The diagnostic species of the new association, *Trisetum flavescens, Helictotrichon pubescens, Rhinanthus freynii, Medicago lupulina, Ranunculus bulbosus* and *Plantago media*, characterise species-rich, cultivated meadows on former fields with relatively shallow soil in the lower montane belt in the region of beech forests from the Illyrian alliance *Aremonio-Fagion*. *Rhinanthus freynii* is an eastern-Alpine-Illyrian species, character species of sub-Mediterranean dry grasslands from the suballiance *Hypochoeridion maculatae* (Aeschimann et al. 2004: 278), which characterise the new association both ecologically and chorologically. The nomenclature type, *holotypus*, of the new association is relevé No. 20 in Table 1. The association is divided into two variants. The variant with *Rhinanthus minor* (its differential species is also *Lychnis flos-cuculi*) comprises the relevés on slightly moister soil and the variant with *Polygala comosa* (its differential species include *Bromopsis erecta, Brachypodium rupestre* and *Lathyrus pratensis*) comprises relevés on slightly drier sites. In terms of their characteristics the stands of the new association represent a transition between the stands of the syntaxa from orders *Poo alpinae-Trisetetalia* and *Arrhenatheretalia elatioris*. The new association is classified into the alliance *Arrhenatherion elatioris*, order *Arrhenatheretalia elatioris* and class *Molinio-Arrhenatheretea*.

Following the classification of Palaearctic habitats (P. Devillers-Terschuren and J. Devillers-Terschuren 1998, 2002) we classify the studied meadows into a new habitat type, southeastern-Alpine-northern-Illyrian lower montane hay meadows – 38.239-S1. The description of the new habitat is as follows:

Lower montane hay meadows are distributed in the foothills of the Alps and in the northern part of the Dinaric Mountains at elevations between (400) 500 m to 800 (1000) m in the belt of Illyrian montane beech forests (alliance *Aremonio-Fagion*) and are an intermediate stage between HT 38.22 and 38.3. The geological bedrock is usually calcareous, but frequently interlayered with silicate (limestone, limestone and marl), in places also glacial material (till). The soil is shallow brown rendzina, brown calcareous soil, eutric brown soil. Meadows are situated on plateaus or gentle slopes, frequently on former fields. They are usually mown once or twice a year, unfertilised or only moderately fertilised and species rich. The dominant grass is *Trisetum flavescens*, while *Arrhenatherum elatius*, *Helictotrichon pubescens* and *Holcus lanatus* have a slightly lower median coverage. More poorly represented or absent from the species composition are the diagnostic species of montane hay meadows. The differential species are some of the species of semi-dry meadows from the class *Festuco-Brometea* (*Medicago lupulina,*

Rhinanthus freynii, Plantago media, Ranunculus bulbosus). In terms of floristics they are more similar to lowland hay meadows from the associations *Ranunculo bulbosi-Arrhenatheretum* and *Centaureo carniolicae-Arrhenatheretum* (HT 38.221) than to montane hay meadows, but are nevertheless substantially different in their entire floristic composition, especially in terms of the presence of different companion species that are characteristic of the (lower) montane belt and in that *Trisetum flasvescens* dominates over *Arrhenatherum elatius*.

Conclusions

Despite the predominant *Trisetum flavescens* the hay meadows on former fields in the lower montane belt of the northwestern and western Slovenia, originally the sites of beech forests from the alliance *Aremonio-Fagion,* are floristically nevertheless more similar to the meadows from the order *Arrhenatheretalia elatioris* than to those from the order *Poo alpinae-Trisetetalia.* They could be classified either into the association *Ranuculo bulbosi-Arrhenatheretum* or *Centaureo carniolicae-Arrhenatheretum*, but their distribution in the lower montane belt and entire species composition justify their classification into the new association *Rhinantho freynii-Trisetetum flavescentis*. The new association indicates an intermediate type of hay meadows of the lower montane belt where *Trisetum flavescens* is the dominating species; their species composition, however, does not yet comprise expressly montane (upper-montane) species, but more species of semi-dry grasslands. They are species rich and less prone to degradation that is caused by intensive fertilisation. These meadows also comprise several protected species (Anon. 2004), such as *Orchis ustulata, O. morio, O. tridentata, Lilium bulbiferum, Listera ovata* and *Dactylorhiza fuchsii*.

Povzetek

V letu 2014 smo pri kartiranju habitatnih tipov v Spodnji dolini v Bohinju med Bitnjami in Ribčevim Lazom označili travnike s prevladujočo vrsto *Trisetum flavescens* kot habitatni tip 38. 31 (srednjeevropski gorski gojeni travniki), vendar se nam ta oznaka ni zdela najbolj ustrezna. Naslednje leto, 2015, smo te travnike fitocenološko popisali. Podobne travnike smo opazili in popisali tudi v hribovju Banjšic nad srednjo Soško dolino in ponekod drugod v prigorju Julijskih Alp in v Trnovskem gozdu. Vse te popise smo uredili v fitocenološko tabelo in njihovo vrstno sestavo primerjali z vrstno sestavo sestojev asociacij *Astrantio-Trisetetum, Pastinaco-Arrhenatheretum* (Petras Sackl et al. 2012), *Ranuncolo bulbosi-Arrhenatheretum* (Čarni 2003) in s sestoji montanske forme asociacije *Centaureo carniolicae-Arrhenatheretum* (Poldini in Oriolo 1994). Primerjavo smo izvedli s hierarhično klasifikacijo, ob upoštevanju frekvence vrst in zgolj prisotnosti ali odsotnosti vrst. Primerjane združbe smo analizirali tudi po sestavi skupin diagnostičnih vrst. Na podlagi navedenih analiz ugotavljamo, da uvrstitev preučenih sestojev v asociaciji *Pastinaco-Arrhenatheretum* ali *Astrantio-Trisetetum* zaradi odsotnosti njunih diagnostičnih vrst ni mogoča. Po prisotnosti diagnostičnih vrst in (ali) celotni floristični podobnosti bi te sestoje mogli uvrstiti v asociacijo *Ranunculo bulbosi-Arrhenatheretum* ali v montansko formo asociacije *Centaureo carniolicae-Arrhenatheretum*. Ob upoštevanju prevladujoče vrste teh travišč, *Trisetum flavescens*, njihove vrstne sestave in razširjenosti v spodnjem gorskem pasu, smo se odločili za njihovo poimenovanje po prevladujoči vrsti in jih uvrščamo v novo asociacijo *Rhinantho freynii-Trisetetum flavescentis*. Označuje vrstno bogata travišča na nekdanjih njivah z razmeroma plitvimi tlemi, v katerih prevladuje rumenkasti ovsenec, a v njihovi sestavi še ni vrst, ki so značilne za zgornji gorski pas, pač pa več vrst polsuhih travišč. Obravnavana travišča so značilna za spodnji gorski pas v območju bukovih gozdov iz ilirske zveze *Aremonio-Fagion*. Diagnostične vrste nove asociacije so *Trisetum flavescens,Helictotrichon pubescens, Rhinanthus freynii, Medicago lupulina, Ranunculus bulbosus* in *Plantago media.* Po tipologiji palearktičnih habitatnih tipov (P. Devillers-Terschuren in J. Devillers-Terschuren 1998, 2002) preučene travnike uvrščamo v nov habitatni tip jugovzhodnoalpski-severnoilirski spodnjegorski gojeni travniki – 38.239-S1.

Acknowledgements

In our field work we were assisted by Branko Zupan and Iztok Sajko. Two anonymous reviewers helped us with valuable improvements and corrections. English translation by Andreja Šalamon Verbič.

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Table 1 (Preglednica1): *Rhinantho freynii-Trisetetum flavescentis*

	Number of relevé (Zaporedna številka popisa)		1	2	3	4	5	6	7	8	9
	Database number of relevé (Delovna številka popisa)		245813	257007	258234	245826	245880	254991	245944	245948	245945
	Author of relevé (Avtor popisa)		ID	ID	ID	ID	ID	ID	ID	ID	ID
	Elevation in m (Nadmorska višina v m)		730	716	620	760	550	500	700	620	695
	Aspect (Lega)		S	SE	SW	SW	NW	0	SE	E	S
	Slope in degrees (Nagib v stopinjah)		10	10	5	5	5	0	10	10	5
	Parent material (Matična podlaga)		DRG	AL	AF	DRG	D	Pr	Gr	ALR	Gr
	Soil (Tla)		Eu	Eu	Eu	Eu	Eu	Re	Re	CC	Re
	Cover of herb layer in % (Zastiranje zeliščne plasti v %):		100	100	100	100	100	100	100	100	100
	Number of species (Število vrst)		53	46	51	44	53	46	51	49	43
	Relevé area (Velikost popisne ploskve)	m^2	20	20	20	20	20	20	20	20	20
	Date of taking relevé (Datum popisa)		6/6/2012	5/31/2015	6/4/2015	6/6/2012	6/13/2012	6/9/2014	6/5/2012	6/5/2012	6/5/2012
	Locality (Nahajališče)		Mrzla Rupa - Pri Štalah	Banjšice - Perrtovti	Banjšice - Kopruščе	Mrzla Rupa-Pri Štalah	Čepovan-Vrata-Gruden	Bohinjska Bistrica - Zagradec	Rut nad Baško dolino	Rut nad Baško dolino	Rut nad Baško dolino
	Quadrant (Kvadrant)		9949/3	9948/1	9948/1	9949/3	9848/4	9749/2	9749/3	9749/3	9749/3
	Coordinate GK Y (D-48)	m	413609	401964	402371	413316	407840	420918	414685	414823	414716
	Coordinate GK X (D-48)	m	5096108	5102318	5105622	5096250	5107424	5126613	5118741	5118173	5118732
	Diagnostic species of the association (Diagnostične vrste asociacije)										
MA	*Trisetum flavescens*	E1	1	1	3	3	3	1	3	3	3
MA	*Helictotrichon pubescens*	E1	.	+	2	2	1	1	4	3	3
FB	*Medicago lupulina*	E1	+	.	.	1	+	1	+	+	+
FB	*Rhinanthus freynii*	E1	1	2	1	3	2	3	+	1	.
FB	*Plantago media*	E1	.	1	+	.	+	+	1	+	1

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Pr.	Fr.
	245946	258389	245949	258230	254787	258515	258517	254984	258507	258513	258510	258512	258514	258516	258511		
	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID		
	715	735	335	630	1030	580	590	920	580	590	575	575	575	575	560		
	SSE	NE	SW	NE	NE	NE	SEE	W	W	N	W	0	NNW	NE	S		
	5	20	3	5	15	5	5	5	2	5	2	0	2	5	2		
	Gr	L	Gr	AL	DL	Mo	Mo	A	Mo	Mo	Mo	Mo	Mo	Mo	Mo		
	CC	Eu	CC	Eu	Eu	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC		
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100		
	41	39	45	28	38	29	33	38	44	45	39	30	37	45	43		
	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20		
5118810	414643	9749/3	Rut nad Baško dolino	6/5/2012													
5108118	402617	9848/3	Banjšice -Levpa - Testeni	5/28/2015													
5114306	413300	9849/1	Koritnica v Baški dolini	6/5/2012													
5105505	402020	9948/1	Banjšice -Kuprušče - Sv. Tomaž	6/4/2015													
5096354	417860	9949/4	Vojsko - Gnezda	6/19/2014													
5125447	418443	9749/2	Bohinjska Bistrica-Dobrava	6/2/2015													
5125214	418418	9749/2	Bohinjska Bistrica-Dobrava	6/2/2015													
5089654	415021	0049/3	Predmeja - Podkaplice-Polh	4/8/2014													
5125300	417973	9749/2	Bohinjska Bistrica-Dobrava	5/19/2015													
5125231	418143	9749/2	Bohinjska Bistrica-Dobrava	5/19/2015													
5125397	418008	9749/2	Bohinjska Bistrica-Dobrava	5/19/2015													
5125180	417754	9749/2	Bohinjska Bistrica-Dobrava	5/19/2015													
5125606	418008	9749/2	Bohinjska Bistrica-Dobrava	5/19/2015													
5125514	418599	9749/2	Bohinjska Bistrica-Dobrava	6/2/2015													
5125127	417494	9749/2	Bohinjska Bistrica-Dobrava	5/19/2015													
																Pr.	Fr.
	3	3	2	3	4	4	3	3	1	1	3	4	1	2	2	24	100
	.	3	2	3	.	+	+	1	2	2	2	2	+	1	2	21	88
	+	+	+	1	.	1	.	.	+	+	1	+	+	2	1	19	79
	+	1	+	.	+	+	1	+	.	+	1	.	.	.	2	18	75
	+	+	+	.	.	.	.	+	+	+	+	.	+	+	+	17	71

	Number of relevé (Zaporedna številka popisa)		1	2	3	4	5	6	7	8	9
FB	*Ranunculus bulbosus*	E1	+	1	.	.	.	+	1	+	+
	Differential species of lower units (Razlikovalnice nižjih enot)										
FB	*Polygala comosa*	E1	.	+	+	+	+	.	1	.	1
FB	*Bromopsis erecta*	E1	+	1	+	+	+	.	1	+	.
FB	*Brachypodium rupestre*	E1	2	+	.	2	1	+	+	+	.
MA	*Lathyrus pratensis*	E1	.	.	1	.	+	.	1	1	.
CU	*Rhinanthus minor*	E1	.	+	.	.	.	1	.	.	.
MA	*Lychnis flos-cuculi*	E1	.	1	.	.	.	.	.	.	.
MA	***Arrhenatheretalia, Molinio-Arrhenatheretea***										
	Leucanthemum ircutianum	E1	1	1	1	1	1	2	1	1	1
	Arrhenatherum elatius	E1	1	.	1	3	4	1	1	3	3
	Ranunculus acris	E1	1	+	+	.	1	1	+	1	1
	Leontodon hispidus	E1	1	+	1	1	1	2	2	2	3
	Rumex acetosa	E1	+	.	+	+	1	1	1	+	1
	Dactylis glomerata	E1	1	.	1	2	2	2	1	1	2
	Galium mollugo agg. *(G. album)*	E1	1	+	+	1	+	+	.	+	+
	Trifolium pratense	E1	1	1	1	+	.	1	2	.	2
	Poa pratensis	E1	.	.	.	1	1	1	1	1	2
	Holcus lanatus	E1	2	4	3	+	1	.	1	1	+
	Knautia arvensis	E1	1	.	.	3	.	2	2	2	3
	Lotus corniculatus	E1	+	1	1	.	.	1	1	1	1
	Plantago lanceolata	E1	+	+	+	+	1	1	+	+	+
	Pimpinella major	E1	.	+	+	.	+	+	2	1	2
	Cerastium holosteoides	E1	+	1	+	1	.	+	1	1	+
	Achillea millefolium	E1	.	+	+	+	1	.	.	+	+
	Festuca pratensis	E1	+	+	+	+	+	2	+	1	+
	Centaurea jacea	E1	2	+	+	1	2	1	.	.	.
	Cynosurus cristatus	E1	+	.	+	+	+	1	.	.	.
	Trifolium repens	E1	+	.	1	+	+	+	+	+	.
	Daucus carota	E1	+	1	+	.	.	+	+	1	+
	Lolium perenne	E1	.	.	.	.	.	.	+	+	+
	Tragopogon pratense subsp. *orientalis*	E1	.	1	1	1	+	+	1	+	.
	Festuca rubra	E1	1	2	1	.	.	.	1	+	.
	Crepis biennis	E1	.	.	+	+	1	1	.	1	.
	Vicia cracca	E1	.	.	1	+	+	.	1	+	+
	Veronica chamaedrys	E1	.	.	.	.	+	.	.	.	+
	Campanula patula	E1	.	1	.	.	+	.	.	.	.
	Taraxacum officinale agg.	E1	.	.	.	.	+	.	.	.	.
	Heracleum sphondylium	E1	.	+	.	.	.	.	+	+	+
	Allium scorodoprasum	E1	.	.	.	.	1	+	.	+	.
	Ajuga reptans	E1	+	.	.	.	.	.	.	.	.
	Stellaria graminea	E1	.	+	+	.	.	.	.	.	.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Pr.	Fr.
.	+	.	.	.	.	.	.	+	.	+	.	+	.	+	11	46
+	.	.	1	.	.	.	.	.	.	.	.	.	+	.	9	38
.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	8	33
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7	29
.	+	.	.	+	.	.	+	.	.	.	.	.	.	.	7	29
.	.	.	.	.	.	.	+	2	1	1	1	2	+	1	10	42
.	.	.	.	+	.	.	.	2	1	1	1	2	1	1	9	38
1	1	1	+	+	+	+	+	1	+	1	1	1	1	1	24	100
4	1	1	3	3	4	3	3	1	2	2	2	+	1	2	23	96
1	2	1	+	+	+	1	1	2	1	2	1	2	2	2	23	96
2	+	2	2	2	1	1	1	1	2	1	.	.	1	2	22	92
1	+	+	+	1	+	+	+	+	1	1	1	.	+	1	22	92
1	1	1	1	2	2	2	2	.	1	1	1	1	2	1	22	92
+	+	+	+	1	1	1	1	.	.	+	1	+	1	+	21	88
2	2	2	3	2	1	1	1	.	2	2	2	2	1	2	21	88
1	2	+	+	.	+	+	+	2	2	1	+	+	+	1	20	83
+	.	2	.	+	.	2	.	3	3	3	2	4	3	2	19	79
2	1	2	.	.	1	1	1	1	1	+	1	1	2	1	19	79
2	.	1	1	1	+	.	+	+	+	1	.	1	+	+	19	79
+	1	+	+	.	+	.	+	+	.	.	+	+	1	.	19	79
1	+	1	.	1	1	+	.	+	1	+	+	+	+	.	19	79
+	1	.	.	1	.	.	.	+	+	1	+	1	1	+	19	76
1	1	+	.	1	1	1	1	+	+	+	+	+	1	.	19	76
.	.	1	+	1	.	+	1	+	.	+	.	.	1	.	17	71
.	.	2	+	1	.	1	1	+	+	1	1	1	+	.	17	71
.	.	1	.	2	+	3	.	3	3	2	2	2	2	1	16	67
1	.	.	1	+	.	1	+	+	.	.	.	.	+	.	14	58
.	.	+	.	.	.	.	.	+	+	+	.	+	+	+	14	58
+	.	1	.	+	1	+	.	1	+	+	1	1	+	.	14	58
.	1	1	1	.	.	.	.	+	+	1	.	.	.	.	13	54
.	.	+	.	1	1	+	1	.	.	.	.	+	2	.	12	50
.	1	1	.	1	.	1	+	.	+	+	.	.	.	.	12	50
1	+	.	.	1	.	.	.	.	.	.	.	.	.	.	9	38
+	1	.	.	.	.	.	.	.	+	+	1	+	.	+	9	38
.	.	.	.	.	.	.	+	.	+	+	.	+	+	1	8	33
.	1	+	.	.	.	.	.	1	2	.	1	1	.	+	8	33
.	+	.	+	.	+	.	.	.	.	.	.	.	.	.	7	29
.	.	.	.	.	+	+	.	.	+	.	.	.	.	+	7	29
.	+	.	.	.	.	.	+	+	+	.	.	+	.	.	6	25
.	1	.	.	1	.	+	+	.	.	.	.	.	.	.	6	25

