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# Classification and diversity of perennial sand-dune vegetation in Serbia

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**Abstract:** Sand dunes are one of the critically endangered habitats in Europe. This vegetation type is under a strong anthropogenic influence. Its natural value is very high since many plant species with a narrow distribution are found there. The classification of Serbian sand-dune vegetation and the status of the described associations has not been validated by numerical analyses. This research presents an evaluation of sand-dune vegetation in Serbia on the basis of literature data, as well as new relevés. Psammophytic vegetation in Serbia was classified into two alliances, annual and ruderal *Bassio laniflorae-Bromion tectori* and perennial *Festucion vaginate*. The aim of this research was the classification of the alliance *Festucion vaginatae,* perennial, drought-tolerant, tussock-forming vegetation on sand. As proposed by OptimClass, Jaccard distance and flexible beta data (β=-0.25) were used for the classification. Classification results were confirmed by detrended correspondence analysis (DCA) ordination. The result of the classification established that only two associations, *Alysso gmelini-Festucetum vaginatae* and *Festucetum vaginatae*, are present in Serbia. These associations are divided into groups that mostly correspond to traditional subassociations.

**Keywords:** sandy vegetation; *Festucion vaginate;* PC-ORD; DCA; vegetation classification

## INTRODUCTION

Vegetation on sand is a unique phenomenon in all stages of its successional development, from pioneer stadia to closed grasslands. Sandy soils are mostly alluvial deposits that are further shaped by aeolian erosion. Sandy substrates can vary in the dimensions of sandy granulations, and soil reactions, but they are always nutrient poor, dry and with extreme variations of temperature. All continental sandy habitats in Europe are strictly protected by Annex I of the European Habitats Directive and represent a significant Natura 2000 habitat type (Council Directive 92/43/EEC). Continental sand vegetation requires intense study and active protection programs that will ensure its survival [1].

In Serbia, sand dunes are distributed along the Danube River. In general, the sandy habitats of Serbia appear in three distinct areas. They include the Subotica-Horgoš Sands, the Deliblato Sands, two protected natural areas, and the third group of sands on the right bank of the Danube river in eastern Serbia (from Ram to Kladovo, and further to the east, along the Danube until its exit from Serbia [2] (Supplementary Fig. S1). All sand dunes in Serbia are characterized by different microclimatic, hydrological and pedological conditions. In addition, geomorphological forms (dunes) are not identical in all sandy habitats and the vegetation of sandy habitats also differs [2].

In Serbia, only basophilic carbonate sands are present. The vegetation in these habitats and surrounding area is most often syntaxonomically classified in one class – *Festucetea vaginatae* Soó 1968 [3-6], while in recent overviews [7,8] this vegetation has been classified within the class *Koelerio glaucae-Corynephoretea canescentis* Klika in Klika et Novák 1941, order *Festucetalia vaginatae* Soó 1968 or *Festuco-Sedetalia acris* Tx. 1951, and alliance *Festucion vaginatae* Soó 1929. This classification correlates with the most recent reviews of the vegetation of the surrounding area and Europe [9,10].

Except for the alliance *Festucion vaginatae* Soó 1929, several authors have pointed to the existence of initial

vegetation on sands belonging to the alliance *Bassio laniflorae-Bromion tectorum* [11,12,5] with the association *Brometum tectorum* Soó 1938. This vegetation type predominantly consists of therophytes and annuals, and the average plant cover in this open community is very low (up to 50%) [5]. This vegetation type implies an initial stage, but also different degradation stages when sand surfaces reopen, most often under the influence of excessive grazing or exploitation of the sand mass.

The central aim of this study was to provide a detailed classification and overview of grasslands dominated by drought-tolerant, tussock-forming perennial grasses such as *Festuca vaginata.*

