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COMPARATIVE HISTOLOGICAL EVALUATION OF THE FRUIT OF *RIBES NIGRUM* AND *RIBES RUBRUM*

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Abstract: To complete the wide range of studies on chemical composition and pharmacological potential of the fruit of *Ribes nigrum* and *Ribes rubrum*, this work aimed to perform a comparative morpho-anatomical characterization of the fruits of both species and complete previously records of black currant as frequently studied species. Microslides of fruits were made with rotation microtome, and then cross sections were stained with toluidine blue. Documentation and evaluation were carried out in microphotos. In *R. rubrum*, the epidermis cells are ovoid to round, while those of *R. nigrum* are rectangular to almost round. Under the epidermis, in both fruits, there are two layers of oval hypodermal cells. Underneath the hypodermal layers, parenchyma cells of different size with thin walls and scattered collateral closed bundles were detected. The seeds are embedded in a gelatinous sheath having large thin-walled cells. Endocarp separates the arillar tissue from parenchyma cells, in addition, a gap can be found among these layers, endocarp, arillar tissue, and seeds. The seed coat consists of sclerenchyma cells in both species. In conclusion, histological differences and similarities were described in the fruits of the selected *Ribes* species, highlighted the first morpho-anatomical description of the berry of *Ribes rubrum*.

Keywords: *Ribes nigrum*, *Ribes rubrum*, fruit, microscopic examination.

1. Introduction

Grossulariaceae family is composed of approximately 195 species (The Plant List, 2018). According to Plant Database (USDA, 2018) there are 65 species of *Ribes* genus including native and introduced plants in the USA. In Europe 16 *Ribes* species are known (Marhold, 2011; EU-NOMEN) involving 8 taxa in Romania namely *R. uva-crispa* L., *R. nigrum* L., *R. alpinum* L., *R. aureum* Pursh, *R. sanguineum* Pursh, *R. petraeum* Wulfen in Jacq., *R. spicatum* Robson, and *R. rubrum* L. (Ciocarlan, 2000). *Ribes nigrum* (black currant) is a perennial shrub which grows in bushy places, groves (Ciocarlan, 2000). It is also widely cultivated for berry production. The species has a wide variety of secondary metabolites. Leaves contain lignoids (Sasaki et al. 2013), glycosides of quercetin and kaempferol as major phenolics (Liu et al., 2014), accompanied by significant amounts of salicylic acid, p-coumaric acid, caffeic and gallic acids (Nour et al., 2014). Mineral and trace elements also were measured in the leaves of different cultivars of the plant (Nour et al., 2014). Fruits contain phenolics: they are especially rich in anthocyanins, e.g. glycosides of delphinidin and cyanidin (Gavrilova et al. 2011). The level of anthocyanins varies depending on the season, degree of maturity, environmental conditions, type of cultivation, and genetic characters (Milivojevic et al., 2012; Zheng et al., 2012; Vagiri et al., 2013; Mikulic-Petkovsek et al., 2015). Among organic acids, citric, fumaric and malic acids are dominant (Mikulic-Petkovsek et al., 2012; Woznicki et al., 2017). The fruits are rich in vitamin C (Häkkinen et al. 1999).

Ribes rubrum (red currant) is cultivated in Romania, which is not the member of the spontaneous flora (Ciocarlan, 2000). In the literature it is known under synonymies *R. vulgare* Lam. and *R. sylvestre* Mert. et Koch (Ciocarlan, 2000; Marhold, 2011; EU-NOMEN). In phytochemical aspect, anthocyanin content – mostly glycosides of cyanidin (Gavrilova et al., 2011; Mikulic-Petkovsek et al., 2015) – is about 10% in the fruit compared to that of black currant (Gavrilova et al., 2011; Mikulic-Petkovsek et al., 2015). Among flavonoids, glycosides of quercetin, myricetin and kaempferol (Mikulic-Petkovsek et al. 2015), as well as citric, fumaric and malic acids were detected in the plant in similar, but shikimic acid in 13 times higher quantity compared to the fruit of black currant (Mikulic-Petkovsek et al. 2012).

In the fruit of both species, fructose and glucose are predominant among sugars (Mikulic-Petkovsek et al., 2012; Woznicki et al., 2017). The seed oil is rich in tocopherol, unsaturated fatty acids, linoleic, α-linolenic, and γ-linolenic acids (Traitler et al., 1984; Goffman and Galetti, 2001; Leskinen et al., 2009; Vuorinen et al., 2016).

In pharmacological aspect, currants were studied for the effect in dietary management of various diseases (Zágoni, 2005; Zdunić et al., 2016). Polyphenolic substances of the fruit and leaf were earlier examined for the anti-inflammatory, antioxidant, antifungal, and anticancer effect (Knekt et al., 1997; Šavikin et al., 2009; Bishayee et al., 2010; Slavin and Lloyd, 2012). The polyphenolic composition of the fruit and the leaf of black currant plays a role in the structure of biological and lipid membranes, highlighted flavonols in the leaf, and anthocyanins in the fruit as dominant antioxidant compounds. The studied extracts protected the membranes against the oxidation and modified the properties of the hydrophilic regions of the membranes (Cyboran et al. 2014). The fruits of black and red currant were tested *in vitro* for potential management of type 2 diabetes and hypertension resulted high α-glucosidase, α-amylase and ACE inhibitor activities in red currant (Da Silva Pinto et al., 2010).

Despite the described phytochemical and pharmacological potential of the selected species, there are few studies on the morpho-anatomical description of the fruits as medicinally used parts in *Ribes* species (Wrońska-Pilarek, 2001; Wrońska-Pilarek, 2002; Kendir et al., 2015). In an earlier report arrilar tissue was observed in several species of the genus. Among them, arils cover the seeds in the slightly fleshy pericarps in *R. nigrum*, which is absent in *R. rubrum* (Corner, 1976).

In the last two decades, only one histological report was published on the fruit of black currant (Glidewell et al., 1999), and according to our best knowledge no other recent study is available concerning the histology of red currant. Thus, this work aimed to perform a comparative histological characterization of the fruit of *R. nigrum* and *R. rubrum* collected in Romania.

2. Materials and Methods

2.1 Plant material

Fruits of *R. nigrum* and *R. rubrum* (10 pieces of each species) were harvested at full maturity from a local farmer's garden in July and August 2016 (Cornești, Mureș County, Romania). Collected samples were frozen and stored at -20°C until analyses.

2.2 Morphological and anatomical study

For microscopic examination, whole fruits were preserved in the mixture of ethanol : glycerine : distilled water (1:1:1). Fruits were dehydrated in 50%, 70% and 96% ethanol (Békési-Kallenberger et al. 2016), then they were embedded in Technovit® 7100 resin (Realtrade Ltd., Hungary). 15 µm thick cross-sections were obtained with a rotary microtome (Anglia Scientific 0325) (Papp et al., 2013). The slides were dried at 50°C for 2 hours, stained in toluidine blue (0.02%) for 5 min, and soaked twice in 96% ethanol (3 min each), isopropanol (4 min), and twice in xylol (3 and 4 min). Sections were studied with a NIKON Eclipse 80i microscope and micrographs were taken with SPOT Basic 4.0 image analysis system.

3. Results and discussions

3.1 Histological features of the fruit of *Ribes nigrum*

In the fruit of *R. nigrum*, the epidermis is formed of rectangular to almost round shape cells covered by cuticle layer. Under the epidermis (epicarp), two layers of oval hypodermal cells can be found (**Fig. 1 A,B**). Under the hypodermal layers the mesocarp parenchyma is composed of different size of thin-walled cells and scattered vascular bundles (**Fig. 1 A,C**). The collateral closed vascular bundles consists of xylem and phloem that are surrounded by sclerenchyma (**Fig. 1 D**). Seeds are embedded in a gelatinous sheath which is constructed from large thin-walled cells (**Fig. 2 A,B,C**), accordingly to a previous study (Glidewell et al. 1999). The arillar tissue is separated from the endocarp by a space (**Fig. 2 B**). The seed coat (testa) consists of sclerenchyma cells (**Fig. 2 D**).

3.2 Histological features of the fruit of *Ribes rubrum*

In *R. rubrum*, the epidermis of the fruit is formed from more ovoid to round cells than those of *R. nigrum*. The double-layered hypodermis contains horizontally thick-walled and elongated cells (**Fig. 3 A, 3 D**). Parenchyma is composed of thin-walled cells in various sizes. The scattered collateral closed vascular bundles (**Fig. 3 A**) have only xylem and phloem elements (**Fig. 3 B**). Endocarp separates the arillar tissue from parenchyma cells; between arillar tissue and seeds a gap was observed (**Fig. 3 C**). Similar to black currant, seeds are embedded in a gelatinous sheath (**Fig. 3 C, Fig. 4 A**) consisting of large cells with slightly sinuous cell walls (**Fig. 4 C**). The seed coat includes sclerenchyma cells (**Fig. 4 B**), while the storage ground tissue consists of thick-walled cells in the seed (**Fig. 4 D**).

Under histological aspect, the stem of some *Ribes* species were described in wood anatomical atlases (Metcalfe and Chalk, 1957; Schweingruber et al. 2011), e.g. a diffuse-porous xylem in *Ribes nigrum* (Schweingruber et al. 2011). In another work, the whole fruit of *Ribes nigrum* was analysed in different developmental stages (Glidewell et al. 1999). Morphological and histological characters of the seed were reported for native species like *R. uva-crispa*, *R. alpinum*, *R. petraeum*, and *R. spicatum* in Poland (Wrońska-Pilarek, 2001; Wrońska-Pilarek, 2002), as well as a surface analysis by scanning microscope on the fruit, seed and pollen of *R. biebersteinii* Berl. ex. DC, *R. uva-crispa*, *R. alpinum*, *R. orientale* Desf., *R. multiflorum* Kit. ex Romer &Schultes, and *R. anatolica* Behçet which are native to

Turkey (Kendir et al., 2015), without detailed histological description of all parts of the fruits. Based on these studies, our work describes the histological features of the whole fruit of *Ribes nigrum* and *R. rubrum* including the seeds from the collected samples from Romania. In this work the fruit of red currant is described in all details here for the first time. Similarities and differences were observed in microscopic preparations of the fruit of both currant species. Among similar features, a thin layer of cuticle, lack of stomata, two layers of hypodermal cells (**Fig. 1 B, 2 A**), scattered collateral closed bundles (**Fig. 1 D, 3 B**), and arillar tissue (**Fig. 2 B**) can be mentioned. The seed coats of both species consist of sclerenchyma cells surrounded by a gelatinous sheath (**Fig. 1 C, 2 C**) which is in accordance with earlier literature data (Glidewell et al. 1999). This sheath, also called sarcotesta, is different in thickness in *Ribes* species. Sometimes it is equal to the width of the seeds (Wrońska-Pilarek, 2001), but it is less than half of those in *R. nigrum* and *R. rubrum* described in this study. In both fruits, major part of the pericarp and the placental region is occupied by medium and large parenchyma cells (**Fig. 1 A,C** and **Fig. 3 A,C,D**), as it was previously described in black currant (Glidewell et al. 1999).

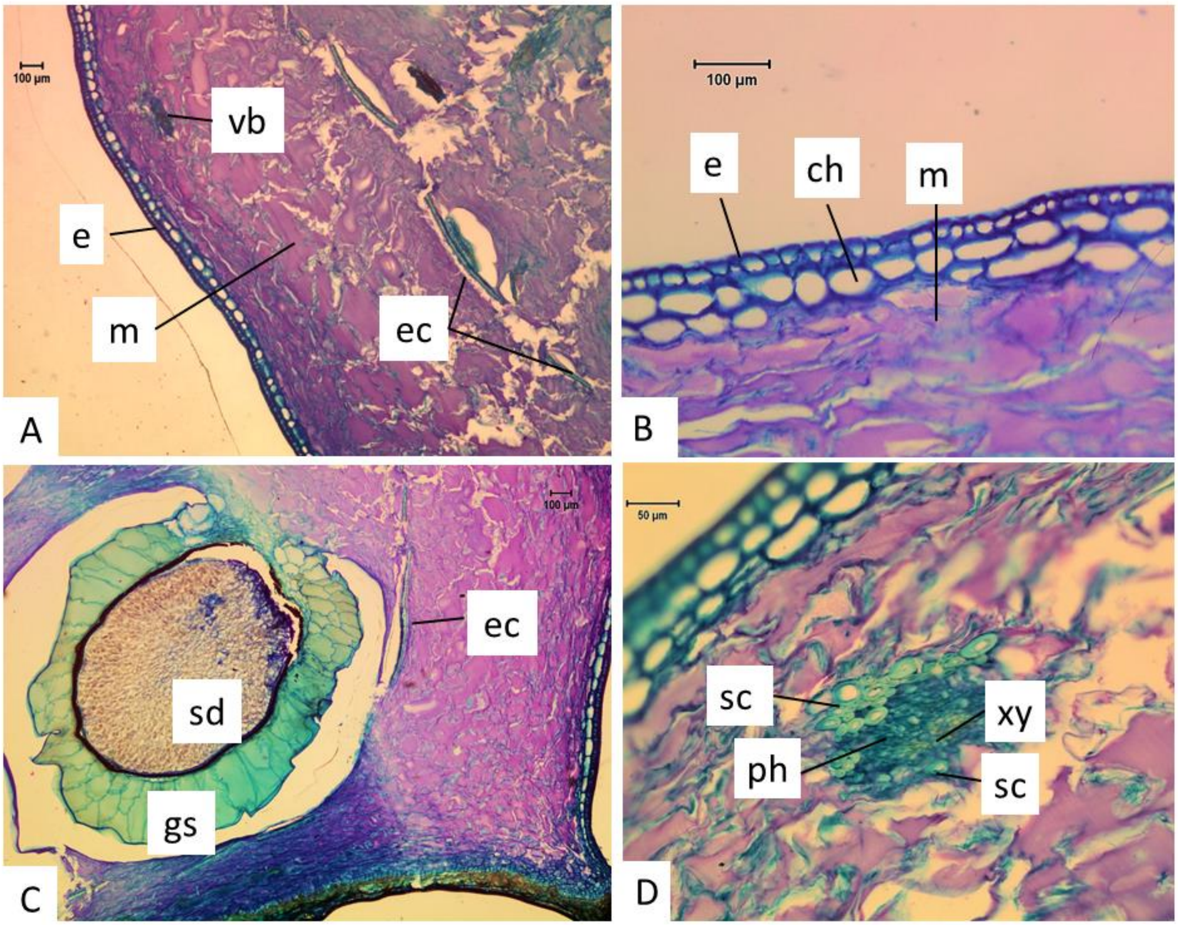

Fig. 1. Histological features of the fruit of *Ribes nigrum*. A-B) fruit wall; C) seed embedded in gelatinous sheath; D) collateral closed vascular bundle; e: epicarp (epidermis), ec: endocarp, ch: collenchymatic hypodermis, gs: gelatinous sheath, m: mesocarp, xy: xylem, ph: phloem, sc: sclerenchyma, sd: seed, vb: vascular bundle

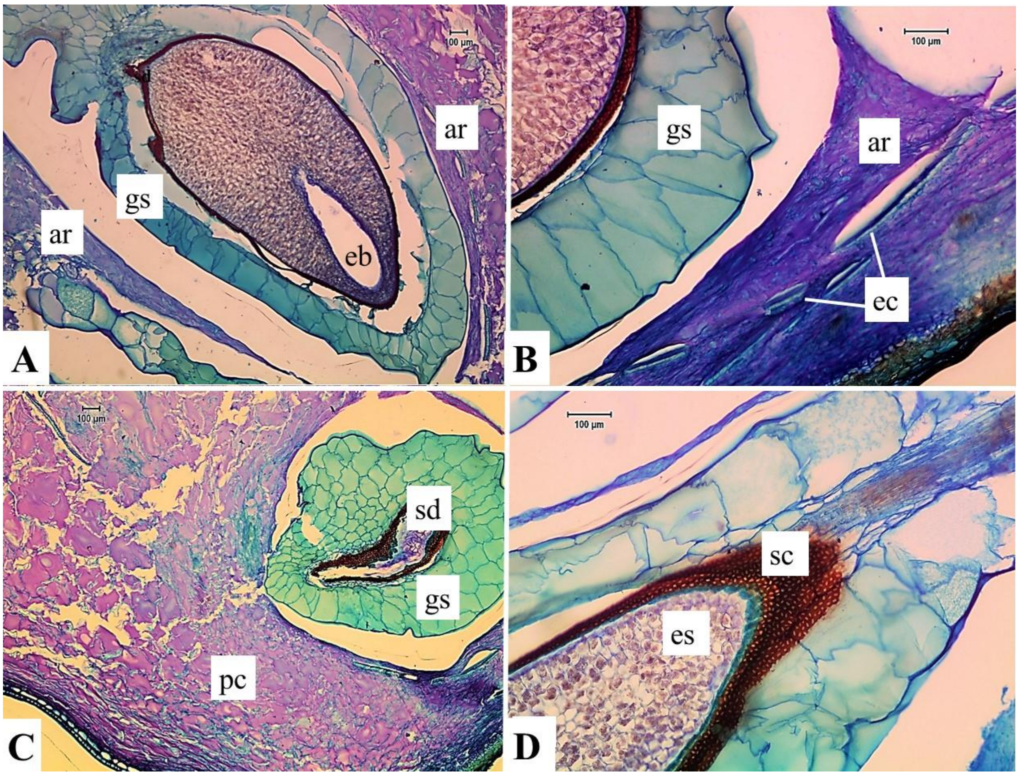

Fig. 2. Histological features of the seed of *Ribes nigrum*. A) seed; B) aril; C) gelatinous cells; D) testa; ar: aril, eb: the place of embryo (tissue is missing), ec: endocarp, es: endosperm, gs: gelatinous sheath, pc: parenchyma cells, sc: sclerenchyma cells, sd: seed

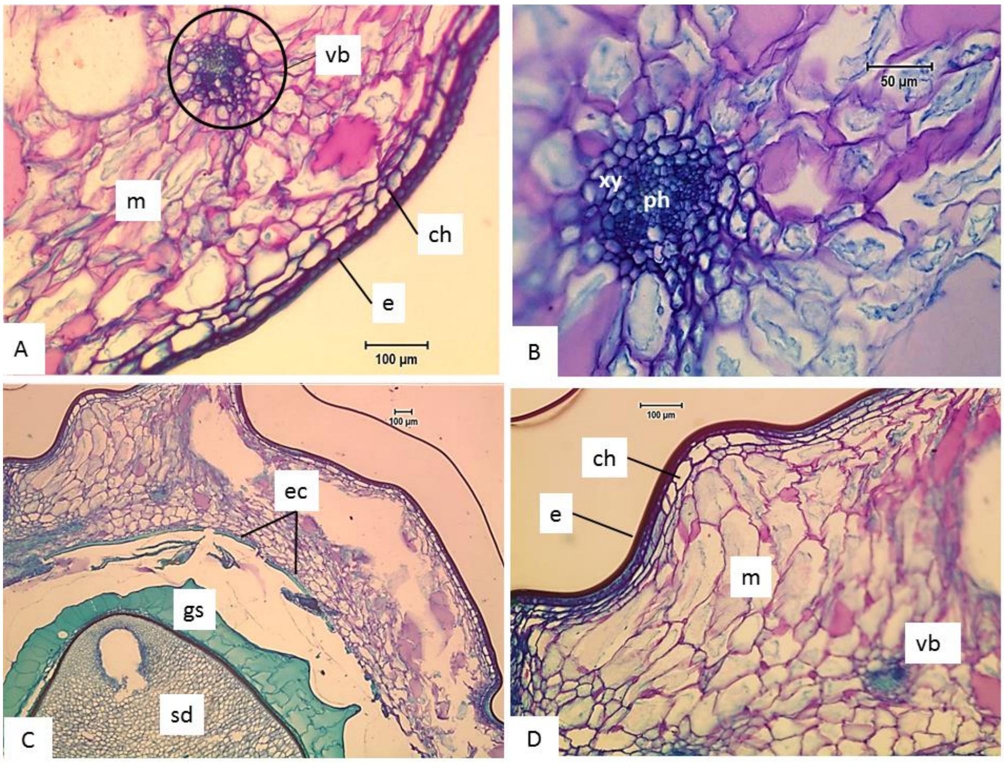

Fig. 3. Histological features of the fruit of *Ribes rubrum*. A) pericarp; B) vascular bundle; C) seed embedded in gelatinous sheath; D) pericarp; e: epicarp (epidermis), ec: endocarp, ch: collenchymatic hypodermis, gs: gelatinous sheath, m: mesocarp, xy: xylem, ph: phloem, sd: seed, vb: vascular bundle

As differences, various shapes of epidermis cells (**Fig. 1 B, 3 A**) and different intensity of dyeing of parenchymal cells were observed: stronger coloration of the pericarp and placenta refer to a higher polysaccharide content in black currant compared to red currant (**Fig. 1 A, 3 A,C**). Contrary to gooseberry's fruit (Williamson et al., 1993), the gelatinous sheath around the seeds is not single-layered, but multi-layered up to six cell layers in the studied species (**Fig. 1 C, 4 A**). In the endosperm, fatty oil drops were detected in *R. nigrum* (**Fig. 2 D**), which does not appear in *R. rubrum* (**Fig. 4 D**). This is in accordance with earlier findings using Sudan IV: coloration was found only in the external layer of the endosperm in red currant (Wrońska-Pilarek 2001).