	Number of relevé (Zaporedna številka popisa)		1	2	3	4	5	6	7	8	9
	Carum carvi	E1	.	.	.	1	.	.	.	+	.
	Prunella vulgaris	E1	+	.	.	1	.	+	.	.	.
	Vicia sepium	E1	.	.	.	+	1	.	.	+	.
	Poa trivialis	E1	.	.	.	.	.	.	.	.	.
	Euphrasia rostkoviana	E1	.	.	.	.	.	.	1	.	1
	Medicago sativa	E1	.	.	.	.	.	.	.	.	+
	Bellis perennis	E1	.	.	.	.	.	.	.	.	.
	Carex hirta	E1	+	.	.	.	.	.	.	.	.
	Bromus hordeaceus	E1	.	.	.	+	.	.	.	.	.
	Achillea roseoalba	E1	.	.	.	.	.	.	.	.	.
	Agropyron repens	E1	.	.	.	.	.	.	.	.	.
	Phleum pratense	E1	.	.	.	.	.	.	.	.	.
	Pastinaca sativa	E1	.	+	.	.	.	.	.	.	.
	Trifolium campestre	E1	.	+	.	.	.	.	.	.	.
	Anthriscus sylvestris	E1	.	.	.	.	1	.	.	.	.
	Alchemilla xanthochlora	E1	.	.	.	.	+	.	.	.	.
	Alopecurus pratensis	E1	.	.	.	.	.	.	.	.	+
	Festuca arundinacea	E1	.	.	.	.	.	.	.	.	.
	Alchemilla vulgaris	E1	.	.	.	.	.	.	.	.	.
	Veronica serpyllifolia	E1	.	.	.	.	.	.	.	.	.
	Trifolium patens	E1	.	.	.	.	.	.	.	.	.
	Ornithogalum umbellatum	E1	.	.	.	.	.	.	.	.	.
Mo	***Molinietalia caeruleae***										
	Colchicum autumnale	E1	1	+	.	1	.	+	+	+	.
	Centaurea carniolica	E1	.	.	.	.	.	.	.	.	.
	Primula elatior	E1	.	.	.	.	+	.	.	.	.
	Primula x digenea	E1	.	.	.	.	+	.	.	.	.
	Equisetum arvense	E1	.	.	.	.	.	+	.	.	.
	Betonica officinalis	E1	.	.	.	.	.	.	.	.	.
PaT	***Poo alpinae-Trisetetalia***										
	Anthoxanthum odoratum	E1	1	2	1	+	+	.	.	+	+
	Agrostis capillaris	E1	.	.	.	+	.	.	+	.	.
	Ranunculus nemorosus	E1	.	.	.	.	.	.	.	.	.
MuA	*Veratrum album* subsp. *lobelianum*	E1	.	.	.	.	.	.	.	.	.
FB	***Brometalia erecti, Festuco-Brometea***										
	Salvia pratensis	E1	.	2	1	3	+	3	3	2	.
	Briza media	E1	2	1	1	.	+	2	1	.	.
	Thymus pulegioides	E1	1	1	+	+	.	1	+	+	+
	Carex caryophyllea	E1	+	+	.	.	.	.	.	+	+
	Pimpinella saxifraga	E1	+	.	+	1	.	.	+	.	.
	Silene vulgaris subsp. *vulgaris*	E1	.	.	.	.	.	+	1	.	1
	Festuca rupicola	E1	.	+	.	.	.	1	1	.	.
	Orobanche gracilis	E1	.	.	.	.	.	+	1	.	+
	Arabis hirsuta	E1	.	.	.	+	.	.	+	.	.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Pr.	Fr.
.	1	1	.	.	.	.	.	+	+	.	.	.	.	.	6	25
.	.	.	.	+	.	.	.	.	.	.	.	.	+	.	5	21
.	+	.	.	.	.	1	.	.	.	.	.	.	.	.	5	21
.	.	.	.	.	+	1	.	.	.	+	1	.	1	.	5	21
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.	2	1	.	1	.	.	.	1	2	2	+	1	+	1	17	71
+	+	.	.	+	.	.	2	.	.	.	.	.	1	.	7	29
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+	1	+	+	.	.	+	.	+	+	1	+	.	+	2	18	75
+	.	+	.	.	.	.	+	+	.	+	.	.	+	.	12	50
.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	9	38
+	.	.	.	.	.	.	.	+	+	.	.	.	.	+	8	33
.	.	.	.	.	.	.	.	.	.	+	.	+	.	+	7	29
+	.	.	.	.	+	+	.	.	.	.	.	.	.	.	6	25
.	.	+	.	.	.	.	.	+	.	.	.	.	.	.	5	21
+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	5	21
+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	4	17

	Number of relevé (Zaporedna številka popisa)		1	2	3	4	5	6	7	8	9
	Sanguisorba minor s. lat.	E1	+	.	+	.	.	.	1	+	.
	Silene nutans	E1	+	.	.	.	+	.	.	.	.
	Buphthalmum salicifolium	E1	.	.	+	.	+	.	.	.	.
	Filipendula vulgaris	E1	.	.	.	.	.	.	.	.	.
	Koeleria pyramidata	E1	1	.	.	.	.	+	.	.	.
	Orchis ustulata	E1	+	.	.	r	.	.	.	.	.
	Hieracium bauhinii	E1	+	.	.	.	.	.	.	.	.
	Linum catharticum	E1	1	.	.	.	.	.	.	.	.
	Knautia illyrica	E1	.	1	1	.	.	.	.	.	.
	Peucedanum oreoselinum	E1	.	.	+	.	.	.	1	.	.
	Orchis tridentata	E1	.	.	+	.	.	.	.	.	.
	Ranunculus polyanthemophyllus	E1	.	.	+	.	.	.	.	.	.
	Trifolium montanum	E1	.	.	+	.	.	.	.	.	.
	Centaurea scabiosa subsp. *fritschii*	E1	.	.	.	2	+	.	.	.	.
KC	*Arenaria serpyllifolia*	E1	.	.	.	+	.	.	.	.	+
	Cirsium erisithales	E1	.	.	.	.	+	.	+	.	.
	Anthyllis vulneraria	E1	.	.	.	.	.	+	.	.	.
	Carex montana	E1	.	.	.	.	.	1	.	.	.
	Thlaspi praecox	E1	.	.	.	.	.	.	1	.	+
	Galium verum	E1	.	.	.	.	.	.	+	.	.
	Danthonia alpina	E1	1	.	.	.	.	.	.	.	.
	Scabiosa triandra	E1	+	.	.	.	.	.	.	.	.
	Genista tinctoria	E1	.	+	.	.	.	.	.	.	.
	Festuca valesiaca	E1	.	+	.	.	.	.	.	.	.
	Campanula rapunculus	E1	.	.	1	.	.	.	.	.	.
	Cirsium pannonicum	E1	.	.	+	.	.	.	.	.	.
	Helianthemum ovatum	E1	.	.	+	.	.	.	.	.	.
	Orchis morio	E1	.	.	+	.	.	.	.	.	.
	Gymnadenia conopsea	E1	.	.	.	r	.	.	.	.	.
	Campanula glomerata	E1	.	.	.	.	+	.	.	.	.
	Campanula rotundifolia	E1	.	.	.	.	+	.	.	.	.
	Medicago falcata	E1	.	.	.	.	.	+	.	.	.
	Euphorbia verrucosa	E1	.	.	.	.	.	.	.	.	.
	Carlina acaulis	E1	.	.	.	.	.	.	.	.	.
	Gentianella ciliata	E1	.	.	.	.	.	.	.	.	.
	Ononis spinosa	E1	.	.	.	.	.	.	.	.	.
CU	***Calluno-Ulicetea***										
	Luzula campestris	E1	+	1	+	.	+	.	+	+	.
	Carex pallescens	E1	2	1	+	.	.	.	.	.	.
	Veronica officinalis	E1	+	+	.	.	.	.	.	.	.
	Chamaespartium sagittale	E1	1	.	.	.	.	.	.	.	.
	Polygala vulgaris	E1	+	.	.	.	.	.	.	.	.
	Potentilla erecta	E1	+	.	.	.	.	.	.	.	.
	Phyteuma zahlbruckneri	E1	.	.	.	.	.	.	.	.	.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Pr.	Fr.
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	Number of relevé (Zaporedna številka popisa)		1	2	3	4	5	6	7	8	9
	Festuca filiformis	E1	.	.	.	.	.	.	.	.	.
TG	***Trifolio-Geranietea***										
	Viola hirta	E1	+	.	.	.	.	+	.	.	.
	Clinopodium vulgare	E1	.	.	.	+	+	.	.	.	.
	Dianthus barbatus	E1	.	.	.	.	.	.	+	+	.
	Hypericum perforatum	E1	.	+	.	.	1	.	.	.	.
	Astragalus glycyphyllos	E1	.	.	.	.	.	.	.	.	+
	Campanula rapunculoides	E1	.	.	.	.	.	.	.	.	+
	Verbascum nigrum	E1	.	.	.	.	.	.	.	.	.
	Thalictrum minus	E1	.	.	.	.	.	.	.	.	.
	Lilium bulbiferum	E1	.	.	.	.	.	.	.	.	.
CD	***Caricetalia davallianae***										
	Carex panicea	E1	.	.	.	.	.	.	.	.	.
SM	***Stellarietea mediae, Galio-Urticetea***										
	Erigeron annuus	E1	.	.	.	+	+	.	.	+	+
	Myosotis arvensis	E1	.	.	.	.	.	.	.	.	.
	Veronica arvensis	E1	.	.	.	.	.	.	.	.	.
	Convolvulus arvensis	E1	.	.	.	.	+	.	.	.	.
	Silene latifolia subsp. *alba*	E1	.	.	.	.	.	.	.	.	.
	Vicia hirsuta	E1	.	.	.	.	+	.	.	.	.
	Aegopodium podagraria	E1	.	.	.	.	.	.	.	+	.
	Rumex obtusifolius	E1	.	.	.	.	.	.	.	.	.
	Plantago major	E1	.	.	.	.	.	.	.	.	.
EA	***Epilobietea angustifolii***										
	Carex muricata	E1	.	.	.	.	.	.	.	.	+
	Myosotis sylvatica	E1	.	.	.	.	.	.	.	.	.
	Cirsium arvense	E1	.	.	.	.	.	.	.	.	.
FS	***Fagetalia sylvaticae***										
	Knautia drymeia	E1	+	.	.	.	1	.	+	+	.
	Carex sylvatica	E1	.	.	.	.	.	.	.	1	.
	Primula vulgaris	E1	.	.	+	.	.	.	.	.	.
	Phyteuma spicatum subsp. *coeruleum*	E1	.	.	.	.	.	.	.	.	.
QF	***Querco-Fagetea***										
	Cruciata glabra	E1	1	+	+	.	.	.	.	.	1
	Carex flacca	E1	+	.	.	.	.	.	.	.	.
	Listera ovata	E1	.	.	.	.	.	.	.	.	.
	Dactylorhiza fuchsii	E1	.	.	.	.	.	+	.	.	.
	Ornithogalum pyrenaicum	E1	.	.	.	.	.	.	.	.	.
O	**Other species (Ostale vrste)**										
	Alchemilla sp.	E1	+	.	.	.	.	.	.	.	.
	Orobanche sp.	E1	.	.	.	.	.	+	.	.	.
	Agrostis sp.	E1	.	.	.	.	.	.	.	+	.
	Knautia sp.	E1	.	.	.	.	.	.	.	.	.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Pr.	Fr.
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Legend - Legenda

ID Igor Dakskobler
MuA Mulgedio-Aconitetea
KC *Koelerio-Corynophoretea*
A Limestone - apnenec
D Dolomite - dolomit
Gr Gravel - grušč
Pr Alluvium - prod
L Marlstone - laporovec
G Claystone - glinavec
R Chert - roženec
Mo Till - til
Eu Eutric brown soil - evtrična rjava tla
CC Chromoc Cambisols - rjava pokarbonatna tla
Re Rendzina - rendzina

Table 2: Synoptic table of *Trisetum flavescens* and *Arrhenatherum elatius* dominating (sub)montane grasslands in Slovenia and NE Italy

Preglednica 2: Sintezna tabela (sub)montanskih travnikov s prevladujočima vrstama *Trisetum flavescens* in *Arrhenatherum elatius* v Sloveniji in severovzhodni Italiji

	Successive number (Zaporedna številka)		1	2	3	4	5
	Number of relevés (Število popisov)		24	10	7	44	47
	Number of taxa (Število taksonov)		166	120	71	191	147
	Sign for the syntaxa (Oznaka sintaksonov)		RfT	CcA	RbA	AT	PA
	Author (Avtor)		DS	PO	Č	PS	PS
	Diagnostic species of the association *Rhinantho freynii-Trisetetum* (Diagnostične vrste asocacije)						
MA	*Trisetum flavescens*	E1	100	90	86	98	91
MA	*Helictotrichon pubescens*	E1	88	50	43	68	15
FB	*Medicago lupulina*	E1	79	30	.	30	45
FB	*Rhinanthus freynii*	E1	75	30	.	84	21
FB	*Plantago media*	E1	71	70	.	48	26
FB	*Ranunculus bulbosus*	E1	46	.	100	25	.
	Diagnostic species of the association *Centaureo-Arrhenatheretum* (Diagnostične vrste asociacije)						
MA	*Pimpinella major*	E1	79	100	43	27	9
MA	*Knautia arvensis*	E1	79	70	57	27	57
Mo	*Colchicum autumnale*	E1	54	60	.	48	9
MA	*Heracleum sphondylium*	E1	33	80	.	25	74
FB	*Silene vulgaris* subsp. *vulgaris*	E1	25	80	.	27	36
Mo	*Centaurea carniolica*	E1	8	50	.	.	.
MA	*Anthriscus sylvestris*	E1	4	60	.	.	.
EA	*Myosotis sylvatica*	E1	4	40	.	.	.
	Diagnostic species of the association *Ranunculo bulbosi-Arrhenatheretum* (Diagnostične vrste asociacije)						
FB	*Salvia pratensis*	E1	75	90	.	75	49
FB	*Ranunculus bulbosus*	E1	46	.	100	25	.

	Successive number (Zaporedna številka)		1	2	3	4	5
FB	*Carex caryophyllea*	E1	33	.	.	.	.
FB	*Pimpinella saxifraga*	E1	29	.	43	55	46
FB	*Silene nutans*	E1	17	10	29	20	30
TG	*Clinopodium vulgare*	E1	13	20	.	16	11
FB	*Carex montana*	E1	8	.	.	5	2
FB	*Linum catharticum*	E1	8	10	.	5	.
FB	*Trifolium montanum*	E1	8	30	14	5	4
FB	*Carlina acaulis*	E1	4	20	.	27	9
KC	*Lychnis viscaria*	E1	.	.	14	.	.
	Diagnostic species of the association *Astrantio-Trisetetum* (Diagnostične vrste asociacije)						
MA	*Astrantia major*	E1	.	.	.	61	2
FB	*Carex montana*	E1	8	.	.	5	2
FB	*Linum catharticum*	E1	8	10	.	5	.
QF	*Listera ovata*	E1	8	10	.	5	.
	Diagnostic species of the association *Pastinaco-Arrhenatheretum* (Diagnostične vrste asociacije)						
MA	*Arrhenatherum elatius*	E1	96	100	100	57	79
MA	*Campanula patula*	E1	33	30	29	27	32
MA	*Pastinaca sativa*	E1	4	40	.	5	87
MA	*Geranium pratense*	E1	.	.	.	5	.
MA	***Molinio-Arrhenatheretea***						
	Leucanthemum ircutianum	E1	100	60	43	89	89
	Ranunculus acris	E1	96	100	100	32	34
	Leontodon hispidus	E1	92	90	57	66	96
	Rumex acetosa	E1	92	100	57	11	15
	Dactylis glomerata	E1	92	70	29	98	85
	Galium mollugo agg. *(G. album)*	E1	88	80	29	66	55
	Trifolium pratense	E1	88	100	86	55	83
	Poa pratensis	E1	83	40	29	18	32
	Holcus lanatus	E1	79	30	86	48	72
	Lotus corniculatus	E1	79	80	57	82	87
	Plantago lanceolata	E1	79	80	57	52	89
	Achillea millefolium	E1	76	80	43	86	55
	Cerastium holosteoides	E1	76	60	29	.	.
	Festuca pratensis	E1	71	70	43	41	68
	Centaurea jacea	E1	71	.	43	84	70
	Cynosurus cristatus	E1	67	10	14	23	28
	Trifolium repens	E1	58	50	14	16	.
	Daucus carota	E1	58	10	.	11	32
	Lolium perenne	E1	58	.	.	5	17
	Tragopogon pratense subsp. *orientalis*	E1	54	80	100	52	62
	Festuca rubra	E1	50	40	86	36	19
	Crepis biennis	E1	50	40	.	34	38
	Lychnis flos-cuculi	E1	38	.	14	23	4
	Vicia cracca	E1	38	90	.	77	91

	Successive number (Zaporedna številka)		1	2	3	4	5
	Veronica chamaedrys	E1	38	70	71	45	13
	Campanula patula	E1	33	30	29	27	32
	Taraxacum officinale	E1	33	50	43	18	13
	Allium scorodoprasum	E1	29	.	.	.	.
	Lathyrus pratensis	E1	29	80	.	57	43
	Stellaria graminea	E1	25	10	14	93	70
	Ajuga reptans	E1	25	10	.	5	38
	Carum carvi	E1	25	70	.	.	.
	Prunella vulgaris	E1	21	20	.	64	40
	Poa trivialis	E1	21	.	14	16	6
	Bellis perennis	E1	21	.	.	93	30
	Vicia sepium	E1	21	40	.	7	.
	Euphrasia rostkoviana	E1	13	10	.	30	.
	Medicago sativa	E1	13	10	.	.	4
	Achillea roseoalba	E1	8	20	.	.	.
	Bromus hordeaceus	E1	8	.	14	7	34
	Carex hirta	E1	8	.	14	.	.
	Phleum pratense	E1	8	.	.	18	62
	Agropyron repens	E1	8	.	.	.	6
	Alchemilla xanthochlora	E1	4	60	.	50	19
	Ornithogalum umbellatum	E1	4	20	.	.	.
	Pastinaca sativa	E1	4	40	.	5	87
	Alopecurus pratensis	E1	4	.	29	20	11
	Festuca arundinacea	E1	4	.	.	.	.
	Plantago major	E1	4	.	.	2	4
	Trifolium campestre	E1	4	.	.	11	23
	Alchemilla vulgaris	E1	4	.	.	.	.
	Trifolium patens	E1	4	.	.	.	.
	Veronica serpyllifolia	E1	4	.	.	.	.
	Viola tricolor	E1	.	30	.	7	2
	Agrostis stolonifera	E1	.	10	.	.	.
	Orobanche minor	E1	.	10	.	.	.
	Senecio gaudinii	E1	.	10	.	.	.
	Moenchia mantica	E1	.	.	14	.	.
	Senecio jacobea	E1	.	.	14	.	.
	Symphytum officinale	E1	.	.	14	.	100
	Astrantia major	E1	.	.	.	61	2
	Deschampsia cespitosa	E1	.	.	.	16	17
	Phleum phleoides	E1	.	.	.	.	47
	Ranunculus repens	E1	.	.	.	.	23
	Lolium multiflorum	E1	.	.	.	.	4
MC	***Molinietalia caeruleae***						
	Betonica officinalis	E1	4	10	14	34	4
	Carex panicea	E1	4	.	.	.	.
	Primula elatior	E1	4	.	.	.	.