## MATERIALS AND METHODS

### Dataset

In the process of data selection and sandy vegetation classification, our data set was compared with relevés from the entire Pannonian plain. In this comparison it was confirmed that psammophytic vegetation in Serbia can be divided into two alliances – *Bassio laniflorae-Bromion tectorum* and *Festucion vaginatae*. The pioneer association *Corispermo nitidi-Polygonetum arenariae* on sands was described in Serbia and classified within the alliance *Festucion vaginatae* [12]. However, this communitiy is heterogeneous and during selection of data its relevés were divided between two alliances – *Festucion vaginatae* and *Bassio laniflorae-Bromion tectorum*. Relevés of this association with a low plant cover and a large share of therophytes/annuals were not taken into consideration. This initial community on sandy habitats, dominated by *Corispermum nitidum*, is described by Stjepanović-Veseličić [12], and is represented by a small number of relevés in the dataset (a total of 11). The author [12] considers it as non-homogeneous association, with a significant ratio of steppe and ruderal species or taxa of other successive vegetation types. Soó [14] in the review of psammophytic communities in the Pannonian-Carpathian basin, identified the autumn aspect of the community *Corispermo nitidi-Polygonetum arenariae* as a *Brometum tectorum* Soó 1938 – geographical race *deliblaticum*. According to this author [14], this community actually belong to a subassociation of *cynodontetosum* of the association *Brometum tectorum*. On the other hand, some relevés have floristic similarities with other *Festucion vaginatae* communities. Also, in selecting the perennial sandy grasslands *Festucion vaginatae* for this research, the lectotype [5] of association *Corispermo nitidi-Polygonetum arenariae* remained within *Festucion vaginatae* Thus, 5 relevés of this association were retained for further classification.

In this research, literature data as well as vegetation relevés collected in the period 2012-2018 were used. The total number of analyzed relevés was 130 from different sources [11-13,15] and 106 are new phytocoenological relevés. The entire data set contained 225 taxa. The taxonomy and nomenclature of plants were checked and harmonized following [16] for vascular plants, [17], for lichens and [18,19] for mosses. Taxa with inconsistent or frequently doubtful determination were combined into aggregates (Supplementary Table S1).

### Additional data

Borhidi indicator values (BIV) for light, soil moisture, temperature, soil reaction and continentality were used for ecological interpretation of the vegetation patterns [20]. For each relevé, we calculated the unweighted mean of species indicator values using JUICE software [21], which gives a rough approximation of the local site conditions.

### Classification

The classification method and the optimal number of clusters were selected using the OptimClass method [22]. There were 20 different possible classification options tested in a combination of different transformation of data (presence/absence, sqrt, log, ordination scale cut levels: 0, 5, 25), indices for distance measurements (Sørensen/Bray-Curtis, relative Sørensen, Jaccard, Euclidean and relative Euclidean), and different group-linkage methods (Flexible beta, β=-0.25 and Ward's method). The OptimClass test showed that the use of Jaccard distance and flexible beta (where β=-0.25) for the classification was optimal. Cover values by Braun-Blanquet [23] were transformed to percentages. For further transformation of data, an ordination scale with cut levels 0, 5 and 25 was selected as the optimal method. The optimum number of clusters according to the OptimClass test was 10. Hierarchical classification analysis was performed in PC-ORD

5.0. For each group, diagnostic species were defined according to the values of the phi coefficient (fidelity measure) ≥0.20 [24]. Species with a frequency ≥20% were defined as constant. Species with cover value ≥25% were defined as dominant. The classification allowed for nomenclature validation and revision of the associations and subassociations within the alliance *Festucion vaginatae* in accordance with the International Code of Phytosociological Nomenclature [25].

### Ordination and ecological analyses of data

In order to explain the floristic differentiation within Serbian sand dunes and to explore the possibilities of separation of vegetation at the association level, the relationship between the ordination of relevés and bioindicator values was analyzed. According to the longest gradient length (6.4 SD), as an adequate technique for ordination of data DCA was selected [26]. DCA was performed using the R (vegan) package used with JUICE software [21]. Differences of established groups and environmental conditions were also shown on the box-whiskers plots using BIV [20].

## RESULTS

An optimal number of clusters was selected using the OptimClass method. Hierarchical classification is presented on the dendrogram in Fig. 1, where two hierarchical groups, associations (A and B), are clearly separated. The associations are further divided into clusters, which mainly correspond to traditional subassociations. Descriptions of groups 1-10 are given in Supplementary Table S2. Perennial sandy vegetation in Serbia is classified into two associations and subassociations (Table 1). The results of the hierarchical classification in this research is presented in Table 1 and Supplementary Table S2.