Generally, in berries (Giongo et al. 2013), as in other fruits such as tomato (Saladie et al. 2007), the texture of the fruit is related to the cell wall structure and to change in ripening phases (Giongo et al. 2013). The structure of the pericarp has a great influence on the storage ability of the ripe fruit and the postharvest management. As expected, epidermal cells in the berry of black and red currant are covered by cuticle which can explain the relative long maintenance of their freshness.

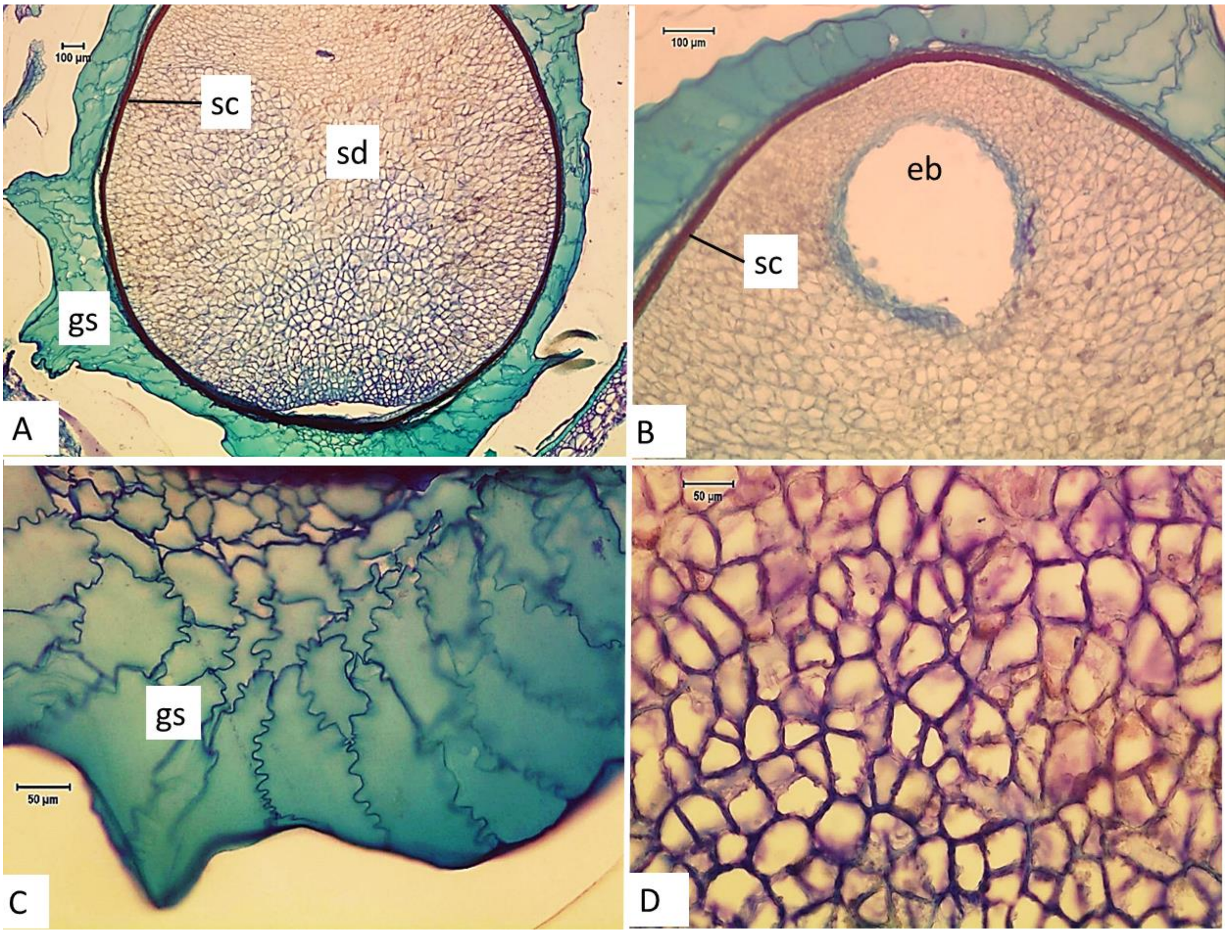

Fig. 4. Histological features of the seed of *Ribes rubrum*. A) Seed embedded in gelatinous sheath; B) seed; C) gelatinous sheath; D) endosperm; eb: the place of embryo (tissue is missing), gs: gelatinous sheath, sc: sclerenchyma cells, sd: seed

Conclusions

The histological determination of the fruit of *Ribes nigrum* and *R. rubrum* complete the reported data of the species. In the fruits of the selected *Ribes* species differences and similarities are described, which highlight the first morpho-anatomic study of the fruit of *Ribes rubrum*. The observed features play an important role in the characterisation, which will be useful providing a reliable basis for identification of the fruits.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Békési-Kallenberger H, Horváth GY, Bencsik T, Balázs Vl, Filep R, Papp N (2016) Comparative Histological and Phytochemical Study of *Fallopia* species. Natural Product Communications 11:251–254.
2. Bishayee A, Háznagy-Radnai E, Mbimba T, Sipos P, Morazzoni P, Darvesh AS, Bhatia D, Hohmann J (2010) Anthocyanin-rich black currant extract suppresses the growth of human hepatocellular carcinoma cells. Natural Product Communications 5:1613–1618.
3. Ciocarlan V (2000) Flora ilustrată a României, Editura Ceres, Bucureşti
4. Corner EJH (1976) The seeds of dicotyledons. Cambridge University Press, Cambridge
5. Cyboran S, Bonarska-Kujawa D, Pruchnik H, Zyłka R, Oszmiański J, Kleszczyńska H (2014) Phenolic content and biological activity of extracts of black currant fruit and leaves. Food Research International 65:47–58. doi: 10.1016/j.foodres.2014.05.037.
6. Da Silva Pinto M, Kwon Yi, Apostolidis E, Lajolo FM, Genovese M, Shetty K (2010) Evaluation of red currants (*Ribes rubrum* L.), black currants (*Ribes nigrum* L.), red and green gooseberries (Ribes uva-crispa) for potential management of type 2 diabetes and hypertension using in vitro models. Journal of Food Biochemistry 34:639–660. doi: 10.1111/j.1745-4514.2009.00305.x
7. EU-NOMEN (n.d.) http://www.eu-nomen.eu/portal/taxon.php?GUID= 3734D1D0-9A3C-41FC-BCB9-20279F1C1502 (accessed 18 sept. 2018).
8. Gavrilova V, Kajdžanoska M, Gjamovski V, Stefova M (2011) Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLC-DAD-ESI-MSn. Journal of Agricultural and Food Chemistry 59(8):4009–4018. doi: 10.1021/jf104565y
9. Giongo L, Poncetta P, Loretti P, Costa F (2013) Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. Postharvest Biology and Technology 76:34–39. doi: 10.1016/j.postharvbio.2012.09.004
10. Glidewell S, Williamson B, Duncan GH, Chudek J, Hunter G (1999) The

development of black currant fruit from flower to maturity: A comparative study by 3D nuclear magnetic resonance (NMR) micro-imaging and conventional histology. New Phytologist 141(1):85–98. doi: 10.1046/j.1469-8137.1999.00319.x

11. Goffman F, Galetti S (2001) Gamma-linolenic acid and tocopherol contents in the seed oil of 47 accessions from several *Ribes* species. Journal of Agricultural and Food Chemistry 49(1):349–354. doi: 10.1021/jf0006729
12. Häkkinen SH, Kärenlampi SO, Heinonen IM, Mykkänen HM, Törronen AR (1999) Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. Journal of Agricultural and Food Chemistry 47(6):2274–2279. doi: 10.1021/jf9811065
13. Kendir G, Güvenç A, Acar A, Çeter T, Pinar NM (2015) Fruits, seeds and pollen morphology of Turkish *Ribes* L. (Grossulariaceae). Plant Systematics and Evolution 301(1):185–199. doi: 10.1007/s00606-014-1064-2
14. Knekt P, Järvinen R, Seppänen R, Hellövaara M, Teppo L, Pukkala E, Aromaa A (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. American Journal of Epidemiology 146(3):223–30. doi: 10.1093/oxfordjournals.aje.a009257
15. Leskinen H, Suomela, Kallio H (2009) Effect of latitude and weather conditions on the regioisomer compositions of α- and γ-linolenoyldilinoleoylglycerol in currant seed oils. Journal of Agricultural and Food Chemistry. 57(9):3920–3926. doi: 10.1021/jf900068b
16. Liu P, Kallio H, Yang B (2014) Flavonol glycosides and other phenolic compounds in buds and leaves of different varieties of black currant (*Ribes nigrum* L.) and changes during growing season. Food Chemistry 160:180–189. doi: 10.1016/j.foodchem.2014.03.056
17. Marhold K (2011) Grossulariaceae. Euro+Med Plantbase. http://ww2.bgbm.org/euroPlusMed/PTaxonDetail.asp?UUID=3734D1D0-9A3C-41FC-BCB9-20279F1C1502 (accessed 18 september 2018).
18. Metcalfe CR, Chalk L (1957) Anatomy of the dicotyledons vol I., Oxford, At The Clarendon Press
19. Mikulic-Petkovsek M, Rescic J, Schmitzer V, Stampar F, Slatnar A, Koron D, Veberic R (2015) Changes in fruit quality parameters of four *Ribes* species during ripening. Food Chemistry 173:363–374. doi: 10.1016/j.foodchem.2014.10.011
20. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Stampar F, Veberic R (2012) Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. Journal of Food Science 77(10):1–7. doi: 10.1111/j.1750-3841.2012.02896.x
21. Milivojevic J, Slatnar A, Mikulic-Petkovsek M, Stampar F, Nikolic M, Veberic R (2012) The influence of early yield on the accumulation of major taste and health-related compounds in black and red currant cultivars (*Ribes* spp.). Journal of Agricultural and Food Chemistry 60(10):2682–2691. doi: 10.1021/jf204627m
22. Nour V, Trandafir I, Cosmulescu S (2014) Antioxidant capacity, phenolic compounds and minerals content of blackcurrant (*Ribes nigrum* L.) leaves as influenced by harvesting date and extraction method. Industrial Crops and Products 53:133–139. doi: 10.1016/j.indcrop.2013.12.022
23. Papp N, Csete S, Farkas Á (2013) Comparative ecomorphology of the cyathial nectaries in eight European *Euphorbia*

species. Acta Biologica Hungarica 64(1):45–59. 10.1556/ABiol.64.2013.1.5

24. Saladie M, Matas AJ, Isaacson T, Jenks MA, Goodwin SM, Niklas KJ, Xiaolin R, Labavitch JM, Shackel KA, Fernie AR, Lytovchenko A, O'neill MA, Watkins CB, Rose JKC (2007) A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiology 144(2):1012–1028. doi: 10.1104/pp.107.097477
25. Sasaki T, Li W, Zaike S, Asada Y, Li Q, Ma F, Zhang Q, Koike K (2013) Antioxidant lignoids from leaves of *Ribes nigrum*. Phytochemistry 95:333–340. doi: 10.1016/j.phytochem.2013.07.022
26. Šavikin K, Zdunić G, Janković T, Tasić S, Menković N, Stević T, Đorđević B (2009) Phenolic content and radical scavenging capacity of berries and related jams from certificated area in Serbia. Plant Foods for Human Nutrition 64(3):212–217. doi: 10.1007/s11130-009-0123-2
27. Schweingruber FH, Börner A, Schulze ED (2011) Atlas of Stem Anatomy in Herbs, Shrubs and Trees vol 1, Springer-Verlag Berlin Heidelberg
28. Da Silva Pinto M, Kwon Y, Apostolidis E, Lajolo FM, Genovese MI, Shetty K (2010) Evaluation of red currants (*Ribes Rubrum* L.), black currants (Ribes Nigrum L.), red and green gooseberries (*Ribes Uva-Crispa*) for potential management of type 2 diabetes and hypertension using in vitro models. Journal of Food Biochemistry 34(3):639–660. doi: 10.1111/j.1745-4514.2009.00305.x
29. Slavin JL, Lloyd B (2012) Health benefits of fruits and vegetables. Advances in nutrition (Bethesda, Md.) 3(4):506–16. doi: 10.3945/an.112.002154
30. The Plant List http://www.theplantlist.org/1.1/browse/A/Grossulariaceae/ (accessed 9.18.18).
31. Traitler H, Winter H, Richli U, Ingenbleek Y (1984) Characterization of gamma-linolenic acid in *Ribes* seed. Lipids 19(12):923–928. doi: 10.1007/BF02534727
32. USDA. (n.d.). PlantsDatabase. https://plants.usda.gov/core/profile?symbol=RIBES (accessed 9.18.18).
33. Vagiri M, Ekholm A, Öberg E, Johansson E, Andersson SC, Rumpunen K (2013) Phenols and ascorbic acid in black currants (*Ribes nigrum* L.): Variation due to genotype, location, and year. Journal of Agricultural and Food Chemistry 61(39):9298–9306. doi: 10.1021/jf402891s
34. Vuorinen Al, Kalpio M, Linderborg KM, Hoppula KB, Karhu ST, Yang B, Kallio HP (2016) Triacylglycerol biosynthesis in developing *Ribes nigrum* and *Ribes rubrum* seeds from gene expression to oil composition. Food Chemistry 196:976––987. doi: 10.1016/j.foodchem.2015.10.010
35. Williamson B, Goodman BA, Chudek JA (1993) The structure of mature gooseberry (*Ribes grossularia*) fruits revealed non-invasively by NMR microscopy. Micron 24(4):377–383. doi: 10.1016/0968-4328(93)90003-J
36. Wojdylo A, Oszmianski O, Milczarek M, Wyetrzyk J (2013) Phenolic profile, antioxidant and antiproliferative activity of black and red currants (*Ribes* spp.) from organic and conventional cultivation. International Journal of Food Science and Technology 48:715–726. doi: 10.1111/ijfs.12019
37. Woznicki Tl, Sønsteby A, Aaby K, Martinsen BK, Heide OM, Wold AB, Remberg SF (2017) Ascorbate pool, sugars and organic acids in black currant (*Ribes nigrum* L.) berries are strongly influenced by genotype and post-flowering temperature. Journal of the Science of Food and Agriculture 97(4):1302–1309. doi: 10.1002/jsfa.7864

38. Wrońska-Pilarek D (2002) Seed morphology of the native species of the genus *Ribes* L. Part 2. The characteristics of individual species. Acta Societatis Botanicorum Poloniae 71(1):5–16. doi: 10.5586/asbp.2002.001
39. Wrońska-Pilarek D (2001) Seed morphology of the polish native species of the genus *Ribes* L. Part 1. General characteristic. Acta Societatis Botanicorum Poloniae 70(4):281–289. doi: 10.5586/asbp.2001.036
40. Zágoni E (2005) A feketeribiszke. Alutus kiadó, Miercurea Ciuc
41. Zdunić G, Šavikin K, Pljevljakušić D, Djordjević B (2016) Black (*Ribes nigrum* L.) and red currant (*Ribes rubrum* L.) cultivars, in: Simmonds, M., Preedy, V. (Eds.), Nutritional Composition of Fruit Cultivars. Academic Press, pp. 101–106.
42. Zheng J, Yang B, Ruusunen V, Laaksonen O, Tahvonen R, Hellsten J, Kallio H (2012) Compositional differences of phenolic compounds between blackcurrant (*Ribes nigrum* L.) cultivars and their response to latitude and weather conditions. Journal of Agricultural and Food Chemistry 60(26):6581–6593. doi: 10.1021/jf3012739.

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CONTRIBUTIONS TO THE STUDY OF THE CHOROLOGY AND CONSERVATION STATUS OF LYCOPODS IN MUREŞ COUNTY

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Abstract: This paper presents the obtained results on the chorology and conservation status of the lycopod species identified in Mureș County. It also represents a basis for further studies on monitoring, and establishes conservation measures for these species that are of community interest, included in the IUCN category LC (least concern). During the study, five lycopod species out of the seven listed in Romania were identified. The species are wide spread, occurring in groups that extend over relatively large areas. The conservation status is predominantly good and very good.

Keywords: lycopods, distribution, specific diversity, conservation.

1. Introduction

The Lycopodiaceae family is an ancient, cosmopolitan family of over 400 species (Øllgaard and Windisch, 2014).

Lycopodiaceae are composed of three genera: *Huperzia*, *Lycopodiella* and *Lycopodium*, which differ in characters of branching patterns, the presence or absence of modified leaves, spore morphology, gametophyte shape, and chromosome number. Many lycopods have underground gametophytes that make an obligatory association with fungi (Merckx et al., 2012).

In Europe, 14 species of Lycopodiaceae are encountered, of which 3 are endemic (García Criado et al., 2017). In Romania, the family Lycopodiaceae comprises 7 species (Sârbu et al., 2013). They are widespread from the mountain area to the alpine area, in the alpine bioregion. They prefer the shady forests, the peat bogs, and the edge of the shady hills, the rocks, and the grassy places at the top of the mountains (Mihăilescu et al., 2015).

Despite the fact that they are widespread, in Europe all species of the genus *Lycopodium* have shown a general decline in abundance and have therefore been included in Annex V of the Habitats Directive 92/43/CEE, and 5A Annex of Romanian Government Ordinance 57/2007. *Lycopodium clavatum* in particular, is also included in Annex D of the Council Regulation

(EC) No 338/97 on the protection of species of wild fauna and flora by regulation their trade. Therefore, it is necessary to establish conservation measures. Knowing the distribution and conservation status of the lycopod species will be vital in establishing these measures.

The present study contributes to the knowledge of lycopod species distribution in the area of Mureș County and it gives us a base for future research about population dynamics and conservation trend.

2. Materials and Methods

Data from the literature as well as the field observations during 2012-2019 were taken into account for the study of the Lycopodiaceae family in Mureş County. Some of the locations specified in the literature have been confirmed. Also, numerous new locations with representative populations of the studied species were recorded using the Global Positioning System (GPS).

The method of transects was applied for the field study. Transects were established on the main river valleys that cross the territory: Mureş Gorge, Gurghiului Valley and Niraj Valley, as well as their tributaries. Each point where the studied species were encountered was noted and their abundance was estimated.

The studied territory, represented by the mountain area of Mureș County (Călimani and Gurghiului Mountains), extends on a vegetation belt, namely the mountain belt. The vegetation studies from these areas highlight the presence of the forests characteristic of this sector, namely beech forests belonging to the Carpino-Fagetea class and spruce forests of the Vaccinio-Piceetea class (Oroian, 1998, Sămărghiţan 2005), which are typical habitats for the development of lycopod species.

In order to establish the frequency at the national level, and the biological and ecological characteristics of the species, the works of Ciocârlan (2009), Oprea (2005) and Sârbu et al. (2013) were taken into account. For each observation point, location data (GPS coordinates), altitude, and abundance data were recorded. In order to evaluate the abundance a 5-level scale was used. In this scale, we considered the range of cover of aboveground shoots/m^2. The 5 levels are: D-dominant (80-100%), A-abundant (60-80%), F-frequent (40-60%), O-occasional (20-40%), and R-rare (1-20%). Considering the altitudinal gradient 9 elevation levels were used: 1 (501-600 m), 2 (601-700 m), 3 (701-800 m), 4 (801-900 m), 5 (901-1000 m), 6 (1001-1100 m), 7 (1101-1200 m), 8 (1201-1300 m), and 9 (> 1300 m).

3. Results and discussions

In Mureş County five out of the seven lycopod species that grow in Romania were identified. These are: *Huperzia selago* (L.) Schrank et Martins (*Lycopodium selago* L.), *Lycopodium alpinum* L., *Lycopodium annotinum* L., *Lycopodium clavatum* L., and *Lycopodium complanatum* L (**Fig. 1-3**).

These species occur in the mountain area of the county, mainly Călimani and Gurghiului Mountains. They are widespread in their characteristic habitats such as woods, and mountain grasslands. Regarding the distribution and frequency at the national level, all 5 studied species are sporadic and none are rare or occasional (**Table 1.**).

In the study area, 201 records of the five species of lycopods were noted. The most common populations are those with *Lycopodium annotinum*, and *Lycopodium clavatum*, which often occur together in the studied territory the two species being identified in several cases at the same locations.

The studied species were found in forests, forest edges, shrubs and meadows. They have clonal growth and are frequently distributed in small groups but spread over a large area.

Fig. 1. Lycopods from Mureș County, Romania: **A.** *Huperzia selago* (Neagra Valley); **B.** *Lycopodium alpinum* (Iodului Valley) (Photos: Silvia Oroian)

Fig. 2. Lycopods in Mureș County, Romania: **A.** *Lycopodium annotinum* (Secuieu Valley); **B.** *Lycopodium complanatum* (Răstolița) (Photos: Mihaela Sămărghițan and Silvia Oroian)

Fig. 3. *Lycopodium clavatum* in Mureș County, Romania: **A.** in Sovata Valley (Mureș County, Romania); **B.** in Stânceni (Photos: Mihaela Sămărghițan and Silvia Oroian)

Huperzia selago (L.) Schrank et Martins (*Lycopodium selago* L.) is widespread in the studied area, being recorded in 30 locations (**Fig. 4.**). It is a characteristic species of the Vaccinio-Piceetea class Br.-Bl. in Br.-Bl. et al. 1939 (Coldea, 2015), being identified in acidophilic spruce forests belonging to this class as well as in Dacian beech forests of Symphyto-Fagion. This species participates in the composition of herbaceous synusia of the associations *Hieracio transsilvanici-Piceetum* Pawłowski et Br.-Bl. 1939 and *Leucanthemo waldsteinii - Fagetum* (Soó 1964) Täuber 1987 sometimes with significant dominance values, up to 20%.