	Successive number (Zaporedna številka)		1	2	3	4	5
	Primula x digenea	E1	4	.	.	.	.
	Equisetum arvense	E1	4	.	14	11	4
	Herminium monorchis	E1	.	.	14	.	.
	Sanguisorba officinalis	E1	.	.	14	.	.
	Cirsium oleraceum	E1	.	.	.	75	21
	Inula salicina	E1	.	.	.	23	2
	Valeriana officinalis	E1	.	.	.	14	.
	Molinia caerulea	E1	.	.	.	7	4
	Alchemilla glabra	E1	.	.	.	5	.
	Succisa pratensis	E1	.	.	.	5	
PaT	***Poo alpinae-Trisetetalia***						
	Anthoxanthum odoratum	E1	71	50	71	80	72
	Agrostis capillaris	E1	29	20	.	.	.
	Ranunculus nemorosus	E1	13	.	.	.	4
	Trollius europaeus	E1	.	70	.	16	.
	Poa alpina	E1	.	10	.	5	.
	Campanula scheuchzeri	E1	.	.	.	66	.
FB	***Festuco-Brometea***						
	Briza media	E1	50	50	57	91	64
	Polygala comosa	E1	38	.	.	2	.
	Thymus pulegioides	E1	38	20	.	48	6
	Bromopsis erecta	E1	33	.	.	86	62
	Brachypodium rupestre	E1	29	20	.	2	4
	Festuca rupicola	E1	21	10	43	.	.
	Orobanche gracilis	E1	21	10	.	.	.
	Arabis hirsuta	E1	17	10	.	.	.
	Sanguisorba minor s. lat.	E1	17	20	.	16	23
	Buphthalmum salicifolium	E1	13	10	.	55	.
	Filipendula vulgaris	E1	13	.	43	20	11
	Koeleria pyramidata	E1	13	10	.	.	.
	Orchis ustulata	E1	13	.	.	2	.
	Anthyllis vulneraria	E1	8	.	.	61	23
	Centaurea scabiosa s. lat.	E1	8	.	.	27	11
	Cirsium erisithales	E1	8	10	.	.	.
	Galium verum	E1	8	.	57	57	43
	Hieracium bauhinii	E1	8	.	14	5	11
	Knautia illyrica	E1	8	.	.	.	.
	Orchis tridentata	E1	8	.	.	.	.
	Peucedanum oreoselinum	E1	8	.	71	.	.
	Ranunculus polyanthemophyllus	E1	8	.	.	.	.
	Thlaspi praecox	E1	8	.	.	.	.
	Campanula rapunculus	E1	4	.	.	.	.
	Campanula glomerata	E1	4	10	.	25	51
	Campanula rotundifolia	E1	4	90	.	2	.
	Cirsium pannonicum	E1	4	.	.	5	2

	Successive number (Zaporedna številka)		1	2	3	4	5
	Danthonia alpina	E1	4	.	.	.	.
	Euphorbia verrucosa	E1	4	.	.	.	.
	Festuca valesiaca	E1	4	.	.	.	.
	Genista tinctoria	E1	4	.	.	.	.
	Gentianella ciliata	E1	4	.	.	.	.
	Gymnadenia conopsea	E1	4	10	.	39	6
	Helianthemum ovatum	E1	4	.	.	30	9
	Medicago falcata	E1	4	.	.	2	2
	Ononis spinosa	E1	4	.	.	5	11
	Orchis morio	E1	4	.	.	.	.
	Scabiosa triandra	E1	4	.	.	14	4
	Gentianella germanica	E1	.	10	.	9	.
	Thesium linophyllon	E1	.	.	43	.	.
	Euphorbia cyparissias	E1	.	.	14	34	4
	Prunella grandiflora	E1	.	.	.	43	9
	Allium carinatum	E1	.	.	.	32	11
	Cuscuta epythimum	E1	.	.	.	25	9
	Dianthus hyssopifolius	E1	.	.	.	25	.
	Asperula cynanchica	E1	.	.	.	18	.
	Scabiosa columbaria	E1	.	.	.	7	2
	Senecio integrifolius	E1	.	.	.	7	.
	Carlina vulgaris	E1	.	.	.	5	2
	Gentiana cruciata	E1	.	.	.	5	.
	Melica ciliata	E1	.	.	.	5	.
	Veronica teucrium	E1	.	.	.	5	34
	Galium lucidum	E1	.	.	.	.	9
	Centaurium erythrea	E1	.	.	.	.	4
KC	***Koelerio-Corynophoretea***						
	Arenaria serpyllifolia	E1	8	.	.	.	.
	Sedum sexangulare	E1	.	20	.	.	.
	Thlaspi perfoliatum	E1	.	10	.	.	.
	Dianthus deltoides	E1	.	.	14	.	.
	Poa compressa	E1	.	.	14	.	.
	Petrorhagia saxifraga	E1	.	.	.	11	4
	Sedum reflexum	E1	.	.	.	.	13
ES	***Elyno-Seslerietea***						
	Scorzonera rosea	E1	.	10	.	.	.
	Acinos alpinus	E1	.	10	.	.	.
	Betonica alopecurus	E1	.	10	.	.	.
	Carduus defloratus	E1	.	10	.	20	.
	Euphrasia picta	E1	.	10	.	20	.
	Galium anysophyllum	E1	.	10	.	.	.
	Phyteuma orbiculare	E1	.	10	.	.	.
	Biscutella laevigata	E1	.	.	.	48	.
	Polygonum viviparum	E1	.	.	.	16	2
	Ranunculus carinthiacus	E1	.	.	.	34	.

	Successive number (Zaporedna številka)		1	2	3	4	5
	Ranunculus montanus	E1	.	.	.	7	.
	Rhinanthus glacialis	E1	.	.	.	5	.
	Knautia longifolia	E1	.	.	.	5	2
CD	***Caricetalia davallianae***						
	Tofieldia calyculata	E1	.	.	.	32	.
	Astrantia carniolica	E1	.	.	.	16	.
	Parnassia palustris	E1	.	.	.	7	.
CU	***Calluno-Ulicetea***						
	Luzula campestris	E1	45	.	71	66	30
	Rhinanthus minor	E1	42	10	57	.	.
	Carex pallescens	E1	21	.	.	18	4
	Veronica officinalis	E1	8	.	.	2	.
	Chamaespartium sagittale	E1	4	.	29	5	9
	Festuca filiformis	E1	4	.	.	.	.
	Phyteuma zahlbruckneri	E1	4	30	.	32	4
	Polygala vulgaris	E1	4	.	.	30	19
	Potentilla erecta	E1	4	10	14	82	17
	Galium pumilum	E1	.	20	.	.	.
	Luzula multiflora	E1	.	20	.	.	.
	Rumex acetosella	E1	.	.	29	52	28
	Carex leporina	E1	.	.	14	.	.
	Hypochoeris radicata	E1	.	.	14	.	.
	Arnica montana	E1	.	.	.	18	.
	Leontodon helveticus	E1	.	.	.	16	4
	Nardus stricta	E1	.	.	.	7	38
	Holcus mollis	E1	.	.	.	7	4
	Carex pilulifera	E1	.	.	.	7	.
	Campanula barbata	E1	.	.	.	5	.
	Antennaria dioica	E1	.	.	.	2	.
	Calluna vulgaris	E1	.	.	.	2	.
TG	***Trifolio-Geranietea***						
	Dianthus barbatus	E1	13	30	.	2	2
	Viola hirta	E1	13	.	.	.	.
	Astragalus glycyphyllos	E1	8	.	.	.	.
	Campanula rapunculoides	E1	8	.	.	.	2
	Hypericum perforatum	E1	8	10	14	18	26
	Lilium bulbiferum	E1	4	10	.	11	2
	Thalictrum minus	E1	4	.	.	.	.
	Verbascum nigrum	E1	4	.	.	.	.
	Vicia incana	E1	.	40	.	.	.
	Anntherícum ramosum	E1	.	10	.	20	4
	Vicia sylvatica	E1	.	10	.	.	.
	Hieracium umbellatum	E1	.	.	14	.	.
	Trifolium alpestre	E1	.	.	.	75	21
	Trifolium medium	E1	.	.	.	70	83

	Successive number (Zaporedna številka)		1	2	3	4	5
	Laserpitium latifolium	E1	.	.	.	14	6
	Vincetoxicum hirundinaria	E1	.	.	.	14	.
	Lilium carniolicum	E1	.	.	.	7	.
	Origanum vulgare	E1	.	.	.	5	4
	Polygonatum odoratum	E1	.	.	.	5	.
	Trifolium rubens	E1	.	.	.	5	.
MuA	***Mulgedio-Aconitetea***						
	Veratrum album subsp. *lobelianum*	E1	4	.	.	11	2
	Carduus carduelis	E1	.	.	.	34	.
	Chaerophyllum aureum	E1	.	70	.	.	.
	Chaerophyllum hirsutum	E1	.	70	.	14	9
	Geranium sylvaticum	E1	.	60	.	.	.
	Hypericum maculatum	E1	.	10	.	.	.
	Lathyrus occidentalis	E1	.	10	.	.	.
	Phyteuma ovatum	E1	.	10	.	45	9
	Silene dioica	E1	.	10	.	.	.
	Thalictrum aquilegiifolium	E1	.	.	.	5	.
EA	***Epilobietea angustifolii***						
	Carex muricata	E1	4	.	.	.	.
	Cirsium arvense	E1	4	.	.	2	4
	Fragaria vesca	E1	.	.	14	.	.
	Carex spicata	E1	.	.	.	5	.
GU	***Galio-Urticetea***						
	Aegopodium podagraria	E1	4	20	.	.	.
	Lamium album	E1	.	20	.	.	.
	Cirsium eriophorum	E1	.	.	.	2	4
	Salvia verticillata	E1	.	.	.	2	6
SM	***Stellarietea mediae***						
	Erigeron annuus	E1	28	.	29	7	26
	Myosotis arvensis	E1	24	.	.	.	.
	Veronica arvensis	E1	20	10	29	.	.
	Convolvulus arvensis	E1	8	.	.	2	32
	Silene latifolia subsp. *alba*	E1	8	10	.	.	.
	Rumex obtusifolius	E1	4	.	14	7	9
	Vicia hirsuta	E1	4	10	.	.	.
	Vicia sativa	E1	.	10	.	.	.
	Capsella bursa-pastoris	E1	.	.	.	14	30
	Vaccaria pyramidata	E1	.	.	.	2	4
	Poa annua	E1	.	.	.	.	32
	Potentilla reptans	E1	.	.	.	.	60
EP	***Erico-Pinetea***						
	Aquilegia atrata	E1	.	30	.	.	.
	Knautia ressmanii	E1	.	10	.	.	.
	Polygala chamaebuxus	E1	.	.	.	.	6

	Successive number (Zaporedna številka)		1	2	3	4	5
VP	***Vaccinio-Piceetea***						
	Deschampsia flexuosa	E1	.	.	.	43	19
	Luzula luzuloides	E1	.	.	.	14	.
	Maianthemum bifolium	E1	.	.	.	9	.
	Calamagrostis arundinacea	E1	.	.	.	7	4
	Gentiana asclepiadea	E1	.	.	.	5	2
	Luzula luzulina	E1	.	.	.	5	.
FS	***Fagetalia sylvaticae***						
	Knautia drymeia	E1	21	.	.	77	87
	Carex sylvatica	E1	8	.	.	.	.
	Phyteuma spicatum subsp. *coeruleum*	E1	4	.	.	.	.
	Campanula trachelium	E1	.	10	.	9	6
	Lilium martagon	E1	.	10	.	.	.
	Helleborus niger	E1	.	.	.	9	.
	Melica nutans	E1	.	.	.	7	2
QF	***Querco-Fagetea***						
	Cruciata glabra	E1	21	20	29	80	23
	Carex flacca	E1	13	.	.	27	17
	Listera ovata	E1	8	10	.	5	.
	Dactylorhiza fuchsii	E1	4	.	.	18	.
	Ornithogalum pyrenaicum	E1	4	.	.	.	.
	Primula vulgaris	E1	4	.	.	32	4
	Aquilegia vulgaris	E1	.	20	.	16	.
	Primula veris	E1	.	20	.	5	.
	Crocus vernus	E1	.	.	.	38	89
	Melampyrum pratense	E1	.	.	.	11	.
	Serratula tinctoria	E1	.	.	.	5	.
	Chamaecytisus supinus	E1	.	.	.	5	.
O	***Other species*** **(Druge vrste)**						
	Agrostis sp.	E1	4	.	.	.	.
	Alchemilla sp.	E1	4	.	.	.	.
	Knautia sp.	E1	4	.	.	.	.
	Orobanche sp.	E1	4	.	.	11	9
	Achillea sp.	E1	.	.	.	30	9
	Sedum sp.	E1	.	.	.	23	.
	Aquilegia sp.	E1	.	.	.	7	.

Legend - Legenda

RfT *Rhinantho freynii-Trisetetum*, this article (ta članek);
CcA *Centaureo carniolicae-Arrhenatheretum* f. *montana*, Poldini and Oriolo 1994, Table 1, rel. 13-22;
RbA – *Ranunculo bulbosi-Arrhenatheretum*, Čarni 2003, Table 1, rel. 1-7;
AT – *Astrantio-Trisetetum*, Petras Sackl et al. 2012, Table 1;
PA - *Pastinaco-Arrhenatheretum*, Petras Sackl et al. 2012, Table 2.
DS Dakskobler, Seliškar
PO Poldini, Oriolo
Č Čarni
PS Petras Sackl et al.

Table 3: Phytosociological structure of *Trisetum flavescens* and *Arrhenatherum elatius* dominating (sub) montane grasslands in Slovenia and NE Italy (relative frequencies)

Preglednica 3: Sestava po diagnostičnih vrstah v (sub)montanskih travnikih s prevladujočima vrstama *Trisetum flavescens* in *Arrhenatherum elatius* v Sloveniji in severovzhodni Italiji (relativne frekvence)

Successive number (Zaporedna številka)	1	2	3	4	5
Number of relevés (Število popisov)	24	10	7	44	47
Sign for the syntaxa (Oznaka sintaksonov)	RfT	CcA	RbA	AT	PA
Author (Avtor)	DS	PO	Č	PS	PS
Molinio-Arrhenatheretea	**62**	**59**	**60**	**40**	**58**
Molinietalia caeruleae	1,9	2,8	2,1	**4,3**	1,1
Poo alpinae-Trisetetalia	2,7	**3,5**	2,6	3,2	1,9
Festuco-Brometea	**22**	**16**	**20**	**24**	**18**
Koelerio-Corynophoretea	0,2	0,7	1,6	0,2	0,4
Elyno-Seslerietea, Caricetalia davallianae	0,1	1,6	0	**4,1**	0,1
Calluno-Ulicetea	**3,2**	2,1	**8,5**	**6,8**	**4**
Trifolio-Geranietea	1,8	**3,1**	1	5,1	4,1
Mulgedio-Aconitetea	0,1	**5,6**	0	2,1	0,5
Epilobietea angustifolii	0,3	0,9	0,5	0,1	0,1
Stellarietea mediae, Galio-Urticetea	2,6	1,9	2,7	0,7	**5,2**
Vaccinio-Piceetea, Erico-Pinetea	0	0,9	0	1,6	0,8
Fagetalia sylvaticae	0,9	0,5	0	2	2,4
Querco-Fagetea	1,2	1,6	1,1	**4,7**	**3,4**
Other species (Druge vrste)	0,4	0	0	1,4	0,5
Skupaj (Total)	100	100	100	100	100

Legend - Legenda

See Table 2 (glej preglednico 2)

ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 61–75

Extraction of DNA from different sample types – a practical approach for GMO testing

Ekstrakcija DNA iz različnih vrst vzorcev -praktični pristop za določanje GSO

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Abstract: Current methods based on DNA targets for the detection, identification and quantification of genetically modified organisms (GMOs) involve extraction of the DNA. Different extraction procedures have been developed for the great variety of samples from food, feed, seeds and particular plant parts. This makes the operation of routine analytical laboratories complex and workloads heavy. Here we present a decision-making system, developed over many years of GMO testing on different samples, that result in the application of only a few extraction methods for the majority of samples. Developed decision-making system enables quicker and more cost effective testing of GMOs. In addition, the performance of DNA extraction resulting from the use of the selected extraction methods is presented for use in subsequent testing of GMOs by real time PCR methods. This approach can be used as a model for similar systems based on nucleic acid analysis in food, feed, seeds and plants.