Group A represents the relevés of the association *Alysso gmelini-Festucetum vaginatae* Stjepanović-Veselič ić 1956. This association was first described on the Deliblato Sands as *Festucetum vaginatae* in the geographical variant *deliblaticum*. The same author in 1956 [11] described the *Alysso gmelini-Festucetum vaginatae* on sands in eastern Serbia, in two geographical variants, *kladovense* and *ramo-požežense*. Stjepanović-Veselič ić [11] mentioned the possibility

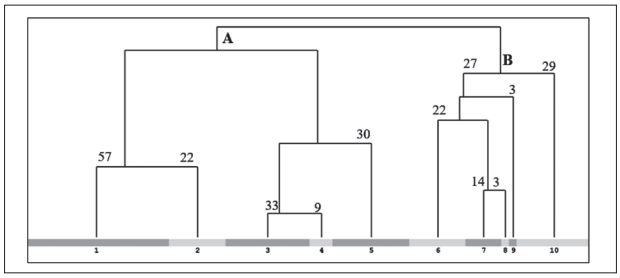


**Fig. 1.** Dendrogram of the hierarchical classification. **A** – association *Alysso gmelini-Festucetum vaginatae* Stjepanović-Veselič ić 1956; **B** – association *Festucetum vaginatae* (Rapaics ex Soó 1929) Borhidi 1996). The number of relevés in the group is presented above each branch. Group 1-10 descriptions are given in Supplementary Table S2.

that all communities of sandy vegetation in southeastern Vojvodina and eastern Serbia are actually one association, and this view was accepted by [7]. Numerical classification in this research confirmed the floristic connection of the communities on the Deliblato Sands and the sands from Ram to Kladovo. These communities are unique and classified as one association (Supplementary Fig. S2A).

Diagnostic species are as follows: *Artemisia campestris, Carex liparocarpos, Centaurea arenaria, Corispermum nitidum, Echinops ritro* subsp. *ruthenicus, Festuca wagneri, Odontarrhena tortuosa, Peucedanum arenarium, Polygonum arenarium, Sedum urvillei, Stipa borysthenica, Syntrichia ruralis, Tragopogon floccosus.*

Constant species are as follows: *Alyssum gmelinii, Artemisia campestris, Centaurea arenaria, Euphorbia seguieriana, Festuca vaginata, Koeleria glauca, Odontarrhena tortuosa, Polygonum arenarium, Stipa borysthenica.*

Dominant species are as follows: *Alyssum gmelinii, Artemisia campestris, Carex liparocarpos, Euphorbia seguieriana, Festuca vaginata, Fumana procumbens, Minuartia glomerata, Odontarrhena tortuosa, Polygonum arenarium, Silene otites, Sedum urvillei, Stipa borysthenica, Syntrichia ruralis, Tortella tortuosa.*

Group B represents the association *Festucetum vaginatae* (Rapaics ex Soó 1929) Borhidi 1996 (Supplementary Fig. S2B). The data in this cluster are from the Subotica-Horgoš Sands, published by Gajić et al. [15] and Parabućski et al. [11]. Gajić [15] described

**Table 1.** Syntaxonomic scheme of sandy vegetation in Serbia.

| | | | |
|---|---|---|---|
| **Class** | COR *Koelerio glaucae-Corynephoretea canescentis* Klika in Klika et Novák 1941 | | |
| **Order** | COR01 *Festucetalia vaginatae* Soó 1968 | | |
| **Alliances** | COR01A *Bassio laniflorae-Bromion tectorum* (Soó 1957) Borhidi 1996 | COR01B *Festucion vaginatae* Soó 1929 | |
| **Associations** | COR01A.1 *Brometum tectorum* Soó 1938 | COR01B.1 *Alysso gmelini-Festucetum vaginatae* Stjepanović-Veseličić 1956 (syn. *Festucetum vaginatae deliblaticum* Stjepanović-Veseličić 1953) | COR1B.2 *Festucetum vaginatae* (Rapaics ex Soó 1929) Borhidi 1996. (syn. *Festucetum vaginatae danubiale* Soó 1929; *Festucetum vaginatae mixtum* Gajić 1986) |
| **Subassociations** | | 1- subass. *stipetosum borysthenicae* (Stjepanović-Veseličić 1953) Butorac&Panjković 2013 nom. mut. prop. (syn.: stipetosum joannis Stjepanović-Veseličić 1953; stipetosum capillatae Stjepanović-Veseličić 1956)<br>2- Typus: Stjepanović-Veseličić 1953, Tab. 2, rel. 35, p:24/25 – lectotypus hoc locosubass. *sytrichietosum ruralis* subass. nov. hoc loco (syn.: *muscetosum*, Stjepanović-Veseličić 1979 nom. inval. [Art. 5, 8]). *Typus*: Stjepanović-Veseličić 1979, Tab. 2, rel. 25, p:40/41 – *holototypus hoc loco*<br>3- subass. *typicum*<br>4- subass. *fumanetosum procumbenti* Stjepanović-Veseličić 1953 Butorac&Panjković 2013 nom. corr. *hoc loco* Typus: Stjepanović-Veseličić 1953, tab. 2, rel. 4, p:24/25 – *lectotypus hoc loco* (initial group with relevés from *Corispermo nitidi-Polygonetum arenariae)* | 1- subass. *stipetosum borysthenicae* Soó 1957 nom. mut. prop. [Art. 45]); (syn.: *stipetosum joannis* Soó 1929 subass. *muscetosum* Gajić 1986; including subass. *fumanetosum* (Magyar 1933) Soó 1939 sensu Gajić 1986). Typus: Gajić 1986, Tab. 8, rel., 23, p:336 – *lectotypus hoc loco*<br>2- subass. typicum (syn. *festucetosum vaginatae* (Magyar 1933) Soó 1955 *typicum* Gajić 1986; including subass. *calamagrostietosum* Soó 1955 sensu Gajić 1986)<br>3- subass. *salicetosum rosmarinifoliae* Hagitai 1940. Typus: Gajić 1986, Tab. 8, rel., 30, p:336 – *lectotypus hoc loco*<br>4- relevés by Parabućski |

