ROMÂNIA
UNIVERSITATEA DIN BUCUREŞTI
GRĂDINA BOTANICĂ "D. BRANDZA"

ACTA HORTI BOTANICI BUCURESTIENSIS
42

editura universității din bucurești®
2015

Publisher: Editura Universităţii din Bucureşti

Acta Horti Botanici Bucurestiensis (text and summaries in English) is published once a year by the Botanical Garden "D. Brandza", University of Bucharest (Şoseaua Cotroceni 32, 060114, Bucureşti, România) and it is available for exchange. Scientific papers are also available online at: http://www.degruyter.com/view/j/ahbb

ISSN 2359-7089 (on line)
ISSN –L 1453-8830

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DE GRUYTER OPEN

Acta Horti Bot. Bucurest. 2015, **42**: 5-22
DOI: 10.1515/ahbb-2015-0004

SYMPHYOTRICHUM CILIATUM AN INVASIVE SPECIES IN THE ROMANIAN FLORA – CONTRIBUTIONS TO THE KNOWLEDGE OF THE VEGETATIVE ORGANS STRUCTURE

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Abstract: *Symphyotrichum ciliatum* (Ledeb.) G.L. Nesom is an adventive plant first reported in Romania in 1967, which has spread rapidly over the last few decades in Moldova, Muntenia and, more recently, in Transylvania. Although the species has been known for a while in Europe and Romania, there is no information about the anatomy of the vegetative organs of this invasive taxon. This paper presents a series of structural aspects of the vegetative body, of *Symphyotrichum ciliatum* collected from a sandy and salty substrate (Sacalin Island – Danube Delta). These demonstrate the ability of this plant to adapt to the environment and its capacity to achieve a wide spread. As such, although this plant is an annual species, the root and stem achieve secondary growth in their width, which offers robustness to the corm; the palisade tissue has an ecvifacial disposition which enhances efficiency in capturing light, especially on sandy soil that reflects light; there are aeriferous and aquiferous formations that ensure efficiency in adapting to a wet and relatively salty environment. These results are documented by original photographs of optical microscopy and a distribution map of the taxon in Romania as at 2011.
Keywords: adventive plant, meso-hygro-halophyte, structural adaptations

Received 8 June 2015 *Accepted 17 September 2015*

Introduction

Symphyotrichum ciliatum (Lebed.) G.L. Nesom [*Brachyactis ciliata* (Ledeb.) Ledeb., *Erigeron ciliatus* Ledeb.] (Fam. Asteraceae) (Sârbu et al. 2013) is an adventive, invasive plant (Anastasiu & Negrean 2007, Sîrbu & Oprea 2011) reported in Romania in 1967 at Şesul Bahlului (Iaşi County) and at Tanacu (Vaslui County) (Beldie & Váczy 1976).

It is an annual herbaceous plant, with erect stems and branched at the bases (up to 60 cm height), yellow-green, frequently with a hint of red, glabrous or with sparse hairs.

Leaves are alternate, simple, sessile, linear or linear-lanceolates. The edge of the lamina is whole and has short tector hairs (ciliated edge – 'ciliatum') and the top is short acuminate. The base leaves usually dry before flowering (Fig. 1).

The plant flowers between August and October, forming numerous heads (calathides), displayed in panicles. Fruits with pappus are easily transported over long distances.

Heliophile, thermophile, eurinitrophile species, it prefers slightly acidic to neutral soils. It grows in relatively wet places (meso-hydrophyte) and more or less

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saline (halophyte), on silt meadows, marshes, disturbed land, along canals and ditches, along roads, train stations, ruderal places, in plains and hills floors (Popescu & Sanda 1998, Sârbu et al. 2001, Ciocârlan 2009, Sîrbu & Oprea 2011, Sârbu et al. 2013).

Fig. 1. *Symphyotrichum ciliatum*, the Sacalin Island – Danube Delta, September 2012 (Photo: Daniela Smarandache)

It is worth pointing out that „the plant can vegetate on dry soils, but it prefers soils with excess moisture and moderate in salt" (Filipaş & Cristea 2006).

The natural range of the species is in Asia (Sârbu et al. 2013), but according to other authors its range also includes North America (Sîrbu & Oprea 2011).

Symphyotrichum ciliatum is recorded as being native to North America and the steppes of North Eurasia and present in the salt marshes (Sîrbu et al. 2015).

In the USA the plant is common in areas with degraded land, especially along motorways, where salt spread during the cold season accumulates. Occasionally the species grows in industrial area, in areas where waste is stored and along roads crossed by lorries (McKinney 2006).

As an adventive plant in Europe, it has been mentioned in Poland, the Republic of Moldova and Romania (Sîrbu & Oprea 2011). As regards its presence in Europe, the taxon was reported in 1987 at Košice, being identified as „a new species in the flora of Czechoslovakia". It was envisaged at that time that „given the ecological necessities of the species, the taxon will spread in the communities of ruderal and coastal plants, and eventually in the agrocenoses from the plain regions in Eastern Slovakia" (Chocholoušková & Pyšek 2003).

Since 1967 the plant has spread relatively quickly in Romania, having been reported in localities from Moldova (Iaşi, Botoşani, Suceava, Neamţ, Vaslui, Galaţi and Vrancea counties), Muntenia (Buzău County), Dobrogea (including the Danube Delta) and, more recently, from Transylvania (Sibiu, Harghita, Cluj - Cluj-Napoca counties) (Sîrbu & Oprea 2011, Filipaş & Cristea 2006, Sîrbu et al. 2015) (Fig. 2).

In Muntenia (Buzău County) the taxon was initially reported along the river Râmnicul Sărat (Ştefan 1980). Subsequently the presence of the species was noted in two other locations from Buzău County: Cocârceni village – the basin of the river Bălăneasa, a tributary of the river Buzău, at an altitude of 300 meters, on gravel and sandy places, and also in the village Vipereşti, in ruderal places (Anastasiu 2010).

As regards the presence of the taxon in the Danube Delta, this was reported in the Sacalin Island, Sf. Gheorghe (Ciocârlan 1994, 2009). Up to 2011, *Symphyotrichum ciliatum* was reported in Romanian in 86 locations from 13 counties.

Taking into account the plant's capacity to develop abundantly and its ecological preferences, the plant is considered particularly dangerous because it can invade meso-hydrophilic and moderately halophilic areas in coastal habitats, which are considered a priority in the strategy for biodiversity conservation (Filipaş & Cristea 2006).

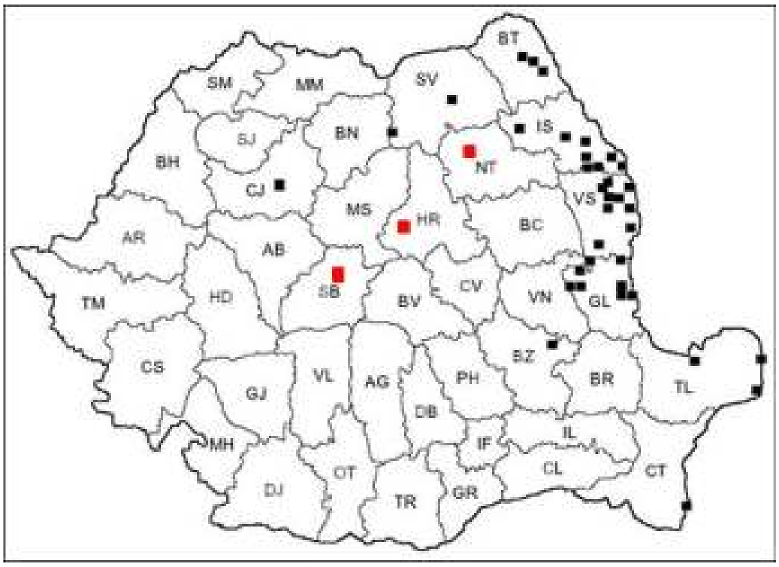

Fig. 2. Symphyotrichum ciliatum, distribution in Romania, according to Filipaş and Cristea 2006, with additions according to Sîrbu and Oprea 2011 (additions after 2006 in red)

Material and methods

The biological material represented by mature plants of 20-25 cm height, at fructification, was collected in September 2012 in the Danube Delta, Sf. Gheorghe locality, the Sacalin Island, during field trips made within the framework of the project "Complex study on allochthonous plants from Danube Delta in order to establish their ecological impact, to assess the risk and to elaborate minimal measures for management" (grant PN-II-ID-PCE, contract no. 970/2009).

The fixing of the biological material was made in 70% ethanol for a period of one year. In order to conduct structural analyses, cross sections were made through the principal root (median third), principal stem (lower, median and superior third), Order I branch (lower, median and superior) and lamina (median third).

The sections obtained were processed according to the double colouration technique (Iodine Green & Carmine Alum), and the highlighting of starch was carried out with IIK (Şerbănescu-Jitariu et al. 1983).

The analysis of the microscopic slides and their micro-photography was made with the Docuval optical microscope.

Results and discussion

The root. The root differentiates a secondary structure which has resulted from the activity of both primary meristems: the cambium and phellogen (Fig. 3).

The phellogen is sub-epidermal, has a reduced activity and produces a few layers of suber, which exfoliate gradually, and a few layers of phelloderm (Fig. 4).

The primary bark is parenchymatous (aeriferous parenchyma), with large cells, between which there are meatuses and aeriferous gaps (Figs 3, 4).

The cortical zone ends with a primary type endodermis, with tangentially elongated cells and with Caspary thickening in the radial walls. The cells of this last stratum of the bark are in contact with the pericycle and have a regular disposition along the entire circumference of the root (Fig. 4).

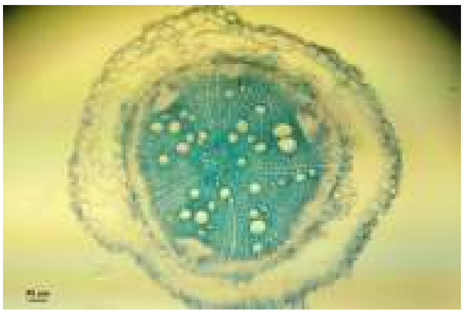

Fig. 3. Symphyotrichum ciliatum, the structure of the root in the median area (cross section, Iodine Greene & Carmine Alum)

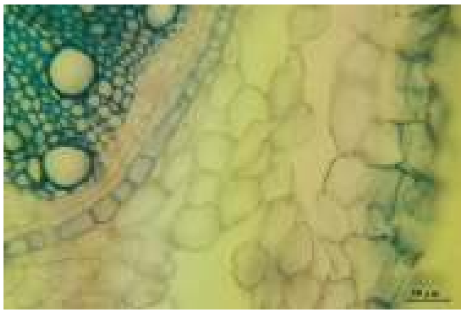

Fig. 4. Symphyotrichum ciliatum, cross section through the root, highlighting the suber, the bark and the external area of the central cylinder (Iodine Greene & Carmine Alum)

The central cylinder is bulky (~70% of the root). The pericycle is unistratified with tangentially elongated cells which generally alternate with the cells of the endodermis (Figs 4, 5).

The secondary vascular tissues are unequally represented in the structure of the root. The secondary phloem is in the form of a narrow ring which includes from place to place packages of sclerenchymatous fibres. The sclerenchymatous fibres have relatively thin and heavily lignified cell walls (Fig. 6).

The secondary xylem is located centrally and has the form of a bulky and compact body. The wood vessels have a different diameter and are dispersed in a mass of libriform (the fibres have heavily thickened and lignified walls) (Fig. 7).

The primary xylem is located centrally and the pith is missing (Fig. 7).

In the structure of the central cylinder there are uni- and pluristratified medullary rays, whose cells highlight uniformly thickened and lignified cell wall (Fig. 7).

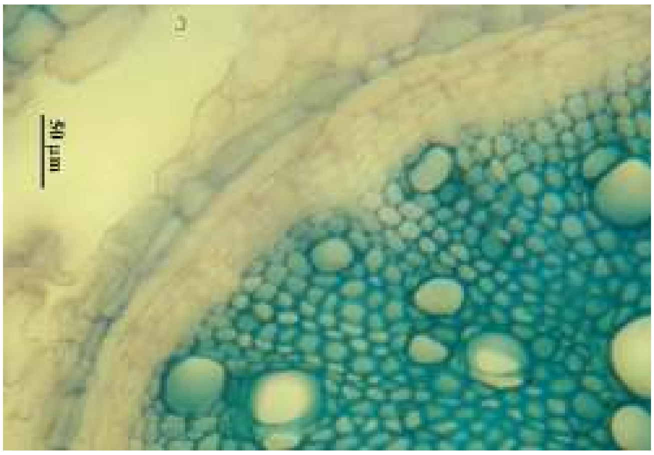

Fig. 5. Symphyotrichum ciliatum, cross section through the root highlighting the central cylinder (Iodine Greene & Carmine Alum)

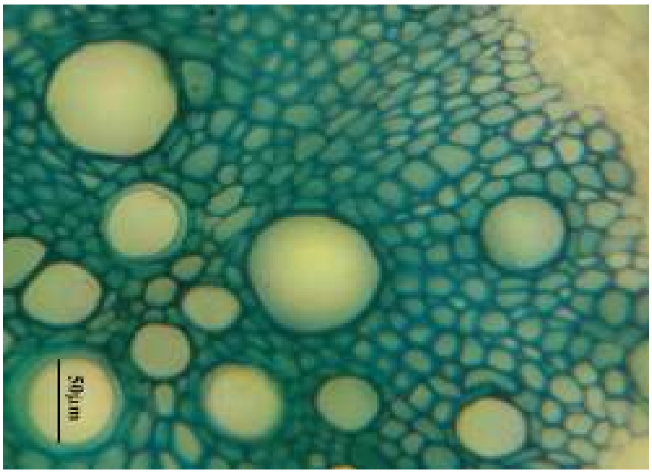

Fig. 6. Symphyotrichum ciliatum, cross section through the root highlighting elements of the central cylinder (Iodine Greene & Carmine Alum)

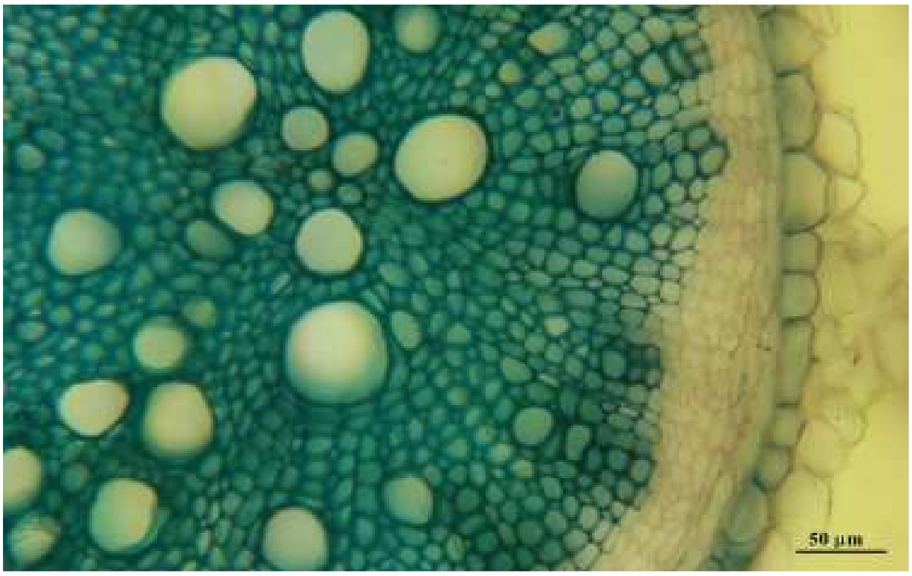

Fig. 7. Symphyotrichum ciliatum, cross section through the root highlighting the compact body of the xylem (Iodine Greene & Carmine Alum)

The stem. The structure is typical secondary at all three levels of sectioning (lower, median and upper third) and only results from the activity of the vascular cambium. The epidermis is unistratified and covered by a noticeable cuticle which differentiates cuticular ridges. The stomata are anomocytic and rare. In the lower third of the stem the bark is represented by an aerenchyma with large, relatively isodiametric cells with meatuses and gaps (Fig. 8). In the median third and the upper third respectively, the dimensions of the bark and those of the aeriferous gaps are reduced. The internal layer of the bark forms an endodermis where no accumulation of starch was found (reaction with IIK) (Figs 8, 9, 10).

Inside the central cylinder, the vascular tissues are displayed in the form of concentric rings (Fig. 9). The cambium produces a thin external area of phloem and an internal thick area of xylem. Both rings of secondary tissues are crossed by medullary rays of different thickness, made of cells with uniformly thickened and lignified walls (Figs 9, 10).

At the external limit of the secondary phloem there are packages of sclerenchymatous fibres. The sclerenchymatous cells have heavily thickened and lignified walls (Figs 9, 10).

In the secondary xylem the wood fibres (libriform) are predominant and feature radial rows of wood vessels of different sizes. Both the elements of the secondary xylem and the wood fibres have lignified cells wall.

At the internal part of the secondary xylem, the primary xylem can be observed. This is formed of wood vessels displayed in packages and separated by parenchymatous cells, with walls slightly thickened and lignified (Fig. 9).

The pith is parenchymatous in the lower third (Fig. 9). In the median and upper thirds the cells formed in the central area of the pith disorganise and thus lead to the creation of two large aeriferous cavities with an irregular contour (Figs 11, 12).

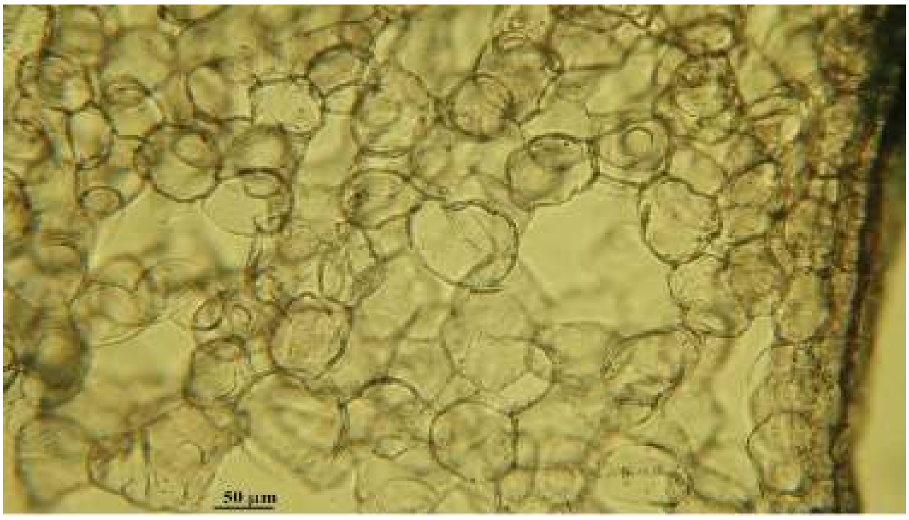

Fig. 8. Symphyotrichum ciliatum, cross section through the base area of the stem highlighting the epidermis and the cortical aerenchyma (Iodine Greene & Carmine Alum, reaction with IIK)

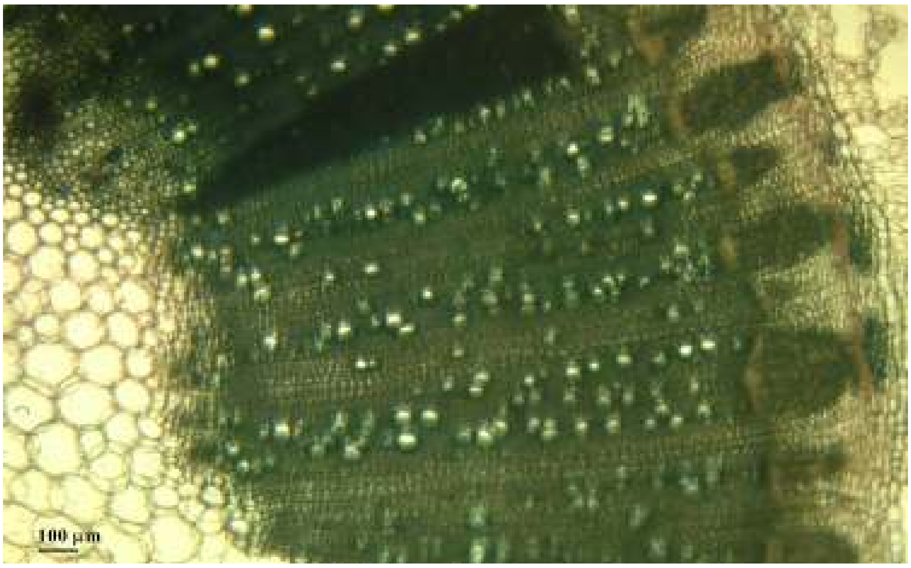

Fig. 9. Symphyotrichum ciliatum, cross section through the base area of the stem highlighting vascular tissues and the pith (Iodine Greene & Carmine Alum, reaction with IIK)

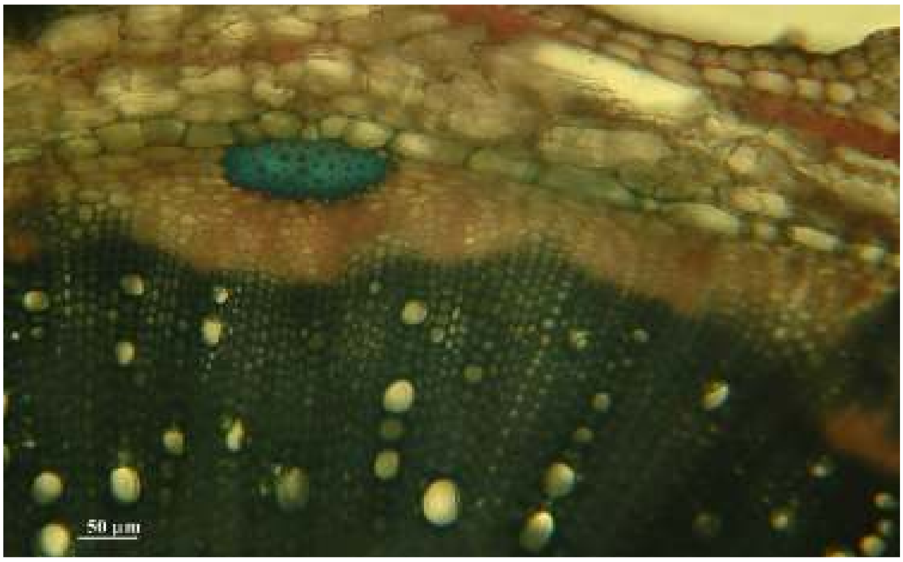

Fig. 10. Symphyotrichum ciliatum, cross section through the median area of the stem highlighting the epidermis, the bark and the vascular tissues (Iodine Greene & Carmine Alum, reaction with IIK)

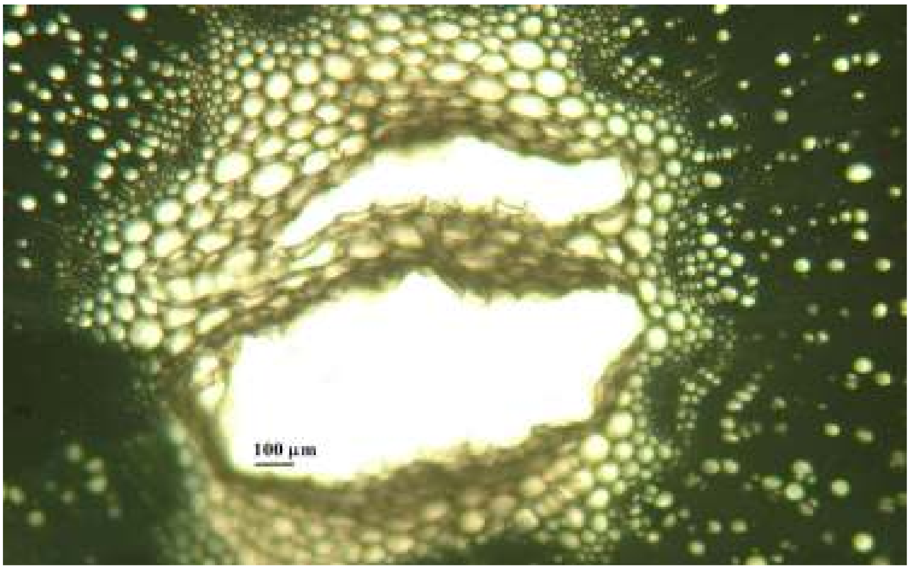

Fig. 11. Symphyotrichum ciliatum, cross section through the median area of the stem highlighting the medullary aeriferous cavities (Iodine Greene & Carmine Alum)

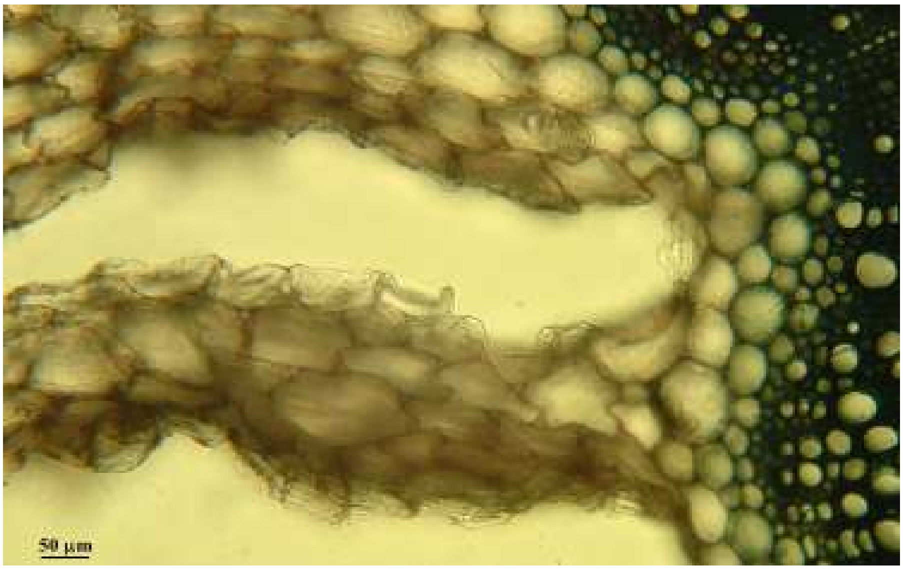

Fig. 12. Symphyotrichum ciliatum, cross section through the median area of the stem highlighting a detail at the level of the medullary aeriferous cavities (Iodine Greene & Carmine Alum)

Order I Branch. In cross section the contour of the branch is almost circular, with 4-5 ribs separated by vallecules. The coastal area becomes more prominent and the vallecules more visible towards the tip of the stem (Figs 13, 14, 15).