Keywords: Extraction methods, Genetically modified organisms (GMO), Decision-making system, GMO testing, NucleoSpin® Food, Cetyltrimethylammonium bromide (CTAB)

Izvleček: Metode za določanje, identifikacijo in kvantifikacijo gensko spremenjenih organizmov (GSO) temeljijo na zaznavanju značilnih zaporedij DNA, zato je ključni del metode ekstrakcija DNA. Za ekstrakcijo DNA iz različnih vrst vzorcev, kot so živila, krma, semena in deli rastlin, so razviti različni postopki. Delo rutinskih laboratorijev je zato zelo kompleksno in obsežno. Tu predstavljamo sistem odločanja, ki smo ga razvili v mnogih letih testiranja GSO v različnih vzorcih. Z uporabo nekaj izbranih metod ekstrakcije lahko analiziramo večino vzorcev na hitrejši in finančno učinkovitejši način. Dodatno podajamo informacijo o uporabi izbranih ekstrakcijskih metod v povezavi z nadaljnjo analizo GSO s PCR v realnem času. Ta pristop se lahko uporabi kot model za podobne sisteme, ki temeljijo na analizi nukleinskih kislin v živilih, krmi, semenih in rastlinah.

Ključne besede: Metode ekstrakcije, Gensko spremenjeni organizmi (GSO), Sistem odločanja, GSO testiranje, NucleoSpin® Food, Cetiltrimetilamonijev bromid (CTAB)

Introduction

Efficient and reliable extraction of nucleic acids is a prerequisite for obtaining accurate results from molecular analyses such as amplification of specific DNA or RNA sequences by polymerase chain reaction (PCR). The extraction method should yield sufficient DNA of adequate structural integrity and purity, independently of the matrix to which it is applied (Codex Committee On Methods Of Analysis And Sampling 2010). Acceptance criteria applicable to DNA extraction methods were recently defined in the new European Network of GMO Laboratories (ENGL) document on Definition of minimum performance requirements for analytical methods of GMO testing (ENGL 2015).

GMOs are already widespread and countries have different regulations and policies for their management (http://bch.cbd.int/). Many countries have labeling requirements for products containing GMOs, demanding methods for their detection and for their analysis in order to control approved and unapproved GMOs. Some countries have also set thresholds for the unintended presence of GMOs in a product, requiring quantitative analysis of GMOs. Testing for genetically modified organisms (GMOs) is based on multiplication of the genetic elements characteristic of the GMOs, therefore a DNA extraction procedure is crucial (Žel et al. 2012). Only DNA of certain quality is amplifiable in further multiplication using molecular methods. Real-time PCR is the technology of choice for detecting GMOs, especially when quantitative analyses are needed (qPCR) (Žel et al. 2012), even PCR is also used by some laboratories. Other methods, such as isothermal multiplication and digital PCR, that are under rapid development, are also dependent on a good quality extraction method (Milavec et al. 2014).

GMOs have been detected in various samples – food, feed, seeds and plants from the field. Each sample may contain only one ingredient, *e.g.* maize seeds, or a number of ingredients, such as feed composed of a variety of grains, *e.g.* maize, rice and soybean. Additionally, particular processed samples, such as corn flakes, can differ, depending on the manufacturing procedure and the DNA can be present in low amounts and also degraded. As early as 2006, Cankar et al. pointed out that extraction technique and sample properties have a crucial influence on the results of GMO detection and quantification (Cankar et al. 2006). Molecules of plant origin or from other sources, even components of DNA extraction solutions can influence PCR reactions. During GMO quantification standard curve using certified reference material is used, therefore similarity of PCR efficiency for the sample and certified reference material is a prerequisite for exact quantification. The appropriate extraction method has to be determined for each sample type. Detailed guidelines have been reported for validating the extraction methods applied prior to PCR examination of food and feed products for the presence of genetically modified material (Waiblinger and Grohmann 2014; ENGL 2011).

Selection of methods for DNA extraction and purification is mostly done on experience-based choice of the user, as noted in ISO 21571:2005 that deals with nucleic acid extraction for detecting GMOs (ISO 21571:2005; ISO 21571:2005/A1 2013). Some extraction methods have already been proposed in this standard, together with their scope and examples of samples for each method. Specific extraction methods have been proposed for challenging samples such as pollen from honey (Waiblinger et al. 2012), soybean lecithin (ISO 21571:2005/A1 2013*)* or choline chloride (Sacco et al. 2014). The influence of extraction method and of sample characteristics on the quality and yield of DNA extracted from different samples has been reviewed (Gryson 2010; Demeke and Jenkins 2010). The suitability of different extraction methods for different samples, the effects of sample processing, the presence of PCR inhibitors in the extraction reagents and in the samples themselves are reviewed, together with the theory behind the effects.

One extraction method can be more suitable for a particular sample than another, but it would be very challenging to test all available methods on all samples. It is worthwhile to mention also that there are different quantification procedures for quantification of extracted nucleic acids however, the methods produce different results, making it unwise to try to compare data obtained with the different methods (Bustin et al. 2009).

Using different extraction methods for different samples is also time and cost wasting for labora-

tories testing many samples daily. It is therefore essential to set up, in the laboratory, a manageable decision-making system that can support selection of the extraction method appropriate for an individual sample.

Here we present a decision-making system for selecting extraction methods for GMO testing on different samples – food, feed, seeds and whole plants. It has been developed over many years of GMO testing at the National Institute of Biology, Ljubljana, where various extraction methods have been tested for individual samples. Additionally, and in line with the decision-making system, data relating the extraction method appropriate for further testing of GMOs with real time PCR to a given sample type, based on practical experience, are tabulated.

Materials and methods

Samples

Samples were taken from routine analyses, including seeds/grains, plant leaves and various food and feed samples (for details see also Tab. 1-6). Each sample (*e.g.* soybean flour, maize corn flakes etc.) was tested at least 5 times from independent consignments.

Homogenization

Most of the samples (seeds, feed, soybean meal, choline chloride, rice, and pasta) were ground with a Retsch ZM200 rotor mill. Some (usually with high fat content, *e.g.* oilseed rape seeds) were cooled with liquid nitrogen before grinding with a Retsch GM200 knife mill. Samples such as sausage or tofu were homogenized using a Bioreba HOMEX 6 homogenizer. Leaf samples were prepared by cutting off small pieces from different leaves, then combining and homogenizing them together as one sample with a FASTprep instrument (MP Biomedicals) using 15ml tubes, a ceramic ball and quartz sand.

DNA extraction

200 mg of homogenized sample was used for all isolations, unless stated otherwise.

Different extraction methods were used (see Tab. 1 to 6).

NucleoSpin® Food (Macherey-Nagel) was used as described by the manufacturer. In lysis step more than 200 mg of sample can be used when needed (e.g. when small amounts of DNA in the sample can be expected) with scaled up lysis buffer volumes and in this case, after lysis, double the amount of supernatant was used for further processing.

Cetyltrimethylammonium bromide (CTAB) method with RNase-A and proteinase-K solutions for removing RNA and proteins from the sample was performed as described in ISO 21571, Annex A.3 – Preparation of PCR-quality DNA using the CTAB-based DNA extraction methods (ISO 21571:2005). Removal of lipid components from chocolate and lecithin was performed using hexane prior the first step of the CTAB method (Solfizzo et al. 1998). For samples with low amount of DNA up to 20 g of sample was used and further steps of DNA extraction were done with larger volumes of chemicals.

Samples of oil were first centrifuged and pellet was used for DNA extraction using NucleoSpin® Food (Macherey-Nagel) (Costa et al. 2010).

The DNeasy Plant Mini Kit (Qiagen) was used to extract DNA from plant leaves, as described by the manufacturer.

All extractions from each sample were carried out in duplicate.

qPCR

The quality of extracted DNA was checked by measuring the efficiency of amplification of reference taxon specific genes by qPCR, using appropriate dilutions of both DNA extractions *e.g.* 10x and 100x (Žel et al. 2012), with the exception of stable sample types (for explanation see results section), for which a second dilution was made only for one extraction. Each dilution of DNA was analyzed in duplicate.

Negative control of extraction and environmental control, no template control and positive control were included. The following qPCR methods were applied: for soybean, the method targeting the lectin Le1 gene (*Le1*) (Pauli et al. 2001), for maize, the method targeting the high mobility group protein gene (*hmga*) (Hernandez

et al. 2004); for oilseed rape the method targeting the phosphoenolpyruvate carboxylase gene (*PEP*) (Zeitler et al. 2002); for rice the method targeting the phospholipase D gene (*PLD*) (Mazzara et al. 2006); for potato and tomato the method targeting the potato and tomato specific metallo-carboxy-peptidase inhibitor gene (*POT, TOM*) (Hernandez et al. 2003) and, for flax, the method targeting the stearoyl-acyl carrier protein desaturase gene (*SAD*) (Genetic ID NA Inc. 2009).

The method targeting the 18S rRNA gene was used for confirmation of DNA extraction when insufficient reference gene was present (Eukaryotic 18S rRNA Endogenous Control, Life Technologies, Part No.:4319413E).

qPCRs were randomly run on the ABI PRISM® 7900 HT sequence detection system, ViiA™ 7 (both Life Technologies) or LightCycler® 480 System (Roche). Reactions were performed in 10 µl reaction mixture, using 2 µl of diluted DNA, in a 384-well plate. All qPCR assays were performed using TaqMan® Universal PCR Master Mix (Life Technologies, Part No.: 4304437). PCR cycling conditions were set to 2 min at 50°C and 10 min at 95°C followed by 45 cycles of 15 s at 95°C and 1min at 60°C.

Table 1: Extractions used for soybean samples.
Tabela 1: Ekstrakcije uporabljene za vzorce soje.

Sample	Stability of sample	Test portion	DNA extraction method[a]	Elution volume (µl)	Volume of water added after elution (µl)	Cq value obtained on reference gene
Seeds/grains						
grains	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	800 µl	24-27
Food						
flour	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	800 µl	24-27
tofu-soybean curd	stable	500 mg	NucleoSpin® Food	100 µl+100 µl	300 µl	21-23
soybean drink	variable	500 ul - 1 ml	NucleoSpin® Food	100 µl+100 µl	50 µl	> 22[b]
Frankfurt sausage, sausage, cold meats	variable	500 mg to 1 g	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
flackes, cracker, crispies	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
soy steaks, burger, medallion	variable	200 mg to 1 g	NucleoSpin® Food	50 µl + 50 µl	/	> 24[b]
soy spread	variable	1 g to 5 g	NucleoSpin® Food	50 µl + 50 µl	/	> 23[b]
soybean meat, soybean peaces,	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 23[b]
soy nuggets	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
biscuits	variable	at least 5 g	CTAB	100 µl	/	> 22[b]
lecithin	Variable	at least 15 g	hexane + CTAB	100 µl	/	> 28[b]
chocolate	Variable	20 g	hexane + CTAB	100 µl	/	> 34[b]
Soya desert	variable	10 g	CTAB	100 µl	/	> 23
Feed						
animal feed	variable	200 mg	NucleoSpin® Food	100 µl+100 µl	300 µl	> 22[b]
soybean meal	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	800 µl	24-27
soy proteins	variable	200 mg	NucleoSpin® Food	100 µl+100 µl	300 µl	> 22[b]

[a] when more than 200mg was used for the NucleoSpin® Food extraction, the starting procedure was modified as described in Materials and Methods
[b] actual value depends on the DNA content and processing

Table 2: Extractions used for maize samples.
Tabela 2: Ekstrakcije uporabljene za vzorce koruze.

Sample	Stability of sample	Test portion	DNA extraction method[a]	Elution volume (µl)	Volume of water added after elution (µl)	Cq value obtained on reference gene
Seeds/grains						
kernels	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	/	20-24
Food						
flour	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	/	20-24
semolina	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	/	20-24
corn cobs	stable	200 mg to 1g	NucleoSpin® Food	100 µl+100 µl	/	20-24
corn flakes	stable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	23-25
chips, tortilla chips	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
tortilla	variable	200 mg till 2g	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
canned corn	stable	1 g	NucleoSpin® Food	50 µl + 50 µl	/	20-27
popcorn chips	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 25[b]
bread	variable	5 g	NucleoSpin® Food	50 µl + 50 µl	/	> 25[b]
crunchy muesli	variable	at least 5 g	CTAB	100 µl	/	> 30[b]
corn gluten	variable	2 g	CTAB	100 µl	/	> 23[b]
Feed						
feed	variable	200 mg	NucleoSpin® Food	100 µl+100 µl	300 µl	> 22[b]
Leaves						
maize leaves	stable	200 mg to 1 g	CTAB / DNeasy Plant Mini kit	100 µl / 50 µl + 50 µl	/	20-27 / 24-30

[a] when more than 200mg was used for the NucleoSpin® Food extraction, the starting procedure was modified as described in Materials and Methods
[b] actual value depends on the DNA content and processing

Table 3: Extractions used for samples with oilseed rape and flax.
Tabela 3: Ekstrakcije uporabljene za vzorce oljne ogrščice in lanu.

Plant species	Sample	Stability of sample	Test portion	DNA extraction method[a]	Elution volume (µl)	Volume of water added after elution (µl)	Cq value obtained on reference gene
seed rape	**Seeds**						
	seeds	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	300 µl	22-26
	Feed						
	Oilseed cake	variable	200 mg	NucleoSpin® Food	100 µl+100 µl	300 µl	> 23[b]
	Leaves						
	leaves	stable	200 mg to 1 g	CTAB / DNeasy Plant Mini kit	100 µl / 50 µl + 50 µl	/	20-27 / 24-30
flax	**Seeds**						
	seeds	stable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	22 - 26

[a] when more than 200mg was used for the NucleoSpin® Food extraction, the starting procedure was modified as described in Materials and Methods
[b] actual value depends on the DNA content and processing

Table 4: Extractions used for samples containing rice.
Tabela 4: Ekstrakcije uporabljene za vzorce riža.

Sample	Stability of sample	Test portion	DNA extraction method[a]	Elution volume (µl)	Volume of water added after elution (µl)	Cq value obtained on reference gene
Seeds/grains						
grain	stable	200 mg	NucleoSpin® Food	100 µl+100 µl	50µl	22-24
Food						
waffels	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 26[b]
rice drink	variable	1 ml	NucleoSpin® Food	100 µl+100 µl	50µl	> 22[b]
rice pudding	variabe	10 g	NucleoSpin® Food	50 µl + 50 µl	/	> 26[b]
spaghetti	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
rice cracker	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 28[b]
boiled rice grains	variable	500 mg to 5g	NucleoSpin® Food	50 µl + 50 µl	/	> 26[b]
rice bread	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 24[b]
choline chloride (rice)	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 27[b]
Feed						
dog food with rice	variable	200 mg	NucleoSpin® Food	50 µl + 50 µl	/	> 24[b]

[a] when more than 200mg was used for the NucleoSpin® Food extraction, the starting procedure was modified as described in Materials and Methods
[b] actual value depends on the DNA content and processing

Table 5: Extractions used for samples with potato.
Tabela 5: Ekstrakcije uporabljene za vzorce krompirja.

Sample	Stability of sample	Test portion	DNA extraction method[a]	Elution volume (µl)	Volume of water added after elution (µl)	Cq value obtained on reference gene
Food						
tuber	stable	0,5 g	NucleoSpin® Food	50 µl + 50 µl	/	> 22[b]
potato puree	variable	1 g	NucleoSpin® Food	50 µl + 50 µl	/	> 30[b]
pommes frites	variable	at least 1 g	NucleoSpin® Food	50 µl + 50 µl	/	> 25[b]
croquettes	variable	at least 1 g	NucleoSpin® Food	50 µl + 50 µl	/	> 28[b]
gnocchi	variable	at least 1 g	NucleoSpin® Food	50 µl + 50 µl	/	> 30[b]
potato starch	variable	1g	CTAB	100 µl	/	> 35 (Ct 18S 25)

[a] when more than 200mg was used for the NucleoSpin® Food extraction, the starting procedure was modified as described in Materials and Methods
[b] actual value depends on the DNA content and processing

Table 6: Extractions used for samples with tomato.
Tabela 6: Ekstrakcije uporabljene za vzorce paradižnika.

Sample	Stability of sample	Test portion	DNA extraction method[a]	Volume of water added after elution (µl)	Expected Cq value obtained on reference gene
Food					
fresh tomato	stable	500 mg	NucleoSpin® Food	/	23-27
concentrate	variable	1 ml	NucleoSpin® Food	/	> 33[b]
ketchup	variable	1 ml	NucleoSpin® Food	/	> 35[b]
peeled tomato (canned)	variable	1 ml	NucleoSpin® Food	/	> 33[b]
Leaves	stable	200 mg to 1 g	CTAB / DNeasy Plant Mini Kit	/	20-27 /24-30

[a] when more than 200mg was used for the NucleoSpin® Food extraction, the starting procedure was modified as described in Materials and Methods
[b] actual value depends on the DNA content and processing

Table 7: Comparison of prices of different extraction methods.
Tabela 7: Primerjava cen različnih izolacijskih metod.

	1 sample			3 samples			10 samples		
Extraction method	Working hours	Chemicals[a]	Final price[b]	Working hours	Chemicals	Final price	Working hours	Chemicals	Final price
NucleoSpin® Food	2,25	6	60	2,5	21	58	6,5	66	94
CTAB	4	5	100	6,5	10	100	9	25	100
CTAB with larger volumes	6	8	127	10	20	203	NA	NA	NA
DNeasy	2,25	10	64	2,5	35	73	6,5	110	109

[a] prices in Slovenia
[b] calculated prices expressed as ratio (CTAB is taken as 100%)

Results and discussion

We divided samples in two categories, known and new sample types, depending on our experiences on their performance during extraction procedure with respect to the efficiency of amplification of reference taxon specific genes by qPCR as explained in Material and Methods section. Known sample types are the ones which we tested already many times and we have experiences on the efficiency of extraction and quality of extracted DNA in contrast with new sample types where no experience have been obtained. Some of the known samples (stable sample types) gave repeatable extraction results, even when obtained from different sources or produced by different manufacturers. Others, (variable sample types), are more variable and unexpected results of DNA extraction can be obtained, so the testing procedure for these samples was adapted.

Some of the samples, known or new, can be also challenging samples, usually containing highly degraded DNA or the presence of inhibitors and they are treated differently.

Testing different extraction methods

Over many years of testing, individual samples were tested in our laboratory by different methods – DNeasy Plant Mini Kit (Qiagen, Valencia, CA), Wizard extraction (Promega, Madison, WI), CTAB based extraction, CTAB extraction and GENESpin kit (GeneScan, Freiburg, Germany) (Cankar et al. 2006), and NucleoSpin® Food. The most important parameter for testing quality of extracted DNA was the qPCR efficiency of amplification of reference taxon specific genes, using appropriate dilutions.

Extraction of DNA from known sample types

For known stable food and feed sample types NucleoSpin® Food was the most successful method, producing the expected yield and quality of DNA. DNA was extracted from leaves with the CTAB method or DNeasy Plant Mini Kit as explained later.