Table 1. Lycopods identified in Mureş County; their frequency and distribution on national level; biological and ecological characteristics.

Species	Frequency on the national level	Distribution on national level	Biological characteristics	Ecological features
Huperzia selago	Sporadic	In the spruce forests belt, wet grassy places, forests, bushes, peatlands	Perennial, Ch, 2n=90, 264, 272, P	Moderate acidophilous oligotrophic, meso-hygrophilous, scyophilous-helio-scyophilous, microthermal, calciphilous
Lycopodium alpinum	Sporadic	In the spruce forests belt, grasslands, shrubs	Perennial, Ch, 2n=46-48-50, P	Oligotrophic, mesophilous
Lycopodium annotinum	Sporadic	In the beech and the spruce belt; in wet places, marshes, hedges, forests, and peatlands	Perennial, Ch, 2n=68, P	acidophilous, oligotrophic, mesophilous-meso-hygrophilous, helio-scyophilous - scyophilous, calcifuge
Lycopodium clavatum	Sporadic	From the oak to the spruce forests belt in shrub land, grasslands, forest fringes	Perennial, Ch, 2n=68, P	Acidofilous, oligotrophic, eury-philous, helio-scyophilous - scyophilous, calcifuge
Lycopodium complanatum	Sporadic	In the beech and the spruce forests belt, in forests and shrubs	Perennial, Ch, 2n=46, P	Oligotrophic, calcifuge

In the study area the highest abundance and frequency values were recorded for the species *Lycopodium annotinum* L. It was identified in 109 locations, sometimes dominant in the grass layer of acidophilic spruce forests (Bătrâna Valley AD2, Lăpuşna AD1, Şirodului Valley AD1). In the study area it appeared most commonly in the coenoses of plant associations *Hieracio transsilvanici-Piceetum* Pawłowski et Br.-Bl. 1939 and *Sphagno girgensohnii-Piceetum* Kuoch 1954. It is also present in the composition of beech forests included in the association *Leucanthemo waldsteinii-Fagetum* (Soó 1964) Täuber 1987. Significant populations were also encountered near the mountain forests of the Vaccinio-Piceetea class; also entering in the composition of habitat 6440. These coenoses dominated by *Deschampsia caespitosa* are distributed linearly along mountain valleys.

The variety of habitats of community interest in which this species is encountered (**Table 2.**) as well as the high density of the above ground shoots observed on large surfaces (a large number of small areas) justifies the affirmation that the species is well represented and frequent in the studied territory.

Along with *Lycopodium annotinum*, sometimes sharing the same area, *Lycopodium clavatum* L. has also been identified. In the study area the populations of *L. clavatum* were distributed at altitudes between 563 and 1371 m asl (Scaunul Domnului Peak). The identified populations are represented by vigorous individuals, well developed on surfaces ranging from a few square meters to dozen of square meters. This species grows very well in the forest cuts, and in the clearings. It was encountered along the studied paths both in forest habitats, spruce forests belonging to the Vaccinio-Piceetea class and in pastures habitats in fragmented micro-areas wide spread on large areas (**Table 2.**).

Lycopodium alpinum L. and *Lycopodium complanatum* L. were present in the studied territory, but have a limited spread. These species are included in the national red lists under the category R (Rare) (Oltean et al., 1994).

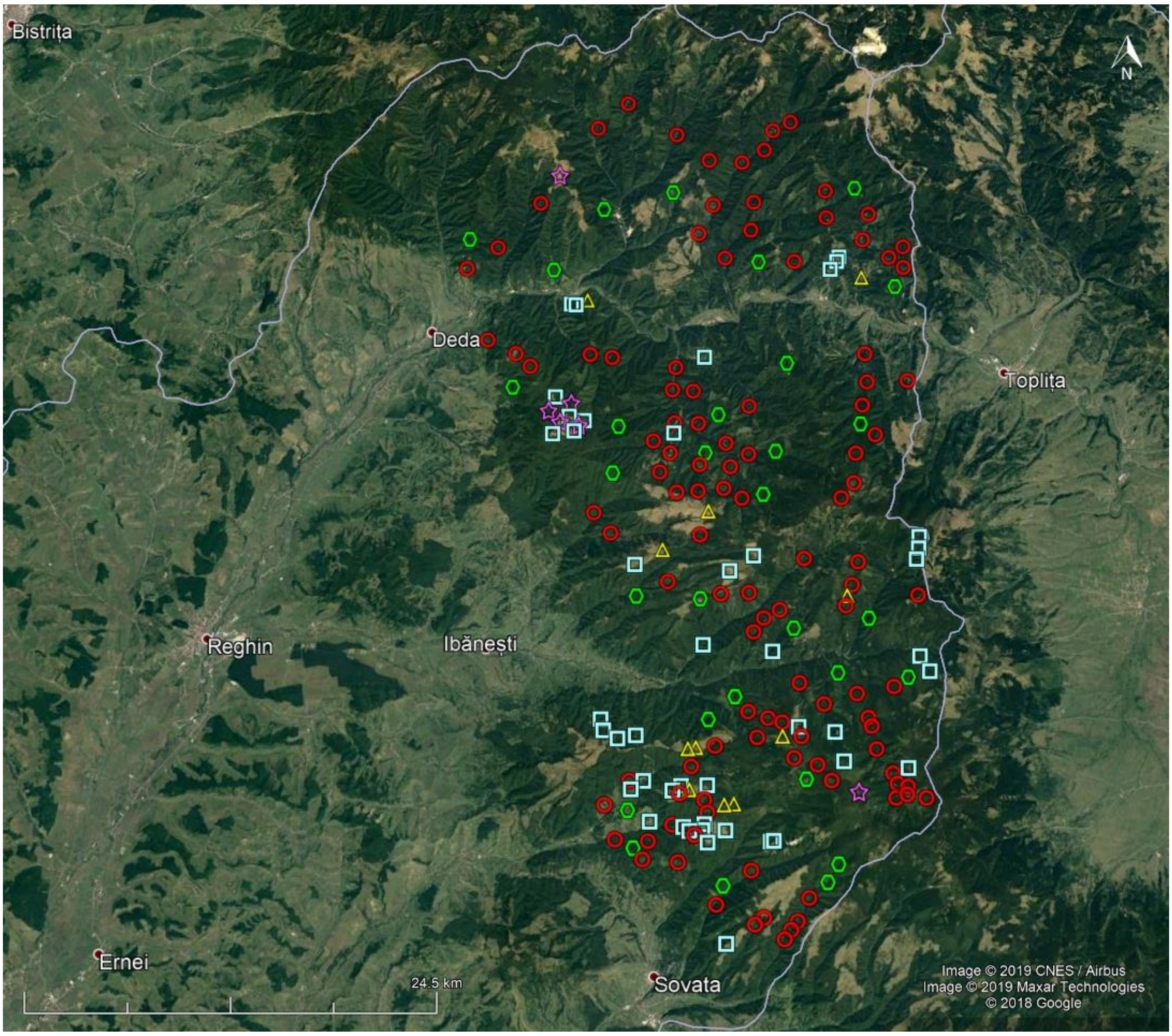

Fig. 4. The distribution map of the studied lycopod species (Mureș County, Romania): O*Huperzia selago*, O*Lycopodium annotinum*, △*Lycopodium complanatum*, □*Lycopodium clavatum*, and ☆*Lycopodium alpinum*

Table 2. The Natura 2000 habitat types in which the studied lycopod species were identified (Mureș County, Romania)

Natura 2000 Habitat	Species
9410 Acidophilous Picea forests from the mountain to the alpine level (Vaccinio-Piceetea)	*Huperzia selago*, *Lycopodium annotinum*, *Lycopodium clavatum*, *Lycopodium complanatum*
91V0 Dacian Beech forests (Symphyto-Fagion)	*Huperzia selago*, *Lycopodium annotinum*
6230* Species-rich *Nardus* grasslands, on silicious substrates in mountain areas	*Lycopodium alpinum*, *Lycopodium clavatum*
6440 Alluvial meadows of river valleys of the Cnidion dubii	*Lycopodium annotinum*, *Lycopodium clavatum*
6520 Mountain hay meadows	*Lycopodium clavatum*, *Lycopodium complanatum*

Populations of *Lycopodium alpinum* were identified in grasslands dominated by *Nardus stricta* located at higher altitudes entering in the floristic composition of the association *Violo declinatae-Nardetum* Simon 1966. The populations occupied limited areas and were represented by vigorous individuals.

The species *Lycopodium complanatum* was rare in the site, and the population numbers are decreasing. It was identified at the edge of spruce forests but also in mountain meadows, in coenoses belonging to the *Festuco rubrae-Agrostietum capillaris* Horvat 1951 association.

Due to the clonal nature of lycopods, it was difficult to estimate the abundance of species in the study plots by estimating the number of individuals on the surface. Therefore, a 5-level abundance scale was used in which the abundance of the aboveground shoots/m^2 was assessed: D-dominant, A-abundant, F-frequent, O-occasional, and R-rare. Analyzing the recorded results, the most abundant populations (60-100% abundance) were identified at elevation level 1001-1300 m. *Huperzia selago* and *Lycopodium annotinum* were recorded with abundant/dominant populations also at altitudes between 801-1000 m asl. In the study area populations of *Lycopodium alpinum* were identified at the elevation level 1100-1300 m. They had the highest abundance values at elevation level 1201-1300 m, where the species was also dominant in the analyzed plots (up to 87.5%). In the other plots, the species was occasionally or rarely (below 17.5%).

Lycopodium annotinum had dominant and abundant populations, especially at altitudes between 1101-1300 m asl, but a significant number of abundant or dominant populations were found also at elevation level 801-1000 m. Abundant populations of *Lycopodium clavatum* were found between 900-1300 m asl. Only the populations of *Lycopodium complanatum* identified at 1001-1300 m asl had higher abundance, at other points the species were occasional or rare (**Fig. 5-8**).

The main threats to which *Lycopodium* species are exposed are generally related to human activities, infrastructure construction, forest clearing, changes in land use, as well as eutrophication and climate change. The abandonment of traditional land use, especially pasture, leads to the succession of vegetation, in the mountain area leading to reforestation. This results in the reduction of lycopod populations in the meadows. Lycopods (eg *Lycopodium clavatum*) are weak competitors because they depend on light and are disadvantaged compared to taller species. Pollution and eutrophication have a negative impact on lycopod species because they can affect mycorrhizae, the symbiotic partners of lycopods.

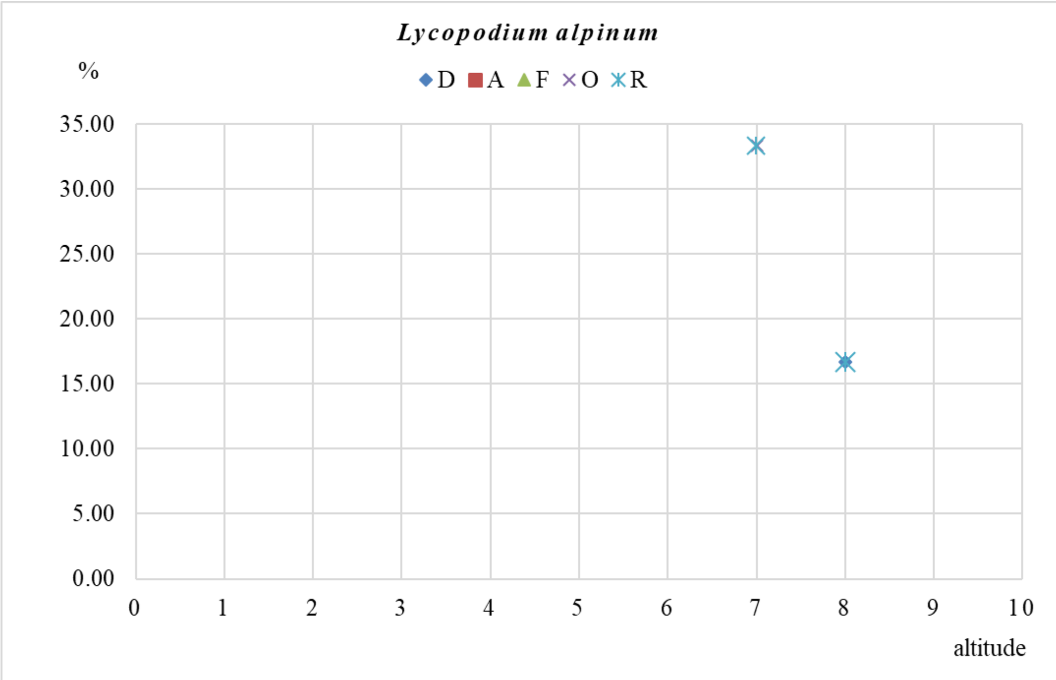

Fig. 5. Variation of the abundance along the elevation gradient in case of *Lycopodium alpinum* (Mureș County, Romania): D-dominant, A- abundant, F- frequent, O-occasional, and R-rare; Elevation levels: 1 (501-600 m), 2 (601-700 m), 3 (701-800 m), 4 (801-900 m), 5 (901-1000 m), 6 (1001-1100 m), 7 (1101-1200 m), 8 (1201-1300 m), and 9 (> 1300 m).

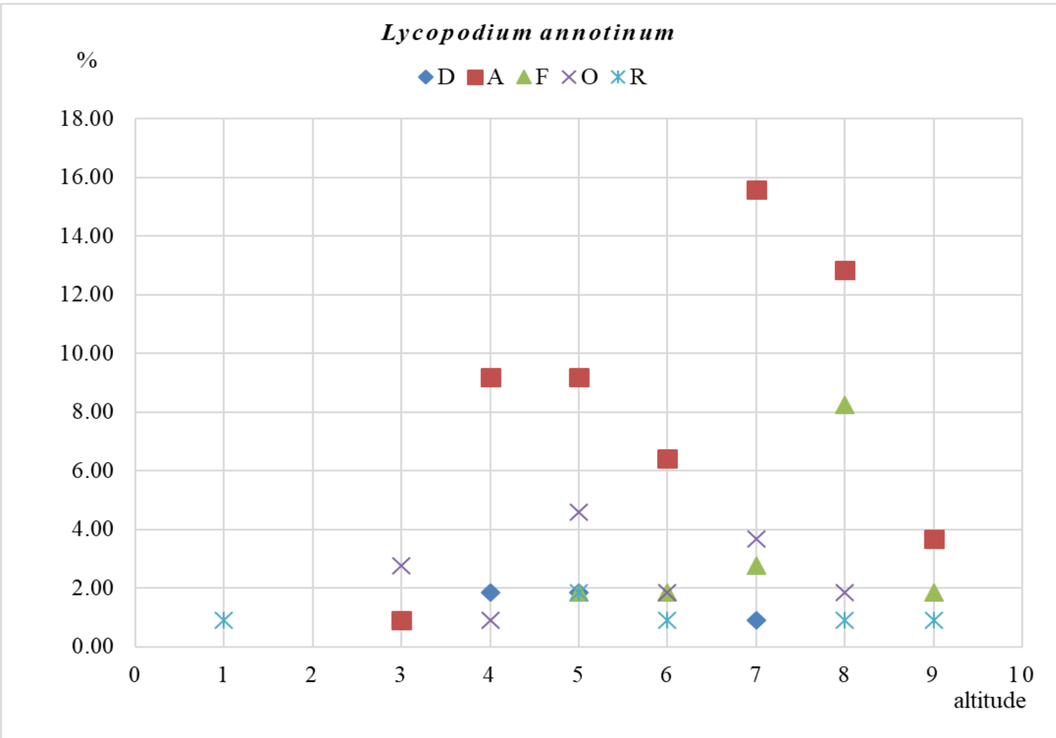

Fig. 6. Variation of the abundance along the elevation gradient in case of *Lycopodium annotinum* (Mureș County, Romania): see abbreviations at **Fig. 5.**

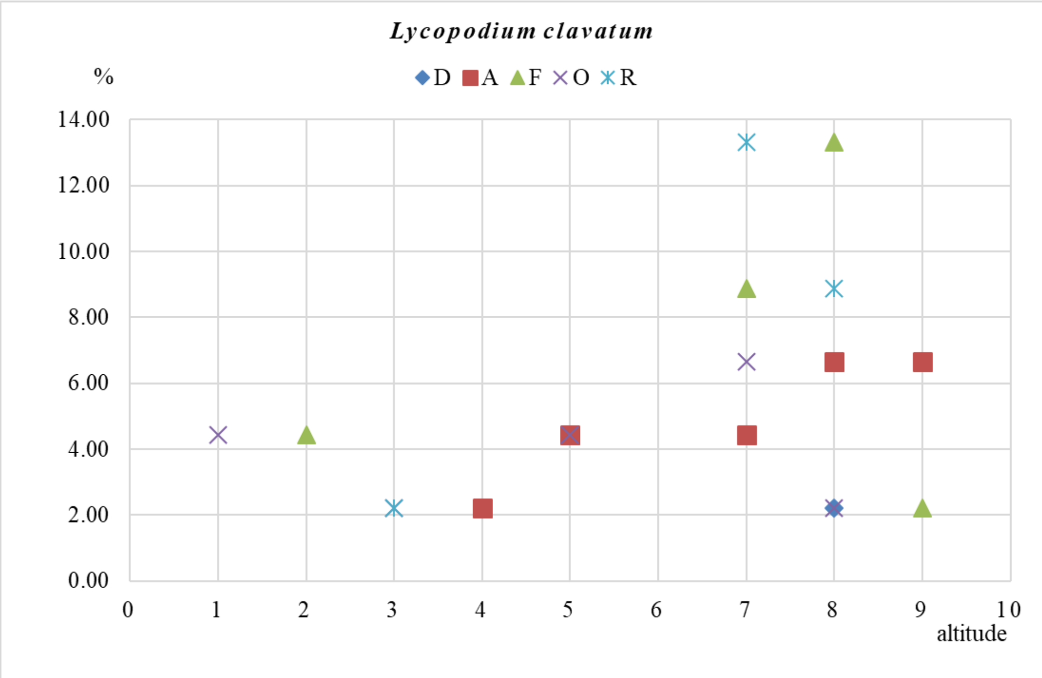

Fig. 7. Variation of the abundance along the elevation gradient in case of *Lycopodium clavatum* (Mureș County, Romania): see abbreviations at **Fig. 5.**

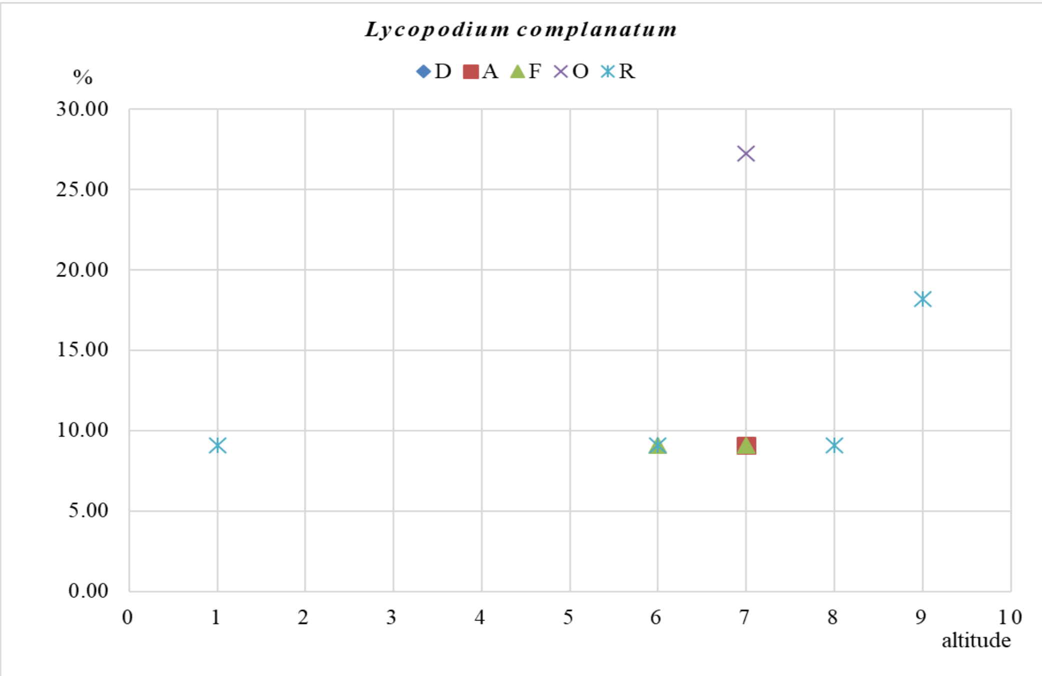

Fig. 8. Variation of the abundance along the elevation gradient in case of *Lycopodium complanatum* (Mureș County, Romania): see abbreviations at **Fig. 5.**

Tourism and animal trampling are factors that exert pressure on lycopods. However, the potential for regeneration of these species is high and therefore are not of high threat. The use of some species in traditional medicine is another threat, due to the irrational collections. Thus, *Huperzia selago* contains narcotic action principles. Selagoline, Huperzina A and serratidine alkaloids were identified in the species composition (Stærk et al., 2004).