At all three levels of the cross sectioning, the branch displays a fasciculate secondary structure, which resulted from the activity of the vascular cambium.

The epidermis is unistratified, with isodiametric cells covered by a thick cuticle which differentiates prominent cuticular ridges. From place to place there are stomata (anomocytic type) generally displayed at the same level as the other epidermal cells (Fig. 16). The primary bark is parenchymatous with palisade external layers (Figs 17, 18, 19). The number of palisade layers increased from the base of the branch (one-two) to the top of the branch (four-five). The internal layers of the bark are formed of large cells, more or less isodiametric, and have rare aeriferous meatuses. At the level of the vallecules, especially in the upper third of the branch, there are subepidermal areas of tabulo-angular collenchyma (Fig. 20).

The last layer of the bark is an endodermoid where no accumulation of starch was identified (reaction with IIK). The dimensions of the central cylinder and the thickness of the secondary xylem decrease from the base of the branch to the top. The central cylinder contains 10-12 vascular bundles of different sizes (predominantly large), with a circular disposition (Figs 18, 19, 21). Each bundle presents a periphloemic cap of lignified sclerenchymatous fibres.

The medullary rays are large, formed of cells with thickened and lignified walls at the secondary xylem (Fig. 18). At all the cross sectioning levels the pith is parenchymatous, meatic, made up of large, polygon cells.

Fig. 13. Symphyotrichum ciliatum, cross section through the Order I branch, in the base area (Iodine Greene & Carmine Alum)

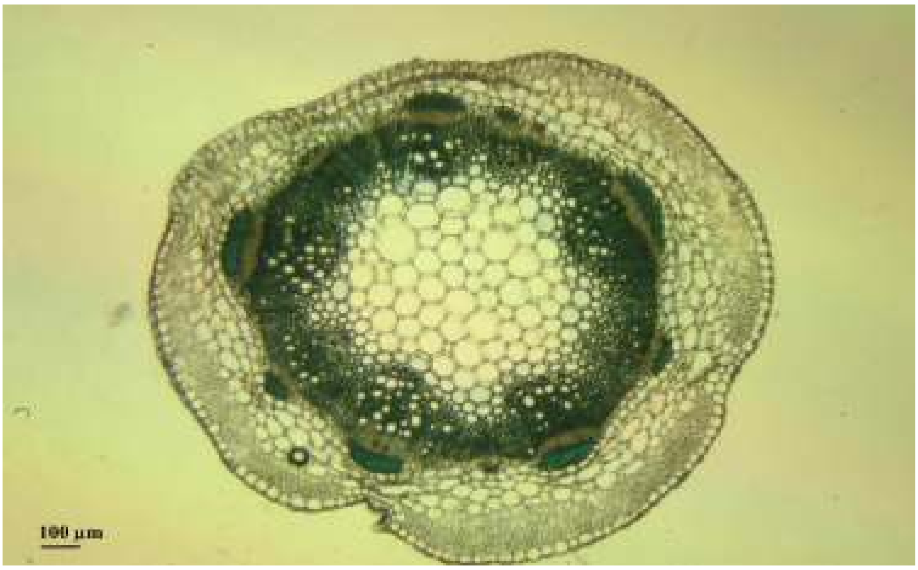

Fig. 14. Symphyotrichum ciliatum, cross section through the Order I branch, in the median area (Iodine Greene & Carmine Alum)

Fig. 15. Symphyotrichum ciliatum, cross section through the Order I branch, in the apical area (Iodine Greene & Carmine Alum)

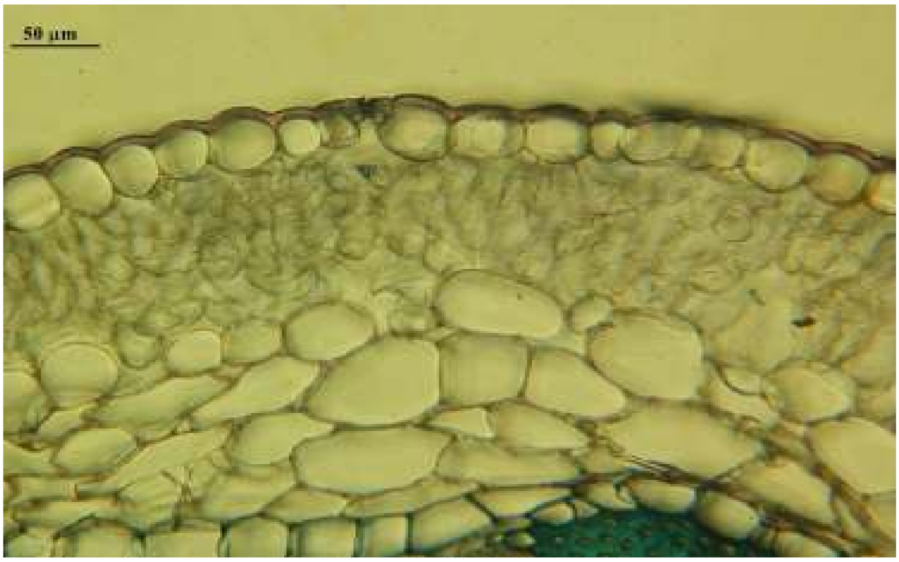

Fig. 16. Symphyotrichum ciliatum, cross section through the Order I branch, in the median area, highlighting the epidermis and the bark (Iodine Greene & Carmine Alum)

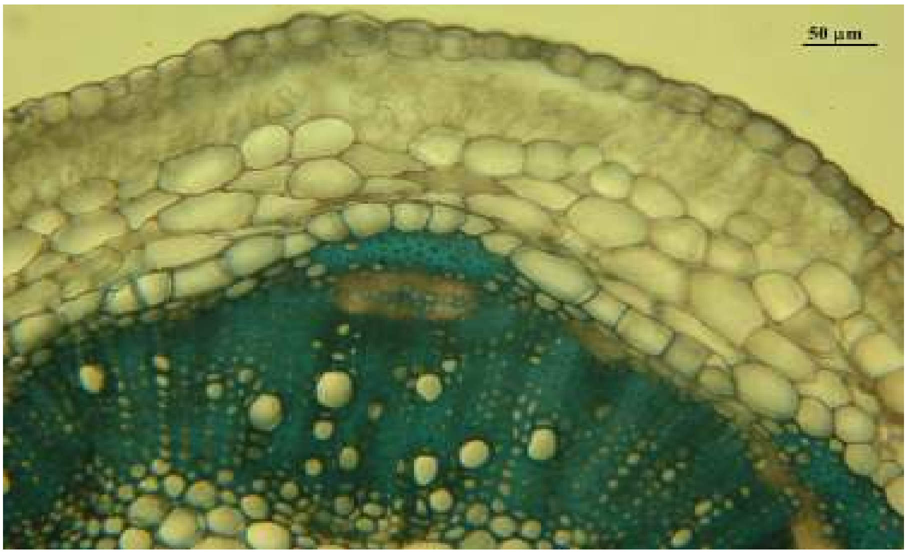

Fig. 17. Symphyotrichum ciliatum, cross section through the Order I branch, in the base area, highlighting the epidermis, bark and vascular tissues (Iodine Greene & Carmine Alum)

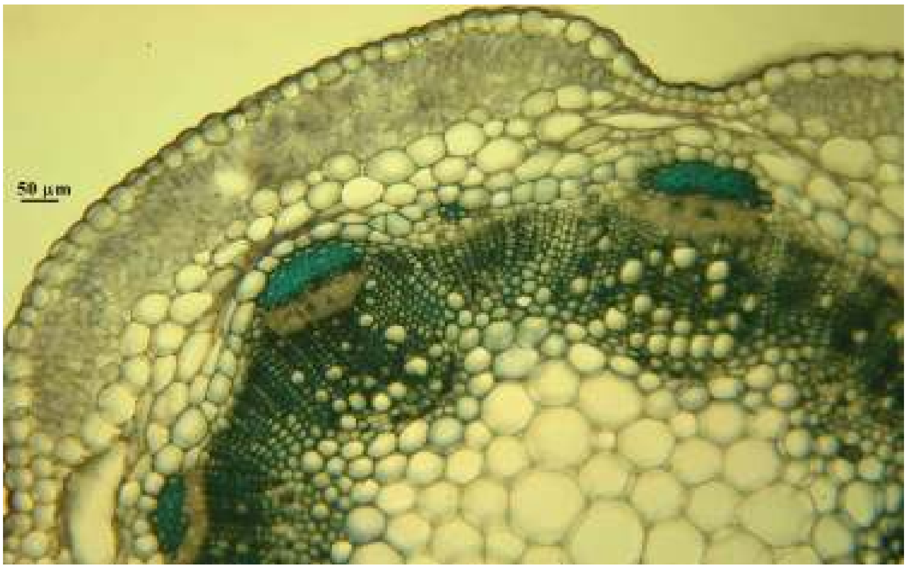

Fig. 18. Symphyotrichum ciliatum, cross section through the Order I branch, in the median area, highlighting the epidermis, bark and central cylinder (Iodine Greene & Carmine Alum)

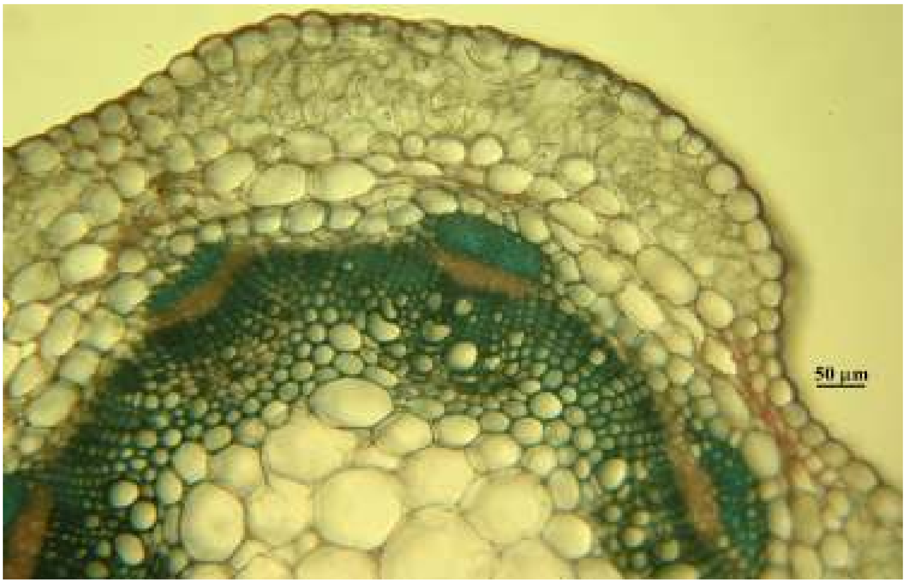

Fig. 19. Symphyotrichum ciliatum, cross section through the Order I branch, in the apical area, highlighting the epidermis, bark and the central cylinder
(Iodine Greene & Carmine Alum)

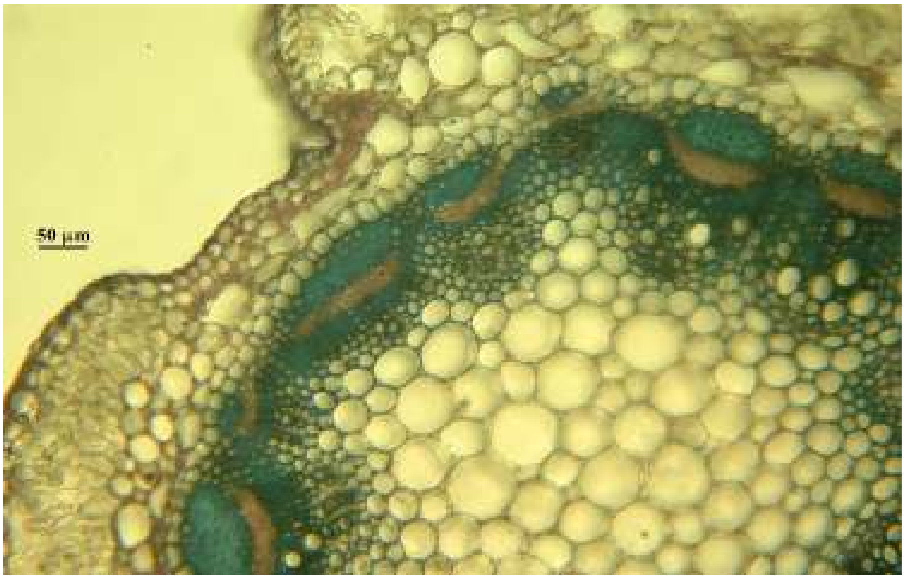

Fig. 20. Symphyotrichum ciliatum, cross section through the Order 1 branch, in the apical area, highlighting the epidermis, bark and central cylinder
(Iodine Greene & Carmine Alum)

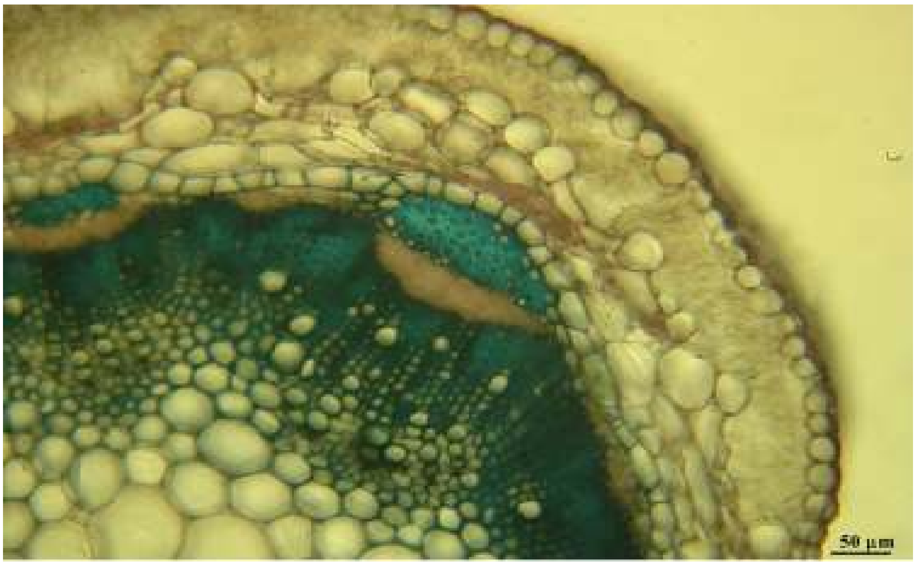

Fig. 21. Symphyotrichum ciliatum, cross section through the Order I branch at the base, highlighting the epidermis, bark and the central cylinder
(Iodine Greene & Carmine Alum)

The lamina. In the lower and median third of the lamina, the contour of the cross section is semi-circular with a flat adaxial face (Fig. 22). In the upper third, the lamina has an elongated, eliptic contour with both sides flat.

At the level of the rounded edges of the lamina there are pluricellular tector hairs (Fig. 23). The epidermis has isodiametric cells, with the external wall thicker than the internal one, and covered by a cuticle with cuticular ridges (Fig. 24).

The lamina is bifacial, amphystomatic, with an ecvifacial structure. The anomocytic stomata are displayed at approximately the same level as the epidermal cells. The mesophyll is formed of two-three layers of palisade cells located under both epidermises and in a narrow central area, formed from relatively isodiametric assimilative cells which leave between them small gaps (dense lacunose tissue) (Fig. 25). Around the nervures the palisade cells have a radial disposition.

The vascular tissues form seven closed collateral vascular bundles. The median bundle is the biggest and is slightly prominent at the level of both epidermises through to hypodermal columns formed of large, isodiametric and colourless cells (Fig. 26).

Xylem and phloem vascular elements are present in the structure of all vascular bundles, but are represented in a higher number in the median bundle. Each vascular bundle is surrounded by a parenchymatous, perifascicular sheath, formed of colourless isodiametric cells of various sizes.

Symphyotrichum ciliatum collected on the sands of the Sacalin Island – Danube Delta can be considered a plant of wet and relatively salty places (a meso-hygro-halophyte). According the ecological requirements, Sârbu et al. (2013) already defined *Symphiotrichum ciliatum* as a meso-hygro-halophyte plant. A series of anatomic-histological aspects related to the plant's vegetative organs can be associated with this status.

In this respect, it is worth noting the presence of the aerenchyma in the structure of the root's primary bark and that of the stem, and also the formation of medullary aeriferous cavities in the median and upper areas of the stem.

Generally hygrophytes form aeriferous tissue in the majority of vegetative organs as an adaptation to the high humidity environment they live in (Toma & Rugină 1998, Sârbu 1999, Toma & Gostin 2000, Ciocârlan 2009).

On the other hand, the presence of meatuses and of the periferous gaps in the structure of the primary bark is considered by many authors a feature characteristic to halophilic plants. As such this characteristic has been noted in other halophytes such as *Aster tripolium* L. (meso-hygrohalophyte up to hygrohalophyte) and *Juncus gerardii* Loisel. (meso-hygrohalophyte) (Grigore & Toma 2010).

Another structural aspect that sustains the preference of this plant for salty soils is the presence in the structure of the lamina of some elements with a role in storing water (aquiferous), represented by the large and colourless parenchymatous cells from the structure of the hypodermal columns located around the median nervures, and also by those of the perifascicular sheaths. Such elements involved in the water economy of halophile plants have also been reported in other Asteraceae plants such as *Aster tripolium* L. and *Artemisia santonicum* L. (xero-mesophyte, halophyte) (Grigore et al. 2014).

The results presented in this paper confirm the ecologic status of *Symphyotrichum ciliatum* as a meso-hygro-halophyte plant.

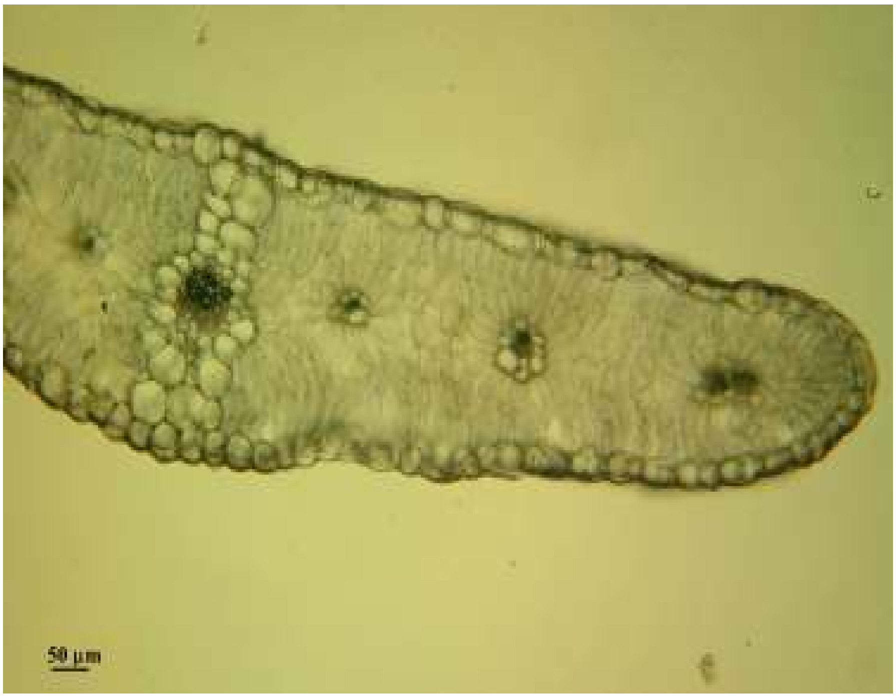

Fig. 22. Symphyotrichum ciliatum, cross section through the lamina, in the median third (Iodine Green & Carmine Alum)

Fig. 23. *Symphyotrichum ciliatum*, cross section through the lamina, in the median third, highlighting the formation of a tector hair (Iodine Green & Carmine Alum)

Fig. 24. *Symphyotrichum ciliatum*, cross section through the lamina, in the median third, highlighting the lower epidermis cells (Iodine Green & Carmine Alum)

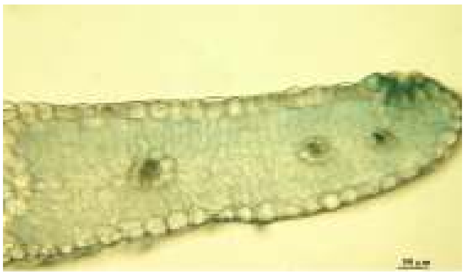

Fig. 25. *Symphyotrichum ciliatum*, cross section through the lamina, in the median third, highlighting the lamina's ecvifacial structure (Iodine Green & Carmine Alum)

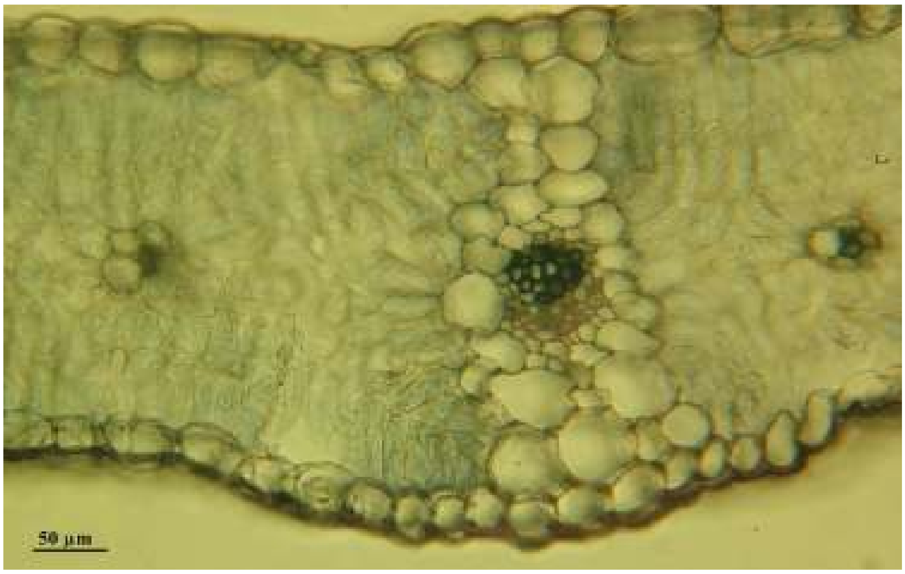

Fig. 26. *Symphyotrichum ciliatum*, cross section through the lamina, in the median third, highlighting the median nervure (Iodine Green & Carmine Alum)

Conclusion

Symphyotrichum ciliatum is an invasive adventive plant, spread across many areas in Romania, more often on relatively wet soils and lightly salted.

The structural characteristics of the vegetative body of the plant analysed in the current paper document the ecological preference of this species, and support the great capacity for spreading of the taxon.

Symphyotrichum ciliatum has at the level of the vegetative organs a particular structure for an annual plant, which enables it to constitute a robust vegetative body well adapted to the environment. In this respect the root, the stem and the branches differentiate a secondary structure, and the ecvifacial bifacial structure of the lamina provides efficiency in capturing the light, especially on sands where light is reflected.

Symphyotrichum ciliatum is a meso-hygrophyte and halophyte, well adapted to the conditions of the wet, salty soils. In this respect, the plant has aeriferous formations at the level of the root and stem, and aquiferous formations at the level of the lamina.

The robust structure of the vegetative body and its adaptations to the environment of wet and salt areas confirm the fact that this plant, already considered invasive in Romania, can have a significant impact over the vegetal communities where they grow, becoming a real danger for coastal habitats.

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Acta Horti Bot. Bucurest. 2015, **42**: 23-40
DOI: 10.1515/ahbb-2015-0003

ADDITIONAL GLACIAL RELICTS IN CAREI PLAIN NATURAL PROTECTED AREA, NORTH-WESTERN ROMANIA

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Abstract: This paper presents new scientific data on the spread of mountain elements in the plains of the northwestern part of Romania, species that managed to survive in this area since the last glacial period. Previous studies revealed that the northwestern part of Romania does not exhibit vegetation specific to the region of forest steppe, hills or low mountain areas, as expected. Many species common to mountainous regions were previously observed not only in Carei Plain, but also in Ier Plain, Tur Plain, Tăşnad Hills or Oaş region across Satu Mare, Bihor and Sălaj counties. The same observations were made in other parts of Carei Plain in Hungary, and conclusions were drawn that the area was a glacial refuge. On the Hungarian side of the Carei Plain, the ecosystems were also better preserved comparative to the Romanian side, where most of the natural ecosystems of the Carei Plain were destroyed due to the conversion to agricultural land starting with the 19th century and culminating in the communist period during the 20th century. The study is also intended to be a complement to the data on "Natura 2000" sites, whose goal is the protection of biodiversity in Europe along with their conservation in the most favorable conditions. An update of these sites is becoming increasingly necessary since in recent decades they have been greatly impacted by human activities.
Keywords: glacial relicts, protected species, Carei Plain, Romania

Received 19 October 2014 *Accepted 28 August 2015*

Introduction

Both the current spread and survival of species present during the last ice age have always intrigued scientists. Most species were forced to migrate southwards in order to survive. It is known that such species have survived in major areas of Europe that were sheltered in the Iberian, Italian and Balkan Peninsulas (Pop 1979). But some species, less susceptible to frost, managed to survive in sheltered places further north up to Central Europe. One such place is the northwestern part of Romania and northeastern Hungary. These assumptions are based both on the species remains that were discovered in the area, especially pollen in peat bogs and swamps, and especially the presence of species that continued living in these locations through and since the last glaciation. While in most lowland areas these species retreated to higher mountainous regions, some have remained due to geographical elements.

In parts of the Western Plains of Romania, these elements comprised wide areas of swamps that maintained a wet and cool climate in the northern part, relative to the

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center and south parts of the Western Plains (Karácsonyi 1987). The extensive riverside shrubby thickets and floodplain forests have substantially reduced evaporation, thereby preventing the withdrawal of the marshes, especially during the warm Boreal Period. The following periods (Atlantic and Subboreal), wetter and colder, further helped the perpetuation of these species in the area (Ardelean & Karácsonyi 2005). With the descent of beech forests into the plains, more mountain low altitude species are arriving here, which, like the previous ones, still have had favorable conditions for survival. The warming during the Subatlantic period seals the fate of the beech forests, which withdrew to the mountains, some species disappearing (Ardelean & Karácsonyi 2005, 2008). But the greatest danger of these relicts is represented by human activities. The widely spread marshes of northwestern Romania were drained and most riverside thickets and forests cleared since the nineteenth century. In addition to deforestation a main contribution was the activity of a society that was aimed to draining the swamps and marshes; followed by the expansion of vineyards and black locust plantations from 1892 (Ardelean & Karácsonyi 2005). With their disappearance, most relicts had no chance of survival, but in partial natural places (islands) they continue to persist.