For variable sample types, giving different quantities and qualities of the extracted DNA, some were treated with NucleoSpin® Food and others, mostly more processed ones, with the CTAB method. Sometimes NucleoSpin® Food as the first choice does not give expected result, then CTAB method is applied on the sample.

The CTAB method has been used widely for extracting DNA from leaves, seeds/grains and processed food/feed samples [ISO 21571:2005)]. It was developed in 1980 (Murray and Thompson 1980) and the various versions or modifications of the CTAB protocol are reviewed in Demeke and Jenkins (2010). The procedure is however time-consuming and uses hazardous chemicals including phenol and chloroform (Demeke and Jenkins 2010). The CTAB method was therefore

not the first choice. The larger amounts of extracted DNA are not a decisive factor, since the yields obtained by NucleoSpin® Food are sufficient for further analyses in most of the samples according to our experiences.

In cases where samples contain only small amounts of DNA, larger test portions can be used for extracting DNA. A limited sample size (*e.g.* 20–200 mg) is generally used for DNA extraction with kits (Demeke and Jenkins 2010), so the CTAB method is more appropriate in the case of large test portions (e.g., >200 mg). NucleoSpin® Food was also used successfully for sample sizes greater than 200 mg, with an adapted starting procedure as described in Materials and Methods.

Experiences for selection of extraction methods for different samples, arranged according to the prior knowledge on the presence of the main plant species ingredient, are described further.

Known sample types are presented in the corresponding Tables 1-6, where it is also indicated whether a sample is stable or variable. Further information on the testing procedures is given on the size of the test portion of the analytical sample used for extraction and on elution volumes. Since the quality and quantity of extracted DNA is checked by measuring the effectiveness of amplification and the quantity of the reference gene, the expected quantification cycles (Cq) values are also provided.

- Soybean (*Glycine max* (L.) Merr. Fabaceae) samples

Stable soybean sample types, like grains, flour, and most of the food variable ones (*e.g.* soybean drink, sausages) can be extracted with NucleoSpin® Food (Tab. 1)**.** Some (*e.g.* biscuits and soya desert) are extracted with CTAB. If the extraction of DNA from variable sample types extracted with NucleoSpin® Food does not result in the appropriate quality or quantity of DNA, the CTAB method is used. Hexane is used as the first step in the CTAB method for lecithin and chocolate samples. It is known that DNA is difficult to extract from lecithin. In our laboratory very different Cq values were obtained for lecithin samples.

NucleoSpin® Food has been used without any problem for all feed samples containing soybean as the prevailing ingredient.

- Maize (*Zea mays* subsp. *mays*, Poaceae) samples

As for soybean, NucleoSpin® Food was used successfully for all unprocessed and for most processed maize-based food samples (Tab. 2). Exceptions are crunchy muesli and corn gluten, for which the CTAB method was used.

NucleoSpin® Food was used without any problem for all feed samples containing maize as the prevailing ingredient.

DNA was extracted from leaves using either CTAB or DNeasy Plant Mini Kit. CTAB gives higher yields but enough DNA for reliable qPCR analyses can be obtained with DNeasy Plant Mini Kit and the extraction is much quicker. DNeasy Plant Mini Kit was used when multiple samples of leaves had to be tested at the same time.

- Oilseed rape (*Brassica napus* L., Brassicaceae) and flax (Linum usitatissimum L., Linaceae) samples

NucleoSpin® Food can also be used for oilseed rape and flax seeds (Tab. 3). Extraction of DNA with NucleoSpin® Food was also successful for samples of rapeseed cake, used as animal feed.

DNA from oilseed leaves is extracted by CTAB or DNeasy Plant Mini Kit as described for maize leaves.

- Rice (*Oryza sativa* L., Poaceae) samples

All rice samples tested so far have been extracted by NucleoSpin® Food (Tab. 4). The presence of rice was also examined in samples of choline chloride, which is used as an additive in feed, rice being used in the manufacturing process as a plant derived carrier. A special schedule was written for testing in choline chloride, where a slightly modified CTAB method was proposed for extracting DNA (Sacco et al. 2014). In accordance with our system we have used NucleoSpin® Food as our primary method and found that it gives satisfactory results.

- Potato (*Solanum tuberosum* L., Solanaceae) sample types

DNA from potato tubers and from most pro-

cessed food can be extracted with NucleoSpin® Food with the exception of potato starch, for which the CTAB method is used (Tab. 5). For potato tubers we use cores from potato heel ends, where there is more DNA than in other parts of the tuber.

- Tomato (*Solanum lycopersicum* L., Solanaceae) samples

DNA from tomato fruits can be extracted by NucleoSpin® Food (Tab. 6). NucleoSpin® Food is also the first choice for more processed tomato samples, such as canned tomato, ketchup or tomato concentrate. Extraction of these challenging sample types is occasionally not effective and CTAB with larger test portions has to be used. However, this approach can fail to extract enough DNA for further testing. DNA from tomato leaves is extracted by CTAB or DNeasy Plant Mini Kit as described above.

Extraction of DNA from new sample types

Experiences from known sample types are used also in the samples not previously tested. The successful use of NucleoSpin® Food suggests it as the first choice for new sample types. The extracted DNA is then analyzed for the reference gene with qPCR. NucleoSpin® Food is then accepted for use if qPCR amplification efficiency and quantity of the extracted DNA were adequate; otherwise the CTAB mehod is applied, sometimes with the addition of hexane, when more lipids are present in the sample. If the CTAB method is not successful the sample is treated as a challenging sample.

Extraction of DNA from challenging samples

Some samples are generally known as very challenging for the extraction of DNA, including those containing lecithin, starch, chocolate, canned tomato or oil, those that are highly processed and/or those in which DNA is present in very low amounts or is degraded. The effect of food processing on plant DNA degradation and PCR-based GMO analysis is reviewed in more detail by Gryson (2010). The samples can vary greatly and inhibitors of PCR reaction may be present. Certain additional steps can be used to minimize inhibition, like the addition of proteinase K to degrade proteins or of hexane to remove lipid components (Gryson 2010, Solfizzo et al 1998, Terry et al. 2002). When testing the final product is too difficult, it is advisable to ask for raw material, if available, because the extraction of DNA from the raw material is usually more successful.

Extraction of DNA from oil samples is also difficult, because the DNA remains only in the leftovers of plant material present in the oil. Therefore prior centrifugation of the sample, using the sediment for further extraction with NucleoSpin® Food, is recommended and has been used in our laboratory (Costa et al. 2010).

When testing the effectiveness of DNA extraction by a method targeting the reference gene, the possibility exists that the extraction from the sample with more ingredients was effective but that insufficient reference gene was present. Since preferred reference genes are usually chosen on the basis of their being only one copy, the 18S rRNA gene which is present in more copies and characteristic for all eukaryotic cells, can be additionally used to assess the level of DNA extraction.

Decision-making system for selecting extraction methods

A decision-making system was developed, based on DNA extraction results for known and new sample types, taking into account also challenging samples (Fig. 1). The system dictates the use of two extraction methods for most samples, with practical implications for fluent workflow in the testing of many different samples. Most of the samples could be tested using NucleoSpin® Food or the CTAB method, sometimes with modification for challenging samples.

Financial aspects

In general, published protocols proved to be cheaper than commercial kits because they do not depend on costly reagents covered by international patents (Pirodini et al. 2010). On the other hand DNA extraction kits generally operate faster than the CTAB method (Demeke and Jenkins 2010). Some financial comparisons have been made by Smith et al. (2005) concerning methods for extracting DNA from potatoes and potato-derived products; only costs of materials were compared (Smith et al. 2005). No other exact economic

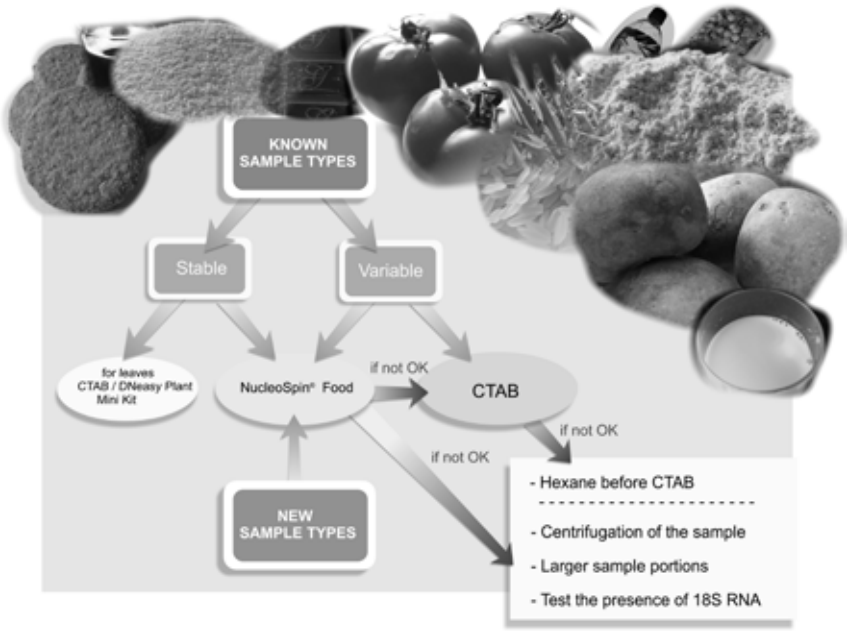

Figure 1: Decision-making system for selection of DNA extractions.
Known sample types – tested many times, knowledge on DNA extraction regarding efficiency is known.
Stable ones give repeatable, while variable ones give unexpected results of DNA extraction.
New sample types – there is no experience on DNA extraction.

Slika 1: Sistem odločanja za izbor metode za ekstrakcijo DNA.
Poznani vzorci - testirani večkrat, poznana je uspešnost ekstrakcije DNA.
Stabilni vzorci – analiza da pričakovan rezultat, medtem ko je pri variabilnh vzorcih lahko nepričakovan rezultat.
Novi vzorci - zanje ni izkušnje glede uspešnosti ekstrakcije DNA.

evaluations have been published. The financial aspect of the methods selected by our decision-making system follows.

As noted above, NucleoSpin® Food and the CTAB method were used for extracting DNA from most samples. Additionally, the DNeasy Plant Mini Kit was used for extracting DNA from leaves. The number of samples tested at the same time influences the final price; one, three and ten samples were tested in parallel – only the CTAB method using larger volumes is limited to test maximum of three samples at the same time. Working hours, chemicals and final prices were each compared, being expressed as ratios, with CTAB being taken as 100% (Tab. 7). Comparison of the final costs of all the methods showed that NucleoSpin® Food is the most cost effective, especially on account of the least number of working hours required to perform extractions. Independently of the extraction method used, the price decreases with increasing number of samples on account of the reduced working hours per sample. With more samples tested in parallel, final prices became more comparable.

Future perspectives

The laboratories dealing with many different samples daily need efficient system for extraction of DNA from these samples to enable high throughput and reliable DNA testing results. Developed decision-making system enables organized and supportive structure for quicker decisions in daily workflow in the laboratory.

Besides experiences described from testing of GMOs in our laboratory, further possibilities exist for more efficient operation of the DNA extraction step in testing procedures. The introduction of automation of DNA extraction using magnetic beads should further speed the process and result in higher throughput (Guertler et al. 2014).

The modular approach, declaring individual steps of the detection procedure, like DNA extraction and qPCR as independent modules, is commonly accepted in the detection of GMOs, but not in all other areas using nucleic acid based testing (Bellocchi et al. 2010; Holst-Jensen and Berdal 2004). In GMO detection the ratio between transgene and reference gene is used. In other areas, however, absolute quantification is needed, so it is even more important that extraction methods used in different laboratories are comparable, therefore decision-making systems can be used also for the purposes of standardization.

New technologies can also enable amplification of DNA without prior intensive extraction, as was shown for leaf and seed tissues of MON 810, using loop-mediated isothermal amplification (LAMP) (Lee et al. 2009) or even qPCR (Mano et al. 2014) and digital PCR (Pavšič et al. 2015) giving further possibilities for even more standardized procedures of testing without influences of the extraction step.

Conclusions

The decision-making system for selecting extraction methods described here enables the use of tested methods for most samples. The advantage of such a system is that different samples coming to the laboratory can be processed in parallel using the same method, thus resulting in rationalization of the workflow, labor and cost. In consequence, fewer methods need to be verified or validated and the education of personnel is more rational. The tables presented in this paper, which relate extraction to sample type, can also be useful for other laboratories testing similar sample types. The decision-making system presented here can also contribute to harmonizing the extraction steps for GMO detection used in different laboratories. The decision-making system can also be adapted for other areas using nucleic acids testing in food, feed, seeds and plants.

Povzetek

Pri analizah živil, krme, semen in rastlin, ki temeljijo na določanju nukleinskih kislin, se uporablja različne metode ekstrakcije DNA. Takšen primer je tudi določanje gensko spremenjenih organizmov (GSO), kjer izvajamo analize na zelo različnih vrstah vzorcev, od rastlin ali semen, do zelo procesiranih vzorcev kot so kosmiči ali čokolada, ki se med seboj lahko zelo razlikujejo, npr. zaradi različnih načinov obdelave pri proizvodnji ali različnih dodatkov ipd. Celo vzorci enake sestave se lahko med različnimi proizvajalci razlikujejo glede kvalitete DNA, ki jo vsebujejo. Razvite so različne metode za ekstrakcijo DNA, ki delujejo uspešno pri nekaterih vrstah vzorcev, pri drugih pa ne. Zato je delo v laboratorijih, ki analizirajo različne vrste vzorcev zelo kompleksno.

Na osnovi naših izkušenj smo razvili sistem odločanja za izbor ekstrakcije DNA za posamezen vzorec. V laboratoriju uporabljamo tri metode za ekstrakcijo DNA. Za večino vzorcev uporabimo NucleoSpin® Food, za nekatere vzorce (npr. gluten, lecitin in čokolada) je najbolj primerna izolacija s cetiltrimetilamonijevim bromidom (CTAB), pri ekstrakciji DNA iz listov pa uporabljamo CTAB ali DNeasy Plant Mini Kit. Vzorce smo glede na pridobljene izkušnje o uspešnosti ekstrakcije razdelili na poznane vzorce, ki smo jih že večkrat testirali, in nove vzorce. Poznane vzorce ločujemo na stabilne vzorce, za katere smo pokazali, da iz njih vedno pridobimo DNA ustrezne kvalitete in v zadostni količini, in na variabilne vzorce, pri katerih je lahko učinkovitost ekstrakcije zelo različna zaradi zgoraj omenjenih razlogov. Novi vzorci so tisti, s katerimi še nimamo izkušnje o učinkovitosti ekstrakcije DNA. Skladno s to razdelitvijo smo naredili shemo odločanja, ki je prikazana na Sl. 1. Obenem smo za vzorce z različnimi vsebnostmi posamezne rastlinske vrste (koruze, soje, lanu, krompirja, paradižnika, oljne ogrščice) podali natančne informacije o rezultatih pridobljenih pri nadaljnji analizi DNA z metodo PCR v realnem času.

Vzpostavljeni sistem odločanja izbora metode ekstrakcije DNA je narejen na primeru določanja GSO, vendar je uporaben tudi za druge podobne analize nukleinskih kislin v obravnavanih vrstah vzorcev.

Acknowledgements

We are grateful to Dr. Roger Pain for critical review of the manuscript. The work was co-financed by the Slovenian Research Agency (contract no. P4-0165) and the Slovenian Ministry of Agriculture and Environment (contract no. 2330-13-000072). The research leading to these results has also received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 613908, relating to the project "Development of Cost-efficient Advanced DNA-based methods for specific Traceability issues and High Level On-site Applications".

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ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 77–92

Prispevek k slovenski anatomski terminologiji: latinsko − slovenski, slovensko − latinski slovar ptičjih kosti

Contribution to Slovenian anatomical terminology: Latin − Slovenian, Slovenian − Latin dictionary of bird bones

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Izvleček: V članku je predstavljeno poimenovanje kosti ptičjega skeleta. Posamezni strokovni izraz je zapisan v mednarodni strokovni nomenklaturi, tj. v latinščini ali latinizirani grščini, večinoma sledi poslovenjeni strokovni izraz in nato slovensko poimenovanje posameznega anatomskega izraza. Zaporedje gesel sledi anatomski regionalizaciji ptičjega telesa. V prvem dodatku so po abecedi navedeni slovenski strokovni izrazi, opremljeni z besednovrstnimi oznakami za končnico v drugem sklonu ednine in za spol, z latinskim izrazom v nadaljevanju. V drugem dodatku so navedena po abecedi latinska anatomska poimenovanja, ki jim sledijo slovenski strokovni izrazi.

Ključne besede: anatomija, ptičje kosti, okostje, skelet, slovenska terminologija

Abstract: The work presents anatomical terminology of bones of a bird skeleton. An individual technical term is written in the international standard form, i.e. in Latin or Latinized Greek, mostly followed by the slovenized technical term and afterwards by the Slovenian expression for the individual anatomical term. The succession of terms follows the anatomical body regionalisation in birds. In Appendix 1, Slovenian technical terms are listed in alphabetical order with marks for the genitive case singular and sex, followed by Latin names. In Appendix 2, the Latin anatomical names are listed in alphabetical order succeeded by Slovenian expressions.

Key words: anatomy, birds bones, skeleton, Slovenian terminology

Uvod

Terminologija (strokovno izrazoslovje) je veda o strokovnih izrazih izbranega področja in v tem delu predstavljamo poimenovanje oz. slovenske izraze za ptičje kosti. Prispevek je zasnovan kot dvojezični strokovni slovar. Zajema poimenovanja posameznih ptičjih kosti in sklope kosti posameznih telesnih področij. Z njim dopolnjujemo in posodabljamo slovensko strokovno izrazje.

Pri uporabi slovenskih ustreznic smo se ravnali po obstoječem splošnem anatomskem izrazju, kot ga navajata Veterinarski terminološki slovar (Brglez in sod. 2013) in Slovenski medicinski slovar (Kališnik 2012). Specifično anatomsko izrazje za ptiče v temeljni slovenski veterinarski literaturi ni zastopano (npr. Rigler 1985 in 1990), povzeli smo ga po novejši monografiji Funkcionalna anatomija ptičev z osnovami ornitologije (Golob 2011).