this association as a mixture of different successive stands and named it *Festucetum vaginatae mixtum* Gajic 1986. Parabućski et al. [13] noted that this association is the same as that in Hungary, *Fesucetum vaginatae danubiale* Soó 1929, which is an ecological and geographical variant of sand vegetation between the Tisza and Danube rivers [14].

Diagnostic species are as follows: *Alkanna tinctoria, Alyssum alyssoides, Apera spica-venti, Arenaria serpyllifolia, Calamagrostis epigejos, Camelina sativa* aggr.*, Cladonia foliacea, Cynoglossum officinale, Dianthus pontederae, Equisetum ramosissimum, Eryngium campestre, Erysimum canum, Erysimum diffusum, Koeleria macrantha, Melica transsilvanica, Noccaea perfoliata, Salix repens* subsp. *rosmarinifolia, Scirpoides holoschoenus, Secale sylvestre, Silene conica, Stipa pennata, Tragopogon pratensis, Viola arvensis.*

Constant species are as follows: *Achillea millefolium* aggr.*, Arenaria serpyllifolia, Asperula cynanchica, Calamagrostis epigejos, Centaurea stoebe* s.l.*, Eryngium campestre, Euphorbia seguieriana, Festuca vaginata, Koeleria glauca, Potentilla incana, Salix repens* subsp. *rosmarinifolia, Silene otites, Stipa borysthenica, Verbascum lychnitis, Tortella tortuosa.*

Dominant species are as follows: *Cladonia foliacea, Elytrigia repens, Euphorbia seguieriana, Festuca vaginata, Fumana procumbens, Koeleria glauca, Melica transsilvanica, Minuartia verna, Phleum phleoides, Potentilla incana, Salix repens* subsp. *rosmarinifolia, Secale sylvestre, Stipa borysthenica, Stipa pennata.*

## Ordination and ecological analyses of data

The results of the ordination analyses follow the results of the classification analysis, which are shown in Fig. 2. Two main groups of cluster analysis are recognized. The pattern of floristic differentiation between two associations reflect the environmental and floristic differences (Fig. 3).

The position of clusters 5 and 9 (Fig. 2) is not clear. Cluster 5 is a group of relevés with a high percentage of widespread species such as *Cynodon dactylon*, *Botriochloa ischaemum*, *Medicago minima*, *Tragus racemosus* and *Setaria viridis* occurring in degraded sandy habitats