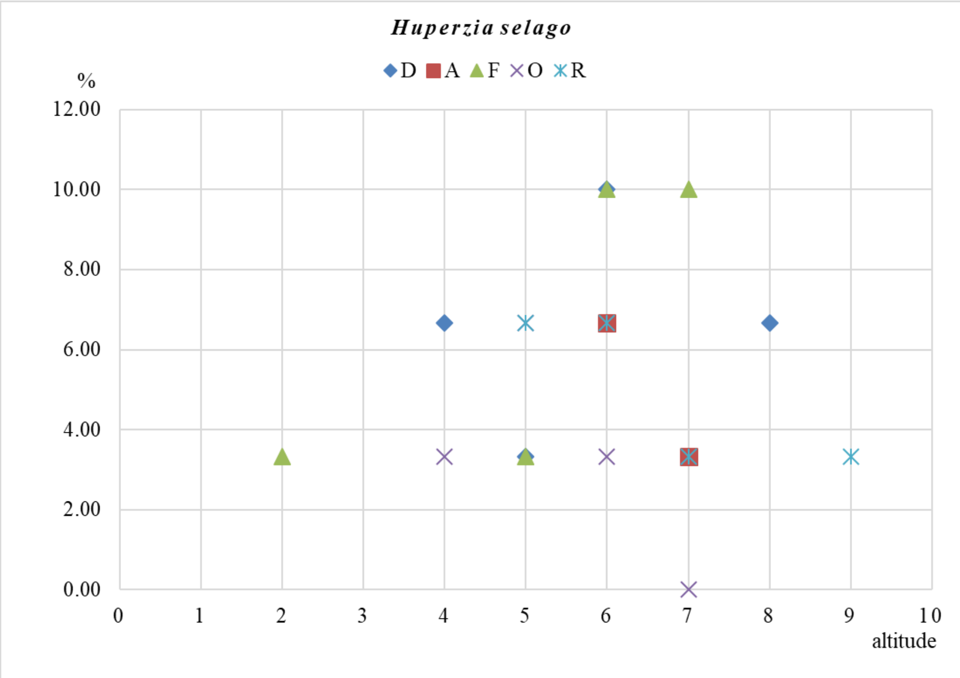

Fig. 9. Variation of the abundance along the elevation gradient in case of *Huperzia selago* (Mureș County, Romania): see abbreviations at **Fig. 5.**

Huperzine A is known to have neuronal protection properties used to improve memory and treatment behavior in Alzheimer's disease (Ma et al., 2007). *Lycopodium clavatum* is used in homeopathy for treatments of aneurysms, constipation, chronic lung and bronchial disorders, and fevers. It also reduces gastric inflammation, simplifies digestion and helps in the treatment of chronic kidney disorders. *Lycopodium* is used to treat various mental conditions such as anxiety, forgetfulness, and also relieves from tiredness and chronic fatigue (Jhilik Banerjee et al., 2014). Extracts from *Lycopodium clavatum* spores are used for the treatment of urinary, digestive, epileptic, pulmonary disorders, as well as rheumatism (Hanif et al., 2015). These two species are the most collected in the study area. The other species of lycopods, are also collected and used by locals as traditional remedies for different diseases.

The state of conservation of the identified populations is predominantly good and very good in the studied territory. Only in a few plots, where the anthropic pressure is higher, the conservation status is bad and populations are decreasing. The fact that the habitats in which these species have been studied are in a predominantly good state of conservation also indicates that the long-term evolution of species conservation state is stable.

The most important conservation measure for lycopod species concerns the conservation of their habitats. Because most of the observation points are in protected areas, the conservation measures provided for these areas also have a positive impact on maintaining the favorable conservation status of the species in the studied territory. Further studies are also required to establish and monitor the dynamics of these populations

Conclusions

In the studied area 5 species of community interest belonging to the Lycopodiaceae family were identified: *Huperzia selago*, *Lycopodium alpinum*, *L. annotinum*, *L. clavatum*, and *L. complanatum*.

The most widespread of the identified species, are: *Huperzia selago*, *Lycopodium annotinum* and *L. clavatum*.

We can appreciate that although at the national level all species are sporadically encountered, in the study area *Huperzia selago*, *Lycopodium annotinum*, and *L. clavatum* can be considered species with high frequency, while *L. alpinum* and *L. complanatum* are rare.

All these species are of community interest and belong to the IUCN LC (Least concern) category.

Lycopodium alpinum and *L. complanatum* are also included in the National Red List as rare species (R).

The populations of the species are predominantly in a good and very good state of conservation, but a decrease of the populations can be expected in the future.

In order to avoid the tendency of declining populations and to maintain their favorable state of conservation, it is necessary to apply conservation measures and monitoring.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Bilz M, Kell SP, Maxted N, Lansdown RV (2011) European Red List of vascular plants. Luxembourg: Publications Office of the European Union.
2. Ciocârlan V (2009) Flora ilustrată a României. Pteridophyta et Cormophyta. Ed. Ceres, Bucureşti
3. Coldea Gh (ed.) (2015) Les association végétales de Roumanie, Tome 3 Les associatios forestiéres et arbustives, Edit. Presa Universitară Clujeană&Accent, Cluj-Napoca
4. Doniţă N, Popescu A, Paucă-Comănescu M, Mihăilescu S, Biriş Iovu A (2005) Habitatele din România, Ed. Tehnică Silvică, Bucureşti
5. Gafta D, Mountford O (coord.) (2008) Manual de interpretare a habitatelor Natura 2000 din România, Ed. Risoprint, Cluj-Napoca
6. García Criado M, Väre H, Nieto A, Bento Elias R, Dyer R, Ivanenko Y, Ivanova, D, Lansdown R, Molina JA, Rouhan G, Rumsey F, Troia A, Vrba J, Christenhusz MJM (2017) European Red List of Lycopods and Ferns. Brussels, Belgium: IUCN. iv + 59pp
7. Hanif K, Kumar M, Singh N, Shukla R (2015) Effect of homeopathic *Lycopodium clavatum* on memory functions and cerebral blood flow in memory-impaired rats. Homeopathy, 104(1):24–28
8. Jhilik Banerjee, Sunipa Biswas, Nithar Ranjan Madhu, Susanta Roy Karmakar, Merckx, V., Freudenstein, J., Kissling, J., Christenhusz, M.J.M., Stotler, R.E., Crandall-Stotler, B., Wickett, N., Rudall, P.J., Maas-van de Kamer, H. and Maas, P.J.M. (2012) Taxonomy and classification. In: Merckx V (ed.), Mycoheterotrophy, the biology of plants living on fungi. Heidelberg: Springer, pp. 19–104
9. Ma X, Tan C, Zhu D, Gang DR, Xiao P (2007) Huperzine A from *Huperzia*

species–an ethnopharmacolgical review. J. Ethnopharmacol, 113:15–34. doi:10.1016/j.jep.2007.05.030

10. Mihăilescu S, Anastasiu P. Popescu A. (2015) Ghidul de monitorizare a speciilor de plante de interes comunitar din România, Ed. Dobrogea, Constanţa
11. Oprea A (2005) Lista critică a plantelor vasculare din România, Ed.Univ. „Al. I. Cuza", Iaşi
12. Oroian S, Giurgiu M (2003) Plante medicinale spontane din Valea Nirajului, Rev. Medico-Chirurgicală a societăţii de Medici Naturalişti din Iasi, Supplement 1, 107(2):124–130.
13. Oltean M, Negrean G, Popescu A, Roman N, Dihoru G, Sanda V, Mihăilescu S (1994) Listă roşie a plantelor superioare din România, Studii, sinteze, documentaţii de ecologie, 1, Acad. Română, Instit. de Biologie, Bucureşti
14. Oroian, S, (1998) Flora şi Vegetaţia Defileului Mureşului între Topliţa şi Deda, Casa de Editură Mureş
15. Øllgaard B, Windisch PG (2014) Lycopodiaceae in Brazil. Conspectus of the family I. The genera *Lycopodium*, *Austrolycopodium*, *Diphasium* and *Diphasiastrum* – Rodriguésia, 2:293−309. http://www.scielo.br/pdf/rod/v65n2/a02v65n2.pdf
16. Sămărghiţan M. (2005) Flora şi vegetaţia Văii Gurghiului, Ed. University Press, Târgu-Mureş
17. Sârbu I, Ştefan N, Oprea A, (2013) Plante vasculare din România, determinator ilustrat de teren. Ed. "Victor B. Victor", Bucureşti
18. Stærk D, Larsen J, Larsen LA, Olafsdottir ES, Witt M, Jaroszewski JW (2004) Selagoline, A new alkaloid from *Huperzia selago*, Natural Product Research, 18(3):197–203. doi: 10.1080/14786410310001620600
19. Surjyo Jyoti Biswas (2014) A better understanding of pharmacological activities and uses of phytochemicals of *Lycopodium clavatum*: A review, Journal of Pharmacognosy and Phytochemistry, 3(1):207–210
20. *** Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein ELI: http://data.europa.eu/eli/reg/1997/338/2017-02-04
21. *** Habitats Directive 92/43/EEC – Concil Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (OJ L 206, 22.7.92)
22. *** Ordonanța de urgență nr. 57/2007 privind regimul ariilor naturale protejate, conservarea habitatelor naturale, a florei și faunei sălbatice

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EVALUATION OF MORPHOMETRIC PARAMETERS IN CASE OF *LEUCOJUM VERNUM* L. FROM THE PERES FOREST IN VELYKA DOBRON WILDLIFE RESERVE, WESTERN UKRAINE

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Abstract: *Leucojum vernum* L. (Amaryllidaceae) is a bulbous geophyte from Central Europe known as medicinal and ornamental plant. Native population of this species can be found in the Peres Forest (parcels number 23-24) from Velyka Dobron Wildlife Reserve (Western Ukraine), monitored continuously since 2014. The aim of this study was the morphometric evaluation of the species and the chemistry analysis of the soil in which the species grow. According to our results the length of leaf sheath was the most variable parameter from the evaluated morphometric indicators (number of leaves, maximum leaf length and width, bract length, leaf sheath length, scape length, and bulb diameter). Correlations were found between maximum leaf length and scape length, but also between maximum leaf length and bract length. Soil chemistry analysis showed that the chemical properties of the soil are favorable for the studied plants. The soil pH was slightly acidic, the humus content and the supply of alkaline hydrolyzed nitrogen were high, and the amount of soluble P_2O_5 was moderate. Differences between the territories were found with respect to the soil agrochemical parameters.

Keywords: *Leucojum vernum* L., morphometric analysis, agrochemical indices, population distribution, Velyka Dobron Wildlife Reserve.

1. Introduction

According to recent molecular studies, only two species belong to the genus *Leucojum* (Amaryllidaceae): *Leucojum vernum* L. and *Leucojum aestivum* L. (**Fig. 1.**) (Lledo et al., 2004). Natural populations of both species can be found in the Ukrainian Transcarpathian region. The genus is also medically important because of galanthamine content of the species. Galanthamine is used for the treatment of Alzheimer's disease (Scott and Goa, 2000). As the alkaloid is obtained from natural sources, there is a need for sustained production of the active principle, together with sustained cultivation of the species. The study conducted by Demir (2014) on the use of galanthamine from *L. aestivum* showed that the cost of producing drugs used to treat Alzheimer's disease is relatively high. Considering the findings of Demir (2014) and also the climate change (IPCC, 2014), it is important to know, monitor and protect the natural populations of *L. vernum* and *L. aestivum*, and to develop sustainable cultivation of both species, where it is necessary. It is known that plant populations respond slowly to changes in the environment, primarily through morphological, physiological, and biochemical responses. The detection of morphological variability within the population allows the identification of species characteristics (which have also taxonomic significance) and makes possible the record of adaptive characters (Кричфалуший and Комендар, 1990).

The object of this study is *L. vernum* (Spring snowflake), a bulbous geophyte from Central Europe. Its natural habitats are present in the following provinces (regions): Lviv, Zakarpatska, Ivano-Frankivsk and Chernivtsi. In Zakarpatska it can be found from the plain region to the upper forest zone, between elevations of 100-1400 m above sea level (asl). In the IUCN Red List of Threatened Species it appears in the Least Concern (LC) category, while in the Ukrainian Red Book is listed in the Data Deficient (DD) category. The study of *L. vernum* population in Velyka Dobron Wildlife Reserve started in 2014. Currently, 30 distribution areas are known in the Zakarpatska (Transcarpathian) region (**Fig. 2.**).

Fig. 1. *Leucojum vernum* L. and *Leucojum aestivum* L. from Velyka Dobron Wildlife Reserve (Western Ukraine) (Photos: Erzsébet Kohut)

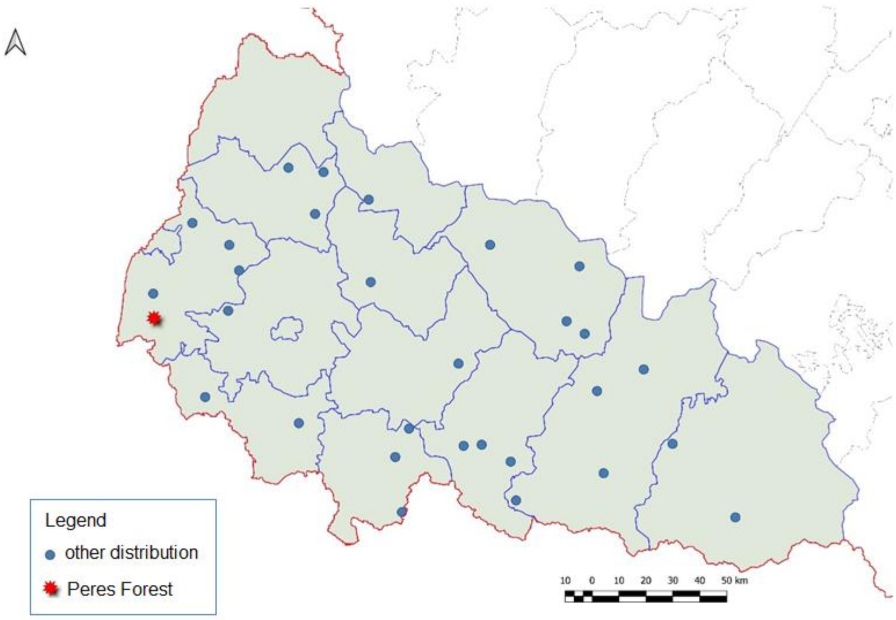

Fig. 2. The distribution of *Leucojum vernum* L. in the Zakarpatska (Transcarpathian) region

2. Materials and Methods

2.1 Studied population

The studied *L. vernum* population is located at 10 km distance from the Chop-Záhony border crossing, in a deciduous floodplain forest with hardwood species (*Fraximus excelsior*, *Quercus robur*, *Acer platanoides*, *Populus alba*, *Populus nigra*, *Ulmus laevis*), on the right bank of the river

Latorica. The three separate territories (were the plants are present) are covering together an area of 238741 m^2 (**Fig. 3.**).

The studied territories along the River Latorica are regularly flooded, and in some years the soil is covered by water for several months, due to the low elevation (110-137 m asl). The density of the species is 75-79 individuals / m^2 (Kohut et al., 2017).

2.2 Morphometric analysis

The morphometric data were collected in 2017. In the generative stage of the plants, from each of the 30 quadrates (sampling points), 10 randomly selected individuals were measured (a total of 300 individuals). The following parameters were analyzed: number of leaves (NL), maximum leaf length (MaxLL) and width (MaxLW), bract length (BL), leaf sheath length (LSHL), scape length (SL, from the inferior part of the scape, to the bract), and bulb diameter (BD). BD was measured in case of 60 individuals (2 plants / quadrates). Statistical analysis of the obtained data was performed using Microsoft Excel according to Злобін et al. (2013).

Determination of the linear correlation coefficient

The simple linear correlation coefficient (r) is used to detect the stochastic relationships (transition between two extremes - fully functional and completely independent) and to express the closeness between two probability variables.

The value of the linear correlation coefficient can vary between +1 and -1. The positive values of the coefficient indicate a clear relationship between the two variables, while the negative values indicate the opposite relationship. In the case of a function relation, its value is +1 or -1, and if there is no relationship between the two variables, its value is 0. A correlation coefficient with a smaller absolute value (near to 0), it means also that between the studied parameters there is no relationship (Fidy and Makara, 2005).

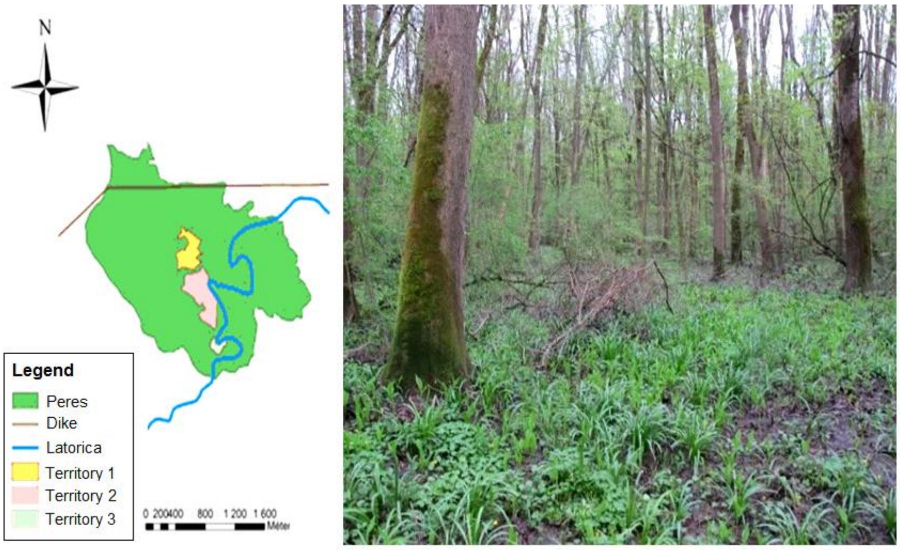

Fig. 3. *Leucojum vernum* L. population in the Peres Forest from Velyka Dobron Wildlife Reserve (Western Ukraine) (Kohut et al., 2017)

Significance of the linear correlation coefficient

The coefficient obtained from the correlation analysis can also be considered as an assumption that there is a relationship between the two variables. Whether this relationship to a specific number (*r*) is realistic can also be determined by assumption testing. Our null hypothesis in this case is that there is no relationship between the two variables ($r = 0$). The rejection of the null hypothesis depends on the magnitude of the coefficient (*r*) and the degree of freedom (*f*) (Fidy and Makara, 2005). Thus, knowing the number of pairs of data (*n*), the absolute value (*t*) of the correlation coefficient can be determined for given levels of significance (reliability), above which the null hypothesis can be rejected, i.e. the correlation coefficient (the true relationship between the two variables) can be considered realistic (Péczely, 1998). To test the significance of *r*, a *t*-statistic of $f = n-2$ is used. The formula for *t*-distribution statistics (Fidy and Makara, 2005) is the following (1):

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \tag{1}$$

2.3 Soil sampling and chemical analysis

Samples were collected at a depth of 0-25 cm after removal of the leaf litter (**Fig. 4.**). The time of sampling, the number and the coordinates (GPS) of the quadrates (sampling points) were recorded.

In case of each soil sample the following parameters were determined: pH value, the content in humus, soluble phosphorus and nitrogen. The measurements were carried out in the Laboratory of Soil Science and Agrochemistry of the Ferenc Rákóczi II. Transcarpathian Hungarian Institute.

F-tests and two-sample Student's *t*-tests on the soil data were carried out using Microsoft Excel's Analysis ToolPak. An acceptance level of $p = 0.05$ was used in both cases.

Working protocols

The soil samples were dried at room temperature. After grinding, the samples were passed through a 2 mm sieve. The pH of the soils was measured in aqueous and 1 mol / dm^3 KCl extract with a soil: solvent ratio of 1: 2.5 (ДСТУ ISO 10390:2007).

The soluble phosphorus content of the soils was measured in 0.2 mol / dm^3 HCl extract according to the Kirsanov method. The soil: solvent ratio was of 1: 5. After shaking for 1 minute and resting for 15 minutes, the suspension was filtered through a paper filter and the filtrate was analyzed (ДСТУ 4405:2005).

Fig. 4. Soil sampling from the studied quadrates with *Leucojum vernum* L. in Peres Forest from Velyka Dobron Wildlife Reserve (Western Ukraine) (Photos: Erzsébet Kohut)

The Tyurin method was used to determine the humus content. In properly prepared soil, the humus is oxidized with potassium dichromate in a highly sulfuric medium. The remaining potassium dichromate is titrated with Mohr's salt solution (ДСТУ 4289:2004).

The values determined during the tests were compared with the accepted values in Ukraine.

The pH value was measured on a WTW Multi 9620 IDS instrument and the phosphorus content was determined on a Libra S21 spectrophotometer.