In Carei Plain, islands with archaic vegetation were found in Ciumeşti (Răchitişul Lung) (Karácsonyi 1992), Sanislău (Vermeş Bog) as well as in numerous other smaller, scattered places. Scărişoara Nouă, Satu Mare County, is one such place where archaic vegetation was recently discovered and published in this paper.

Material and methods

1. *Species identification*

Plant species have been observed and identified in the field at Scărişoara Nouă, Satu Mare County. Unknown species were collected, preserved as herbarium specimens and their identity was determined using taxonomic keys (Ciocârlan 2009, Sârbu et al. 2013). Pictures were also made for each species and habitat, available on the website “mybiosis.info”. A phytocoenology survey was used in order to find the percentage of dominant and relict species. The scientific names of plants are in accordance with the accepted names by the website ”The Plant List” and they are taxonomically assigned to the APG III Classification System. The identification of present habitats was also performed according to the manual of the Romanian habitats (Doniţă et al. 2005).

The data presented here were collected during field research in the years of 2012, 2013 and 2014. Field research was conducted throughout the growing season (fall, spring and summer) to capture as many species as possible.

2. *Description of the site*

The sands from the northwestern part of the country form a distinct geomorphological unit in the high plains of the western lowlands. Its characteristic landscape is made by the presence of rows of sand dunes with a north (northeastern) – south (southwestern) orientation, alternating with low interdune lands, sometimes occupied by large swamps. In some parts of the region yellow, flying sands are still present. The wetlands are mostly covered with dark brown sands (Benedek 1969).

The site is located on the stretch of an interdune through which flows a stream, which at that time was dry, but in following years the water level has returned to normal. This interdune is covered entirely by riverside thickets and natural osiers (*Salix* spp.), who apparently escaped the deforestation nearby. The sand dunes that border the site, unfortunately are covered with planted black locust, forming two compact corridors on south-north direction. The black locust forest on the north side stretches to the edge of the site, but without negative influences, forming a veritable curtain of protection. On the south side are large areas of wet meadows, almost in a natural, preserved state, as well as sand dunes with their characteristic vegetation from the Pannonian region with *Corynephorus canescens*. Nearby there are other interdunes with swamps that could be the object of further investigations. The coordinates of the newly found place are 47°37'20" N and 22°12'56" E, at an altitude of about 142 m. The place is depicted on a map within Europe in Figure 1 (Fig. 1).

Fig. 1. The new site with glacial relicts near Scărișoara Nouă, Satu Mare County (Google Earth) seen at European level (yellow mark)

Results and discussion

The new location with glacial relicts was documented following a field research, on 23 September 2012. Due to the unusual drought that year, the marshes throughout the northwest had dried up, therefore this new location was discovered because it became more accessible. Also, the location was noticeable from afar due to the presence of tree species, otherwise unusual in the plains.

The tree layer of the site consists strictly of birch (*Betula pendula*), downy birch (*Betula pubescens* subsp. *carpatica*) and aspen (*Populus tremula*). Other woody species are represented by natural riverside thickets consisting of various willow species (e.g. *Salix alba, Salix fragilis*) and white poplar (*Populus alba*). The three species of trees at the site are characteristic of the mountain regions, especially the birches. *Betula pubescens* subsp. *carpatica* are not usually encountered below the beech forest altitude (600-1200 m), also this subspecies is endemic to the Carpathian Mountains. *Betula pendula* and *Populus tremula* are also not usually growing below the sessile oak elevations (Ciocârlan 2009). In some places a few other tree species are appearing represented by one individual tree, such as: grey poplar (*Populus canescens*), narrow-leafed ash (*Fraxinus angustifolia* subsp. *danubialis*), the pedunculate oak (*Quercus* *robur*), and adventitious trees like the swamp oak (*Quercus palustris*) and the American ash (*Fraxinus americana*), probably from nearby plantations.

The shrub layer is extremely rich in marsh species. The most remarkable are the willows encountered at much lower altitudes than normal: eared willow (*Salix aurita*) and purple-osier willow (*Salix purpurea*). The dominant species is grey willow (*Salix cinerea*), accompanied by alder buckthorn (*Frangula alnus*), guelder rose (*Viburnum opulus*) and common dogwood (*Cornus sanguinea*). In the shrub layer there are also occasionally found species such as hawthorn (*Crataegus monogyna*), dog rose (*Rosa canina*) and dewberry (*Rubus caesius*). Another adventitious shrub, with invasive character is the black cherry (*Prunus serotina*), which also comes from the nearby plantations. Remarkable is the mistletoe (*Viscum album*) parasitizing the birches. The main vines encountered are: hops (*Humulus lupulus*), hedge bindweed (*Calystegia sepium*) and bittersweet nightshade (*Solanum dulcamara*), as well as the adventive North American vine (*Vitis vulpina*).

The herbaceous layer present amongst the clusters of trees and shrubs as well as the grasslands also contain many mountain species, presented in Table 1. Patches are dominated by reed (*Phragmites australis*), various sedges (*Carex acuta, Carex acutiformis, Carex hirta, Carex nigra*), the marsh fern (*Thelypteris palustris*), velvet bent (*Agrostis canina*), woodclub rush (*Scirpus sylvaticus*) and soft rush (*Juncus effusus*).

The moss layer is very rich, many species usually growing in the mountains, a very notable characteristic of the site: *Cirryphyllum piliferum, Brachythecium rutabulum, Eurhinchium (Oxyrrhynchium) hians, Plagiomnium rostratum, Plagiomnium undulatum, Amblystegium serpens, Atrichum undulatum, Leptodyctium riparium, Climacium dendroides, Plagiomnium ellipticum*, and on the sandy dunes *Polytrichum piliferum*.

The wet meadows from the interdune present an exceptional floristic diversity, growing here many species included on the national Red Lists, listed in Table 2 (Boşcaiu et al. 1994, Oltean et al. 1994, Dihoru & Negrean 2009). Also the present plant associations are very diverse for such a small area. The main dominant species that make up the associations are: purple moor grass (*Molinia caerulea*) (a grass typical of mountain regions), creeping bentgrass (*Agrostis stolonifera*), tufted hairgrass (*Deschampsia cespitosa*), tall fescue (*Festuca arundinacea* subsp. *orientalis*), meadow fescue (*Festuca pratensis*). These habitats are home to a wide range of rare species and glacial mountain relicts for the region (Tables 1 and 2), playing a part of the archaic vegetation of the past time. This study also reports plant species that were not previously known to occur in the Carei Plain area such as *Luzula multiflora, Danthonia decumbens, Silene otites* subsp. *hungarica, Trifolium dubium, Lathyrus nissolia, Lathyrus palustris, Salix aurita, Achillea ptarmica, Mentha arvensis* subsp. *austriaca, Rumex thyrsiflorus, Festuca arundinacea* subsp. *orientalis.*

Within the site nine types of habitats were identified, a particularly high number for a single interdune, a character that underlines once again the diversity of flora and fauna of Carei Plain in particular in the preserved natural islands, and the necessity of increased efforts to protect these oases of biodiversity. Of these, six belong to the wet meadows and pastures as well as the marsh vegetation, two types of alluvial forests and swamp thickets and one xeric habitat characteristic to the sand dunes bordering the valley. These nine types of habitats are as follows:

R3710 Dacian *Molinia caerulea* grasslands. It is probably one of the last standing areas of significant surface in northwestern Romania, *Molinia caerulea* habitats being continuously diminished due to marsh draining and inappropriate use of the grasslands. In literature, these types of habitats are not recorded below 300 m altitude (Doniţă et al. 2005), while patches of *Molinia caerulea* reported from the plains of northwestern Romania being at very low altitudes, around 100 m. Conservation value is presented as moderate (Doniţă et al. 2005), but this Carei (Nir) Plain relict habitats require protection. The main species that make up the habitat are*: Molinia caerulea, Angelica sylvestris, Cirsium rivulare, Serratula tinctoria, Lathyrus pratensis, Succisa pratensis, Lychnis flos-cuculi, Carex ovalis, Galium uliginosum, Euphorbia lucida, Sanguisorba officinalis* etc. Among the outstanding species found here are *Angelica palustris, Salix rosmarinifolia, Achillea ptarmica, Dianthus superbus* and *Scirpoides holoschoenus*.

R3712 Dacian communities with *Deschampsia cespitosa* and *Agrostis stolonifera.* A habitat also found here at a lower altitude than usual, is affected due to marsh draining. The main species (*Deschampsia cespitosa*) is in decline, but its place is taken remarkably by *Festuca arundinacea* subsp. *orientalis*, which also form compact meadows particularly biodiverse. According to Doniţă et al. (2005) the conservation value of this habitat is low, but as in previous habitat (R3710 Dacian *Molinia caerulea* grasslands) these habitats are some of the last remnants of tall herb vegetation from the ancient wetlands of northwestern Romania and we consider that it needs protection.

The main species encountered along with *Deschampsia cespitosa* and *Agrostis stolonifera* are: *Festuca arundinacea* subsp. *orientalis, Alopecurus pratensis, Juncus conglomeratus, Juncus inflexus, Briza media, Trifolium pratense, Phleum pratense, Euphorbia lucida, Cynosurus cristatus, Holcus lanatus, Thalictrum lucidum* and *Dianthus superbus*.

R3715 Danubian-Pannonic meadows of *Agrostis stolonifera.* It occupies the areas surrounding tall grasslands. Their conservative value is low (Doniţă et al. 2005), but in the Carei Plain they host many rare and low altitude mountain species, that require protection. The main species found here are: *Agrostis stolonifera, Alopecurus pratensis, Poa trivialis, Ranunculus repens, Trifolium fragiferum, Trifolium pratense, Eleocharis palustris, Ranunculus acris, Poa pratensis, Equisetum palustre, Carex panicea, Carex flacca, Veratrum album* etc.

R3716 Danubian-Pontic grasslands of *Poa pratensis, Festuca pratensis* and *Alopecurus pratensis.* It occupies large areas in Carei Plain, but is becoming increasingly scarce in species due to mowing and overgrazing. In Romania they have a moderate conservation value (Doniţă et al. 2005). Within the interdune of the site presented by this study this habitat is very rich in species diversity, the most representative are: *Festuca pratensis, Alopecurus pratensis, Poa pratensis, Dactylis glomerata, Juncus effusus, Lotus corniculatus, Lysimachia nummularia, Pulicaria dysenterica, Ononis arvensis, Galium palustre, Carex hirta, Cirsium rivulare, Briza media, Leucanthemum vulgare, Thalictrum simplex* subsp. *galioides* etc. Among the rare species stands out *Iris sibirica, Orchis laxiflora* subsp. *elegans Achillea aspleniifolia* and *Taraxacum palustre*.

R4407 Danubian forests of *Salix alba* with *Rubus caesius.* Forms narrow belts of vegetation along the creek that runs through the site. This habitat have a high conservation value (Doniţă et al. 2005), an increased conservation value which further increases due to the rare and relict species contained in this habitat. Although the habitat is mainly formed by *Salix alba*, in some places *Populus tremula* alongside with *Betula pendula* and *Betula pubescens* subsp. *carpatica* become dominant species. Other trees found here are: *Populus alba, Salix fragilis*, with rare specimens of *Fraxinus angustifolia* subsp. *danubialis* and adventive species such as *Fraxinus americana* and *Quercus palustris*. The shrub layer is dominated by: *Frangula alnus, Cornus sanguinea, Salix cinerea, Viburnum opulus, Rubus caesius* that are occasionally covered with creepers like *Humulus lupulus, Calystegia sepium* and *Solanum dulcamara*. The dominant grasses are: *Agrostis canina, Agrostis stolonifera, Lysimachia nummularia, Glechoma hederacea, Polygonum hydropiper, Scutellaria galericulata, Lycopus europaeus*, sometimes dominating *Phragmites australis* with *Thelypteris palustris*. These damp woods shelter important rare species such as *Urtica kioviensis, Peucedanum palustre* and *Carex nigra*.

R4419 Southeast Carpathian thickets of *Salix cinerea* with *Calamagrostis canescens.* This habitat is particularly interesting, being reported only in mountainous regions and Carei Plain, its conservation value is high (Doniţă et al. 2005) primarily because shelters relict species in all areas of the country where it occurs, and also this thickets are found rarely in the northwestern part of Romania. The main associations between the defining species of this type habitat were found in the marshes at the borders of the villages Sanislău and Ciumeşti (Ardelean & Karácsonyi 2005). The habitat consists of the following species, which contains the following relicts: *Calamagrostis canescens, Betula pubescens* subsp. *carpatica, Salix aurita, Carex nigra, Carex cespitosa, Carex echinata, Peucedanum palustre, Salix rosmarinifolia, Angelica palustris*. The main dominant shrubs alongside *Salix cinerea* are: *Frangula alnus, Viburnum opulus* and *Cornus sanguinea*. The herbaceous layer consists of the *Carex* species mentioned above as well as *Carex acuta* or *Carex acutiformis, Poa trivialis, Lysimachia vulgaris, Scirpus sylvaticus, Urtica kioviensis, Urtica dioica, Angelica sylvestris, Lythrum salicaria, Lycopus europaeus, Galium palustre, Iris pseudacorus, Solanum dulcamara, Thelyperis palustris, Symphytum officinale, Oenanthe aquatica, Stachys palustris* etc. Special attention should be given to the fact that in one of the adjacent valleys near the investigated area presented here was reported another glacial relict in the Romanian flora: *Spiraea salicifolia*, a basic component of the habitat described by Ardelean & Karácsonyi (2005). The species here is of uncertain origin. The relict species *Calamagrostis canescens* and the impressive *Calamagrostis stricta* are endangered throughout the northwest of the country due to marsh draining, being already extinct from the most of the localities reported in the literature.

R5305 Danubian communities with *Typha angustifolia* and *Typha latifolia.* This habitat occupies a silted area that once was probably a former lake, the conservative value being generally low (Doniţă et al. 2005). The main species found here are: *Typha latifolia, Typha angustifolia, Schoenoplectus lacustris, Lythrum salicaria, Glyceria maxima, Alisma plantago-aquatica, Carex riparia, Carex acutiformis, Epilobium hirsutum, Lysimachia vulgaris, Mentha arvensis, Bolboschoenus maritimus* etc.

R5310 Dacian-Danubian communities with *Carex elata, Carex rostrata, Carex riparia* and *Carex acutiformis.* These tall sedges occupy depressions with especially excessive humidity. Their conservation value, otherwise moderate throughout Romania

(Doniţă et al. 2005), increases considerably in the plains of the northwestern part of the country, where it forms large areas of archaic wetlands, which often escaped grubbing and drainage works because of their high content in forage species. The dominant species of this particular habitat present at the site presented by this study includes *Carex acuta, Carex acutiformis, Carex riparia*, with *Carex elata* occuring only in minor clusters. The accompanying main species found here are: *Equisetum palustre, Lythrum salicaria, Peucedanum palustre, Rorippa sylvestris, Galium palustre, Eleocharis palustris, Lathyrus palustris, Phalaris arundinacea, Poa palustris, Lysimachia vulgaris, Thalictrum lucidum, Trifolium hybridum* subsp. *elegans, Sonchus arvensis* subsp. *uliginosus* etc.

R6401 Pannonian grasslands of *Corynephorus canescens* and *Festuca vaginata.* The habitat that is characteristic exclusively to the Carei (Nir) Plain throughout Romania, still preserves large areas of sand occupied by species having a priceless scientific value. It is one of the habitats with high conservative value in Romania (Doniţă et al. 2005). The sand dunes bordering the studied valley are in a continuously stage of decay, being planted with black locust forests or turned into agricultural lands, thereby being plowed. The habitat was identified in low clusters in the areas escaped from the attention of the farmers. Among the few remaining sandy species and particularly adapted to this habitat were identified the following: *Corynephorus canescens, Anthemis ruthenica, Jasione montana, Petrorhagia prolifera, Dianthus giganteiformis* subsp. *pontederae, Trifolium arvense, Rumex thyrsiflorus, Rumex acetosella, Silene otites* subsp. *hungarica* etc.

The research site described above has not yet been reported in previous works on the flora of Nir Plain (Carei Plain). This is due to its location very close to the border with Hungary, an area forbidden to research or any other human activities during the communist period, which also might have contributed to its preservation. The new species found here reinforce the assumption that this region was a glacial refuge as they are among the last withstanding species of those periods. The new relict species for the sandy region includes *Salix aurita, Luzula multiflora* and *Achillea ptarmica. Salix aurita* was indicated only in older works from the Ecedea moor, north of Carei city, which was one of the largest forested floodplain wetland transformed into agricultural land. The vegetation of this old swamp was characterized by the presence of many mountain species, such as *Pedicularis palustris, Carex flava, Carex davalliana* or *Cirsium palustre* (Karácsonyi 1987). One of the most important remnants of the postglacial period is *Angelica palustris*, a relict in the flora of Romania. Its presence in the swamps of Carei Plain at a considerable distance from other populations scattered throughout the Carpathians, brings a new argument for the relict character of the region. The newly discovered populations of *Betula pubescens* subsp. *carpatica, Carex nigra, Carex echinata, Betula pendula* etc. indicates they were quite common in the Nir region, and their disappearance was probably caused primarily by human activities.

To better highlight the relict character of the site presented in this paper, in the autumn of 2012 the following floristic analysis was made for the grove with birch trees and aspens. The plant species found strictly in this grove are shown in Table 3 together with their environmental requirements (U - humidity, T - temperature, R - soil pH), considering the importance of analyzing these species in the context of their bioforms and geoelements (Sanda et al. 1983).

Table 1. The glacial relicts discovered at Scărișoara Nouă (Carei Plain)

Nr.	Family	Species
	Relicts that are not usually found below the beech forest floor (most of these species are low altitude mountain elements, not being real glacial relicts at the country level)	
	Family	**Species**
1.	Betulaceae	*Betula pubescens* subsp. *carpatica* (Waldst. & Kit. ex Willd.) Asch. & Graebn.
2.	Apiaceae	*Angelica palustris* (Besser) Hoffm.- glacial relict in the Romanian flora
3.		*Peucedanum palustre* Moench
4.	Salicaceae	*Salix aurita* L.
5.	Asteraceae	*Cirsium rivulare* (Jacq.) All.
6.	Melanthiaceae	*Veratrum album* L.
7.	Cyperaceae	*Carex echinata* Murray
8.		*Carex nigra* (L.) Reichard
9.	Poaceae	*Calamagrostis canescens* (G.H.Weber)Roth - glacial relict in the Romanian flora
	Relicts that are not usually found below the sessile oak forest floor, found here at some of the lowest altitudes ever reported	
10.	Athyriaceae	*Athyrium filix-femina* (L.) Roth
11.	Dryopteridaceae	*Dryopteris carthusiana* (Vill.) H.P.Fuchs
12.	Betulaceae	*Betula pendula* Roth
13.	Rosaceae	*Potentilla erecta* (L.) Raeusch.
14.	Salicaceae	*Populus tremula* L.
15.		*Salix purpurea* L.
16.	Caprifoliaceae	*Succisa pratensis* Moench
17.	Asteraceae	*Achillea millefolium* L.
18.	Juncaceae	*Luzula multiflora* (Ehrh.) Lej.
19.	Cyperaceae	*Carex cespitosa* L.
20.		*Carex flacca* Schreb.
21.		*Carex ovalis* Gooden.
22.	Poaceae	*Agrostis canina* L.
23.		*Cynosurus cristatus* L.
24.		*Danthonia decumbens* (L.) DC.
25.		*Holcus lanatus* L.
	Other species that are usually encountered especially in mountainous areas, but here descend also at the oak forest floor level, with some exceptions even in the plains	
26.	Thelypteridaceae	*Thelypteris palustris* (A. Gray) Schott
27.	Caryophyllaceae	*Dianthus superbus* L.
28.	Rosaceae	*Sanguisorba officinalis* L.
29.	Salicaceae	*Salix rosmarinifolia* L.
30.	Asteraceae	*Achillea ptarmica* L.
31.	Iridaceae	*Iris sibirica* L.
32.	Cyperaceae	*Carex disticha* Huds.
33.		*Carex panicea* L.
34.	Poaceae	*Deschampsia cespitosa* (L.) P. Beauv.
35.		*Molinia caerulea* (L.) Moench

Table 2. Rare and protected species

Nr.	Family	Species	Observations
Rare and protected plant species. Species found within the studied site and the outlying sand dunes, listed in the Red Book (Dihoru & Negrean 2009) (A), Red Lists [(Oltean et al. 1994 (B), Boşcaiu et al. 1994 (C)], Carpathian Red List of endangered species (Witkowski et al. 2003) (D) and Natura 2000 (E)			
Species from national red lists			
1.	Urticaceae	*Urtica kioviensis* Rogow.	A, B
2.	Polygonaceae	*Rumex thyrsiflorus* Fingerh.	A, B, C
3.	Apiaceae	*Angelica palustris* (Besser) Hoffm.	A, B, C, D, E
4.		*Oenanthe aquatica* (L.) Poir.	C
5.		*Peucedanum palustre* Moench	C
6.	Salicaceae	*Salix rosmarinifolia* L.	B
7.	Asteraceae	*Achillea aspleniifolia* Vent.	D
8.		*Achillea ptarmica* L.	B
9.	Iridaceae	*Iris sibirica* L.	C, D
10.	Orchidaceae	*Orchis laxiflora* subsp. *elegans* (Heuff.) Soó	B
11.	Poaceae	*Corynephorus canescens* (L.) P. Beauv.	A, C, E
Rare and endangered plant species from Carei Plain (rare – rare species in the Romanian flora, R – glacial relicts and low altitude mountain elements in the plains of north-western Romania, EN – endangered in Carei Plain and northwestern Romania, T – typical to the sandy region of Carei Plain)			
12.	Thelypteridaceae	*Thelypteris palustris* (A. Gray) Schott	R, EN
13.	Betulaceae	*Betula pendula* Roth	R, EN, T
14.		*Betula pubescens* subsp. *carpatica* (Waldst. & Kit. ex Willd.) Asch. & Graebn.	R, EN
15.	Caryophyllaceae	*Dianthus giganteiformis* subsp. *pontederae* (A.Kern.) Soó	T
16.		*Dianthus superbus* L.	R, EN
17.		*Silene otites* subsp. *hungarica* Wrigley	rare, T
18.	Fabaceae	*Lathyrus palustris* L.	EN
19.	Euphorbiaceae	*Euphorbia lucida* Waldst. & Kit.	EN
20.		*Euphorbia palustris* L.	EN
21.	Violaceae	*Viola stagnina* Kit.	EN
22.	Salicaceae	*Salix aurita* L.	R, EN
23.	Lamiaceae	*Mentha arvensis* subsp. *austriaca* (Jacq.) Briq.	rare
24.	Rubiaceae	*Galium uliginosum* L.	EN
25.	Campanulaceae	*Jasione montana* L.	rare, T
26.	Asteraceae	*Sonchus arvensis* subsp. *uliginosus* (M.Bieb) Nyman	rare
27.		*Taraxacum palustre* (Lyons) Symons	rare, EN
28.	Melanthiaceae	*Veratrum album* L.	R, EN
29.	Cyperaceae	*Carex acuta* L.	EN
30.		*Carex cespitosa* L.	R, EN
31.		*Carex echinata* Murray	R, EN
32.		*Carex elata* All.	EN
33.		*Carex nigra* (L.) Reichard	R, EN
34.		*Scirpoides holoschoenus* (L.) Soják	T
35.	Poaceae	*Calamagrostis canescens* (G.H.Weber) Roth	R, EN
36.		*Festuca arundinacea* subsp. *orientalis* (Hack.) Tzvelev	EN
37.		*Molinia caerulea* (L.) Moench	R, EN

Table 3. List of species found at the relict site of Scărişoara Nouă

Family	Species	Ecological indices
Equisetaceae	*Equisetum arvense* L.	G, Cosm;U3 T3 R0
Thelypteridaceae	*Thelypteris palustris* Schott	HH, Cp; U4 T0 R3
Dryopteridaceae	*Dryopteris carthusiana* (Vill.) H. P. Fuchs	H,Cp; U4 T3,5 R0
Cannabaceae	*Humulus lupulus* L.	H, Eua; U3,5 T3 R4
Urticaceae	*Urtica kioviensis* Rogow.	H,Pont; U4,5 T3,5 R4
Fagaceae	*Quercus robur* L.	MM,Eur;U3,5 T3 R0
Betulaceae	*Betula pendula* Roth	MM, Eua; U3 T2 R2
	Betula pubescens Ehrh. subsp. *carpatica* (Waldst. et Kit. ex.Willd.) Asch. et Graeb.	MM-M, Eua; U4,5 T2 R2
Polygonaceae	*Polygonum lapathifolium* L.	Th,Cosm; U4 T0 R3
Rosaceae	*Crataegus monogyna* Jacq.	M, Eur; U2,5 T3 R3
	Potentilla erecta (L.) Raeusch.	H,Eua;U0 T0 R0
	Rosa canina L.	N,Eur;U2 T3 R3
	Rubus caesius L.	H,Eua;U4,5 T3 R4
	Sanguisorba officinalis L.	H,Eua(bor); U3 T3 R0
Fabaceae	*Lathyrus palustris* L.	H,Cp; U5 T0 R4,5
	Vicia cracca L.	H,Eua;U3 T0 R3
Lythraceae	*Lythrum salicaria* L.	H,Cosm;U4 T3 R0
Cornaceae	*Cornus sanguinea* L.	M,Euc;U3 T3 R4
Santalaceae	*Viscum album* L.	Ch (N), Eua; U3,5 T3 R0
Rhamnaceae	*Frangula alnus* Mill.	M, Eua; U4 T3 R3
Apiaceae	*Angelica sylvestris* L.	H, Eua; U4 T3 R3
	Peucedanum palustre (L.) Moench	H,Eua; U5 T3 R0
Hypericaceae	*Hypericum tetrapterum* Fr.	H,Eur; U4 T3 R4
Salicaceae	*Populus canescens* (Aiton) Sm.	MM-M, Eua; U3,5 T3 R3
	Populus tremula L.	MM,Eua;U3 T2 R2
	Salix cinerea L.	M, Eua; U5 T3 R3
	Salix rosmarinifolia L.	M, Eua; U4 T0 R0
Primulaceae	*Lysimachia vulgaris* L.	H,Eua;U5 T0 R0
Oleaceae	*Fraxinus angustifolia* Vahl subsp. *danubialis* Pouzar	MM,Pont-Pan; U4,5 T4 R4,5
Convolvulaceae	*Calystegia sepium* (L.) R.Br.	H,Eua;U4 T3 R4
Boraginaceae	*Symphytum officinale* L.	H, Eua, U 4 T 3 R 0
Lamiaceae	*Lycopus europaeus* L.	HH, Eua; U5 T3 R0
	Prunella vulgaris L.	H,Cp;U3 T3 R0
	Scutellaria galericulata L.	H,Cp; U4 T3 R4
	Stachys palustris L.	H(G), Cp; U4 T3 R4
Plantaginaceae	*Veronica chamaedrys* L.	H-Ch,Eua;U3 T0 R0
Solanaceae	*Solanum dulcamara* L.	Ch(N), Eua; U4,5 T3 R4
Rubiaceae	*Galium palustre* L.	H, Cp; U5 T3 R0
	Galium uliginosum L.	H,Eua; U4,5 T3 R4
Adoxaceae	*Viburnum opulus* L.	M, Cp; U4 T3 R4
Caprifoliaceae	*Valeriana officinalis* L.	H,Eua(Med);U4 T3 R4
	Succisa pratensis Moench	H,Eua; U4 T3 R0
Asteraceae	*Achillea millefolium* L.	H,Eua;U3 T0 R0
	Bidens tripartita L.	Th, Eua; U 4,5 T 3 R 0
	Cirsium rivulare (Jacq.) All.	H,Euc; U4 T3,5 R0
	Eupatorium cannabinum L.	H,Eua;U4 T3 R0

Juncaceae	*Juncus effusus* L.	H, Cosm; U4,5 T3 R3
Cyperaceae	*Carex acuta* L.	HH-G, Eua; U5 T3 R0
	Carex acutiformis Ehrh.	HH, Eua(Med); U6 T3 R4
	Carex hirta L.	G, Eur(Med); U0 T3 R0
	Scirpus sylvaticus L.	HH(G), Cp; U4,5 T3 R0
Poaceae	*Agrostis canina* L.	H,Eua; U3,5 T3 R3
	Leersia oryzoides (L.) Sw.	HH, Cp; U6 T3 R0
	Molinia caerulea (L.) Moench	H, Eua; U4 T3 R0
	Phragmites australis (Cav.) Steud.	HH, Cosm; U5 T0 R4
	Poa palustris L.	H, Cp; U5 T3 R4
BRYOPSIDA		
Polytrichaceae	*Atrichum undulatum* (Hedw.) P. Beauv.	Cp
Mniaceae	*Plagiomnium rostratum* (Schrad.) T.J. Kop.	Cp
	Plagiomnium ellipticum (Brid.) T.J.Kop.	Cp
	Plagiomnium undulatum (Hedw.) T.J. Kop.	Cp
Amblystegiaceae	*Amblystegium serpens* (Hedw.) Schimp.	Cp
	Leptodictyum riparium (Hedw.) Warnst.	Cp
Brachyteciaceae	*Brachytecium rutabulum* (Hedw.) Schimp.	Cp
	Cirriphyllum piliferum (Hedw.) Grout	Cp
	Eurhynchium hians (Hedw.) Sande Lac.	Cp
Climaciaceae	*Climacium dendroides* (Hedw.) F. Weber & D. Mohr	Cp

Abbreviations: (MM - megaphanerophytes, M - mesophanerophytes, N - nanophanerophytes, Ch - chamaephytes, H - hemicryptophytes, HH - helohidatophytes, G - geophytes, Th – annual therophytes, Eur - European , Eua - Eurasian Euc - Central European, Pont - Pontic, Pont-Pan - Ponto-Pannonian, Cp (Circ) - Circumpolar, Cosm. – Cosmopolitan, Adv – Adventive, U2-U2,5 - xero-mesophyle, U3-U3,5 - mesophyle, U4-U4,5 - mesohydrophyle, U5- hygrophyle, U6 – hydrophyle, U0 - amphitolerant, T2 - microtherm, T3-T3,5 - micromesothermic, T4 - moderately mesotherms, T0 – amphitolerant, R2 - acidophiles, R3 - acid-neutrophile, R4 - R4,5 - weak acid-neutrophile, R0 - amphitolerant).