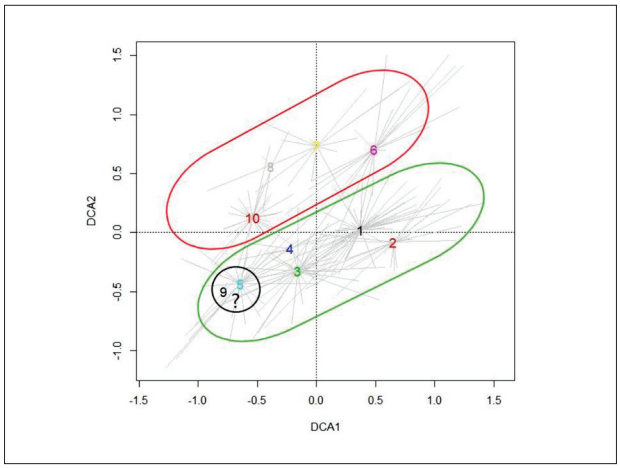


**Fig. 2.** Ordination (DCA) of two associations of alliance *Festucion vaginatae* Soó 1929 in Serbia. **Green** (or **A** in the classification results) – sandy vegetation on Deliblato Sands and sandy habitat from Ram to Kladovo; **red** (or **B** in the classification results) – sandy vegetation on Subotica-Horgoš Sands; **black** – subass. *cladonietosum* (9) and *Tribulo-Tragetum racemosi* (5).

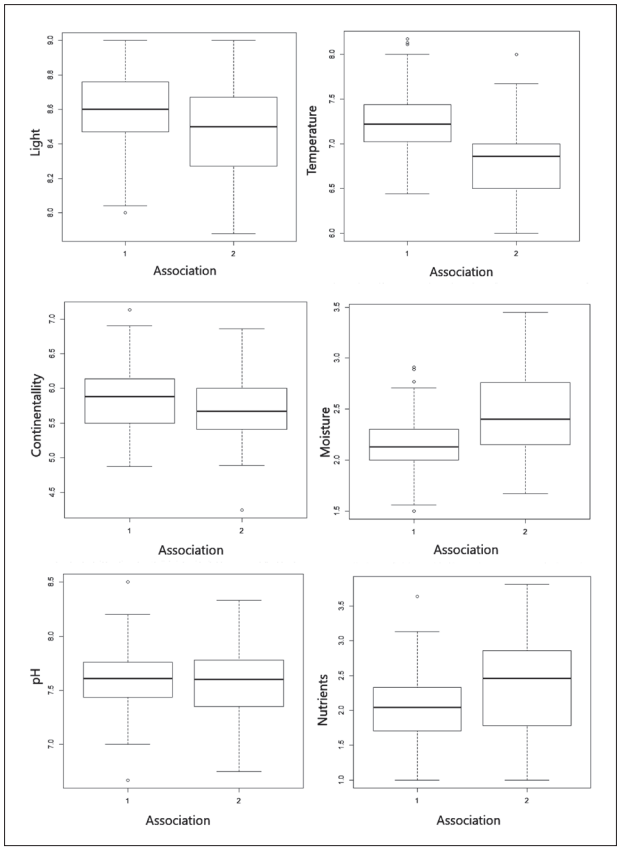


**Fig. 3.** Analysis of average bioindicator values of each relevé in two associations of sandy vegetation of Serbia (for light, temperature, continuity, humidity and amount of nutrients in the substrate, as well as pH substrates): **1** – Ass. *Alysso gmelini-Festucetum vaginatae*; **2** – Ass. *Festucetum vaginate.*

and can be identified as a transitional group to the association *Tribulo-Tragetum racemosi* Soó & Timár in Timár [27,28]; it is obvious that this can appear in all sand dunes in Serbia. Cluster 9 represents three relevés dominated by *Cladonia foliacea*. These relevés were observed only on the Subotica-Horgoš Sands. In the relevés there are only one or two species. They are defined as a special subassociation of *cladonietosum* Gajić 1986. These two problematic groups together contain 15 relevés and were removed from further analysis.

## DISCUSSION

The result of the classification shows that within the alliance *Festucion vaginate*, only two associations were present: *Alysso gmelini-Festucetum vaginatae* and *Festucetum vaginatae*. Relevés originally attributed to the more developed stages of the pioneer association of sandy habitats (*Corispermo nitidi-Polygonetum arenariae*) were not classified as a separate association but were unified with the initial stages of the community *Alysso gmelini-Festucetum vaginatae*.

A species that defines the contested community, *Corispermum nitidum*, has a very limited distribution on the Deliblato Sands, since the areas of semi-open and open sands are reduced to small local areas. These stands always appeared after fires [29] but were quickly replaced by the successive stages of vegetation, i.e. communities dominated by *Festuca vaginata*. According to field observations, the classification is justified.