Geselske iztočnice in njihovo zaporedje smo povzeli po Baumelu in sod. (1993), primerjali smo jih z Nickelom (2000). Zaporedje gesel sledi regionalizaciji ptičjega telesa po telesnih delih oz. področjih telesa: lobanja z osnim skeletom, hrbtenica in prsni koš, privesni skelet s plečnim obročem in kostmi peruti ter medenični obroč s kostmi noge. Poleg zaporedja, ki sledi anatomski regionalizaciji, so v dodatku še v abecednem zaporedju gesla v latinskem in slovenskem jeziku ter v slovenskem in latinskem jeziku. V prvem dodatku so v abecednem zaporedju gesel slovenske iztočnice zapisane v imenovalniku ednine ali množine, dodane so besednovrstne oznake za končnico v rodilniku in oznaka za spol (*m, ž, s*).

Geselska iztočnica je eno- ali večbesedna v latinskem jeziku, sledijo poslovenjena in slovenska ustreznica oz. več ustreznic. Pri nekaterih geslih so navedene še vedno uporabljane sopomenke, te so označene z okrajšavo sin. (sinonim). Slovenske ustreznice temeljijo na rabi v sodobnem strokovnem jeziku. Kjer slovenskih ustreznic do sedaj nismo imeli, smo latinsko poimenovanje glasovno poslovenili. Sledijo slovenske sopomenke in ponekod še opisne oblike izrazov. Latinske iztočnice so zapisane v krepkem tisku (sinonimi v navadnem tisku), sledijo slovenske ustreznice v navadnem tisku.

Prispevek predstavlja prvine sporazumevalnega jezika stroke. Navedene so kosti, ki jih imajo ptiči. Nekatere med njimi so značilne samo za določene skupine, npr. tilna kost (*os nuchale*) za kormorane in kačjevratnike, ali pa štrleča kost (*os prominens*) za kanje, sokole in sove. Nasploh je pri ptičih precejšnja raznolikost v obliki in velikosti skeletnih elementov.

Latinsko-slovenski anatomski izrazi ptičjega okostja

SKELETON AXIALE – AKSIALNI SKELET, OSNO OKOSTJE
CRANIUM – KRANIJ, LOBANJA

ossa cranii – kosti kranija, kosti lobanje
os occipitale – okcipitalna kost, zatilnica
os basioccipitale – baziokcipitalna kost, osnovna zatilnica
os exoccipitale – eksokcipitalna kost, stranska zatilnica
os supraoccipitale – supraokcipitalna kost, zgornja zatilnica
os sphenoidale – sfenoidna kost, zagozdnica
os basisphenoidale – bazisfenoidna kost, osnovna zagozdnica
os laterosphenoidale, sin. os orbitosphenoidale – laterosfenoidna kost, orbitosfenoidna kost, stranska zagozdnica
os parasphenoidale – parasfenoidna kost, obzagozdnica
ossa temporalia – temporalne kosti, senčnične kosti
os squamosum – skvamozna kost, luskasta kost
os oticum – otična kost, ušesna kapsula
os epioticum – epiotična kost, nadušesna kost

os opisthoticum – opistotična kost, ušesni stebriček
os prooticum – prootična kost, predušesna kost
os metoticum – metotična kost, priušesna kost
pila otica, sin. opisthotic, sin. columella, sin. pila prootica – kolumela, stebrc
os parietale – parietalna kost, temenica
os frontale – frontalna kost, čelnica
os ethmoidale – etmoidna kost, sitkina kost
os mesethmoidale – mezetmoidna kost, sredinska sitkina kost
os ectethmoidale – ektetmoidna kost, zunanja sitkina kos**t**
os lacrimale, sin. os prefrontale – lakrimalna kost, solznica, predčelnica

ossa maxillae et palati – kosti maksile in palatinuma, kosti zgornje čeljusti in neba
os nasale – nazalna kost, nosnica
premaxilla, sin. rostrum maxillae – premaksila, kljun zgornje čeljustnice, predčeljustnica, medčeljustnica
maxilla, sin. os maxillare – maksila, zgornja čeljustnica
os palatinum – palatinalna kost, nebnica, nebna kost
vomer – vomer, lemežnica, ralo
os pterygoideum – pterigoidna kost, krilna kost, krilatka
os jugale – jugalna kost, jarmova kost
os quadratojugale – kvadratojugalna kost, kvadratnojarmova kost
os quadratum – kvadratna kost, štirikotnica

ossa mandibulae, kosti mandibule, kosti spodnje čeljusti
os dentale – dentalna kost, zobna kost, zobnica
os angulare – angularna kost, kotna kost
os articulare – artikularna kost, sklepna kost
os coronoideum – koronoidna kost, kronasta kost
os prearticulare – preartikularna kost, predsklepna kost
os spleniale – splenialna kost, žmulasta kost
os supraangulare – supraangularna kost, nadkotna kost

ossa accessoria cranii – dodatne kosti lobanje
anulus tympanicus – bobničev obroč
os nuchale – nuhalna kost, tilna kost
ossa sclerae – beločnične kosti
os siphonium – cevasta kost
os suprajugale – suprajugalna kost, nadjarmova kost
ossa supraorbitalia – supraorbitalne kosti, nadočnične kosti
os lacrimopalatinum – solzničnonebna kost
ossa suturarum – šivne kosti
os uncinatum – kavljasta kost

apparatus hyobranchialis, sin. apparatus hyoideus – hiobranhialni aparat, jezičnoškržni aparat, jezični aparat, jezičnica
paraglossum, sin. entoglossum – paraglosum, objezična kost, objezičnica, entoglosum, znotrajjezična kost, znotrajjezičnica
basihyale, sin. basihyoideum – bazihioid, telo jezičnice
urohyale, sin. urohyoideum, sin. basibranchiale – urohioid, rep jezičnice, telo škržnega dela jezičnice
cornu branchiale – rog jezičnice, rog jezičnega aparata

columna vertebralis – hrbtenica
vertebrae cervicales – cervikalna vretenca, vratna vretenca
atlas – atlas, nosač
axis, sin. epistrophaeus – aksis, okretač

vertebrae thoracicae, lumbicales sacrales et caudales – torakalna, lumbalna, sakralna in kavdalna vretenca, prsna, ledvena, križna in repna vretenca
notarium, sin. os dorsale – notarij, hrbtna kost, hrbtnica

synsacrum, sin. os lumbosacrale – sinsakrum, sokrižnica
vertebrae synsacrales – sinsakralna vretenca, vretenca sokrižnice

vertebrae caudales liberae – prosta repna vretenca
pygostylus – pigostil, križni opornik, mrdenica, jurična kost

skeleton thoracis – torakalni skelet, okostje prsnega koša
costae – rebra
costa vertebralis – vertebralno rebro, vretenčno rebro
costa sternalis – sternalno rebro, prsnično rebro
costae completae verae – prava popolna rebra
costae completae spuriae – neprava popolna rebra
costae incompletae – nepopolna rebra
costae fluctuantes – prosta rebra, plavajoča rebra

sternum – sternum, prsnica

SKELETON APPENDICULARE – APENDIKULARNI SKELET, PRIVESNO OKOSTJE

skeleton membri thoracini – skelet torakalne okončine, okostje prsne okončine
ossa cingula membri thoracini – kosti obroča torakalne okončine, kosti obroča prsne okončine, kosti plečnega obroča, kosti ramenskega obroča

furcula, sin. clavicula – furkula, klavikula, vilice, ključnica
scapula – skapula, plečnica, (lopatica)
coracoideum, sin. os coracoideum – korakoid, krokarnica

skeleton alae – okostje peruti
skeleton brachii – skelet brahija, okostje nadlakti
humerus – humerus, nadlahtnica

skeleton antebrachii – skelet antebrahija, okostje podlakti
ulna – ulna, komolčnica, podlahtnica
radius – radius, koželjnica

skeleton manus – skelet roke, okostje roke
ossa carpi – karpalne kosti, zapestnice
os carpi radiale – radialna karpalna kost, koželjnična zapestnica
os carpi ulnare – ulnarna karpalna kost, komolčnična zapestnica
os prominens – prominentna kost, štrleča kost

carpometacarpus – karpometakarpalna kost, zapestnodlančna kost, zapestnodlančnica
os metacarpale alulare, sin. metacarpus pollicis – alularna dlančnica, perutkina dlančnica
os metacarpale majus – večja dlančnica
os metacarpale minus – manjša dlančnica

ossa digitorum manus – prstnice roke
phalanx digiti alulae – prstnica perutke
phalanx proximalis digiti majoris –zgornja prstnica večjega prsta
phalanx distalis digiti majoris – spodnja prstnica večjega prsta
phalanx digiti minoris – prstnica manjšega prsta

skeleton membri pelvini – okostje medenične okončine

ossa cingula membri pelvini – kosti obroča medenične okončine
pelvis – medenica
os coxae – kolčnica
os ilium – črevnica
os ischii – sednica
os pubis – dimeljnica

skeleton femoris – okostje stegna
os femoris – femur, stegnenica
patella – patela, pogačica

skeleton cruris – okostje goleni
tibiotarsus – tibiotarzus, goleničnonartna kost, golenonartnica
fibula – fibula, mečnica

skeleton pedis – okostje stopala
ossa tarsi – nartne kosti, nartnice
os tibiale, sin. astragalus – astragalus, golenična nartna kost
os fibulare, sin. calcaneum – kalkaneum, mečnična nartna kost
os tarsi distale – distalna tarzalna kost, spodnja nartna kost
tarsometatarsus – tarzometatarzus, nartnostopalna kost, nartostopalnica
os metatarsale I (primum), sin. os hallucis – metatarzlana kost I, prva stopalnica
os metatarsale II (secundum) – metatarzalna kost II, druga stopalnica
os metatarsale III (tertium) – metatarzalna kost III, tretja stopalnica
os metatarsale IV (quartum) – metatarzalna kost IV, četrta stopalnica
ossa digitorum pedis – prstnice stopala
phalanx (mn. phalanges) digitorum pedis – prstnica (prstnice) prstov stopala
phalanx ungualis – prstnica kremplja, krempeljnica

Označevanje prstov na medenični okončini smo povzeli po Baumelu in sod. (1993): palec ima oznako 1 (*hallux*), notranji prst 2 (*digitus secundus*), srednji prst 3 (*digitus tertius*) in zunanji prst oznako 4 (*digitus quartus*). V splošnem imajo ptiči naslednje število prstnic v posameznem prstu: palec

dve prstnici, notranji prst tri prstnice, srednji prst štiri prstnice in zunanji prst pet prstnic. Končna oz. distalna prstnica (*phalax ungualis* – krempeljnica) je pri večini ptičev zavita v obliki kavlja in skupaj s keratinskim ovojem (ramfoteko) tvori krempelj, pri ponirkih je sploščena. Število prstnic v posameznem prstu noge zapišemo s prstnično formulo (*phalangeal formula*), npr. 2-3-4-5.

Razprava

Kljub homolognim kostem pri ljudeh in tetrapodih (štirinožcih) obstajajo precejšnje razlike v anatomskem poimenovanju kosti med biološko, medicinsko in veterinarsko stroko. Npr. v medicinski literaturi se za izraz **scapula** dosledno uporablja lopatica, v veterinarski pa plečnica. Po drugi strani pri ptičji osteološki terminologiji še vedno niso povsod upoštevana homoplazna stanja nekaterih kosti prednje in zadnje okončine, predvsem zeugopodija in autopodija. Tako npr. večina domače strokovne literature sploh ne obravnava ptičjega tibiotarzusa in tarzometatarzusa.

Med anatomskimi posebnostmi ptičje lobanje velja omeniti vsaj nekatere, npr. enojni okcipitalni čvrš, gibljivost kvadratne kosti in krilatke ter čeljustni sklep med mandibulo in kvadratno kostjo. Za lobanjo je značilna tudi razmeroma obsežna orbita, katere dno pri večini ptičev ni koščeno, ampak ga večinoma oblikujejo čeljustne mišice. Kinezo lobanje omogočajo sklepi med nekaterimi kostmi viscerokranija, pa tudi stanjšanja kosti, ki predstavljajo upogibna področja (*zonae flexoriae*).

Maxilla – maksila (sin. *os maxillare*) je ena od kosti zgornje čeljusti, z izrazom **maxilla** pa poimenujemo tudi kompleks kosti, ki tvorijo zgornjo čeljust. Skratka, v prvem primeru gre za individualno kost, v drugem za kompleks (sestav) kosti (Baumel in sod. 1993). Zato se pri navedbi kosti **os maxillare** držimo načela, da gre za eno od kosti maksile (tj. maksilarno kost). Je pa res, da se v veterinarski terminologiji sesalcev in v medicinski terminologiji ne uporablja izraza *os maxillare*, ampak maxilla v pomenu (1) para votlih kosti v obraznem delu lobanje in (2) zgornje čeljusti.

Poseben premislek namenjamo slovenjenju termina **os jugale**, jugalna kost. Ta kost se nahaja na osrednjem delu jugalnega loka (*arcus jugalis*): kavdalno je koščeno zraščena s kvadratojugalno kostjo (*os quadratojugale*) in rostralno z jugalnim podaljškom maksilarne kosti (*os maxillare: processus jugalis*). Jugalni lok povezuje zgornjo čeljust s štirikotnico (*os quadratum*). Izraz *jugalis* (lat. *iugalis* vprežen, povezujoč; *iugo* spojiti, zvezati; *iugum* jarem) opredeljuje položajno lastnost objekta (vezno, spojno), ki ima obliko in funkcijo jarma. Pri slovenjenju tega termina se je mogoče nasloniti na izraze jârmnica ali jármovka (trta, vitra pri jarmu) v Pleteršnikovem Slovensko-nemškem slovarju, zap. št. 5240 in 5241 (Fran 2015a); ter geslo jarem v Slovenskem pravopisu iz leta 1950 (Ramovš in sod. 1950); ter jarmski (nanašajoč se na jarem), jarmov (npr. j. lok) in jarmnik (klin, s katerim se povežeta zgornji in spodnji del jarma) iz SSKJ (Fran 2015b)). Izraz **jarmov lok** (*arcus jugalis*) je sprejemljiv, nekoliko manj **jarmova kost** (*os jugale*), saj so jarmove vse tri kostne sestavine jarmovega loka. Je pa mogoče, če za to obstojijo posebni razlogi, kost natančno opredeliti kot jarmova kost v ožjem pomenu besede (*os jugale proprium*).

Za jezični skelet, tj. **jezičnoškržni aparat** (*apparatus hyobranchialis*) je v strokovni literaturi vrsta sopomenk, ki imajo različen izvor svojega nastanka. V novejšem času se uveljavljajo predvsem izrazi, ki označujejo pripadnost bodisi podjezičnemu (hioidnemu) loku ali škržnim (branhialnim) lokom. Podjezični (hioidni) del jezičnice obsega tri sestavine. Spredaj je **objezična kost** ali **objezičnica** (*paraglossum*, sin. *entoglossum*, sin. *glossohyale*), ki ima pri večini ptičev obliko puščice; kost nastopa v paru z kavdolateralno ležečim **rogu podobnim koncem** (*ceratohyale proper* – rogu podobna jezičnica v ožjem pomenu besede, tudi *epihyale proper* – končni del jezičnice v ožjem pomenu besede); tretja sestavina podjezičnega dela je **telo jezičnice** (*basihyale*, sin. *basihyoideum*). Škržni del jezičnega aparata izhaja iz prvega in drugega škržnega loka. Iz prvega nastaneta **rogu podobna škržna kost** (*ceratobranchyale*) in **končna škržna kost** (*epibranchiale*), ki skupaj tvorita (večji) **rog jezičnega aparata**, tj. **rog jezičnice**. Z drugega škržnega loka izhaja **urohioid** (*urohyale,* sin. *urohyoideum*, sin. *basibranchiale* – **rep jezičnice** ali **telo škržnega dela jezičnice**), ta pa se pri odraslih ptičih zraste s pred njim ležečim telesom

jezičnice (bazihioidom) (Baumel in sod. 1993, Fine Dictionary 2013). Jezičnoškržni aparat ima vrstno značilne modifikacije, tako sta npr. rogova jezičnice pri žolnah in detlih izredno dolga. Pri izrazu urohioid gre za določeno nejasnost, kajti kost ne izvira iz podjezičnega (hioidnega) loka, ampak iz drugega škržnega (branhialnega) loka, na kar opozarja sopomenka *basibranchiale* (telo škržnega dela jezičnice).

Zaradi dvojnosti rabe izrazov lopatica in plečnica pri živalih v našem naravoslovju ni odveč navesti naslednje. Za poimenovanje izraza **scapula** se pri slovanskih narodih večinoma uporablja *lopatica* oz. *lópatka*, v srbskem jeziku tudi *plećka*, pri zahodnoevropskih pa npr. *omoplate* (fr.) / *omoplato* (šp.) / *das Schulterblatt* (nem.) / *skulderblad* (šved.) oz. *shoulderblade* (angl.). Pri zahodnoevropskih narodih je torej dosledno opredeljeno področje te kosti (pleča, rame), medtem ko pomenijo izrazi *blade / blatt / blad / plate / plato* rezilo (npr. noža, sablje), tudi rezilo omotače ali lopate (tj. lopatico). Domneva se, da izraz **scapula** izvira iz grškega **'skaptein'**, kar pomeni kopati (Wikipedia 2013). Pri ptičih je skapula večinoma ozka in rahlo ukrivljena (sabljasta), pri pingvinih pa lopatasto razširjena, podobna kot pri sesalcih. V našem prispevku smo dali prednost izrazu plečnica, ker je anatomsko smotrno glede na pleča (področje trupa med plečnicama). Nenatančnost se je prenesla tudi v SSKJ (Fran 2015b), kjer so opisana pleča kot »zgornji del človeškega ali živalskega trupa med lopaticama«, plečnica pa kot »parna ploščata kost na spodnjem stranskem delu prsi«. Zaradi istih in podobnih vzrokov smo dali prednost tudi izrazu plečni obroč (in ne ramenski obroč).

Eno od posebnih vprašanj zadeva medenično okončino, predvsem golenico, nartnice in stopalnice. Ker se posamezne kosti oz. njihove koščene zasnove pojavijo med embrionalnim razvojem ptičev, uporabljamo vse navedene izraze, glede na stanje pri odraslih ptičih pa tudi nova izraza **tibiotarzus** (golenonartnica: golenica je zraščena z dorzalnima nartnicama astragalusom in kalkaneumom) in **tarzometatarzus** (nartostopalnica: stopalnice II, III in IV so zraščene med seboj in z distalno nartnico, *os tarsi distale*).