The analysis of the bioforms is particularly important because it provides information about the balance of the phytocoenoses with the entire environmental conditions and the specificities of the sites, giving us paleoecological information upon the flora and vegetation of the region, but also revealing the influence of the human factor (Cristea et al. 2004). In Figure 2 we notice the predominance of the hemicryptophytes (H) with a percentage of 51.72%, which is characteristic of the temperate zone within which the research site is located. Also, the large number of woody species (24.12%) indicates a natural place, characterized by very little disturbance as a result of human activity. The importance of woody species increases significantly if we consider both their unusual environmental requirements in the lowland and that most of these natural riverside thickets are endangered in Europe. The percentage of 12.06% of helohidatophytes (HH) emphasises the excessive moisture of this place. The extremely low number of annual therophytes (Th) – 2, also indicates an unspoiled, well preserved natural place, they of course, at this location, are aquatic (*Bidens tripartita* and *Polygonum lapathifolium*).

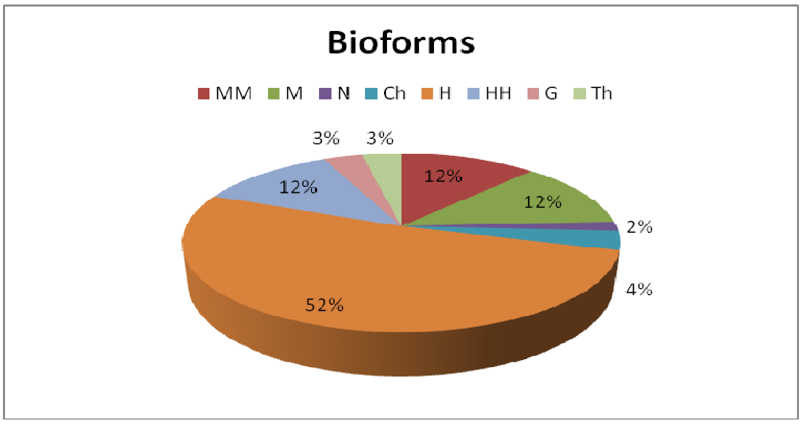

Fig. 2. Analysis of bioforms of flora near Scărişoara Nouă
(abbreviations same as in Table 3)

The geoelements are categories of plant species that are more or less phylogenetically distant, but in the process of speciation have occupied the same geographical region, following specific routes of migration and the phytocoenosis integration leading to the present species composition of the researched site. The evolutionary implications of this study are particularly interesting as the species presented here are either: far away from their original homeland, have survived in the studied region from long gone geological eras, arrived in more recent times, or they evolved locally (Cristea 1991). The species found at this site also provide information about the macro- and microclimates in which the species have developed and bring further arguments in support of protecting these areas with special phytogeographical significance (Cristea et al. 2004).

To learn more about the history of this location we also took into account the moss species found here, which later confirmed the relict character of the location. Figure 3 provides data for this purpose. The predominance of the Eurasian species (Eua - 46.96%), normal for the region, was observed. In contrast stands the unusually high number of circumpolar species (Cp - 30.30%) from a station in the plains. These species are characteristic of mountain regions, finding shelter here in the ambience created by the woody species or in the microclimates created along watercourses or streams. The swamps of the Nir Plain interdunes appear to be almost perfect for the conservation of the above mentioned species. The conservational importance of this site is increased due to the presence of the pontic species (Pont) *Urtica kioviensis*, now extremely rare in our country's flora, and the woody ponto-pannonian (Pont-Pan) subspecies *Fraxinus angustifolia* subsp. *danubialis* that provides clues about the floristic region in which we are, namely the pannonian region. Again, the small number of adventive species is due to the low impact of human activities on the site presented by this study. There are only two species (*Prunus serotina, Quercus palustris*) that were probably brought over by animal seed dispersers, due to their nutritious fruits. Both species have similar ecological requirements at the studied site and adapt easily, but the wild black cherry (*Prunus serotina*) was also found in the permanent wetland areas as noted in other places in Nir Plain (Szatmari 2012). I notice this issue since, within the most native areas, the wild black cherry is behaving differently, avoiding water ponding (Marquis, 1990). At the Scărişoara Nouă station the wild black cherry becomes invasive, although currently most individuals are seedlings, but they can threaten the biodiversity in the future (Anastasiu & Negrean 2007). It may cause unfavorable competition with the relict species, which has been reported from other parts of Europe (Robakowski & Bielinis 2011), and in Hungary (Juhász Kocsis & Bagi 2007, Zoltan-Botta & Balogh 2008).

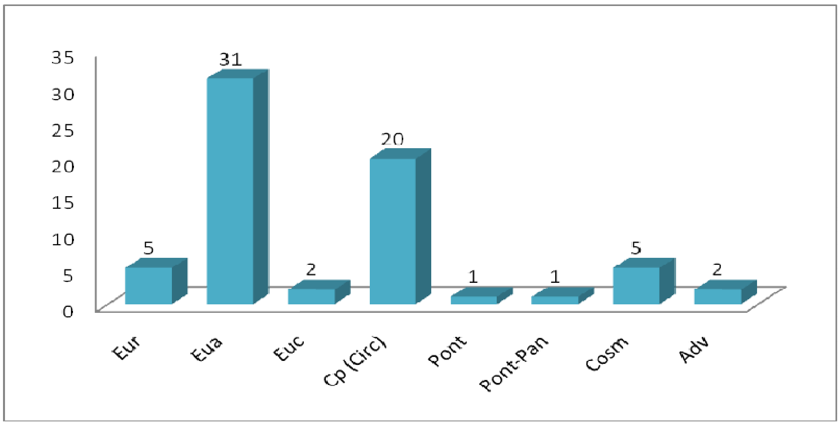

Fig. 3. Analysis of geoelements of flora near Scărişoara Nouă (abbreviations same as in Table 3)

The ecological indices from Figure 4 provides new data about the researched site. Thus, the behavior of the species towards humidity (U) indicates the predominance of the mesohydrophyle species (U4-U4.5) (48.21%). They are joined by mesophyle (U3-U3.5) and hydrophyle (U5) species. The many moisture-loving species indicates that groundwater is near the surface. According to temperature (T) preferences, the predominant species are the micromesothermic (T3-T3.5), which prefer moderate temperatures. These indicator species prefer moist or partially shaded places. Most species are typical to marshes and forests. The second place is occupied by eurythermic species (T0) that supports wide temperature oscillations. Among them are: *Thelypteris palustris, Salix rosmarinifolia, Potentilla erecta* or *Achillea millefolium* which usually prefer mountain regions. On the third place are the microtherm species (T2-T2.5), truly remarkable in lowland areas. The most unusual is that the three microtherm species form the upper floor of the canopy, represented by tree species: *Betula pendula, Betula pubescens* subsp. *carpatica* and *Populus tremula*, an irrefutable argument for the relict origin of the studied site. According to soil pH reaction (R), the investigated flora has a mainly weak acid-neutrophil character (R4-R4.5) - 30.35%. Their number is exceeded by species that tolerate a wide range of soil pH - R0) - 44.64%, which have been adapted to a wide ecological range of this parameter. Typically, these species may have an invasive character, but not at the moment because the amphitolerant species that are found here, are either swamp or mountain species at a lower altitude. If we consider that argument, the idea of survival of the mountain species in the lowlands is quite plausible.

They, not being related to a particular type of soil, had more chance of surviving if moisture remains constant throughout the year. Among these we have noticed *Peucedanum palustre, Dryopteris carthusiana, Cirsium rivulare, Salix rosmarinifolia* or *Molinia caerulea*. They are followed in smaller percentages by the acid-neutrophil species (R3-R3.5 - 19.64%) and the acidophiles (R2-R2.5 - 5.35%). Once again, the fact that the dominant woody or herbaceous species that fall within this category raises some questions (*Betula pendula, Betula pubescens* subsp. *carpatica, Populus tremula* – R2, *Salix cinerea, Frangula alnus, Thelypteris palustris, Agrostis canina, Juncus effusus* etc. – R3). The soil acidity formed under the influence of shallow groundwater facilitated the perpetuation of these mountain species. So, the combination of several factors together has created a true refuge for the mountain species which continue to survive here since the last glacial period.

The conclusion would be that since this ecological niche could not have been exploited by other tree or herbaceous species, the competition between them became almost zero, so the mountain species have managed to grow unhindered.

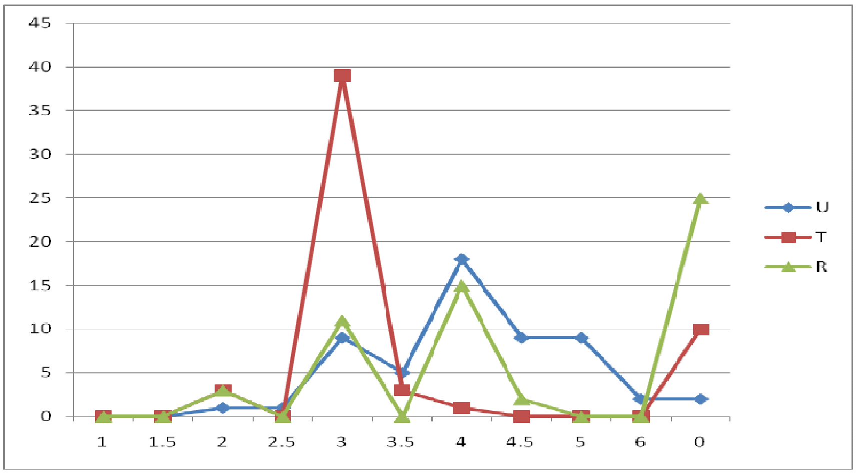

Fig. 4. The ecological analysis of the flora from the place at Scărişoara Nouă with relict character (Abbreviations same from Table 3. + U1-U1.5 – xerophytes,T1-T1.5 – cryophiles, T2.5 – microtherms, T4.5 – moderately mesotherms, T5 – thermophiles, R1-R1.5 – strong acidophiles, R2.5 – acidophiles, R3.5 – acido-neutrophiles, R5 – basiphilous-neutrophiles)

Another element to observe at the unusual species in the lowlands is that the study of their areas and the spread limits in our country. Thus, here we have species that are not usually found below the altitude limit of the spread of beech and sessile oak forests (Ciocârlan 2009). The region in which Scărişoara Nouă, and almost all of the Nir (Carei) Plain are within the forest steppe zone between 50-150 m altitude. The oak sublevel starts at 100 m (Doniţă et al. 2005). In Carei Plain there are exceptions regarding the spread of oak forests, which once occupied a large part. Therefore, there is a gap in the northwestern part of the country, many woody species defining the forests at lower elevations. For this reason, many forest remnants are composed of a mixture of species from the forest steppe and the oak floor. Instead the species of sessile oak sublevel (300-600 m) or beech forests (600-1200 m) (Ciocârlan 2009) were unable to take hold in the studied area by subsequent colonization, but only by their perpetuation since the ice age. Since the station at Scărişoara Nouă is located at 142 m altitude, the colonization of mountain species as adventive plants in the area is excluded. In the first table (Table 1) are shown the areas of distribution of the species considered mountain relicts in the lowlands, according to the *Flora Ilustrată a României* (Ciocârlan 2009).

The character of a relict station is highlighted also by the remarkable age of the species of birch and aspen, or white poplar and willow species nearby. Also the associations edified by *Carex acuta* and *Molinia caerulea* describe an archaic vegetation, yet unaltered in the region. The presence of the species *Molinia caerulea, Succisa pratensis* and *Sanguisorba officinalis* indicates the final stage of the marshes with permanent water, namely the lowering groundwater. Instead, the presence of *Carex* species, *Thelypteris palustris, Cirsium rivulare* and of *Betula* spp., suggests a significant amount of groundwater that reaches the surface, where these species exist. The fauna of this place is probably also remarkable, requiring some more detailed studies in the future. The presence of the mountain lizard (*Zootoca vivipara*) at this location, further underlines the fact that these plants did not reach this area later, and they remained here since the last ice age. Also in the nearby Vermeş Swamp there are two relict species of amphibians: *Rana lessonae* and *Rana arvalis* (Covaciu-Marcov et al. 2009).

Conclusion

The Carei Plain (Nir Plain) is a very special geographical region, profoundly different from the surrounding areas. Across the region are found a whole range of rare, protected or unusual plants, animals, fungi etc. This is why the plain received the status of Natura 2000 protected site (Ardelean & Karácsonyi 2005). Among the unusual plant and animal species stand out the steppe and glacial relicts, which coexist here together. This paper brings new data and arguments upon the spread and survival of these species. They managed to survive here until today because they had similar ecological conditions in the mountains, the swamps surrounded by forests maintaining the coolness and humidity they needed to remain alive. Noteworthy is the fact that they live here in places with much higher humidity that in the mountains, thus compensating the lower rainfall (Karácsonyi 1987).

The assumption of their coming as pioneer species is less likely because of the significant distance from the nearby mountain regions and because of their problematical ecological conditions, like the peat moss. Most likely during the warm period after the glaciation not all the marshes completely dried out in the region, forming islands of relict vegetation around them. These pockets of vegetation spread out rapidly across the region after the beech forest descended to the plains and also they retreated back to the marshes after the new warming period.

As mentioned in Table 1, the species listed here are not glacial relicts at the country level, only to the plains of northwestern Romania. Some authors call them low altitude mountain elements (Karácsonyi 1987). The nationwide glacial relicts, found here, are only *Angelica palustris* and *Calamagrostis canescens*, but the rest of the plain conserves numerous other glacial relicts from Romania (Pop 1976). The others are species found almost exclusively in the mountain regions, encountered only in exceptional cases under the beech or sessile oak forests floors.

In some swamped interdunes near Scărişoara Nouă were also found remarkable plant species, which grow probably near the previously described place, as the mountain or glacial relicts in Romania and Hungary (Karácsonyi 1987). Among them stands the glacial relict *Spiraea salicifolia* which is found only in the Carpathian Mountains at high altitudes, but here is of uncertain origin. Another low station for *Spiraea salicifolia* that I found was near Livada in Oaş, recorded also by Carol Karácsonyi (Karácsonyi 1995). The number of low-altitude mountain species, reported from the area of Scărişoara Nouă is astounding: *Menyanthes trifoliata, Epilobium roseum, Galium boreale, Carex appropinquata* and *Calamagrostis stricta* [the last two plants are glacial relicts in Romania (Pop 1976, Karácsonyi 1987)], *Carex hartmanii, Carex rostrata, Carex viridula, Calamagrostis canescens* etc. (Ardelean & Karácsonyi 2005). Numerous other relict species are found in this plain: peat moss (*Sphagnum* spp.), *Trollius europaeus, Alnus incana, Eriophorum angustifolium, Eriophorum latifolium, Equisetum fluviatile* etc., some of them forming mountainous plant associations in the lowlands such as: *Carici-Calamagrostetum neglectae* Soó (1938) 1971, unique in the country, *Carici-Menyanthetum* Soó (1938) 1955, *Salici cinereae-Sphagnetum recurvi* (Zólyomi 1931) Soó 1954, *Calamagrostio-Salicetum cinereae* Soó et Zólyomi 1955 etc. (Karácsonyi 1987). This suggests that in the area are swamps which still preserve the archaic and relict vegetation of the northwestern part of Romania, and indeed the vegetation is less impacted by human activities than in other parts of Nir (Carei) Plain. Therefore, further research studies are required within the area, both from botanical and zoological points of view, and stricter protection by the authorities to prevent further damage like the wildfires that took place in spring 2012.

Acknowledgements. Our study was supported by the Foundation of Freies Europa Weltanschauung, the custodian of the Carei Plain Natural Protected Area, to which we thank this way, as well as the team of university professors from the Biology Department of the University of Oradea (Dr. Severus-Daniel Covaciu-Marcov, Dr. Alfred-Ştefan Cicort-Lucaciu, Dr. István Sas) and especially to Dr. Carol Karácsonyi, with his help I could pursue the researches in the area. I also want to thank to Dr. Ioana Popescu from the Biology Department at Drury University, Springfield, Missouri, for the many suggestions provided. The study is part of the researches dedicated to the inventory of biodiversity of the Carei Plain Natural Protected Area, the results obtained and presented here will serve to protect the natural area included in the Natura 2000 network.

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Acta Horti Bot. Bucurest. 2015, **42**: 41-55
DOI: 10.1515/ahbb-2015-0005

FRITILLARIA MELEAGROIDES IN ROMANIA

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Abstract: The presence in the spontaneous flora of Romania of the species *Fritillaria meleagroides* is reported in this paper. It was identified in the North of the village Cotu Morii (Popricani Commune, Iaşi County), on (meso-) hygrophilous, slightly halophilous meadows, placed on the both sides of an old meander of the Jijia river. This place is at the western limit of natural area of *F. meleagroides*, which stretches from the Central Asia to the Eastern Europe (European Russia, Ukraine and Bulgaria). Since *F. meleagroides* is an endangered species in large part of its natural range, we consider that urgent actions to protect its populations from Romania are necessary.
Keywords: botanical description, identification key, marginal populations, new records, vascular flora

Received 26 November 2015 *Accepted 05 December 2015*

Introduction

The vascular flora of Romania has been studied of approx. two centuries by numerous botanists. However, the research of the flora still can give us nice surprises and new recordings of species, both indigenous and adventive, have been done in the last decades, increasing the inventory of the vascular flora of Romania (Oprea 2005, Ciocârlan 2009, Sârbu et al. 2013).

One of the genera from the vascular flora of Romania is *Fritillaria* L. (Liliaceae) (Zahariadi 1966, Ciocârlan 2009, Sârbu et al. 2013). This genus comprises between 100 and 160 species (depending on the points of view of different authors), occurring in most temperate regions of the Northern Hemisphere (Zahariadi 1966, Rønsted et al. 2005, Tomović et al. 2007, Türktaş et al. 2012, Karakaş Metin et al. 2013).

In the most recent classification, which is supported by the molecular phylogenetic data, eight subgenera of *Fritillaria* have been separated (Rønsted et al. 2005). Among these, the subgenus *Fritillaria* (with subglobose bulbs, usually consisting of two fleshy scales, more or less tunicated by the translucent remains of the previous years scales) is the largest one, widely distributed from the Western Europe and the Mediterranean region to Eastern Asia (Rønsted et al. 2005, Peruzzi et al. 2008). According to Rønsted et al. (2005), species of this subgenus with a trifid style are

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included in the section *Fritillaria*, while species with an undivided style are included in the section *Olostylea* Boiss.

Two species of the genus, both of them belonging to the section *Fritillaria*, have been known in the spontaneous flora of Romania, till now, namely (Zahariadi 1966, Sârbu et al. 2013): *F. meleagris* L. and *F. montana* Hoppe ex. W. D. J. Koch (syn.: *F. degeniana* J. Wagner; *F. orientalis* auct., non Adam; *F. tenella* auct., non M. Bieb.).

Fritillaria meleagris occurs in the wild both in Europe (from the Great Britain and Central Russia south to the Southern Alps and Western Balkans) (Lozina-Lozinskaya 1935, Zahariadi 1966, Rix 1980, Sârbu et al. 2013) and Asia (Western Siberia and Altai) (Vlasova 1987). It has been mentioned in Romania since the first decades of the 19th century (Baumgarten 1816). It is a (meso)-hygrophilous species (Sârbu et al. 2013) spread sporadically, from the steppe zone, up to the spruce forest belt, through meadows, thickets, coppices or open deciduous forests (Zahariadi 1966, Csergő & Frink 2003, Oprea 2005; Sârbu et al. 2013), in the structure of plant communities from various alliances, such as: *Potentillion anserinae* R. Tx. 1947 (Ularu & Parascan 1970, Sămărghiţan & Oroian 2011, Sârbu et al. 2013), *Molinion* W. Koch 1926, *Calthion* Tx. 1937, *Magnocaricion* W Koch 1926 (Csergő & Frink 2003), *Alnion incanae* Pawłowski, Sokołowski *et* Wallisch 1928 (Bujorean & Grigore 1965, Maloş et al. 1967, Ularu & Parascan 1970), or *Salicion cinereae* Müller *et* Görs *ex* Passarge 1961 (Bujorean & Grigore 1965, Ularu & Parascan 1970).

Its populations have been greatly affected by the massive collecting during flowering time, as well as by the anthropogenic disturbance of habitats (Pop & Sălăgeanu 1965, Csergő & Frink 2003). Therefore, in order to protect its populations, the species was declared a "monument of nature" in Romania, being protected by law (Pop & Sălăgeanu 1965, Zahariadi 1966).

Fritillaria montana is native in the South-Eastern Europe (Zahariadi 1966, Peruzzi et al. 2008, Mancuso et al. 2012) or in the Pontic-Mediterranean area (Sârbu et al. 2013). It has been reported in Romania (under various names) since the first half of the 19th century (1844) (Simonkai 1886). Like in other European countries (see comments in Peruzzi et al. 2008, Peruzzi & Bartolucci 2009), this species has a complicated taxonomic history in Romania. Authors from the 19th century (Heuffel 1858, Fuss 1866, Schur 1866, Kanitz 1879-1881, Brândză 1879-1883, Simonkai 1886, Grecescu 1898) mentioned it using the misapplied name of *F. tenella* M. Bieb., for which they gave several synonyms (including *F. montana*). Prodan (1939) noticed for the first time that the plant from Romania is not *F. tenella* M. Bieb., reporting it as "*F. degeniana* J. Wagner (*F. tenella* auct.)". The name of *F. montana* was for the first time used correctly in the Romanian literature by Zahariadi (1966). This author also demonstrated, by analyzing herbarium specimens, that *F. montana* Hoppe and *F. orientalis* Adam (syn. *F. tenella* M. Bieb.) are two distinct species, as they actually were also treated in the Flora of USSR (Lozina-Lozinskaya 1935), and that the latter (an East Pontic species from the Caucasus Mountains) do not grow in Romania. Subsequently, however, with some exceptions (Sârbu et al. 2013), the species continued to be erroneously reported in Romania, either as *F. tenella* Bieb. (Beldie 1979) or as *F. orientalis* Adams (Ciocârlan 2009), probably (in the second case) under the influence of the erroneous taxonomic treatment of the Flora Europaea (Rix 1980).

Fritillaria montana is a mesophilous species spread sporadically in Romania, through shrubs and deciduous forests, from the forest steppe zone, up to the beech forest belt (Zahariadi 1966, Sârbu et al. 2013). It grows in plant communities of the orders *Quercetalia pubescentis* Br.-Bl. (1931) 1932 and *Orno-Cotinetalia* Jakucs 1961 (Chifu et al. 2006, Sârbu et al. 2013), or of the alliances *Alnion incanae* Pawłowski, Sokołowski et Wallisch 1928 and *Lathyro hallersteinii-Carpinion* Boşcaiu 1979 (Chifu et al. 2006).

Although both *F. meleagris* and *F. montana* are included in some national red lists, as vulnerable or rare (e.g. Oltean et al. 1994, Sârbu & Chifu 2001) and the latter is included in the European list of threatened species which are present in Romania (Sârbu et al. 2003), however, none of them are listed in The Red Book of vascular plants of Romania (Dihoru & Negrean 2009).