The alkaline hydrolyzing nitrogen content of the samples was determined from 1.0 mol / dm^3 NaOH extract using the Kornfield method. The soil: NaOH solution ratio was of 1: 2.5. The samples were kept in a thermostat for 48 hours at 28° C with sodium hydroxide, and then the ammonium absorbed with boric acid was titrated with sulfuric acid (ДСТУ 4405:2005).

3. Results and discussions

3.1 Morphometric analysis

The The seeds of the harvested capsules (**Fig. 5.**) were weighed on an analytical balance. The weight of one thousand seeds was equal with 16.19 g. The data used for the morphogram (**Fig. 6**) are summarized in **Table 1**.

Fig. 5. Capsules and seeds of *Leucojum vernum* L. from Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine) (Photo: Erzsébet Kohut)

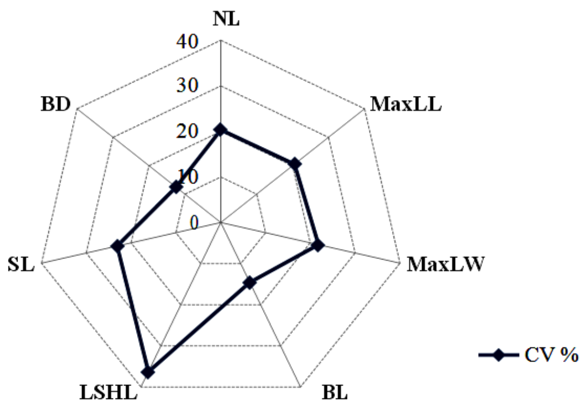

Fig. 6. The morphogram of *Leucojum vernum* L. collected from Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine): NL-number of leaves; MaxLL-maximum leaf length; MaxLW-maximum leaf width; BL-bracts length; LSHL-Leaf sheath length; SL-scape length; BD-bulb diameter

The coefficient of variation (CV) ranged from 12.5% to 36.3% (**Fig. 6.**). The most variable parameter was the leaf sheath length (CV = 36.3%, CV≥26% - high), and the other parameters were found to be less or moderately variable (CV≤15% - low, 16≤CV≤25% - moderate). The least variable parameter was the bulb diameter, with a CV of 12.5%. The results obtained in this study, regarding the morphometric parameters, were similar with those published by Ljubinyec (Любинець) (2013) from Yavorivskyi National Park (Ukrainian Roztocze). In their three years study (2006, 2007 and 2011) the most variable parameter was also the length of the leaf sheath with a CV of 24.6%, 19.1% and 25.8%, respectively.

3.2 Comparison of morphometric parameters

Relationships between the 7 studied morphometric parameters were analyzed. For this, a linear correlation test was performed. The examined 300 individuals were collected from 30 quadrates (10 plants/quadrates). In the sampling quadrates, the measured values of each parameters were averaged, so for the comparison, 30 data pairs (n = 30) were obtained for each parameter. The obtained values of the coefficient (r) from the correlation analysis are summarized in **Table 2**. Our null hypothesis was that there is no real relationship between two compared variables (no morphometric parameter affects the other). Confidence (significance) of the decision was fixed at 5% (p = 0.05) acceptance level. Pairing the indicators, a total of 21 cases were examined. Of these, in 7 cases significant correlations were found. The strongest significant linear relationship was found between MaxLL and BL (r = 0.765) and MaxLL and SL (r = 0.908) (**Fig. 7 and 8**).

The coefficient of determination for MaxLL and BL is R^2 = 0.585 (**Fig. 7.**) and for MaxLL and SL it is R^2 = 0.824 (**Fig. 8.**). Thus, 58.5% of the total variation in the maximum leaf length is due to the linear relationship with the bract length, and 82.4% with the scape length, while the remaining 41.5% and 17.6% are other factors, for example random variation or measurement error.

Table 1. Statistical values of the examined morphometric parameters in case of *Leucojum vernum* L. collected in Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine)

Evaluated parameters	$\bar{x}$	σ	**SE**	$\bar{x}$ **+ SE**	**CV (%)**
NL	4.3	0.9	0.1	4.3 ± 0.1	20.4
MaxLL (cm)	32.1	6.6	0.4	32.1 ± 0.4	20.6
MaxLW(cm)	15.4	3.4	0.2	15.4 ± 0.2	21.8
BL (cm)	38.6	5.6	0.3	38.6 ± 0.3	14.6
LSHL (cm)	27.2	9.9	0.6	27.2 ± 0.6	36.3
SL (cm)	24.3	5.6	0.3	24.3 ± 0.3	23.3
BL (cm)	20.3	2.5	0.3	20.3 ± 0.3	12.5

Note: NL-number of leaves; MaxLL-maximum leaf length; MaxLW-maximum leaf width; BL-bracts length; LSHL-Leaf sheath length; SL-scape length; BD-bulb diameter

Table 2. Statistical values of the examined morphometric parameters in case of *Leucojum vernum* L. collected in Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine)

Evaluated parameters	NL	MaxLL (cm)	MaxLW (cm)	BL (cm)	LSHL (cm)	SL (cm)	BD (cm)
NL		**0.497**	0.419	0.368	0.179	0.425	0.061
MaxLL (cm)			**0.604**	**0.765**	0.158	**0.908**	0.181
MaxLW (cm)				**0.513**	0.020	**0.517**	0.132
BL (cm)					0.044	**0.732**	0.134
LSHL (cm)						0.067	0.007
SL (cm)							0.180
BD (cm)							

Note: NL-number of leaves; MaxLL-maximum leaf length; MaxLW-maximum leaf width; BL-bracts length; LSHL-Leaf sheath length; SL-scape length; BD-bulb diameter

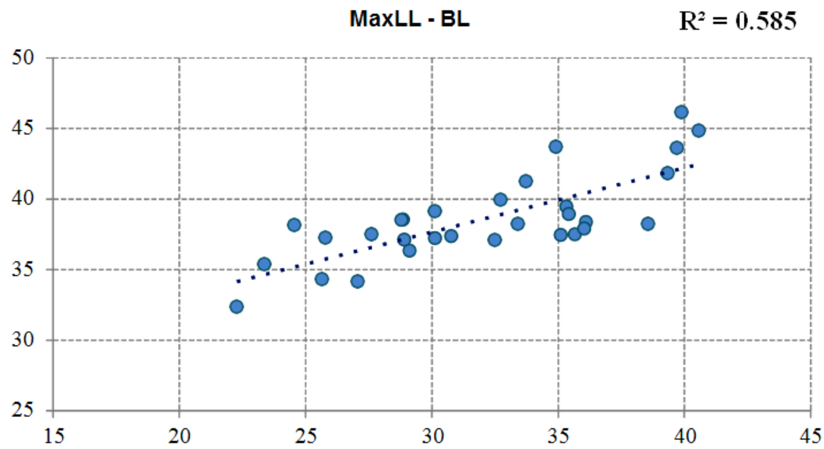

Fig. 7. Correlation between maximum leaf length (MaxLL) and bract length (BL) of *Leucojum vernum* L. collected from Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine)

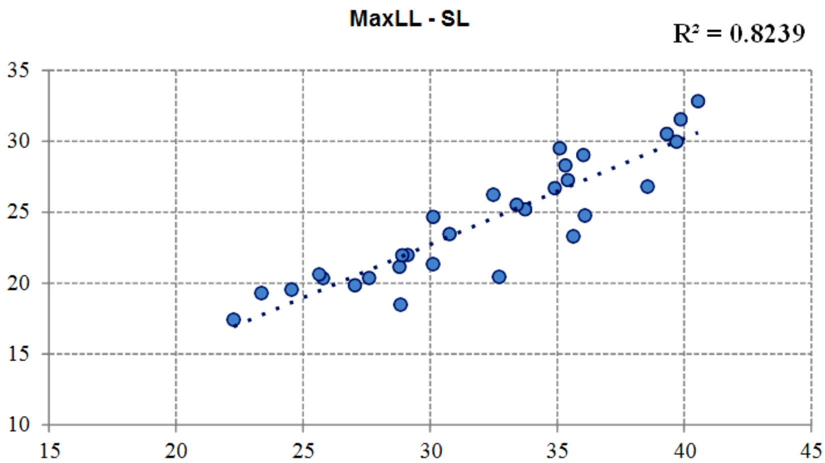

Fig. 8. Correlation between maximum leaf length (MaxLL) and scape length (SL) of *Leucojum vernum* L. collected from Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine)

3.3. Soil chemical analysis

Initially, to analyze the chemical properties of the soil samples we divided the samples into two groups. The first group contained the samples from Territory 1 (T1), while the second group consisted of the samples of Territory 2 and Territory 3 (T2+T3).

The reasoning behind this classification was that T2 and T3 are spatially much closer to the Latorica River, hence the soil of these two territories might have different chemical properties as a result of the enhanced influence of the river. Moreover, the samples were taken in an approximate equidistant fashion to ensure a uniform sampling over the whole investigated territory, which inherently led fewer samples from T3 than T1 or T2 due to the smaller area of T3. Thus, separate analysis and comparison of the three regions would have provided much less reliable statistical data.

The following agrochemical parameters were measured on the two groups of samples: the alkaline hydrolyzing nitrogen content, pH with two methods, soluble P_2O_5 content and the humus content (**Table 3**).

After the evaluation of the measurements the data were analyzed with two-sample Student's *t*-tests to determine if there are significant differences between the sample means of the two groups, with respect to the agrochemical parameters. Beforehand, F-tests were used to validate if the variance of the two groups are equal regarding the different parameters (acceptance level was $p=0.05$), which is a prerequisite to carry out two-sample Student's *t*-tests. The results of the F-tests showed that the variances are in fact the same with respect to the measured properties so Student's *t*-tests were applied (**Table 3**). From the results it can be seen, that there are significant differences between the means in four of the five measured parameters. Especially high differences can be identified in the alkaline hydrolyzing nitrogen content (312.05 vs. 240.80 which both represent very good nitrogen supply) and soluble P_2O_5 content (58.67 vs. 101.19 representing a medium supply). Besides the potential impact of the Latorica River, this can also be reasoned by the different altitude, age and density of the forests between the two territories, which gives us the opportunity for a future research in the subject.

Table 3. The agrochemical indices of the examined soil samples from Peres Forest, Velyka Dobron Wildlife Reserve (Western Ukraine)

Indices	Studied territory	Mean	Standard dev.	Sample size	p (F-test)	p (t-test)	Significant?
Alkaline hydrolyzing nitrogen (mg / kg)	T1	312.05	29.34	15	0.435665	0.000001	YES
	T2+T3	240.8	30.64	14			
pH (H_2O)	T1	6.33	0.25	15	0.10409	0.002347	YES
	T2+T3	6.6	0.17	14			
pH (KCl)	T1	5.44	0.16	15	0.198592	0.007075	YES
	T2+T3	5.64	0.2	14			
Soluble P_2O_5 (mg / kg)	T1	58.67	22.87	15	0.464275	0.000035	YES
	T2+T3	101.19	23.4	14			
Humus content (%)	T1	6.15	1.19	15	0.06613	0.087411	NO
	T2+T3	5.48	0.78	14			

The mean values of pH in the aqueous solution were 6.33 and 6.60 for the two groups and 5.44 and 5.64 in the KCl extract. Thus, in all cases the soil has a slightly acidic or near neutral pH, which is generally favorable for the nutrient uptake of plants. The humus content was the only parameter which didn't show significant difference between the two groups and both of them considered to be good according to the limits of Ukraine.

Conclusions

According to morphometric results, the most variable parameter was the length of the leaf sheath and the less variable was the bulb diameter, which is the same as reported in the literature of Ljubinyec (Любинець) in 2013. The correlation analysis showed that there is a strong positive relationship between the maximum leaf length and the bract length, and also between the maximum leaf length and

scape length. One thousand seeds weighted 16.19 g. The alluvial type soil had significant accumulation of organic matter and high level of humus content. The soil pH values (obtained with both methods) are favorable for the growing and development of plants. The soil is of medium supply based on the amount of soluble P_2O_5, while alkaline hydrolyzing nitrogen has a high supply. However, there are significant differences between the agrochemical parameters of Territory 1 and Territory 2, 3 (except the humus content), which can be explained by multiple factors and hence will be the subject of a future research.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Demir A (2014) Medical Resource Value Appraisal for Leucojum aestivum in Turkey. American Journal of Alzheimer's Disease & Other Dementias 29(5):448–451. doi.org/10.1177/1533317514535334
2. IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland. https://www.met.hu/downloads.php?fn=/metadmin/attach/2018/07/bf121478e6e4bb976886646893cd71ff-ipcc-jelentes-2014.pdf. Accessed 01 Dec 2019
3. Fidy J, Makara G (2005) Biostatisztika, InforMed Kiadó, Budapest
4. Kohut E, Höhn M, Fülöp M, Kopor Z, Ljubka T, Molnár F, Takács G (2017) Előzetes eredmények a *Leucojum vernum* L. populációjának állapotáról a Peresben. II. RF KMF – Limes 4:9-22.
5. Lledo DM, Davis AP, Crespo MB, Chase MW & Fay MF (2004) Phylogenetic analysis of *Leucojum* and *Galanthus* (*Amaryllidaceae*) based on plastid matK and nuclear ribosomae spacer (ITS) DNA sequences and morphology. Plant

Systematics and Evolution 246(3):223–243. doi: 10.1007/s00606-004-0152-0
6. Péczely Gy (1998) Éghajlattan. Nemzeti Tankönyvkiadó, Budapest
7. Scott LJ, Goa KL (2000) Galantamine: a review of its use in Alzheimer's disease. PubMed. 60(5):1095–1122. doi: 10.2165/00003495-200060050-00008
8. Зайцев ГН (1973) Методика биометрических расчетов. Математическая статистика в экспериментальной ботанике. Наука, Москва
9. Злобин ЮА, Скляр ВГ, Клименко АА (2013) Популяции редких видов растений: теоретические основы и методика изучения. Монография. Університетська книга, Суми
10. Кричфалуший ВВ, Комендар ВИ (1990) Биоекология редких видов растений. Свит, Львов
11. Любинець І (2013) Стан і динамічні тенденції ценопопуляції *Leucojum vernum* L. на території Яворівського національного природного парку. Вісник Львівського університету. Серія біологічна, 63:51–61.
12. http://publications.lnu.edu.ua/bulletins/index.php/biology/article/download/8236/8191
13. ДСТУ ISO 10390:2007 Якість ґрунту. Визначення pH
14. ДСТУ 4289:2004 Якість ґрунту. Методи визначання органічної речовини
15. ДСТУ 4362:2004 Якість ґрунту. Показники родючості ґрунтів
16. ДСТУ 4405:2005 Якість ґрунту. Визначання рухомих сполук фосфору і калію за методом Кірсанова в модифікації ННЦ ІГА

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PLANT AQUAPORINS

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Abstract: This mini-review briefly presents the main types of plant aquaporins, highlighting their importance for different plant species and for plant cellular functions. Aquaporins (AQPs), families of water channel proteins (WCPs) are transmembrane proteins that are present in prokaryotes, animals, plants, and humans. The plant aquaporins are part of the Major Intrinsic Proteins (MIPs) family which resides in the following plant organs: roots, stems, leaves, flowers, fruits, and seeds. According to the sub-cellular localization, to their sequence homologies and to their phylogenetic distribution, plant aquaporins have been divided in five subgroups: (a) plasma membrane intrinsic proteins (PIPs); (b) tonoplast intrinsic proteins (TIPs); (c) Nodulin26-like intrinsic membrane proteins (NIPs); (d) small basic intrinsic proteins (SIPs) and (e) uncharacterized intrinsic proteins (XIPs). Different subclasses of the plant aquaporins allow several types of transport using: water, glycerol, urea, hydrogen peroxide, organic acids, ethanol, methanol, arsenite, lactic acid, and gaseous compounds. Plant aquaporins have a significant role in cell response to cold stress, photosynthesis, plant growth, cell elongation, reproduction, and seed germination.

Keywords: plant aquaporins, water transport, plasma membrane proteins, tonoplast membrane proteins, nodulin26-like membrane proteins, small basic proteins, uncharacterized proteins.

1. Introduction

Aquaporins (AQPs), families of water channel proteins (WCPs) are transmembrane proteins that form a membrane pore and allows water to rapidly pass through biological membranes by osmosis (Agre et al., 1993; Agre, 2004; Benga, 2012). The first WCP, discovered in the red blood cell membrane by Benga's research group (Benga et al., 1986 a,b), was purified by Agree's group scientists and was named aquaporin 1 (AQP1) (Agre et al., 1993; Agre, 2004).

AQPs are present in prokaryotes (Tanghe et al., 2006), in various animals (Benga, 2013), plants (Maurel et al., 1993; Maurel et al., 2008; Sutka et al, 2017) and humans (Benga and Huber, 2012; Papadopoulos and Verkman, 2012; Verkman, 2013).

Some studies have shown that tomato, soybean, corn and spinach leaf aquaporins have a certain homology with human aquaporin-4 (Lambert et al., 2019).

The first plant WCP was discovered by Maurel et al. (1993) in the vacuolar membrane of *Arabidopsis thaliana*, named AQP (γTIP or *At*TIP1;1) (Maurel et al., 1993; Wudick et al., 2009). Comparatively to the animals/humans, plants contain a much higher number of AQPs (between 30 and 70) (Wudick et al., 2009; Deshmukh et al., 2016; Kapilan et al., 2018).

The plant aquaporins (AQPs) are part of the Major Intrinsic Proteins (MIPs) family that are integral α-helical proteins and allow passive water and neutral solute bidirectional transport across biological membranes and, also have a significant role in cell response to cold stress (Rouge and Barre, 2008; Hernandez-Sanchez et al., 2019) and to osmotic stress (Balarynová et al., 2018), also are important during leaf and petal movements, cell elongation, reproduction, and seed germination (Maurel, 2007).

The plant aquaporins have typical structural particularities: the six transmembrane core as α-helical domains with N- and C-terminal ends towards on the cytoplasmic side of the membrane, linked by five loops (A-E), that define an aqueous transmembrane pore; the pore stability and specificity is given by two hydrophobic motifs Asp-Pro-Ala (NPA) of the B and E loops that are embedded in center of the membrane. The high specificity for substrate is determined by an aromatic/Arg (ar/R) motif. AQPs monomers function as water channel within membrane and four AQPs monomers tend to form homotetramers by hydrogen bonds among the monomer loops; tetrameric structure of the holoproteins gives protein stability and functionality (Shapiguzov, 2004; Wudick et al, 2009; Kaldenhoff et al., 2014; Kapilan et al., 2018).

Aquaporin`s permeability depends on water structure. However, aquaporin permeability is the same for both distilled water and original tap water (Kozumi and Kitagawa, 2016).

The plant aquaporins are present in the following plant organs: roots, stems, leaves, flowers, fruits and seeds (Kapilan et al., 2018). The phosphorylation level of the AQPs subunits determines their sub-cellular localization (Kapilan et al., 2018). AQPs are located in different intracellular places: plasma membrane, vacuoles (tonoplast), plastids, endoplasmic reticulum, and, in some species, interact with symbiotic organisms (Maurel et al., 2015).

Some studies have described several types of transport using different subclasses of the plant aquaporins: water, glycerol, urea, hydrogen peroxide (H_2O_2), organic acids, ethanol, methanol, arsenite, lactic acid and gaseous compounds (oxygen – O_2, ammonia – NH_3, carbon dioxide – CO_2 (Shapiguzov, 2004; Wudick et al., 2009; Kapilan et al., 2018). Some AQPs are sensitive to inhibitors that contain mercury, which induces conformational changes in AQPs and block water passage through the channel (Shapiguzov, 2004).

According to the substance type transported, plant aquaporins have been classified in three subgroups: (a) aquaporins specific for water transport (AQPs); (b) aquaporins GlpFs specific for small carbohydrates (glycerol) transport and (c) aquaglyceroporins (AQP3s) specific for both water and small non-ionic dissolved molecules transport (Rouge and Barre, 2008). According to the sub-cellular localization, to their sequence homologies and to their phylogenetic distribution, plant aquaporins have been divided in five subgroups: (a) plasma membrane intrinsic proteins (PIPs); (b) tonoplast intrinsic proteins (TIPs); (c) Nodulin26-like intrinsic membrane proteins (NIPs); (d) small basic intrinsic proteins (SIPs), and (e) uncharacterized intrinsic proteins (XIPs) (Rouge and Barre, 2008; Deshmukh et al., 2016; Hernandez-Sanchez et al., 2019). The phylogenetic classification takes into account the characteristics and functions of each AQPs (Deshmukh et al., 2016).

2. Plasma membrane intrinsic proteins (PIPs)

The plants PIPs are the largest proteins subgroup with high variability of terminal domains and are located in the plasma membrane, but also into other structures as vascular tissues, guard cells, and flowers. There are 13 isoforms in *Arabidopsis*, which have been subdivided into two phylogenetic subgroups: PIP1 and PIP2, containing similar amino acid sequences, but with different water transport ability. N-terminal tails of PIP1 are longer compared to PIP2 and PIP1 C-terminal tails are shorter (Shapiguzov, 2004; Wudick et al., 2009; Kapilan et al., 2018).