Comparison of phytosociological data from three different sandy areas in Serbia with non-metrical multidimensional scaling (NMDS), showed that the floristic composition of sandy vegetation on the Deliblato Sands and sandy areas from eastern Serbia, from Ram to Kladovo, made one association, which is different from the sandy association of the Subotica-Horgoš Sands. Box-whiskers plots (Fig. 3) for each of the ecological indicators showed that the association *Alysso gmelini-Festucetum vaginatae* prefers open and dry habitats. According to all parameters, the community from the Subotica-Horgoš Sands, *Festucetum vaginatae*, occurs in more closed, moister and richer habitats. Considering the specificity of the high dunes on the Deliblato Sands, the results of the ecological analysis

of the classification groups confirmed the previously proposed explanations of differentiation of Serbian sandy vegetation [2,5,12,31].

Differentiation between two sandy associations of the alliance *Festucion vaginatae* is reflected in their floristic composition and different microecological conditions. Subotica-Horgoš sand dunes are 137 m high [15] and the groundwater is close to the surface. These characteristics have a greater resemblance to the sands of southern Hungary than to the sandy habitats of Deliblato and Ram-Kladovo.

In order to compare the sandy associations in Serbia and their equivalents in neighboring Hungary, the floristic compositions of these three groups of relevés were analyzed. We compared 221 relevés from Serbia (initial dataset, without outlaying groups 5 and 9) and 209 relevés from Hungary. The similarity of the community *Festucetum vaginatae* on the Subotica-Horgoš Sands to Hungarian sands' vegetation is clear and logical in view of the geographical proximity [11-13,15,31-33].

If we compare the floristic inventory on all three sandy areas in Serbia and Hungary, we can find the following only on Deliblato and Ram-Kladovo sands: *Peucedanum arenarium, Astragalus dasyanthus, Festuca wagneri, Silene subconica, Echinops banaticus* (the species *Echinops ritro* subsp. *ruthenicus* [34]). *Onosma viridis* and *O. pseudoarenaria*, which were not found on the both analyzed localities, but were recorded in the relevés of these sandy habitats.

The species characteristic for the Subotica-Horgoš perennial sandy grassland are: *Stipa pennata, Scirpoides holoschoenus* and *Salix repens* subsp. *rosmarinifolia*. The taxonomic status of *Stipa pennata* in the relevés is doubtful and it is probably *Stipa borysthenica*, which is found on all sands of the Pannonian Plain. On the other hand, Gajić [15] within *Festucetum vaginatae* distinguished the subassociation *salicetosum rosmarinifoliae*, while on the sands in Hungary [6,35] and on the Deliblato Sands [12] *Salix repens* subsp. *rosmarinifolia* built separate associations. The same is also valid for *Scirpoides holoschoenus* that occurs in the association *Salicetum rosmarinifoliae* and can also be a part of separate communities [6,15].

Species that occur on perennial sandy grasslands in Hungary, but not in Serbia, are: *Achillea ochroleuca* and *Ephedra distachya*, as well as *Astragalus varius* and *Gypsophila fastigiata* (typical subsp. and subsp. *arenaria*), although for the last two there are some sporadic localities on the Subotica-Horgoš Sands. The same also applies to *Helichrysum arenarium*, for which there exist floristic localities on Serbian sands, but which is not recorded in phytocoenological relevés.

Species thriving on the Subotica-Horgoš and Hungarian sands but not on other sands in Serbia are *Alkanna tinctoria, Dianthus serotinus, Secale sylvestre* and *Onosma arenaria*. There is floristic differentiation between *Alysso gmelini-Festucetum vaginatae* and *Festucetum vaginatae* as some species appear only on the Subotica-Horgoš Sands, such as *Astragalus varius, Gypsophila fastigiata* subsp. *arenaria*, *Iris humilis* and *Inula sabuletorum*, although these species have not been recorded in relevés.

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**Author contributions:** Mirjana Ćuk contributed with original data, provided the study design, data organization and analyses, field research, plant material collection and determination and manuscript writing. Miloš Ilić participated in field research and manuscript writing. Ružica Igić supervised the activities and reviewed several drafts of the manuscript. Tijana Šikuljak participated in field research and data collection. Dragana Vukov participated in field research and data analysis. Andraž Čarni supervised the activities and reviewed the manuscript. The publication of this article was approved by all authors.

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## Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/Cuk%20et%20al%20_4493_Supplementary%20Material.pdf