In the spring of 2015, we identified another spontaneous species of *Fritillaria* unknown in Romania till now, which is reported in this paper.

Material and methods

The species was recorded during our recent field work (2015), in the North East Romania. Specimens collected on the field were deposited in the herbarium of the University of Agricultural Sciences and Veterinary Medicine Iaşi (IASI), as well as in the herbarium of the Botanic Garden "Anastasie Fătu", University "Alexandru Ioan Cuza" of Iaşi (IAGB). The geographic coordinates were recorded using an eTrex Legend HCx GPS system. The species was identified using several standard floras (Lozina-Lozinskaya 1935, Yanev 1964, Rix 1980, Chen & Mordak 2000), but it was also checked in other references (Ledebour 1830a, b, 1853, Pachoskiy 1914, Artiushenko 1979, Prokudin et al. 1987, Hill 2008, 2010) and compared to its original diagnosis (Schultes & Schulthes 1829). Morphological characters of 10 specimens were analyzed both on the field and in the above mentioned herbaria. Seed sections were prepared and analysed using an Optica B-350 microscope, in order to examine the membranous edge of the seed. Biological and ecological features of the species were noted in the field and compared with data from references. The nomenclature of the cited plant taxa follows Sârbu et al. (2013), except the species for the first time reported in the flora of Romania (Rix 1980).

Results and discussion

Fritillaria meleagroides Patrin ex Schult. & Schult. fil., unknown in the flora of Romania until now, has been identified (on April, 23rd, 2015), in the North-Eastern Romania, to the North of the village Cotu Morii (Popricani commune, Iaşi county), and to the East of the fish farm "Acvares", on both sides of an old meander of the Jijia river (Fig. 1 and 2). This meander, forming two unequal sinuosities (a smaller one toward the Jijia river, and a larger one, on the opposite side), is located between a dike delimiting eastward the ponds of the "Acvares" farm and the collector channel, ± parallel to the dike. On that area, we have identified three populations of *F. meleagroides*. A smaller one of approx. 50 specimens has been located on the meadow between the smaller sinuosity of the meander and the collector channel (N47º19'0.05", E27º33'4.88"). Other two populations of several hundred specimens each, have been located northward, on the one side (N47º19'23.59", E27º33'6.23"), respectively on the other side (N47º19'35.58", E27º33'9.01") of the terminal sinuosity of the meander.

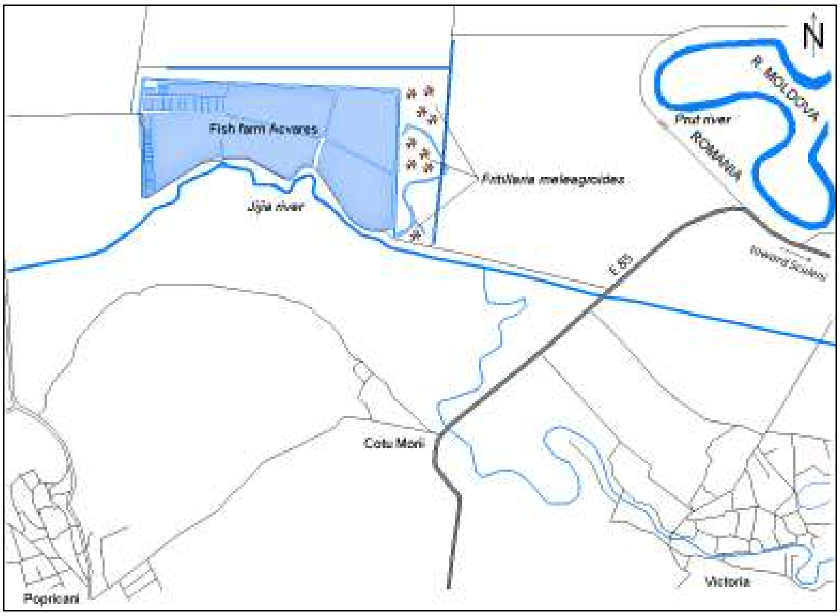

Fig. 1. Map indicating the location of *F. meleagroides* in the NE Romania

Fig. 2. The old meander of Jijia river, within the studied area

Fig. 3. *Fritillaria meleagroides* from NE Romania: A-general habitus; B-the flower.
Scale bar: 1 cm (A), 0.5 cm (B)

Fig. 4. Flowers of *F. meleagroides* from herbarium specimens. Scale bar: 1 cm

Botanical description. The following description of the species is based on the specimens examined on the field and herbarium specimens (IASI 17977; IAGB 47674, 47675; leg. A. Oprea, C. Sîrbu; det. M. Peregrym, C. Sîrbu, A. Oprea) collected from the above mentioned locality.

Perennial. Bulb ± globose, 10-12 mm in diameter, with a whitish-brown, translucent tunic. Stems 28-50 cm long, glabrous, smooth, green-glaucous, sometimes purple spotted, internodes very unequal. Leaves 5-7, alternate, sessile, linear, attenuate toward apex, glabrous, the lowermost 11-14 × 5-8 mm, the above gradually smaller.

Flower (Figs 3, 4) solitary, campanulate, nodding; peduncle 2.9-9 cm long. The ratio between the length of the peduncle and the bracteant (upper) leaf is variable, between 0.5 and 2. Tepals 25-28 mm long, sub equal, blackish-purple outside, greenish inside, obscurely (faintly) tessellated, the outer ones oblong-elliptic, 8-11 mm wide, the inner ones obovate, wider (12-18 mm). Nectaries (on the adaxial surface of tepals) greenish yellow, linear, of 12-18 × 1-1.5 mm, placed at 4-5 mm above the base of the tepals. The outer gibbosity of tepals, corresponding with the inner nectariferous fovea, weak developed. Stamens slightly longer than 1/2 of tepals; filaments papillose, 7-11 mm long, slightly widened towards the base; anthers yellow, basifixed, 4-9 × 1.5-2 mm (the open anthers are shorter than the ones unopened yet !). Ovary 3-sided, 7-9 mm long; style 5-7 mm long, papillose, 3-branched, the branches 3-4 mm long, papillose. Capsule (Fig. 5) erect at ripening, obtusely 3-angled, wingless, 15-35 × 8-12 mm, strongly narrowed at the bottom (on a length of 1-4 mm). Seeds (Fig. 6) numerous, in two rows per locule, yellowish brown, triangular-ovate, 4-6 × 3-4 mm, flattened (0.3-0.4 mm thickness, in the central region), with a membranous swollen edge, filled with air, as a tire.

General distribution. The natural range of *F. meleagroides* stretches from Northwest China (Xinjiang), through Central Asia (Altai, Kazakhstan, Western Siberia), Northwest Caucasus, to Eastern Europe (Lozina-Lozinskaya 1935, Yanev 1964, Chen & Mordak 2000, Korotchenko & Orlov 2009). In the neighbourhood of Romania, *F. meleagroides* has been reported in the central, eastern and southern regions of Ukraine (Lozina-Lozinskaya 1935, Rix 1980, Prokudin et al. 1987, Korotchenko & Orlov 2009), as well as in western Bulgaria (Yanev 1964, Rix 1980, Andreev 1993, Assyov & Petrova 2006, Petrova & Vladimirov 2009, Ivanova 2015).

The presence of this species in the Republic of Moldova (Geideman 1986, Negru 2002, 2007, Ciubotaru et al. 2007), although very probable, has been recently invalidated by Pînzariu & Sîrbu (2014). Both in the "Plant world of the Republic of Moldova", IV, p. 33 (Ciubotaru et al. 2007) and "The Red Book of the Republic of Moldova", p. 78 (Negru 2002), the description and iconography of the plant named *F. meleagroides* are rather suitable for *F. montana* (by upper leaves opposite or verticillate). In addition, the "Identification book of plants from the Republic of Moldova" (Negru 2007) contains the name "*F. meleagroides*" in the alphabetical index of taxa, but on the page indicated in the text, instead of *F. meleagroides*, another species (*F. meleagris*) is mentioned. Therefore, without other reliable data, it seems reasonable that, according to Pînzariu & Sîrbu (2014), *F. meleagroides* has been erroneously referred from this country, at least in some publications, by confusion with *F. montana* or with *F. ruthenica*.

Plant variability. Table 1 (columns 1-3) presents some data on the morphological variability of the species *F. meleagroides* resulted from the analysis of

10 specimens from Romania. Data from botanical references were also presented in the table (columns 4-9), for comparison. According to our data, the next morphological characters of *F. meleagroides* populations from Romania had a more pronounced variability (i.e. CV% > 15%): the plant height, the length of stem internodes, the width of lowermost leaf, the ratio between the length of the peduncle and the bracteant leaf, the length and width of capsule, the length of the narrowed basal region of the capsule.

The morphological variation of specimens analyzed from Romania generally fall within the limits described in the botanical references (Table 1), with the next interesting exceptions:

- tepals are wider as against data from references: 8-11 mm instead of 5-9 mm (outer tepals), respectively 12-18 mm instead of 7-12 mm (inner tepals);
- nectaries of variable length, between 12 and 18 mm (compared with 15 mm, data from the literature) and narrower (1-1.5 mm) than 2 mm (data from the literature);
- seeds are smaller (4-6 mm × 3-4), as against the literature data (6-6.6 × 4.8-5.2 mm).

Despite these differences, we are convinced that the plants identified by us belong to the species *F. meleagroides*. Populations from Romania are localized, as we shown, at the western border of the natural range of the species. Therefore, as in the case of many other species, a large morphological variability of the marginal populations, against of those from the inside of the distribution range, can be expected [see, for instance, Tomović et al. (2007), or for an extensive description of the topic, Crawford (2008)].

Taxonomic notes and identification key. *Fritillaria meleagroides* has been described in 1829 by Schultes & Schultes fil., in Roemer & Schultes, Systema Vegetabilium, 7: 395-396. Type of *F. meleagroides* (collector Patrin, 1791, in montibus Altai) is stored on the Muséum National d'Histoire Naturelle (Paris), P00730799 (GPD 2015).

It seems that the first reliable illustration of this species has been made by Ledebour (1830b), in the "Icones plantarum ... ", under the name of *F. minor*. In the Flora of USSR, Vol. 4, pp. 306, Lozina-Lozinskaya (1935) treated the name *F. minor* Ledeb. as a synonym for *F. meleagroides*, according to the principle of priority. However the diagnosis published by Ledebour (1830a) in "Flora Altaica" for his *F. minor* includes some characters which cannot be recognized at the plant illustrated by the same author in the "Icones plantarum ... " (Ledebour 1830b) and which are specific rather for *F. ruthenica* Wikst.: "Folia …, apice plerumque incurvo …; duo inferiora nonnunquam subopposita …". For this reason, probably, in the Flora Europaea, Vol. 5 (Rix 1980), the name *F. minor* is accepted as a synonym not for *F. meleagroides*, but for *F. ruthenica*. However, by its upper leaves (bracts) verticillate and twisted (with a tendril-like apex) (Lozina-Lozinskaya 1935, Rix 1980), *F. ruthenica* obviously differs from the iconography of "*F. minor*" published by Ledebour (1830b), as well as from the "*F. minor*" specimens collected by the same author from Altai (see herbarium specimens digitized by the GPD 2015). In the meantime, reliable iconography of the species *F. meleagroides* has been enriched by the contributions of Yanev (1964), Hill (2008, 2010), Buko & Maltseva (2012), Ivanova (2015) etc. We have to note, however, that some references give erroneously, for *F. meleagroides*, photos or drawings which actually represent other related species, such as: *F. meleagris* (Korotchenko & Orlov 2009) or *F. montana* (Negru 2002, Ciubotaru et al. 2007).

Fig. *5*. Capsules of *F. meleagroides*. Scale bar: 2.5 mm

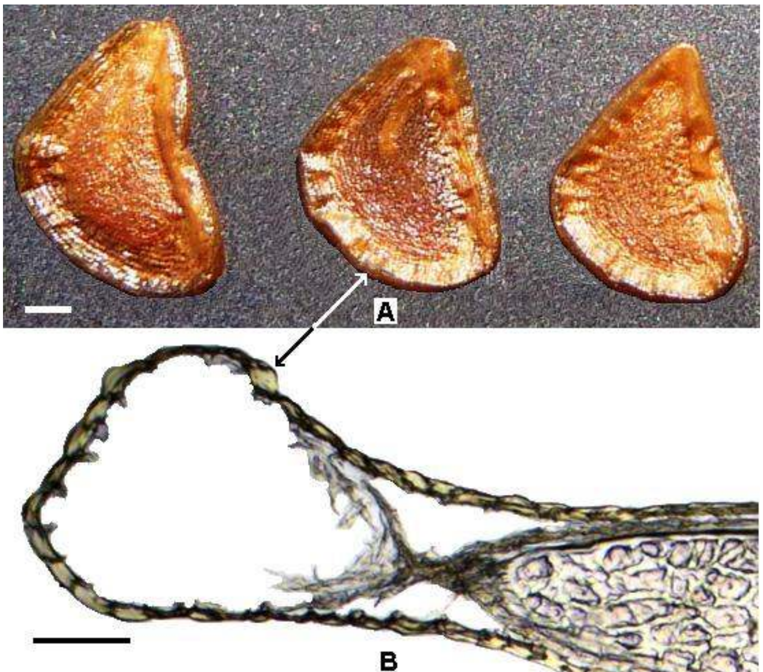

Fig. *6*. Seeds of *F. meleagroides* (A-general view; B-cross-section). Arrows indicate the membranous swollen edge of the seed. Scale bar: 1 mm (A), 0.1 mm (B)

In the identification key below, we show the main characters by which *F. meleagroides* can be easily distinguished from the other congeneric species previously known in the flora of Romania (Zahariadi 1966, Ciocârlan 2009, Sârbu et al. 2013), including the cultivated species *F. imperialis*:

1a Lowermost and middle leaves of 25-40 mm width. Flowers orange-red, (2) 3 to 6 (8) in a false umbel with erect bracts arranged in a tuft over the flowers. Tepals not tessellated. Nectaries more or less circular, white, at the base or slightly above the base of tepals. Plants cultivated in gardens ………................................... ***F. imperialis***

1b Leaves of 2-10 mm width. Flowers purple, blackish- or greenish-purple, pink or white, solitary or in racemes. Tepals more or less tessellated (marked with alternating squares of light and dark colour). Nectaries linear, starting at the point of inflection of tepals (4-12 mm above the base of the tepals). Plants spontaneous ....… **2**

2a Upper (and / or the lower) leaves opposite or verticillate (in a whorl of 3), the intermediate leaves alternate. Plants mesophilous ....……………. ***F. montana***

2b All leaves alternate. Plants ± hygrophilous …..............................…...............….. **3**

3a Tepals of 30-45 (50) mm long, purple, pink or (rarely) white, obviously regular tessellated, the inner ones elliptical-elongate (wider at the middle). Nectaries at 8-12 mm above the base of tepals. The outer gibbosity of tepals, corresponding with the inner nectariferous fovea, very pronounced, ± angular. Plants do not grow on salty soils ....…... ***F. meleagris***

3b Tepals less than 30 mm long, blackish-purple outside, greenish inside, less obviously (obscurely) tessellated, the inner ones obovate (wider in the upper third). Nectaries at ca. 4 mm above the base of tepals. The outer gibbosity of tepals, corresponding with the inner nectariferous fovea, less obvious, rounded. Plants tolerate (slightly) salty soils ………... ***F. meleagroides***

According to Rix (1980), nectaries are of 7-10 × 1.5 mm at *F. meleagris* and 15 × 2 mm at *F. meleagroides*. This would be another important criterion for distinction between the two species. However, this criterion is not quite sure, since according to Stpiczyńska et al. (2012), at *F. meleagris*, nectaries are of 9.12 - 14.43 mm long (11 mm, on average) and according to our data, at *F. meleagroides,* nectaries are rather variable in length and narrower (12-18 × 1-1.5 mm).

Biology. According to Korotchenko & Orlov (2009) and Ivanova (2015), *F. meleagroides* flourishes in April-May, is pollinated by insects and the fruits ripen in June. This is consistent with our observations on the field. In the northwestern China, it seems that the plant flourishes later on, in May-June (Chen & Mordak 2000). It reproduces both by seeds and vegetative (bulbs), but the latter way is less effective (Korotchenko & Orlov 2009, Ivanova 2015). We suppose that the swollen edge of the seed, filled with air (as a tire) is an adaptation for both anemochorous and hydrochorous seed dispersal. Although we have not done a detailed study on this issue, we noticed on the field that the seeds are easily carried by the wind. We have also found in the laboratory that the seeds float on the water for several days without sinking.

Ecology, habitats and plant communities. Throughout its natural area, *F. meleagroides* grows in wet habitats, temporary inundated (Golub & Saveljeva 1991, Andreev 1993, Chen & Mordak 2000, Korotchenko & Orlov 2009, Didukh 2011, Ivanova 2015), often with an excess of salts (on solonetzs) (Lozina-Lozinskaya 1935, Artiushenko 1979, Prokudin et al. 1987, Golub & Saveljeva 1991, Golub 1994). Plant communities in which the species usually grows, sometimes as a constant species, belong either to the halophilous wet meadows (e.g. the class *Asteretea tripolium* Westhoff et Beeftink in Beeftink 1962, partly synonymous with *Festuco-Puccinellietea* Soó 1968) (Golub 1994) or to the wet meadows of the class *Molinio-Arrhenatheretea* R. Tx. 1937 em. Mirk. et Naumova 1986 (Golub & Saveljeva 1991, Korotchenko & Orlov 2009).

According to our preliminary data, the populations of *F. meleagroides* from Northeastern Romania grow under similar conditions, on wet meadows (Fig. 7), with temporary excess of moisture, which are mainly dominated by *Agrostis stolonifera*, *Elymus repens* and *Alopecurus pratensis* (alliance *Potentillion anserinae*), with an important presence of some halophilous species, characteristic for the class *Festuco-Puccinellietea* Soó 1968 (e.g. *Cerastium dubium*, *Dianthus pratensis* subsp. *racovitzae*, *Iris brandzae*, *Iris halophila*, *Juncus gerardi*, *Limonium gmelinii*, *Puccinellia distans* subsp. *limosa*, *Scorzonera cana*, *Rhaponticum serratuloides* etc.). However, further research is needed for more detailed knowledge of the populations of *F. meleagroides* from Romania, concerning their structure, distribution, ecology, biology and phytosociology. Unfortunately, part of the territory occupied by this species in the northeastern Romania is currently anthropogenically disturbed, mainly by ploughing for agricultural crops. By its tolerance to soil salinity, *F. meleagroides* is clearly distinguishable from the two other congeneric species from the flora of Romania, *F. meleagris* and *F. montana*.

Fig. 7. Meadow with *F. meleagroides* (NE Romania)

Sozological importance. The inclusion of Romania on the distribution map of the species *F. meleagroides* at the western boundary of its area is an important fact in phyto-geographical terms, especially since in the European part of its area this species is found in various stages of endangerment, being included in the Red Book of vascular plants both in Ukraine (as vulnerable) (Korotchenko & Orlov 2009), and in Bulgaria (as critically endangered) (Ivanova 2015), or even in the Republic of Moldova (as vulnerable) (Negru 2002) (see however the comments above on the presence of *F. meleagroides* in this country). Since *F. meleagroides* is an endangered species in large parts of its natural range, we consider that urgent actions are necessary for preservation of its population in Romania (i.e., carrying out research on populations in order to establish their sozological status; establishment, by official authorities, of the area in which it grows in Romania as protected area; declaring in the official legislation as a protected species; imposing the mowing regime on meadows and so on).

Conclusions

The presence of *Fritillaria meleagroides* in Romania is documented for the first time in this paper. It grows in wet meadows, ± salinized, situated in Northeastern Romania, North of the village Cotu Morii, on the alluvial plain of the Jijia river, near the border with the Republic of Moldova.

Fritillaria meleagroides differs from the other two congeneric from the vascular flora of Romania, as follows: i) compared to *F. meleagris*, wherewith it resembles through the alternate leaves, it is distinguished mainly by the smaller flowers, with darker tepals (blackish-purple), obscurely tessellated; by outer gibbosity of tepals (corresponding with the inner nectariferous fovea) less obvious; by nectaries longer, at ca. 4 mm above the base of tepals; ii) compared to *F. montana*, which has quite similar flowers, it clearly differs through all leaves alternate; iii) it is distinguished by both species through its tolerance to soil salinity.

Fritillaria meleagroides is an endangered species in large parts of its natural range and urgent actions are necessary for preservation of its population in Romania. For this purpose, further research is needed for a more detailed knowledge on the size of populations of this species in Romania, their structure, distribution, ecology, biology and phytosociology.

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Table 1 Morphometric data on *F. meleagroides*. Data from Romania is based on measurements taken from 10 specimens (*med*.-medium size, *min*.-*max*. - minimum-maximum size, CV% - coefficient of variation)

Characters	**Romania**			**Data from references**					
	med.	min.-max.	CV%	Schultes & Schultes 1829	Lozina-Lozinskaya 1935	Yanev 1964	Rix 1980	Chen & Mordak 2000	Bojnanský & Fargašová 2007
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Plant height (cm)	37.6	28-50	20.1		25-60	25-60	25-60	20-40	25-60
Length of internodes (cm)	5.8	4.7-7.1	15.2						
No. of leaves	5.9	5-7	14.8	ca. 4	3-7	3-7(-9)	3-6(-7)	3-7	
Lowermost leaf length (cm)	12.0	11-14	7.9	14	5-15	4-15	7-16	5-15	
Lowermost leaf width (mm)	6.2	5-8	15.4	ca.5	up to 7	up to 7	up to 7	up to 5	
Number of flowers	1	-	-	1	1	1 (2-3)	1	usually 1	1
Peduncle / bracteant leaf ratio	0.9	0.5-2.1	56.6		> 1	< 1	ca. 1	variable	
Tepal length (mm)	26.4	25-28	5.1	29	20-30	20-30	20-25	20-30	
Outer tepals width (mm)	10.1	8-11	8.4	8.4-9.5 (indistinct)		6-7 (-9)	7-9	5-8	
Inner tepals width (mm)	15.1	12-18	13.7		up to 12	7-12	10	7-12	
Position of nectaries (mm, from the base of tepal)	4.3	4-5	10.0				4		
Nectaries length (mm)	14.3	12-18	11.4				15	15	
Nectaries width (mm)	1.5	1-1.5	10.9				2	2	
Stigma branches length (mm)	3.2	3-4	10.9				3-4		
Style length (mm)	6.2	5-7	14.8				7	4-8	
Stamens / tepals ratio	0.57	0.5-0.6	9.9	ca. 0.5	0.5	0.5		0.66	
Capsule length (mm)	22.5	15-35	19.4		20	20			
Capsule width (mm)	10.3	8-12	19.0		10	10			
Length of narrowed region at the bottom of capsule (mm)	2.3	1-4	36.7						
Seed length (mm)	5.0	4-6	13.1						6-6.6
Seed width (mm)	3.6	3-4	11.6						4.8-5.2

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Acta Horti Bot. Bucurest. 2015, **42**: 57-62
DOI: 10.1515/ahbb-2015-0002

NEW CHOROLOGICAL DATA FOR RARE VASCULAR PLANTS FROM ROMANIA

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Abstract: New chorological data about seven rare vascular plant taxa are reported in the present paper: *Conringia austriaca*, *Jurinea multiflora*, *Linaria arvensis*, *Nonea pallens*, *Ophrys apifera*, *Ophrys scolopax* subsp. *cornuta*, *Saponaria officinalis*. For *Linaria arvensis*, previously considered doubtful in the absence of the herbarium material, we confirm its presence in Romania. The report of *Nonea pallens* is the first for Dobrogea, while the report of *Jurinea multiflora* is the first for Muntenia region of Romania.
Keywords: chorological data, flora, rare plants, Romania

Received 9 March 2015 *Accepted 3 April 2015*

Introduction

During various field studies in the period 2010–2014, new data on the occurrence of some native vascular plant species have obtained. They are rare species (Oltean et al. 1994), generally with a very limited distribution not only in Romania, but also in Europe (Tutin et al. 1964-1980, Tutin et al. 1993).

The nomenclature is according to The Plant List (2013). Beside the new chorological data, the geographic coordinates (decimal degrees, WGS 1984 system) are given, as well as a discussion about their distribution in Romania. Furthermore, the voucher specimen is indicated, the herbarium code being according to Index Herbariorum (Thiers, continuously updated).

Results and disscusion

Conringia austriaca (Jacq.) Sweet (Fam. Brassicaceae)

Dobrogea, Constanţa County: Şipotele E, near the village, bushes with *Paliurus spina-christi*, 19.04.2010; Şipotele S, Izlaz Hill, bushes, 44.026147°N, 27.950989°E, 30.04.2010; Şipotele E, near village, rocky grassland, 44.050405°N, 27.965562°E, 30.04.2010; Şipotele E, edge of bushes with *Paliurus spina-christi*, 21.05.2010 [CL 661637; CL 661638].

According to Oprea (2005), the plant has previously been reported only from a few counties, as follows: Bihor County – in the natural reserve Defileul Crişului Repede; Cluj County – Cheile Turzii; Sibiu County – Bungard, Şura Mare, Slimnic; Mehedinţi County – Gura Văii, Vârciorova to Porţile de Fier and Gura Slătinicului; Tulcea County – Atmagea on Ghiubelca valley. This last specification is the only existing one to date in Dobrogea.

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Distribution in Europe: SE & EC Europe, extending westwards to Austria and Sardinia (Ball 1993, Marhold 2011a).

Jurinea multiflora (L.) B.Fedtsch. [syn. *Jurinea linearifolia* (DC.) DC.] (Fam. Compositae) (Fig. 1)

Muntenia, Buzău County: Balta Albă S, 45.278428°N, 27.291308°E, ca. 45 m alt., 02.07.2013 and 19.07.2013. The plants (three flowering individuals) have been recorded in dry grassland with *Festuca valesiaca*, *Agropyron cristatum* s.l., *Tanacetum millefolium*, *Teucrium chamaedrys*, *Bassia prostrata*, *Stipa capillata*, *Astragalus austriacus*, *Linum perenne*, *Bothriochloa ischaemum*. In the same habitat with *Jurinea multiflora* we also identified four rare species for Romania (Oltean et al. 1994): *Allium denudatum* (syn. *Allium albidum*), *Astragalus dasyanthus*, *Goniolimon besserianum*, *Tanacetum millefolium*.