Proces zraščanja kosti je prisoten tudi v peruti, ki privede do nastanka **karpometakarpusa** (zapestnodlančnice: zrast zapestnic in dlančnic). Med embrionalnim razvojem nastanejo osifikacijska jedra šestih zapestnih koščic, od katerih ena nazaduje in izgine, dve se izoblikujeta v samostojni zapestnici (*os carpi radiale* – koželjnična zapestnica in *os carpi ulnare* – komolčnična zapestnica), tri pa se vključijo v proksimalni del dlančnih kosti. Nastane sestavljena kost zapestnodlančnica, pri kateri razlikujemo tri dlančne sestavine: perutkino dlančnico (*os metacarpale alulare*), večjo dlančnico (*os metacarpale majus*) in manjšo dlančnico (*os metacarpale minus*). Večja in manjša dlančnica sta proksimalno zraščeni med seboj, s perutkino dlančnico in z omenjenimi tremi zapestnicami; nato potekata ločeno, med njima je dlančnični prostor (*spatium metacarpale*), na distalnem koncu pa sta sinostozno zraščeni (Baumel in sod. 1993). Opisana anatomska značilnost je pomembna sestavina peruti, saj predstavlja podlago za pripetje primarnih letalnih peres.

Veterinarski terminološki slovar prej navedenih izrazov ne obravnava, prav tako ne obravnava dosledno za ptiče značilnih diferenciacij hrbtenice in posebnosti skeleta lobanje. V praksi se izrazi sicer uporabljajo, predvsem v pedagoške namene na univerzah v Sloveniji, navaja pa jih tudi Golob (2011). Izraz **sinsakrum** opredeljuje Veterinarski terminološki slovar (Brglez in sod. 2013) kot kost iz zraščenih kavdalnih prsnih, ledvenih, križnih in nekaj repnih vretenc ptičev, ne navaja pa slovenskega imena. Osnovo izraza predstavlja **sakrum**, tj. križnica (lat. *os sacrum*), predpona **sin-** pa pomeni zlitje ali zraščenost (gr. *syn*: s, z, skupaj, hkrati, v sestavljankah s so- (Verbinc, 1968)) prej navedenih vretenc. Sinsakrum torej lahko slovenimo kot **kost(i) sokrižja, sokrižna zrast** ali **sokrižnica**. Pri tem je treba upoštevati, da je križnica sesalcev zraščena iz nekaj (štiri do pet pri človeku) križnih vretenc. V obeh primerih gre torej za zraščenost vretenc v področju križa, le da je ta pri ptičih obsežnejša in predstavlja evolucijsko prilagoditev njihovemu načinu gibanja.

Razložiti velja tudi slovensko poimenovanje izraza **pygostylus** (pigostil). Le-ta zadeva končno kost hrbtenice pri ptičih, ki nastane s postnatalnim zlitjem zadnjih štiri do osem vretenc. Izraz ima grški izvor in pomeni **križni opornik** (gr. *pyge* del telesa pred repom, križ; sokrižnica vključuje tudi prva repna vretenca; *stylos* steber, opornik), tj.

opornik repnega dela ptičjega telesa in še posebej repnih letalnih peres. Za razumevanje tega izraza si pomagajmo z razlago izraza **uropigij** (gr. *oura* rep, *pyge* križ), tj. zadnji del telesa, ki obsega rep in križno področje. V slovenščini označujemo to področje ptičjega telesa tudi s pogovornim izrazom (kurja) škofija. Pigostil je torej kost v področju (kurje) škofije, t. i. **mrdina** (Brglez in sod. 2013). Vendar se izraz mrdina ni uveljavil v naši strokovni literaturi, za kar je najbrž več vzrokov, predvsem ta, da kot izpeljanka iz **mrde** (Fran 2015b, Snoj 1997) ne ustreza dovolj tvornosti našega tehničnega izrazja na področju osteologije (prim. čelo → čelnica, pleče → plečnica, nadlaket → nadlahtnica, stegno → stegnenica itn.). Sprejemljivejši bi bil izraz (kost) **mrdenica**. Pigostil ima v strokovni literaturi dve sopomenki, urostil in kokciks. Izraz **urostil** (gr. *oura* rep, *stylos* steber) se uporablja predvsem v osteologiji rib in žab, **coccyx** (gr. *kokkyx* kukavica, gre za podobnost končne hrbtenične kosti iz zraščenih vretenc s kukavičjim kljunom (Merriam-Webster 2013)) pa predvsem v osteologiji človeka in človeku podobnih opic (slov. **trtica**). Slovenjenje izraza pigostil s trtico torej smiselno ne ustreza izvirniku, prav tako ne poimenovanje drugih delov ptičjega telesa z izpeljankami iz trtice (npr. trtična žleza; *gl. uropygii*, dobesedno repnokrižna žleza).

Posebej je treba omeniti tudi **ključnici** oz. njuno koščeno zraščenost v **furkulo**, vilice (lat. *furca*). V naše strokovno in splošno izrazje se je kot sopomenka furkuli vrinila **kobilica**. Vendar gre v tem primeru za poimenovanje, ki ga ni mogoče nasloniti na furkulo. Gredelj ali kobilica je namreč na prsnici (*carina sterni*).

Pojasniti je treba tudi vprašanje uvajanja slovenskih anatomskih izrazov. Obstoječe uveljavljene izraze smo vnesli v gradivo, sicer pa smo predvsem slovenili mednarodno sprejete termine. Tako je npr. pri opisu vretenc mogoče govoriti o njihovem sprednjem in zadnjem koncu, vendar se v sodobni anatomski terminologiji tetrapodov (štirinožnih živali) opuščata izraza anteriorni (sprednji) in posteriorni (zadnji). Uveljavila sta se izraza kranialni (tj. bližji lobanji) in kavdalni (bližji repu). V navedenem primeru sta izraza sprednji in zadnji (primerni) splošni opisni sopomenki, ki pa terminološko ne ustrezata izrazoma kranialen (bližji lobanji) oz. kavdalen (bližji repu). Poleg tega pri vretencih dvonožnih živali in človeka pomeni sprednji (anteriorni) ventralno (trebušno) in zadnji (posteriorni) dorzalno (hrbtno) smer.

Zahvala

Za skrben jezikovni pregled besedila in izboljšav se zahvaljujemo Gregorju Fazarincu iz Veterinarske fakultete Univerze v Ljubljani, Cvetani Tavzes iz Inštituta za slovenski jezik Frana Ramovša Slovenske akademije znanosti in umetnosti, Tonetu Novaku iz Fakultete za naravoslovje in matematiko Univerze v Mariboru in Viktorju Majdiču. Prispevek je bil delno podprt s sredstvi Agencije RS za raziskovalno dejavnost iz raziskovalnega programa Biodiverziteta (P1-0078) in projekta Prazgodovinska kolišča na Ljubljanskem barju, Slovenija: kronologija, kultura in paleookolje (L6-4157).

Dodatek 1: Abecedno zaporedje gesel v slovenskem in latinskem jeziku. Slovenskim iztočnicam je dodana končnica v rodilniku in spol (*m, ž, s*).
Appendix 1: Alphabetical list of terms in Slovenian and Latin language. Marks for genitive case and sex (m, ž, s) are added to Slovenian terms.

beločnična kost -e -i *ž*	os sclerae
bobničev obroč -ega -a *m*	anulus tympanicus
cevasta kost -e -i *ž*	os siphonium
čelnica -e *ž*	os frontale
četrta stopalnica -e -e *ž*	os metatarsale IV
črevnica -e *ž*	os ilium
spodnja nartna kost -e -e -i *ž*	os tarsi distale
dimeljnica -e *ž*	os pubis
druga stopalnica -e -e *ž*	os metatarsale II
golenična nartna kost -e -e -i *ž*	os tibiale
goleničnonartna kost -e -i *ž*	tibiotarsus
hrbtenica -e *ž*	columna vertebralis
hrbtna kost -e -i *ž*	notarium
jarmova kost -e -i *ž*	os jugale
jezičnica -e *ž*	apparatus hyobranchialis
kavljasta kost -e -i *ž*	os uncinatum
ključnica -e *ž*	furcula
kolčnica -e *ž*	os coxae
komolčnica -e *ž*	ulna
komolčnična zapestnica -e -e *ž*	os carpi ulnare
kotna kost -e -i *ž*	os angulare
koželjnica -e *ž*	radius
koželjnična zapestnica -e -e *ž*	os carpi radiale
krempeljnica -e *ž*	phalanx ungualis
krilatka -e *ž*	os pterygoideum
križni opornik-a -a *m*	pygostylus
križno vretence -ega -a *s*	vertebra sacrales
krokarnica -e *ž*	coracoideum
kronasta kost -e -i *ž*	os coronoideum
kvadratnojarmova kost -e -i *ž*	os quadratojugale
ledveno vretence -ega -a *s*	vertebra lumbicales
lemežnica -e *ž*	vomer
lobanja -e *ž*	cranium
luskasta kost -e -i *ž*	os squamosum

manjša dlančnica -e -e *ž*	os metacarpale minus
mečnica -e *ž*	fibula
mečnična nartna kost -e -e -i *ž*	os fibulare
nadjarmova kost -e -i *ž*	os suprajugale
nadkotna kost -e -i *ž*	os supraangulare
nadlahtnica -e *ž*	humerus
nadočnične kosti -ih -- *ž*	ossa supraorbitalia
nadušesna kost -e -i *ž*	os epioticum
nartnostopalna kost -e -i *ž*	tarsometatarsus
nebnica -e *ž*	os palatinum
neprava popolna rebra -ih -ih reber *s*	costae completae spuriae
nepopolno rebro -ega -a *s*	costa incompleta
nosač -a m	atlas
nosnica -e *ž*	os nasale
objezičnica -e *ž*	paraglossum
obzagozdnica -e *ž*	os parasphenoidale
okretač -a m	axis
osnovna zagozdnica -e -e *ž*	os basisphenoidale
osnovna zatilnica -e -e *ž*	os basioccipitale
perutkina dlančnica -e -e *ž*	os metacarpale alulare
plečnica -e *ž*	scapula
pogačica -e *ž*	patella
prava popolna rebra -ih -ih reber *s*	costae completae verae
predčeljustnica -e *ž*	premaxilla
predsklepna kost -e -i *ž*	os prearticulare
predušesna kost -e -i *ž*	os prooticum
priušesna kost -e -i *ž*	os metoticum
prosto rebro -ega -a *s*	costa fluctuans
prosto repno vretence -ega -ega -a *s*	vertebra caudalis libera
prsno vretence -ega -a *s*	Vertebra thoracica
prsnica -e *ž*	sternum
prsnično rebro -ega -a *s*	costa sternalis
prstnica manjšega prsta -e -- -- *ž*	phalanx digiti minoris
prstnica perutke -e -- *ž*	phalanx digiti alulae
prstnica prstov stopala -e -- -- *ž*	phalanx digitorum pedis
prva stopalnica -e -e *ž*	os metatarsale I
rebro -a *ž*	costa
rep jezičnice -a -- *m*	urohyale

repno vretence -ega -a *s*	vertebra caudalis
rog jezičnice -a -- *m*	cornu branchiale
sednica -e *ž*	os ischii
senčnične kosti -e -- *m*	ossa temporalia
sitkina kost -e -i *ž*	os ethmoidale
sklepna kost -e -i *ž*	os articulare
sokrižnica -e *ž*	synsacrum
solznica -e *ž*	os lacrimale
solznično nebna kost -e -i *ž*	os lacrimopalatinum
spodnja prstnica večjega prsta -e -e -- -- *ž*	phalanx distalis digiti majoris
sredinska sitkina kost -e -e -i *ž*	os mesethmoidale
stebrc -a *m*	pila otica
stegnenica -e *ž*	os femoris
stranska zagozdnica -e -e *ž*	os laterosphenoidale
stranska zatilnica -e -e *ž*	os exoccipitale
šivna kost -e -i *ž*	os suturarum
štirikotnica -e *ž*	os quadratum
štrleča kost -e -i *ž*	os prominens
telo jezičnice -esa -- *ž*	basihyale
temenica -e *ž*	os parietale
tilna kost -e -i *ž*	os nuchale
tretja stopalnica -e -e *ž*	os metatarsale III
ušesna kapsula -e -e *ž*	os oticum
ušesni stebriček -ega -čka *ž*	os opisthoticum
večja dlančnica -e -e *ž*	os metacarpale majus
vratno vretence -ega -a *s*	vertebra cervicalis
vretence sokrižne zrasti -a -- -- *ž*	vertebra synsacralis
vretenčno rebro -ega -a *s*	costa vertebralis
zagozdnica -e *ž*	os sphenoidale
zapestnodlančnica -e *ž*	carpometacarpus
zatilnica -e *ž*	os occipitale
zgornja čeljustnica -e -e *ž*	os maxillare
zgornja prstnica večjega prsta -e -e -- -- *ž*	phalanx proximalis digiti majoris
zgornja zatilnica -e –e *ž*	os supraoccipitale
zobna kost -e -i *ž*	os dentale
zunanja sitkina kost -e -e -i *ž*	os ectethmoidale
žmulasta kost -e -i *ž*	os spleniale

Dodatek 2: Abecedno zaporedje gesel v latinskem in slovenskem jeziku.
Appendix 2: Alphabetical list of terms in Latin and Slovenian language.

anulus tympanicus	bobničev obroč
apparatus hyobranchialis	jezičnica
atlas	nosač
axis	okretač
basihyale	telo jezičnice
carpometacarpus	zapestnodlančnica
columna vertebralis	hrbtenica
coracoideum	krokarnica
cornu branchiale	rog jezičnice
costa sternalis	prsnično rebro
costa vertebralis	vretenčno rebro
costae	rebra
costae completae spuriae	neprava popolna rebra
costae completae verae	prava popolna rebra
costae fluctuantes	prosta rebra
costae incompletae	nepopolna rebra
cranium	lobanja
fibula	mečnica
furcula	ključnica
humerus	nadlahtnica
maxilla	zgornja čeljustnica
notarium	hrbtna kost
os angulare	kotna kost
os articulare	sklepna kost
os basioccipitale	osnovna zatilnica
os basisphenoidale	osnovna zagozdnica
os carpi radiale	koželjnična zapestnica
os carpi ulnare	komolčnična zapestnica
os coronoideum	kronasta kost
os coxae	kolčnica
os dentale	zobna kost
os ectethmoidale	zunanja sitkina kost
os epioticum	nadušesna kost
os ethmoidale	sitkina kost
os exoccipitale	stranska zatilnica

os femoris	stegnenica
os fibulare	mečnična nartna kost
os frontale	čelnica
os ilium	črevnica
os ischii	sednica
os jugale	jarmova kost
os lacrimale	solznica
os lacrimopalatinum	solzničnonebna kost
os laterosphenoidale	stranska zagozdnica
os mesethmoidale	sredinska sitkina kost
os metacarpale alulare	perutkina dlančnica
os metacarpale majus	večja dlančnica
os metacarpale minus	manjša dlančnica
os metatarsale I	prva stopalnica
os metatarsale II	druga stopalnica
os metatarsale III	tretja stopalnica
os metatarsale IV	četrta stopalnica
os metoticum	priušesna kost
os nasale	nosnica
os nuchale	tilna kost
os occipitale	zatilnica
os opisthoticum	ušesni stebriček
os oticum	ušesna kapsula
os palatinum	nebnica
os parasphenoidale	obzagozdnica
os parietale	temenica
os prearticulare	predsklepna kost
os prominens	štrleča kost
os prooticum	predušesna kost
os pterygoideum	krilatka
os pubis	dimeljnica
os quadratojugale	kvadratnojarmova kost
os quadratum	štirikotnica
os siphonium	cevasta kost
os sphenoidale	zagozdnica
os spleniale	žmulasta kost
os squamosum	luskasta kost

os supraangulare	nadkotna kost
os suprajugale	nadjarmova kost
os supraoccipitale	zgornja zatilnica
os tarsi distale	spodnja nartna kost
os tibiale	golenična nartna kost
os uncinatum	kavljasta kost
ossa accessoria cranii	dodatne kosti lobanje
ossa alae	kosti peruti
ossa carpi	zapestnice
ossa cingula membri pelvini	kosti obroča medenične okončine
ossa cingula membri thoracini	kosti obroča prsne okončine
ossa cranii	kosti lobanje
ossa digitorum manus	prstnice roke
ossa digitorum pedis	kosti prstov stopala
ossa mandibulae	kosti spodnje čeljusti
ossa maxillae et palati	kosti zgornje čeljusti in neba
ossa membri pelvici	kosti medenične okončine
ossa metatarsalia	stopalnice
ossa pedis	kosti stopala
ossa proximalia tarsi	bližnje nartne kosti
ossa sclerae	beločnične kosti
ossa supraorbitalia	nadočnične kosti
ossa suturarum	šivne kosti
ossa tarsi	nartne kosti
ossa temporalia	senčnične kosti
paraglossum	objezičnica
patella	pogačica
pelvis	medenica
phalanx digiti alulae	prstnica perutke
phalanx digiti minoris	prstnica manjšega prsta
phalanx digitorum pedis	prstnica prstov stopala
phalanx distalis digiti majoris	spodnja prstnica večjega prsta
phalanx proximalis digiti majoris	zgornja prstnica večjega prsta
phalanx ungualis	krempeljnica
pila otica	stebrc
premaxilla	predčeljustnica
pygostylus	križni opornik

radius	koželjnica
scapula	plečnica
skeleton alae	okostje peruti
skeleton antebrachii	okostje podlakti
skeleton appendiculare	privesno okostje
skeleton axiale	osno okostje
skeleton brachii	okostje nadlakti
skeleton cruris	okostje goleni
skeleton femoris	okostje stegna
skeleton manus	okostje roke
skeleton membri pelvini	okostje medenične okončine
skeleton membri thoracini	okostje prsne okončine
skeleton pedis	okostje stopala
skeleton thoracis	okostje prsnega koša
sternum	prsnica
synsacrum	sokrižnica
tarsometatarsus	nartnostopalna kost
tibiotarsus	goleničnonartna kost
ulna	komolčnica
urohyale	rep jezičnice
vertebrae caudales	repna vretenca
vertebrae caudales liberae	prosta repna vretenca
vertebrae cervicales	vratna vretenca
vertebrae lumbales	ledvena vretenca
vertebrae sacrales	križna vretenca
vertebrae thoracicae	prsna vretenca
vomer	lemežnica

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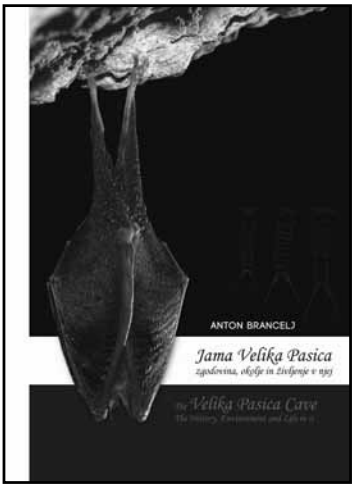
ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2015 Vol. 58, Št. 2: 93–95

Brancelj, Anton, Jama Velika Pasica/ The Velika Pasica Cave
Zgodovina, okolje in življenje v njej/ The History, Environment and Life in it
Prva izdaja, Založba ZRC in Nacionalni inštitut za biologijo, LJUBLJANA 2016



Bibliografski podatki o knjigi: knjiga obsega 110 strani, vključuje 25 slik, 9 tabel in 55 izvirnih fotografij. Knjiga s strokovno recenzijo in lektoriranjem je napisana v slovenskem in angleškem jeziku.