PIPs are important proteins in regulating the water transport, photosynthesis, plant growth, development and restriction of root water uptake, regulated by low pH values (Agre, 2004; Wudick et al., 2009; Zhu and Ming, 2019). PIPs own an important role in the regulation of water transport under abiotic stress (Ding et al., 2019).

The PIP1 subgroup consists of five members: PIP1;1 to PIP1;5. All the PIP1 proteins have low water permeability because some of them cannot act one with another and they must form heterotetramers with PIP2 monomers (Kapilan et al., 2018). PIP1 trafficking to the plasma membrane depends on PIP2 interaction. These facts suggest a functional interaction between PIP1 and PIP2 and, also support roles for PIP1 in water transport. When expressed alone, some PIP1 do not localize to the plasma membrane. PIP1-PIP2 interactions represent post-translational regulatory mechanisms that influence intrinsic permeability of PIPs (Yaneff et al., 2015). It is also considered that PIPs phosphorylation has an important role in the gating mechanisms and sub-cellular dynamics (Verdoucq et al., 2014; Pawłowicz and Masajada, 2019) and in redox-dependent modulation of osmotic water permeability in plasmalemma from roots (Piotrovskii et al., 2019). An example is phosphorylation of Ser or Thr residues of the cytoplasmic C-terminal tail of the *Arabidopsis* root PIP2s (Maurel et al., 2009). Within *Arabidopsis*, the tetramer structure of the AtPIP2;1 is essential for water permeability. In the plasma membrane, inter-transmembrane interactions between monomers played an important role in tetramer formation of the AtPIP2;1 (Yoo et al., 2016).

The PIP2 subgroup consists of eight members: PIP2;1 to PIP2;8 than, are more efficiently compared to PIP1 group members (Kapilan et al., 2018; Nada and Abogadallah, 2019). PIP2s are organized in homotetramers that translocate to the plasma membrane by interacting with SNARE proteins (Yaneff et al., 2015).

An important aspect regarding the PIPs response to various environmental conditions is transcriptional and post-transcriptional regulation mechanisms. In the secretory pathways, PIPs are synthesized within the rough endoplasmic reticulum (RER). Then, PIP2 proteins are associated with PIP1 proteins and form PIP oligomers that transit Golgi apparatus and then, are loaded into secretory vesicles, being transported to the plasma membrane, where it merges through SNARE proteins. PIPs internalization it depends on environmental factors or signaling molecules and is achieved in constitutive pathway through clathrin-coated vesicles (Hachez et al., 2013).

AQPs, in particular PIPs, support more post-translational modifications: deamination, phosphorylation, methylation, acetylation, that influence their activity and trafficking. So, PIP and NIP residues phosphorylation plays a key role in the gating of the pore and regulation of the protein sub-cellular localization (Fox et al., 2017). The plant aquaporins gating (opening and closing of the water channel pore) may be regulated by phosphorylation, protons and divalent cations. Phosphorylation was first proposed for spinach SoPIP2;1 and pea PvTIP3;1 (Li et al., 2014). In addition, it is considered that PIPs gating is regulated by cytosolic pH and the intracellular Ca^{2+} concentration (Tan et al., 2018).

Some studies showed that heterotetramerization, cytosolic acidification and loop B serine phosphorylation affect aquaporin gating. Loop B serine phosphorylation affects pH gating of FaPIP2;1 but not of FaPIP1;1 from *Fragaria* x *ananassa* (Yaneff et al., 2016).

Based on the expression level, it is considered that for rice, the ability of PIP1 to allow water diffusion was relatively low or absent, compared to a PIP2, that was high. Within rice, three PIP1s (PIP1;1-PIP1;3) were highly expressed in roots (Ding et al., 2019) and the expression of PIP2;1 was the highest among the six PIP2s (Ding et al., 2019). Also, enhancing AQPs activity, increased the ability of root water uptake and drought tolerance of rice supplied with NH_4^+ (Ding et al., 2015).

Regarding the effect of high root temperature on the water uptake capability of broccoli was observed increase in osmotic water permeability at higher temperature. This process was not due to changes in the PIPs abundance, but was probably due to increase of permeability through lipid bilayer and changes in plasma membrane fluidity (Iglesias-Acosta et al., 2010).

A recent study showed that during flooding a rapid decrease of cytosolic pH it takes place, due to anoxia. This process leads to a simultaneous aquaporin closure in the plasma membrane, mechanism where it is involved a conserved histine on cytosolic loop D of the aquaporin (Frick et al., 2013).

Aquaporins have been used as target proteins in genetic manipulation, to improve plant water relations under environmental stress. For example, *Vicia faba* PIP1 (VfPIP1) expression in transgenic *Arabidopsis thaliana* improved drought resistance by reducing water loss through transpiration (Martinez-Ballesta and Carvajal, 2014).

For *Nicotiana tabacum*, a member of PIP1 subgroup (NtAQP1) decreased plant resistance to water stress. Some members of PIPs (NtAQP1) are involved in water transport and can participate in carbon dioxide transport, thus contributing to photosynthesis (Pawłowicz and Masajada, 2019).

In *Pisum sativum*, PIP1 has an important role in water absorption during seed water uptake. (Kapilan et al., 2018).

The plant protection against dehydration stress depends on the interaction between specific proteins such dehydrins and aquaporins, that protect other proteins from damage during dehydration. Dehydrins are intrinsically disordered proteins that accumulate during abiotic stress conditions. It is considered that two classes of proteins behave simultaneously for AQPs denaturation and inactivation (Hernandez-Sanchez et al., 2019).

In *Saccharum ssp.*, three aquaporins (ShPIP2;1, ShPIP2;5 and ShPIP2) replied to water deficit conditions and their expressions were depending on genotype, experimental conditions and duration of drought stress (Mara de Andrade et al., 2016).

The relevance of aquaporin root water transport at low temperatures has been demonstrated by over-expressing of PtdPIP2;5 (PtdPIP2;5ox) at transgenic *Populus tremula* x *alba*. Over-expression of PtdPIP2;5 was efficient in attenuation of the effect at low root temperature, on hydraulic conductivities and gas exchange (Ranganathan et al., 2016).

Some members of the PIP1 subgroup had a relevant role in glycerol and CO_2 transport (Kapilan et al., 2018). High rates of photosynthesis are provided by PIP1 aquaporins, using CO_2 channels that are important for the mesophyll and stomatal CO_2 conductance in leaves (Yaneff et al., 2015).

For maize, the pairs ZmPIP1;5-ZmPIP2;5 and ZmPIP1;1-ZmPIP2;1 are functional units whitin roots, important in salt stress cell response and during root development (Yaneff et al., 2015). For *Saccharomyces cerevisiae*, some PIP2 transport H_2O_2, while in maize, ZmPIP1;2 does not allow peroxide transport (Yaneff et al., 2015). For stability and increase of water permeability, maize ZmPIP1s and ZmPIP2s interact; similar results were observed for *Nicotiana tabacum*, *Beta vulgaris*, *Mimosa pudica*, *Vitis vinifera*, *Hordeum vulgare*, *Triticum turgidum* (Bienert et al., 2014). Using a yeast system, it was observed that ZmPIP1;2 is not permeable to hydrogen peroxide, comparatively to wild type ZmPIP2;5 (Bienert et al., 2014). Differences in H_2O_2 permeability between various AQP isoforms are due to differences in open mechanism of channels and to miss targeting to cellular membranes other than plasma membrane (Bienert and Chaumont, 2014). The oxidative stress responses were investigated in the *Arabidopsis* roots, through redistribution of PIPs from plasma membrane to intracellular membranes. AtPIP2;1 analyses with endomembrane markers showed that H_2O_2 determined AtPIP2;1 accumulation in the late endosomal compartments. The high stability of PIPs was maintained under oxidative stress conditions due to the intracellular redistribution of PIPs without degradation (Wudick et al., 2015).

In case of *Chrysanthemum morifolium*, CmPIP1 and CmPIP2 differ in their response expression to salt, presenting higher expression in the leaves and lower in stems and flowers. Their role is very important: stomatal opening and closing, transpiration and photosynthesis (Zhang et al., 2019).

3. Tonoplast intrinsic proteins (TIPs)

The The tonoplast intrinsic proteins (TIPs) are the most abundant aquaporins in the vacuolar membrane (tonoplast). Some TIPs isoforms are found in small vacuoles and in the membrane surrounding the protein storage vacuoles (PSV), having a storage function for protein and play a role in protein degradation (Wudick et al., 2009; Kapilan et al., 2018). TIPs are the first water transporting proteins that have been identified in tonoplast of *Arabidopsis thaliana* (AtTIP1;1) (Maurel et al., 1993; Wudick et al., 2009; Kapilan et al., 2018). According to their sequence homologies, in maize, rice, and *Arabidopsis*, TIP group consists of five subgroups: TIP1-TIP5. For example, ten tonoplast intrinsic protein (TIP) homologues are present in *Arabidopsis* (Wudick et al., 2009) and 17 TIPs are present in *Populus trichocarpa* (Kapilan et al., 2018).

The tonoplast TIPs play a key role in transporting small solutes (diffusion of water, urea, ammonia, H_2O_2) and gases, in maintenance of cell turgor pressure, and TIPs genes become under-expressed after drought stress (Abascal et al., 2014; Fox et al., 2017; Kapilan et al., 2018). Water permeability of the tonoplast is higher than that of the plasma membrane, due to the abundance of the aquaporines in the tonoplast (Kapilan et al., 2018).

TIPs isoforms can have different physiological functions. For example, in *Mesembryanthemum crystallinum*, TIPs may play an important role in stress responses, therefore when the plant was exposed to salt stress, the abundance of TIP1;2 decreased to 75% (Kaldenhoff and Fischer, 2006; Wudick et al., 2009; Kapilan et al., 2018).

TIPs proteins are aquaporin synthesized during seed maturation and found in vacuolar membranes of cotyledons. TIPs that function in seed drying, cytoplasmic osmoregulation, seed rehydration can be regulated by phosphorylation (Daniels et al., 1999; Li et al., 2014).

TIPs may play a role in equilibrating urea concentrations between the vacuole and the cytoplasm (Kaldenhoff and Fischer, 2006).

An implication of TIPs in urea transport was first described in tobacco suspension cells, where, in accordance with NtTIPa expression, vesicles showed higher permeability to urea. In *Arabidopsis*, almost all TIP subclasses (AtTIP1;1, AtTIP1;2, AtTIP1;3, AtTIP2;1, AtTIP4;1, AtTIP5;1) transport urea. In plants, TIPs may allow a non-saturable and pH-independent transport of urea during nitrogen metabolism or nutrition (Wudick et al., 2009).

NH_3 transport was first described for TIP2 members from wheat (TaTIP2;1) and *Arabidopsis* (AtTIP1;2, AtTIP2;1, AtTIP2;3). TIPs play a function in sub-cellular partitioning of NH_3 and contribute to the detoxification of NH_3 excess amounts in the cytosol (Wudick et al., 2009). Expression of AtTIP2;1 in roots was up regulated in response to nitrogen starvation (Jahn et al., 2004).

For tomato, the over-expression of SlTIP2;2 attenuated the reduction of plant transpiration under stress, ensuring an adequate balance between CO_2 uptake and water and nutrient supply (Martinez-Ballesta and Carvajal, 2014).

A key role of the tonoplast is to maintain turgor pressure against the cell wall. Recent studies have shown that in grapevine tonoplast, TIP2;1 activity is regulated by turgor induced membrane tension, changing from an opened to a closed state. Membrane tension may induce water channels closure, preventing fast water loss. This turgor that depends on TIP2;1 gating may be a mechanism to regulate vacuolar size and shape in grapevine (Leitao et al., 2014).

Furthermore, a specific role for PIPs and TIPs in primary root elongation and development of lateral roots was showed that may contribute to improving drought resistance (Zargar et al., 2017).

In drought conditions, the expression of PIPs and TIPs from tea plant remained relatively high after leaf rehydration, but they were repressed in the roots. CsPIP and CsTIP genes play an essential role in stress response, as well as in flower development and opening process. More, some CsNIPs, CsSIPs and CsXIPs can regulate flower development and opening process (Yue et al., 2014).

4. Nodulin26-like intrinsic membrane proteins (NIPs)

Nodulin26-like intrinsic membrane proteins (NIPs), aquaporins that have been initially localized in peribacteroid membrane of nitrogen-fixing root nodules of leguminous plants, are present in plasma membrane of the non-symbiotic plants too (Wudick et al., 2009; Kapilan et al., 2018). Aquaporins identified as nodulin26 proteins were first characterized in soybean and then in other rhizobia-symbiotic vegetables (Rouge and Barre, 2008).

Nodule formation is a result of symbiotic relationship between plant and nitrogen fixing bacteria. During the process of nodule formation, nodulin proteins are expressed by plants and transferred to the membranes. Nodulin 26 proteins are expressed at the symbiosome membrane and have similar sequences to NIP proteins (Kapilan et al., 2018).

NIPs have been classified in five phylogenetic groups (NIP1-NIP5) and in three functional groups (NIP I, NIP II, NIP III), based on similarity of the aromatic/arginine constriction (Mateluna et al., 2018).

Comparatively with other aquaporins, NIPs play a similar role as transporters of water, but in contrast with PIPs and TIPs, NIPs have lower water transport activity (Maurel et al., 2015; Kapilan et al., 2018). However, besides glycerol and water (Kruse et al., 2006), NIPs are permeable to small organic solutes and mineral nutrients and mediate diffusion of metalloids (boric acid, silicon and selenium) and toxic elements (arsenic) (Martinez-Ballesta and Carvajal, 2014; Maurel et al., 2015; Fox et al., 2017). NIPs transport functions have been established by direct mutagenesis and by expression in oocytes (Martinez-Ballesta and Carvajal, 2014). NIP I proteins are permeable to water, glycerol and ammonia, NIP II proteins transport urea and metalloid nutrients (boron), but have lower water permeability and NIP III proteins transport larger molecules (silicic acid, urea) (Mateluna et al., 2018).

Some structural particularities in NPA motif (alanine is replaced with serine, glycine or valine) might render NIPs capable of transporting substrates other than water (Wudick et al., 2009). NIP2 proteins have a wider pore than those of NIP1 and are permeable to large solutes (urea). Also, AtNIP2;1 is expressed in the endoplasmic reticulum of roots, whereas AtNIP5;1 is expressed in plasma membrane of roots elongation zones. The AtNIP2;1 mediates lactic acid transport, being very important to the adaptation to lactic fermentation under anaerobic stress (Gomes et al., 2009). When NIPs phosphorylation is increased, NIPs have an important role in maintaining plant water balance and in drought and salt stress responses (Martinez-Ballesta and Carvajal, 2014; Kapilan et al., 2018).

NIPs proteins have multifunctional transport properties of water and/or uncharged solutes as glycerol. NIP gene transcripts were found in seed coat, shoot and roots (Kaldenhoff and Fischer, 2006).

5. Small basic intrinsic proteins (SIPs)

As the TIPs proteins, SIPs proteins are small, but very basic, and can be found in seed plants and mosses (Abascal et al., 2014; Kapilan et al., 2018). Their small sizes are due to the very short cytosolic N-terminal region (Kapilan et al., 2018). SIPs proteins are associated with intracellular membranes, particularly with endoplasmic reticulum, having a key role for facilitating the intracellular water movement. Also, SIPs participate in the passage of water through the endoplasmic reticulum membranes and regulate the morphology of the organelle (Gomes et al., 2009).

SIPs proteins comprises four classes, of which in *Arabidopsis* there are three members of the SIPs (Wudick et al., 2009). In case of cotton, based on NPA sequence, SIPs include SIP1 subgroup that is divided into SIP1;1 and SIP1;2 (Gomes et al., 2009; Kapilan et al., 2018). Different SIP isoforms have different solute permeability, due to different sites of characteristic residues. So, in SIPs of cotton, alanine residues present in the first NPA motif was converted to tyrosine (Kapilan et al., 2018). From a functional point of view, although SIPs have an original pore conformation, it ensures only a moderate water transport (Maurel et al., 2015).

Three SIPs were identified in potato and were included in the SIP1 subgroup. Some of the potato aquaporins (PIP1;4, PIP2;1, TIP2;4, SIP1;1a, SIP1;1b and NIP5;1) were abundant in stolons, swollen stolons and tubers, suggesting that these aquaporins may play a potential role in the tuberization process (Venkatesh et al., 2013).

6. Uncharacterized intrinsic proteins (X intrinsic proteins - XIPs)

XIPs have been characterized in protozoa, fungi, mosses and dicots. There have been 19 XIP members: five XIPs in *Populus trichocarpa*, ten in dicots other than *Populus*, three from moss and one from protozoa (Kapilan et al., 2018). XIPs are absent in Brassicaceae, monocots and in certain dicots such as *Arabidopsis* (Li et al., 2014; Mara de

Andrade et al., 2016; Kapilan et al., 2018). XIP homologues have also been found in flowering plants (dicot species), in Solanaceae species (*Solanum lycopersicum*), *Glycine max* and *Jatropha curcas* (Wudick et al., 2009; Pawłowicz and Masajada, 2019). The potato contains more XIPs comparatively with other dicot species (cotton, *Vitis*). Except XIP1, which was less expressed in root tissues, but higher expressed in leaves, XIP2, XIP3;1 and XIP4;1 were highly expressed in potato root. The rapid transport of inorganic solutes and metabolites can make possible a higher expression of aquaporins in roots (Venkatesh et al., 2013).

The same as PIPs and NIPs, XIPs are localized in the plasma membrane (Yaneff et al., 2015; Pawłowicz and Masajada, 2019). It is considered that XIPs are multifunctional channels permeable to water and metalloids (Maurel et al., 2015). In grapevine, VvXIP1 play a key role in osmotic regulation in addition to H_2O_2 transport and metal homeostasis (Kapilan et al., 2018).

Another two sub-families were discovered in moss (*Physcomitrella patens*), and not in vascular plants: GIPs (GlpF-like intrinsic protein) and HIPs (hybrid intrinsic proteins) (Wudick et al., 2009; Abascal et al., 2014; Li et al., 2014; Yaneff et al., 2015; Pawłowicz and Masajada, 2019).

Conclusions

In plants, aquaporins are very abundant and are important for whole plant, for water transport to and from the vascular tissues. At the cellular level, plant aquaporins have key roles in control of the osmotic fluctuations. Due to the regulated abundance and activity of aquaporins, plants have the ability to control water transport. Plasma membrane proteins are important in regulating water transport, photosynthesis, transpiration, plant growth, development and restriction of roots water uptake regulated by low pH values. Tonoplast membrane proteins play key roles in transporting small solutes and gases, maintaining cell turgor pressure. Nodulin26-like membrane proteins are permeable to small organic solutes and mineral nutrients and mediate diffusion of metalloids and toxic elements. Small basic proteins participate in the passage of water through the endoplasmic reticulum membranes and regulate the morphology of the organelle. Uncharacterized proteins play key roles in osmotic regulation besides H_2O_2 transport and metal homeostasis.