Jurinea multiflora is a perennial species, woody at base, with lanceolate or linear-lanceolate, shortly acuminate, usually with revolute margin, arachnoid-tomentose leaves. Capitula are small (5-20 mm), cylindrical, with involucral bracts shortly acuminate, white or pale green below, pink or reddish-purple distally, with scarious apex. Achenes are tetragonal, ribbed, glabrous, with pappus ca. 3 times as long as achene (Kožuharov 1976). This taxa is very closed to *Jurinea tzar-ferdinandii*, but the latter has involucral bracts white or pale green distally. It is also related to *Jurinea stoechadifolia*, but this species has larger capitula (27-30 mm). Both *J. tzar-ferdinandii* and *J. stoechadifolia* have been reported for Romania.

Jurinea multiflora is a continental element (Dihoru & Negrean 2009, Sârbu et al. 2013), with general distribution in S & SE parts of former U.S.S.R., SE Romania, growing in steppes and semideserts (Kožuharov 1976, Greuter 2006+).

This species has been reported for Romania only in two counties in the E-SE part of the country, as follows: Constanţa County, Nistoreşti (Horeanu 1972), Medgidia (Prodan 1957); Galaţi County, Galaţi city, near Brateş Lake (Herbarium C. Zahariadi according to Dihoru & Negrean 2009). Sârbu et al. (2013) mention it as a rare species in the steppe area, dry grassland and stony hills, in *Agropyro-Kochietum prostratae* association. The status of *Jurinea multiflora* has been appreciated as critically endangered (Dihoru & Negrean 2009) or vulnerable (Oprea 2005).

The field research in Nistoreşti and Brateş Lake area does not confirm the presence of the plant for these locations. The presence of the species in Medgidia is difficult to verify because the area is quite large, and the bibliography does not indicate a specific location. We note that there are no herbaria specimens collected in Romania.

Our information is the first one on the presence of the species in Muntenia; choronyms of Tulcea and Galaţi, mentioned in the literature, are located in Dobrogea, respectively in Moldova regions. At the same time this data confirms the presence of the plant in the Romanian Flora.

Linaria arvensis Desf. (syn. *Antirrhinum arvense* L.) (Fam. Plantaginaceae)

Transylvania, Hunedoara County: Sălaşu de Sus, the natural reserve Fânaţele cu narcise, in ruderal places (wayside), with only few accompanying species (*Polygonum aviculare*, *Persicaria hydropiper*, *Erigeron annuus* s.l.), 45.500739° N, 22.947352°E, ca. 515 m alt., 11&12.08.2013 [BUC 402223; BUC 402224].

Banat, Caraş-Severin: Coronini, 44.671233°N, 21.751248°E, ca. 400 m alt., 29.08.2014. The plants have been identified in disturbed soil on the platform around a wind turbine, accompanied by the following species: *Dactylis glomerata* s.l., *Anthyllis vulneraria* s.l., *Fragaria vesca*, *Medicago falcata*, *Cerastium fontanum* s.l., *Hypericum elegans*, *Stellaria graminea*, *Galium verum*, *Bothriochloa ischaemum*, *Elymus repens* s.l., *Chondrilla juncea*, *Geranium lucidum*, *Trifolium arvense*, *Scabiosa arvensis*, *Plantago lanceolata*, *Salvia verticillata*, *Leucanthemum vulgare*, *Carduus acanthoides*, *Lotus corniculatus*, *Achillea setacea*, *Linaria vulgaris*, *Trifolium pratense*, *Potentilla argentea*, *Convolvulus arvensis*, *Teucrium chamaedrys*, *Tanacetum vulgare*, *Rumex acetosella*, *Dianthus carthusianorum*, *Centaurea phrygia*, *Agrimonia eupatoria*, *Rubus caesius*, *Trifolium repens*. According to Sârbu et al. (2013), it grows in sandy, ruderal and cultivated places, in plains and hills, in *Centauretalia cyani*.

Linaria arvensis is a Central European element (Ciocârlan 2009, Sârbu et al. 2013), with large distribution in European countries (Marhold 2011c). The presence in Romania was considered uncertain in the absence of herbarium material (Chater et al. 1972, Oprea 2005, Ciocârlan 2009, Sârbu et al. 2013). However, the plant was mentioned in several counties: Satu Mare, Cluj, Mureş, Harghita, Braşov, Alba, Sibiu, Iaşi, Suceava, Botoşani, Tulcea (Marian 2000, Oprea 2005, Ciocârlan 2009, Sîrbu & Oprea 2011, Sârbu et al. 2013). Sîrbu & Oprea (2011) consider this species as allochtonous for Romania.

The two choronyms indicated above confirm the presence of the species in the country and are the first reports for Hunedoara and Caraş-Severin counties.

Nonea pallens Petrovič (Fam. Boraginaceae)

Dobrogea, Constanţa County: Şipotele N, grassland near the road, 44.062817°N, 27.971017°E, 19.04.2010; Şipotele SE, secondary grassland, near the road Şipotele-Tufani, 44.028067°N, 27.971000°E, ca. 140 m alt., 29.04.2010 [CL 661664]; Sevendic N-NE, grassland, 44.019683°N, 27.950200°E, 30.04.2010; Deleni N-NE, Cişmeluţei Valley, grassland, 22.05.2010; Zorile W-SW, Ghiolpunar Hill, grassland, 44.075567°N, 27.910067°E, 23.05.2010; Zorile W-SW, Ghiolpunar Hill, edge of agriculture field, 07.06.2010; Deleni, Refugiului Valley, stony grassland, 44.104933°N, 28.002250°E, 07.06.2010.

According to Ciocârlan (2009) and Sârbu et al. (2013), *Nonea pallens* is a Ponto-Balkan element. It is spread in SE Europe, from Albania to S Ukraine (Chater 1972, Valdés 2011), native in most of countries, except Republic of Moldova where it is considered an introduced species (Valdés 2011). From Romania, it was reported from the SW of the country, in Mehedinţi County, from Gura Văii to Vârciorova (Grecescu 1898) and in grasslands from Gura Oglănicului to Gura Slătinicului (Roman 1974). The first record in Dobrogea was communicated at a national scientific symposium in 2010 (Anastasiu 2010a) and subsequently some further information ("Constanţa: Şipotele către păd. Deleni") was published by Sârbu et al. (2013). The occurrence of *Nonea pallens* in SE of the country demonstrates a continuity of the distribution area between Bulgaria and Ukraine.

Ophrys apifera Huds. subsp. ***apifera*** (Fam. Orchidaceae) (Fig. 2)

Muntenia, Buzău County: Cocârceni, grassland, 45.363671°N, 26.486291°E, ca. 440 m alt., 01.06.2014, two mature individuals. *Ophrys apifera* subsp. *apifera* is an Atlantic-Mediterranean taxon (Sârbu et al. 2013), previously reported only from a few counties in Romania: Sibiu, Hunedoara, Mehedinţi, Dâmboviţa, Prahova (Oprea 2005, Sârbu et al. 2013). This taxon is appreciated as critically endangered at national level

(Dihoru & Negrean 2009), but as "least concern" in the IUCN Red List (Bilz et al. 2011). This report is the first one for Buzău County.

Fig. 1. Jurinea multiflora, Balta Albă, Buzău County, 02.07.2013 (photo: P. Anastasiu)

Fig. 2. Ophrys apifera subsp. *apifera*, Cocârceni, Buzău County, 01.06.2014 (photo: P. Anastasiu)

Ophrys scolopax Cav. subsp. ***cornuta*** (Steven) Camus (Fam. Orchidaceae)

Muntenia, Buzău County: Ulmet, bushes with *Hippophaë rhamnoides* s.l., 45.368787°N, 26.465205°E, ca. 470 m alt., 09.06.2014 [BUC 401676]. This is the fifth report from Buzău County, the plant having been previously reported from Cislău (Nyárády & Beldie 1972, Oprea 2005, Dihoru & Negrean 2009), Pătârlagele (Popova et al. 1981 in Oprea 2005), Căneşti (Anastasiu et al. 2008) and Cocârceni (Anastasiu 2010b). *Ophrys scolopax* subsp. *cornuta* is very rare in Romania and critically endangered (Dihoru & Negrean 2009). It has been reported from Banat, Oltenia, Muntenia, Moldova (Oprea 2005, Sâbu et al. 2013).

Saponaria glutinosa M.Bieb. (Fam. Caryophyllaceae)

Dobrogea, Constanţa County: Şipotele E, at the edge of Ponto-Sarmatic deciduous thickets, 44.050417°N, 27.965000°E, 21.05.2010 [CL 661639]; Dumbrăveni, at the edge of forest, 02.06.2011. Considered a Mediterranean species (Dihoru & Negrean 2009), *Saponaria glutinosa* is distributed in SE Europe and Spain (Chater 1993, Marhold 2011b). It is rare in Romania, reported from a few places in Mehedinţi

County and Constanţa County – Dumbrăveni, Canaraua Fetii (Arcuş 1998). Its status in Romania has been appreciated as being at Low Risk (Dihoru & Negrean 2009).

Acknowledgements. I am indebted to Cristina Bogdan who verified the presence of *Jurinea multiflora* in Galaţi County, as well as to Mr. Negrean Gavril for the helpful comments on the manuscript.

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Acta Horti Bot. Bucurest. 2015, **42**: 63-66
DOI: 10.1515/ahbb-2015-0007

BIOLOGICAL ACTION OF PLANT EXTRACTS ON A FUNGAL PLANT BIOSTIMULANT STRAIN OF *TRICHODERMA VIRIDE*

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Abstract: The antifungal activity of nine plant extracts manufactured by Hofigal Import Export S.A. Romania against the biocontrol fungal agent *Trichoderma viride* Pers. (isolate Tv 82) was assessed *in vitro* for the first time in Romania. In general, the development (mycelial growth and sporulation) was not inhibited by the six plant extracts (*Satureja hortensis*, *Achillea millefolium*, *Allium sativum*, *Mentha* sp., *Hyssopus officinalis*, *Artemisia dracunculus* 'Sativa'), with three exceptions (*Rosmarinus officinalis*, *Valeriana officinalis*, *Tagetes patula*), applied in a concentration of 20%. Among these, the extract of *Tagetes patula* has inhibited the Tv 82 development, applied as lower concentrations (10% and 5%), efficacy being 54.3% and 50%, respectively. In addition, the tested plant extracts of *Satureja hortensis*, *Achillea millefolium*, *Mentha* sp. proved stimulative effect on Tv 82 development. This approach add to the early studies on the selectivity of *Trichoderma* spp. to chemicals used in plant protection, new data referring to the use of antagonistic fungi, like *Trichoderma* spp., as a protective mean against phytopathogens. Also, these data sustain the possibility of applying plant extracts as an alternative in plant protection or to apply together chemical (pesticides) and biological means (plant extracts) especially to protect ecological crops, as vegetables, medicinal plants a.o.
Keywords: *Trichoderma viride*, plant extracts, biocontrol

Received 17 November 2015 *Accepted 11 December 2015*

Introduction

Trichoderma species (Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreales, Hypocreaceae, *Index Fungorum*, Kirk, 2015) are effective biocontrol agents against many plant pathogens. The antifungal activity of nine plant extracts manufactured by Hofigal S.A. against a plant biostimulant strain of *Trichoderma viride* Pers., isolate Tv 82, from the Collection of Research – Development Institute for Plant Protection (RDIPP) Bucharest, was assessed *in vitro*, based on the literature considerations (Harman et al. 2004, 2012, Schuster & Schmoll 2010, Hermosa et al. 2012, Saba et al. 2012, Sofo et al. 2012 a.o.).

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Material and methods

Fungal material: one isolate of *Trichoderma viride* (Tv 82) from the Collection of RDIPP Bucharest.

Biological material: nine hydroalcoholic plant extracts from: *Achillea millefolium* L., *Allium sativum* L., *Artemisia dracunculus* L. 'Sativa', *Hyssopus officinalis* L., *Mentha* sp., *Rosmarinus officinalis* L., *Satureja hortensis* L., *Tagetes patula* L., *Valeriana officinalis* L., prepared by S.C. Hofigal Import Export Bucharest Romania (from fresh biomass as stems, leaves, flowers, sprouts, bulbs).

***In vitro* tests**: the method of including plant extracts in PDA medium at 20%, 10%, and 5% concentrations, in three replicates for each extract, has been used. Discs of 5 mm from 7 days *T. viride* Tv 82 cultures have been placed in the Petri plates with plant extracts included in the PDA agarized medium.

Evaluation of biological action has been achieved by the following parameters: inhibition rate of mycelial growth (mm) compared to untreated control (%); effective concentrations EC50 and EC90, the concentrations which reduced mycelial growth by 50 and, respectively, 90%).

Results and discussion

Development (mycelial growth and sporulation) of *T. viride*, isolate Tv 82, was not inhibited by the six plant extracts (*S. hortensis*, *A. millefolium*, *A. sativum*, *Mentha* sp., *H. officinalis*, *A. dracunculus* 'Sativa'), with three exceptions (*R. officinalis, V. officinalis*, *T. patula*) applied at 20% concentration (Figs 1, 2).

Among these, the extract of *Tagetes patula* has inhibited the Tv 82 development, applied as lower concentrations (10% and 5%), inhibition being 54.3% and 50%, respectively. In addition, some of the tested plant extracts (*Satureja hortensis*, *Achillea millefolium*, *Mentha* sp.) proved a stimulative effect on Tv 82 development.

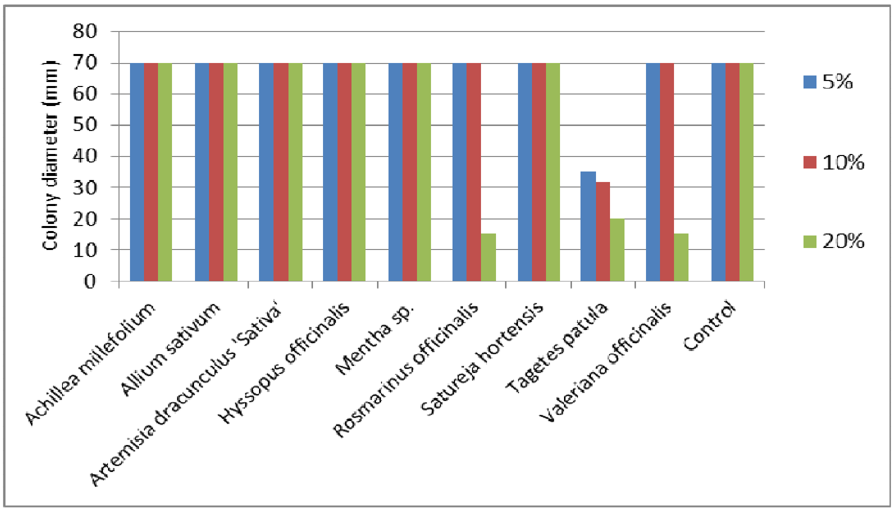

Fig. 1. Colony diameter (mm) of *Trichoderma viride*, isolate Tv 82, after *in vitro* treatments with plant extracts

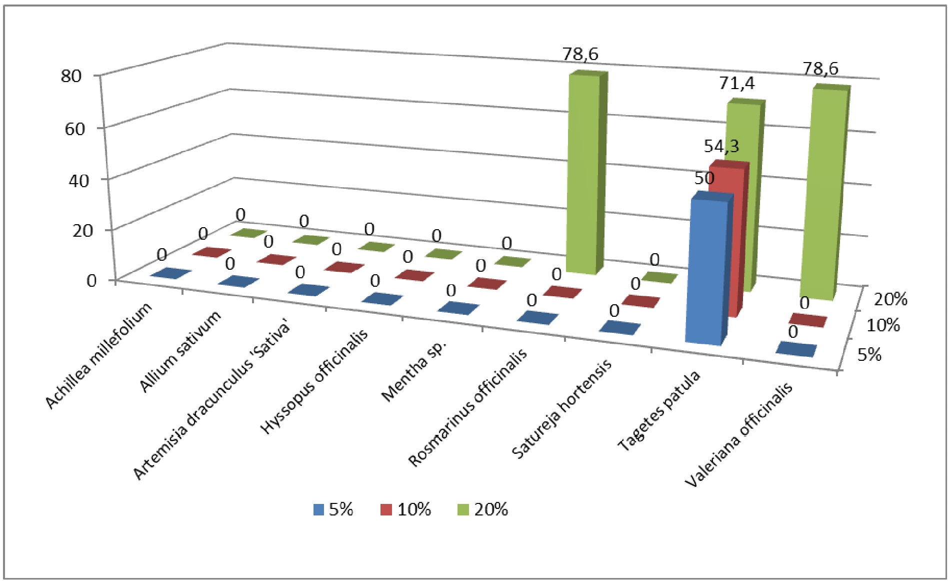

Fig. 2. Biological action of the *in vitro* plant extracts treatments on the development of *Trichoderma viride*, isolate Tv82

Table 1

In vitro level of sensitivity for *Trichoderma viride*, isolate Tv 82

Variant (plant extract)	Level of sensitivity (%)	
	EC50 (%)	EC90 (%)
1. *Rosmarinus officinalis*	15.9	23.0
2. *Valeriana officinalis*	23.3	36.4
3. *Tagetes patula*	5.0	33.1
4. *Satureja hortensis*	> 20	> 20
5. *Achillea millefolium*	> 20	> 20
6. *Allium sativum*	> 20	> 20
7. *Mentha* sp.	> 20	> 20
8. *Hyssopus officinalis*	> 20	> 20
9. *Artemisia dracunculus* 'Sativa'	> 20	> 20

The level of sensitivity of Tv 82 isolate to the tested plant extracts has been also evaluated and the results were expressed as effective concentrations EC50 and EC90. As shown in the Table 1, with regard to mycelial growth response to plant extracts, Tv 82 isolate was no sensitive to *S. hortensis*, *A. millefolium*, *Allium sastivum*, *Mentha* sp., *H. officinalis* and *A. dracunculus* 'Sativa' extracts, with EC90 values over 20%. By contrast, the tested isolate seems to be more sensitive to *T. patula, R. officinalis* and *V. officinalis* extracts, with EC90 values ranging between 23.3% and 36.4%.

Further studies are needed to assess the possibility of combining plant extracts and biocontrol agents to provide a novel approach for inhibiting the pathogenic fungi in nutraceutical crops in Romania.

Conclusions

Our present approach add to the early studies on the selectivity of *Trichoderma* spp. to chemicals used in crop treatments (Şesan et al. 1998, Şesan 2002) new data referring to the use of plant biostimulant fungi, like *Trichoderma* spp., as a new mean for crop treatments. Also, these data sustain the possibility of applying plant extracts as an alternative for crop treatments, especially on organically managed vegetables or medicinal/nutraceutical plants.

Acknowledgments. This research was financially supported by the projects PN-II-PT-PCCA-2013-4-0995 (MAIA) and PN-II-PT-PCCA-2013-4-0846 (CERES).

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Acta Horti Bot. Bucurest. 2015, **42**: 67-86
DOI: 10.1515/ahbb-2015-0001

ANALYSIS OF THE SAXICOLOUS LICHEN COMMUNITIES IN MĂCIN MOUNTAINS NATIONAL PARK

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Abstract: The assemblage of saxicolous lichenized fungal communities in Măcin Mountains National Park was assessed during a biodiversity study developed between 2006 and 2008. Fifty three species of saxicolous lichenized fungi were identified on Hercynic granites and granitoid outcrops characterized by intense weathering process. Apparently, competition was not the main mechanism in community assemblage as calculated C score showed (non-significant difference between mean calculated and simulated score). Niche overlap assessment showed that lichens avoided competition by spatial niche partition (mean Pianka index of 0.07 for sampling quadrats and 0.20 for locations). The estimation of nestedness index (N=0.63 at local scale and N=0.88 at sampling quadrat scale) indicated that local communities were subsets of a larger, regional scale metacommunity. Similarities in community composition across locations were assessed by means of Ward algorithm, results indicating that the most dissimilar communities were encountered at Pietrele Mariei, a residual inselberg and Suluc foothill. Conservation of saxicolous communities containing endangered species such as *Umbilicaria grisea,* critically endangered *Ramalina obtusata* and vulnerable *Acrocordia gemmata, Pertusaria hemisphaerica, Pertusaria pertusa* will be challenged in the future by anthropogenic impact coming from agriculture, sheep grazing and quarries operating in the proximity of the reserve area.
Keywords: community assemblage, saxicolous lichenized fungal community, Hercynic granites, C-score, niche overlap, nestedness, conservation of endangered species

Received 27 November 2014 *Accepted 02 February 2015*

Introduction

One of the central goals of ecology is to identify the mechanisms that govern community assembly and structure (Horner-Devine et al. 2007, Beaudrot & Marshall 2011). Whether the co-occurrence of species is random or dictated by species interaction or abiotic factors is still a matter of debate. Assembly rules were advocated as driving the community composition, at the present day state of art, being understood as any constraint imposed to species association to establish a community (Diamond 1975, Connor & Simberloff 1979, Weiher & Keddy 1999, Maestre et al. 2008, Götzenberger et al. 2012) and describe co-occurrence patterns. In a larger context, metacommunity models advocate two categories of structuring forces: a) ecological niche differentiation through species interaction sand species sorting along ecological gradients and b) dispersal limitation (Leibold & Norberg 2004). Another structural

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feature of any community is species richness, maybe the simplest way to describe community and regional diversity (Magurran 1988). Species richness and species co-occurrence within communities are both modeled by environmental envelope, dispersion and species interactions.

Nestedness is a particular case of beta diversity exploring the level of among site variation in terms of species richness (Ulrich & Almeida-Neto 2012). However, nestedness and co-occurrence patterns estimated by some metric are both important in the quest of community assembly mechanisms and devised to be used as alternatives to explain the site or regional variation in species richness.

Saxicolous lichens are intimately linked to geochemical composition of the rocks they colonize as substrates, rock composition playing an important role in lichen community assembly (Purvis & Halls 1996) but other forces as competition or parasitism can be tested for community structuring. In a large area such as a mountain range, saxicolous lichens establish a metacommunity (*sensu* Leibold et al. 2004), with distinct local patches or local communities, Măcin Mountains National Park serving as an example for this concept.

Măcin National Park is a heterogeneous area with a variety of unique biotopes surrounded by agricultural landscape. A highly fragmented land use types represent the matrix of the reserve mosaic of biotopes. One may refer to the reserve as to an island in an agricultural ocean. The protected biotopes are of climax type or close to a climax state (Hansson & Angelstam 1991) therefore sensitive to anthropogenic stress. Măcin Mountains National Park margin represents a sharp transition to different types of anthropogenic ecosystems. Consequently, the various annual agricultural crops as wheat, barley, sunflower, canola and corn represent a threat rated as of medium risk due mostly to nitrate pollution (Badiu et al. 2014).

The present study addresses the topic of saxicolous lichens richness, community structure and the assembly rules leading to the current structure of spatially distinct communities developing on acidic substrate represented by granitic and granitoid rocks in Măcin Mountains National Park in South-Eastern Romania and establishing a metacommunity at regional scale. The study of saxicolous lichen communities was performed between 2006 and 2008 in the frame of GEF/UNDP project: *Strengthening the National System of Protected Areas in Romania by Demonstrating the Best Management Practices in the Măcin Mountains National Park.* The analysis should indicate whether sets of spatially distinct local communities were established by competition or some other species segregation/aggregation mechanism as main driving force (Diamond 1975) or/and are nested meaning that the assemblages in less species-rich sites form non/random subsets of those at progressively species richer sites, a model proposed initially in island biogeography (Patterson & Atmar 1986). Both hypotheses on species assembly rules were tested for statistical significance using a hierarchy of null models which generally help in understanding which factors contribute mostly to the observed pattern in species assemblages as compared to a null, randomly assembled community (Feeley 2003). Two scales were employed in the analysis: the small scale of sampling quadrats and the scale of local communities covering different types of habitats within the investigated area (steppe versus woodland boulders and outcrops embedding vegetation, the foothills versus mountain ridges).

A sound ecological theory concerning community structure could serve for sustainable conservation of saxicolous lichen communities in Măcin Mountains National Park in the context of rare habitats established by the oldest rocks in Europe.

Material and methods

1. Description of the site and sampling

Măcin Mountains correspond to Northern Dobrogea orogenesis with Hercynic foundation of diverse geological composition dominated by Permo-Carboniferous formations (Carpelit strata) Hercynic granites and granitoid outcrops are characteristic for this area (Seghedi 2012) which harbor specific saxicolous lichen communities adapted to acidic substrate, with high silica content. Măcin Mountains unfold as parallel ridges oriented in North-Western to South-Eastern direction generating a characteristic ruiniform landscape. An important feature is the advanced erosion of the cliffs and rocks which determined the modest contemporary altitude of these mountains, the highest peak reaching only 467 m (Ţuţuiatu Peak) composed of ridges, pyramidal peaks, and steep slopes resulted from erosion. The geology of the area is characterized by Paleozoic weakly metamorphosed rocks, intruded by various igneous rocks in the Western part and by Mezosoic rocks predominating in the Eastern part of the range. The Paleozoic schists are protruded by intrusive granite and granodiorite rocks (Gavrilă 2012). Precambrian rocks are represented by amphibolites, schists and quartzites. Within the main basin, inselbergs represent an important geomorphologic feature.

The climate is temperate continental with hot summers, frequent droughts and cold winters with little snow. The average annual temperature is 11-11.5°C and average annual rainfall varies between 350-400 mm/m^2/year. The main vegetation covers are represented by forests (forested steppe, mesophilic forests and Mediterranean forests) and xerophilous grasslands (Popescu & Doniță 2010, Gavrilă 2012).

Other protected habitats within this area are: transition between steppe and woodland areas and wetlands. However, most remarkable habitats are established by granitic and granitoid outcrops which harbor specific saxicolous associations such as *Koelerio lobatae-Sempervivetum ruthenicae* Popescu & Doniță, *Moehringio grisebachi-Alyssetum saxatile* Popescu & Doniță and *Diantho nardiformis – Campanulo romanicae* Popescu & Doniță (Popescu & Doniță 2010) and lichen communities.

Locations of sampling sites were selected within the area of Măcin Mountains National Park (45°05'- 45°16'N: 28°12'-28°16'E). Sampling on rocks and boulders occurred in 14 locations, both in forested areas and on peaks where steppe vegetation prevails: A – Osmanului Ridge: B – Căpușa Peak: C - Arsu Peak: D – Caramalău Peak: E – Șerparu Saddle: F – Suluc foothill: G – Stănilă Hill: H – Cheia Peak: I – Vraju Peak: J – Chediu Canyon: K – Chediu Plateau: L – Pietrele Mariei Inselbergs: M-Sulucu Mare Peak: N – Crapcea Peak).