ISBN 978-961-254-820-9 (Založba ZRC) 281110784

Prof. Antona Branclja, avtorja knjige o zgodovini in življenju v jami Velika Pasica v bližini vasi Gornji Ig na Krimu, poznam že od študentskih let, ko se je njegovo zanimanje za svet podzemlja kazalo kot navdušenje in strast do odkrivanja zanimivosti in razsežnosti jamskega okolja ter življenja v njem. Pogosti obiski kraškega podzemnega sveta od dijaških let pa vse do danes so avtorju prinašali nova spoznanja o biologiji in razširjenosti jamskih organizmov ter prispevali k razvoju slovenske speleobiologije. Kot dolgoletni član Društva za raziskovanje jam se je avtor udeleževal sprva številnih ekskurzij, pozneje pa tudi jamarskih odprav z namenom raziskovanja in poučevanja. Kot pedagoški delavec je znanje o življenju v jamah posredoval generacijam študentov in jih navduševal za delo na tem področju.

Pričujoče delo je dokaj obsežna monografija, ki vključuje dober zgodovinski pregled dogodkov, povezanih z naključnimi in načrtovanimi obiski, meritvami in odkritji v jami Velika Pasica v obdobjih od sredine 19. stoletja pa vse do danes. V katastru Društva za raziskovanje jam Ljubljana (DZRJL) je shranjena večina starejših zapisnikov o obiskih Velike Pasice. Iz zapisnika DZRJL je razvidno, da je bila jama 15. maja 1927 registrirana kot "VELIKA PASICA pri Zgornjem Igu".

Avtor v uvodu opiše geografsko lego jame s slikovitimi in nazornimi navodili za preprost dostop, ki spodbudijo bralca, da bi se nemudoma odpravil na pot in poiskal to znamenitost. Ko prispemo do vhoda naletimo na železno rešetko, ki zapira vhod v jamo. Pred leti so domačini jamo zavarovali z vrati, saj je bila v preteklosti občasno izpostavljena tudi nezaželenim obiskom in vandalizmu. Na seznamu naravnih vrednot, ki imajo lastnosti jame v skladu z zakonom, ki določa varstvo podzemnih jam, ima Velika Pasica status odprte vodoravne jame z nadzorovanim vstopom. Po temeljitem očiščenju jame je postala jama dostopna in primerna tudi za obiskovalce, ki si želijo ogledati to naravno zanimivost osrednjeslovenske regije.

Jamo Velika Pasica uvrščamo v kategorijo hidrološko neaktivnih jam, ker v njej ni tekoče vode. Knjiga vsebuje natančen opis velikosti jame, jamskega reliefa in prikaz kapniških struktur, dopolnjen s kvalitetnimi fotografijami in izvirnimi risbami. Fotografije jamarjev v barvitih oblačilih popestrijo vsebino in pričajo o tem, da je življenje v jami lahko občasno tudi zelo živahno, in hkrati vzbujajo optimizem o prihodnosti raziskovanja in ohranjanja slovenskega kraškega podzemlja.

Knjiga vsebuje množico podatkov o klimatskih razmerah v jami in njeni okolici, ki izvirajo iz dolgoletnih in natančnih meritev temperature vode in zraka ter pretoka prenikajoče vode. Knjiga

vključuje tudi grafične prikaze izvirnih rezultatov meritev, ki so podpora za razprave o hidroloških procesih v jami. Prikazan je tudi vpliv večjih nalivov na povečan pretok prenikle vode in spremembe temperature na merilnih mestih. V knjigi so podrobno predstavljeni hidravlični procesi v jami v povezavi z različnimi padavinskimi režimi. Rezultati hidroloških in kemijskih meritev so prikazani in razloženi z vidika ekoloških razmer, v katerih živijo vodni organizmi epikrasa, najvišjega sloja krasa, ki sega do površja in zajema preperino in kraško kamnino.

Strokovna kakovost dela temelji na obsežni in natančni navedbi virov, na predstavitvi številnih izvirnih podatkov fizikalnih in kemijskih meritev vode in zraka ter skrbno načrtovanih poskusih. Zelo natančno so opisane naprave za zbiranje vzorcev materiala, ki pronica v jamo z vodnimi curki, prikazani pa so tudi shranjevalniki podatkov. Bralec sledi opisom posameznih živalskih vrst skozi zgodovino vse do današnjih časov, ko lahko opisane primerke opazujemo v njihovem naravnem okolju. Avtor opisuje različne skupine živali in njihovo prilagoditev na življenje v jamah; od občasnih obiskovalcev jam do stalnih prebivalcev v jamskih vodnih in kopenskih habitatih.

V okolici jame prevladuje jelovo-bukov gozd, ki je zaradi pogoste sečnje že precej redek. Ob vhodu v jamo so skale porasle s praprotjo, mahovi in jetrnjaki in algami. V vhodni dvorani so ostanki lesa prerasli s plesnimi, kamnine pa so večinoma pokrite s kolonijami bakterij, ki so pokrite z vodnimi kapljicami, ki se ob osvetlitvi zlato ali srebrno zableščijo in jih zato jamarji imenujejo »jamsko zlato«.

V knjigi so s fotografijami in slikovitimi opisi predstavljene najpogostejše živali, ki živijo v jami, od migetalkarjev, polžev, rakov, predvsem ceponožcev in dvoklopnikov, do paščipalcev, kačic in hroščev. Prav med temi organizmi je bilo opisanih trinajst novih vrst in podvrst. Zgodovinsko pomembni so opisi jamskih hroščev, polžev in paščipalcev, več kot pet novih vrst je bilo opisanih iz te jame v obdobju med drugo polovico 19. in prvo polovico 20. stoletja. Avtor knjige je opisal štiri nove vrste rakov ceponožcev iz talnih lužic v jami. Pozimi se v vhodnih delih jame pogosto zadržujejo tudi netopirji mali podkovnjaki. Iz najdenih iztrebkov gre sklepat, da so občasni gostje v jamah tudi kune, polhi in gozdne miši. Fotografije primerkov živali iz jame, ostankov njihovih iztrebkov ali znakov aktivnosti nekaterih živali v preteklosti (okostje jamskega medveda) prispevajo k zanimivosti knjige.

Kopenski živalski svet v jami je zelo bogat, še bogatejši pa je svet vodnih organizmov, ki živijo v različnih tipih talnih lužic, predvsem v bližini stalnih curkov. Glede na število opisanih podzemnih vrst živali (31) uvrščamo jamo Velika Pasica na deveto mesto na svetovni lestvici. Prisotnost epikraških vrst v jami z visoko nadmorsko višino je posebnost, ki jo je avtor zelo podrobno opisal v knjigi in dokumentiral tudi z navajanjem velikega števila vzorčenih osebkov v štirih curkih iz plavja v povezavi s hidrokemijskimi in hidrološkimi parametri v osemletnem obdobju (2006–2013). Avtor ugotavlja, da je glede na številnost osebkov verjetno, da je epikras za opisane vrste primarno okolje, od koder se v obliki plavja pomikajo v nižje ležeče predele. Posebnost telesnih oblik organizmov iz epikrasa, ki se kažejo predvsem kot močni in zaobljeni trni na okončinah in zadnjem telesnem členu ceponožcev iz jame Velika Pasica, je pomembna prilagoditev na življenje v ozkih špranjah, kjer se pretaka voda.

Vredno je izpostaviti dejstvo, da je v knjigi opisana velika raznolikost življenja v povezavi z razmerami v specifičnem epikraškem okolju. Kot navaja avtor, je to verjetno tudi posledica dolgoletnih in podrobnih raziskav in opisov živalstva v jami Velika Pasica. Knjiga je zanimiva tako za strokovnjake kot za širšo javnost. Delo je strokovno korektno in vključuje številne podatke, ki temeljijo na dolgoletnem raziskovalnem delu, objavljenem v znanstvenih člankih v kvalitetnih revijah. Primerna je kot obštudijsko gradivo za študente biologije, znanosti o okolju in naravovarstva in za študente naravoslovja. Avtorju je uspelo jamo Veliko Pasico predstaviti v obliki, ki bo navdušila tudi širši krog bralcev, saj s slikovito in razumljivo besedo naravnost vabi v njen svet. Tudi pri mlajših bralcih bo knjiga z odličnimi ilustracijami zagotovo vzbudila zanimanje za speleologijo in biologijo podzemlja.

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INSTRUCTIONS FOR AUTHORS

1. Types of Articles

SCIENTIFIC ARTICLES are comprehensive descriptions of original research and include a theoretical survey of the topic, a detailed presentation of results with discussion and conclusion, and a bibliography according to the IMRAD outline (Introduction, Methods, Results, and Discussion). In this category ABS also publishes methodological articles, in so far as they present an original method, which was not previously published elsewhere, or they present a new and original usage of an established method. The originality is judged by the editorial board if necessary after a consultation with the referees. The recommended length of an article including tables, graphs, and illustrations is up to fifteen (15) pages; lines must be double-spaced. Scientific articles shall be subject to peer review by two experts in the field.

REVIEW ARTICLES will be published in the journal after consultation between the editorial board and the author. Review articles may be longer than fifteen (15) pages.

BRIEF NOTES are original articles from various biological fields (systematics, biochemistry, genetics, physiology, microbiology, ecology, etc.) that do not include a detailed theoretical discussion. Their aim is to acquaint readers with preliminary or partial results of research. They should not be longer than five (5) pages. Brief note articles shall be subject to peer review by one expert in the field.

CONGRESS NEWS acquaints readers with the content and conclusions of important congresses and seminars at home and abroad.

ASSOCIATION NEWS reports on the work of Slovene biology associations.

2. Originality of Articles

Manuscripts submitted for publication in *Acta Biologica Slovenica* should not contain previously published material and should not be under consideration for publication elsewhere.

3. Language

Articles and notes should be submitted in English, or as an exception in Slovene if the topic is very local. As a rule, congress and association news will appear in Slovene.

4. Titles of Articles

Title must be short, informative, and understandable. It must be written in English and in Slovene language. The title should be followed by the name and full address of the authors (and if possible, fax number and/or e-mail address). The affiliation and address of each author should be clearly marked as well as who is the corresponding author.

5. Abstract

The abstract must give concise information about the objective, the methods used, the results obtained, and the conclusions. The suitable length for scientific articles is up to 250 words, and for brief note articles, 100 words. Article must have an abstract in both English and Slovene.

6. Keywords

There should be no more than ten (10) keywords; they must reflect the field of research covered in the article. Authors must add keywords in English to articles written in Slovene.

7. Running title

This is a shorter version of the title that should contain no more than 60 characters with spaces.

8. Introduction
The introduction must refer only to topics presented in the article or brief note.

9. Illustrations and Tables
Articles should not contain more than ten (10) illustrations (graphs, dendrograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided in electronic form. Tables should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2; Tab. 1 and Sl. 1). A full name is used in the legend title (e.g. Figure 1, Table 2 etc.), written bold, followed by a short title of the figure or table, also in bold. Subpanels of a figure have to be unambiguously indicated with capital letters (A, B, …). Explanations associated with subpanels are given alphabetically, each starting with bold capital letter (**A**), a hyphen and followed by the text.

10. The quality of graphic material
All the figures have to be submitted in the electronic form. The ABS publishes figures either in pure black and white or in halftones. Authors are kindly asked to prepare their figures in the correct form to avoid unnecessary delays in preparation for print, especially due to problems with insufficient contrast and resolution. Clarity and resolution of the information presented in graphical form is the responsibility of the author. Editors reserve the right to reject unclear and poorly readable pictures and graphical depictions. The resolution should be 300 d.p.i. minimum for halftones and 600 d.p.i. for pure black and white. The smallest numbers and lettering on the figure should not be smaller than 8 points (2 mm height). The thickness of lines should not be smaller than 0.5 points. The permitted font families are Times, Times New Roman, Helvetica and Arial, whereby all figures in the same article should have the same font type. The figures should be prepared in TIFF, EPS or PDF format, whereby TIFF (ending *.tif) is the preferred type. When saving figures in TIFF format we recommend the use of LZW or ZIP compression in order to reduce the file sizes. The photographs can be submitted in JPEG format (ending *.jpg) with low compression ratio. Editors reserve the right to reject the photos of poor quality. Before submitting a figure in EPS format make sure first, that all the characters are rendered correctly (e.g. by opening the file first in the programs Ghostview or GSview – depending on the operation system or in Adobe Photoshop). With PDF format make sure that lossless compression (LZW or ZIP) was used in the creation of the *.pdf file (JPEG, the default setting, is not suitable). Figures created in Microsoft Word, Excel, PowerPoint etc. will not be accepted without the conversion into one of the before mentioned formats. The same goes for graphics from other graphical programs (CorelDraw, Adobe Illustrator, etc.). The figures should be prepared in final size, published in the magazine. The dimensions are 12.5 cm maximum width and 19 cm maximum height (width and height of the text on a page).

11. Conclusions
Articles shall end with a summary of the main findings which may be written in point form.

12. Summary
Articles written in Slovene must contain a more extensive English summary. The reverse also applies.

13. Literature
References shall be cited in the text. If a reference work by one author is cited, we write Allan (1995) or (Allan 1995); if a work by two authors is cited, (Trinajstić and Franjić 1994); if a work by three or more authors is cited, (Pullin et al. 1995); and if the reference appears in several works, (Honsig-Erlenburg et al. 1992, Ward 1994a, Allan 1995, Pullin et al. 1995). If several works by the same author

published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc.: (Ward 1994a,b). If direct quotations are used, the page numbers should be included: Toman (1992: 5) or (Toman 1992: 5–6).The bibliography shall be arranged in alphabetical order beginning with the surname of the first author, comma, the initials of the name(s) and continued in the same way with the rest of the authors, separated by commas. The names are followed by the year of publication, the title of the article, the international abbreviation for the journal (periodical), the volume, the number in parenthesis (optional), and the pages. Example:

Mielke, M.S., Almeida, A.A.F., Gomes, F.P., Aguilar, M.A.G., Mangabeira, P.A.O., 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of Genipa americana seedlings to soil flooding. Experimental Botany, 50 (1), 221–231.

Books, chapters from books, reports, and congress anthologies use the following forms:

Allan, J.D., 1995. Stream Ecology. Structure and Function of Running Waters, 1st ed. Chapman & Hall, London, 388 pp.

Pullin, A.S., McLean, I.F.G., Webb, M.R., 1995. Ecology and Conservation of *Lycaena dispar*: British and European Perspectives. In: Pullin A. S. (ed.): Ecology and Conservation of Butterflies, 1st ed. Chapman & Hall, London, pp. 150-164.

Toman, M.J., 1992. Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1-7.

14. Format and Form of Articles

The manuscripts should be sent exclusively in electronic form. The format should be Microsoft Word (*.doc) or Rich text format (*.rtf) using Times New Roman 12 font with double spacing, align left only and margins of 3 cm on all sides on A4 pages. Paragraphs should be separated by an empty line. The title and chapters should be written bold in font size 14, also Times New Roman. Possible sub-chapter titles should be written in italic. All scientific names must be properly italicized. Used nomenclature source should be cited in the Methods section. The text and graphic material should be sent to the editor-in-chief as an e-mail attachment. For the purpose of review the main *.doc or *.rtf file should contain figures and tables included (each on its own page). However, when submitting the manuscript the figures also have to be sent as separate attached files in the form described under paragraph 10. All the pages (including tables and figures) have to be numbered. All articles must be proofread for professional and language errors before submission.

A manuscript element checklist (For a manuscript in Slovene language the same checklist is appropriately applied with a mirroring sequence of Slovene and English parts):
English title – (Times New Roman 14, bold)
Slovene title – (Times New Roman 14, bold)

Names of authors with clearly indicated addresses, affiliations and the name of the corresponding author – (Times New Roman 12)
Author(s) address(es) / institutional addresses – (Times New Roman 12)
Fax and/or e-mail of the corresponding author – (Times New Roman 12)
Keywords in English – (Times New Roman 12)
Keywords in Slovene – (Times New Roman 12)
Running title – (Times New Roman 12)
Abstract in English (Times New Roman 12, title – Times New Roman 14 bold)

Abstract in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Introduction – (Times New Roman 12, title – Times New Roman 14 bold)
Material and methods – (Times New Roman 12, title – Times New Roman 14 bold)
Results – (Times New Roman 12, title – Times New Roman 14 bold)
Discussion – (Times New Roman 12, title – Times New Roman 14 bold)
Summary in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Figure legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, figure designation and figure title – Times New Roman 12 bold)
Table legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, table designation and table title – Times New Roman 12 bold)
Acknowledgements – (Times New Roman 12, title – Times New Roman 14 bold)
Literature – (Times New Roman 12, title – Times New Roman 14 bold)
Figures, one per page; figure designation indicated top left – (Times New Roman 12 bold)
Tables, one per page; table designation indicated top left – (Times New Roman 12 bold)
Page numbering – bottom right – (Times New Roman 12)

15. Peer Review

All Scientific Articles shall be subject to peer review by two experts in the field (one Slovene and one foreign) and Brief Note articles by one Slovene expert in the field. With articles written in Slovene and dealing with a very local topic, both reviewers will be Slovene. In the compulsory accompanying letter to the editor the authors must nominate one foreign and one Slovene reviewer. However, the final choice of referees is at the discretion of the Editorial Board. The referees will remain anonymous to the author. The possible outcomes of the review are: 1. Fully acceptable in its present form, 2. Basically acceptable, but requires minor revision, 3. Basically acceptable, but requires important revision, 4. May be acceptable, but only after major revision, 5. Unacceptable in anything like its present form. In the case of marks 3 and 4 the reviewers that have requested revisions have to accept the suitability of the corrections made. In case of rejection the corresponding author will receive a written negative decision of the editor-in-chief. The original material will be erased from the ABS archives and can be returned to the submitting author on special request. After publication the corresponding author will receive the *.pdf version of the paper.