A better understanding of the AQPs in plants and evaluation of various distribution models of aquaporins, depending on the degree of cell compartmentation, can lead to a better knowledge of cellular functions and to the development and management of plants better adapted to changing environmental conditions.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Abascal F, Irisarri I, Zardoya R (2014) Diversity and evolution of membrane intrinsic proteins. Biochimica et Biophysica Acta 1840:1468–1481. http://dx.doi.org/10.1016/j.bbagen.2013.12.001
2. Agre P (2004) Aquaporin water channels (Nobel lecture). Angew. Chem. Int. Ed. Engil. 43:4278–4290. doi: 10.1002/anie.200460804
3. Agre P, Sasaki S, Chrispeels MJ (1993) Aquaporins: a family of water channel proteins. Am J Physiol. 265 (3 Pt 2):F461. doi:10.1152/ajprenal.1993.265.3.F461
4. Balarynová J, Danihlik J, Fellner M (2018) Changes in plasma membrane aquaporin gene expression under osmotic stress and blue light in tomato. Acta Physiol Plant 40:27. https://doi.org/10.1007/s11738-017-2602-7
5. Benga G (2012) On the definition, nomenclature and classification of water channel proteins (aquaporins and relatives). Molecular Aspects of Medicine 33:514–517. http://dx.doi.org/10.1016/j.mam.2012.04.003
6. Benga G (2013) Comparative studies of water permeability of red blood cells from humans and over 30 animal species: an overview of 20 years of collaboration with Philip Kuchel. Eur. Biophys. J. 42:33–46. doi: 10.1007/s00249-012-0868-7
7. Benga G, Popescu O, Borza V, Pop VI, Muresan A, Mocsy I et al (1986a) Water permeability in human erythrocytes: identification of membrane proteins involved in water transport. Eur. J. Cell Biol. 41:252–262. doi: 10.1021/bi00355a011
8. Benga G, Popescu O, Pop VI, Holmes RP (1986b) p-(Chloromercuri) benzenesulfonate binding by membrane proteins and the inhibition of water transport in human erythrocytes. Biochemistry 25:1535–1538. doi: 10.1021/bi00355a011
9. Benga O, Huber V J (2012) Brain water channel proteins in health and disease. Mol. Aspects Med. 33:562–578. doi: 10.1016/j.mam.2012.03.008
10. Bienert GP, Chaumont F (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. Biochimica et Biophysica Acta 1840:1596–1604. http://dx.doi.org/10.1016/j.bbagen.2013.09.017
11. Bienert GP, Heinen RB, Berny MC, Chaumont F (2014) Maize plasma membrane aquaporin ZmPIP2;5, but not ZmPIP1;2, facilitates transmembrane diffusion of hydrogen peroxide. Biochimica et Biophysica Acta 1838:216–222. http://dx.doi.org/10.1016/j.bbamem.2013.08.011
12. Daniels MJ, Chrispeels MJ, Yeager M (1999) Projection structure of a plant vacuole membrane aquaporine by electron cryo-crystallography. Journal of Molecular Biology 294(5):1337–1349. https://doi.org/10.1006/jmbi.1999.3293
13. Deshmukh RK, Sonah H, Bélanger RR (2016) Plant Aquaporins: Genome-Wide Identification, Transcriptomics, Proteomics, and Advanced Analytical Tools. Front Plant Sci. 7:1896. doi: 10.3389/fpls.2016.01896
14. Ding L, Gao C, Li Y, Li Y, Zhu Y, Xu G, Shen Q, Kaldenhoff R, Kai L, Guo S (2015) The enhanced drought tolerance of rice plants under ammonium isrelated to aquaporin (AQP). Plant Science 234:14–21. http://dx.doi.org/10.1016/j.plantsci.2015.01.016

15. Ding L, Uehlein N, Kaldenhoff R, Guo S, Zhu Y, Kai L (2019) Aquaporin PIP2;1 affects water transport and root growth in rice (*Oryza sativa* L.). Plant Physiology and Biochemistry 139:152–160. https://doi.org/10.1016/j.plaphy.2019.03.017
16. Fox AR, Maistriaux LC, Chaumont F (2017) Toward understanding of the high number of plant aquaporin isoforms and multiple regulation mechanisms. Plant Science 264:179–187. http://dx.doi.org/10.1016/j.plantsci.2017.07.021
17. Frick A, Järvå M, Törnroth-Horsefield S (2013) Structural basis for pH gating of plant aquaporins. FEBS Letters 587:989–993. http://dx.doi.org/10.1016/j.febslet.2013.02.038
18. Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F (2009) Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochimica et Biophysica Acta 1788:1213–1228. doi: 10.1016/j.bbamem.2009.03.009
19. Hachez C, Besserer A, Chevalier AS, Chaumont F (2013) Insights into plant plasma membrane aquaporin trafficking. Trends in Plant Science, 18 (6):344-352. http://dx.doi.org/10.1016/j.tplants.2012.12.003
20. Hernandez-Sanchez IE, Maruri-Lopez I, Molphe-Balch EP, Becerra-Flora A, Jaimes-Miranda F, Jimenez-Bremont JF (2019) Evidence for in vivo interactions between dehydrins and the aquaporin AtPIP2B. Biochemical and Biophysical Research Communications 510:545–550. doi: 10.1016/j.bbrc.2019.01.095
21. Iglesias-Acosta M, Martínez-Ballesta CM, Teruel JA, Carvajal M (2010) The response of broccoli plants to high temperature and possible role of root aquaporins. Environmental and Experimental Botany 68:83–90. doi: 10.1016/j.envexpbot.2009.10.007
22. Jahn TP, Møller ALB, Zeuthen T, Holm LM, Klærke DA, Mohsin B, Kuhlbrandt W, Schjoerring JK (2004) Aquaporin homologues in plants and mammals transport ammonia. FEBS Letters 574:31–36. doi: 10.1016/j.febslet.2004.08.004
23. Kaldenhoff R, Fischer M (2006) Functional aquaporin diversity in plants. Biochimica et Biophysica Acta 1758:1134–1141. doi: 10.1016/j.bbamem.2006.03.012
24. Kaldenhoff R, Kai L, Uehlein N (2014) Aquaporins and membrane diffusion of CO_2 in living organisms. Biochimica et Biophysica Acta 1840:1592–1595. http://dx.doi.org/10.1016/j.bbagen.2013.09.037
25. Kapilan R, Vaziri M, Zwiazek JJ (2018) Regulation of aquaporins in plants under stress. Biological Research 51:4. https://doi.org/10.1186/s40659-018-0152-0
26. Kozumi T, Kitagawa Y (2016) Water structure changes induced by ceramics can be detected by increased permeability through aquaporin. Biochemistry and Biophysics Reports 5:353–358. https://doi.org/10.1016/j.bbrep.2016.01.002
27. Kruse E, Uehlein N, Kaldenhoff R (2006) The aquaporins. Genome Biology 7(206) doi:10.1186/gb-2006-7-2-206
28. Lambert J, Mejia S, Vojdani A (2019) Plant and human aquaporins: pathogenesis from gut to brain. Immunol Res 67(1):12–20. https://doi.org/10.1007/s12026-018-9046-z
29. Leitao L, Prista C, Loureiro-Dias MC, Moura TF, Soveral G (2014) The grapevine tonoplast aquaporin TIP2;1 is a pressure gated water channel. Biochemical and Biophysical Research Communications 450:289–294.

http://dx.doi.org/10.1016/j.bbrc.2014.05.121

30. Li G, Santoni V, Maurel C (2014) Plant aquaporins: Roles in plant physiology. Biochimica et Biophysica Acta 1840:1574–1582. http://dx.doi.org/10.1016/j.bbagen.2013.11.004
31. Mara de Andrade L, Macedo Nobile P, Vasconcelos Ribeiro R, Nebó Carlos de Oliveira JF, Vargas de Oliveira Figueira A, Tadeu Marques Frigel L, Nunes D, Perecin D, dos Santos Brito M, Célia de Matos Pires R, Guimarães de Andrade Landell M, Creste S (2016) Characterization of PIP2 aquaporins in Saccharum hybrids. Plant Gene 5:31–37. http://dx.doi.org/10.1016/j.plgene.2015.11.004
32. Martinez-Ballesta M del C, Carvajal M (2014) New challenges in plant aquaporin biotechnology. Plant Science 217-218:71–77. http://dx.doi.org/10.1016/j.plantsci.2013.12.006
33. Mateluna P, Salvatierra A, Solis S, Nuñez G, Pimentel P (2018) Involvement of aquaporin NIP1;1 in the contrasting tolerance response to root hypoxia in Prunus rootstocks. Journal of Plant Physiology 228:19–28. https://doi.org/10.1016/j.jplph.2018.05.001
34. Maurel C (2007) Plant aquaporins: Novel functions and regulation properties. FEBS Letters 581:2227–2236. doi: 10.1016/j.febslet.2007.03.021
35. Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in plants. Physiol Rev. 95(4):1321 New challenges in plant aquaporin biotechnology. Plant Science 1358. doi: 10.1152/physrev.00008.2015
36. Maurel C, Reizer J, Schroeder JL, Chrispeels MJ (1993) The vascular membrane protein gamma-TIP creates water specific channels in *Xenopus oocytes*. EMBO J. 12:2241–2247
37. Maurel C, Santoni V, Luu D-T, Wudick MM, Verdoucq L (2009) The cellular dynamics of plant aquaporin expression and functions. Current Opinion in Plant Biology 12:690–698. doi: 10.1016/j.pbi.2009.09.002
38. Maurel C, Verdoucq L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. Annu. Rev. Plant Biol. 59:595–624. doi: 10.1146/annurev.arplant.59.032607.092734
39. Nada RM, Abogadallah GM (2019) Contrasting root traits and native regulation of aquaporin differentially determine the outcome of overexpressing a single aquaporin (OsPIP2;4) in two rice cultivars. Protoplasma 1–13. https://doi.org/10.1007/s00709-019-01468-x
40. Papadopoulos MC, Verkman AS (2012) Aquaporin 4 and neuromyelitis optica. Lancet Neurol. 11:535–544. doi: 10.1016/S1474-4422(12)70133-3
41. Pawłowicz I, Masajada K (2019) Aquaporins as a link between water relations and photosynthetic pathway in abiotic stress tolerance in plants. Gene 687:166–172. https://doi.org/10.1016/j.gene.2018.11.031
42. Piotrovskii MS, Lapshin NK, Andreev IM, Trofimova MS (2019) Role of PIP-Aquaporin phosphorylation in redox-dependent modulation of osmotic water permeability in plasmalemma from roots of pea seedlings. Russ J Plant Physiol 66:637–645. https://doi.org/10.1134/S1021443719040113
43. Ranganathan K, Kayal WE, Cooke JEK, Zwiazek JJ (2016) Response of hybrid aspen over-expressing a PIP2;5 aquaporin

to low root temperature. Journal of Plant Physiology 192:98–104. https://doi.org/10.1016/j.jplph.2016.02.001

44. Rouge P, Barre A (2008) A molecular modeling approach defines a new group of Nodulin 26-like aquaporins in plants. Biochemical and Biophysical Research Communications 367:60–66. doi: 10.1016/j.bbrc.2007.12.079
45. Shapiguzov YA (2004) Aquaporins: Structure, Systematics, and Regulatory Features. Russian Journal of Plant Physiology 51 (1): 127–137. doi: 10.1023/B:RUPP.0000011313.02617.49
46. Sutka M, Amodeo G, Ozu M (2017) Plant and animal aquaporins crosstalk: what can be revealed from distinct perspectives. Biophys Rev 9 (5):545–562. https://doi.org/10.1007/s12551-017-0313-3
47. Tan X, Xu H, Khan S, Equiza MA, Lee SH, Vaziriyeganeh M, Zwiazek JJ (2018) Plant water transport and aquaporins in oxygen-deprived environments. Journal of Plant Physiology 227:20–30. https://doi.org/10.1016/j.jplph.2018.05.003
48. Tanghe A, Van Dijck P, Thevelein JM (2006) Why do microorganisms have aquaporins? Trends Microbiol. 14:78–85. doi: 10.1016/j.tim.2005.12.001
49. Venkatesh J, Yu J-W, Park SW (2013) Genome-wide analysis and expression profiling of the *Solanum tuberosum* aquaporins. Plant Physiology and Biochemistry 73:392-404. http://dx.doi.org/10.1016/j.plaphy.2013.10.025
50. Verdoucq L, Rodrigues O, Martiniere A, Luu D-T, Maurel C (2014) Plant aquaporins on the move: reversible phosphorylation, lateral motion and cycling. Current Opinion in Plant Biology 22:101–107. http://dx.doi.org/10.1016/j.pbi.2014.09.011
51. Verkman AS (2013) Aquaporins. Curr Biol. 23(2): R52–R55. doi: 10.1016/j.cub.2012.11.025
52. Wudick MM, Li X, Valentini V, Geldner N, Chory J, Lin J, Maurel C, Luu D-T (2015) Subcellular redistribution of root aquaporins induced by hydrogen peroxide. Mol. Plant. 8:1103–1114. http://dx.doi.org/10.1016/j.molp.2015.02.017
53. Wudick MM, Luu D-T, Maurel C (2009) A look inside: localization patterns and functions of intracellular plant aquaporins. New Phytologist 184 (2):289-302, https://doi.org/10.1111/j.1469-8137.2009.02985.x
54. Yaneff A, Sigaut L, Gómez N, Fandiño CA, Alleva K, Pietrasanta LI, Amodeo G (2016) Loop B serine of a plasma membrane aquaporin type PIP2 but not PIP1 plays a key role in pH sensing. Biochimica et Biophysica Acta 1858:2778–2787. http://dx.doi.org/10.1016/j.bbamem.2016.08.002
55. Yaneff A, Vitali V, Amodeo G (2015) PIP1 aquaporins: intrinsec water channels or PIP2 aquaporin modulators? FEBS Letters 589:3508–3515. http://dx.doi.org/10.1016/j.febslet.2015.10.018
56. Yoo Y-J, Lee HK, Han W, Kim DH, Lee MH, Jeon J, Lee DW, Lee J, Lee Y, Lee J, Kim JS, Cho Y, Han J-K, Hwang I (2016) Interactions between transmembrane helices within monomers of the aquaporin AtPIP2;1 play a crucial role in tetramer formation. Mol. Plant. 9:1004–1017. http://dx.doi.org/10.1016/j.molp.2016.04.012
57. Yue C, Cao H, Wang L, Zhou Y, Hao X, Zeng J, Wang X, Yang Y (2014) Molecular cloning and expression analysis of tea plant aquaporin (AQP) gene family. Plant

Physiology and Biochemistry 83:65–76. http://dx.doi.org/10.1016/j.plaphy.2014.07.011

58. Zargar SM, Nagar P, Deshmukh R, Nazir M, Wani AA, Masoodi KZ, Agrawal GK, Rakwal R (2017) Aquaporins as potential drought tolerance inducing proteins: Towards instigating stress tolerance. Journal of Proteomics 169:233–238. http://dx.doi.org/10.1016/j.jprot.2017.04.010
59. Zhang B, Xie L, Sun T, Ding B, Li Y, Zhang Y (2019) *Chrysanthemum morifolium* aquaporin genes CmPIP1 and CmPIP2 are involved in tolerance to salt stress. Scientia Horticulturae 256 (108627):1-8. https://doi.org/10.1016/j.scienta.2019.108627
60. Zhu F, Ming R (2019) Global identification and expression analysis of pineapple aquaporins revealed their roles in CAM photosynthesis, boron uptake and fruit domestication. Euphytica 215:132. https://doi.org/10.1007/s10681-019-2451-0

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PHYTOSOCIOGICAL RESEARCH ON WET MEADOWS DOMINATED BY *SCIRPUS SYLVATICUS* L. IN CĂLIMANI AND GURGHIULUI MOUNTAINS (MUREȘ COUNTY)

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Abstract: Phytosociological structure of the communities dominated by *Scirpus sylvaticus* was studied in the mountain area of Mureş County with focus on vegetation community organization, floristic composition and habitat conservation. The vegetation sampling and data analysis were done following standard procedures. The study of wet meadows from the Călimani and Gurghiului Mountains carried out in field during 2015-2019, highlights the presence of hygrophilous coenoses belonging to the *Scirpetum sylvatici* Ralski 1931 plant association. These wet meadows grow on alluvial, gleyic and acidophilous soils. The identified communities belong to Natura 2000 habitat 6430 Hydrophilous tall-herb fringe communities of plains in the montane to alpine levels. The conservation status of the habitat is good and very good and the floristic composition emphasizes a rich floristic diversity.

Keywords: plant association, habitat, conservation status, Mureş County.

1. Introduction

Phytosociological analysis indicates the organization and structure of the vegetation in a particular habitat which determines the distribution pattern of individuals from other species, and it is important for understanding the functioning of any community.

The diversity of semi-natural communities along the rivers beds and valley streams is determined by the variety of the habitat conditions both in terms of water content and soil fertility.

The study area is represented by the valleys of the following rivers: Mureș, Gurghiu, Niraj, Sebeș and Sovata. These are mountainous valleys stretching along the Gurghiului and Călimani Mountains in Mureș County. On the river side, plots with tall herbs and wet meadows occur. They belong to the Natura 2000 habitat 6430 Hydrophilous tall-herb fringe communities of plains in the montane to alpine levels.

In the present investigation an attempt has been made to document the structure of plant communities, composition and diversity of some meadows from Gurghiului and Călimani Mountains. A phytosociological survey of the wet meadows dominated by *Scirpus sylvaticus* in the study area was made during 2015-2019.

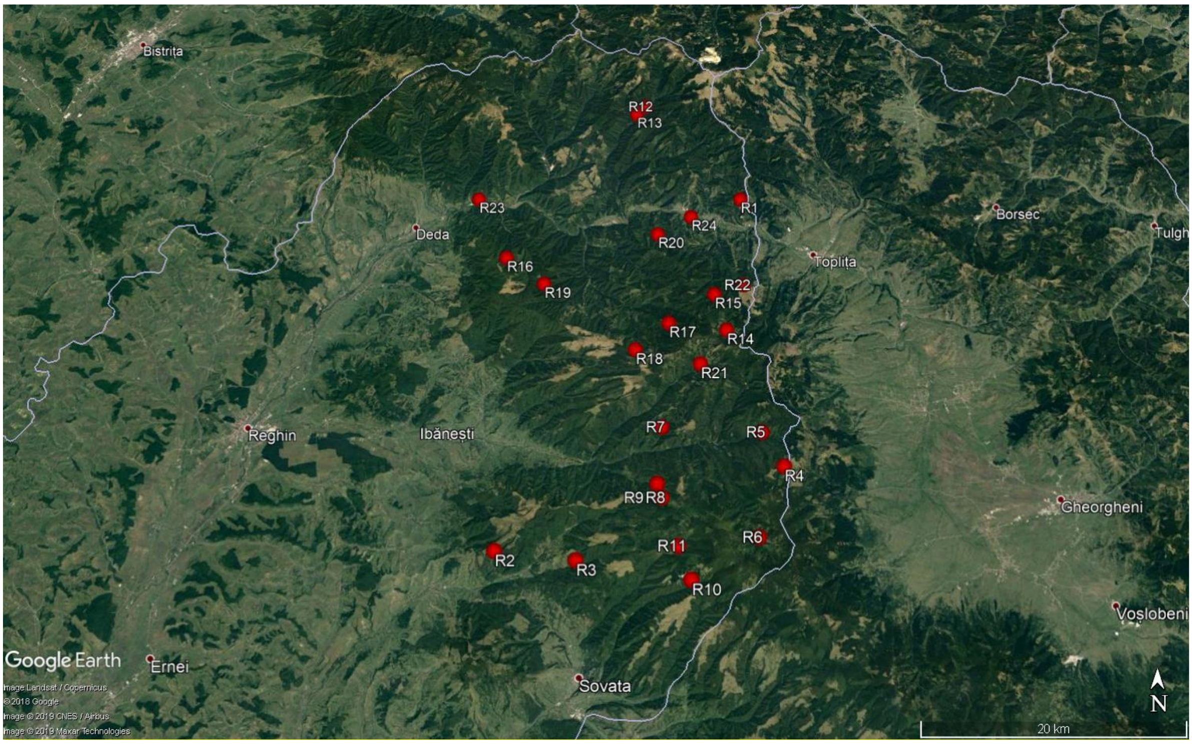

Fig. 1. The map with the location of relevées from Gurghiului and Călimani Mountains (Romania)

They were classified in the association *Scirpetum sylvatici* Ralski 1931. This association is wide spread in the East Carpathians; it was described from Bistriţa Aurie Valley by Pascal and Mititelu (1971) and Gergely and Raţiu (1973) within the Ciuc Basin. Asoltani L. (2008) mentions this association in Călimani Mountains (Suceava County).

In Mureș County, previous studies (Oroian, 1998; Sămărghițan, 2005) record this association in the valleys of Mureș and Gurghiu rivers and on their tributaries. The coenoses with *Scirpus sylvaticus* are widespread in the study area. The places for the conducted 24 phytosociological surveys were chosen to cover all the study area (**Fig. 1.**). In the research area these communities are fragmentarily distributed, on flat land, isolated and are not used as pastures or hayfields. The studied coenoses have a low conservative value and low fodder resource being grazed by animals only at an early stage. However, they are important as ecotones that provide shelter and feeding places for many invertebrates and small mammals, so these habitats must be protected.

2. Materials and Methods

In order to analyze the plant communities, vegetation was sampled and phytosociological surveys were elaborated using the standard Central European method (Braun-Blanquet, 1964, Cristea et al., 2004). The data have been gathered during 2015-2019 in 24 vegetation surveys (phytocoenologic relevées). For each survey the species composition was noted. In order to assess the dominance of species in the plant communities the Braun-Blanquet scale was used. The life forms, geoelements, and ecological indicators, as well as the name of the species were based on Sârbu et al. (2013). The affiliation to higher syntaxa was made according to Coldea (2012) and Mucina et al. (2016). In order to describe the Natura 2000 habitat type two scientific works were used: *Habitats in Romania* (Doniţă et al., 2005), and *A handbook for interpreting NATURA 2000 habitats in Romania* (Gafta and Mountford, 2008). The classification of threatened species in the study area is based on IUCN Red list (Biltz et al., 2011) and National Red List (Olteanu et al. 1994).

3. Results and discussions

In the studied area 24 phytosociological surveys were conducted, each on 100 m^2 surface (**Table 1.**). These studies show a high species diversity (98 species were identified). The phytosociological relevées were carried out on alluvial gleyic soils with moderately acidic pH from the hydrographic basins of the following rivers: Mureș, Gurghiu, Niraj and Târnava Mică. The land here is characterized by the stagnant water present almost all the year and the soil well soaked with water. The syntaxonomic scheme of vegetation is:

Cls. **Molinio-Arrhenatheretea R. Tx.1937**
Ord. Molinietalia caeruleae W.Koch 1926
All. Calthion palustris Tx. 1937
Ass. *Scirpetum sylvatici* Ralski 1931

The surveys were taken on the mountain belt with altitudes between 504 and 1310 m above sea level (asl). The climate records an average annual temperature between 7° C and -5° C and rainfalls between 650-1056 mm / year. The soils are alluvial, and rich in nutrients. The phytocoenoses have a good coverage (97-100%); the structure is stratified, the higher species form the upper level, which exceeds 1 m in height. In the floristic composition of the association, 98 taxa were identified. Along with the edifying and characteristic species *Scirpus sylvaticus* (**Fig. 2.**), numerous species belonging to the coenotaxons that subordinate the association have been identified. The species composition of this association in the study area is similar to those of the rest of Europe (Balátová-Tulácková 1987, Hájek et al., 2005, Hájková and Hájek, 2007, Malovcová-Staníková, 2009.