Caramalău and Vraju are residual peaks developed on granitoids containing the entire spectrum of weathering morphologies such as boulders, tors and exfoliated rocks (Gavrilă & Anghel 2013). Chediu canyon was formed by Chediu creek activity and is characterized by a milder local climate being composed of conglomerates and sandstone of Carboniferous origin. Pietrele Mariei is a specific type of geomorphosite for Măcin Mountains, consisting in isolated and eroded formations (inselbergs) which dominate the surrounding steppe (Fig. 1A). Caramalău Peak is characterized by steep slopes and advanced erosion of rocks generating a ruiniform landscape with tors (Gavrilă 2012). Same ruiniform geomorphology is encountered in Sulucu Mare ridge and Stănilă Hill.

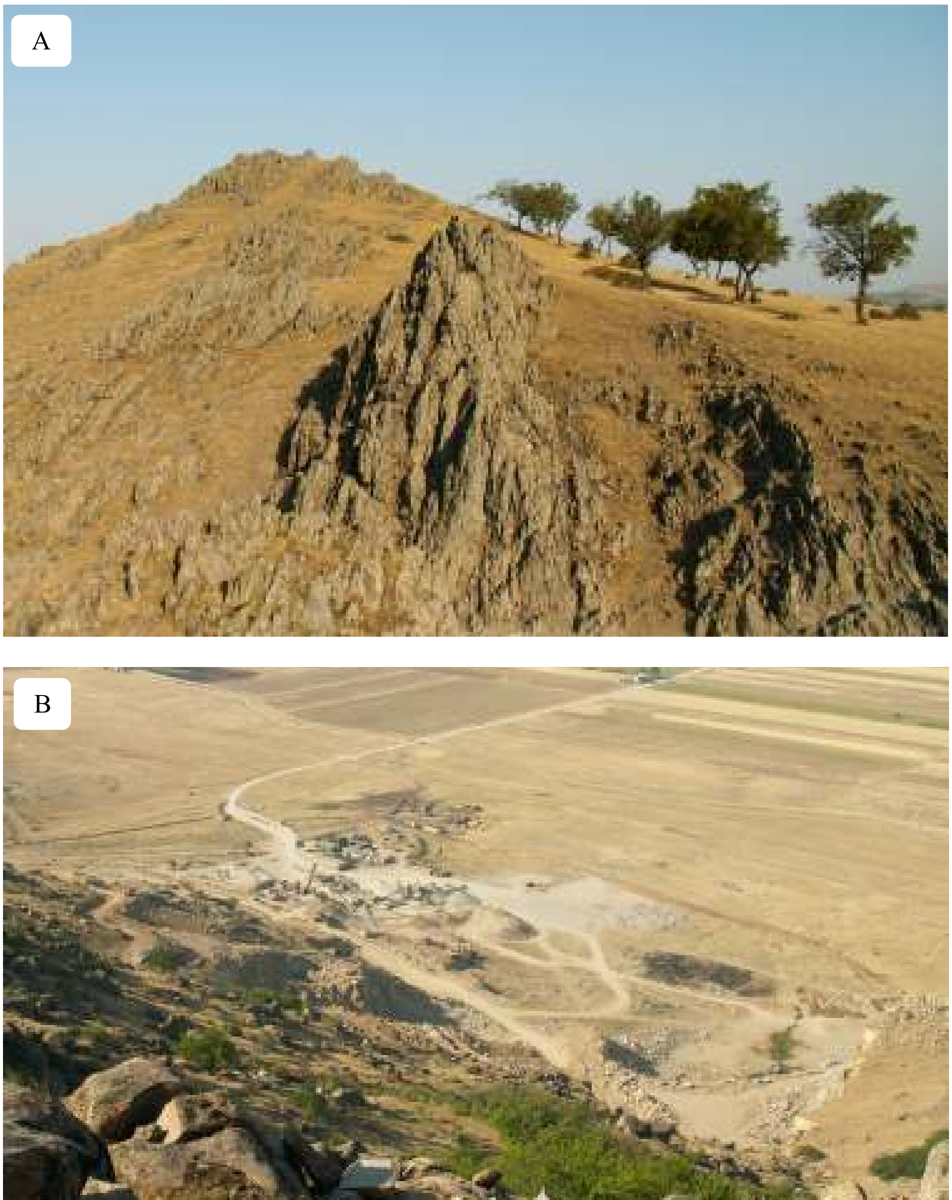

Fig. 1. Pietrele Mariei inselbergs (A); Granite quarry seen from Vraju Peak (B)
(Photos Ovidiu Hâruța)

2. Stressful factors affecting the protected area

The activity of several granite quarries (Fig. 1B) in the past represented the main disturbance in the area to which agriculture and sheep breeding contributed as stressful factors.

3. Species identification and matrix construction

Sampling was performed at random in the above mentioned locations and separately, in sampling quadrats *in situ*, also on photographs and collected material (rock fragments).

The identification was performed in laboratory conditions, mainly based on microscopic analysis and color reactions. For lichen identification various keys were employed and confronted with existing Romanian literature synthesized by Ciurchea (1998). A series of photographic images was taken to asses lichen species as additional identification tool. Nomenclature follows *Index Fungorum* specifications.

Photographs of standard areas were employed and observations from all mentioned locations were merged for the construction of the incidence matrix. The considered area for each plot corresponded to a microhabitat with homogenous lichen distribution, varying between $1m^2$ and $0.25cm^2$ (in accordance with Hale 1982), approximately 5 sampling quadrats per site. Another matrix was assembled for separate locations merging all observation made *in situ* and on photographs. The species resolution is generally high when sampling quadrats are considered still giving an incomplete projection of local community composition while inventory of species on random walk, in different locations has a low resolution but permits a more inclusive species inventory as a part of metacommunity composition.

4. Quantitative analysis

In order to make robust inferences about community structure and following previous recommendations (Feeley 2003), we employed a co-occurrence metric (C score) in combination with nestedness metric (incidence matrix temperature) to which additional information provided by ordination methods (non-metric multidimensional scaling and cluster analysis) was added. A hierarchy of null models, with increasing constraint degree was employed to better explore the significance of community structure testing results. The null models were introduced by Connor & Simberloff (1979) to test for non-random co-occurrence patterns. Incidence data were used to assess the sampling effort and completeness of species richness account.

4.1. Species accumulation curve

Sample rarefaction depicted by the species accumulation curve performed on incidence data is a tool for the assessment of sampling effort (Colwell et al. 2004) and species potential richness in the area. The sample based rarefaction computes the number of species *s* when a number of *m* samples are drawn at random from a set of samples (locations) (Gotelli & Colwell 2001) preserving the spatial aggregation or segregation between species. The illustrating accumulation curve in which on *x* axis are represented the samples or locations and on *y* axis, the accumulated number of species permits a graphical display of increasing number of species as function of area. The matrix with species identified in locations was employed for the assessment of the species accumulation curve.

The analytical solution is given by Mao’s tau with standard deviations.

$$\tau(h) = S_{obs} - \sum_{j=1}^{H} \alpha_{ih} S_j$$

where: S_{obs} stands for number of observed species, j for samples, S_1 the number of species found exactly in one sample, etc. The calculations and graphical representation were performed in PAST (Hammer et al. 2001). For species richness estimation, nonparametric Chao2 index was used devised for incidence data and more or less homogenous samples.

$$S_{Chao2} = S_{obs} + \frac{q_1^2}{2q_2}$$

Where: q_1 stands for number of species found exactly in one sample, q_2 is the number of species found exactly in 2 samples.

4.2. Co-occurrence C score

Presence-absence matrices (incidence matrices) are considered as fundamental units of analysis in community ecology (McCoy & Hech 1987), accordingly the present analysis was developed based on the presence- absence matrix of lichen species in selected locations across the national park.

The co-occurrence C score (Stone & Roberts 1990, Gotelli 2001) was employed to test the hypothesis of aggregation/segregation of the saxicolous lichens in the study area. However, initially, the index was devised for testing competition as driving force, yet there are opinions that a better interpretation of the results should be focused on aggregation due to some habitat characteristic or facilitation (Götzenberger et al. 2011). This algorithm was previously employed for lichens composing the biotic crusts of gypsum outcrops in Central Spain (Maestre et al. 2008). Co-occurrence was calculated over all possible pairs of species assuming the null model of random species assembly. If the number of non-co-occurring species combinations differs from the observed co-occurrences, the assembly is considered non-random and structured by competition (Sanderson et al. 1998) or other force driving specific species aggregation. C score represents the number of checkerboard units (CU) in species presence/absence matrix:

$$CU = (r_i - S)(r_j - S)$$

where: S is the number of shared sites (sites containing both species) and r_i and r_j are the row totals for species i and j. The C-score is the average of all possible checkerboard pairs, calculated for species that occur at least once in the matrix. The checkerboard units form sub-matrices that are exchanged using a swap algorithm (Gotelli & Enstminger 2001a).

A presence-absence matrix of sampling quadrats was constructed for the calculation of C score, rows corresponding to species and columns to unit samples. Another matrix was constructed on data collected from different locations across the investigated protected area.

Significance of calculated C score indices was tested using different null models. The null model is conceived as one that should generate a stochastic pattern with respect to an ecological process of interest such as community assembly (Moore & Swihart 2007). Models with insufficient structure are rejected as non-significant.

EcoSim software ver. 7 was employed (Gotelli & Enstminger 2001b) on 5000 simulated matrices to test the fixed rows and fixed columns sequential swap algorithm, rff (Connor & Simberloff 1979). Swapping creates a new matrix configuration without altering the row and column totals (Gotelli & Entsminger 2001a). Software R, package vegan (Oksanen et al. 2013) was employed to test alternative null models. The employed null models were: r00 null model - maintains the number of presences but fills them anywhere so that neither species nor sites are preserved, a maximally relaxed constrain on 1000 simulated matrices is known to be the least restrictive of the null models employed also in testing nestedness results (Wright et al. 1998); r0 - fills the presences anywhere on rows with no respect to species frequencies, assuming that species are observed with similar frequencies, testing mostly for species commonness; r1 - uses column marginal frequencies as probabilities which are proportional to observed species incidence frequencies, and r2 – fixes the row sums and assigns presences to each columns with probabilities proportional to squared incidence frequencies (Jonsson 2001). There was criticism in literature on r1 model as being prone to type I error (Simberloff & Martin 1991) which has led to r2 model by Jonsson in 2001, supposed to be a better model with respect to significance testing errors. Null communities (Gotelli & Graves 1996) are generated in this manner and test statistic or index is calculated for each generated null community. Null model is tested against the hypothesis that community is structured by interspecific competition or other form of aggregation triggered by some environmental factor.

A measure of departure from the null model of the observed index is given by SES (standardized effect size) which is basically the difference between calculated index (I_{obs}) and the mean of simulated indices (I_{sim}) obtained by sequential swap of the sub-matrices of the form 01/10 divided by the standard deviation of the simulated indices, $(I_{obs}-I_{sim})/SD_{sim}$. Significant departure from null model means that SES must be different from 0 (Sanders et al. 2003) with the probability generated by simulations reported.

4.3. *Niche overlap*

It is a community attribute that can be estimated using different indices, among which Pianka (1973) is one of the most utilized indices. Niche overlap predicts the degree of species interaction in sharing or competing for a food resource and was initially introduced by McArthur & Levins (1967). It is a symmetric index taking values in the range (0:1). EcoSim is testing the calculated index over 1000 simulations providing SES and a probability for assessing the significance of the calculated index using rff as null model, under the sequential swap algorithm.

4.4. *Nestedness*

It is a community univariate metric that illustrates a particular type of asymmetry in species interactions (Bascompte et al. 2003, Vásquez et al. 2005) and is one of the proposed assembly rules which states that subsets of species extracted from samples or local communities are nested within a larger community, at larger spatial scales (Patterson & Atmar 1986). Nestedness is calculated as T or the matrix temperature, a measure of how the presence/absence pattern departs from perfect nestedness; it is testing concomitantly for species incidence and species composition. The basic idea behind nestedness calculation is to assess the state of ordering (Ulrich & Gotelli 2007, Podani 2000) being performed for the present work with software Binmatnest

(Rodríguez-Gironés & Santamaría 2006) which finds the best minimum temperature matrix using a genetic algorithm. Matrix temperature is a normalized sum of squared distances of absences above and presences below the hypothetical isocline that separates occupied from non-occupied area in a perfectly nested matrix. Nestedness is a non-dimensional index and is calculated as N=100-T/100 for a matrix which contains lichen species on rows and sample units on columns. The matrix of interest is ordered according to marginal row and column totals, with most common species placed in the upper rows and species-rich sites placed in the left hand column. The nestedness is expressed as the concentration of presences in the left triangle of the matrix (Ulrich et al. 2009).

Statistical validation was performed against the null matrices using r00, r0, r1 and r2 (for explanations see table 2) in R package *vegan* version 2.0-10 (Oksanen et al. 2013). Null models were considered to exclude the constraint of interest in community assemblage (Gotelli 2001a) being liberal models such as r00 and with increasing degree of constraint in r0, r1 and r2. The interaction matrix was considered nested if the matrix temperature of the observed matrix was below or above the mean temperature of the 1000 simulated random matrices generated under the specified null model (Rodríguez-Gironés & Santamaría 2006).

4.5. Species ordination

NMDS: ordination of locations with respect to species composition was performed using non metric multidimensional scaling in PAST software. The method is based on a distance matrix such Euclidean distance and places the points iteratively in 2D in such a way that ranked differences are preserved (does not take absolute distances into account).

Clustering: similarities in species composition across unit samples and separately across different locations were analyzed using clustering, Ward algorithm (dendrogram generation and all the calculations were performed in PAST). In Ward algorithm the clusters are joint to minimize the within group variance, Euclidean distance measure being inhered in the algorithm.

Results

1. Identified species

Granite containing substrates generally harbor highly acidophilic crustose, umbilicate, fruticose and foliose lichens (Wolseley et al. 2006). Rocks can be compared to islands considering space discontinuities. The composition differs according to lichens positioning on the rock (southward as opposed to northward, on sunny expositions of the rock as opposed to shadowed expositions). However, the main characteristic is the rock type and the plots in this study were selected from granitic or granitoid rocks, rich in silica. Local saxicolous lichen communities are parts of the metacommunity established on granitic rocks within the Măcin Mountains range.

Species richness estimation produced different results at two scales of investigation: at microsite scale, using random sampling quadrats (not more than 5-7 per location) there were identified 34 species and at location scale, using observations made on random walk track, 53 species were identified (Table 1). Sample rarefaction indicates that more intense sampling should be performed at location scale (Fig. 2).

Table 1

Saxicolous lichen species identified in Măcin Mountains National Park, 2006-2008

Species	**Sampling locations***
Acarospora badiofusca (Nyl.) Th. Fr.	C I L M
Acarospora bullata Anzi	H K L
Acarospora cervina (Ach.) A. Massal	I
Acarospora fuscata (Nyl.) Th. Fr	C E F H K L
Acarospora smaragdula (Wahlenb.) A. Massal.	C I L
Aspicilia caesiocinerea (Nyl. ex Malbr.) Arnold	D E F G I K L M
Aspicilia cinerea (L.) Körb	B C F G H I J K
Caloplaca crenularia (With.) R. Laudon	C L
Caloplaca holocarpa (Hoffm ex Ach.) Wade	L
Candelariella reflexa (Nyl.) Lettau	A
Candelariella vitellina f. *vitellina* (Hoffm.) Müll.Arg	A B C D E F G H I J K L M N
Chrysotrix chlorina (Ach.) J.R. Laundon	J
Cladonia foliacea (Huds.) Willd	A C
Collema flaccidum (Ach.) Ach.	J
Dermatocarpon miniatum var. *miniatum*(L.)W. Mann	A C E H I K
Dimelaena oreina (Ach.) Norman	D N
Diploschistes actinostomus (Ach.)Zahlrb.	C
Diploschistes scruposus (Schreb.) Norman	D E F H I K L
Haematomma ochroleucum var. *ochroleucum* (Neck.) J.R. Laudon	F
Lecanora argopholis (Ach.) Ach	A E H
Lecanora gangaleoides Nyl.	A E F K L
Lecanora garovaglioi (Körb.) Zahlbr.	E L
Lecanora muralis (Schreb.) Rabenh	F G H J N
Lecanora silicea var. *silicea* Gyeln.	K
Lecidea fuscoatra (L.) Ach.	E F K L
Lepraria incana (L.) Ach	I K M
Melanelia panniformis (Nyl.) Essl.	A C
Melanelia stygia (L.) Essl.	G
Melanelia tominii (Oxner) Essl.	A B D E F G H I K M
Micarea erratica (Körb.) Hertel, Rambold & Pietschm.	L
Parmelia saxatilis (L.) Ach.	B E F G H I K L M
Pertusaria lactea (L.) Arnold	A F H I L
Pertusaria pertusa (L.) Tuck	D H
Physcia caesia (Hoffm.) Hampe ex Fürm.	F L M
Physconia grisea (Lam.) Poelt	A
Protoparmelia badia (Hoffm.) Haffelner	E H
Ramalina farinacea (L.) Ach.	F
Ramalina fastigiata (Pers.) Ach.	B E G H
Ramalina obtusata (Arnold) Bitter	C G H
Ramalina pollinaria (Westr.)Ach.	C F I L
Ramalina polymorpha (Lilj.) Ach.	F
Ramalina sp.	N
Rhizocarpon geographicum (L.) DC	A B C D E F G H I L
Squamarina cartilaginea (With.) P.James	C

Tephromela atra (Huds.) Hafellner	L N
Umbilicaria grisea Ach.	A
Umbilicaria polyphylla (L.) Baumg.	E
Umbilicaria pustulata (L.) Hoffm	A C
Umbilicaria spodochroa Erh. ex Hoffm.	D E M
Xanthoparmelia conspersa (Ehrh. ex Ach.) Hale	A B F H I L
Xanthoparmelia pulla (Ach.) O. Blanco, A. Crespo, Elix, D.Hawksw. & Lumbosch	B D F H
Xanthoria elegans (Link.) Th. Fr.	J
Xanthoria parietina (L.) Beltr.	J L

*Sampling locations: A - Osmanului Ridge: B - Căpușa Peak: C - Arsu Peak: D - Caramalău Peak: E - Șerparu Saddle: F - Sulucu Mare foothill: G - Stănilă Hill: H - Cheia Peak: I - Vraju Peak: J - Chediu Canyon: K - Chediu Plateau: L - Pietrele Mariei Inselbergs: M- Sulucu Mare Peak: N - Crapcea Peak

Mean number of species in a sampling quadrat was 2.09 and, mean species number per location was 12.71.

Several of the identified species are included in the IUCN list of vulnerable and endangered species:

- regionally extinct in several countries in Europe is *Umbilicaria grisea.*
- critically endangered: *Ramalina obtusata*
- vulnerable: *Acrocordia gemmata, Pertusaria pertusa*

The compiled lichen lists in Măcin are very similar to lists reported from Turkey for Bursa district (Yazici & Aslan 2006) and Şirvan Mountain (Halici & Askoy 2006). In this context, *Melanelia stygia* is cited as being rare for Romania, while *Rhizocarpon geographicum* and *Xanthoparmelia pulla* considered being widespread (Cobanoğlu et al. 2011).

2. Species accumulation curve (sample rarefaction)

The accumulation curve of locations' matrix showed that species richness did not reach an asymptote, several infrequent or rare species still being absent from performed censuses (Fig. 2). Sample rarefaction is recommended to be used when smaller samples or sets of species are nested subsets of larger assembly of species (Gotelli & Colwell 2001).

Since asymptote was not reached, additional sampling would bring new species, contributors to regional species pool. In conjunction with Chao 2 species richness estimator which provides a stopping rule for species richness sampling, our data indicate that further studies will be needed to census fully saxicolous lichen diversity in the investigated area. Chao 2 index yielded a result that indicated that the species richness of the area should have been reached 65.91±8.12 saxicolous lichen species under a more extensive sampling effort.

3.Species co-occurrence

Species co-occurrence estimation at two scales, sampling quadrats and locations indicated that the restrictive *rff* null model discarded competition or other type of species interaction as the mechanism in community assembly (species co-occur randomly) (Table 2).

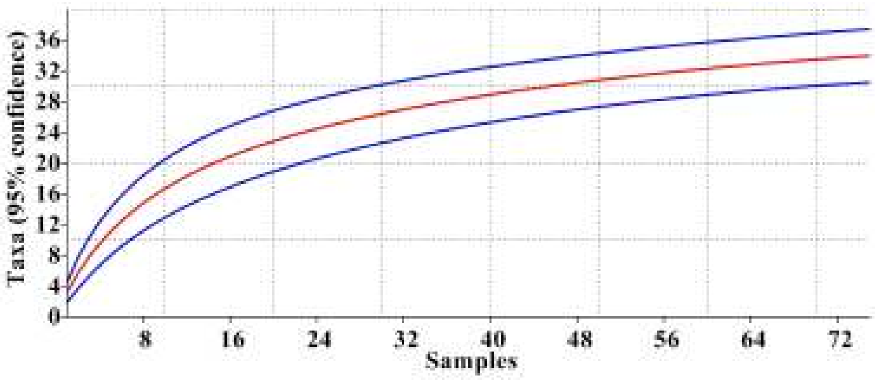

Fig. 2. Sample rarefaction curve of saxicolous lichen communities in Măcin Mountains National Park performed on locations matrix. Graphical representation generated in PAST. Blue curves correspond to confidence interval.

Table 2

Results of C-score index calculation at small scale of sampling quadrats and larger scale of local communities across the Măcin Mountains National Park

C-score of local communities (incidence matrix)				
Null model	Calculated C score	Mean simulated C score	Z score (SES)	P_{simul}
Rff	3.68	3.69	-0.27	0.57NS
R00	52.62	88.05	-14.18	0.01**
R0	52.62	54.24	0.50	0.67NS
R1	52.62	53.64	-0.39	0.59NS
R2	52.62	43.75	2.85	0.01**
C-score of sampling quadrats (incidence matrix)				
Rff	34.69	34.78	-0.29	0.58NS
R00	5.29	7.07	-18.53	0.01**
R0	5.29	7.08	-19.99	0.01**
R1	5.29	4.76	3.32	0.01**
R2	5.29	7.04	3.97	0.01**

On the contrary, null model *r00*, the least restrictive of the employed null models yielded a different result: species co-occur at small and local scale driven by competition or some aggregation promoting interaction factor (SES values were negative), a result yielded by the more restrictive *r2* model also. The restricted null model being considered the most appropriate for assessing C-score results due to its robustness in type I errors (Ulrich et al. 2009) competition or other biotic interactions can be considered as less important mechanisms in community assembly of saxicolous lichens. Species interaction and habitat filtering are more likely to select species at microsite level since the different null models display stable significant results. Being pioneer organisms, lichens colonize the favorable substrata obeying to the preemptive model of competition, first colonizers arriving in a random sequence.

4. Niche overlap

Under the null model rff, niche overlap estimated by Pianka index yielded the observed mean index of 0.07 for sampling quadrats (simulated mean of 0.063 after 5000 iterations, SES=4.09, $p \leq 0.001$), and of 0.20 for locations (simulated mean of 3.69, SES=-0.27, $p \leq 0.006$). Both results are significant and indicate a relative separation of species' niches, more clearly in the case of microsite level observations. The results support the non-competitive nature of community assembly (still, the presumed mechanism to be considered, preemptive competition).

5. Nestedness

Nestedness temperature ($N_{local\ com}$=0.63; $N_{sampling\ quad.}$=0.88; see table 3 for nestedness temperature values) reflects the fact that at microsite level communities appear to be more structured than at local communities' level (Fig. 3A). However, the results are unstable according to employed null models (Table 3). The recommended r2 null model for testing nestedness significance (Jonsson 2001) yielded a significant result in the case of local communities and highly significant in the case of sampling quadrats. The richest local community was located at Pietrele Mariei inselbergs while the poorest, appeared to be at Stănilă Hill (Fig. 3B). Pietrele Mariei inselbergs are massive, relatively continuous rock formations while other locations are characterized by fragmented rocks, tors, exfoliated and weathered rocks supporting different abundance of saxicolous lichen species. The most frequently encountered species were: *Candelariella vitellina, Parmelia saxatilis, Rhizocarpon geographicum, Lecanora gangaleoides, Pertusaria lactea, Acarospora bullata, Physcia caesia, Ramalina polynaria, Acarospora smaragdula, Tethromela atra, Xanthoria parietina, Lecanora garovaglioi, Caloplaca crenularia* and *Caloplaca holoplaca.*

Table 3

Results of nestedness temperature calculation at small scale of sampling quadrats and larger scale of local communities across the Măcin Mountains National Park

null model	$NT_{calc.}$	Mean simulated NT	Z score (SES)	Matrix fill	P_{simul} (1000 iterations)
Nestedness temperature of local communities incidence matrix					
R00	36.908	53.23	-3.17	0.23	0.0009***
R0	36.908	22.202	4.116	0.23	0.0009***
R1	36.62	33.49	0.77	0.23	0.46 NS
R2	36.94	22.75	4.22	0.23	0.02**
Nestedness temperature of sampling quadrats incidence matrix					
R00	11.03	25.25	-5.74	0.09	0.0009***
R0	11.13	10.12	0.78	0.09	0.44NS
R1	11.19	7.64	3.64	0.09	0.0009***
R2	11.11	5.34	7.19	0.09	0.0009***

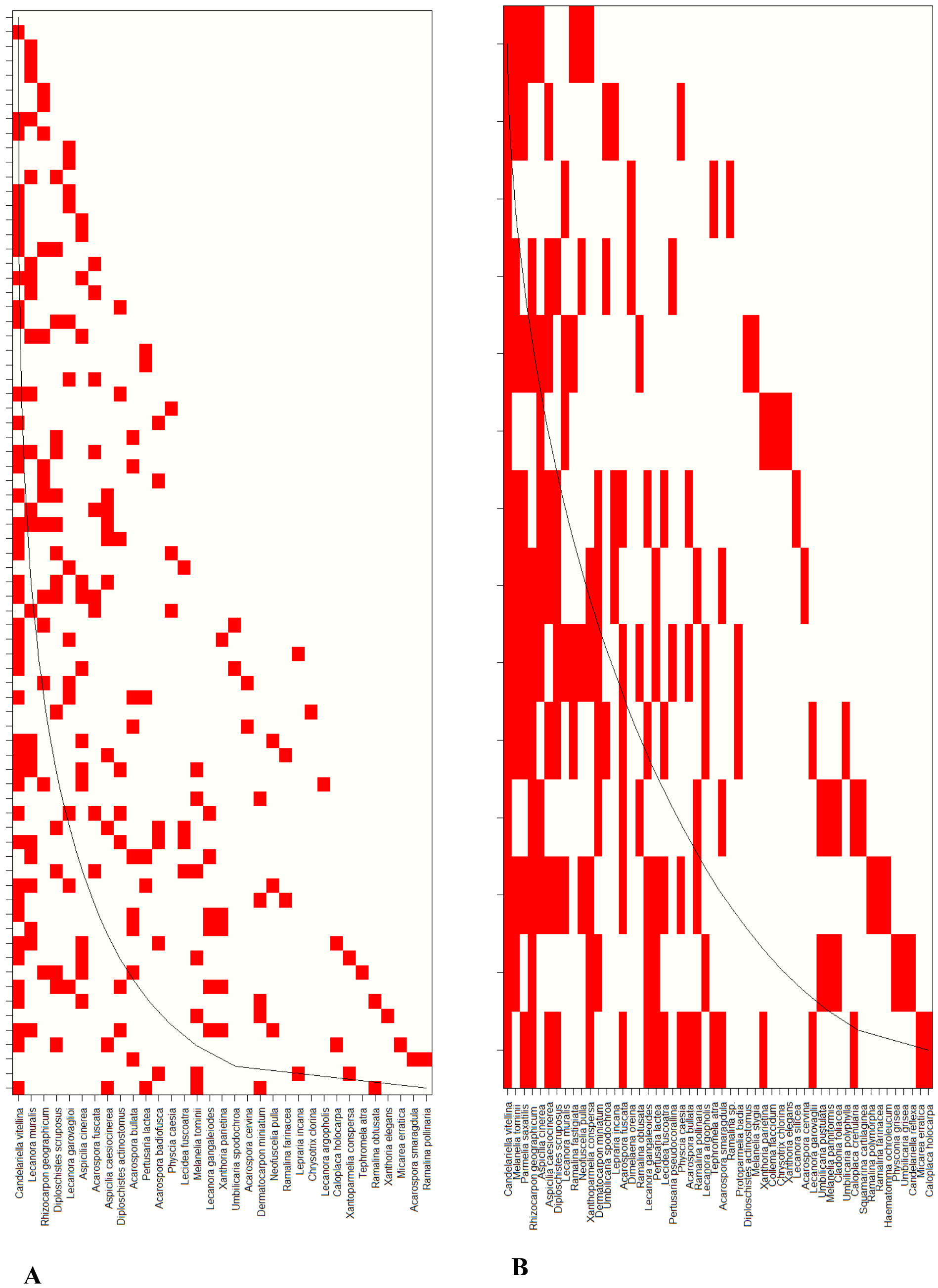

Fig. 3. Nested matrices of sampling quadrats (A) and local communities (B). Curves correspond to isoclines of perfect nestedness

6. Species ordinations: NMDS and clustering

Locations characterized by dissimilar lichen communities according to the performed ordinations (clustering analysis and non-metric multidimensional scaling, were: Osmanului Ridge, Arsu Peak, Pietrele Mariei Inselbergs, Sulucu Mare foothill, Cheia Peak, Chediu Canyon and Șerparu Saddle. The site differences are indeed significant: Chediu Canyon is more humid and harbors abundant woody vegetation interspersed with cliffs and outcrops, other locations are more xerophytic, with steppe and sparse woody vegetation, with exposed rocks and various degrees of anthropogenic influences. The dissimilar locations in terms of species composition cluster in two separate clusters merging Pietrele Mariei inselbergs with Sulucu Mare Foothill and a separate cluster merging Osmanului Ridge and Arsu Peak characterized by xerophytic steppe vegetation and sparse woody vegetation exposed to anthropogenic pressure from surrounding agricultural lands, Chediu Canyon and Crapcea Peak with alternate mesophytic forests and steppe interspersed with sparse woody vegetation (Figs 4, 5).

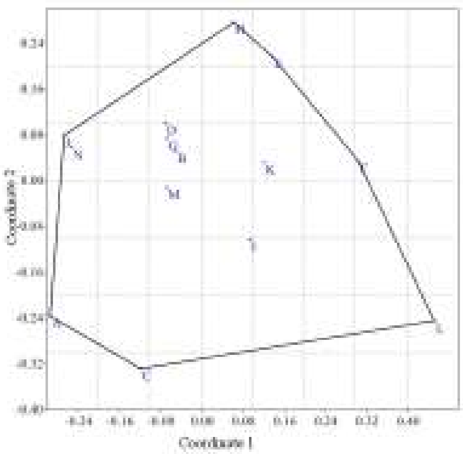

Fig. 4. NMDS ordination (using Euclidean distance) performed on saxicolous lichen communities identified in 14 different locations in Măcin Mountains National Park. Notations same as in Table 1

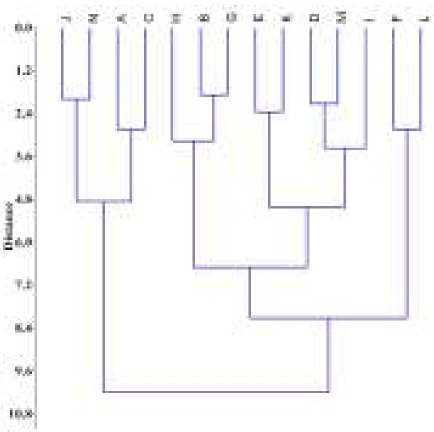

Fig. 5. Clustering of locations according to saxicolous lichen species composition in Măcin Mountains (Ward agglomerative algorithm). Notations as in Table

The remaining locations harbor lichen communities sharing many common species under more similar environmental conditions (exposed peaks with ruiniform landscape, scattered outcrops and steppe vegetation).

Discussion

Saxicolous lichens in Măcin Mountains establish a metacommunity (sensu Leibold et al. 2004), with local patches whose composition vary according to substrate (various granitic rocks), by the nature of the rock, light availability, competition with other lichen species or plants, moisture level (Will-Worf et al. 2004), and various anthropogenic influences such as eutrophication. Metacommunities are characterized by species with unequal local abundances, with more frequent species in the area to be more likely to colonize a new patch of habitat, larger sites harboring many species (Ulrich et al. 2009). Same pattern was observed in the case of saxicolous lichens in Măcin Mountains range.

A section of granitic boulders harbors 10-15 species of lichens as cited for Canada (Hale 1982). It is considered that lichens occupy surface at scales corresponding to microhabitat variation. At scales larger than 0.01-0.25 m^2 the distribution of lichens will be heterogeneous. Our observations performed on quadrats *in situ*, *ex situ* (on photographs) and on small rock fragments indicated that the average number of species was lower, around 2.09. Also, community composition differed strongly among microhabitats, the degree of structuring being correlated to higher nestedness highlighting the non-random assembly of species at small scale.

The local communities displayed nestedness considering that rocks and boulders represent a highly fragmented habitat. It is a pattern of species co-occurrence intrinsically related to species aggregation but different mechanisms can lead to nested patterns, stochastic and deterministic (Ulrich et al. 2009), lichens displaying the both. Propagula dispersed by wind, random arrival on a substrate and then the influence of preemptive competition between thalli are the mechanisms leading to community establishment on rocks. The demonstrated competition in lichen guilds (Armstrong & Welch 2007) is mostly preeemtive (Hestmark et al. 2007) in nature but it is not the only species interaction observed among lichen thalli. Competition is largely overrated since biotic interactions include other negative and positive types such as parasitism or facilitation. *Diploschistes scruposus*, *Candelariella vitellina* and *Rhizocarpon geographicum* are initially lichenicolous, parasitizing other lichens (Will-Wolf et al. 2004). So far, these species are among the most frequently identified on granitic rocks of Măcin Mountains. At small scale, community appears to be assembled by dispersal and the ability to establish given the abiotic combination of factors, a mechanism observed in other types of communities also (Lindo et al. 2008) being an explanation for the nested community structure. Modest overlap of species niches is consistent with non-significant competition in the case of saxicolous lichens, the most probable competition mechanism to be considered being preemptive competition.

Testing nestedness under the assumptions of several null models with increasing constrain in randomness generating process in order to comply to some biological criterion indicated that r00 null model, the least restrictive provided significance to nestedness result in both matrices, one to summarize species richness at local scale and the one based on sampling quadrates. R0 null model test the community structure with focus on species commonness, and nestedness of the empirical matrices again departed significantly from random. This null model generates pattern under the assumption that species vary in terms of commonness and rarity but sites are characterized by the same species richness (Jonsson 2001). Null models that incorporate constraints (r1 and r2) on row and column totals address more subtle structural characteristics than previous models namely the mechanisms generating the actual composition of community such as competition, niche partition and nestedness. The most conservative of the models with fixed row and column sums was successfully applied to test the significance of co-occurrence metrics (C-score is one example) (Gotelli 2001a) and more recently nestedness (Moore & Swihart 2007). However, in testing C-score the rff null model is largely employed because it is robust to type I errors and in testing nestedness r00 and r0 are the preferred null models to be tested. According to the more stringent rff model, saxicolous lichen communities in Măcin area are not driven by competition but are significantly nested at both scales (under the null model r2).

Lichens are considered stress tolerant organisms according to Grime classification (1979) and competition plays an important role in community structure (Armstrong & Welch 2007) but co-occurrence analysis has failed to extract competition mechanism as important for saxicolous lichen communities. If equiprobable null models are used, nestedness and co-occurrence scores such as C-score produce similar, significant results; under less liberal null models this correlation does not hold (Ulrich et al. 2009) as our results indicate too.

Cliffs and rocks support diverse lichenological guilds together with rare and diverse vascular plants (Matthes et al. 2004). Umbilicate lichens as *Umbilicaria spodochroa, Umbilicaria grisea, Umbilicaria pustulata* or *Dermatocarpon miniatum* are prone to destruction due to climbers (on rocks) and sheep grazing (on boulders). Among the crustose lithophilous lichens, species display different degrees of tolerance toward anthropogenic stress: *Acarospora fuscata* is tolerant, the infrequent *Melanelia panniformis* is sensitive (Paukov & Trapeznikova 2004).

Among fruticose lichens building extended covers on boulders and rocks was observed *Xanthoparmelia conspersa* at Șerparu Saddle, a species known to react with the substrate, leading to the rock exfoliation (Schatz 1963), one of important geomorphological characteristics of the area.

For lichens vegetating on acidic rocks, one of the most threatening factors are nitrogen deposits. Nitrogen enrichment is caused by grazing sheep and their excrements. Frequently, in saxicolous lichen thalli were found parasitical Ixodidae demonstrating the intense grazing in the boulders' vicinity. Agricultural activities influence lichen guilds composition directly by destruction or indirectly by eutrophication; consequently lichen communities in agricultural landscapes are severely impoverished (Motiejûnaitë & Faútynowicz 2005). Most diverse saxicolous lichen communities are associated in Măcin with peaks and plateau rocky environment were grazing pressure is not so high. For instance species from genera such as *Parmelia* and *Ramalina* are relatively indifferent to levels of acidification or nitrogen addition, *Xanthoria parietina* is nitrophyte (Sparrius 2004) and is found on rocks in the majority of locations being accordingly, tolerant to stresses induced by anthropogenic activities in the area. However, *R. geographicum* is one of the nitrophilous and acidophilous species encountered also on man-made substrates such as walls, consequently tolerant to anthropogenic disturbance. *Parmelia saxatilis* is acidophilous and *Candelariella vitellina, Acarospora fuscata, Tephromela atra* are nitrophilous their association being linked elsewhere with granite boulders in pastures and arable lands (Wolseley et al. 2006). The nitrophilous associations could be used as indicators for agricultural pressure on adjacent natural habitats harboring saxicolous lichen communities. It was previously stressed that the agriculture based on ploughing is a serious threat to protected areas in Măcin Mountains (Badiu et al. 2014) due to the specificity of the landscape: the mountains are immersed in a matrix of cultivated land and are intensely grazed by sheep lately.

Because of the slow growth and the environmental sensitivity of most lichen species, a habitat approach to their conservation is a practical approach (Knudsen & Magney 2006). Lichen guilds cover the substrate in various proportions which is sometimes an indication of successional stage in rock colonization. The cover for different crustose species on rocks depends on life history, initial growth rate, maximal size possible to be attained, maturation, death rate and propagule type. Covers in the investigated area differ significantly depending also on exposition and disturbance regime. High mortality was observed on top areas of the boulders (*Melanelia tominii, Xanthoparmelia saxatilis* thalli) where the desiccation is most severe, proving once more that rocks represent an extreme environment.

It is worth to make efforts toward saxicolous lichens' conservation for their multiple functions: increase of structural complexity, influence on nutrient cycles, providing habitats for various invertebrates and nesting material for mammals and birds (Perez et al. 2004).

Advocating lichens' conservation, one argument refers to their scenic beauty, argument that Knudsen & Magney (2006) consider that has been neglected in the past and must be taken in consideration in the present in conservation plans.

Acknowledgments. The present study is dedicated to those who taught me during my student years at the Biology Faculty of the University of Bucharest, among them the celebrated Professor, Mr. Ioan Cristurean. Also I am deeply grateful to the workers at the Măcin Mountains National Park administration for their support during the study which was developed in the framework of the GEF/UNDP project*: Strengthening the National System of Protected Areas in Romania by Demonstrating the Best Management Practices in the Măcin Mountains National Park* (2006-2008). The majority of the photographs were taken by Ovidiu Hâruța, from the University of Oradea. He also helped with sampling in the field and constructed the map of lichens' location in Măcin Mountains National Park, for which I am very grateful.

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Acta Horti Bot. Bucurest. 2015, **42**: 87-96
DOI: 10.1515/ahbb-2015-0006

THE OCCURRENCE OF MARINE ALGA *ENTEROMORPHA INTESTINALIS* IN WATER COURSES FROM SALT AREA MELEDIC PLATEAU, VRANCEI SUBCARPATHIANS (ROMANIA)

STRAT Daniela[1]

Abstract: Three new sites of *Enteromorpha intestinalis* have been found in the drainage basin of the Slănic River located in the Subcarpathians region, salt Meledic Plateau. *E. intestinalis* is a cosmopolitan macro green alga species with tubular thali that is primarily found in the coastal zone, including the Romanian Black Sea coast. Due to its salt tolerance this alga it was found in some inland waters, both fresh and saline waters courses and limnic waters that are often positively correlated with cultural eutrophication. These new reported localities of *E. intestinalis* in inland waters from this saline region contribute new and essential information about the distribution of this originally marine species on the inland area of Romania.
Keywords: *Enteromorpha intestinalis* (L.) Nees 1820, water courses, salt diapir, salt karst, Meledic Plateau, Slănic River

Received 13 October 2015 *Accepted 11 December 2015*

Introduction

Enteromorpha Link (1820), the gut weed, is one of the best known marine green algae genus. Its habit is easily recognizable: tubular thallus, gas or liquid-filled, with the wall of the tube made by a single cell layer. Initially, *Enteromorpha* grows attached to the substrate but then, mature thalli, filled with air, can become free floating. The thallus can be branched or unbranched and in case of some species it can reach until two meters in length and 1-1.5 cm wide (Horincar et al. 2011).

According to Index Nominum Algarum there are 135 *Enteromorpha* species (Hayden 2003). Additionally, there are subspecies, varieties and forms but they are very difficult to identify because morphological differences between taxa are often slight (Tan et al. 1999, Hayden et al. 2003, Leskinen & Pamilo 1997, Leskinen et al. 2004).

Enteromorpha grows attached to the substrate by a disc like holdfast and the thallus of some species can reach until two meters in length (Horincar et al. 2011). Although, based on the gross morphology – tubular monostromatic thallus – *Enteromorpha* was considered for a long time a separate genus of green algae family Ulvaceae, molecular and culture data (Tan et al. 1999) fallowed by nuclear ribosomal internal transcribed spacer DNA analyses have provided strong evidence that *Ulva* and *Enteromorpha* genera are not distinct evolutionary entities and should not be recognized as separate genera. For this reason *Enteromorpha* must be reduced to synonymy with *Ulva*

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as originally circumscribed by Linnaeus (1753) (Tan et al. 1999, Hayden et al. 2003). After the *Enteromorpha* genus was merged with *Ulva* by Hayden et al. (2003), in the last decades, an increasing number of authors in their papers has used synonym *Ulva* instead of *Enteromorpha* genus.

Few species of *Enteromorpha,* with distinctive hollow cylindrical thalli, can proliferate in inland waters (slow-flowing rivers, ponds and lakes) as a response to cultural eutrophication (Taft 1964, Pfiester et al. 1976, John et al. 2002, Messyasz & Rybak 2009, Rybak et al. 2011). Also, few of them are cosmopolitan euhalinity species with a wide distribution in marine, freshwater and brackish water environments throughout the world like *E. intestinalis* (Black & Weeks 1972, Pfiester et al. 1976, Vladimirescu 2007, Messyasz & Rybak 2008, Messyasz 2009, Moore 2009), *E. compressa* (Messyasz 2009) and *E. flexuosa* subsp. *pilifera (*Mareš 2011). *E. flexuosa* subsp. *pilifera* (Kützing) M.J. Wynne 2005*,* which is a dominating taxon from the *Enteromorpha* genus in freshwater ecosystems of Europe (Messyasz et al. 2015), has been reported as an exclusively freshwater alga in Europe (Christensen 1994) and North America (John & Rindi 2015). It is often present in nutrient-rich (eutrophic) ponds and lakes, commonly as free-living floating and irregularly wrinkled tubes with short uniseriate branches (Burkholder 2010). However, distinctly, all freshwater members of *Enteromorpha* genus are elongated, tubular and usually branched (John & Rindi 2015).

Some studies emphasize that the occurrence of *Enteromorpha* species in different inland water bodies are very often associated with anthropogenic sodium chloride pollution and nutrient enrichment (Zimmermann-Timm 2007, Messyasz & Rybak 2009, Messyasz & Rybak 2011). Furthermore, during 20th century, freshwater *Enteromorpha* populations located in inland ecosystems that are not in contact with salt waters have been recorded in at least eight countries from worldwide (Messyasz & Ryback 2011).

Also, marine species of *Enteromorpha* genus, especially *E. intestinalis* were reported in inland athalassic salty lakes (Savage 1964, Taft 1964, Pfiester et al. 1976, Conner et al. 1978, Hammer 1981, Reinke 1981, Hammer & Heseltine 1988, Eimanifar & Mohebbi 2007, Kaštovský et al. 2010, Gheorghievici et al. 2015, Messyasz et al. 2013), hypersaline springs and rivers (Velasco et al. 2006, Messyasz & Rybak 2011, Millán et al. 2011), streams and lagoons receiving effluents from salt mines (John & Rindi 2015). However, based on literature review, the most widespread species of *Enteromorpha* genus in inland waters is *E. intestinalis*. Both field and laboratory observations have showed that key chemical factors affecting colonization and growth of *Ulva* species in inland waters as well as the length, the wide and branching thallus are sodium chloride, phosphate and nitrite concentrations (Messyasz & Rybak 2011). High concentrations of sodium chloride inhibit the development of *Enteromorpha* sp. while high nitrite concentrations were positively correlated with an increase in the number of thalli and the thalli length (Messyasz & Rybak 2011).

In Romania, apart from the Black Sea coast (Cărăuşu 2012, Nastac et al. 2014), the occurrence of *E. intestinalis* was recorded both in inland freshwaters (Vladimirescu 2007, Cojocaru 2012) and athalassic (Gheorghievici et al. 2015) and anthroposaline lakes (Horotan 2010).

In this paper we report occurrence of macroalga *Enteromorpha* sp., tubular form thallus, in two rivers and one stream that drain a salt diapir from Vrancei Subcarpathians. A brief description of the habitat where it has been found is presented.

Material and methods

In August and September 2014, during a geomorphological field work on Meledic Plateau, it were found typical vividly green tube shaped thalli of *Enteromorpha* in the Slănic River and the Meledic Stream. A year later, on 3 August, it was found *Enteromorpha* thalli in the superior course of the Jghiab Stream bed, a right side tributary of the Slănic River. For each occurrence geographical coordinates were recorded with GARMIN GPSMAP 64s. Macroscopic observations related to length, diameter, ramification, color, and general appearance of thalli were performed. Also, abiotic environment observations were made. A hand lens (18 mm – 10 X) was used to for complementary examination of thalli in the field.

Results and discussion

The Meledic Plateau Salt is located in the Vrancei Subcarpathians. From geological point of view, the plateau belongs to the Mânzăleşti salt formation that was accumulated in Early and Middle Miocene (Săndulescu 1984). The Meledic Plateau was cropped from a salt massif by four valleys: the Slănic River valley in the south, the Meledic Stream valley in the north, the Jghiab River valley in the east, and the Sărata Stream valley in the west (Fig. 1). On the south-west side of the Meledic Plateau, which corresponds to the right slope of the Slănic River valley, the salt massif or diapire outcrop is named Muntele de Sare. From it, salt springs that flow to the Slănic River generate salt crusts on the geographical left river bed side which are washed out during high flows. The salt is mixed with clays and salt breccia and covered by clays and other soft sedimentary rocks.

During rainy events, all salt outcrops are subjected to runoff and dissolution. The water with high dissolved salt load is collected by streams and drained to the marginal rivers and salt caves. Some caves drain directly to the margins of the plateau, which are the local base level of drainage. Except east edge of the plateau, from all other sides emerge brine springs that are directly and indirectly collected by Slănic River. Underground salt dissolution and suffusion has been generated caves and dolines which change the surface topography of the plateau. Above ground, many dolines have become freshwater lakes and small wetlands with a great biodiversity.

Because salt deposits are quite impure they are not economically valuable for exploitation. However, in the whole area the human pressures are low thus a great diversity of genuine salt karst features exists. Due to geological, geomorphological (Giurgiu 2010, Mărunţeanu & Ioane 2010, Irimia & Irimuş 2012) and biodiversity value (Sava et al. 2010) the west side of Meledic Plateau and a small section of Jghiab Valley, an area who covers 151 ha, was designed national protected area according to Law 5/2000 (Official Monitor 152/12.04. 2000). Later, in 2008, around 81 % (136 ha) of the protected area became part of Site of Community Importance Platoul Meledic (ROSCI0199 Platoul Meledic), mainly because of the lepidoptera *Lycaena dispar* (Haworth 1803) and Ponto-Sarmatic deciduous thickets habitat, 40C0* code Natura 2000 (OM MMDD nr. 1964/2007).

The altitude of Meledic Plateau ranges between 450 and 600 m. The climate is middle temperate. Average annual air temperature is 11°C and annual sum of precipitation is around 565 mm with torrential episodes during the summer.

Fig. 1. The Meledic Plateau and the geographical position of sites with *Enteromorpha intestinalis.* 1 – The Slănic River; 2 – The Meledic Stream; 3 – The Jghiab River

Occurrence sites of *Enteromorpha intestinalis* (L.) Nees 1820

Green tube-shaped thalli of *Enteromorpha* sp. were found in three water courses from Meledic Plateau area (Fig. 1) that is located in Vrancei Subcarpathians. Based on gross observation of thalli, abiotic environment assessment (geological and geomorphological characteristics) and literature review we suggest this alga is *E. intestinalis*. The occurrence sites of this alga are:

1. The Slănic River site. Between Lopătari village and the confluence with the Jghiab River, the Slănic is a braided river. It is shallow and flows in two or more channels around alluvial (gravel and sand) exposed and submerged bars. *Enteromorpha* thalli were found in a subsidiary channel with slow flow, very close to right river bank that is made by salt. Because in vicinity of site there is a sheepfold, probably there is an input of nitrogen as a result of nitrate leaching from manure**.** Dense, branched and submerged thalli were attached to cobbles and pebbles (Fig. 2). Only few of them were free-floating and they have looked like intestinoid tubes with light green-yellow color. Most of thalli reached a length of up 50 cm and around 0.3 – 0.5 cm wide. Geographical coordinates of site with the occurrence of *Enteromorpha* are: 45°29'01" N, 26°36' 35"E, 433 m.a.s.l., opposite to the Săreni village.

2. The Meledic Stream site. In the Meledic stream (Figs 1, 3) *Enteromorpha* thalli were found along 200 m of course water in sheltered areas. There were found both types of thalli: attached to the substrate and as well as free-floating intestinoid clusters (Figs 4, 5) in sheltered site sections with very slow flowing, almost stagnant, and soft river bed. Thalli were not branched. Their length ranged up to 1 m and the diameter was frequently 0.5 cm. Thalli of *Enteromorpha intestinalis* occurred in water curse section when the Meledic Stream crosses salt deposits and salt springs flowing into its channel. In the upper catchment of the Meledic stream, upstream to occurrence of *Ulva intestinalis*, there are two sheepfolds (in area of Trestioara village) which contaminate water with manure. Geographical coordinates of the site (Fig. 1) are: 45°30'13"N, 26°37' 65"E, 488 m.a.s.l.

3. The Jghiab River site. Free-floating and attached *Ulva* thalli were found in milky water of the Jghiab River from its upper catchment, right upstream to the confluence with Bisocuța Stream (Fig. 1). River bed is covered by cobbles, boulders and clay. In this area, the Jghiab River has dissected a salt diapir that was exposed by erosion as outcrops on both sides of valley. Springs salt flow in river thereby contributing to change of sodium chloride concentrations of water. All thalli were poorly branched and rarely exceeded 1 m in length (Fig. 6). Geographical coordinates of the site are: 45°31'86"N, 26°38'51"E, 483 m.a.s.l.

Fig. 2. Enteromorpha intestinalis attached to cobbles from river bed of subsidiary arm of the Slănic River. The amphibian is a *Bombina* sp.

Conclusions

Enteromorpha tubular thalli were found in three water courses from Vrancei Subcarpathians, Slănic upper drainage basin, the salt Meledic Plateau region. All sites are located in sections of water courses where they are naturally supplied with sodium chloride derived from dissolution of geological substratum (salt and saline rocks). Based on gross morphology, the tubular algal thalli belong to *Enteromorpha intestinalis* species and its occurrence in this area is supported by sodium chloride concentrations of water due to the chemical characteristics of the physical environment. Vectors who have contributed to migration of this marine species into inland waters from this area are unknown. However, observations on microscopic anatomy of thallus are further required in order to confirm the species identification. The occurrence of this marine macro alga in Meledic Plateau region adds value to the biodiversity of the two protected areas that were designed within – The Meledic Plateau Natural Reserve and the Natura 2000 site ROSCI0199 Meledic Plateau – that are partially overlapped.

Fig. 3. The Meledic Stream valley – upstream view. The steep slope is an outcrop of salt diapir. During the time without rains, especially in the warm season, a salt crust appears along to the salty springs that flow into Meledic Stream

Fig. 4. Mat of *Enteromorpha intestinalis* thalli in Meledic Stream

Fig. 5. Close view of *E. intestinalis* that was found in the Meledic Stream

Fig. 6. Attached and free-floating thalli of *E. intestinalis in* the Jghiab River in the area where its water is enriched with natrium chloride provided by salt springs

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