Fig. 2. *Scirpetum sylvatici* association on Mureș Gorge (photo Silvia Oroian)

Considering the affiliation to various coenotic groups a dominance of the species belonging to higher syntax can be noted. Thus, some species are characteristic to Calthion alliance, such as: *Caltha palustris*, *Chaerophyllum hirsutum*, *Crepis paludosa*, *Juncus articulatus*, *Myosotis scorpioides*, *Trifolium hybridum* etc. Species with high frequency were identified from the Filipendulion alliance (*Cirsium oleraceum*, *Epilobium parviflorum*, *E. hirsutum*, *Filipendula ulmaria*, *Geranium palustre*, *Lysimachia vulgaris*, *Lythrum salicaria*, *Mentha longifolia*, and *Valeriana officinalis*) and the Molinietalia and Molinion alliance (*Cirsium erisithales*, *Cirsium palustre*, *Dactylorhiza majalis*, *Galium uliginosum*, *Juncus effusus*, *Lychnis flos-cuculi*, *Lycopus europaeus* etc.). Beside the characteristic species of Molinio-Arrhenatheretea class, in the studied communities the following taxa were present: *Achillea millefolium*, *Agrostis capillaris*, *Alopecurus pratensis*, *Holcus lanatus*, *Potentilla reptans*, *Prunella vulgaris*, *Stellaria graminea* etc. In the floristic composition of the association some rare and threatened plants were identified such as *Angelica archangelica* (LC) and *Dactylorhiza majalis* (LC). Their presences give a higher conservative value for the habitat.

In the coenoses with higher anthropic pressure the presence of *Erigeron annuus* ssp. *annuus*, an alien invasive species was noticed.

For the association the analysis of geoelements and bioforms were made. The Eurasian element was dominant (51.55%), followed by the Circumboreal which was well represented in the studied communities (20.62%). The European elements were in a proportion of 12.97%. This distribution shows the Central-European origin of *Calthion* vegetation with the corresponding habitat conditions (climate, relief). There is a distinct regional peculiarity because of the presence of Alpine-Carpathian, Carpathian and Carpathian-Balkan elements (4.12%) (**Fig. 3.**).

According to the analysis of bioforms (**Fig. 4.**), the hemicryptophytes prevail (75.52%), followed by geophytes (10.20%) and helophytes (4.08%). The chamaephytes represent 3.06% of the bioforms while the phanaerophytes 2.01%, due to closeness with the forest belt. The percentage of therophytes was comparatively high (5.10%), which is not typical for this kind of vegetation.

The stationary conditions in which this association develops favor a large number of light-loving species that grow generally in well-lit places, but also occurring in partial shade (L_7-48.45%), alongside with some light-loving species (L_8-21.65%). The thermal regime is favorable for the development of the mesothermophilous species (21.65%) and euriterms (55.67%). The Ellenberg indices were used to reveal the necessary moist conditions for the species within the association. Thus meso-hygrophilic species with preference for moderately wet soils (U_6=20.62%) and those which prefer wetter soils (U_7=17.53%, U_8=15.46%) are dominant. In terms of preference to soil reaction most species of this association are eurionic (56.7%) or indicator of moderate acidity, occurring rarely in strongly acidic or in neutral to alkaline soil conditions (19.59%). They occur in nutrient-poor sites more frequently than in sites with moderate nutrient supply and exceptionally in sites with rich nutrients (N_3-15.46%) (**Fig. 5.**).

The phytocoenoses analyzed in this study belong to Natura 2000 Habitat 6430 Hydrophilous tall-herb fringe communities of plains in the montane to alpine levels. This habitat is wide spread in the area; the herb layer is usually tall and the cover is highly variable depending on the mechanical action of water and level fluctuations.

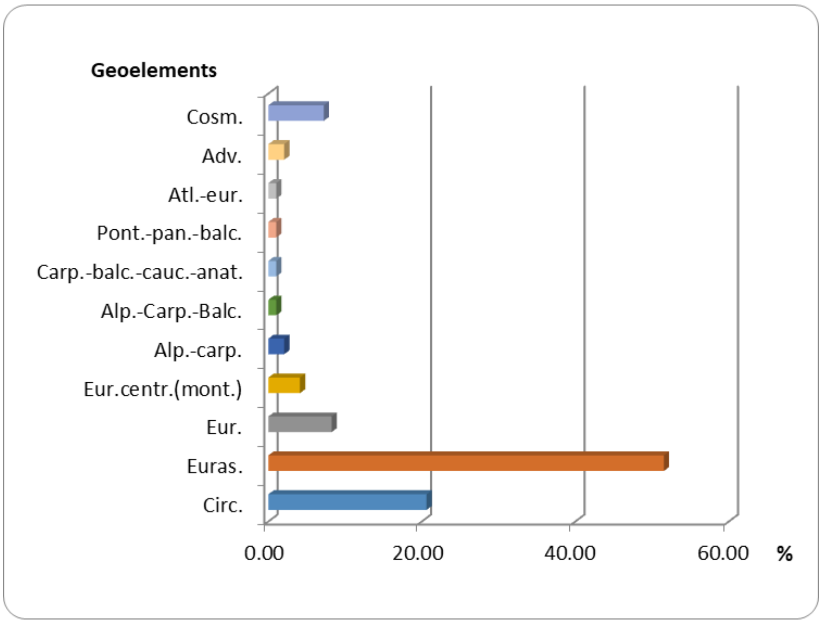

Fig. 3. Geoelements of *Scirpetum sylvatici* association

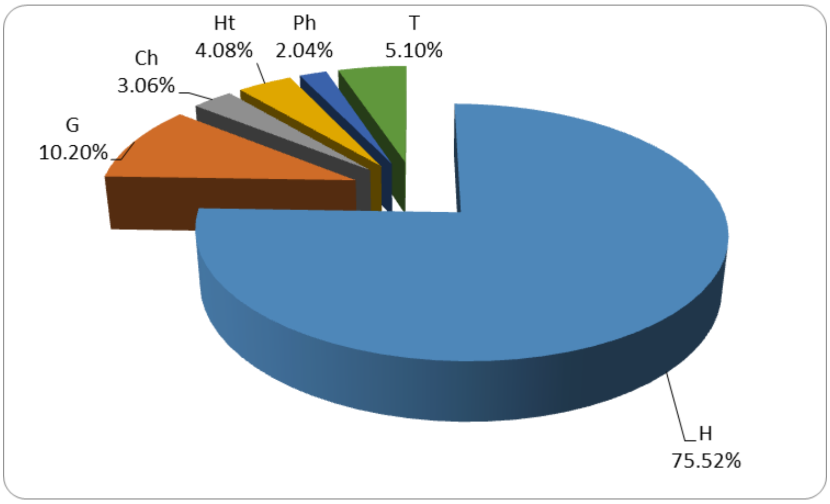

Fig. 4. Bioforms spectrum of *Scirpetum sylvatici* association

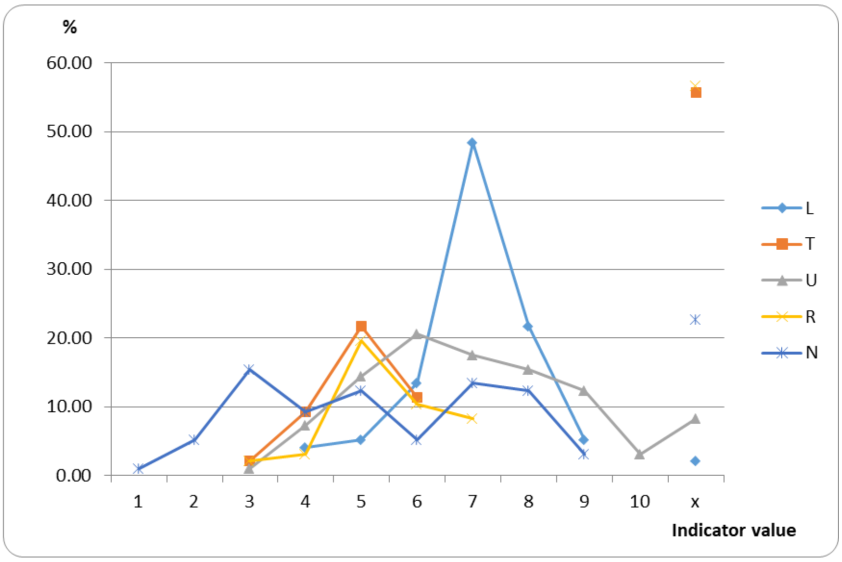

Fig. 5. Ecological indices of *Scirpetum sylvatici* association

The anthropogenic threats are numerous, generally affecting the biodiversity. The main pressures/threats estimated from the field study refer to forest management and use. In the Călimani-Gurghiului mountains was noticed the transport of the exploited wood with trucks or heavy equipment. These affect the banks of the Mureş tributaries, where these phytocenoses occur, leading to the damages on large areas of the habitat. It also causes the settlement of the wet soil, favoring the erosion processes that in time create free spaces on the ground for the installation of weeds and invasive alien species. Also moist soil settlements might affect the underground part (rhizomes) of some plants that ensure their perennial and vegetative reproduction, thus leading in time to the reduction of the population. Therefore, it is recommended to supervise the activity of wood exploitation by the companies involved. Drought and reduced rainfall in recent years have led to the reduction of phytocenoses dominated by *Scirpus sylvaticus*.

Regarding the conservation status, it can be mentioned that the habitat is in very good (45.84% of the surveys carried out) or good conservation condition (45.84% of the surveys carried out) and only in 8.34% of the surveys, the conservation status of the habitat is satisfactory. As such, the following short term impact of risk factors can be mentioned: the restriction of characteristic plant communities in favor of ruderal ones, and changes in the structure and composition of the vegetation due to the proliferation of invasive species. Therefore, permanent monitoring is required to obtain information on the evolution trends of the habitat in order to preserve the habitat conservation status.

Conclusions

In the study area, the hygrophilous plant communities dominated by *Scirpus sylvaticus* were included in the association *Scirpetum sylvatici* Ralski 1931. These stands belong to Natura 2000 Habitat 6430 Hydrophilous tall-herb fringe communities of plains in the montane to alpine levels. The 24 phytosociological surveys highlight the biodiversity of these communities that comprise 98 plant species. The floristic composition of the association is similar with those described in Europe. The diagnostic and characteristic species of association are present and have a good coverage up to 100%. In these phytocoenoses some threatened species were identified such as *Angelica archangelica* (LC) and *Dactylorhiza majalis* (LC). In the study area these phytocoenoses are mostly in a good and very good state of conservation and they can be considered frequent in the area.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Asoltani L. (2008) Contributions to the study of paludal vegetation from the Neagra Șarului river's basin (Suceava county), Analele ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi, Tomul LIV, fasc. 1, s. II a. Biologie vegetală, Iași
2. Balátová-Tulácková E, (1987) Beitrag zur Kenntnis der Feuchtwiesen des Gebirges Hostynské vrchy. Tuexenia 7:199–213 http://publikationen.ub.uni-frankfurt.de/files/38065/balatovatulackova_1987_hostynskevrchy.pdf

3. Bilz M, Kell SP, Maxted N, Lansdown RV (2011) European Red List of Vascular Plants. Luxembourg: Publications Office of the European Union
4. Braun-Blanquet J (1964) Plant sociology. The study of plant communities. Ed.3. Wien-New York: Springer Publishing
5. Coldea Gh (ed.) (2012) Les association végétales de Roumanie, Tome 2 Les associations anthropogènes, Edit. Presa Universitară Clujeană, Cluj Napoca
6. Cristea V, Gafta D, Pedrotti F (2004) Fitosociologie. Edit. Presa Universitară Clujeană, Cluj-Napoca
7. Doniţă N, Popescu A, Paucă-Comănescu M, Mihăilescu S, Biriş I.A (2005) Habitatele din România, Ed. Tehnică Silvică, Bucureşti
8. Gafta D, Mountford O (coord.) (2008) Manual de interpretare a habitatelor Natura 2000 din România, Ed. Risoprint, Cluj-Napoca
9. Gergely I, Raţiu F (1973) Vegetaţia mlaştinilor eutrofe din bazinul superior al Ciucului, Contrib. Bot. Cluj:143–165.
10. Hájek M, Tzonev R T, Hájková P, Ganeva AS, Apostolova I I (2005) Plant communities of subalpine mires and springs in the Vitosha Mt. Phytologia Balcanica, 11:193–205.
11. Hájková P, Hájek M (2007): TDF08 *Scirpetum sylvatici* Ralski 1931. In: Chytrý M. (ed.), Vegetace České republiky. 1. Travinná a keříčková vegetace [Vegetation of the Czech Republic. 1. Grassland and Heathland Vegetation]. Academia, Praha, pp. 264-266.
12. Kulik M, Baryła R, Warda M, Stamirowska-Krzaczek E. (2016) Vegetation changes of the Molinio-Arrhenatheretea class in the Bystra valley, eastern Poland. Acta Agrobot, 69(4):1689. http://dx.doi.org/10.5586/ aa.1689.
13. Malovcová-Staníková M (2009) Communities *Scirpetum sylvatici* and *Epilobio palustri-Juncetum effusi* in the Borská nížina Lowland, Bull. Slov. Bot. Spoločn., Bratislava, 31(2):35–39.
14. Mucina L, Bültmann H, Dierßen K, Theurillat J P, Raus T, Čarni A, Šumberová K, Willner W, Dengler J, García RG, Chytrý M, Hájek M, Di Pietro R, Iakushenko D, Jens Pallas J, Daniëls FJA, Bergmeier E, Santos Guerra A, Ermakov N, Valachovič M, Joop H.J. Schaminée JHJ, Lysenko T, Didukh YP, Pignatti S, Rodwell JS, Capelo J, Weber HE, Solomeshch A, Dimopoulos P, Aguiar C, Hennekens SM, Tichý L (2016) Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen and algal communities. Applied Vegetation Science 19, Suppl. 1:3–264.
15. Oltean M, Negrean G, Popescu A, Roman N, Dihoru G, Sanda V, Mihăilescu S (1994) Listă roşie a plantelor superioare din România, Studii, sinteze, documentaţii de ecologie, 1, Acad.Română, Instit. de Biologie, Bucureşti
16. Oroian S (1998) Flora şi Vegetaţia Defileului Mureşului între Topliţa şi Deda, Casa de Ed. Mureş
17. Pascal P, Mititelu D (1971) Contribuţie la studiul vegetaţiei din bazinul Bistriţei Aurii (Jud. Suceava), Comunic Şti. Inst. Pedagog., Iaşi, pp. 331–363.
18. Sămărghiţan M (2005) Flora şi vegetaţia Văii Gurghiului, Ed. University Press, Târgu-Mureş
19. Sârbu I, Ştefan N, Oprea A, (2013) Plante vasculare din România, determinator ilustrat de teren. Ed. "Victor B. Victor", Bucureşti

Table 1. *Scirpetum sylvatici* Ralski 1931 association from Gurghiului and Călimani Mountains (Romania)

Relevées	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	
Altitude (m asl)	748	748	822	1106	937	1230	986	1054	1019	1123	1191	984	943	1310	1063	991	1139	1195	991	971	1111	1031	504	769	
																									K
Car.ass																									
Scirpus sylvaticus	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	V
Calthion																									
Caltha palustris	-	-	-	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	IV
Chaerophyllum hirsutum	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	-	+	II
Cirsium rivulare	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Crepis paludosa	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	II
Juncus articulatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	I
Myosotis scorpioides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	V
Trifolium hybridum	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	+	-	-	-	+	+	-	-	+	III
Filipendulion																									
Epilobium parviflorum	-	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	II
Epilobium hirsutum	-	-	-	+	-	-	+	-	-	+	-	+	-	+	+	+	+	+	+	+	-	+	-	-	III
Filipendula ulmaria	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	V
Geranium palustre	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	I
Lysimachia vulgaris	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	+	+	III
Lythrum salicaria	-	+	+	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	-	+	+	-	+	III

Mentha longifolia	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	IV
Valeriana officinalis	+	-	-	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	II
Molinietalia (inc. Molinion)																									
Cirsium canum	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	I
Cirsium palustre	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	I
Deschampsia cespitosa	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	IV
Equisetum palustre	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	-	+	II
Galium uliginosum	+	-	+	-	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	+	+	+	III
Juncus conglomeratus	+	-	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-	-	+	+	-	+	-	III
Juncus effusus	-	+	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	+	IV
Lychnis flos-cuculi	+	-	-	+	-	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-	+	+	-	+	III
Lycopus europaeus	+	+	+	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	-	II
Stachys officinalis	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	I
Symphytum officinale	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	II
Molinio-Arrhenatheretea																									
Achillea millefolium	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	I
Agrostis capillaris	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Alopecurus pratensis	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	I
Carex hirta	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	+	-	+	+	-	-	-	II
Cerastium holosteoides	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	II
Euphrasia rostkoviana	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	I
Festuca pratensis	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Holcus lanatus	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	+	-	-	-	+	II

Lathyrus pratensis	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Lysimachia nummularia	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-	-	-	+	IV
Poa trivialis	+	-	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	II
Prunella vulgaris	+	-	-	+	-	+	+	-	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	IV
Ranunculus repens	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	II
Stellaria graminea	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	II
Trifolium pratense	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	I
Epilobium palustre	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	I
Scutellaria galericulata	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	I
Variae Syntaxa																									
Agrostis stolonifera	-	-	-	-	-	+	-	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	-	+	III
Campanula patula	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	II
Carex distans	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Carex leporina	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	I
Carex rostrata	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	I
Carex vulpina	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	II
Centaurea phrygia	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	I
Dactylis glomerata	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	+	-	+	-	+	-	II
Eleocharis palustris	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	I
Equisetum arvense	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	I
Erigeron annuus	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	II
Galium aparine	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	I
Hypericum maculatum	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	+	+	-	-	-	II
Impatiens noli-tangere	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	II
Juncus inflexus	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Leucanthemum	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	I

vulgare																								
Mentha arvensis	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	I
Poa palustris	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	I
Potentilla erecta	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	I
Salix cinerea	+	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	-	II
Stachys sylvatica	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-	-	-	I
Stellaria nemorum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	II
Trifolium repens	+	-	+	-	-	-	+	-	+	-	+	+	+	+	-	+	+	+	-	-	+	-	+	-	III
Urtica dioica	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	I

Species present in one relevé: *Polygonum bistorta* (2); *Potentilla reptans* (2); *Alnus glutinosa* (3); *Dactylorhiza majalis* (4); *Galium palustre* (5); *Plantago lanceolata* (6); *Poa pratensis* ssp. *angustifolia* (7); *Carex pallescens* (7); *Cirsium waldsteinii* (8); *Alchemilla vulgaris agg.* (10); *Cynosurus cristatus* (10); *Petasites hybridus* (10); *Rhinanthus rumelicus* (10); *Angelica archangelica* (11); *Aconitum napellus ssp. tauricum* (11); *Chamerion angustifolium* (11); *Doronicum austriacum* (11); *Lotus corniculatus* (11); *Telekia speciosa* (11); *Cardamine pratensis* (15); *Veratrum album* (18); *Heracleum spondylium* (18); *Cirsium erisithales* (18); *Achillea distans* (20); *Mentha × verticillata* (21); *Cirsium oleraceum* (21); *Ranunculus acris* (22); *Calystegia sepium* (23);*Veronica officinalis* (23); *Eupatorium cannabinum* (24).

Place and date of relevé: R1 - Ciobotani-Mermezeu, 06.15.2015; **R2** - Câmpul Cetății - Pârâul Cald, 06.20.2015; **R3** - Câmpul Cetății – Nirajul Mic, 06.21.2015; **R4** – Lăpușna - Gurghiu (Pârâul Mocirlos), 07.05.2016; **R5** - Lăpușna - Gurghiu (Pârâul Mijlociu), 07.05.2016; **R6** – Lăpușna - Pârâul Secuieu, 07.07.2016; **R7** - Lăpușna – Pârâul Negru, 07.07.2016; **R8** – Ibănești -Șirodul Mare, 07.10.2016; **R9** - Ibănești -Șirodul Mic, 07.10.2016; **R10** – Sovata –Sebeș, 08.04.2017; **R11** – Sovata, 08.06.2017; **R12** – Lunca Bradului – Valea Ilva, 08.11.2018; **R13** - Lunca Bradului – Valea Ilva Mare 08.11.2018; **R14** – Stânceni – Gudea Mare, 08.12.2018; **R15** – Stânceni – Gudea Mică, 08.12.2018; **R16** – Răstolița – Valea Rusu, 08.13.2018; **R17** – Sălard – Gropușoara Mică, 08.16.2018; **R18** - Sălard – Hidegag, 08.16.2018; **R19** – Sălard - Țâba Mică, 08.18.2018; **R20** – Neagra – Pârâul Neagra, 08.23.2019; **R21** – Ibănești – Fâncel, 08.26.2019; **R22** – Ciobotani, 08.29.2019; **R23** –Răstolița, 06.09.2019; **R24** – Meștera, 08.20.2019.

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